Nutrient Composition and Medicinal Properties of Plant Foods Consumed by Ethnic Groups in Chittagong Hill Tracts

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I dedicate this thesis to my beloved family for their endless support, constant encouragement and unconditional love.

Certificate

This is to certify that the thesis entitled Nutrient Composition and Medicinal Properties of Plant Foods Consumed by Ethnic Groups in Chittagong Hill Tracts has been completed sincerely and satisfactorily by Parveen Begum, Registration No. 68 Session 2011-2012 enrolled in University of Dhaka, Dhaka – 1000, Bangladesh for the fulfillment of the degree of Doctor of Philosophy (PhD) in Nutrition and Food science was supervised by us and can be submitted to the examination committee for evaluation.

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Preface

Bangladesh is an agriculture based country. Agriculture produces around 90% of its food including cereals and vegetable (WFP, 2010).Vegetables are important for nutritional, financial and food security in Bangladesh. However, the present consumption of fruits and vegetables in Bangladesh is 126 g/day/capita (23g leafy vegetables, 89 g non-leafy vegetables and 14 g fruit), which is far below the minimum average requirement of 400 g/day/capita (FAO/WHO 2003).As our country is so fertile, so that varieties of vegetables grown in Bangladesh all over the year.

Food habits of people in different regions of the world depend on the available local foods and social behaviors. The ethnic people of Chittagong Hill Tracts (CHTs) of Bangladesh consume almost all of the tender leaves, and foods those consumed by monkey and birds, this help them in selecting edible and poisonous foods and thus, foods they eat are naturally screened. Epidemiological and clinical studies document the relationship between diet and health. Plant foods are rich in micronutrients and people consuming it have a lower incidence of diseases.

Reliable data on the nutrient composition of foods consumed by people are critical in many areas such as health assessments, formulation of appropriate institutional and therapeutic diets, nutritional education, training and research, plant breeding and food manufacturing. A food composition database (FCD) provides essential information on the nutritive value of foods. It provides values for energy and nutrients (e.g. protein, Fat, Carbohydrate, vitamins and minerals) and other important food components or bioactive compounds that are important for human nutrition. On the whole FCD provides the basis for planning food, nutrition and health related policy tools. The earliest known food composition table was produced in 1818 (Somogyi, 1974). The current knowledge of nutrition is still incomplete, and studies are still required, often at ever increasing level of sophistication, into the composition of foods and the role of these components and their interactions in health and diseases (Greenfield and Southgate, 2003a).

The association between human beings and plants possibly started from the first day of the advent of human beings. Plants not only provide human beings with essential nutrition, but also provide medicinal values. The indigenous people of various countries of the world, through their living amid nature, have first-hand knowledge of the various benefits provided by plants, even though they may not be aware of the rationale or the causes behind the benefits provided by any particular plant. From time immemorial, indigenous people have used plants not only for treatment of various ailments, but also have used plants as preventive measures against occurrence of various ailments. Modern science has recognized this in recent times, and various plant-derived products are now available commercially and are known as functional foods, nutraceuticals, preventive medicines, or pharma foods. In fact, a functional food is generally defined to be 'foods that provide health benefit beyond its traditional content of nutrients". Consumption of these plant products are presumed to provide nutritional, therapeutic and preventive benefits, besides avoiding the side-effects of modern drugs, which drugs may be effective from the sense of healing a disease but can cause at the same time numerous deleterious consequences (Rahmatllah *et al*, 2011).

Indigenous and ethnic plants that are widely used in folk-medicines are numerous and diverse. In Bangladesh, 787 plants species have been identified as medicinal plant having therapeutic properties (Yusuf *et al*., 2009). Despite development of modern medicine, many rural people of

Bangladesh still depend on plant products and herbal remedies for treatment of their ailments. Although reports of antibacterial activity of indigenous plants have been published from many regions (Nadkarni, 1908; Dhar *et al*., 1968), they have not been systematically conducted, except in a few cases, thereby leading to confusion in drawing meaningful conclusions (Padmaja *et al.,* 1993; Ndamba *et al*., 1994; Vijaya *et al.,* 1995). Researchers are increasingly turning their attention to folk medicine looking for new leads to develop better drugs against viral and microbial infections (Hoffmann *et al.,* 1993). Although hundreds of plant species have been tested for antimicrobial properties, the vast majority have not yet been adequately evaluated (Tshikalange *et al*., 2005; Dalmarco *et al.*, 2010).

The medicinal properties of plants are due to some chemical substances that produce certain definite physiological action on the human body (R K Shah & R.N.S. Yadav, 2015).

Nutrition Science attempts to isolate biologically active principles in foods with potential to improve health and to reduce the risk of diseases. Food Composition Table (FCT) for Bangladesh has very recently been published (Shaheen *et al*, 2013). Preparation Food Composition Database (FCD) and FCT have been launched (FAO, 2010; Islam *et al*, 2012). Comprehensive Food Composition Survey (CFCS) and Focus Group Discussions (FGDs) data document that ethnic people uses a variety of their foods in treatment of many ailments (Islam *et al*, 2010). By far, none of these ethnic foods has been explored for their medicinal principles. Medicinal plant foods provide this opportunity to improve the health status, reduces health care costs and support economic development in rural communities.

This study aimed to analyze nutrient composition and medicinal properties of some selected ethnic foods. In line of its objective, it has been designed to

Analyze Nutrient Composition of selected ethnic vegetables and screening of medicinal properties of some selected ethnic plant foods. In accordance research work has been designed into three sections:

Section-A: Nutrient composition of selected ethnic plant foods.

Section-B: Medicinal properties of some selected ethnic plant foods.

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Abstract

In Bangladesh, the use of traditional medicinal is widespread among most of the ethnic people and village dwellers (IUCN,2011) The use of natural product or natural products based medicine is increasing all over the world especially in the developing countries like Bangladesh, India, China and The Middle East. About 25% of prescribed drugs in the world are of plant origin (Abul Khair *et al*, 20014. According to WHO, any plant could be medicinal that contain substances which can be apply for the production of useful drugs (Junaid *et al*, 2006). The medicinal properties of plants are due to some chemical substances that produce certain definite physiological action on the human body (R K Shah & R.N.S. Yadav, 2015).

Ethnic people are healthy and hardworking, have less morbidity and higher life expectancy. This may be because they consume almost all of the wild plant and animal foods, particularly those consumed by monkey and birds. This helps them in selecting edible and poisonous foods and thus, makes the natural screening of edible foods. The wilds foods are rich in health promoting nutrients.

This study aims to investigate nutrient composition and medicinal properties of selected plant foods consumed by ethnic people. Twenty five plant foods comprising 19 leafy and 6 non-leafy foods were included; particular emphasis was given in selection of the foods, which are reported and using in their different ailments. Comprehensive food consumption survey (CFCS) and Focous group discussions (FGDs) data were used in this grouping. The selected ethnic foods were collected from weekly local markets at Bandarban Rangamati and Khagrachari. Three food samples were collected for each food from every market. Taxonomic study was performed to confirm the identification of the collected foods. In the analysis of nutrient composition, contents of micronutrients and proximate nutrient were estimated employing standard methods. Carotenoid, vitamin C, total phenol, phytate contents were estimated by spectrophotometry, carotene profile and B vitamins were analysed by HPLC and mineral content was determined by atomic absorption spectrophotometry. Proximate composition was estimated by AOAC methods.

It was noted that Mrolapying (*Manihot esculenta* Gantz) contained highest amount of carotenoid (9337µg/100g edible); Missayanu (*Sarcochlamys*) possessed highest amount of both β-carotene and α-carotene equivalent to 5673 and 703.9 µg/100g edible. Highest amount of vitamin C was present in Mori shak (*Foeniculum vulgare*) (68mg/100g edible). Highest content of vitamin B_1 (0.72mg/100g edible), B_2 (0.82mg/100g edible) and B_3 (5.77mg/100mg edible) were possessed in Mo alu (*Dioscorea bulbifera L.*), Mrolapying (*Manihotesculenta* Gantz), and Fala (*Alpinia nigra (Gaertn)*) respectively. Kiokokkro (Not Known) contained highest amount of calcium (1338mg/100g edible) and iron (14.6mg/100g edible). Forash dal (*vigna grahamiana*) contained highest amount of copper (138mg/100g edible), zinc (3.8mg/100g edible) and phosphorus (382mg/100g edible). In proximate analysis, it was indicated that highest value of protein (23.85g/100g), fat (2.56g/100g), ash (2.41g/100g), dietary fiber (5.06 g/100g) respective in Forash dal (*Phaseolus vulgaris*), Kamino (*Caesalpinia digyna* Rottler), Forah dal (*vigna grahamiana*), Missayanu (*Sarcochlamys*).Total phenol content in Kanimo, Missayanu, Mo alu was around 200mg GAE/100g. Phytate content was noted highest (72.18mg/100g) in Forash dal and lowest in Balapata (*Pouzolzia hirta (Blume)*) (5.09mg/100gedible).

In the case of medicinal property screening antibacterial activity was estimated by disc diffusion method (Islam *et al*., 2002) 10 plants foods were randomly selected for antbcterial analysis. Fourteen strains of pathogenic bacteria (6 gm+, 8 gm-, including *bacillus, shigella*, *klebsiella*, *E-coli*, *salmonella*, *staphylococcus* etc.) and antibiotic "Ciprofloxacin" was used as standard in this regard. 'Kamino' (*caesalpinia digyna*), 'khoro pata' (*cissus repens*), and 'chikipung' (*rumex vesicarius*) in fresh extract were shown high sensitivity against all organisms in compare to those in ethanol extract. However, 'Yangfu' (*feics benghalensis*), 'ozon shak *(Spilanthes calva*)', 'tak

begun' (*Solanum virginanum*), 'Kochi amm pata' (*magnifera indica*) were also shown remarkable 'zone of inhibition (mm)' but only in fresh extract. In contrast, Bala pata (*pouzolzia hirta*) in both fresh and ethanol extract did not show any sensitivity reaction against all organisms.

While screening the hypoglycemic activity in alloxen-induced diabetic mice, the serum glucose level was estimated by glucose oxidize method (Trinder, 1969; Glynn, 1991) using commercial kit [Human, Germany with the help of ELISA plate reader (Labsystem, Finland). Eighty four white albino mice (25-35 gm) each of both sexes were procured from the animal house of Jahangir Nagar University. They were housed at standard environmental conditions of temperature and dark/light cycle, and were fed with commercial pellet diet and drinking water. The animal were fasted for 12 hours before the experiments, but had free access to water. Ethical guidelines in animal handling and use were adequately maintained during the study. Baseline, 0 day, 07 days, 14 days, 21 days and 28 days mean serum blood glucose were analyzed using paired sample t-test in SPSS software (version 17). Among ethanol and fresh extract groups (6 groups: 3 fresh vs 3 ethanol groups of, Kochi aam pata, ozon shak and khoro pata ozon shak and khoro pata were significantly different on reducing glucose level of alloxen-induced diabetic mice. On the other hand, no significance difference was observed between ethanol and fresh extract of Kochi aam pata in reducing blood glucose level of Swiss mice.

Contents

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List of Abbreviations

AMDR	Acceptable Macronutrient Distribution Range
AI	Adequate Intake
ADA	American dietetic Association
AOAC	Association of Official Analytical Chemists
AAS	Atomic Absorption Spectrophotometer
BBS	Bangladesh Bureau of Statistics
CHT	Chittagong Hill Tracts
CV	Co-efficient of Variance
CFCS	Comprehensive Food Consumption Survey
DRI	Daily Reference Intake
DAE	Department of Agricultural Extension
EP	Edible Portion
ES	External Standard
FGDs	Focus group discussions
FAO	Food and Agriculture Organization of the United Nations
FCDB	Food Composition Database
FCT	Food Composition Tables
HKI	Helen Keller International
HDL	High Density Lipoprotein
HPLC	High Pressure Liquid Chromatography
HH	House Hold
INFS	Institute of Nutrition and Food Science
IS	Internal Standard
LDL	Low Density Lipoprotein
NFCD	National Food Composition Database
NPNL	Non Pregnant Non Lactating
NPN	Non Protein Nitrogen
ND	Not Available
NK	Not Known
P Hc	Population and Housing census
QAP	Quality Assurance Program
RDA	Recommended Dietary Intake
RE	Retinol Equivalents
SD	Standard Deviation
SEM	Standard Error of Mean
SRM	Standard Reference material
TDF	Total Dietary Fiber
TK	Traditional Knowledge
WFP	World Food Program
WHO	World Health Organization

Nutrient Composition of selected Ethnic Plant Foods

CHAPTER 1

Introduction

1. Introduction

1.1 Overview

Plants have been utilized by human beings from time immemorial not only to satisfy hunger and meet nutritional needs, but also for purposes of medicine and prevention against diverse ailments. Plants contain hundreds of secondary metabolites; lately, a number of these metabolites have been recognized by modern science as to possess ability for preventing occurrence of diseases like cancer, arthritis, and cardiovascular disorders. Such plants and their secondary metabolites are described as preventive medicines, functional foods, and nutraceuticals or pharma foods. A food composition table (FCT) contains essential information on the nutive values of key foods consumed by the mass population. Bagladesh is in the process of revisiting and updating the food composition database (FCDBs) by analyzing the nutrient composition of indigenous and ethnic foods.

This study aims to investigate nutrient composition and medicinal properties of selected ethnic plant foods consumed by ethnic people. Twenty five plant foods comprising nineteen(19) leafy and six(6) non-leafy foods were included; particular emphasis was given in selection of the foods, which are reported and using in their different ailments. Comprehensive food consumption servey (CFCS) and focus group discussions (FGDs) data were used in this grouping. The selected ethnic foods were collected from weekly local markets at Bandarban, Rangamati and Khagrachari. Three food samples were collected for each food from every market. Taxonomic study was performed to confirm the identification of the collected foods. In the analysis of nutrient composition, contents of micronutrients and proximate nutrients were estimated employing standard methods. Carotenoid, vitamin C, total phenol, phytate contents were estimated by spectrophotometry, carotene profile and B vitamins were analysed by HPLC and mineral content was determined by atomic absorption spectrophotometry. Proximate composition was estimated by AOAC methods.

Plant foods consumed by ethnic people are rich in micronutrients. Making awareness of consuming ethnic foods will ensure health benefit. Incorporation of the nutrient data would enrich the existing FCT and FCD.

1.2 Background

The indigenous peoples, through living in a natural state for thousands of years, have developed a first-hand knowledge of plants that can be used both for therapeutic as well as preventive purposes (Rahmatullah2011).

Human focused more on domesticated cultivars and gave less attention to wild species, plants that once offered important flavor, texture satisfaction and supplied essential nutrients to the diet declined in popularity. As humans changed, economically and technologically from hunter-gatherer encampments to settlements, and ultimately to urban living, diets changed significantly in two ways. First, human food patterns reflected increasing intake of fewer domesticated plant staples and second, edible wild species that once sustained health and nutritional status began to be reduced, and then eliminated from the diet (Grivetti, 1976, 1978, 1981). Plants have sustained human populations in each of the inhabited continents. The agricultural revolution that began more than 10,000 years ago created a dramatic shift in the human food supply (Isaac *et al*, 1970; Heiser, 1973; Grivetti, 1980). One result was a significant reduction in dietary diversity.

Food is essential component for human survival. Good health needs balanced diet. To have it, nutrient composition of consumed foods has to be made well-known and available to the people. Ethnic people are healthy, strong and hardworking, have less morbidity and higher life expectancy. This may be because of that they consume almost all of the wild plant and animal foods that make them active and healthy.The wilds foods are rich in health promoting nutrients. Ethnic people consume almost all of the tender leaves, and foods those consumed

by monkey and birds; *that help them in selecting edible and poisonous foods and thus, foods they eat are naturally screened.* Epidemiological and clinical studies document the relationship between diet and health. Plant foods are rich in micronutrients and people consuming it have a lower incidence of diseases.

Bangladesh is a densely populated country of South East Asia that has a rich tribal presence. There are about 58 tribes living in different parts of the country. Bangladesh has 1.2 million tribal people, which is just above 1 percent of the total population. Whatever the population they differ in their social organizations, marital customs, rites and rituals, food and other customs from the people of the rest of the country. These ethnic people use forest products as a source of food and medicine. These communities often suffer from improper and imbalanced diet. For getting a balanced diet, these communities routinely get food from wild source like honey, some wild fruits, flowers and wild vegetables. Although the principal role of these plants is to supplement the food cultivated in home gardens and other forms of agriculture, many of the species grown or wild-harvested are reported to have both therapeutic and dietary functions (ogle *et al*, 2003) Use of large number of wild species to meet their diverse requirements is largely due to the prevalence of diversity of vegetation in the area. Wild edible plants not only supplement the food quantity but also make significant contribution to the populations' nutrition throughout the year (Grivetti& Ogle, 2000;Herzog *et al*;Angami, 2006).The use of wild plants is integral part of their strong traditional & cultural systems and practice that have developed and accumulated over generations. Food plants are traditional in the sense that they are accepted by rural communities by custom, habit and tradition as appropriate and desirable food. People are used to them; they know how to cultivate and prepare them and enjoy the dishes made from them. They are grown for food within the farming systems operating in any particular locality or gathered as wild or semi-wild products. There is no universally accepted short list of such plants. Communities have evolved their own preferences and food habits.

These systems form the basis of local-level decision-making in agriculture, food production, human and animal health and natural resource management (Angami, 2006). World over,ethnic/ tribal population still stores a vast knowledge on utilization of local plants as food material and other specific uses (Sundriyal *et al*,1998). The tribal communities draw their sustenance mainly from the forests, which provide them food plants and other material requirement. Their lives are much dependent on forest or natural plant wealth (Arora&Pandey, 1996). The biological wealth is so intrinsically important to the life style and systems of the indigenous communities that wild plants make an important contribution for sustenance of local communities. In recent years, these plants which are collected from forest or fields are available in local market (called 'Hat'). The sale from the surplus of their collection also adds to their income significantly. Research in several regions has also illustrated that many wild plants that are retained in local food cultures are inseparable from traditional therapeutic systems (Fleuret, 1986). Emphasis on the conservation and management of those plants will help enhance and maintain the region's biodiversity with little adverse impact on the biodiversity (Ogle, 2001)

Bangladesh is not only an alluvial plain land; about 12% of its territory is occupied by hills. These are located mainly in the south-east and north east part of the country. Two main kinds of hilly land characterize the country are,

High hill ranges: such as Sitakunda range north of Chittagong, whose highest point mainly lies between 300 and 1000 m above mean sea level. The highest point in Bangladesh, 954 m (3141 ft), lies on the border between Bangladesh and Myanmar.
Low hills: such as Lalmai Hills near Comilla, whose crest generally lies below 150 m. The original sediments have been uplifted, folded, faulted and dissected to form long hill ranges or areas of complex hill relief. Most slopes are very steep.

A survey of Chittagong hill tracts (CHT) showed that more than 70% of the land outside the Forest Reserves has slopes steeper than 40 percent (regarded as the safe limit for

cultivation); the proportion in the forest reserves is probably even higher. Only 3% of the unreserved area, mainly in the valleys, has slopes less than 5% (Forestal, 1966).

The hill areas of Bangladesh include districts of Chittagong, CHT, Noakhali, Comilla, Sylhet, Mymensingh and Jamalpur. Tropically, CHT is the only hill intensive area of Bangladesh. The district alone covers 80.24% of the total hill areas of Bangladesh.The CHS is the combination of three districts namely Rangamati, Khagrachori & Bandarban and occupies a narrow inland strip of parallel ranges along the Indian and Myanmarese frontiers.

Districts of Chittagong Hill Tracts

Bandarban

Bandarban was originally a sub-division of Chittagong Hill Tracts district. It was upgraded to a district on October, 1981. Bandarban district is full of hills and forests. The total area of the district is 4479.01 sq.km of which 2653.54 sq.km. is under forest. Bandarban district is bordered by Rangamati district to the north, Arakan (a state of Myanmar) and Naf River to the south, Indian border and Rangamati district to the east, Chittagong and Cox's bazaar districts to the west. According to 2011 population and housing census total population of Bandarban districts was 3,88,335, among them 2,03,350 were male and 1,84,985 were female. The annual growth rate of Bandarban districts was 2.64%. Bandarban is the lowest densely populated district in Bangladesh where 85 persons lives in per sq km (Tuku Talukder, 2014) Marma, Murong, Tripura, Bawm, TanchangaChakma, Chak, Khyang, Khumi, Lushei and the Pankho are main ethnic groups of this district. (P and Hc; 2011).The majority of ethnic people of Bandarban district are Marma.

Khagrachori

Khagrachhari was formerly a sub-division of Chittagong Hill Tracts. It was previously the headquarters of Ramgarh sub-division and became a sub-division in 1970 and was upgraded to a district in 1983. It is bounded on the north by India, on the east by Rangamati district, on the south by Chittagong and Rangamati districts and on the west by India and Chittagong.

The area of Khagrachori is 2749.16 Sq. km. There are three municipalities in Khagrachori named Khagrachhari, Ramgor and Matiranga. The number of sub district in Khagrachori district is nine, named- Khagrachhari, Mohalchori, Manikchori, Panchori, Luxmichori, Dighinala, Matiranga, Ramgor and Merung.

Khagrachhari is a valley. It has three rivers namely Chengi, Kasalong and Maini. Chengi is the longest river in Khagrachhari. Most of the land of Khagrachhari are hilly areas**.**[16]The total population of Khagrachari district is 6, 13,917, of which male are 3, 13,793 and female are 3, 00,124 (PHc; 2011, BBS; 2011). The sex ratio is 105:100. Demographically here 52% are tribal population and 48% are non tribal people. Among the total population there are Bengalees and three major tribes namely Chakma, Marma and Tripura. Ethnicity-wise population distributions of Khagrachori are Chakma-1,460,45; Tripura-67,342, Marma-55,844, non tribal community 2,48,559 and others 673 (P Hc; 2011). The density of population is 225 per square kilometre and annual growth rate is 1.08 (Tuku Talukder, 2014)**.**

Rangamati

Rangamati became a sub-division of former Chittagong Hill Tracts District in 1891. It was upgraded to a district in 1983 (BBS; 2011). Rangamati Hill District lies to the south-east of Bangladesh. The total area of the district is 6,116.11 sq. km. (2361.44 sq. miles), of which 4768.49 sq. km. is forest. There are ten Upazilas in the district. The district is bounded to the

north by Tripura, east by Mizoram, south by the Bandarban district and west by Khagrachhari and Chittagong district. The area covers vast forest land, wide range of hills and alluvial valley bottoms.

According to the population census 2011, the total population of the district is 5, 95,979 of which male 3, 01,376 and female 2, 82,903. The population growth rate is 1.58 and population density per sq. km is 97. Many types of ethnic people live here; such as Chakma, Marma, Tripura, Tanchangya, Lushai, Khyang, Pankhoa, Kuki etc. According to population census 2011, of the total population of Rangamati; 3, 56,153 were ethnic people. Among them 2, 60,445 were Chakma, 51,235 were Marma, 27,052 were Tanchangya and 17,421 belong to other ethnic groups[1]

In Chittagong hill district about 50% of the population is ethnic (*They are tribal people having distinct life style in terms of social, cultural and behavioral characteristics and food habits*) living mostly in the peripheral specific regions of Bangladesh. They represent a minor proportion, about 1% of the total population of Bangladesh and mainly the followers of Theravada Buddhism. Forty eight per cent (48%) of the inhabitants are Bengali Muslim settlers (*Virtual Bangladesh, 2011*). The remaining are followers of Hinduism, Christianity and Animism. The indigenous peoples, collectively known as the *Jumma*, include the Chakma,Marma, Tripura, Tenchungya, Chak, Pankho, Mru, Murung, Bawm, Lushai, Khyang, Gurkha,Assam and Khumitribes.[2]

Ethnic communities of Bangladesh

For centuries, Bangladesh has been the dwelling place of different ethnic groups. They live in different pockets of the hilly zones and some areas of the plane lands of the country. Twenty eight tribes comprising 2, 33,417 numbers of households have been living in Bangladesh (BBS survey, 1991). Among them, the tribes which have at least 1.5% representation in the total ethnic households living in Bangladesh are the 11 tribes that had ≥5% representation in the total tribal population living in Bangladesh. These tribes included *Marma, Chakma, Tanchangya, Tripuri, Bam,Murang, Monipuri, Khashia, Shaotal, Garo*and *Hajong*, which comprises 1,64,667 households representing 70.54% of total ethnic households living in Bangladesh.(*Islam et.al. 2010*).Chittagong hill tracts are inhabited by a variety of tribes, at least 6 tribes are predominantly recorded (*Islam et. al. 2010*). Their historical background, economic activities, social structure, religious beliefs, food habit and festivals make them distinctive. Here describe the four major tribal communities of Chittagong Hill Tracts.

The Chakma

The Chakma are the largest ethnic tribal minority in Bangladesh. They are concentrated in the central and northern parts of the Chittagong Hill Tracts where they live a midst several other ethnic groups. According to the 1991 population census, there were about 2, 53,000Chakma. More than 90 percent of them are concentrated in Rangamati and Khagrachhari districts.

The Chakma are divided into different clans (Ghosti) that maintain cordial relations among themselves. A sheaf (Gosa) is made up of a number of clans. The people of a clan or a sheaf are considered hear relatives of each other. This social lifestyle is called relation-based. The head person of the Chakma tribe is called the Chakma Raja (King). He is the only judge presiding over all sorts of activities of the Chakma.

[1] http://en.wikipedia.org/wiki/Khagrachari_District
[2] http://en.wikipedia.org/wiki/Chittagong_Hill Tracts

Chakma can get married within or outside their clan/sheaf. Traditionally they sing and dance before the day of marriage and arrange 'Chungulong' worship (puja) on the day of marriage. The Chakma call their village the 'Adam'. The Chief of an Adam is called 'Karbari'.

Most of the Chakma of rural areas live on bamboo-made platforms with huts on them also made of bamboos. They use ladders for climbing up and down. The Chakma language has its own alphabet. They have their own dance, songs and literature. They use their own system of numbers for counting. A good number of Chakma are engaged in weaving cotton fabrics and producing bamboo-made baskets.

The vast majority of Chakma are Buddhists, and they form the largest Buddhist population in Bangladesh. Integrated in their Buddhist practice are older religious elements, such as worship of the power of nature. One of their annual highlights is the Bizu festival held in Chaitra, the last month of the Bengali year.

The male Chakma wear dhuti (*a narrow piece of cloth wound round the waist between the legs with a fringed end hanging down from the rear*) or lungi, panjabi and shirt while the women wear short saree and blouse. The women wear the saree almost like a lungi. The Chakma wear very colorful clothes during their festivals.

The Chakma possess good health and are physically fit as a result of climbing hills. They are very hardworking people. Nowadays more and more of them are becoming literate and many are studying in schools, colleges and universities.[3]

The Marma

The Marma are the second largest ethnic minority in Bangladesh. Most Marma live in the three hill districts of Rangamati, Bandarban and Khagrachari. However, some Marma live in the coastal districts of Cox's Bazar and Patuakhali.

According to the 1991 census, the number of Marma in Bangladesh was 1, 57,301. Marma belong to the Mongoloid race. They are relatively short and have prominent cheekbones. They have a yellow complexion, black hair, small eyes and snub noses.

They speak an Arakanese dialect and their language is written in Burmese characters. In recent times, Marma in urban areas and nearby settlements have learnt to speak the local dialect of Chittagong.

The houses of the Marma people are made of bamboo, wild grass and straw. These are built on elevated bamboo or wooden platforms (machang). Every room is a bedroom cum store. The space underneath the machang is used for various purposes such as keeping livestock and storing fuel wood. Some of their houses, however, are made of mud and built without a machang.

Marma men and women typically wear thami and angi. However, the angi used by men is more a waistcoat than a blouse. Marma make their own clothes using traditional weaving technology, although many Marma now purchase Bengali dresses from the market. Kitchen utensils in a Marma family are mostly earthen or made of bamboo and wood.

The nuclear family is predominant in the Marma community. Although the husband is the head of the household, the wife also has a significant role in the family. Agriculture is the main occupation of the Marma and jhum cultivation is their primary agricultural pursuit.

Weaving is a very common activity of the Marma women. Recently they have become involved in trade and commerce. What they produce is sold mostly through middlemen.

[3] *http://en.wikipedia.org/wiki/Marma_people*

Marma believe that their birth, death and all activities in life take place under the influence of a supernatural power. They celebrate Buddhist religious festivals and also perform various forms of ritual worship to placate different gods. Dreams have a very strong influence in decision making in their everyday life.

Marriage is a very important part of the social life of Marma people. Cross-cousin marriage and monogamy are predominant features of this society. Polygamy is also allowed. Child marriage is practically forbidden. Premarital love is common.[4]

The Tripuri (also **Tipra** or **Tipperah**)

They are another large ethnic group in the Chittagong Hill Tracts (CHT) region. At present they live in CHT, especially in Ramgarh and Khagrachhari. It is also believed that Tripuri people currently living in Bangladesh originally came from the Indian state of Tripura. The number of Tripuri in CHT areas was close to 80,000 in 1991, and it has no doubt increased considerably by this time.

Tripuri call their society Dafa. Among the Tripuri community, all the groups and subgroups have their own dialects, dresses and ornaments. This tribal group does not have a uniform lineage system. In some groups, sons draw their lineage from the father's side while daughters draw their lineage from the mother's side.

Kokborok, the language of the Tripuri, belongs to the Bodo group which had its origin in the Assam branch of the Tibeto-Burma family. Kokborok was widely used in writing letters, performing magic and preparing lists of indigenous medicines. But due to lack of use, their script is on the verge of extinction.

Tripuri are mainly Hindus though their beliefs and religious practices are different from those of caste Hindus in many aspects. They worship the god Shiva and the goddess Kali along with 14 other gods and goddesses. They also believe in a number of evil spirits, incorporeal beings and demons, who have their domicile in jungles and who do harm to people by inflicting diseases. They sacrifice animals and birds in the name of their gods and goddesses.

The Tripuri build their houses on hilltops. They also build stairs to climb into their houses. Their houses lie somewhat scattered throughout their villages. The traditional dress of the Tripuri man includes dhuti and a Khaban (turban). During the winter they wear a ruggedly sewn jacket. Both men and women wear crescent-shaped silver ear rings. The women wear necklaces made of beads and shells, nose skewers and ornaments on the hair, neck, wrist and ankle.

This ethnic community follows a custom of arranged marriage which is traditionally not allowed within one's group. The father of the bridegroom has to pay the expenses for the bride's dress and ornaments. Before marriage the bridegroom takes up residence in the bride's home for two years and becomes a member of her family.

The most important social festival of the Tripuri is the Baisuk that lasts for three days. It commences from the penultimate day of the Bengali calendar. On the first day of the festival called haribaisuk, children decorate homes with flowers, wear clean clothes and visit neighbors. Elders also visit neighbors and are treated to drinks. A group of about 15 dancers performs folk dances and are offered chicken, rice and drinks by the householders they visit. Their dances are really colorful and enjoyable.[5]

[3] *http://en.wikipedia.org/wiki/Marma_people*
[5] *http://en.wikipedia.org/wiki/Tanchangya_people*

The Tanchangya[4]*http://en.wikipedia.org/wiki/Tanchangya_people*

The Tanchangya are a small ethnic community living in the Chittagong Hill Tracts. In terms of population they rank 5th among the ethnic communities of Bangladesh. According to the 1991 census, their number was 21,057 and the number of Tanchangya households was 4,043.

Tanchangya live in the Hill districts of Rangamati, Bandarban and Khagrachhari in Ranguniaupazilla in Chittagong district and in Ukhia and Teknaf areas of Cox's Bazar district. Like other tribal, Tanchangya build their houses on the forested slopes of hills.

Tanchangya also live in the southeastern regions of Tripura, Mizoram and Manipur States of India, as well as in the Arakan region of Myanmar. In Arakan they are known as 'dounnak'. Anthropologically, they belong to the Mongoloid group. They speak Pali, Prakrit and ancient Bengali, all belonging to the Indo-Aryan group of languages.

Tanchangya wear traditional costumes. Their women look very attractive in their costumes. Tanchangya women used to wear more colorful clothes and ornaments than all other hilly women. The men of this tribe wear simple clothes without designs. Tanchangya men usually wear long-sleeved shirts.

Tanchangya are Buddhists and observe such religious rites as worshipping. Gautam Buddha, listening to sermons, Kathin Chibor Dan, Maghi Purnima, etc. They have Buddhist Viharas in their localities. They celebrate 'biju' to mark the end and beginning of the Bengali year.

Their laws of inheritance reflect the values of a patriarchal society. The daughters cannot claim any share of the property except when they have no brothers.

Tanchangya are modest in nature. They have seven clans. Agriculture is their main occupation. They cultivate crops and practice horticulture on hill slopes.

There are 3 types of marriages among Tanchangya - the groom is taken to the bride's house; the lovers elope and marry; and widows remarry. Their word for marriage is Sanga.[6]

These ethnic people use forest products as a source of food & medicine. These communities often suffer from improper & imbalanced diet. For getting a balanced diet, these communities routinely get food from wild source like honey, some wild fruits, flowers and wild vegetables. The use of wild plants is integral part of their strong traditional & cultural systems and practice that have developed and accumulated over generations. Wild edible plants not only supplement the food quantity but also make significant contribution to the populations' nutrition throughout the year (Grivetti& Ogle, 2000; Angami, 2006). World over tribal population still stores a vast knowledge on utilization of local plants as food materials and other specific uses (Sundriyal *et al,* 1998). For these, their food habit should be evaluated and these ethnic food values should be enlisted on the FCT of our country.

The Role of Indigenous Plant Biodiversity as a Food Resource

Tribal societies, through a process of trial and error experimentation in their environment, acquired immense amounts of knowledge on (the use of) fauna and flora around them (Fox and Norwood, 1982). From this diversity of life they relied on a high proportion of wild plants as food. These food plants formed a very broad resource base. In Africa, until way into the 19th century; indigenous food plants played an important role in the traditional diets of African people (Flueret 1979, Fox and Norwood, 1982).

Yeung (1985) recorded more than 1000 indigenous food plants in southern Africa alone. The significant role played by this diverse food base is however diminishing. Gomez (1988) has

[4]*http://en.wikipedia.org/wiki/Tanchangya_people*

claimed that economic and technological growth have a debilitating effect on traditional cultural values and food habits which lead to a shift away from traditional food resources. Maundu (1995) cited westernized markets, formal education, urbanization and change in food preferences as the factors contributing to loss of traditional knowledge on edible plant species. One of the interests of this study is to investigate possible factors that could have led to a change in the use of indigenous food plants by the tribal community.

Taking an overall picture of the world at large, a similar trend as above can be observed. Approximately 500,000 flowering plant species are known to exist on earth (Fox and Norwood 1982). 75,000 are believed to be edible and of these about 150 are recognized worldwide as food plants, nearly all of which were discovered by 'primitive' man (Wehmeyer*et al.* 1969). Only about 30 of the latter make a significant contribution towards human nutrition at present (Kochhar and Singh 1989, Koopowitz and Kaye 1990, Walters and Hamilton 1993), with the cereals maize, wheat and rice making up the major dietary staples (Koopowitz and Kaye 1990). This signifies a great reduction in the use of available food plants in the world.

Impact of Agriculture on Plant and Food Diversity

Biodiversity loss in Bangladesh is as a result of several factors. These include inappropriate agricultural practices, over-harvesting of plant resources, commercial land use practices, climatic changes, pollution, population and migration pressure, and many more.

The current agricultural system has brought about clearance of natural vegetation to support vast hectares of uniform stands of biotechnologically produced crops in an effort to satisfy the demands of the world's economic markets at the expense of indigenous food plants better suited to the local conditions. The cultivation of monocultures of hybrid crop varieties has required manipulation of local conditions through the use of irrigation and heavy fertilizer and agro-chemical applications (Slikerveer 1995).

These bring about significant changes to the local environment, which have serious repercussions on plant biodiversity and on continued food security (Gomez 1988, Shiva 1995). Effects of agro-chemicals include release into the soil of toxic matter and increased soil acidity caused by ammonia-based fertilizers which in the long run lead to diminishing yields, environmental degradation and pollution of water resources. Pesticides and herbicides are not highly selective and usually lead to loss of beneficial organisms.

With regards to genetically modified crop cultivars, these are generated for crop uniformity in order to increase yields. If a disease or pest were to appear the entire crop would be wiped out. This has been evidenced by such catastrophes as the Irish potato blight, the southern corn blight of America and the Asian rice incidence, to name a few (Koopowitz and Kaye 1990, Shiva 1995). Such incidences have resulted in dire food insecurity for the affected nations, highlighting the danger of relying on a very narrow food base. Through the promotion of such crops modern agriculture is actually supporting the decline in food varieties, and hence biodiversity, as more and more people rely on fewer and fewer crops (Slikerveer 1995, Shiva 1995). This approach to agriculture is in itself the greatest single threat to biodiversity. Currently the seed industry, specifically the seed company Monsanto, has developed the genetically modified 'terminator' maize seed which produces non-viable seed (Macleod 1999). In addition to creating dependency of the farmer on the seed and therefore agrochemical industry, such genes can be a potential threat to biodiversity if they escape into wild plant populations thereby affecting plant reproduction in nature. Commercial agriculture has also perpetuated the view of any competing wild plants appearing in the uniform field to be considered as 'weeds', despite some of them being useful to humankind (Shiva 1995).

Most communities have been turned to rely on commercial crops to the detriment of their local food sustenance. Slikerveer (1995) has claimed that until the sixties Africa was self-sufficient in its food production. The introduction of high yielding commercial crops marginalized the

growing of indigenous food crops. People began to rely on sales of commercial crops to buy food which they previously grew for themselves. The resultant high yields from commercial crops are however short-lived (due to land degradation and negative impact of agro-chemicals) and in most cases market fluctuations in agricultural produce can have a catastrophic impact on the farmers. Such incidences have occurred in several African countries whose economies are based on agricultural exports. Hobhouse (1999) commented on the aftermath of this when he wrote *"Few islands in the Caribbean have made a concerted effort at self-sufficiency, and despite the fact that the Caribbean has more food plants than Europe, people even in some of the favored agricultural areas would starve but for imports, usually from Canada or the United States"*. What this demonstrated was that the development of agriculture, while it usually increases food production levels, reduces resistance to unpredictable environmental conditions to which the people were previously cushioned from by a broad food resource base.

Indigenous People and Indigenous Plant-Use Knowledge

Global interest in and recognition of indigenous knowledge -"the local knowledge that is unique to a given culture or society" (Warren *et al.* 1995) - is on the increase with the continued realization of how such knowledge can benefit the world. The International Forum of Non-Governmental Organizations held in Rio de Janeiro (*International Council for Adult Education 1992*) stated as one of its principles that "*Environmental Education must recover, recognize, respect, reflect and utilize indigenous history and local cultures, as well as promote cultural linguistic and ecological diversity*". In support of acknowledging knowledge possessed by tribal peoples, Chapter 22 of Agenda 21 (*UNESCO-UNEP 1992*) proclaimed that "*Over many generations, tribal people have evolved holistic, traditional scientific knowledge of their land, natural resources and the environment. Their ability to practice sustainable agriculture has been limited by economic, social and historical factors. Tribal people should actively participate in the shaping of national law and policies on the management of resources or other developmental processes that affect them*".

Tribal people, through a long period of interaction with their surrounding environment, are vested with comprehensive knowledge on the potential use of local plants in their environment. Balick and Cox (1996) have claimed that *"the relationships between plants and people are often clearer in Tribal societies than in our own, since the link between production and consumption is more direct*".

Several writers recognize the impeding threats to tribal peoples' knowledge on the use of indigenous plants. Warren (1995) raised this concern when he said "*Of equal concern (to global awareness concerning the conservation of biodiversity) to many world citizens is the uncertain status of indigenous knowledge that reflects many generations of experience and problem solving by thousands of ethnic groups across the globe. Very little of this knowledge has been recorded, yet it represents an immensely valuable database that provides humankind with insights on how numerous communities have interacted with their changing environment, including floral and faunal resources".*

On the use of indigenous food plants Warren (1995contended that "*More serious than the physical decline and loss of traditional food resources…is the loss of vast and ancient knowledge in identifying and recognizing these resources and of the often elaborate technologies of their utilization".* In southern Africa whilst there is increasing interest in indigenous knowledge relating to plant use, this has mainly been in relation to medicinal plants. Documentation on indigenous medicinal plants includes the works of Gilges (1955), Watt and Breyer- Brandwijk (1962), Kokowaro (1976), Gelfand*et al.* (1985) and Van Wyk*et al* (2008). Cunningham *et al* (1992) and Dakora (1996) confirm this lack of coverage of indigenous food plants, marking it as a knowledge gap that needs urgent attention. Some work done recently in the southern Africa however includes the study of indigenous food

plants. The focus of the present study is solely on the use of rare edible plants as food by ethnic people of Bangladesh.

Ajesh *et al*. 2012 conducted a research work on Ethnobotanical Documentation of Wild Edible Fruits Used ByMuthuvan Tribes of Idukki, Kerala- India. This study highlighted the significance of wild fruit species as a source of nutrients for tribals. The study suggested that wild fruit plants can be included in agro and farm-forestry and reforestation programme. Multiplication of its population through advanced techniques be tried and introduced in ecologically rich areas and botanical gardens to increase the accessibility of the species.

Misraet. al. 2014 suggested that some of the underutilized leaves may be useful as food and medicine that are required in small quantities to cure some of the diseases the tribal and rural poor suffer from. This study found that *Commelina benghalensis* contain 92.6% of moisture, 20.97mg/g of crude protein, 23.53mg/g of total sugar, 0.004mg/g of fat and no vitamin C. And *Glinus oppositifolius* contain 80.6% of moisture, 25.83mg/g of crude protein, and 129.89 of total sugar, 0.006mg/g of fat and 0.353mg/g of vitamin C.

Kar *et. al* (2008) conducted a research on Wild Vegetables of Karbi - Aglong District, Assam. The study provides information regarding wild vegetables of Karbis. 56 species of angiosperm and 1 species of gymnosperm are recorded in the study. It suggested that wild edible vegetables need be popularized as many of them have nutritional and medicinal value. Biochemical analysis of wild vegetables to work out their nutrient value. The authors also emphasize more studies in this field which will add new findings regarding wild vegetables.

Choudhury *et. al.* (2012) found that people in north-east India, Nepal and Bhutan consume various wild and domesticated bamboo tender shoots. The study reveals that bamboo shoots are low in fat and cholesterol content but very high in potassium, carbohydrate and dietary fiber content. Many nutritious and active materials such as vitamins, amino acids and anti-oxidants such as flavones, phenols and steroids are present in bamboo shoots.

Forestry and Environmental Science, Shahajalal University Science Technology, Sylhet suggested that edible bamboo shoot is delicious and nutritious. Many over populated countries like China, Japan and India are using this shoots as vegetables. So bamboo shot could be a welcome addition to the diets of many populations in Bangladesh. In the present population-explosive world, this vegetable could play an important role for solving the global hunger problem.

Mahmudet.al. (2012) observed that *Premnaesculenta* Roxb. (FamilyVerbenaceae) is a shrub used by the ethnic people of Chittagong Hill Tracts of Bangladesh for the treatment of hepatocellular jaundice. The findings of the study indicate that the leaf extract of *P. esculenta* showed a potential hepatoprotective activity and the protective action might have manifested by restoring the hepatic SOD, catalase, and peroxidase levels. The results justify the traditional use of this plant in liver disorders.

Velayudhan et. al. (2012) showed that turmeric is highly useful due to its manifold uses and close association with social, cultural, religious, folk and classical art forms beside its medicinal, cosmetic and ethno botanical uses in human beings. It is an important plant with greater antiquity than many other cultivated crops. The study suggested that there is an urgent need to do more research work on the value addition of turmeric in view of its insecticidal, fungicidal and medicinal properties.

Nutrients in Food

Man needs a wide range of nutrients to perform various physiological functions in the body and to lead a healthy life. They obtain nutrients from their ingested foods. Nutrient is a substance that provides nourishment for growth or metabolism. Nutrients are used to build and repair tissues, regulate body processes and converted to and used as energy. A nutrient

is said to be “essential” if it must be obtained from an external source, either because the organism cannot synthesize it or produces insufficient quantities. Nonessential nutrients are dose-dependent and shortages are called deficiencies.

Nutrients needed in very small amounts are micronutrients and those that are needed in larger quantities are called macronutrients. There are three primary macronutrients defined as being the classes of chemical compounds, humans consume in the largest quantities and which provide mass energy. These are protein, fat, and carbohydrate, all of which humans consume in the largest quantities. The micronutrients are minerals and vitamins. However, currently there are forty six recognized nutrients and six classes into which nutrients are categorized comprising carbohydrates, fat, proteins, vitamins, minerals and water. The foods containing these nutrients which are consumed daily are classified as cereals, legumes (pulses), nuts and oil seeds, vegetables, fruits, milk and milk products and flesh foods (fish, meat and poultry).

Macronutrients

The macronutrients (excluding fiber and water) provide structural constituents such as amino acids from which proteins are made, lipids from which cell membranes and some signaling molecules, and energy are prepared. Some of the structural material can be used to generate energy internally. And in either case it is measured in joules or kilocalories (often called “Calories”). Carbohydrates and proteins provide 17 kJ approximately (4 kcal) of energy per gram, while fats provide 37 kJ (9 kcal) per gram, though the net energy from either depends on factors such as absorption and digestive effort, which vary substantially from instance to instance.

Protein

Protein should account for 10% to 15% of the calories consumed each day. Protein is essential for growth, and for the repair of body tissue. It is also needed for the structure of red blood cells, for the proper functioning of antibodies resisting infection, for the regulation of enzymes and hormones.

- **Protein requirements at different life stages**

  Protein requirements are highest in the growing years. Up until 3 years of age, we require more protein (about 1 to 1.5 grams of protein per kilogram of body weight) than do older children and adults (about 0.85 to 0.95 grams of protein per kilogram of body weight). Pregnancy and lactation also increase protein needs (1.1 to 1.3 grams of protein per kilogram of pre-pregnancy weight).

- **Protein requirements are increased in certain conditions**

  As noted above, severe illness may cause protein deficiency. These illnesses include liver and kidney diseases, burns, severe infections, and major surgery.

- **Too much protein can be harmful**

  Although a deficiency of dietary protein is clearly harmful, many chronic diseases may be caused or worsened by too much protein, particularly animal protein. These diseases include: osteoporosis, kidney stones, kidney failure, gout, and possibly certain cancers. Food from plant sources supply protein in the amount and quality appropriate for all ages.

Food source of protein include

- **Animal protein:** Meat, poultry, fish, eggs, milk, cheese and yogurt provide high biological value proteins, because they contain all the essential amino acids.
- **Plant proteins:** Plants, legumes, grains, nuts, seeds and vegetables provide low biological value proteins. However, combining proteins from different plant sources in the same meal

often results in a mixture of higher biological value. Examples of such combinations are: beans with rice, chickpeas with bread, lentils with potatoes, vegetables with cereals.

Adequate Intakes (AIs) in ordinary type followed by an asterisk . RDAs and AIs may both be used as goals for individual intake. Acceptable Macronutrient Distribution Range (AMDR) is the range of intake for a particular energy source that is associated with reduced risk of chronic disease while providing intakes of essential nutrients. If an individual consumes in excess of the AMDR, there is a potential of increasing the risk of chronic diseases and/or insufficient intakes of essential nutrients.

Fats

Foods contain combinations of *saturated* and *unsaturated* fats. Saturated fat is found in high quantities in dairy products, eggs, and meats, for example, while vegetable oils are particularly high in unsaturated fats. Unsaturated fats are either *monounsaturated* (found in olive and canola oils) or *polyunsaturated* (found in nuts, seeds, and seed oils).

Fat should account for 30% or less of the calories consumed daily, with saturated fats accounting for no more than 10% of the total fat intake. Fats are a concentrated form of energy which helps maintain body temperature, and protect body tissues and organs. Fat also plays an essential role in carrying the four fat-soluble vitamins: A, D, E, and K.

Excess calories from protein and carbohydrates are converted to and stored as fat. Even if you are eating mostly "fat free" foods, excess consumption will result in additional body fat. Fat calories in food are readily stored, while it takes energy to transform protein and carbohydrates to body fat. The only proven way to reduce body fat is to burn more calories than one consumes.

Fats that are in foods are combinations of four main types

a. Saturated Fat

These fats provide a concentrated source of energy in the diet and building blocks for cell membranes and a variety of hormones and hormone-like substances. An excess of these fats in the diet however, is believed to raise the cholesterol level in the bloodstream. These fats consist of fatty acid chains that have no double bonds between the carbon atoms of the chain. They are called saturated because they are fully saturated with hydrogen atoms and cannot incorporate more. They are solid at room temperature and are most often of animal origin.

Function: tends to increase blood cholesterol levels

Source: found mostly in meat and dairy products, as well as some vegetable oils, such as coconut and palm oils (tropical oils).Butter is high in saturated fat, while margarine tends to have more unsaturated fat. Most saturated fats tend to be solid at room temperature, with the exception of tropical oils.

b. Polyunsaturated Fat

These fats are composed mostly of fatty acids such as linoleic or linolenic acids which have two or more double bonds in each molecule. They are also liquid at room temperature.

Polyunsaturated fats are thought to reduce the risk of coronary heart disease. The omega-3 forms are believed to have a positive impact on heart health and to play an important role in brain and eye function.

Function: tends to lower blood cholesterol levels
Sources: found mostly in plant sources. (Sunflower, soybean, corn, safflower, cotton seed).

C. Monounsaturated Fat

These are composed mostly of monounsaturated fatty acids, meaning molecules with one double-bonded carbon. They are liquid at room temperature. They appear to protect against heart disease, in that they reduce blood cholesterol levels.

Function: tends to lower LDL cholesterol (the "bad" cholesterol)

Source: found in both plant and animal products, such as olive oil, canola oil, peanut oil.

d. Trans fatty acids

Unsaturated fats come in different chemical structures: a bent *cis* form or a straight *trans* form. When they adopt the *trans*form, they are called *trans*fatty acids. They are produced by the partial hydrogenation of vegetable oils and present in hardened vegetable oils, most margarine, commercial baked foods, and many fried foods. An excess of these fats in the diet is thought to increase the risk of heart disease.

Carbohydrates

Carbohydrates provide the body with a source of fuel and energy that is required to carry out daily activities and exercise. Any extra energy is stored in the body until it is needed.

Our bodies need a constant supply of energy to function properly and a lack of carbohydrates in the diet can cause tiredness or fatigue, poor mental function and lack of endurance and stamina.

Carbohydrates are also important for the correct working of our brain, heart and nervous, digestive and immune systems.

Source of dietary carbohydrates include

- Monosaccharides: fruits, vegetables and honey.
- Disaccharides: table sugar, sugar beet, sugar cane and fruits.
- Polyols: Isomalt
- Oligosaccharides: grains and vegetables
- Starch polysaccharides: cereals, whole grains, rice, potatoes, peas, pasta, corn and legumes.
- Non-starch polysaccharides: dietary fiber such as cellulose, hemicelluloses, pectins and gums.

It represents Recommended Dietary Allowances (RDAs) in bold type, Adequate Intakes (AIs) in ordinary type followed by an asterisk (*). RDAs and AIs may both be used as goals for individual intake.

Acceptable Macronutrient Distribution Range (AMDR) is the range of intake for a particular energy source that is associated with reduced risk of chronic disease while providing intakes of essential nutrients. If an individual consumes in excess of the AMDR, there is a potential of increasing the risk of chronic diseases and/or insufficient intakes of essential nutrients.

Dietary Fiber

A variety of definition of fiber exists. In an attempt to develop one definition of fiber that everyone can use, the Food and Nutrition Board assembled a panel that came up with the following definitions:

- Dietary fiber consists of no digestible carbohydrates and lignin that are intrinsic and intact in plants. This includes plant no starch polysaccharides (for example, cellulose, pectin, gums, hemicelluloses, and fibers contained in oat and wheat bran), oligosaccharides, lignin, and some resistant starch.

- Functional fiber consists of isolated, no digestible carbohydrates that have beneficial physiological effects in human. This includes no digestible plant (for example, resistant starch, pectin, and gums), chitin, chitosan, or commercially produced (for example, resistant starch, polydextrose, inulin, and indigestible dextrins) carbohydrates.

- Total fiber is the sum of dietary fiber and functional fiber. It’s not important to differentiate between which forms of each of these fibers are getting in diet.

Types of Dietary Fiber

Dietary fiber or sometimes roughage is the indigestible part of plants having two main components: soluble fiber and Insoluble fiber.

a. Soluble fiber

This type of fiber does not absorb water but it dissolves in it and forms gummy or gel-like substances. Soluble fiber is readily fermented in the colon into gases and physiologically active byproducts.

Soluble dietary fiber includes pectin, beta-glucans, fructans, oligosaccharides, some hemicelluloses and gums. Due to its higher degradability rate by the bacteria in the large intestine, soluble dietary fiber has higher calorie content compared to insoluble dietary fiber. It can help lower blood cholesterol and glucose levels.

b. Insoluble fiber

This type of fiber absorbs water and is not fermented by the bacteria in large intestine. Insoluble fiber is metabolically inert but it promotes the movement of material through the digestive system and increases stool bulk. So, insoluble fiber can be of benefit to those who struggle with constipation or irregular stools. Insoluble dietary fiber includes hemicelluloses, cellulose and lignin.

Source of Dietary Fiber

Food sources of dietary fiber are often divided according to whether they provide soluble or insoluble fiber. Dietary fiber is found in plants. Plants food contains both types of fiber in varying degrees, according to the plant's characteristics.

Fiber-rich plant can be eaten directly. Or, alternatively, they can be used to make supplements and fiber-rich processed foods. The American dietetic Association (ADA) recommends consuming a variety of fiber-rich foods.

Some plants contain significant amounts of soluble and insoluble fiber. For example, plums (or prunes) have a thick skin covering a juicy pulp. The plum's skin is an example of an insoluble fiber source, whereas soluble fiber sources are inside the pulp.

Soluble fiber is found in varying quantities in all plant foods, including

- Legumes (peas, soybeans, and other beans)
- Chira, barley oats, rye,
- Some fruits and fruit juices (including plums, berries, bananas, and the insides of apples and pears)
- Certain vegetables such as carrots, broccoli etc.
- Root vegetables such as potatoes, sweet potatoes, and onions (skins of these vegetables are sources of insoluble fiber)

Source of insoluble fiber include

- Whole grain foods
- Wheat and corn bran
- Nuts and seeds
- Potatoes skins
- Flax seed
- Lignins
- Vegetables such as green beans, cauliflower
- The skins of some fruits, including tomatoes.

These are a few example forms of fiber that have been sold as supplements or food additives. These may be marketed to consumers for nutritional purposes, treatment of various gastrointestinal disorders, and for such possible health benefits as lowering cholesterol levels, reducing risk of colon cancer and losing weight.

Nutritional functions and benefits of Dietary Fiber

- **Fiber for weight control**

  High fiber diets may be useful for people who wish to loss weight. High fiber diets tend to be less "energy dense", which means they have fewer calories. Yet fiber can provide a "full" feeling because of its water-absorbing ability. High-fiber foods generally require more chewing time, which gives our body time to register when we are no longer hungry, so we are less likely to overeat. Also, a high-fiber diet tends to make a meal feel larger and longer. So we stay full for a greater amount of time. For example, an apple is more filling than a half cup of apple juice that contains about the same calories.

  One of the reasons that fiber may have an impact on body weight is its ability to slow the movement of food through the intestines. The gel-like substances that soluble fibers form when they dissolve in water cause things to swell and move slower in the intestines.

- **Fiber for preventing heart disease**

  Low blood cholesterol levels (below 200 mg/dl) have been associated with a reduced risk of coronary heart disease. The body eliminates cholesterol through the excretion of bile acids. Water-soluble fiber binds to bile acids in the small intestine, making them less likely to enter the body; this in turn lowers cholesterol levels in the blood. Soluble fiber also reduces the absorption of sugar, sugar response after eating, normalizes blood lipid levels by lowering low-density lipoprotein, or "bad" cholesterol levels and, once fermented in the colon, produces short-chain fatty acids as by products with wide-ranging physiological activities.

  Some types of fiber, however, appear to have a greater effect than others. The fiber found in rolled oats is more effective in lowering blood cholesterol levels than the fiber found in wheat. Pectin has a similar effect in that it, too, can lower the amount of cholesterol in the blood.

- **Fiber for controlling diabetes**

  Dietary fiber helps to control blood sugar levels. Particularly soluble fiber can slow the digestion and absorption of carbohydrate, thus can stabilize the blood sugar level. Therefore, diabetes patient can reduce their dependencies on oral drug by consuming more dietary fiber. Although insoluble fiber is associated with reduced diabetes risk, the mechanism by which this occurs is unknown.

  To keep our blood sugars stable is a goal that we would all benefit from. High fiber diet could be the way to keep it under control. The best time to address type 2 diabetes is before it has developed. Research has shown that high-fiber diet can help prevent this form of diabetes. The most recent study done on overweight and obese men and women without diabetes showed reductions in blood sugar and insulin with the use of a high soluble fiber supplement.

- **Fiber for treating constipation**

  Dietary fiber normalizes bowel movements. Fiber increases the weight and size of the stool and softens it. Insoluble fiber binds water, making stool softer and bulkier. A bulky stool is easier to pass, thus decreasing the chance of constipation. If someone has loose, watery stools, fiber may also help to solidify the stool because it absorbs water and adds bulk to stool. Therefore, fiber, especially which found in whole grain products, is helpful in the prevention of constipation.

  Both soluble and insoluble fibers are necessary for regular bowel movements. Oftentimes, people use over-the-counter supplements to assist with regularity. Unfortunately, these supplements only provide soluble fiber. Studies support the benefits of the combination of soluble and insoluble fiber in alleviating constipation, but only with the consumption of an adequate fluid intake.

- **Fiber for bowel disorders**

  Dietary fiber helps to maintain bowel integrity and health. A high-fiber diet may lower the risk of developing hemorrhoids and diverticulosis. Divertcula are pouches of the intestinal wall that can become inflamed and painful. Although the mechanism by which fiber may be protective against diverticulitis is unknown, several hypotheses have been proposed. For example, some scientists report that fiber helps by decreasing transit time, increasing stool weight, and

decreasing pressure within the colon. The same has been found for Irritable Bowel Syndrome (IBS). The current guidelines for the treatment of IBS include following a high-fiber diet. The bulk that fiber provides is thought to help in preventing the painful spasms often associated with IBS and aid in comfortable regularity.

- **Fiber for preventing colorectal cancer**

  Dietary fiber may help in reducing the risk of some cancers, especially colon cancer. This idea is based on information that insoluble fiber increases the rate at which wastes are removed from the body. This means the body may have less exposure to toxic substances produced during digestion.
  For the bowels to work properly, a lifelong daily intake of 25-30 grams, or about one ounce of dietary fiber is required. After the digestion of all proteins, fats and carbohydrates and the absorption of water and other nutrients in the small intestine, the colon receives approximately one pint of liquid stool together with the undigested fiber.

- **Fiber for preventing breast cancer**

  Studies in diverse populations around the world have reported a lower risk of breast cancer in association with higher intake of dietary fiber and complex carbohydrates. Diets high in fiber may be protective against breast cancer, perhaps due to inhibition of the intestinal re-absorption of estrogen excreted via the biliary system. Fiber may also inhibit the activity of human estrogen syntheses, which in turn reduces production of estrogen.
  Researchers say that fiber may also reduce absorption of toxic chemicals, which may be another mechanism by which dietary fiber reduces the breast cancer risk. A high fiber diet has also been found to be associated with reduced incidence of mammary cancer in animals.
  Study suggests that to have a protective affect against breast cancer, a woman needs to eat 30 grams or more dietary fiber per day (Anderson JW *et. al,* 2008).

- **Intake of dietary fiber and its side effects**

  Although it may seem contradictive, exaggerated amounts of dietary fiber have also been shown to cause several side effects. Such as

  a. Excessive intake of dietary fiber can cause a fluid imbalance, leading to dehydration. Individuals who decide to suddenly double or triple their fiber intake are often advised to double or triple water intake for this reason. Persons eating more than 50 gm of fiber per day may experience an intestinal obstruction, but in the majority of the individuals their physiologic health of their bowel is greatly improved despite the minor side effect.

  b. Dietary fiber in large intestine (colon) absorbs water. If there is not enough water stool will be hard and dense, it may also cause constipation. To minimize fiber effect and optimize elimination of bi-products of metabolism at least 2 liters of water ought to be provided.

  c. Although most fiber remains undigested, some fiber is broken down by bacteria in the intestine and methane gas is released in the process. This can cause bloting and flatulence. In some people food products contain much dietary fiber especially the wheat one, may irritate stomach.

  d. Large amounts of fiber especially of the non fermentable kind taken as a supplement may interfere with absorption of minerals like iron, magnesium, phosphorus, zinc and calcium and possibly cause a mineral deficiency in the long run, or during periods of increased need such as lactation, pregnancy or adolescence.

  e. High dietary fiber may lower efficiency of other medicines e.g. oral contraceptives and medicines decreasing cholesterol concentration. One should keep a two hour interval between a meal and such medicine taking.

Guidelines for fiber intake

Current recommendations from the United States National Academy of Science, Institute of Medicine suggest that adult should consume 20-35 gm of dietary fiber per day. The ADA recommends a minimum of 20-35 gm/day for a healthy adult depending on calorie intake (e.g. a 2000 Kcal diet should include 25

gm of fiber/day). The ADA's recommendation for children is that intake should equal age in years plus 5gm/day (e.g. four years old should consume 4+5=9gm/day). Patients with current constipation, vomiting and abdominal pain should see a physician. Certain bulking agents are not commonly recommended with the prescriptions of uploads because the slow transit time mixed with larger stools may lead to severe constipation, pain, or obstruction. The British Nutrition Foundation has recommended a minimum intake of 18 gm/day for healthy adults.

In industrialized countries the average consumption of dietary fiber is about 15 grams per day (the average American only consumes 14 grams of dietary fiber per day). In non-industrializedsocieties, in Africa daily dietary fiber consumption amounts to 60gm. The effect of it is very low morbidity on tumor disease of colon cancer and rectal cancer. The world health organization (WHO) recommends consumption (RDA) of 20-40 gm of dietary fiber a day. Also fiber intake may vary depending on age and gender.

In 2002, the Food and Nutrition Board of the National Academy of Sciences Research Council issued Dietary Reference Intakes (DRIs) for fiber. Previously, no national standardized recommendation existed. The new DRIs represent desirable intake levels established using the most recent scientific evidence available.

Large intestine digestion of dietary fiber

When dietary fiber reaches the large intestine, the bacteria present release enzymes that cause the fiber to be broken down into smaller molecules such as butyric acid ($CH_3CH_2CH_2COOH$). The intestinal bacteria and the cells lining the large intestine can then use these smaller molecules as an energy source.

In addition to the production of these smaller molecules, the bacterial fermentation process releases gases such as carbon dioxide, hydrogen, methane and hydrogen sulfide. If not used by the bacteria, these gases can build up and are finally excreted as flatus through the anus.

Fiber and calories

Calorie or kilojoules (as used on nutrition labels) are intended to be a measure of how much energy is available from the food source. This energy can be used immediately, for example allowing the body to move during exercise, or to make the heart beat. Energy that is not used immediately is stored as sugars in the short term and later converted to fats, which act as energy reserves.

Energy is extracted from food in a chemical reaction. Because of the principle of conservation of energy, energy can only be extracted when the chemical structure of food particles is changed. Since insoluble fiber particles do not change inside the body, the body should not absorb any energy (or Calories/kilojoules) from them.

Because soluble fiber is changed during fermentation, it could provide energy (calories/kilojoules) to the body. As of 2009 nutritionists have not reached a consensus on how much energy is actually absorbed, but some approximate around 2 Calories (8.5 kilojoules) per gram of soluble fiber.

Regardless of the type of fiber, the body absorbs fewer than 4 Calories (16.7 kilojoules) per gram of fiber, which can create inconsistencies for actual product nutrition labels. In some countries, fiber is not listed on nutrition labels, and is considered 0 Calories/gram, when the food's total Calories are computed. In other countries all fiber must be listed, and is considered 4 Calories per gram, when the food's total Calories are computed because chemically fiber is a type of carbohydrate and other carbohydrates contribute 4 Calories per gram). In the US, soluble fiber must be counted as 4 Calories per gram, but insoluble fiber may be (and usually is) treated as 0 Calories per gram.

Micronutrients

Micronutrients are nutrients required by humans and other living things throughout life in small quantities to orchestrate a whole range of physiological functions but which the organism itself cannot produce. For human they include dietary trace minerals in amounts generally less than 100 mg per day. Micronutrients also include vitamins which are organic compounds required as nutrient tiny amounts. Micronutrients deficiencies are wide spread. Iodine, vitamin A and iron are most important in global public health terms; their lack represents a major threat to

the health and development of populations the world over, particularly children and pregnant woman in low income countries.

Natural sources of micronutrients are abundant. Micronutrients are vitamins and minerals mostly present in green yellow fresh fruits and vegetables, flesh foods and in animal and vegetable fats.

a) Vitamins

Vitamins assist the enzymes that release energy from carbohydrates, proteins and fats. Vitamins and minerals are widely available from the natural foods we eat. Vitamins analyzed in this study included carotenoids and carotene profile vitamin B and C.

1. Carotenoids

Carotenoids are red, yellow and orange pigments that are found in plant-based foods; they are not commonly present in animal-based foods such as dairy, eggs and meats. Foods containing beta-carotene include bell peppers, spinach, mangoes, pumpkin, carrots, and sweet potatoes.

Beta-Carotene is probably the most well known of the carotenoids, which are highly pigmented (red, orange, and yellow) fat-soluble compounds naturally presented in many fruits and vegetables. Beta-carotene is considered a pro-vitamin because they can be converted to active vitamin A. Vitamin A serves several biological functions including involvement in the synthesis of certain glycoprotein, which is a molecule, composed of a protein and a carbohydrate. Vitamin A deficiency leads to abnormal bone development, disorders of the reproductive system, and ultimately death. Beta-carotene is also converted to retinol, which is essential for vision. More recently beta-carotene has been claimed to prevent a number of diseases, including cystic fibrosis and arthritis, Foods rich in Beta-carotene protect your cells from the damaging effects of free radicals, provide a source of vitamin A, enhance the functioning of your immune system and help your reproductive system function properly.

Beta-carotene has many benefits, such as, it is necessary for growth and repair of body tissue, it helps maintain smooth, soft disease-free skin, it helps protect the mucous membranes on the mouth, nose, throat and lungs, thereby reducing susceptibility to infections, it protects against air pollutants, it counteracts night-blindness and weak eyesight, and it aids in bona and teeth formation. Current medical research shows that food rich in Beta Carotene will help reduce the risk of lung cancer and certain oral cancers. Also, unlike Vitamin A from fish liver oil, beta-carotene is non-toxic. Some symptoms that may show if you don’t have enough beta carotene in your diet include an increased susceptibility to infections, rough, dry, scaly skin, loss of smell and appetite, lack of tearing, and defective teeth and gum growth.

2. Vitamin C

Vitamin C is water-soluble, and probably the most famous of all the vitamins. It is one of the most important vitamins for our body. It prevents a lot of diseases, and increases our body's immune system. Vitamin C is widely distributed in fruits and vegetables. Citrus fruits, peppers, green vegetables and fruits like strawberries, guava, and mango are particularly rich sources.

Functions of vitamin C

- Immune stimulating The most prominent role of vitamin C is its immune stimulating effect, which is important for the defense against infections such as common colds.

- Anti-allergic- It acts as an inhibitor of histamine, a compound that is released during allergic reactions.
- Anti-oxidant- As a powerful antioxidant it can neutralize harmful free radicals and aids in neutralizing pollutants and toxins. Thus it is able to prevent the formation of potentially carcinogenic nitrosamines in the stomach (due to consumption of nitrite-containing foods, such as smoked meat). Importantly, vitamin C is also able to regenerate other antioxidants such as vitamin E. Due to these functions vitamin C, especially in combination with zinc, is important for the healing of wounds.
- Vitamin C contributes to the health of teeth and gums, preventing hemorrhaging and bleeding. It also improves the absorption of iron from the diet.
- Finally, vitamin C is also a crucial factor in the eye's ability to deal with oxidative stress, and can delay the progression of advanced age-related macular degeneration (AMD) and vision-loss in combination with other antioxidant vitamins and zinc.

3. B Vitamins

In East Asia, where polished white rice was common staple food of the middle class, beriberi resulting from lack of vitamin B_1 was endemic. In 1884, Takaki kanehiro, a British trained medical doctor of the imperial Japanese Navy, observed that beriberi was endemic among low-ranking crew who often ate nothing but rice, but not among officers who consumed a western-style diet. With the support of the Japanese Navy, he experimented using crews of two battleships; one crew was fed a diet of meat, fish, barley, rice, beans. The group that ate only white rice documented 161 crew members with beriberi and 25 deaths, while the latter group had only 14 cases of beriberi and no deaths. This convinced Takaki and the Japanese Navy that diet was the cause of beriberi, but mistakenly believed that sufficient amounts of proteins prevented it (McCormick DB, 1998). That diseases could result from some of dietary deficiencies was further investigated by Christian Eijkman, who in 1897 discovered that feeding unpolished rice instead of the polished variety of chickens helped to prevent beriberi in the chickens. The following year, Fredrick Hopkins postulated that some foods contained “acces sory factors” –in addition to proteins, carbohydrates, fats- that were necessary for the function of the human body (Shaw GM, *et al*. 1995) Hopkins and Eijkman were awarded the novel prize for physiology or medicine in 1929 for their discovery of several vitamins (Rosenfeld. L.1997). In 1910, the first vitamin complex was isolated by Japanese scientist Umetaro Sujuki who succeeded in extracting a water-soluble complex of micronutrients from rice bran and named it acerbic acid (later Orizanin). He published this discovery in a Japanese scientific journal (Jack Challem 1997). When the article was translated into German, the translation failed to gain publicity. In 1912 Polish biochemist Casmir Funk isolated the same complex of micronutrients and proposed the complex be named “vitamine” (a portmanteau of “vita l amine”) (Carpenter, K. 2004). The name soon became synonymous with Hopkins’ “accessory factors)” and b y the time it was shown that not all vitamin were amines, the word was already ubiquitous. In 1920, Jack Cecil Drummond proposed that the final “e” be dropped to deemphasize the “a mine” reference, after researchers began to suspect that not all “vitamins” (particularly vitami n A) had an amine component (Rosenfeld. L.1997; Tokyo K, 1911). As their important and functions were clarified, US government began recommending daily intake levels to promote and maintain good health. The current recommended levels are known as dietary reference intakes (DRIs).

B vitamins are a group of water-soluble vitamins that play important role in cell metabolism. The B vitamins are necessary to

- Support and increase the rate of metabolism
- Maintain healthy skin, hair and muscle tone
- Enhance immune and nervous system function
- Promote cell growth and division, including that of the red blood cells that help prevent anemia
- Reduce the risk of pancreatic cancer- one of the most lethal forms of cancer when consumed in food, but not when ingested in vitamin tablet form.

All vitamins are water-soluble, and are dispersed throughout the body. Most if the B vitamins must be replenished regularly, since any excess is excreted in the urine. B vitamins have also been hypothesized to reduce the symptoms of attention deficit hyperactivity disorder. However, taking large doses of certain B vitamins may produce harmful effects. Several vitamin deficiency diseases may result from the lack of sufficient B-vitamins.

Source of vitamin B

Certain fruits, vegetables, nuts and legumes are good sources of vitamin B; however, they do not contain significant amounts of B12 that is found in meat and dairy foods. Pomegranate, dates, watermelon, and some berries are especially high in B-complex vitamins. Leafy greens and vegetables such as amaranth, bok choy, Brussels sprouts, potatoes, squashes and parsnips also contain significant amounts. Most legumes have a lot of B vitamins; however, soy beans, black-eye peas and edamame contain the highest amounts of B9, also known as folate.

Table 1. 1: B vitamins & its deficiency

Vitamin	Deficiency
Vitamin B_1	Deficiency causes beriberi, symptoms of which involve nervous system including weight loss, emotional disturbances, Warnicke's encephalopathy (impaired sensory perception), weakness and pain in the limbs, periods of irregular heartbeat, and edema. Heart failure and death may occur in advanced cases. Chronic thiamine deficiency can also cause Korsakoff's syndrome, an irreversible psychosis characterized by amnesia and confabulation.
Vitamin B_2 (riboflavin)	Deficiency causes ariboflavinosis, symptoms of which may include cheilosis (cracks in the lips), high sensitivity to sunlight, angular cheilitis, glossitis (inflammation of the tounge), seborrheic dermatitis or pseudo-syphilis (particularly affecting the scrotum or labia majora and the mouth), pharyngitis (sore throat) hyperemia, and edema of the pharyngeal and the oral mucosa.
Vitamin B_3 (niacin)	Deficiency, along with a deficiency of tryptophan causes pellagra. Symptoms included aggression, dermatitis, insomnia, weakness, mental confusion, and diarrhea. In advanced cases pallegra may lead to dementia and death; 4Ds: dermatitis, diarrhea, dementia and death.
Vitamin B_5 (pantothenic acid)	Deficiency can result in acne and paresthesia, although it is uncommon.
Vitamin B_6 (pyridoxine)	Deficiency may lead to microcytic anemia, depression, dermatitis, hypertension, edema , and elevated levels of homocysteine.

Minerals

Minerals are inorganic micronutrients. We need them in small amounts to help our body function properly. There are 16 minerals which are essential nutrients and must be supplied by the diet. These minerals are divided into two groups: major and trace minerals. Major minerals, like calcium, chloride, magnesium, phosphorus, potassium, sodium and sulfur, are found in our body in amounts larger than 5 grams. Trace minerals, like chromium, copper, fluoride, iodine, iron, manganese, selenium and zinc, are found in our body in amounts less than 5 grams.

a. Calcium

Calcium, an essential dietary element, plays a major role in keeping bones healthy and regulating nerve and muscle functions. It also helps regulate the passage of nutrients in and

out of the cell walls, lowers blood pressure, is important to normal kidney function and reduces blood cholesterol levels. It is the most abundant mineral in the body. Nearly all the calcium in your body remains in the bones and teeth, with just a small amount in the bloodstream. If we lack calcium in our diet, our body removes it from your bones and teeth to use in the bloodstream. In combination with phosphorus it forms calcium phosphate, the dense, hard material of the teeth and bones.

A deficiency may result in arm and leg muscles spasms, softening of bones, back and leg cramps, brittle bones, rickets, poor growth, osteoporosis, tooth decay and mental depression.

b. **Phosphorus**

It is after calcium the second most abundant mineral in the body. It is a principal mineral of bones and teeth. The main inorganic component of bone is calcium phosphate salts. Phosphorus is involved in most metabolic actions in the body, including kidney functioning, cell growth and the contraction of the heart muscle. It is also involved in converting food to energy.

A deficiency is unusual, but may have symptoms varying from painful bones, irregular breathing, fatigue, anxiety, numbness, skin sensitivity and changes in body weight. It is important that the calcium and phosphorus levels of the body are in balance. Higher levels of phosphorus relative to calcium can cause low blood calcium levels, which may result in increased risks of high blood pressure and bowel cancer.

c. Iron

Iron is an essential mineral. Its major function is to combine with protein and copper in making hemoglobin, the component of the blood that carries oxygen from the lungs to the tissues throughout the body. People with iron-poor blood tire easily because their bodies are starved for oxygen. Iron builds up the quality of blood and increases resistance to stress and disease. Iron is also part of myoglobin, which is found only in muscle tissue and helps muscles store oxygen.Iron which comes from fruits and vegetables is well regulated by the body, so overdose is rare and usually only occurs when people take supplements. Contrary to popular belief, fruits and vegetables can be good sources of iron, in addition; vitamin C foods which are mostly fruits and vegetables, help increase the absorption of iron into the body. The current percent daily value for iron is 18 milligrams (mg).
A deficiency may result in weakness, fatigue, paleness of the skin, constipation and anemia.

d. Zinc

Zinc is vital to immune resistance, wound healing, digestion, reproduction, physical growth, diabetes control, taste and smell and maintaining normal Vitamin A levels and usage. Zinc can be found in almost every cell of the body and serves as part of more than 70 enzymes that control body processes.

A deficiency may result in poor growth, acne-like rash, hair loss, diarrhea, delayed sexual maturation, impotence, sterility, eye lesions, loss of appetite, reduced sense of taste and smell, skin lesions and inflammation, poor wound healing, reduced resistance to infections, mental confusion, poor learning ability, changes in hair and nails and anemia. An excess of zinc is rare but possible. It is usually caused by over supplementation. Excess zinc can cause a copper deficiency, diarrhea, cramps, nausea, vomiting, suppressed immune function, impaired formation of red blood cells and reduced levels of HDL ("good") cholesterol.

e. Copper

Copper is involved in the absorption, storage and metabolism of iron. It is important in the formation of red blood cells and keeps bones, blood vessels, nerves and the immune system healthy.

The symptoms of a copper deficiency are similar to iron deficiency. The average level of copper stored in the body is between 50 and 120 mg, most of this in the liver. Excess dietary copper is rare but may occur and can cause liver damage. An excess is usually caused by over supplementation and can lead to symptoms such as weakness and nausea.

Other nutrients

Phenolic compounds

Phenolic compounds, which are synthesized primarily from product of the shikimic acid pathway, are ubiquitous constituents of higher plants found in a wide range of commonly consumed plant foods such as fruits, vegetables, cereals and legumes, and in beverage of plant origin, such as wine, tea, and coffee. The general definition of phenolic compounds is any compound containing a benzene ring with one or more hydroxyl group.

Food source

Apples, Broccoli, Beans, Berries, blackberries, cabbage, carrot, cauliflower, cruciferous vegetables (broccoli, Brussels sprouts, kale, turnips, watercress), cucumber, garlic, orange, potatoes, soybeans, tea, Spinach are rich source.

Among the edible plant materials, remarkable high antioxidant activity and high total phenolic content (GAE > 20 mg/g) were found in berries, especially aronia and cowberry. Apple extract (two varieties) showed also strong antioxidant activity even though the total phenolic contents were low (GAE < 12.1 mg/g). (Kähkönen *et al*, 1999)

Function of phenolic compound

- They have potentially protective factors against human chronic degenerative diseases (cataracts, muscular degeneration, neurodegenerative diseases and diabetic mellitus), cancer and cardiovascular diseases.
- Their consumption has been associated with positive health benefits such as antioxidant, antiviral, anti-allergic, cardioprotective, and anti-carcinogenic effects.

Various epidemiological studies have shown an inverse association between the consumption of polyphenols or polyphenol rich foods and risk of CVD A meta-analysis including seven case control and ten cohort studies suggested a reduction of the risk of myocardial infarction of 11% upon consumption of three cups of tea per day (Peters *et al*, 2001). One more recent prospective study and three cross sectional studies tend to confirm these protective effects of tea against CVD risk. (Yu *et al*, 1995; Rimm *et al*, 1996; Hertog, 1995; Knekt *et al*, 1995)

Antioxidant effects of polyphenols have been reviewed (Delgado *et al*, 2008; Scalbert *et al*, 2005). The most recent human intervention trials showed that when the source of polyphenol was consumed for 1-12 weeks, an increase in the plasma antioxidant capacity or in the concentration of antioxidants such as vitamin-E, Vitamin C, β-carotene and uric acid was observed in some studies, whereas no change were observed in other studies. The same contrasting results were obtained for lipid oxidation products in plasma and low density lipoprotein.

Polyphenols have a variety of anti-inflammatory and anti-modulating effects that may be of relevance of atherosclerosis. Supplementation with black tea, green tea, green tea

polyphenoles isolate, red wine, grape juice and cocoa affected only a few blood parameter related to inflammation such as circulating adhesion molecules, cytokines and mediators.(Badía, *et al*, 2004; De Maat, 2000;Jalil & Ismail, 2008; Hodgson *et al*, 2001; Scalbert *et al*, 2005).

Several types of polyphenols (phenolic acids, hydrolysable tannins, and flavonoides) show anticarcinogenic and antimutagenic effects. Polyphenols might interfere in several of the steps that leads to the developement of malignant tumors, inactivating carcinogens, inhibiting the expression of mutant genes and the activity of enzymes involved in the activation of procarcinogens and activating enzymatic system involved in the detoxification of xenobiotics.(Manach *et al*, 2005).

Phytic acid:

Phytic acid (myo-inositol hexa-phosphoric acid, IP6) is the major phosphorus storage compound of most seeds and cereal grains, it may account for more than &0% of the total phosphorus. Phytic acid has a strong ability to chelate multivalent metal ions, specially zinc, calcium and iron. The binding can result in very insoluble salts with poor bioavailability of minerals (Rhou& Erdman, 1995). Besides its well-known negative properties IP6, by complexing iron, may bring about a favorable reduction in the formation of hydroxyl radicals in the colon (Graf & Eaton, 1993), also positive effect against carcinogenesis have been shown with in vitro cell culture systems, mice, rats and guinea pigs, but the mechanism of action is not understood (Harland & Morris, 1995). Others have suggested that this substance may serve as a store of phosphorus of cations, of glucuronate (a cell wall precursor), or of high energy phosphoryl groups, which can be metabolized by phytase and phytate-nucleotide diphosphatephosphotransferases during germination to support early events in plant development. However, many other metabolites may also supply energy, glucuronate, cations, and phosphorus, suggesting that phytate may subserve additional presently unknown functions.

Metabolism: Role of phytase

The phosphate ester linkages in phytic acid are quite stable. Natural degradation is almost impossible and chemical hydrolysis in the laboratory is very slow (Tunner*et,al,.* 2002). However, the enzyme phytase found in the blood of calves, birds, reptiles and fishes (Haefiner*et,al,.* 2005), in roots, vegetative storage organs and in pollen (Rayboy, 2003), in mature seeds (Williams, 1970) or secreted by variety of microbes-can rapidly breakdown phytate (Mullaney and Ullah, 2003). Phytases are hydrolyses that initiate the step-wise removal of phosphate from phytate (Lei and Porres, 2003; Feng*et. al.,* 2009). To date, four classes of phytases have been characterized:

1. Histidine acid phosphatase (HAP),
2. Cysteinphytase,
3. Purple acid phosphatase and
4. Beta-propeller phasphatase (BPP) (Lei *et. al.,* 2007).

A comparison of the properties of these phytase indicated that only BPP has a neutral optimum pH (6.0 to 7.5) for activity, whereas, the other enzymes have an acidic optimum pH (2.5 to 5.5) (Cheng and Lim, 2006). While hydrolyzing phytate, HAP produces myo-inositol monophosphate as the final product, whereas, alkaline phytase produces myo-inositol triphoaphate as the final product (Oh *et. al.,* 2004). Monogastric and agastric animal do not produce intestinal phytase and thus they require phytases derived from intestinal microbes for efficient phytate hydrolysis in the neutral pH environment of intestine (Ringe*et. al.,* 1995) to facilitate optimal growth of the animals (Oh *et. al.,* 2004).

Phytases are added to animal feedstuff to reduce phosphate pollution in the environment. Dietary phytase increase minerals bioavailability. Phytase application can reduce phosphorus

excretion by up to 50%. Grossly, phytase supplementation of in feed can be beneficial as they can reduce feed costs by the reduction of additional supplementation of phosphorus, reduced the threat of environment pollution from excessive of waste products and the improved utilization rate of other nutrient.

A number of phytase genes and proteins have been identified from plants and microbes including bacteria, yeast and fungi. Expression of phytases in plants is underway to develop plant cultivars that would produce enough transgenic phytase to avoid additional supplementations to the feedstuffs (Mullaney et. al., 2000).

Biological functions

Phytic acid consists of a myo-inositol ring with six-phosphate moieties attached (Graf and Eaton, 1993). Phytic acid is believed to control blood sugar and may reduce the incidence of kidney stones. It acts as a natural anti-oxidant by chelating and reducing the catalytic activities of many divalent transition metals (Rickared and Thompson, 1997). Phytic acid also possesses properties of well known antioxidants.

In contrast, phytic acid is considered to be an antinutrient because it inhibits the absorption of minerals and proteins in the intestine of the human and other non-ruminants, and thus make them indigestible (Oloffs*et. al*., 2000). The physiological effects of phytic acid are applicable to human and animal nutrition and metabolism, as they have been reported to regulate the process of digestion by binding to some digestive products which may in turn delay the onset of diabetes and hyperlipidaemia. In animals, it has been association with reduced absorption of certain minerals especially iron. Populations that depend on wheat as staple food, consume diet rich in phytic acid, bear severe consequences such as anemia, complication in pregnancy, and poor growth, most probably due to chelating property of phytic acid

Phytic acid: A Natural Antioxidant

The catalysis by iron of radical formation and subsequent oxidative damage has been well documented. Although many iron-chelating agents potentiate reactive oxygen formation and lipid peroxidation, phytic acid (abundant in edible legumes, cereals, and seeds) forms an iron chelate which greatly accelerates Fe2+-mediated oxygen reduction yet blocks iron-driven hydroxyl radical generation and suppresses lipid peroxidation. Furthermore, high concentrations of phytic acid prevent browning and putrefaction of various fruits and vegetables by inhibiting polyphenol oxidase. These observations indicate an important antioxidant function for phytate in seeds during dormancy and suggest that phytate may be a substitute for presently employed preservatives, many of which pose potential health hazards.

$$Fe^{2+} + ROOH \text{ --------> } Fe^{3+} + RO^{\cdot} + OH^{-}$$

Phytate, by virtue of chelating free iron, is a potent inhibitor of iron-driven hydroxyl radical (OH) formation. Hydroxyl radical generation mediated by iron requires the availability of at least coordination site that is open or occupied by a readily dissociable ligand such as water. Although most chelates retain a reactive coordination site, Fe^{3+} -phytate does not consequently fails to support $^{\cdot}OH$ generation.

Cancer protection

Inositol hexakisphosphate (IP6) or phytate has been reported to have significant in vivo and in vitro anticancer activity against numerous tumors. However, the molecular mechanism of the effect IP6 has been fully elucidated. The results of some studies show that IP6 is a strong inducer of differentiation (cytostatic effect) and a moderately strong inducer of apoptosis (cytotoxic effect). Evidence has been provided to show that the growth inhibitory effects of IP6 are mediated through the modulation of key signaling pathways. The anti-cancer effect of IP6 was found to be associated with the modulation of multiple genes involved in immunity, Wnt and IGF pathways, IP3 kinase signaling and apoptosis. It protects against several types of cancers, for instance, breast cancer, and cancerous tumors. Fiber having phytic acid is known to be protective against colon cancer; however, it is unclear that the anti-cancerous effect is due to the fiber or the phytic acid (Jenab and Thompson, 1998). Later on, it became obvious that pure phytic acid added to low fiber diet can significantly increase the rate of apoptosis and degree of differentiation in the distal colon (Jenab and Thompson, 2000).

It is a negatively charged molecule and capable of binding proteins and starches present in the diet and affect their solubility, digestibility, function and absorption (Rickard and Thompson, 1997) that may affect the colon environment by stimulating short chain fatty acid production from fermentation of the trapped

starches (Jenab and Thompson, 1998). Also, chelating ability of phytic acid has been suggested to suppress iron-mediated oxidation in the colon, thereby reducing colon cancer risk (Shamsuddin, 1992).Knowledge of the mechanisms through which phytic acid blocks angiogenesis is yet to be explored. Maybe, it blocks sprouting of new capillaries around newly forming uncontrolled cell growth by making phosphorus and other minerals less bio-available, as angiogenesis is an energy consuming process and may only flourish if required nutrients are available. It is likely that in addition to the inhibition of iron-catalyzed oxidative damage, other pathways such as the activation of immune competent cells (Baten et. al., 1989), as well as the participation of inositol phosphates in signal transduction, cell division and differentiation (Shamsuddin, 1992) are also responsible for beneficial effects of dietary phytate in cancer prevention (Rimbach and Pallauf, 1998).

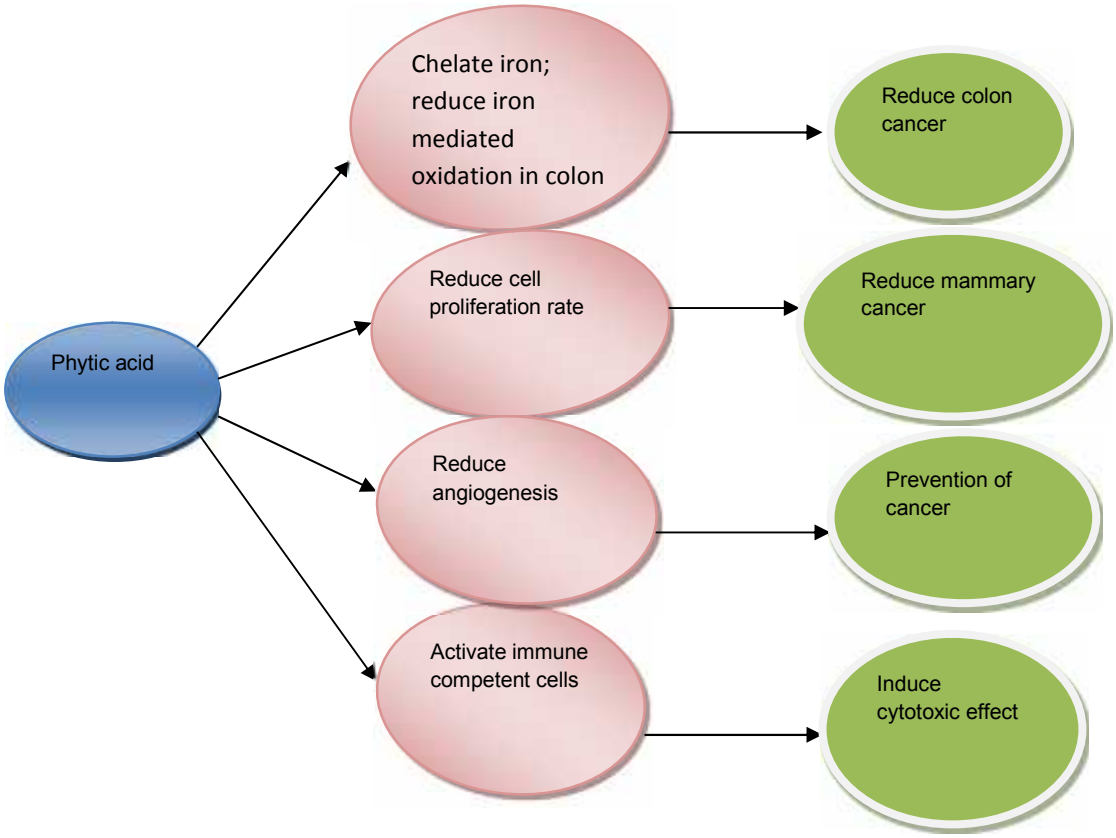

Fig 1.1: Postulated anticancerous effects of phytic acid

Role in Diabetes

Phytic acid has health benefits for diabetes patients. Phytic acid is believed to control blood sugar and may reduce the incidence of kidney stones. It lowers blood glucose response by reducing the rate of starch digestion and slowing the gastric empting. Dolworth*et. al*.,(2005), revealed that phytic acid extract consumption from sweet potato and commercial phytic acid plus zinc supplement lowered blood glucose levels. It causes no significant change in the activity of 6-phosphogluconate dehydrogenase or in pyruvate kinase. It causes a significant increase in the activity of glucose-6-phosphate dehydrogenase in the groups supplemented with phytic acid extract. The activities of malic enzyme and ATP-citrate lyase also were not significantly altered.

Antioxidant activity (DPPH and FRAP assay) and type ll diabetes-related enzyme inhibition property (α-glucosidase inhibition activities) of phytic acid in the raw and processed samples were analyzed according to standard methods. The phytic acid extracted revealed 59-89% of DPPH radical scavenging capacity, 27-3526 mmol Fe (ll)/g of reducing power, 20-72% of α- amylase inhibition activity and 8-91%

of α-glycosidase inhibition activity. Cooking of vegetables and roasting of grains improved the functional properties of phytic acid.

Phytic Acid Prevents Oxidative Stress in Seeds

Phytic acid is a good candidate for protecting the embryo oxidative processes. Oxidative stress is a pervasive and ubiquitous environment related problem that plant cells must cope with. Because of photosynthetic activity, it is particularly frequent in the green parts of the plant, but also affects seeds, usually in the last phase of maturation when seed tissues undergo dehydration which is often accompanied by oxidative stress (Bailly, 2004). The viability of plant seeds is severely and primarily influenced by the degree of oxidative stress Fe^{3+}, alone has been shown to cause the production of reactive oxygen species and lipid peroxidation to Fe^{3+}, which is relative inert even in the presence of oxygen and polyunsaturated lipids.

In seed tissues, as a polyanion at physiological pH, phytic acid is an effective chelator of positively charged cations of important macro-nutrients including K, Mg, Ca, Fe, Zn and Mn, forming phytate (also called phytin), which is sequestered in specialized vacuoles termed protein bodies or protein storage vacuoles (Lott, 1984; Wada and Lott, 1997; Raboy, 2002). Phosphorus and mineral cation reserves deposited in the phytate molecule are essential for germination and for the growth and development of seedlings (Lott, 1984). Phytic acid, by virtue of its ability to chelate iron, is a potent inhibitor of the iron-driven formation of reactive oxygen species (Graf *et. al*., 1984) and of lipid peroxidation in vitro (Graf *et. al*., 1987; Graf and Eaton, Empson*et, al*., 1991).

Therapeutic Uses:

- Phytic acid may be considered a phytonutrient, providing an antioxidant effect (EN, BM Group). Phytic acid's mineral binding properties may also prevent colon cancer by reducing oxidative stress in the lumen of the intestinal tract (Vucenik 1, *et. al*., 2003).
- It has been shown that phytic acid is protective against parkinson's disease *in vitro* (XU, Q, *et.al.*, 2008).
- The compound significantly decreased apoptotic cell death induced by 1-methyl 1-4phynylpyridinium in a cell culture model.
- Phytic acid crosses the blood-brain barrier, (grases F, *et. al.,* 2001) and so, there is a strong possibility that neuroprotective occurs *in vitro* as well.
- Ironically it has also been shown that phytic acid is a required cofactor for *YopJ*, a toxin from Yersinia pestis (Mittal R, et. al., 2010). It is also a required cofactor for the related toxin *AvrA* from *Salmonella typhimurium* (Mital R, *et. al*., 2010).
- Phytic acid's chelating effect may serve to prevent, inhibit, or even cure some cancers by depriving those cells of the minerals (especially iron) they need to reproduce.

Physiological Effects

High-phytate diets results in mineral deficiencies. In population where cereal grains provide a major source of calories, rickets and osteoporosis are common. Interestingly; the body has some ability to adapt to the effects of phytate in the diet. Several studies shows that subjects given high levels of whole wheat, at first excrete more calcium than they take in, but after several weeks in this diet, they reach a balance and do not excrete excess calcium.

The zinc and iron blocking effect of phytic acid can be as serious as the calcium blocking effects. For example, one study showed that a wheat roll containing 2g phytic acid inhibited zinc absorption by 18%; 25g phytic acid in the roll inhibited zinc absorption by 64%; and 250g inhibited zinc absorption by 82%. Nuts have a marked inhibitory action on the absorption of iron due to their phytic acid content.

Over the long terms, when the diets lack minerals or contain high levels of phytate or both, the metabolism goes down, and the body goes into, minerals-starvation mode. The body then sets itself up to use as little of these minerals as possible. Adults may get by for decades on a high-phytate diet, but growing children run into sever problems. In a phytate rich diet, their bodies will suffer from the lack of calcium and phosphorus with poor bone growth, short

stature, rickets, narrow jaws and tooth decay; and for the lack of zinc and iron with anemia and retardation.

Environmental Effects

Phosphorus, which is bound in grain phytates as an insoluble salt, cannot be absorbed by humans and other non-ruminant animals like pigs and chickens, because of the lack of the digestive enzymes, phytase, required separating phosphorus from the phytate molecule.

In most commercial factory farms, non-ruminant livestock such as pigs and poultry are fed a diet rich in soybeans and corn. Because the phytate from these foods cannot be broken down, it passes through the gastrointestinal tract of these animals and elevates the amount of phosphorus in the manure excreted.

Plant Foods of Bangladesh

Bangladesh is now recognized as a developing country but in fact it is still regarded by many as under-developed and under poverty level a large number of people live in poverty. Once the leafy vegetables, popularly and locally known as “Shak” were the major consumable item to be taken with rice by the poor people of Bangladesh. At the time of famine, they sometimes become the only source of food/edible items for the people living below poverty level. None of the consumer knew their nutritional composition or in other words they did not take them as nutrient supplement but to survive somehow.

With the discovery of the nutritional values of the leafy vegetables, specially as regards to the vitamins and minerals and the necessity of these for human being to remain in good health rich people, educated people and the town dwellers’ became interested to include some leafy vegetables as one of the food items with their meals. Doctors and nutritionists are also suggesting their patients to take some leafy vegetable every day. The town dwellers depends for their required vegetables on the market supplies, the village people (of plain land) depends on supplies from their own crop field, fallow waste lands as well as on market, whereas the tribal people living in remote hilly areas depend almost entirely on the wild vegetation.

Uncultivated plants are typically plucked rather than uprooted so that they will regenerate and provide food again. They are in this sense similar to another category of uncultivated foods recognized by local people, that is, cultivated plants that provide edible leaves and shoots in addition to the primary product of cultivation. This includes species such as mustard which is cultivated for the oil seed and jute which is cultivated for its fibrous stalk. The leaves of both plants are also plucked for food (as vegetable). In fact, plucking the leaves in the early part of the growing season is considered by many farmers to be good for the plant and is therefore allowed when with the leaves of pumpkins, sweet gourd, white gourd, and other vegetables grown in the homestead and plucked for vegetables in small quantities almost everyday before the they start fr. Consumptions of such food materials is confined to the people living in the areas where they grow. Recognizing the need for identification of such green leafy vegetables, which are believed to be nutritious, may help in achieving nutritional (Micronutrient) security (Gupta *et al*., 2005).

The number of uncultivated species used as food is also very large. Some 102 species of leafy greens associated which agricultural fields, homesteads and common areas were identified by local people as food, mainly herbs, creepers, aquatic plants, shrubs and trees (Schmid, 2005). They are most abundant during the monsoon, when other food plants are not ready for harvest but pre-monsoon periods.

Vegetable is important for nutritional, financial, and food security in Bangladesh. However, the availability of vegetable is only about 1/5th of the recommended requirement of 200 g/person/day. Based on the growing season, vegetables are categorized as summer/rainy, winter and all season vegetables. The major winter vegetables are cabbage, cauliflower, tomato, brinjal, radish, hyacinth bean, bottle gourd, etc while major summer vegetables are pumpkin, bitter gourd, teasle gourd, Bitter gourd, ladies finger, ribbed gourd, ash gourd, okra, yard-long bean, and Indian spinach,these non-leafy vegetables are carried number of health benefits for human health. Despite of those vegetables,there are numbers of non leafy ethnic plants grows in hilly area, which are almost unknown to mass people are really nutrient rich and beneficial to human health.

Leafy vegetables are among the most nutritious vegetables on a fresh weight basis and are also among the world's most productive plants in terms of nutritional value per unit area, in part because they grow rapidly, allowing several crops or harvests in a season. Although some of the constituents are lost during cooking, they still contribute significant amounts of pro vitamins A and C and several minerals.Leafy vegetables are always green (except for those that have been blanched, such as Belgian, Endive), but some are distinguished further by being called "Dark Green Leafy Vegetables." The darker the leaf, the more Vitamin A, Vitamin C, and Calcium the leaves will have. As well the darker the leaf, the more "vigorous" the taste, which is why it's more of a battle to get people to eat them.

Dark green leafy vegetables are good sources of many vitamins and minerals which body needs to stay healthy, such as vitamins A, C and K, foliate, iron and calcium. They are also great sources of fiber. Research suggests that the nutrients found in dark green vegetables may prevent certain types of cancers and promote heart health.

Despite the importance of leafy and non-leafy vegetables in the present day human lives, no systematic work has been carried out in Bangladesh to identify and document the plant species, their ethno botanical use and also the nutra-medicinal properties of a large number of leafy vegetable items, especially those are used in the remote areas

1.3 Rationale of the study

Nutrition Science attempts to isolate biologically active principles in foods with potential to improve health and to reduce the risk of diseases.FCT for Bangladesh has very recently been published (Shaheen *et al*, 2013). Preparation FCD and FCT have been launched (FAO, 2010; Islam *et al*, 2012).CFCS and FGDs data document that ethnic people uses a variety of their foods in treatment of many ailments (Islam *et al*, 2010).

Nutrient composition of ethnic foods is by far not available, even is not yet explored. Thus, this study attempted to look at a systematic approach to analyze the nutrient composition of some specific vegetables growing in tribal regions of Bangladesh. It is important for promoting production and consumption of available foods for improving both the quality and quantity of the diets of the ethnic / tribal population.

Nutrient composition of indigenous foods grown and consumed in the Chittagong Hill Tracts (CHTs) and other tribal areas is not known. To prepare dietary guidelines and to determine standard dietary intake, the nutrient content of these foods needs to be known.

Moreover, this study will play an important role in conservation and preservation of the ethnic/tribal plant food resources. Exploring the nutritional value of these ethnic/tribal plants will help tribal communities overcoming many diseases. Consequently, this outcome will help to aware the tribal people to lead a healthy life. This will reduce the burden on healthcare and improve the socioeconomic of the ethnic people.

Furthermore, the food chain of the country has been modified during the last decades. Nutritive values of these ethnic food items need to be analyzed and incorporated in the food composition database.

It is time to prepare a Food Composition Database with nutrient data through analysis of ethnic and relatively newer foods. Such a Food Composition Database will help in formulating dietary guidelines for ethnic people to meet their nutrient, consequently health requirements. This is also in line with one of the key areas of intervention of the National Food Policy Plan of Action (2008-2015). *(Islam et al. 2010).*

By far, none of these ethnic foods has been explored for their medicinal principles.A comprehensive nutrient composition database is one of the key in nutritional epidemiology for assessing intakes of nutrients and for informing policies looking at improving nutrition.

1.4 Objective of the study

Most ethnic communities have traditionally relied on a very broad food base, comprising of mainly wild food plants, which was nutritionally excellent. This study aimed to analyze nutrient composition and medicinal properties of some selected ethnic foods. In line of its objective, it has been designed to

Analyze Nutrient Composition of selected 25 ethnic vegetables that included,

a. *Proximate nutrients: moisture, protein, fat, ash, total dietary fibre (TDF).*
b. *Micronutrients:*
    - *Vitamin C, carotenoids, carotene profile and B vitamins (B_1, B_2, B_3, B_6),*
    - *Mineral: Cu, Zn, Fe, Ca, P.*
c. *Phytonutrients: Total phenol and Phytic acid.*

CHAPTER 2

Materials and Methods

2. Methods of analysis

2.1 Sampling of Plant Food

A sampling plan is the predetermined procedure for selection, collection, preservation, transportation and preparation of the analytical portion to be used from a lot as samples. A sampling plan should be a well organized document for program objectives (Proctor *et al*, 2003).

Foods are biological materials and exhibit variation in composition, particularly prone to variation in water, carbohydrate and vitamin contents. This variation is related to a number of factors such as cultivation place (cultivated, wild, garden), geographical location, seasons, state of maturity, cultivar and breed, etc. Therefore, collection of food sample needs to be specific in terms of timing and frequency to reflect these variations.
Food sampling is one of the most important aspects for compositional analysis. It determines the analytical data quality that needs to provide representative nutrient valueto the users. However, food sampling is difficult because of its variability and heterogeneity in composition. The primary objective in food sampling is to collect arepresentative food sample and to ensure that changes in nutrient composition do notoccur between collection and analysis (Greenfield and Southgate, 2003c).Sampling error arises with using a part of total food sample. It is because ofheterogeneity nature of foods. Taking small portions at the primary sampling stage can lead to sampling error. Sampling error is also associated with poor labeling &documentation, non-conforming sample use, incorrect mixing, and also in appropriate storage. In practice, 100-500g represents a convenient sample size. The larger sample size the more reliable the sampling; however, sample size is limited by time, cost,sampling methods and logistics of sample handling, analysis and data processing.Therefore, replicate samples of representative amount must always be taken when estimating the composition of food.

Ethnic food sampling

Ethnic food items were collected from local weekly markets at Rangamati, Bandarban and Khagrachori. Two food samples for each food item were collected from each market. Every two food samples were pooled together to make three analytes (test sample), which were analyzed for their nutrient profile. A few ethnic foods were collected during September through December, 2011 but most of the ethnic foods were collected during January-March, 2012. The ethnic food sampling plan is depicted in figure 2.1.

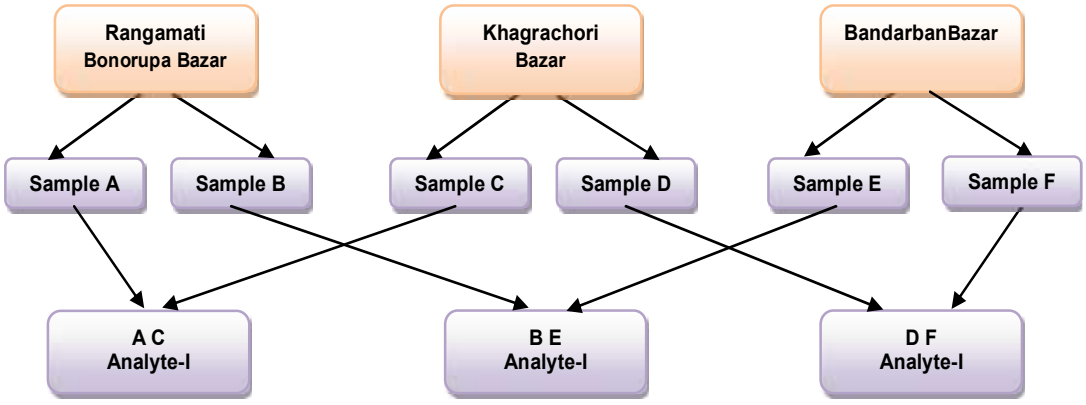

Fig 2.1: Sampling Plan for Ethnic food

Procedure for food sample collection

Ethnic food samples were collected from weekly wholesale markets at Rangamati, Khagrachori and Bandarban district. Two samples for each food items of approximately 1.5kg were purchased from each market. The samples were water sprayed and packed into new clean plastic poly bags for transportation to the lab.

Image. 2.1

2.2 Identification of collected food samples

The food items (vegetable and fruit) were categorically identified and certified by personnel of Department of Agricultural Extension (DAE) and Maksuda Khatun (Ph.D) taxonomist of the Department of Botany, Dhaka University. Food samples were purchased from the weekly wholesale markets with the help of local ethnic DAE staff, who confirmed its identity. After taking the food sample to the lab, the taxonomic expert further identified it for its scientific and English name.

Collection of fresh materials has been made from local markets, village areas and forest lands. Prior to collections assistance from local informants has been taken regarding the use of plant as leafy vegetables. Local vegetable markets have also been surveyed to record marketable items.

The collected samples were made into herbarium sheets following the standard herbarium technique (Hyland, 1972).

Image.2.2

Table 2.1: List of selected ethnic food samples

Sl	Local name	English name	Scientific name	Collection area
Leafy vegetables				
1	Kochi aam pata	Mango leaf	*Mangifera indica* L	CHTs
2	Kamino	Not known	*Caesalpinia digyna* Rottler	CHTs
3	Moroi shak	Funnel.	*Foeniculum vulgare*	CHTs
4	Amsurothi	Not known	Not known	CHTs
5	Noyalongbikrongi	Trailing Smartweed.	Not known	CHTs
6	Monjuri	Not known	Not Known	CHTs
7	Yangfo	Banyan Tree	*Feics benghalensis* L	CHTs
8	Missayanu	Not known	*Sarcochlamys*	CHTs
9	Felong dal sak	Common Bean	*Phaseolus vulgaris* L.	CHTs
10	Gaiboma	Not known	*Polycarpan prostratum*	CHTs
11	Chikipung	Rosy Dock, Dock Sorrel,	*Rumex vesicarius* L.	CHTs
12	Ambush	Not known	*Blumea lacera*	CHTs
13	Mrolapang	Bitter Cassava, Cassava,	*Manihotesculenta* Gantz	CHTs
14	Projukti shak	Arrow leaf False Pickereweed	*Monochoria hastata (L)*	CHTs
15	Khoro pata	Not known.	*Cissus repens Lam.*	CHTs
16	Katoldingi	Arum	*Lasia spinosa (L.) Thw*	CHTs
17	Kasani	Heartshape False Pickereweed	*Monochoria vaginalis*	CHTs
18	Saimya	Lime, Sour Lime, Common Lime	*Citrus antiifolia (chrstm)*	CHTs
19	Bala pata	Pouzolzia	*Pouzolzia hirta (Blume)*	CHTs
Non leafy vegetables				
20	Fala /Tara	Not known	*Alpinia nigra (Gaertn)*	CHTs
21	Forash dal	Kidney been	*Phaseolus vulgaris*	CHTs
22	Kiokokro	Not known	Not Known	CHTs
23	Kortolik	Not known	Not Known	CHTs
24	Mo aloo	Yam	*Dioscorea bulbifera L.*	CHTs
25	Ranga jhum aloo	Greater Yam, Water Yam	*Dioscorea alata*	CHTs

2.3 Taxonomical description ofethnic plant food sample:

Taxonomy considered as the pedestal upon which biology is built deals with identification, nomenclature and classification of organisms. Plant taxonomy is one of the oldest branch of sciences and termed as the mother of all other branches of botany. With the large variety of plants surrounding us, it is very much essential to pinpoint a particular plant of our interest which is done with the consideration of the similarities or differences with other plants. A plant sample one collect from the field to be identified first, then to provide a correct name so that one can communicate ones ideas about it and assigning the plant to a family. The main objective is to provide a convenient method of identification and communication about a taxon and provide a classification which is based on natural affinities of plants.

The vegetables do not form any specific plant group; rather they occur throughout the plant kingdom. The vegetables are among the most nutritious vegetables on a fresh weight basis and are also among the world's most productive plants in terms of nutritional value per unit area. At the present time the medical doctors, the nutritionists, the educated persons all prefer inclusion of some vegetables in their daily food.

Taxonomical description and image of the selected plant foods consumed by ethnic groups in CHTs are presented from the pase 37.

1. Kochi amm pata

Image. 2.3

Scientfic name: *Mangifera indica*L

Family: Anacardiaceae

Mangifera indica L., Sp. Pl.: 200 (1753); Prain, Beng. Pl.1: 248 (1903, rep. ed. 1963).

Local names: *Aam* (Beng.), *Tharaapang* (Rakhain).

English Name: Mango leaf.

Description: A large tree, with bole up to 45 m tall and 1 m in diameter, crown dark green, dense and bushy, bark grey-brown, shallow fissured and scaly. Leaves spirally arranged, simple, 8-40 × 2-10 cm, narrowly elliptic to lanceolate or oblong, base acute, apex blunt, margin often faintly wavy, lateral veins 12-30 pairs, visible on both surfaces, intercostals veins net-like, distinct below, petioles 1.5-10.0 cm long with swollen base. Inflorescence a pyramidal panicle, pseudo-terminal or axillary. Flowers bisexual. Sepals 5. Petals 5, cream to pinkish with 3-5 ridges on the inner face. Stamen 1, fertile, staminodes 4. Ovary sessile, oblique, 1-locular, style lateral, stigmas simple. Fruit a dr[4]*http://en.wikipedia.org/wiki/Tanchangya_people* upe, varies greatly in shape and size, up to 30 x 10 cm, skin yellowish-green to purplish when ripe, pulp pale yellow to orange, with or without fibres, with a sweet to sour taste. Seed 1 per fruit. Flowering and fruiting: January - July.

Habitat: Homesteads, roadsides, plain lands and agricultural lands.

Distribution in Bangladesh: It is cultivated throughout the country.

2. Kamino

Image 2.4

Scientific name: *Caesalpinia digyna*

Family: Caesalpiniaceae

Caesalpinia digyna Rottler, Gerlin. Schrift. 4: 200, t.3 (1803).

Local names: Kamino, Umulkuchi.

English name: Not known.

Description: A large, straggling, scandent shrub, with dark brown red bark and recurved prickles, branches glabrous or slightly downy. Leaves compound, bipinnate, alternate, stipulate, stipules linear-anceolate, awl-shaped, c2 mm long,caduceus, rachis 15-20cm long, glabrous orsparsely pubescent, pinnae 6-12 pairs, up to 5cm long, with a pair of stipular throns at the base, leaflets 8-12 pairs ,8-12x3-4mm, opposite,subsessile, oblong, obtuse to rounded at the apex, slightly unequal at the base, pale beneath. Inflorescence supra-axillary to terminal panicles, 16-30 cm long, hairy whenyoung. Flowers bright yellow, 8-10mm across, bracts awl-shaped, c5mm long, canducous, pedicels slender,1.5-2.5cm long, glabrous, not jointed near the base of the flower. Calyx tube very short, salver-shaped, sepals 5, oblong, obtuse, dotted, the lowest one hood-shaped, glabrous. Petals 5, inserted on the lip of the calyx tube, obovate, oblong or orbicular, the standared petal constricted at the middle, hairy inside along the margin of the claw, reflexed.Stamens 10, free, felaments dilated at thebase and densely woolly in lower part. Ovary glabrous or slightly hairy on the suture. Fruit a pod, 4-6x1.5-2.0 cm elliptic-oblong, fleshy, turgid, slightly constricted between the seeds, thickened along with the margin of both sutures, beaked, twisted, shortly stalked, indehiscent. Seeds 2-4 per pod, 1.2-2.0 x0.7-0.9 mm, sub-globose. Flowering and fruiting: July-January.

Habitat: Clearing, thickets, forest fringes, sometimes slope of hills and open dry places.

Distribution of Bangladesh: It is found in CHTs, Dhaka, Thakurgaon districts.

3. Moroi shak

Image 2.5

Scientific name: *Foeniculum vulgare*

Family: Apiaceae

Foeniculum vulgare Miller, Gard. Diet. Ed. 8, no. 1 (1768); C. B. Clarke in Hook. f., Fl. Brit. Ind. 2: 695 (1879). *Anethum foeniculum* L., Sp. Pl.: 263 (1753).

Local Name: Pan-mohuri (Beng.), Moroi shak (Marma, Chakma)

English Name: Funnel.

Description: A robust, perennial, glabrous, aromatic herb, to 2 m tall. Stem erect, terete, longitudinally striate, profusely branched, internodes hollow when older. Leaves alternate, decompounds, sheathed, lower leaves largest, leaf sheath forming an open cylinder, at the base embracing the stem, margin white scarious, petioles subterete, up to 10 cm long, longer than the sheathing part, longitudinally striate, blade triangular in outline, acute, lobes blue-green, primary pinnae odd-numbered. Inflorescence a terminal, compound umbel, peduncles up to 15 cm (rarely up to 24 cm) long, primary rays 5-30 (rarely up to 70) per umbel, unequal in length, the shortest ones in the centre, secondary rays 10-30 per umbel, up to 1 cm long, unequal in length, involucres and involucels absent. Calyx vestigial at the top of the ovary. Petals 5, distinct, subovate in outline, with strongly inflexed, notched apex, yellow. Stamens 5, c 1.5 mm long. Pistil 1. Ovary inferior, bilocular, styles 2, each with a stylopodium at the base and stigma at the top. Fruit an ovoid-cylindrical, usually slightly curved schizocarp, light green to yellow-brown. Seeds with testa adnate to the pericarp. Flowering and fruiting: November - February.

Habitat: Cultivated in the fields and also in kitchen gardens.

Distribution in Bangladesh: It is cultivated in almost all districts of the country.

4. Amsurothi

Image 2.6

Scientific name: Not Known

5. Noyaliong bikrong

Image -2.7

Scientific name*: Ampelygonum chinense*

Family: Polygonaceae

Ampelygonum chinense (L.) Lindley, Bot. Reg. 24: 63 (1838). *Polygonum chinense* L. (1753); Hook. f., Fl. Brit. Ind. 5: 44 (1886); Prain, Beng. Pl. 2: 664 (1903, rep. ed. 1963).

Local name:,*Mohicharan Sak, Kaker Pantabhat* (Beng.), *Mono-eja-dar* (Chakma).

English Name: Trailing Smartweed.

Description : A perennial herb, with main stem prostrate, growing up to 3 m or more in a year, often scandent on supports. Main stem c 6 mm thick, usually glabrous, branches erect or ascending, up to 60 cm long. Leaves variable in size and shape, petioles c 1 cm long, usually glabrous or hairy beneath, sometimes with 2 auricles at the base, ovate-lanceolate, glabrous, usually glandular, base usually truncate, apex acute to acuminate, margin entire, sometimes crenullate, upper leaves often amplexicaul. Ochrea up to 4 cm long, membranous, unilaterally prolonged, glabrous, eciliate. Inflorescence capitate, peduncles branched, dichotomous or corymbose, mostly with stalked glands, rarely eglandular. Flowers 3.0-3.5 cm long, pedicels up to 1 mm long. Tepals 5, white, accrescent and fleshy, and translucent in fruits. Fruits baccate, fleshy, fruiting perianth c 2 mm thick, white and transluscent outside with a blackish layer inside. Nut trigonous, reticulate, black with a very thin adherent layer of fleshy perianth. Flowering and fruiting: October - February, often continuing up to March.

Habitat: Usually marshy areas.

Distribution in Bangladesh: This species has been reported from Panchagarh, Sylhet, Chittagong, Mymensingh, Netrakona, Faridpur, Narayangonj, Rangamati and Sunamganj districts.

6.Monjuri

Image 2.8

Scientific Name: Not known

7. Yangfo

Image 2.9

Scientific name: *Ficus benghalensis*

Family: Moraceae

Ficus benghalensis L., Sp. Pl.: 1059 (1753); Hook. f., Fl. Brit. Ind. 5: 499 (1885); Prain, Beng. Pl. 2: 735 (1903, rep. ed. 1963).

Local names: Bot, Botgachh, Jalong.

English name: Banyan Tree.

Description: A large, evergreen to semi-deciduous tree, up to 28 m tall, with wide leafy crown, branches much spreading, up to 100 m or more with strong prop roots and accessory trunks. Young shoots white puberulous. Leaves simple, alternate, petiolate, petioles hairy, dorsiventrally compressed, 3-5 cm long, leaf blade ovate, ovate-elliptic to rhomboid, coriaceous, variable in size, usually broadest near the base, glabrous, base obtuse, rounded or subcordate, apex obtuse, margin entire, pinnately reticulate with most commonly 3 main veins from tip of the petioles, stipules stout, caducous, acute. Inflorescence a hypanthodium, produced in axillary pairs on young shoots, depressed-globose, green when very young, then red. Male flowers: ostiolar, numerous, short pedicelled, sepals 2-3, stamen solitary. Female flowers: numerous, mixed with gall flowers, sepals 3-4, ovary with 1-sided elongated styles and bifid stigmas. Fruit a fig, depressed-globose, pinkish-red. Flowering and fruiting: May - August.

Habitat: Plain lands.

Distribution in Bangladesh: It is very common throughout the country.

8. Missayanu

Image 2.10

Scientific Name: *Sarcochlamys pulcherrima*

Family: Urticaceae

Sarcochlamys pulcherrima Gaudich., Voy. Bot.: t. 89 (1826)

Local Name: Jangallya shak, Maricha.

English Name: Not known

Description: It has pubescent branchlets, covered with soft hair, leaves alternate, narrowly lanceolate, toothed, caudate acuminate, membranous rugose, shining and rough above, white beneath, strongly three nerved; inflorescence spike; flowers dioecious; male in slender interrupted spikes and female in stouter spikes; fruit achene, enclosed in fleshy perianth. Flowering and fruiting: June to February.

Habitat: Along the moist shady steeps, bank of streams and evergreen forests.

Distribution in Bangladesh: It is found in the forests of Sylhet, Chittagong and Cox's Bazar districts.

9. Felong dal shak

Image 2.11

Scientific name : *Phaseolus vulgaris*

Family : Fabaceae

Phaseolus vulgaris L., Sp. Pl. 1: 723 (1753); Hook. f., Fl. Brit. Ind. 2: 200 (1876); Prain, Beng. Pl. 1: 275 (1903, rep. ed. 1963). *Phaseolus esculentus* Salisb. (1796).

Local Names: Felong dal (Marma, Chakma, Tripura).

English Names: Common Bean, French Bean, Kidney Bean.

Description: A climbing annual herb. Leaves alternate, trifoliolate, often hairy, petioles 4-9 cm long, grooved above, pulvinus at the base, leaflets ovate, entire, acuminate, 8-16 × 5-11 cm, lateral leaflets asymmetric, stipules small, acute, basifixed, stipels minute, lanceolate. Flowers in axillary, lax raceme, usually shorter than leaf, pedicels 3-10 mm long. Calyx leafy, ovate, pubescent, 3-4 mm long, with 1 upper and 3 lower teeth. Corolla white, yellowish, pink or violet, emarginate, claws of wings 5-6 mm long, keels spirally twisted. Stamens diadelphous, anthers small, globose, uniform, basifixed. Styles twisted, hollow, stigmas capitate. Fruit a pod, slender, 4-6 seeded, glabrous. Seeds variable in shape, size and colour. Flowering and fruiting: November - March.

Habitat: Cultivated.

Distribution in Bangladesh: It is cultivated all over the country, mainly in Bandarban and Khagrachari districts.

10. Gaiboma

Image 2.12

Scienific name: *Polycarpon prostratum*

Family: Caryophyllaceae

Polycarpon prostratum (Forssk.) Aschers. &Schweinf., Oesterr. Bot. Zeitscher 39: 128 (1889). *Alsine prostrata* Forssk. (1775); *Polycarpon loeflingiae* (Wight & Arn.) Benth. & Hook. f. (1862); Edgeworth in Hook. f., Fl. Brit. Ind. 1: 245 (1874); Prain, Beng. Pl. 1: 160 (1903, rep. ed. 1963).

Local names:*Ghimashak* (Beng.), *Beng-bong-jathong* (Koach).

English Name: Not known

Description: A dichotomously branched herb. Stem glabrous or pubescent. Leaves apparently in whorls, sessile, blade linear-oblong, obovate or spathulate, cuneate, entire. Inflorescence axillary, congested cymes. Flowers c 7 mm long, bracts ovate, scarious. Sepals 5, 1.5-3.0 mm long with scarious margin. Petals 5, linear-lanceolate, 1 mm long, dentate. Stamens 3, anthers ovoid. Ovary 1-locular, styles 3-fid. Fruit an ovoid or subglobose capsule, dehiscing by 3 valves. Seeds numerous, 0.3-0.6 mm long, pale brown. Flowering and fruiting: March - September.

Habitat: Damp and marshy places and sandy river banks.

Distribution in Bangladesh: In many parts of the country.

11. Chikipung

Image 2.13

Scienific name: *Rumex vesicarius*

Family: Polygonaceae

Rumex vesicarius L., Sp. Pl. 1: 336 (1753); Hook. f., Brit. Ind. 5: 61 (1886); Prain, Beng. Pl. 2: 665 (1903, rep. ed. 1963).

Bengali/Vernacular Name: Takpalong, Chukapalong, Amlabetom.

English Name: Rosy Dock, Dock Sorrel, Bladder Dock.

Description of the plant:

Description: An annual, glabrous herb. Stem much branched from the base, green, herbaceous when young, turning brown and woody when older, glabrous. Leaves simple, alternate, ochreate, petiolate, petioles about as long as the lamina or longer, lamina triangular, elliptic, ovate or oblong, base cuneate, rarely cordate or hastate. Inflorescence a dense axillary or terminal raceme or panicle. Flowers pedicellate, pedicels slender, jointed about the middle. Male and female flowers on the same plant. Tepals 6, with prominent reticulate venation. Stamens 6, filaments short, anthers oblong. Carpels 3, united. Fruit a nut, trigonous, brown, covered with enlarged reticulate inner tepals. Flowering and fruiting: December - March.

Habitat: Highlands in drier areas where the plant is cultivated, also it spreads as an escape.

Distribution of Bangladesh: It is grown in many districts in CHTs.

12. Ambush

Image 2.14

Scienific name: *Blumea lacera*

Family: Asteraceae

Blumea lacera (Burm. f.) DC. in Wight, Contr. Bot. Ind.: 14 (1834); Hook. f., Fl. Brit. Ind. 3: 263 (1881); Prain. Beng. Pl. 1: 438 (1903, rep. ed. 1963). *Conyza lacera* Burm. f. (1768).

Local names:*Barokukshim, Baraokoksing, Barosaksang, Kukurshunga, Kuksung* (Beng.), *Leikhamal* (Manipuri).

Description: An annual aromatic herb, 40-75 cm tall, glandular. Stem simple or branched, erect, terete, glandular pubescent, interspersed with a few eglandular hairs especially in the inflorescence. Leaves elliptic, obovate-oblong or oblanceolate, 1.0-6.5 × 0.5-3.0 cm, entire, serrate or variously lyrate or lobed, generally with sharp teeth, attenuate and subpetiolate at the base or petiolate, acute, obtuse or apiculate at the apex, having numerous glands and hairs on both surfaces. Inflorescence a capitulum, heterogamous, 3-4 mm in diameter, involucre cylindric. Bracts narrow, many-seriate, hairy, acuminate, outer ones 2-3 mm long, herbaceous, linear to lanceolate, densely hairy, inner ones up to 5 mm long, mostly linear, often acuminate, subscarious or scarious, finely ciliate, especially at the apex, receptacle naked, glabrous. Flowers yellow. Corolla of the female florets 3.5 mm long, 3-lobed, glabrous, that of hermaphrodite florets 4.0-4.5 mm long, lobes glandular-puberulous, 5-lobed, tubular. Fruit a cypsela, up to 1 mm long, linear or oblong, sparsely puberulous. Pappus white, hairy, c 4 mm long. Flowering and fruiting: November - July.

Habitat: Waste places, roadsides, footpaths and open fields.

Distribution in Bangladesh: It is fairly common in all parts of the country.

13. Mrolapang

Image 2.15

Scienific name: *Manihot esculenta*

Family: Euphorbiaceae

Manihot esculenta Crantz, Inst. 1: 167 (1766). *Manihot utilissima* Pohl. (1827); Prain, Beng. Pl. 2: 704 (1903, rep. ed. 1963).

Local names: *Simul-alu* (Garo), *Kepalli Nolpai* (Marma).

English names: Bitter Cassava, Cassava, Tapioka Plant.

Description: A glabrous or sparingly pubescent shrub, up to 5 m tall, root tuber terete, up to 50 cm long, bark smooth, reddish. Leaves stipulate, stipules triangular-lanceolate, 5-7 mm long, entire or with 1-2 bristly segments, petioles 5-20 cm long, palmately 3-9 lobed, shallowly cordate, sometimes slightly peltate, leaf blade oblanceolate to narrowly elliptic, acuminate-attenuate to the base, 6-18 × 1-5 cm, acuminate at the apex, entire, dark green above, glaucous beneath, lateral veins up to 18 pairs. Inflorescence terminal or axillary racemes, 5-10 cm long. Bracts oblong-lanceolate. Male flowers: pedicels 4-6 mm long, decurved, sepals c 6 × 4 mm, oblong-ovate, subacute, greenish, tinged orange and crimson in colour, hairy inside, stamens 10, filaments 3-7 mm long, slender, anthers 1.5 mm long, white-pubescent at the apex, disc concave, 10-lobed. Female flowers: pedicels c 6 mm long, decurved, extending and thickening in fruits, sepals c 10 × 5 mm, triangular-ovate, disc shallowly 5-lobed, ovary ovoid, 2 × 2 mm, with 6 longitudinal angles, styles c 1.5 mm long, stigmas recurved. Fruit a capsule, ellipsoid, with 6 longitudinal wings. Seeds slightly triangular. Flowering and fruiting: September - January.

Habitat: Cultivated in well-drained soil.

Distribution in Bangladesh: This species is cultivated by defferent ethnic communities in different parts of the country.

14. Projukti pata

Imge 2.16

Scienific name*: Monochoria hastata*

Family: Pontederiaceae

Monochoria hastata (L.) Solms in A. DC., Monogr. Phaner. 4: 523 (1883). *Monochoria hastaefolia* Presl, (1827); Hook. f., Fl. Brit. Ind. 6: 362 (1892); Prain, Beng. Pl. 2: 812 (1903, rep. ed. 1963); *Pontederia hastata* L. (1753).

Local names: *Baranukha* (Beng.), Chichir (Garo), Projukti shak (Tripura).

English name: Arrow leaf False Pickereweed.

Description: A perennial robust herb with often long rhizome, covered with the remains of old leaf sheaths. Stem up to 45 cm high, erect or obliquely erect. Leaves 9.5-15 × 5.5-11.0 cm, hastate, many-nerved, basal lobes divergent, petioles of radical leaves longer, broad and sheathing at the base, of the floral leaves shorter, tumid above and embracing the short scape. Inflorescence shortly stalked, dense, erect or suberect, pedicels 3.5-4.5 cm long. Flowers in racemes or subumbellate, perianth segments pale blue, 3 inner obovate, the 3 outer lanceolate, all with 3 strong parallel median nerves. Stamens 6, filaments subequal, the longer one with spur, white, anthers linear-oblong, the large one blue, the others yellow. Ovary ovoid, styles oblique, top densely patently hairy, stigmas obscurely 3-lobed. Capsule c 1.6 cm long, ellipsoid. Seeds oblong, rounded at each end, pale, with many fine brown ribs. Flowering and fruiting: Throughout the year.

Habitat: Shallow water and mud, near canals and ditches.

Distribution in Bangladesh: It is found all over the country.

15. Khoro pata

Image 2.17

Scienific name*: Cissus repens*

Family: Vitaceae

Cissus repens Lamk., Encycl. Math. Bot. 1: 31 (1783). *Cissus cordata* Roxb. (1820); *Vitis repens* (Lam.) Wight & Arn. (1833); Prain, Beng. Pl. 1: 237 (1903, rep. ed. 1963).

Local names:*Marnaria-pata* (Beng.), *Choto Marmaria-lata* (Eth.)

English name: Not known.

Description: A large herbaceous climber. Stem glabrous. Leaves tri-foliolate, leaflets lanceolate or elliptic lanceolate, glabrous, membranous, acute, base cuneate, petioles up to 7 cm long, stipules triangular, tendrils leaf-opposed, slender, simple. Flowers almost sessile, yellowish-green, dioecious. Fruit a spherical berry, bright red when ripe, smooth, 1or 2-seeded. Seeds globose. Flowering and fruiting: March - August.

Habitat: Primary forests.

Distribution in Bangladesh: It occurs in Chittagong, Cox's Bazar, Rangamati and Sylhet districts.

16. Katoldingi

Image 2.18

Scientific name: *Lasia spinosa*

Family: Araceae

Lasia spinosa (L.) Thwait., Enum. Pl. Seyl. 1: 336 (1864). Benne, Fl. Howrah Dist. 1: 92 (1979); *Dracontium spinosum* L. (1753); *Lasia heterophylla* Schott, Melet. 1: 21 (1832); Hook. f., Fl. Brit. Ind. 6: 550 (1893); Prain. Beng. Pl. 2: 840 (1903,rep. ed. 1963).

Local names: *Kanta-kachu* (Beng.), *Bonadia* (Garo).

Description: Perennial stout herb, rhizome up to 1.5m long, continued above into a prostrate or ascending stem, stem up to 0.5m long, 2-6cm thick, clothed with up to 1cm long broad-based spines, inside white, turning brown on exposure. Leaves with petioles about 75-150cm long, sheathing for about 15-20cm at the base, coriaceous, persistent, aculeate with slightly upturned spines all over, geniculate at the apex, hastate or sagittate, older variously pinnately lobed,the anterior lobe 35-45 × 37-47cm, posterior lobe 17-18 × 5-10cm, pinnately lobed leaves with 4-7 pairs of simple, oblong or linear, acute to acuminate lobes and the basal lobes sometimes bi-or trifid, the veins very prominent on the lower surface and diameter, solitary, axillary and spiny. Spathes fleshy, tube ovate-oblong, 6-10cm long when spread, limb linear-lanceolate, 10-30cm long, convolute and twisted, forming an erect dark purplish-green outside, inside yellow green, finally deciduous. Spadix pale crimson, sessile, cylindric, 2-6 × 0.8-1cm, elongating to 10-12 in fruits, raddish, dense-flowered, appendage absent. Flowers compactly arranged, bisexual, perianth segments 4-6, obovate with incurved tips, 1.5-3.0 mm long, stamens 4-6, filaments short, flat, anthers c 0.5mm long, dehiscing by longitudinal slits, ovaries ovoid, 1.5-2.5mm long, 1-loculed, ovule solitary, hanging from the apex, style stout, short, 0.5-1.0 mm long, stigma depressed-globose, c 1mm broad. Fruit a berry, obovoid, hexagonal, top muricate, about 1cm long, 1-seeded. Seeds compressed, rugose, exalbuminous. Flowering and fruiting: January-November.

Habitat: Shady and moist places in forests growing as undergrowth and in village thickets.

Distribution in Bangladesh: It is very common and found throughout the country.

17. Kasani

Image 2.19

Scientific name: *Monochoria vaginalis*

Family: Pontederiaceae

Monochoria vaginalis (Burm. f.) Presl, Rel. Haenk. 1: 128 (1827). Hook. f., Fl. Brit. Ind. 6: 363 (1892); Prain, Beng. Pl. 2: 812 (1903, rep.ed. 1963); Fl. Malay. Penins. 4: 346 (1924); Backer in Steenis, Fl. Males. 1, 4: 256 (1951); *Pontederia vaginalis* Burm. f. (1768); *Pontederia plantaginea* Roxb. (1832).

Local names: *Nukha, Sarkachu* (Beng.), Kusrisha (Khasia).

English Name: Heartshape False Pickereweed

Description: A slender perennial herb. Stem erect or obliquely erect, rootstock short, suberect, spongy. Leaves extremely variable in size and shape, 2.0-5.0 × 0.8-2.5 cm, usually acuminate, petioles of lower leaves long, stout, terete. Inflorescence 4-7 cm long, soon deflexed, sometimes subumbelliform. Flowers 1.0-1.5 cm long, blue, globose in bud and the elongating as the flowers expand, the termimal flower opening first, perianth 1.0-1.3 cm long, outer 3 linear-oblong, inner 3 obovate, purplish-blue. Filaments of large anther with spur, those of the smaller filliform. Ovary ellipsoid, glandular, stigmas 3-lobed. Capsule c 1.3 cm long, glandular outside. Seeds oblong, pale, with many thin brown ribs. Flowering and fruiting: May-January.

Habitat: Shallow water and mud in rice fields, jute fields, along streams and ditches.

Distribution in Bangladesh: It is found in different areas of the country.

18. Saimya

Image 2.20

Scientific name: *Citrus aurantifolia*

Family: Rutaceae

Citrus aurantifolia (Christm. & Panzer) Swingle, J. Wash. Acad. Sci. 3: 465 (1913).

Local names: Lebu, Pati lebu.

English names: Lime, Sour Lime, Common Lime.

Description: An evergreen, densely and irregularly branched, small, spiny tree, up to 5 m tall. Leaves alternate, elliptic-oblong, crenate, petioles narrowly winged. Inflorescence of short axillary racemes. Flowers white, small, bisexual and staminate. Fruit a globose-ovoid berry, shortly mamillate, greenish-yellow when ripe. Seeds small, ovoid, pale, smooth with white embryos. Flowering and fruiting: March-September.

Habitat: Gardens.

Distribution in Bangladesh: It is found all over the country.

19. Bala pata

Image 2.21

Scientific name: *Pouzolzia hirta*

Family: Urticaceae

Pouzolzia hirta L. *Pouzolzia zeylanica* (L.), Benn., Pl. Jav. Rar.: 67 (1838).

Local name: Kullaruki

English name: Pouzolzia.

Description: A monoecious perennial herb, up to 40 cm tall. Stem ascending, basal part creeping and rooting with erect branching. Leaves opposite in lower portion, upper leaves alternate, shortly petioled, petioles 4-15 mm long, lamina broadly ovate or elliptic-ovate, entire, apex acute, base rounded to broadly cuneate. Flowers greenish-white, in axillary, sessile clusters, bracteoles lanceolate, scarious, strigose without. Male flowers shortly pedicellate. Female flowers sessile, perianth tubular with prominent ribs. Fruit an ovoid or elliptic achene and black. Flowering and fruiting: June-December.

Habitat: Damp open forests, brushwood, arable lands, grasslands and disturbed habitats.

Distribution in Bangladesh: This species is found almost all over the country.

20. Fala

Image 2.22

Scientific name: *Alpinia nigra*

Family: Zingiberaceae

Alpinia nigra (Gaertn.) Burtt. Not. Roy. Bot. Gard. Edinb. 35: 213(1977)

Local name: Tara

English name: Not known

Description: Leafy stem 2-5 m high. Leaves sessile or subsessile, lamina oblong-lanceolate, acuminate, glabrous and glossy on both the surfaces excepting few pubescence on either side of the midrib below, coriaceous, rounded, entire, pubescent. Inflorescence terminal, paniculate, lax, rachis pubescent, 2 long linear involucral bracts enclosing the young inflorescence. Capsules globose, black or brownish-black, very brittle, glabrescent. Seeds angular, black. Flowering and fruiting: May- December.

Habitat: Swamps, low-lying areas and bank of streams.

Distribution in Bangladesh: The species is available throughout the country.

21. Forash dal

Image 2.23

Scientific name: *Vigna grahamiana*

Family : Fabaceae.

Vigna grahamiana (Wight & Arn.) Verdc., Kew Bull. 24: 562 (1970).

English name : French bean, common bean or haricot bean.

Description: A climbing, glabrous, annual herb, taproot with large, globular nodules. Leaves trifoliolate, leaflets 6.5-15.0 cm long, entire, broadly or narrowly ovate, the lateral ones oblique, petioles stout, grooved, stipules ovate to lanceolate, persistent. Inflorescence many-flowered, distinctly peduncled raceme. Corolla reddish, twice the calyx. Fruit a pod, straight or nearly so, many-seeded, 7.5-10 cm, glabrous. Flowering and fruiting: November-March.

Habitat: Cultivated.

Distribution in Bangladesh: CHTs.

22. Kiokokro

Image 2.24

Scientific name: Not known

23. kortolic

Image: 2.25

Scientific name: Not known

24. Mou alu

Image 2.26

Scientific name: *Dioscorea bulbifera*

Family: Dioscoriaceae

Dioscorea bulbifera L. var. **Sativa** (Hook.f.) Prain, Bengal Pl. 2: 801 (1963).

Local names: Mou Alu, Mom Alu.

English name: Yam.

Description: A twining herb, tuber usually solitary, globose, stem twinging to the left, glabrous, sharply angled, lacking prickles. Bulbils abundant, large, smooth. Leaves aternate, blade cordiform with the long-acuminate apex, glabrous, slightly glossy or dull on both surfaces, primary veins campylodromous, veins almost straight, parallel, margins distinct, petioles shorter than the blade. Male inflorescence axillary or terminal, paniculate or spicate. Male flowers sessile. Female inflorescence racemose or spike-like. Female flowers much less in number, perianth lobes fleshy. Fruit a capsule. Seeds flattened, margins winged. Flowering and fruiting: August- October.

Habitat: Flat land in dry areas.

Distribution in Bangladesh: It is found Cox's Bazar, Noakhali and Bandarban district.

25. Ranga Jhum alu

Img: 2.27

Scientific name: *Dioscorea alata* L.

Family: Dioscoreaceae

Dioscorea alata L., Sp. Pl.: 1033 (1753).

Local names: Chupri Alu, Guraniya Alu, Kham, Kham Alu.

English Names: Greater Yam, Water Yam, Winged-yam, White Yam.

Description: A twining annual herb, tubers polymorphic, direct at the base of stem, long slender, sometimes clavate or globose, stem twining to the right, glabrous, commonly 4-angled, angles conspicuously wavy-winged, green to purplish, more or less reddish-brown when dried, unarmed or rarely with weak blunt prickles at the base. Bulbils sparse to abundant, globose, ovoid. Leaves opposite, blade ovate or deltoid-ovate. Flowers laxly arranged, almost sessile at anthesis. Fruit a capsule Flowering and fruiting: October-December.

Habitat: Homestead gardens.

Distribution in Bangladesh: It is common in Khagrachari, Rangamati, Bandarban, Cox's Bazar, Chittagong districts.

2.4 Sample preparation for analysis

After receiving samples in the laboratory, it was rinsed with tape water followed by washing with distilled water, then gently swabbed with tissue paper and air dried. Care should be taken to separate the edible portion and non-edible portion. The cleaned air-dried sample was diced or cut into small pieces (peeled where needed) using a cleaned stainless knife on a cleaned plastic cutting board. Hand gloves were used throughout the process. The diced food sample was taken to a stainless steel bowl and mixed with a plastic spatula. Then samples wrapped with aluminum foil and put into the Ziploc brand plastic freezer bags.
To limit the degradation of ß-carotene by oxygen during storage, as much air as possible was squeezed from the sample bag before the Ziploc was closed. Store the samples at -80ºC until analysis is complete.

In case of vitamin analysis these operations were performed very fast in dim light to avoid any degradation by oxygen and light. Where required, the clean air dried samples were homogenated with a lab blender, and the required portion from the homogenated material stored in the freezer.

Analytical sampling

The vegetables and fruits were processed for analytical samplings and stored in multiple portions as –

(a) 3x5g taken for carotenoids analysis
(b) 3x5g taken for vitamin C analysis
(c) 3x10g taken for minerals analysis
(d) 3x10g for dietary fiber analysis
(e) 3x10g taken for nitrogen analysis
(f) 3x25g taken for moisture analysis and
(g) Remaining portion in multiple units

Frozen and stored at -20^{o}C & -40^{o}C depending on nutrient to be analyzed.

Chemicals

All chemicals and reagents used in the analysis of the nutrient profile were of analytical grade and were purchased from Merck (Darmstadt, Germany, BDH (UK), Sigma Chemical Co (St. Louis, MO, USA). Ascorbic acid & β-carotene were procured from Sigma Chemical Co. (St. Louis, MO, USA).

Methods of nutrient analysis

Use of appropriate and accurate methods employing skilled analysts can only ensure reliable data for preparation of a food composition database. However, the choice of analytical methods is limited to equipment facilities and also the fund constraint and time limitation.

In the analysis of nutrient composition, contents of micronutrients and proximate nutrient were estimated employing standard methods. Carotenoid, vitamin C, total phenol, phytate contents were estimated by spectrophotometry, carotene profile and B vitamins were analysed by HPLC and mineral content was determined by atomic absorption spectrophotometry. Proximate composition was estimated by AOAC methods.

Table 2.2: Analytical techniques employed for Nutrients analysed

Nutrient Class	Nutrients	AOAC and Standards methods
Proximate analysis	Moisture	Drying in Air oven at 100 -105°c (AOAC, 1998a)
	Protein	Micro-Kjeldahl method (AOAC, 1998b)
	Fat	Soxhlet extraction (Raghuramulu *et al*, 2003a)
	Ash	Muffle furnace (AOAC, 199c)
	Dietary fiber	Sigma Kit (AOAC, 1998d; Sigma TDF- 100A)
	Carbohydrate	By calculation (Rand *et al*, 1991)
Micronutrients		
1.Vitamins	Total Carotenoids	Spectrophotometry (Roriguez-Amaya and Kimura, 2004; Rahman *et al* 1990)
	Carotene profile(ß-carotene, Leutin, Lycopen,α carotene)	HPLC (Roriguez-Amaya and Kimura, 2004)
	Vitamin C	Spectrophotometry (AOAC, 1998e)
2. 2. Minerals a) Macro mineral b) Micro mineral	Ca, P Cu, Zn, Fe ,	Atomic Absorption Spectrophotometry (Petertsen, 2002).
3. Other nutrient	Total phenol Phytic acid	Folin-Ciocalteau method (Bettini V etal,1985) Spectrophotometry (Wheefer and Ferral, 1971)

Quality assurance programme (QAP)

Method standardization and validation were carried out with internal standard (IS), external standard (ES), intra and inter lab analysis of particular food and percentrecovery. Data quality was maintained by precision (co-efficient of variance, CV), accuracy (Standard Reference material, SRM) and well documented foods, standard error of mean (SEM).

Definition and Expression of Nutrients

All foods, including leafy or non-leafy, are presented per 100 g edible portion.

Edible Portion is the part of the part of food that is customarily eaten by the people depending on their culture or food habit. It is the proportion of edible matter in the food as collected or purchased, express on the basis of weight. It is obtained using formula

Edible weight = As Purchased (A.P.) weight – Refuse Weight (R)

$$\% \text{ Edible Portion (E.P.)} = \frac{\text{Edible weight}}{\text{As Purchased (A.P.) weight}} \times 100$$

The values per nutrient have been standardized and are expressed in fixed maximal number of decimal points, i.e. no decimal points were added but values with higher decimal points

were truncated to the maximal number of decimal. The values reported are mean values derived from foods as well as the standard deviation (Mean± SD).

Table 2.3: Units of expression

Sl no	Nutrients	Units/100g Edible portion
1	Moisture	g
2	Protein	g
3	Fat	g
4	Total Dietery Fibre(TDF)	g
5	Total Minerals/Ash	g
6	Carbohydrate	g
7	Energy	K calorie
9	Viamin-C	mg
10	Total carotenoids	µg
11	B-carotene	µg
12	α-carotene	µg
13	Leutine	µg
14	Lycopene	µg
15	Thiamin(B_1)	mg
16	Riboflavin(B_2)	mg
17	Niacin(B_3)	mg
18	Pyridoxin(B_6)	mg
19	Copper	µg
20	Zinc	µg
21	Iron	mg
22	Calcium	mg
23	Phosphors	Mg
24	Total phenol	Mg
25	Phytic acid	mg

Moisture:

The values per nutrient have been standardized and are expressed in fixed maximal number of decimal points, i.e. no decimal points were added but values with higher decimal points were truncated to the maximal number of decimal. The values reported are mean values derived from foods as well as the standard deviation (Mean± SD).

Moisture content is one of the most variable components. This variable may affect the food composition as a whole. Therefore, the moisture value has a great consideration in food composition. The moisture content of food was determined by estimating the amount of water removed from the food upon heating. Approximately 10g of fresh sample was taken in a crucible (pre-washed and dried at 105°C). It was then kept 100-105°C temperature in an oven for 5 hours and coyoled in desiccators and weighted again. Heating, cooling and weighing were continued until a constant weight was obtained

Calculation

$$\text{Moisture \%} = \left\{\frac{\text{Initial weight} - \text{final weight}}{\text{weight of sample}}\right\} \times 100$$

Here,

Initial weight = Sample weight + crucible weight (before heating)

Final weight = Sample weight + crucible weight (after heating)

Estimation of protein

Protein content in food item was determined by indirect method estimating total nitrogen in the food. It was calculated by multiplying the total nitrogen using the respective factor (6.75) as estimated by Micro-Kjeldahl method. This is referred to as crude protein content since the non protein nitrogen (NPN) present in the material is not taken into consideration. True protein nitrogen can be determined by subtracting NPN from the total nitrogen. The estimation of nitrogen is done Kjeldahl method which depends upon the H2SO4 with the organic nitrogen when digested in present of catalyst (selenium oxide, mercury or copper sulfate) is converted into $(NH4)_2SO_4$. Ammonia liberated by making the solution alkaline is distilled into a known volume of a standard acid, which is then titrated.

Reagent preparation

- Digestion mixture

Potassium sulphate (K2SO4) and copper sulphate (CuSO4.5H2O) were powdered with mortar and pestle and mixed well in a ratio of 98: 2.

- Sodium hydroxide solution (40%)

Sodium hydroxide (40gm) was dissolved in distilled water and volume was made up to 100ml.

- 0.1N H_2SO4
- 0.1N Na_2CO3
- 0.1N NaOH
- Conc H2SO4
- Disti water
- Methyl red indicator (3-4 drops)

Procedure

The Kjeldahl method consists of the following steps:

1. Digestion of the sample
2. Distillation
3. Titration

1. Digestion

Each of the selected samples (2-3gm) was taken in weighing paper and measured accurately. This sample was poured into a 500ml clean and dry Kjeldahl flask, to which 5g of digestion mixture and 25ml of concentrated H2SO4 were added. To avoid frothing and bumping, a glass rod was placed inside the flask. A blank was carried with all reagents except sample material for the comparison.

The flask were heated in Kjeldahl digestion chamber initially at low temperature (40°C) until the mixture no longer froths and then heating was increased to 400°C and heating was continued until the solution became colorless. At the end of digestion period, the flask were cooled and diluted with 100ml distilled water. A small piece of litmus paper was placed in the solution and the reaction was found to be acidic.

2. Distillation

The distillation set of Kjeldahl apparatus was thoroughly washed with distilled water before starting the distillation. 25ml of 0.1N H2SO4 was taken into a 250ml receiving conical flask. In a measuring cylinder, 75ml of 40% NAOH was taken and it was carefully poured down the side of the ground joint flask. The mouth of the flask was closed with a stopper containing connective tube, which was ultimately connected to the ammonia-receiving flask containing 0.1N H2SO4. The mixture was boiled at such a rate that water and ammonia distilled over at a steady moderate rate. The heating was not too slow so that the H2SO4 solution might be sucked into the Kjeldahl flask and not too fast so that the distilling ammonia did not escape the sulphuric acid without absorption.

3. Titration

The ammonia absorbed in the receiving flask containing untreated 0.1N sulphuric acid was titrated against 0.1N sodium hydroxide using 2 drops of methyl red as indicator. Upon neutralization the pink color will disappear which indicates the end point of reaction. Similarly a reagent blank was distilled and titrated.

Calculation

The protein content of sample on the percentage basis was calculated by the following formula

Protein % = $\{(c-b) \times 1.4 \times d \times 6.25 \times 100/(a \times 1000)\}$

Where,

a = sample weight (g)
b = volume of NAOH required for the back titration and to neutralize 20 ml of 0.1 N H2SO4 (for sample)
c = volume of NAOH required for the back titration and to neutralize 20 ml of 0.1 N NH2SO4 (for blank)
d = normality of NAOH used for titration.

The conversion factor of nitrogen to protein is 6.25 and atomic weight of nitrogen is 14.

Estimation of total fat

Powderedfood was subjected to extraction with mixture of chloroform and methanol (Raghuramulu *et al*., 2003).

Procedure

Five gram powdered sample was taken in a conical flask, mixed with chloroform: methanol (2:1). It was subjected to occasional shaking for 24hours at room temperature. It was then filtered until the colour of food substance became clear. Filtrate was taken in a conical flask of known weight with boiling cheap and the flask was heated until the solvent is evaporated. Then it was dried in an oven at 105°C for 3-4 hours. Weight of the conical flask was taken.

Calculation

$$\text{Percentage of fat} = \frac{\text{Final weight of flask} - \text{Initial weight of flask}}{\text{Sample weight}} \times 100$$

Estimation of ash content

In ash estimation, dried food sample is ignited at 600^{o}C to burn out all organic materials. The inorganic material which is ignited at this temperature is the ash.

In this study, ash in the food sample was estimated by heating the dried sample in a Muffle furnace at 600^{o}C for 3h (AOAC, 1998c). Ash content was calculated from weight difference.

Procedure

The temperature of the muffle furnace was set at 600°C and porcelain crucible was heated for 1hour in a heater and transferred into a desiccators, cooled to room temperatures and weighed (W_1). About 2.0g sample was weighed into the crucible of known weight (W_2). The sample was ignited at 600°C for about 2hrs. The crucibles were transferred into the desiccators and cooled to room temperature and weighed (W_3). It was weighed immediately to prevent moisture absorption. The incineration was repeated until constant weight was obtained. The sample was preserved for mineral analysis, if needed.

Calculation

Weight of the sample taken = $W_2 - W_1$
Weight of the ash obtained = $W_3 - W_1$

$$\text{Percentage of ash} = \frac{\text{Weight of the ash}}{\text{Weight of the sample}} \times 100 = \frac{W3 - W1}{W2 - W1} \times 100$$

Analysis of Dietary fiber

Dietary fiber was analyzed by AOAC method (1998d) using total dietary fibre assay kit (TDF-100, Sigma Chemical Co., Saint Louis, Missouri, USA). In this method, a combination of enzymatic and gravimetric techniques was used.

Dried fat free sample was gelatinized with heat stable α-amylase, then enzymatically digested with protease and amyl glycosidase to remove the protein and starch present in the food sample. Ethanol was added to precipitate the soluble dietary fiber. The residue was filtered and washed with ethanol and acetone. After drying, half of the residue was analyzed for protein and half for ash. Total dietary fiber was the weight of the residue minus the weight of the protein and ash.

Reagents

Use distilled or de ionized water to prepare solutions.

- 78% Ethanol
- Place 207 ml of water into a one liter volumetric flask. Dilute to volume with 95% ethanol. Mix and bring to volume again with 95% ethanol if necessary. Mix.
- Phosphate Buffer, 0.08 M, pH 6.0
- Dissolve 1.4 g of Na2HPO4 (Product Code S 0876) and 8.4 g of NaH2PO4, anhydrous (Product Code S 0751) in approximately 700 ml of water. Dilute to one liter with water. Check pH and adjust if necessary with either NaOH or H3PO4. Store in tightly capped container at room temperature.
- Sodium Hydroxide Solution, 0.275 N
- Dilute 275 ml of 1.0 N NaOH solution (Product Code 930-65) to one liter with water in a volumetric flask. Store in a tightly capped container at room temperature.
- Hydrochloric Acid Solution, 0.325 M

- Dilute 325 ml of 1.0 M HCl solution (Product Code 920-1) to one liter with water in a volumetric flask. Store in a tightly capped container at room temperature.

Procedure

- Weigh out four 1-gram samples of each material to be tested into tall form beakers. Sample weights should not differ by more than 20 mg. Record weights to 0.1 mg.
- Add 50 ml of pH 6.0 phosphate buffer to each beaker.
- Add 0.10 ml α-Amylase (Product Code A 3306) to each beaker and mix well.
- Cover each beaker with aluminum foil and place in a boiling water bath. Agitate beakers gently at 5 minute intervals. Incubate for 15 minutes after the internal temperature of the beakers reaches 95 °C.
- Allow solutions to cool to room temperature.
- Adjust the pH of the solutions to 7.5 ± 0.2 by adding 10 ml of 0.275 N NaOH to each beaker. Check pH; adjust if necessary with either NaOH or HCl.
- Immediately before use, make a 50 mg/ml solution of Protease (Product Code P 3910) in phosphate buffer. Pipette 0.1 ml (5 mg Protease) into each beaker.
- Cover each beaker with aluminum foil and place in 60 °C water bath. With continuous agitation, incubate for 30 minutes after the internal temperature of the beakers reaches 60 °C.
- Allow solutions to cool to room temperature.
- Adjust the pH of the solutions to between pH 4.0 and 4.6 by adding 10 ml of 0.325 M HCl to each beaker. Check pH, adjust if necessary with either NaOH or HCl.
- Add 0.1 ml of Amyloglucosidase (Product Code A 9913) to each beaker.
- Cover each beaker with aluminum foil and place in 60 °C water bath. With continuous agitation, incubate for 30 minutes after the internal temperature of the beakers reaches 60 °C.
- Add 4 volumes of 95% ethanol to each beaker.
- Let solutions set overnight at room temperature to allow complete precipitation.
- Filtration: Wet and redistribute the bed of Celite in each crucible using 78% ethanol. Apply gentle suction to draw Celite onto frit as an even mat. Maintain gentle suction and quantitatively transfer the precipitate and suspension from each beaker to its respective crucible. Wash the residue with three 20 ml portions of 78% ethanol, two 10 ml portions of 95% ethanol, and two 10 ml portions of acetone. A gum may form with some samples, trapping liquid. Breaking the surface film with a spatula will improve the rate of filtration. Be sure to rinse any material adhering to the spatula into the crucible. The time for filtration and washing will vary from 0.1 to 6 hours per crucible, averaging about 0.5 hour per crucible.
- Dry crucibles containing residues overnight in a 105 °C air oven or 70 °C vacuum oven.
- Cool all crucibles in a desiccators, weigh to nearest 0.1 mg, and record this weight as “Residue + Celite + Crucible Weight" or W2
- Analyze the residues from two samples and two blanks for protein by Kjeldahl nitrogen analysis as specified in the AOAC procedure.5 Use 6.25 as the factor to convert ammonia determined in the analysis to protein except where nitrogen content in the protein sample is known.
- Ash the residue in the crucibles from two samples and two blanks for 5 hours at 525 ºC. Cool in desiccator, weigh to nearest 0.1 mg and record this weight as "Ash + Celite + Crucible Weight" or W3.

Calculations

Residue Weight = W2-W1 Ash Weight = W3-W1

B = R BLANK – P BLANK - A BLANK

% TDF = [R SAMPLE – P SAMPLE – A SAMPLE - B)/SW] X 100

Where,

TDF = Total Dietary Fiber

R = Average Residue Weight (mg)

P = Average Protein Weight (mg)

A = Average Ash Weight (mg)

SW = Average Sample Weight (mg)

Calculation of Carbohydrate content

The content of available carbohydrate in the food sample was determined by difference. Carbohydrate was calculated by subtracting the sum percentage of moisture, protein, fat, ash, crude and dietary fibre (Rand *et al*., 1991).

Calculation

Carbohydrate content was calculated using the following formula:

Gram percent of available carbohydrates

= 100–{moisture (g%)+Crude protein (g%)+Total fat(g%)+Crude fiber (g%)+Total ash (g%)}

Where,

(g %)= gram per 100 gram leafy vegetable

Calculation of Calorie value

The calorie content of the sample was calculated by multiplying 4 with carbohydrates, 9 with total fat and by 4 with total protein value (Osborne and Voogt, 1987). From the summation of these values total calorie was estimated.

Calculation

Total calorie value (Kcal/100g sample)

=Available carbohydrate (g) ×4+Protein (g) ×4+Fat (g) ×9.

Analysis of vitamin C

Principle

Oxidation of ascorbic acid to dehydroascorbic acid, and subsequent transformation of the latter to diketogulonic acid, followed by coupling with acidic 2,4-dinitrophenylhydrazine (DNP) under carefully controlled conditions to give stable red-colored osazones with an absorption maximum of 500-550 nm 5. The absorbance of the color formed is proportional to the quantity of ascorbic acid (plus dehydro ascorbic acid) present in the solution before oxidation

Preparation of reagents

a. 5% metaphosphoric acid in 10% acetic acid solution:

50g metaphosphoric acid was dissolved in distilled water then 100 ml glacial acetic acid was added to the solution and the volume was made 1000 ml with distilled water.

b. **9N sulphuric acid**

One volume of concentrated sulphuric acid was added to three volumes distilled water.

c. **2,4 dinitrophenyl hydrazine**

2, 4–dinitrophenyl hydrazine (2 g) was dissolved in 9N sulphuric acid and then 4g thiourea was added, shaked until dissolved and the final volume was made 100 ml with 9N sulphuric acid. The mixture was filtered to remove any insoluble materials and stored at 4°C until used.

d. **Acid washed norit**

50g norit (Black charcoal powder) was placed in a flask and 200 ml of 10% hydrochloric acid was added to the norit. Heated to boiling and was filtered with suction. The norit cake was removed to another flask.Then 1000 ml distilled water was added and stirred thoroughly and again filtered. Finally the norit cake was transferred to a beaker and kept in an oven at 110°C-120°C until the norit become dried.

e. **85%Sulphuric acid**

900 ml concentrated sulphuric acid was added carefully to 100 ml cold distilled water. During the preparation the volumetric flask was kept in an ice bath.

Preparation of ascorbic acid standard

25 mg or 0.025 g standard ascorbic acid (Sigma chemical co. USA) was dissolved in 25 ml of 5% metaphosphoric acid in 10% acetic acid solution (AOAC, 16th edition). The concentration was 1 mg /ml.

Preparation of working standard

Multiple standard preparations processed during every day analysis. From the stock standard Solutions, a series of standard containing various concentrations had been made.

a. 20 µgm / ml
b. 40 µgm / ml
c. 60 µgm / ml

a. 20 µmg / ml working standard solution

20 µmg / ml working standard solution of ascorbic acid was prepared by pipetting 0.5 ml of the stock solution in to 25 ml volumetric flask and was made up to the mark with 5% metaphosphoric acid in 10% acetic acid solution. For norit oxidation acid-washed norit (1g) was added to 25 ml working standard solution and mixed thoroughly. After mixing, the solution was filtered through acid-washed filter paper.

b. 40 µmg / ml working standard solution

40 µmg / mlworking standard solution of ascorbic acid was prepared by pipetting 1 ml of the stock solution in to 25 ml volumetric flask and was made up to the mark with 5% metaphosphoric acid in 10% acetic acid solution. For norit oxidation acid-washed norit (1g) was added to 25 ml working standard solution and mixed thoroughly. After mixing, the solution was filtered through acid-washed filter paper.

c. 60 µmg / ml working standard solution:

60 µmg / mlworking standard solution of ascorbic acid was prepared by pipetting 1.5 ml of the stock solution in to 25 ml volumetric flask and was made up to the mark with 5% metaphosphoric acid in 10% acetic acid solution. For norit oxidation acid-washed norit (1g) was added to 25 ml working standard solution and mixed thoroughly. After mixing, the solution was filtered through acid-washed filter paper.

Preparation of standard calibration curve and estimation of ascorbic acid

The fresh fruit sample was taken and its clean by tissue then cut into small pieces,and the sample was mixing together, from them, 3-5 gm sample was taken and homogenized in a morter with a pastle using 10 ml of 5% metaphosphoric acid in 10% acetic acid solution and the mixture was then filtered by suction. The extract was collected in a conical flask and 1.5 gm acid washed norit was added to it and stirred for 10 minutes. The mixture was then filtered

by sintered glass filter. This extracted sample solution was become into water color. This extracted solution was made 25 ml volume by the 5 % metaphosphoric acid in 10% acetic acid solution. Then 2 ml of norit filtrate was taken in each of two test tubes and in a third test tube 2 ml norit treated standard ascorbic acid (All of different concentration like 0.5 ml for 20 µgm, 1.0 ml for 40 µgm, 2.0 ml for 80 µgm and 2.5ml for 120 µgm), 2 ml of 5% metaphosphoric acid in 10% acetic acid solution in another test tube 2 ml metaphosphoric acid solution (as blank) were taken in different test tubes which were marked. Then 0.5ml of 2,4 – dinitrophenyl hydrazine was added to each of the test tubes containing sample extract, standard ascorbic acid and blank solution. The tubes were then placed in a water bath at 60°C for 1 hour. After the end of incubation the tubes were removed and placed in a ice bath, 2.5 ml of 85% sulphuric acid was added drop wise and slowly to each test tube. All the tubes were vortexed thoroughly and carefully and left at room temperature for 30 minutes. Timing is very important to maintain throughout the analysis. The absorbance was measured at 520 nm in spectrophotometer (UV-1201, UV-VIS, and Shimadzu, Japan). The reading was taken against the sample blank .The standard curve was calibrated by plotting the standard absorbance against different concentrations.

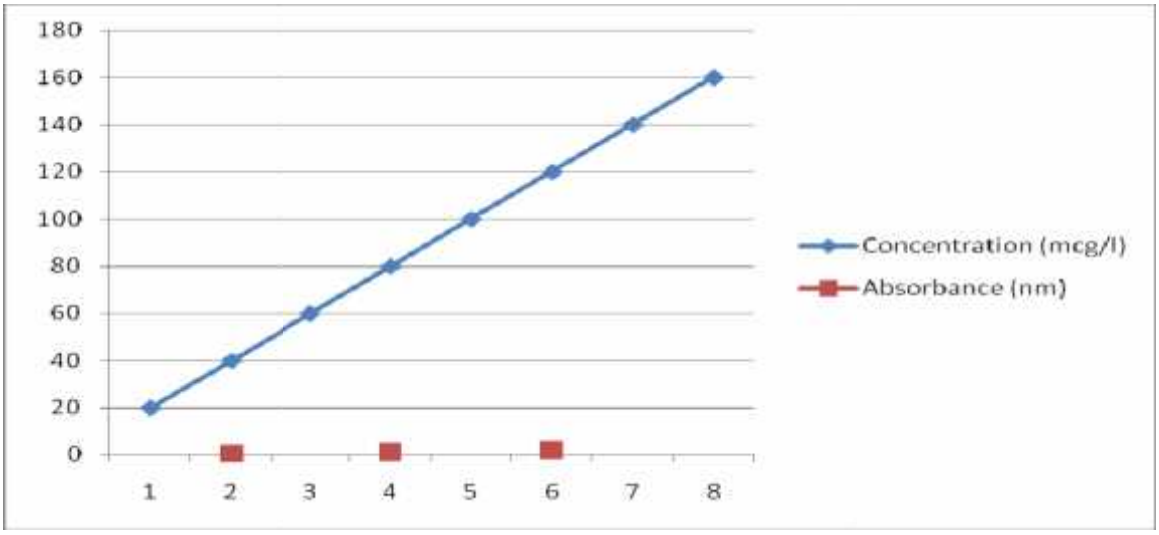

Fig.2.2: Standard curve for vitamin C estimation

Calculation

The ascorbic acid content is calculated using the following formula

$$\text{Ascorbic acid content (mg /100g)} = \frac{\text{Graph factor} \times \text{Absorbance of sample extract} \times \text{Final volume} \times 100}{\text{Sample extract taken for analysis} \times \text{Sample weight} \times 1000} \times 100$$

Analysis of total carotenoids

Carotenoid content in the vegetable or fruit sample was determined by acetonepetroleum-ether extraction followed by spectrophotometric measurement (Roriguez- Amaya and Kimura, 2004). Extraction of carotenoid was performed by *grinding* of processed food sample in mortar and pestle, filtration through sintered glass filter under vacuum and *separation* from acetone to petroleum ether.

When the color of the eluent is orange like, it was read at 450nm in a spectrophotometer (UV-1601, UV-Visible, Shimadzu) for concentration of total carotenoids; when it was green color containing chlorophyll, the extract was passed through a column packed with activated 1:1 alumina and sodium anhydrous to remove the green pigments. The column eluent was then read at 450nm. All preparative and extractive procedures were performed in dim light to avaoid phoyosensitive damage.

Extraction with acetone using a mortar and pestle

- ~5-10 g of the sample is taken in a mortar and pestle and homogenized until mashed and well mixed.
- Three samples were weighed; each weighing ~0.5- 5.0 g from the mashed sample from the step one, and the weight was recorded. Carefully the weighed samples were transferred to 3 small mortar and pestles. Then covered in foil and 2 and 3 no. samples were placed in the refrigerator.
- The remaining mashed sample was placed from step 1 back in the storage bag it was taken from initially and stored at –20° C.
- For each sample beginning with sample 1, the following procedures were followed:
- 25 ml of cold acetone and 100µL of the 1 mg/ml solution of BHT in acetone was added. Then homogenized carefully for about 3 minutes by hand.

- The homogenate was carefully transferred to a Buchner funnel under vacuum using a glass transfer pipette and then the acetone extract was collected into the vacuum flask. The mortar and pestle was washed with small amount of acetone.

- And added to the Buchner funnel to filter. More acetone was added to the Buchner funnel until the acetone was colorless.

- 20 ml of petroleum ether was added to the separatory funnel. The acetone extract was added and the vacuum flask was rinsed with a few ml of acetone.

- 7.150 ml of deionized water was slowly added to the separatory funnel, allowing the water to gently flow down the inside of the funnel (If the water is added too quickly an emulsion will be formed.) The layers were then allowed to separate. Then the lower aqueous layer was discarded.

- The petroleum ether extract was washed 3-4 more times with 100 ml of deionized water until the water was completely clear to remove residual acetone. The lower phase was discarded completely.

- The petroleum ether extract was collected through a funnel that contains a small amount of anhydrous sodium sulfate, into a 25 ml volumetric flask wrapped in foil. The separatory funnel was rinsed with small amount (~2mL) of petroleum ether using a transfer pipette. Then the solution was brought to volume (25 mL) using petroleum ether. The flask was capped and gently mixed and stored in the dark until analysis.

Preparation of chromatographic column

Carotenoids were separated from leafy vegetable sample extracts using a chromatographic column (25×40 cm) with a sintered glass at bottom. To prepare the column, equal proportion of alumina (activated aluminium oxide) and anhydrous sodium sulphate by weight was ground in a mortar with n-hexen to remove air bubbles. The column was packed with the mixture and made the length 15cm, and n-hexen was passed through the column for few hours to make it stable.

Pass through column to remove green pigment

- Put the alumina and sodium sulphate in a beaker and add enough of the elution solvent to make slurry of the alumina and sodium sulphate.
- Put a small plug of cotton wool in the bottom of the column.
- Add the slurry of solvent to the column.
- Drain solvent through the column until the mixture of alumina and sodium sulphate is packed and level. Avoid or remove cracks or bubbles by tapping the column. These will hurt the consistency of the column and therefore make the separation less successful. Let the level of the solvent dip below the top of the mixture. Drain a fair amount of solvent through the column to pack it, but you can re-use the pure solvent that is drained through.
- Once the column is packed, leave a small amount of solvent (~ 5 mm) above the level of the mixture. Do not let the column drain dry.
- With a pipette, add the carotenoids PE solution into the column and let the sample layer go down almost to the surface of the packing material. Carefully add one mL PE to the column, and drain it again.
- Now carefully add enough solvent to fill the funnel without disturbing the column pack and start draining solvent.
- Continue to add PE until the yellow band is eluted, collecting it in a 25 mL volumetric flask.

Estimation of total carotenoids

Carotenoids show characteristic absorbance spectra, for example β-carotene has an absorption maximum of 450 nm in n-hexan with a molecular extinction coefficient of 2592. Therefore, to estimate the total carotenoid in the eluent, absorbance of it was read on a spectrophotometer at 450 nm using a 1 m cell.

Calculation

$$\% \text{ of total Carotenoids (in µg)} = \frac{\text{Absorbance(Unknown)} \times \text{Final Volume(ml)}}{2592 \times \text{Sample wt(g)}} \times 10^6$$

Where,

2592= absorbance 1% cm pet ether β- carotene (Absorbance (Unknown) = Absorbance in spectrophotometer at 450 nm using a 1 m cell

Precaution are taken during analysis of carotenoids

- The samples were protected from exposure to light, oxygen and heat.
- The analysis was completed in one session as quickly as possible to prevent losses of carotenoid from exposure to air, light and heat.
- All procedures were carried out in dim light; containers containing carotenoid solutions were wrapped in foil as added protection .

Analysis of carotene profile

Acetonitrile**:** methanol: 2-propanol (85**:**15**:**33) with 0.01% of ammonium acetate.Concentrate the extract in a rotary evaporator, dry under N2, and immediately before injection, re dissolve in 500µl of mobile phase (for very concentrated samples, it may be necessary to increase the solvent volume), and inject 25µl into the HPLC. Sample and standard should be injected in the same volume.

HPLC System include

Column	VYDAC reverse C18 column with 5 µm particle size
Integrator	SHIMADZU Chromatopac C-R8A
Mobile Phase	acetonitrile**:** methanol: 2-propanol (85**:**15**:**33) with 0.01% of ammonium acetate
Detector	SHIMADZU UV-VIS Detector SPD-10A vp
Pump	SHIMADZU Solvent Delivery Module LC-10ATvp
Injector	Rheodyne 7202 50 µl syringe
Flow rate	1.7ml /min.
Injection volume	25 µl

UV-Visible spectrophotometer (UV-1601, Shimadzu, and Tokyo, Japan) was used to determine the concentrations of standard solution by using reference extinction co-efficient. The standard curve was set using set of 4 standards (10.8, 21.6, 32.4, and 54.0) µg/dl.

B-Vitamin Analysis with an Integrated HPLC System

In present study a reliable instrument was used to test the purity of raw materials and the composition of the final product. Shimadzu's LC-2010 is a fully integrated HPLC system quaternary pump, high speed auto sampler, column oven, and detector in one unit. Samples were filtered through a 0.45µm filter (Millipore) and in jected to HPLC vial. Then HPLC analysis was carried out on a Shimadzu's LC-2010 HPLC system [60]

Reagents and Solvents

- All the chemicals and reagents used were of analytical grade.
- HPLC Methanol (Merck)
- Acetonitrile HPLC (Merck)
- Glacial Acetic acid (BDH)
- Triethylamine (RDH)
- Orthophosphoric acid (BDH)
- Hexane Sulphonic Acid Sodium Salt (Merck)
- Water was distilled and deionized by using Millipore direct q system

Standard Preparation

Standard stock solution for vitamin B2 (riboflavin) was prepared by dissolving 6.9mg ofriboflavin in 100ml of extraction solution because the extraction solution has limit to dissolve 7mg of riboflavin[60].Standard stock solution for vitamin B3 (Nicotinamide) was prepared by dissolving 41.5 mg of Niacin in 25 ml of double distilled water. Standard stock solution for vitamin B5 (Calcium salt of Pantothenic acid) was prepared by dissolving 21.4 mg of Calcium d-pantothenate in 25ml of double distilled water. Standard stock solution for vitamin B6 (pyridoxine) was prepared by dissolving 20.8mg of pyridoxine hydrochloride in25ml of double distilled water.41

Preparation of Working Standards

The working standards were prepared as follows:

For vitamin B_1

I. 0.069mg/ml and 5.1 ml was taken in 10 ml volumetric flask,
II. For B_2,1.66 mg/ml and 60.5 µl was taken in 10 ml volumetric flask,
III. For B_3, 0.856 mg/ml and 292 µl was taken in 10 ml volumetric flask,
IV. For B_6, standard 0.832 mg/ml and 72.2 µl was taken in 10ml volumetric flask.

Preparation of buffer

For buffer preparation, 0.1.08 gm of hexane sulphonic acid sodium salt and 1.36 gm of potassium dihydrogenate phosphate were dissolved in 940 ml HPLC water and 5 ml of triethylamine was added to it and the pH was adjusted to 3.0 with orthophosphoric acid [60]. To prepare the mobile phase, buffer and methanol were mixed with a ratio of 96:4 and filterd through 0.45 µ membrane filter and degassed by using helium gas.

Preparation of extraction solutions

Extraction solution was made by mixing 50 ml of acetonitrile with 10 ml of glacial acetic acid and the volume was finally made up to 1000 ml with double distilled water.

Preparation of samples

10 gm of each sample was weighed and made into homogenized in mortar with pestle and transferred into conical flasks and 25 ml of extraction solution was added, kept on shaking water bath at 70°C for 40 min. Thereafter, the sample was cooled down, filtered and finally the volume was made up to 50 ml with extraction solution. Then sample filtered through 0.45µm filter tips and aliquots of 20µL from this solution was injected into the HPLC by using auto-sampler.

Analytical Conditions

For HPLC analysis, a waters symmetry C18 column (4.6 x 150 mm, 5µm) was used with a linear gradient of Buffer: methanol (96:4) at a constant flow rate of 1 ml/min with 2300 pressure by using waters pump (1515 isocratic) and a UV (2487) detector was employed for the detection of peaks, using channels at a wavelength of 210 nm, a bandwidth of 5 nm. Prior to injection into the chromatographic system, all analytical solutions were degassed by sonication. All the prepared sample solutions were first chromatographed to ensure that interfering peaks were not present. 20µL aliquots of the standard solutions and sample solutions were injected.

Estimation of B-vitamins

Four different concentration levels (ex. 5µg/mL, 10µg/mL, 20µg/mL and 40µg/mL) wereprepared from standard solutions of each by diluting with HPLC water. Then 20µl from each diluted solution was injected into HPLC using auto-sampler and the analyses were concentrations. The linearity of the proposed method was evaluated by using calibration curves to calculate coefficient of correlation, slope and intercept values.monitored at 210nm and repeated three times. The average peak areas were plotted against

The content of B-vitamins (x) was calculated by using the plotted peak areas (y) of three samples of the each leafy vegetables slope (m) and intercept (c) from the calibration curves of B-vitamins standards in this equation, $y = mx + c$, Then result was multiplied by dilution factor.

Analysis of mineral profile

Dried food sample was subjected to wet digestion with nitric acid and per chloric acid in an auto- digester at 125°c. The digested sample after appropriate dilution, calcium (Ca), Phosphorus (P), zinc (Zn), iron (Fe), copper (Cu) were determined by AAS, were determined by flame photometry and phosphorus was determined by spectrophotometry. Absorbance was noted for standard solution of each element and samples The calibration curves obtained for concentration vs. absorbance of the standards. Results were calculated based on that standard curve.

Preparation of reagent and standard

1M ammonium acetate

- Transferred 77.086g ammonium acetate (CH_3COONH_4) to a 1l volumetric flask. 500ml de-ionized water was added. After complete dissolution of the salt, made the volume with de-ionized water
- **$Lacl_3$ –solution (3.25%)**
- Weighed 87g $Lacl_37H_2O$into a beaker. Added 20 ml 5M $HNO_3$and 80ml water. Heat gently until the salt is dissolved. After cooling add 60 ml 5M HNO_3 more and transfer the solution to a 1l volumetric flask. Make the volume with de-ionized water and mix. The solution contains 3.25% $Lacl_3$.

5M nitric acid (HNO_3): Transferred 350 ml concentrated HNO3 (65%) to a 1l volumetric flask and made the volume with de-ionized water.

1:20 diluted nitric acid (HNO_3): Transfer 100ml 68% HNO3 to a 2000ml volumetric flask, made the volume with de-ionized water.

Calcium stock solution

Weight 2.502 g $CaCO_3$ into a 1000ml volumetric flask. Some water was added and then add 20 mL HCl drop wise until the $CaCO_3$ was dissolved. After complete dissolution of the $CaCO_3$, make the volume with water. The solution contains 50 mmol Ca per liter.

Calcium stock solution-2: 100 ml Ca stock solution was taken into 500 ml volumetric flask and made the volume with de-ionized water. This solution contains 10 mmol Ca per liter.

Calcium standard solution: Prepared working standards by pipetting 0 ml, 5 ml, 10 ml, 15 ml and 20 ml aliquots of the stock solution-2 into successive 200ml volumetric flasks. Add 20 ml $Lacl_3$ solution and 20ml 1M ammonium acetate to each flask. Filled to the mark with de-ionized water. The solutions contain 0.0, 0.25, 0.5, 0.75, 1.0 mmol Ca per liter. These diluted solutions must be prepared fresh each day. Aspirate the diluted standards into the flame and record the absorbance.

Phosphorus stock solution-1 (100mg/l): Weight 0.4394 g KH_2PO_4 into a 1000ml volumetric flask. Approximate 500 ml water was added. After complete dissolution of the salt, make the volume with water.

Phosphorus stock solution-2 (1.0mg/l): 10 ml Phosphorus stock solution-1 was taken into 1000 ml volumetric flask and made the volume with de-ionized water.

Phosphorus working standard solution: Transferred 5ml 0.5 M $NaHCO_3$ and 5ml 0.3 M H2SO4 into each of 5 50 ml volumetric flasks. Mix and shake frequently during 30 minutes to complete effervescence. Prepared working standards by pipetting 0-, 5-, 10-, 15- and 20-ml aliquots of the Phosphorus stock solution-2 into above 50mL volumetric flasks and made the volume with deionized water.

Copper stock solution-1(200mg/l): Weight 0.7858 g $CuSO_4.5H_2O$ into a 1000ml volumetric flask. Approximate 500 ml 1:20 diluted HNO_3 was added. After complete dissolution of the salt, make the volume with 1:20 diluted HNO.

Copper stock solution-2 (40mg/l): 100 ml copper stock solution-1 was taken into 500 ml volumetric flask and made the volume with 1:20 diluted HNO.

Copper working standard solution

Prepared working standards by pipetting 0 ml, 5 ml, 10 ml, 15 ml and 20 ml aliquots of the copper stock solution-2 into successive 200mL volumetric flasks and made the volume with 1:20 diluted HNO. The solutions contain 0.0, 1.0, 2.0, 3.0, 4.0 mg/l copper. These diluted solutions must be prepared fresh each day.

Zinc stock solution-1(200mg/l): Weight 0.879 g $ZnSO_4.7H_2O$ into a 1000ml volumetric flask. Approximate 500 ml 1:20 diluted HNO_3 was added. After complete dissolution of the salt, make the volume with 1:20 diluted HNO.

Zinc stock solution-2 (40mg/l): 100 ml zinc stock solution-1 was taken into 500 ml volumetric flask and made the volume with 1:20 diluted HNO.

Zinc working standard solution: Prepared working standards by pipetting 0 ml, 5 ml, 10 ml, 15 ml and 20 ml aliquots of the zinc stock solution-2 into successive 200mL volumetric flasks and made the volume with 1:20 diluted HNO. The solutions contain 0.0, 1.0, 2.0, 3.0, 4.0 mg/l zinc. These diluted solutions must be prepared fresh each day.

Iron stock solution-1(1000mg/l): Weight 0.879 g $ZnSO_4.7H_2O$ into a 1000ml volumetric flask. Approximate 500 ml 1:20 diluted HNO_3 was added. After complete dissolution of the salt, make the volume with 1:20 diluted HNO.

Iron stock solution-2 (100mg/l): 50 ml iron stock solution-1 was taken into 500 ml volumetric flask and made the volume with 1:20 diluted HNO.

Iron working standard solution: Prepared working standards by pipetting 0 ml, 5 ml, 10 ml, 15 ml and 20 ml aliquots of the Iron stock solution-2 into successive 200mL volumetric flasks and made the volume with 1:20 diluted HNO. The solutions contain 0.0, 2.5, 5.0, 7.5, 10.0 mg/l iron. These diluted solutions must be prepared fresh each day.

Digestion

Weight 0.5 g dried plant material into digestion tube. The two tubes were used as blank. Add 5 ml 68% nitric acid to each tube of all tubes. Mixed the content in each tube and leave the tube overnight. Place the tubes in the digester and cover the tubes with the exhaust manifold. Set the temperature to 125°c. Turn on the digester and continue the digestion for 4 hours after boiling has started. Observe that no tubes become dry.

After cooling, transferred the digestion mixture with distilled water to a 100ml volumetric flask. Made the flask to a volume with water and mixed. Filter on a dry filter into a dry bottle, which can be closed with a screw cap. Keep the filtrate in the closed bottle. Ca, Fe, Zn, Cu, P were determined in the filtrate.

Determination of Calciam and Phosphorus

Using a pipette transferred 20 ml filtrate to a 100ml volumetric flask. Made the flask to volume with distilledwater and mix.

Measurement of Calciam: Transferred 20 ml diluted filtrate into a 50ml volumetric flask.5ml LaCl3 solution were added and made the volume with de-ionized water. Measured the Ca content by AAS. If the sample readings were higher than the reading of highest standard solution made a larger dilution, e.g. 10 ml filtrate into a 50ml volumetric flask and then again measured by AAS.

Measurement of Phosphorus: Transferred 5 ml diluted filtrate into 50ml volumetric flask.30ml (approximate) de-ionized water was added. Then 10ml ammonium molybdate-ascorbic acid solution was added and made the volume with de-ionized water. After15 minutes measured the absorbance on a spectrophotometerat 890 nm. If the sample readings

were higher than the reading of highest standard solution, repeated the procedure using a smaller amount of filtrate.

Measurement of Iron, Zinc and Copper: Measured the content of these elements by AAS directly in the un-diluted filtrate.

Calculation of minerals

mg/100g edible portion of Ca, Mg, K, and P

$$= \frac{a \times 2500}{b \times c \times \text{dry factor}}$$

Where,

a = mg/l Ca and P measured on atomic absorption spectrophotometer

b=ml diluted filtrate transferred into the 50 ml volumetric flask for determination of Ca and P

c = g plant material weighed into the digestion tube

Dry factor

$$= \frac{\text{Total fresh weight of sample}}{\text{Total dry weight of sample}}$$

µg/100g edible portion Cu, Zn and Fe

$$= \frac{d \times 100 \times 100}{c \times \text{dry factor}}$$

d =mg/l Cu, Znand Fe measured on atomic absorption spectrophotometer

c = g plant material weighed into the digestion tube

Estimation of Total Polyphenol

Total polyphenol of different samples was estimated by Folin-Ciocalteu method (Bettini V, Fiori A, Martino R, Mayellaro F. 1985). Solutions of various types of phenols behave as weak reducing agents and can be oxidized to quinine type compounds by many oxidizing agents, phenol reagent, davised by Folin-Ciocalteu, oxidizes phenolic compounds under alkaline condition; it is reduced from its initial golden yellow color to a deep blue. This reagent is used to measure phenolic compounds. The absorbance was measured spectrophotometrically at 750 nm wavelength.

Extraction of Samples

Solvent for extraction

- Hexane : Dichloromethane (1 : 1)
- AWA – Acetone : Water : Glacial Acetic acid (70 : 29.5 : 0.5)

Procedure

In a 15 ml screw cap test tube, 500 mg of grinded sample was taken. To that 12.5 ml of Distilled Hexane: Dichloromethane (1:1) solvent was added. It is then allowed to shake for overnight in the shaker at a rate of 100 rpm. After completion of shaking, the mixture was

centrifuged at 5000-7000 rpm for 15 minutes. Then the supernatant was discarded and the precipitate was dried for 5 minutes at 60˚C to evaporate the left over solvent. To the precipitate, 12.5ml of AWA (Distilled Acetone: Distilled Water: Glacial Acetic Acid =70:29.5:.5) was added and sonicated for 15 minutes at 110˚C and 40 rpm using the the sonication bath for disrupting the cell matrix of the sample for maximum extraction. After sonication, the samples were again centrifuged at 5000-7000 rpm for 15 minutes. The supernatant was then separated from the precipitate and stored in a refrigerator at 4˚C for analysis.

Preparation of Standard Curve and Estimation of Total Phenol

i) Preparation of Standard Curve
Preparation of reagents

- Gallic acid stock solution: 1 g/100 ml
- 2 % Sodium carbonate

Procedure

1 g of Gallic acid was dissolved in 10 ml of absolute ethanol and the volume was made up to 100 ml with distilled water in a 100 ml volumetric flask. This was Stock-1. An aliquot of 5 ml of Stock-1 solution was added to 5 ml of AWA to make Stock-2. 1 ml of this Stock-2 was mixed with 9 ml of AWA. This was the working standard solution. Finally, from the working standard solution, 100µl, 200µl, 300µl, 500µl and 1ml was taken and the final volume of each of these was made 1ml with AWA. From each of these diluted solutions, 10µl was taken for the preparation of standard curve.

ii) Estimation of Total Phenol
Reagent

- Folin-Ciocalteu Reagent (FCR)
- 2 % Sodium carbonate solution

Procedure

150µl of sample was taken into test tube. Then, 900µl of water was added to the test tube. Then 225µl of diluted FCR (1 ml FCR + 1ml distilled Water ≈ 2 times dilution) was added to the sample as well as standard solution tubes and let stand for 5 minutes at room temp. Then 1125µl of 2% Sodium Carbonate solution was added and mixed well each time. Then kept it for 15 minutes at room temperature. Finally the absorbance was measured at 750nm by UV-VIS spectrophotometer.

Calculation:

Step 1 By plotting Gallic acid concentration on abscissa and absorbance on ordinate a standard curve was constructed. The concentration of total phenol in sample was calculated from standard curve.

Step 2 Estimation of Total phenol content (µg/ml) of the samples by the following formula:

$$X = \frac{Y + 0.134}{0.003}$$

Where,

X = Total phenol content (µg/ml)
Y = Absorbance at 750nm

Step 3 The obtained result in Step 2 was multiplied by 12.5 (as the final volume of the extracted sample was 12.5 ml), which was µg of total phenol per 0.5g of dry weight sample.

Step 4 Then the value was converted to µg of total phenol per gram of dry weight sample by multiplying with 2.

Step 5 The obtained result was converted from dry weight to fresh weight basis using the dry factor and the results were presented as mg of GAE/100g dry weight as well as mg of GAE/100g of fresh weight sample.

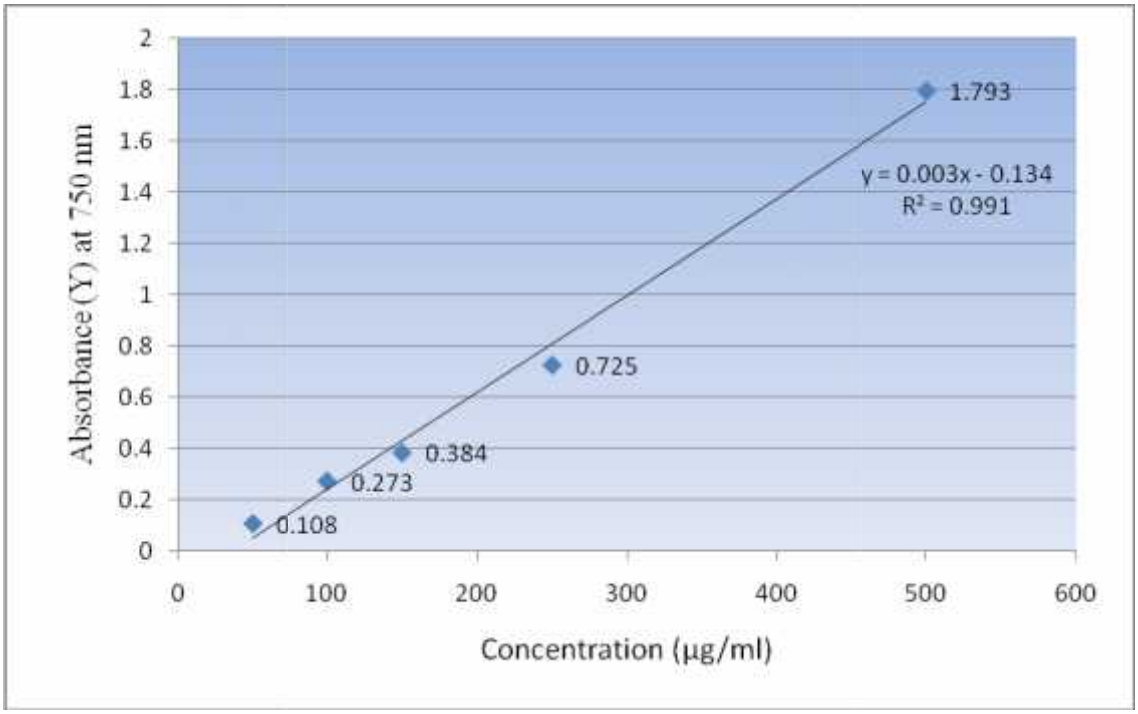

Figure 2.3: Folin Coi-Calteu Gallic Acid Standard curve

Estimation of Phytic acid

Preparation of samples for analysis

- **Drying**
  At first the edible and non-edible portion of the samples were separated. Then the samples were soaked by tissue paper. Then samples were dried below at 50^{o}C for moisture free

- **Grinding**
  After moisture free then samples were blended by the blender machine.
- **Defatted**
  The samples were defatted by methanol: chloroform (2:1) solution.

Preparation of reagents

- **Trichloroacetic acid (TCA), 5%**
  5 gm of TCA dissolved in 100 ml water.
- **Potassium thiocyanate, 20%**
  20 gm of potassium thiocyanate was dissolved in the volume was made up to 100 ml with water.
- **Ferric chloride**
  25 mg dissolved in 100 ml water to make 100 ml of final solution

Procedure of Phytic acid estimation

- Weight 0.25gm defatted sample in a screw tube.
- Add 7.5ml, 5% while mixing on a vortex mixture.
- Incubate it for 10 min at 60^{o}C.
- Centrifuge at 5000 RPM for 10min.
- Transfer the supernatant into a 25ml volumetric flask.
- Repeat the steps ii-iv with residue and after a total of three extractions, pool the supernatant and make up the volume of supernatant to 25ml.
- Pipette out 20ml aliquot in a 75ml Technicone tube and add 5ml $FeCl_3$ solution.
- Heat the tubes in a block digestor Whatman filter paper.
- Cool and filter through Whatman filter paper.
- Pipette out 2.5 filtrate and add 2ml KSCN and 5ml water.
- Read absorbance at 485nm against a water blank.

Preparation of Standard Solution

- Take 5 ml $FeCl_3$ and 20ml water followed by 2ml of 5% TCA in a Technicon tube.
- Heat in a block digestor for 45min at 95^{o}C.
- Cool and filter the solution.
- Pipette out different volumes from the filtrate 0.00 to 5ml in different tubes.
- Add 2ml KSCN in each tube
- Add water according to the following calibration tables :

Standard solution (ml)	Water (ml)	Potassium thiocyanate (ml)
0.0	7.5	2
1.0	6.5	2
2.0	5.5	2
3.0	4.5	2
4.0	3.5	2
5.0	2 .5	2

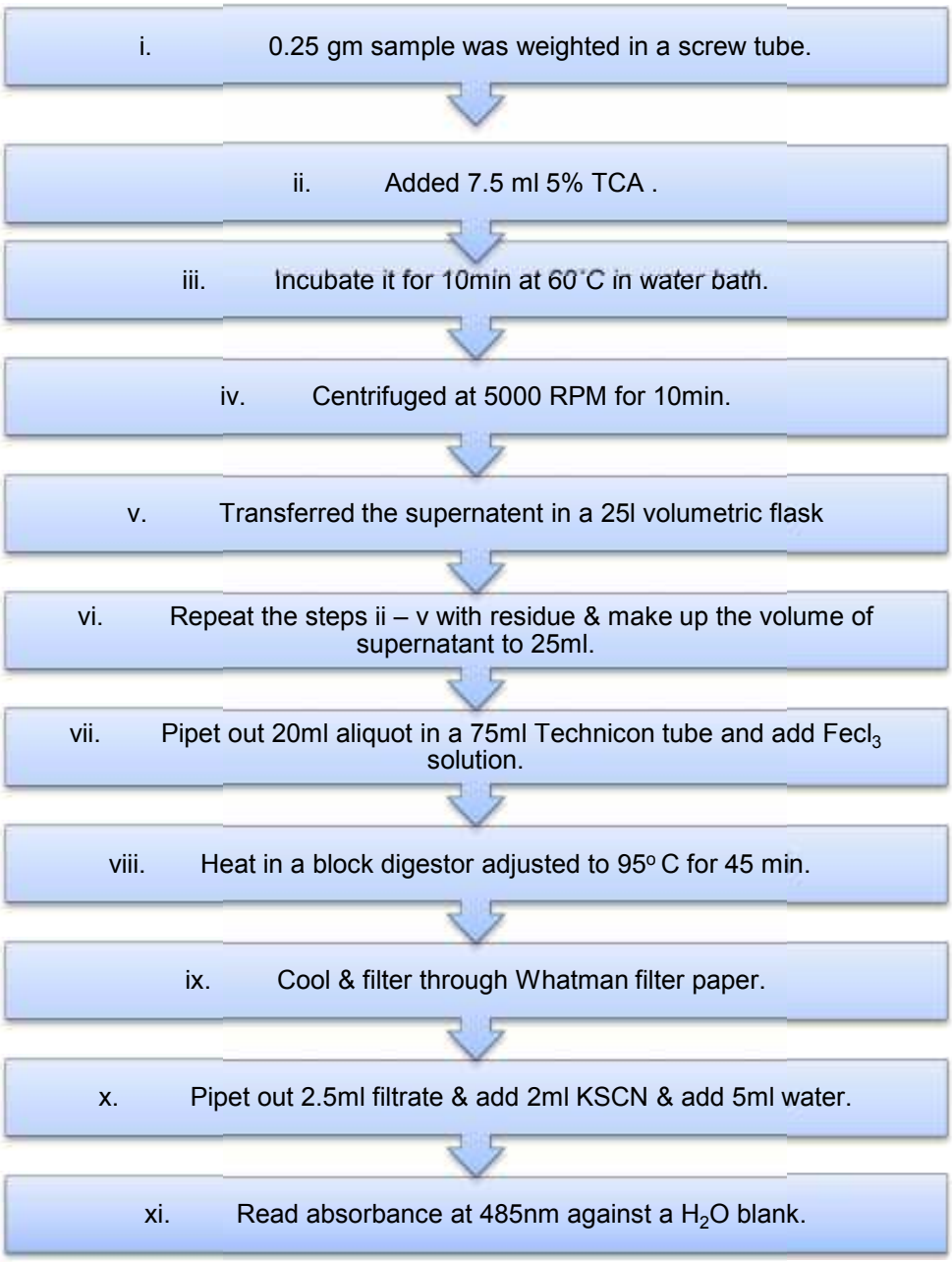

Figure – 2.4: Flow chard of phytic acid determination

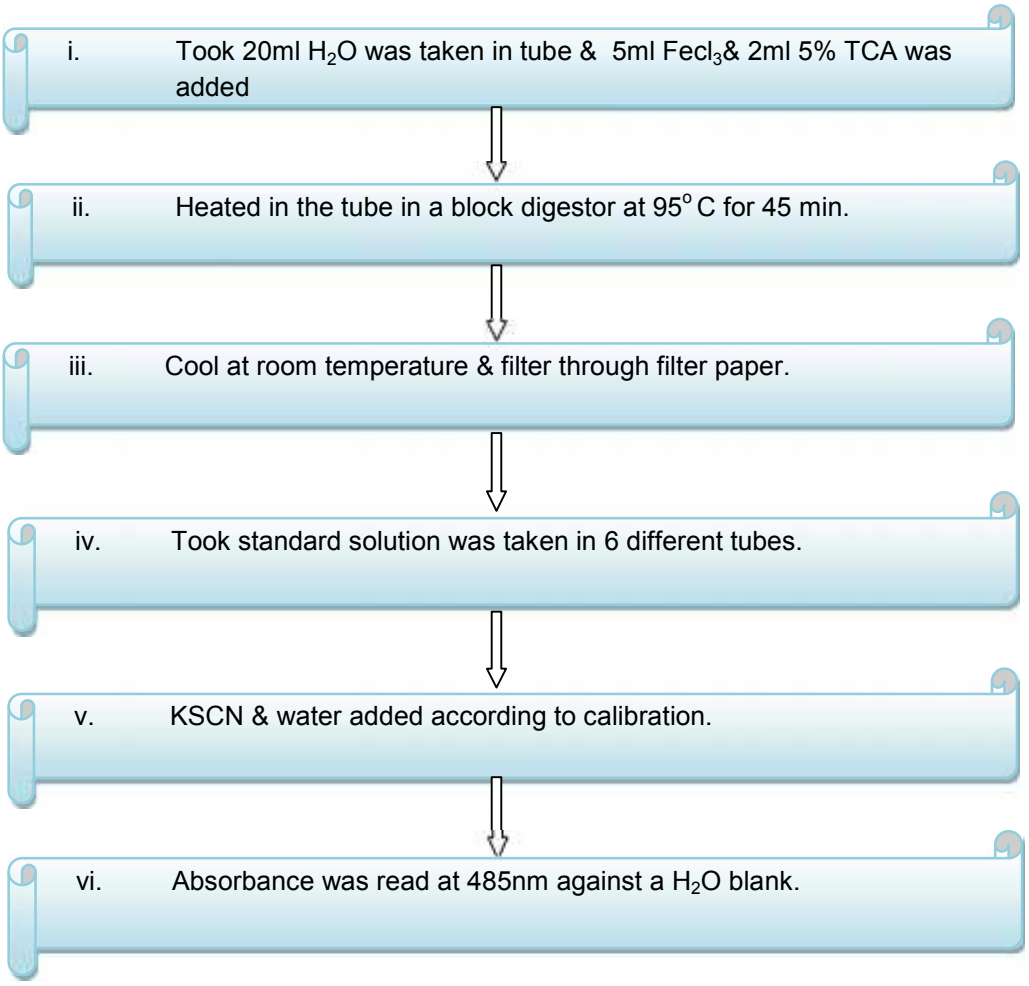

Figure – 2.5: Flow chart of phylic acid standard preparatian

CHAPTER 3

Results and Discusion

3.1 Estimation of nutritent composition selected ethnic plant food

Vegetables are rich source of micronutrients. Vegetables are most commonly consumed in our country. In order to improve the intake of and introduce to micronutrients rich food to mass people, this study has investigated 25 selected vegetables consumed by ethnic people including leafy and non-leafy, nutrients were estimate and analyzed by using high accuracy of technologies. Micronutrients are often referred to as “magic wands” which enable the body to produce enzymes, hormones and other substances essential for proper growth and development (*http://www.who.int/nutrition/topics/micronutrients/en/).*

Micronutrients are natural wonder drug. And they are also cheap when added to the existing food supply *(http://projecthealthychildren.org/why-food-fortification/micronutrients/)*. Hence, the present study has to focus the micronutrient content of selected food items consumed by ethnic communities. And it is noted that the ethnicl food samples contain virtuous amount of micronutrients.

In the present study among 25 vegetables total carotenoids contained ranged from (9337.24 to 48.36 µg/100g edible portion).The highest amount was present in leafy vegetable Mrolapiong (*Manihot esculenta)* (9337.24 µg/100g edible portion) and relatively high in Chikipung (8489.65 µg/100g) whereas lowest amount present in Projukti pata/shak(*Monochoria hastata* L) (655.72 µg/100g edible portion). Among non leafy vegetables, highest amount of total carotenoids was found in Ranga Jhum Alu (*Dioscorea alata) (*2257.76 µg/100g edible portion) and lowest in Fala (*Alpinia nigra)* (48.36 µg/100g).

Table 3.1: Total Carotenoid of selected ethnic vegetables (µg/100 g edible)

Sl	Local name	English name	Scientific name	Total carotinoid
Leafy vegetable				
1	Kochi aam pata	Mango leaf	*Mangifera indica* L	1015.81
2	Kamino	Not known	*Caesalpinia digyna* Rottler	3576.19
3	Moroi shak	Funnel.	*Foeniculum vulgare*	5032.77
4	Amsurothi	Not known	Not known	1947.74
5	Noyalongbikrongi	Trailing Smartweed.	*Ampelygonum chinense*	1367.17
6	Monjuri	Not known	Not Known	5482.92
7	Yangfo	Banyan Tree	*Feics benghalensis*	6319.1
8	Missayanu	Not known	*Sarcochlamys pulcherrima*	7180.9
9	Felong dal sak	Common Bean	*Phaseolus vulgaris .*	2304.1
10	Gaiboma	Not known	*Polycarpan prostratum*	3907.7
11	Chikipung	Rosy Dock, Dock Sorrel,	*Rumex vesicarius .*	1430.61
12	Ambush	Not known	*Blumea lacera*	8489.65
13	Mrolapang	Bitter Cassava, Cassava,	*Manihot esculenta.*	9337.24
14	Projukti shak	Arrow leaf False Pickereweed	*Monochoria hastata*	655.72
15	Khoro pata	Not known.	*Cissus repens*	2004.81
16	Katoldingi	Arum	*Lasia spinosa*	8270.62
17	Kasani	Heartshape False Pickereweed	*Monochoria vaginalis*	709.89
18	Saimya	Lime, Sour Lime, Common Lime	*Citrus aurantiifolia*	1266.93
19	Bala pata	Pouzolzia	*Pouzolzia hirta*	4575.59
Non leafy vegetable				
20	Fala /Tara	Not known	*Alpinia nigra (Gaertn)*	48.36
21	Forash dal	Kidney been, Feench been	*Vigna grahamiana*	82.32
22	Kiokokro	Not known	Not Known	79.84
23	Kortolik	Not known	Not Known	195.99
24	Mo aloo	Yam	*Dioscorea bulbifera L.*	81.61
25	Ranga jhum aloo	Greater Yam, Water Yam	*Dioscorea alata*	2257.76

Table 3.2 presents the carotene profile of some selected vegetables. Among them Missayano (*Sarcochlamys*) contains the highest amount of β-Carotein (5673.1 µg/100g) and α-carotein (703.9 µg/100g) Felong dal sak(*Phaseolus vulgaris* L) contain 2003.3 µg/100g β-Carotein and α-carotein 4.9 µg/100g Khoro pata contain 1407.7 µg/100g β-Carotein and 189.6 µg/100g.

Table 3.2: Carotene profile ((µg per 100 g edible) of some selected ethnic vegetables

Sample name	Scientific Name	Carotenoid	β-carotene	α-Carotene	Leutin	Lycopene
Monjori	*Not known*	5482.92	2647.2	9.6	nd	nd
Yangfo	*Banyan Tree*	6319.1	621.5	16.2	nd	nd
Missayanu	*Sarcochlamys*	7180.9	5673.1	703.9	nd	nd
Felong dal shak	*Phaseo lus vulgaris.*	2304.1	2003.3	4.9	nd	nd
Gaiboma	*Polycarpan prostratum*	3907.7	2741.3	nd	nd	nd
Ambush	*Blumea lacera*	1430.61	1658.1	nd	nd	nd
Mrolapiong	*Manihotesculenta*	9337.24	1089.9	11.3	nd	nd
Projukti shak	*Monochoria hastata*	655.72	182.8	nd	nd	nd
Khoro pata	*Cissus repens .*	2004.81	1407.7	189.6	nd	nd
Kortolik	Nk	195.99	68.8	33.8	nd	nd

Note: Nk= not known, nd= not detectable

In the present study, the maximum amount of vitamin C was present in Mori shak *(Foeniculum vulgare)* (68mg/100g edible). Aproximately in Mrolapiong and Felong dal shak good amount of vitamin C 64mg/100mg and 53mg/100mg were found trespectively. The lowest amount was present in Ranga jhum alu (*Dioscorea alata)* 0.6 mg/100g edible. Highest content of vitamin B_1 (0.72mg/100g edible), B_2 (0.82mg/100g edible) and B_3 (5.77mg/100mg edible) were possessed in Mo alu (*Dioscorea bulbifera L)*, Mrolapying *(Manihotesculenta*), and Fala *(Alpinia nigra)* respectively. Vitamin B_6 was not detected in these selected samples.

Table 3.3: Vitamin-C & B-vitamin content of some selected ethnic vegetables (mg per 100g edible)

Sl	Sample name	Vitamin C	Vitamin B_1	Vitamin B_2	Vitamin B_3	Vitamin B_6
1	Kamino shak	4.9	0.38	0.12	3.6	nd
2	Mouri shak	68	0.09	0.26	1.8	nd
3	Felong dal shak	53	0.016	0.042	2.23	nd
4	Mrolapiong	64	0.58	0.82	2.81	nd
5	Fala	7.8	0.053	0.042	5.77	nd
6	Forash dal	0.013	0.45	0.25	0.76	nd
7	Mo Alu	0.82	0.72	0.04	0.84	nd
8	Ranga jhum alu	0.6	0.35	0.54	0.79	nd
9	Chikipung	13.3	0.036	0.057	0.27	nd

Minerals content of different selected vegetables were analyzed by Atomic Absorption Flame photometer and Atomic Absorption Spectrophotometer.Micro minerals –copper, zinc, iron and macro minerals calcium, and phosphorous were estimated in this study.

Table 3.4 depicts the highest quantity of copper and zinc content are present in Foras dal (*Vigna grahamiana*) 138 and 3.80 µ g/100g edible) and lowest in Kamino (Caesalpinia digyna Rottler (0.05 and 0.12 µ g/100g edible µ g/100g edible). In this table iron content ranged between (0.49-14.46 mg/100g edible portion). Highest in Kiokokro 14.46 Mg/100g edible portion and lowest in Ranga Jhum alu 0.49 Mg/100g edible portion.

Calcium amount in the selected vegetables ranged from 27 to 1338 mg/100g edible portion. Kiokokkro contain highest amount of calcium (1338 Mg/100g edible). Moroi shak (*Foeniculum vulgare)* 347 Mg/100g edible Foras dal *(Vigna grahamiana)* 278 Mg/100g edible Felongdal shak173 Mg/100g edible Mrolapiong (*Manihotesculenta)* 139 Mg/100g edible portion Kamino(*Caesalpinia digyna* Rottler) 135 Mg/100g edibleportion.

Foras dal (*Phaseolus vulgaris)* poses highest amount phosphorus 382 Mg/100g edible portion and the lowest in Chikipung (*Rumex vesicarius* L.) 15.5 Mg/100g edible portion. Felongdal shak (*Phaseolus vulgaris* L), Kamino *(Caesalpinia digyna* Rottler) and Moroi shak (*Foeniculum vulgare)* poses 189, 133, 114 Mg/100g edible portion respectivel

Table 3.4: Mineral Contents some selected ethnic vegetables

Sl	Sample name	Cupper	Zinc	Iron	Calcium	Phosphors
		µ g/100g edible		Mg/100g edible		
1	Mrolapiong	0.58	1.07	3.22	139	93
2	Kamino	0.05	0.12	6.5	135	133
3	Moroi shak	0.08	0.26	9.3	347	114
4	Felongdal shak	0.09	0.19	8.21	173	189
5	Chikipung	0.06	0.32	1.18	69	15.5
6	Fala	0.83	1.76	2.9	42	49
7	Foras dal	138	3.8	4.9	278	382
8	Kiokokkro	nd	nd	14.6	1338	121
9	Mo alu	0.23	0.37	0.86	27	43
10	Ranga jhum alu	0.09	0.32	0.49	38	69

Table 3.5 shows thetotal phenol and. Phytate content in Kanimo, Missayanu, Mo alu was around 200mg GAE/100g. Phytate content was noted highest (72.18mg/100g) in Forash dal almost 70%. 20-30% of pytatic acid found in Saimya (31.39mg/100gm), Kamino (28.58mg/100mg), Mo alu (25.21mg/100mg) and Amsurothi contains (22.93mg/100mg). Also <10% of phytic acid containing ethinic vegetables named Kasani, khoro pata, kiokokro, Chikipung, Ranga jhum alu and the lowest Balapata (5.09mg/100mg) were found.

Table3.5: Total phenol and phytate content in selected ethnic vegetables.

Sl	Sample name	TP content (mg GAE/100g fresh)	Phytic acid (mg/100g)
Leafy			
1	Kochi aam pata	167.06±0.33	10.51±0.58
2	Kamino	210.41±0.58	28.53±0.82
3	Moroi	122.62±0.45	11.81±0.93
4	Amsurothi	86.79±0.40	22.93±0.33
5	Noyalong bikrongi	92.25±0.34	16.55±0.38
6	Monjuri	191.84±0.44	13.85±0.91
7	Yangfo	60.37±0.51	10.66±0.66
8	Missayanu	219.80±0.28	16.31±0.28
9	Felong dal sak	176.49±0.63	14.34±1.09
10	Gairoma	63.27±0.56	19.49±0.79
11	Chikipung	74.92±0.27	7.32±0.73
12	Ambush	183.12±0.28	12.82±0.85
13	Mrolapiong	153.50±0.57	12.53±0.73
14	Projukti shak	93.82±0.30	13.97±0.53
15	Khoro pata	103.34±1.24	8.48±0.69
16	Katol dingi	154.85±1.86	12.09±0.79
17	Kasani	104.31±0.67	8.52±0.48
18	Saimya	108.80±0.60	31.39±0.83
19	Balapata	176.76±0.66	5.09±0.66
Non leafy			
20	Fala	113.09±0.63	15.8±0.81
21	Forash dal	89.63±0.57	72.18±0.56
22	Kiokokro	19.52±0.53	8.21±0.29
23	Kortolik	179.59±0.72	12.48±1.12
24	Mo alu	189.47±0.79	25.21±0.89
25	Ranga Jhum alu	114.90±0.47	6.50±0.32

Proximate analysis is carried out to determine the nutrient content of these leafy and non leafy vegetables under investigation. The proximate analysis revealed the values of carbohydrate, fat, crude protein, dietary fibre, ash and moisture contents of the selected food items.The principal proximate nutrients are protein, fat and carbohydrate. They are oxidized in the body to give energy. In addition to providing energy, the primary function of protein is to supply amino acids for building body proteins. Fats, besides being a concentrated source of energy, provide essential fatty acids having vitamin like function in the body. Water is an essential element, with which the proximate principles form the bulk of the diet. Dietary fibers are indigestible complex molecules, contribute to the bulk and have some important function in the digestive tract.

The proximate composition of all the food analyzed is given in table 3.6. The moisture content of twenty five selected vegetables (both leafy and non-leafy) ranged between 14.91 ± 0.34 and 91.65 ± 0.76g/100 g edible portion. In this study, it was indicated that highest value of protein (23.85g/100g edible portion), fat (2.56g/100g edible portion), ash (2.41g/100g edible portion), dietary fiber (5.06 g/100g edible portion) respective in Forash dal, Kamino, Forah dal and Missayanu.

Table 3.6: Proximate nutrient composition of selected ethnic vegetables (g/100g edible)

Sl	Sample name	Moisture	Protein	Fat	Ash	TDF	CHO	Energy (Kcal)
Leafy vegetables								
1	Kochi aam pata	82.42±0.40	3.39±0.17	2.18±0.34	1.29±0.14	4.36±0.26	6.36	67.30
2	Kamino	80.24±0.39	4.77±0.21	2.56±0.17	1.19±0.11	1.89±0.04	9.35	83.33
3	Moroi shak	86.95±0.11	3.92±0.21	1.46±0.13	1.88±0.08	1.44±0.11	4.36	49.09
4	Amsurothi	80.48±0.27	4.83±0.15	1.89±0.12	1.71±0.16	1.76±0.13	9.34	77.18
5	Noyalong bikrongi	86.02±0.22	2.94±0.07	0.48±0.10	1.56±0.2 8	1.37±0.00	7.63	49.31
6	Monjori	87.57±0.56	3.39±0.22	0.64±0.13	2.21±0.28	1.10±0.13	5.08	41.88
7	Yangfo	85.93±0.33	3.17±0.25	0.61±0.11	1.24±0.28	2.15±0.01	6.90	50.08
8	Missayanu	86.22±0.14	2.24±0.24	0.71±0.13	1.58±0.10	5.06±0.09	4.20	42.23
9	Felong dal shak	83.46±0.50	5.05±0.35	1.08±0.13	2.07±0.25	1.89±0.21	6.45	59.51
10	Gaiboma	88.23±0.71	2.23±0.28	0.46±0.24	1.08±0.24	1.96±0.14	6.04	41.18
11	Chikipung	91.21±0.08	1.86±0.06	0.38±0.06	0.97±0.12	2.15±0.16	3.42	28.84
12	Ambush	89.11±0.54	1.75±0.15	1.06±0.09	1.88±0.06	2.07±0.13	4.13	37.21
13	Mrolapiong	79.36 ± 0.24	4.24 ± 0.34	1.82 ± 0.17	2.18 ± 0.22	2.43 ± 0.40	9.97	78.09
14	Projukti pata	91.65 ± 0.76	1.4 ± 0.33	0.79 ± 0.17	1.54 ± 0.13	1.66 ± 0.17	2.96	27.9
15	Khoro pata	91.61 ± 0.92	1.96 ± 0.09	0.85 ± 0.09	0.94 ± 0.10	1.18 ± 0.25	3.47	31.71
16	Katoldingi	90.76 ± 0.38	2.01 ± 0.17	2.11 ± 0.31	2.03 ± 0.16	1.61 ± 0.24	1.48	36.14
17	Kasani	92.15 ± 0.59	1.44 ± 0.13	1.16 ± 0.41	1.75 ± 0.10	1.88 ± 0.21	1.63	26.45
18	Saimya	85.34 ± 0.39	1.09 ± 0.06	2.18 ± 0.66	2.37 ± 0.02	2.05 ± 0.1	6.97	55.98
19	Balapata	85.84 ± 0.61	0.88 ± 0.04	1.8 ± 0.13	1.62 ± 0.16	1.25 ± 0.08	8.62	56.65
Non leafy vegetables								
20	Fala	81.78 ± 0.66	1.98 ± 0.16	0.87 ± 0.10	1.85 ± 0.17	2.42 ± 0.24	11.11	64.94
21	Forash dal	14.91 ± 0.34	23.85± 0.30	1.82 ± 0.25	2.41 ± 0.78	2.65 ± 0.37	54.37	334.52
22	Kiokokro	16.25 ± 0.86	11.3 ± 0.72	1.88 ± 0.25	2.11 ± 0.16	0.73 ± 0.14	3.04	75.69
23	Kortolik	88.68 ± 0.83	1.36 ± 0.12	0.28 ± 0.10	1.8 ± 0.25	2.27 ± 0.27	5.61	34.95
24	Mo alu	68.97 ± 0.56	2.24 ± 0.25	0.95 ± 0.08	0.94 ± 0.09	1.65 ± 0.13	25.26	121.82
25	Rangajhum alu	65.6 ± 0.75	2.71 ± 0.27	1.98 ± 0.12	2.1 ± 0.10	1.84 ± 0.04	25.77	135.46

3.2 Comparison of some selected ethnic plant foods in different food composition table.

Nutrient levl in foods are available. In the case of plant foods mineral leve may be affected by factors such as the varity of produce item, time of harvest, maturity,climate,soil conditions including fertilizer and pestisides a pplication, storage and marketing system. As biological system plant foods are also subject to random variation in mineral content (Greenfield and Southgate 1992, Torelm and Danialsson 1998).

Here, present study compare analyzed value of some foods with national DKPM (Deshio khaddar Pushtiman ,INFS;1992) and I F C T (Indian Foods Composition Table, 1995).

In table 3.7 Forash dal (*Vigna grahamiana)* Compare with IFCT Rajma and DKPM Field bean (dry) here we show the macro nutrient and micro nutrients contents of Forash dal are nearly similar.

Table 3.2.1: Comparison of "Farash Dal" with National and International FCT.

Nutrients	Present Study	Nutritive value of 'Indian Foods'	Deshio khadder Pushtiman
Macro- nutrients (g/100g edible)	Farash dal	Rajma	Field bean (dry)
	Vigna grahamiana	*Vigna grahamiana*	*dolichos lablab*
Moisture	14.91	12	9.6
Protein	23.85	22.9	24.9
Fat	1.82	1.3	0.8
Ash	2.41	3.2	3.2
TDF	2.65	4.8	1.4
CHO	54.37	60.6	60.1
Energy(Kcal)	334.52	346	347
Micro-nutrients			
VIT B_1 (mg %)	0.45	0.32	0.52
VIT B_2 (mg %)	0.25	0.56	0.16
Iron (mg %)	4.5	5.1	2.7
Calcium (mg %)	278	260	60
Phosphorus (mg %)	382	410	nf

Table 3.2.2 :Comparison of "Kamino" with National and International Food Composition Table (FCT)

Nutrient composition	Present Study	Nutritive value of 'Indian Foods'	Deshio khadder pushtiman
Macro- nutrients (g/100g edible)	*Kamino*	*Tamarind leaves*	*Tamarind leaves*
	Caesalpinia digyna	*Tamarindus indica*	*Tamarindus indica*
Moisture	80.25	70.5	70.5
Protein	4.77	5.8	5.8
Fat	2.56	2.1	2.1
Ash (g%)	1.19	1.5	1.5
TDF	1.89	10.6	1.9
CHO	9.35	18.2	18.2
Micro-nutrients (mg/100g edible)			
Vit-C (mg%)	4.9	03	03
Vit-B1(mg%)	0.038	0.24	0.24
VIT-B2(mg%)	0.12	0.17	0.17
Iron(mg%)	6.5	0.030	5.2
Calcium(mg%)	135	101	101
Phosforus	163	140	nf

Note: nf =Not found

Table3.2.3: Comparison of "chikipong" with National and International Food Composition (FCT)

Nutrient composition	Our Study	Nutritive value of 'Indian Foods'	Deshio khadder pushtiman
Macro- nutrients (g/100g edible)	Chikipung	Ambat chuka	Talk palong
	Rumex vesicarius	*Rumex vesicarius*	*Rumex vesicarius*
Moisture	91.21	95.2	93.5
Protein	1.86	1.6	2.9
Fat	0.38	0.3	0.1
Ash	0.97	0.9	1.4
TDF	2.15	3.2	0.6
CHO	3.42	1.4	2.1
Micro-nutrients (mg/100g edible)			
Vitamin C	13.86	12	15
Vit B1	0.053	0.03	0.1
Vit B2	0.073	0.06	0.09
Iron	1.44	0.75	8.7
Calcium	62.53	60	79
Phosphorus	15	17	nf
Carotenoids (µg%)	8489.65	9400	794o

Note: nf=Not found

3.3 **Out standing and excellent foods:**

Among the selected 25 vegetables Forash dal (*Vigna grahamiana)* ,Ranga Jhum Alu, **leafy** vegetable 'Kamino' Spices"Fala" were found to be out standing foods because of their rich nutrient content compare to others (Table 3.10 to 3.13).

Table 3.3.1: Outstanding food 'Farash Dal' (*Vigna grahamiana)*

Nutrients	Nutritive value (ep)	% RDA covered
Protein(g%)	23.85	42.59
Fat(g%)	1.82	6.62
CHO(g%)	54.82	42.17
Energy(g%)	334.52	13.49
Iron(mg%)	4.5	56.25
calcium(mg%)	278	37.07
Phosphorus (mg%)	382	54.57

Table 3.3.2: Excellent leafy vegetable 'Kamino'

Nutrients	Nutritive value(ep)	% RDA covered
Protein(g%)	4.77	8.52
Fat(g%)	2.56	9.48
CHO(g%)	9.35	7.19
Vit-C(mg%)	4.90	5.44
Iron(mg%)	6.5	81.25
Calcium(mg%)	135	13.50
Phosphorus(mg%)	163	23.29

Table 3.3.3: Excellent non leafy vegetable "Ranga Jhum Alu"

Nutrients	Nutritive value(ep)	% RDA covered
Protein (g%)	2.71	4.84
Fat (g%)	1.98	7.33
CHO (g%)	25.77	19.82
vitamin C (mg%)	11.6	12.9
Iron (mg%)	0.49	6.13
Calcium (mg%)	38	3.8

Table-3.3.4: Excellent non leafy Spices"Fala"

Nutrients	Nutritive value(ep)	% RDA covered
Protein (g%)	1.98	3.54
Fat (g%)	0.87	3.22
CHO (g%)	11	8.46
vit.--C (mg%)	7.8	8.66
Calcium (mg%)	42	4.2
Iron (mg%)	2.9	36.25

3.4 Discussion for nutritent composition of selected ethnic plant food

Food is essential component for human survival. Good health needs balanced diet. To have it, nutrient composition of consumed foods has to be made well-known and available to the people. Ethnic people are healthy, strong and hardworking, have less morbidity and higher life expectancy. This may be because of that they consume almost all of the wild plant and animal foods that make them active and healthy.

In the present study twenty five ethnic vegetables were selected, some of which are traditionally used by the ethnic people in the treatment of different illness. CFCS and FGDs listed 113 exclusive ethnic foods (identity of which has been confirmed by taxonomic study). Of these, nutrient composition of eighteen foods were analyzed and incorporated in the database (FAO, 2010), some were analyzed by other MS and M. Phil Students and published in FCT and database for Bangladesh (Islam *et al*, 2012).

Epidemiological and clinical studies document the relationship between diet and health. Plant foods are rich in micronutrients and people consuming it have a lower incidence of diseases.This study conducted on ethnic vegetables of Chittagong hill tract.

It is known that people who live in hill tracts have to work hard to survive. Their geo graphic area is totaly different and food habit. They consume some ethnic foods those are different from normal food. In the present study 25 vegetables were taken to see the nutritive value and the difference.

The consumption of phytochemicals such as carotenoids and polyphenols within whole vegetables has been associated with decreased incidence of various inflammation and oxidative stress related chronic diseases, which may be due to direct antioxidant effects, or indirect mechanisms such as affecting signal transduction/gene expression. (Table 3.1) Total carotenoid content varied considerably between the different vegetables ranged from 9337.24 to 48.36 µg/100g edible portion. The highest amount was present in Mrolapang (*Manihot esculenta*) (9337.24 µg/100g edible portion) and relatively high in Chikipung (8489.65 µg/100g) whereas lowest amount present in Projukti pata/shak (655.72 µg/100g edible portion). Among non leafy vegetables, highest amount of total carotenoids was found in Ranga Jhum Alu (2257.76 µg/100g) and lowest in Fala (48.36 µg/100g).

The analyzed vegetables samples contain appreciable amount of total carotenoids in Mrolapang (*Manihot esculenta*) (9337.24 µg/100g edible portion) . FAO funded project "*Preparation of food composition database with special reference to Indigenous and Ethnic foods"* showed that our national vegetables Spinach (Palong shak) contain 4350 µg/100g edible portion of carotenoid and shabuj kochu shak contain 8350 µg/100g edible portion. Boon alu contain 565 µg/100g edible portion of carotenoid on the other hand Ranga jhum Alu contain 2257.76 µg/100g edible portion of carotenoid. So we can say ethnic vegetsbles contain appreciable amount of carotenoids which can prevent the vitamin A deficiency disorder of the people of Bangladesh.

Vitamin C enhances iron absorption from non-heme iron. Several human absorption studies conducted by several investigators indicate that each main meal should preferably contain at least 25 mg to 50 mg of ascorbic acid. Higher ascorbic acid intakes should be considered if meals contain higher amounts of factors inhibiting iron absorption, such as phytates and tannins (FAO/WHO 2002). The totality of evidence from human studies reviewed by Carr and Frei (1999) suggest that a dietary of intake of 90 mg to 100 mg of vitamin C per day is associated with reduced risk of cardiovascular disease and cancer. In the present study, (table 3.3) the maximum amount of vitamin C was present in Mori shak (*Foeniculum vulgare*) (68mg/100g edible). Aproximately in Mrolapiong and Felong dal shak good amount of vitamin C 64mg/100g and 53mg/100g edible were found respectively. In FAO funded project

Preparation of food composition database with special reference to Indigenous and Ethnic foods showed that our national leafy vegetable pat shak & Spinach (Palong shak) contain (54.43 mg/100g edible) &22.44mg/100g edible portion vitamin C respectively.

So in every day diet with an approximate portion size of this vegetable if consumed by the ethnic people can meet their daily requirement of vitamin C.

The FAO/WHO (2002) recommendations for these ages were rather similar to the DRI Committee's recommendations. The recommended intake was 1.2 mg/day for males, and 1.1 mg/day for females. In the present study, higest amount of vit B_1 was found in Mo alu (0.72mg/100g edible). To meet daily requirement concumption should be more then 100g/day is needed.

Also (Table 3.3) vit B_2 and vit B_3 found in a good amount in Mrolapying, and Fala. Micronutrient content is importent in daily diet. This study indicates the content of B vitamins varies in different leafy and non- leafy vegetables.

Vegetables are the major functional foods because they are the main sources of nutraceuticals such as vitamins, minerals and phenolic compounds (Tomás-Barberán and Espin, 2001; Szeto *et al*., 2002; Rupasinghe and Clegg, 2007). Epidemiological studies have shown the existence of a significant correlation between the intake of fruits and vegetables and the decrease of mortality and morbidity due to degenerative processes caused by oxidative stress (Birt *et al*., 2001; Dragsted *et al*., 2004). In the present study highest quantity of copper is in Foras dal (*Phaseolus vulgaris)* 138 µ g/100g edible and lowest quantity in Kamino (*Caesalpinia digyna* Rottler) (0.05 µ g/100g edible).

Kiokokkro contain highest amount of calcium (1338 Mg/100g edible). Moroi shak (*Foeniculum vulgare)* 347 Mg/100g edible Foras dal 278 Mg/100g edible Felong dal shak173 Mg/100g edible Mrolapion (*Manihotesculenta)* 139 Mg/100g edible Kamino 135 Mg/100g edible.

Foras dal (Phaseolus vulgaris) poses highest amount phosphorus 382 Mg/100g. Total phenol content in Kanimo, Missayanu, Mo alu was around 200mg GAE/100g. Phytate content was noted highest (72.18mg/100g) in Forash dal almost 70%. Brat *et al*. (2006) have also determined total phenolics of green and red bell peppers (8.2 and 26.8 mg/100 g FW respectively), citrus genus fruits (30 to 45 mg/100 g), onion (76.1 mg/100g FW), garlic (59.4 mg/100 g, FW) and eggplant (65.6 mg/100 g, FW). This study results shows that compaired to local foods most of the slected ethnic food contain two or three time high phenols and phytic acid.

Protein content of local leafy vegetables ranges from a highest value of 5.2±0.95 g/100g edible portion in pat shak and a lowest value of 1.5±0.65g/100g edible portion in pui shak.
Plant foods that provide more than 12.0% of its calorific value from protein are considered good source of protein (Pearson, 1976). As we know vegetables are not a good protein source. Pulses contain a good amount of protein. In the present study (Table 3.6) indicated that highest value of protein (23.85g/100g) was found in forash Dal. But leafy vegetables content of protein average 3 to 4 g/100gm.

The total content of mineral salt as ash in fruits and vegetables varied from 0.2g/100 g edible portion to 1.5g/100 g edible portion (Gardner *et al.* 1939). Forah Dal from selected foods contains highest ash (2.41g/100g). Also a good amount of ash containing ethnic food found in this study like Saimya(2.37g/100g) , Kiokokro (2.11g/100g), Monjori (2.21g/100g)

As well as vegetables are not a good source of fat a minimum and highest fat content was found in kamino (2.56g/100g).
As a nutritive value of food, fibers in the diet are necessary for digestion and for effective elimination of wastes (Vadivel & Janardhanan, 2005). Highest dietary fiber content (5.06 g/100g) was found in Missayanu. (table 3.5)

Tongco et.al. (2014) conducted a research on Nutritional and phytochemical screening, and total phenolic and flavonoid content of *Diplaziumesculentum (Retz.) Sw*.from Philippines. The proximate analysis of D. esculentum using standard AOAC methods showed that the fresh plant samples contain 91.82 ± 0.43 % moisture, 1.42 ± 0.10 % ash, 0.28 ± 0.004 % crude fat, 0.87 ± 0.004 % crude protein, and 0.72 ± 0.05 % crude fiber while oven dried plant samples contain 17.39 ± 0.82 % ash, 3.40 ± 0.05 % crude fat, 10.67 ± 0.05 % crude protein, and 9.06 ± 0.67 % crude fiber. Fresh *D. esculentum* is very high in water content. Drying removes the water present in the plant tissues, making it easier to quantify the various components of the plant. The analysis shows that D. esculentum is high in inorganic minerals (ash content). Additionally, the study showed that D. esculentum is high in fiber and protein contents.

The Tubers of several species of Yams (Dioscorea spp.) are edible and are counted just after potato in its food value. In fact, species like D. alata,D. pentaphylla and D. bulbifera are the most worldwide cultivated true yams for their tubers which are of rich source of tarch that form an important dietary supplement. Apart from starch the root tubers of Dioscorea also contain protein, fats, fibers and among minerals nutrients Potassium, Sodium, Phosphorus, Calcium, Magnesium, Copper, Iron, Manganese, Zinc and Sulphur containing amino acids (Gopalan, 1996)

Ethnic vegetables are excellent sources of nutrients, required for substance of health, even to prevent or reduce many fatal diseases. People should be empowered and aware enough to consume the nutrient rich ethnic plant foods as well as sources of additional organic nutrients in daily meals. Some fruits and vegetables have medicinal values not restrict to treat disease but also improve overall health due to their vitamin and other nutrient content.

It is evident that ethnic fruits and vegetables grown in Bangladesh are rich in nutrient content. Therefore, ethnic foods need analysis for exploring their nutrient composition to improve nutrition and health status of the ethnic and mass people of Bangladesh. It will help in designing balanced diets and food based dietary guidelines. It could also provide benefit to food security. Incorporating ethnic or tribal food in the food manu would improve our daily food value.

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Medicinal Properties of Ethnic Plant Foods

CHAPTER 1

Intriduction

1.1 Over view

In Bangladesh, the use of traditional medicinal is widespread among most of the ethnic people and village dwellers (IUCN,2011) The use of natural product or natural products based medicine is increasing all over the world especially in the developing countries like Bangladesh, India, China and The Middle East. About 25% of prescribed drugs in the world are of plant origin (Abul Khair *et al*,20014.According to WHO, any plant could be medicinal that contain substances which can be apply for the production of useful drugs (Junaid *et al*, 2006). The medicinal properties of plants are due to some chemical substances that produce certain definite physiological action on the human body (R K Shah & R.N.S. Yadav, 2015).

Medicinal properties analysis Antibacterial activity was estimated by disc diffusion method (Islam *et al*., 2002) 10 plants foods were randomly selected for antimicrobial analysis. Fourteen strains of pathogenic bacteria (6 gm+, 8 gm-, including *bacillus*,*shigella*,*klebsiella*, *E-coli*, *salmonella*, *staphylococcus*etc.) and antibiotic "Ciprofloxacin" was used as standard in this regard.

Alloxen-induced diabetic mice:Serum glucose level was estimated by Glucose oxidase method (Trinder, 1969; Glynn, 1991) using commercial kit [Human, Germany with the help of ELISA platereader (Labsystem,Finland)]

Seventy two white albino mice (25-35 gm) each of both sexes were procured from the animal house of Jahangir Nagar University. They were housed at standard environmental conditions of temperature and dark/light cycle, and were fed with commercial pellet diet and drinking water.

1.2 Background

Bangladesh, a country of fertile deltaic land has a rich diversity of flora of medicinal plants scattered throughout the forests, crop fields, roadsides, gardens and wastelands. However, population and over extraction of resources is a harsh reality for the country and like other resources, medicinal plants are also nearing extinction. Unfortunately, we do not have detailed information and complete inventories on such plants, which makes it more challenging to conserve them. The plants genetic resources are lost at an alarming rate due to degradation of natural habitat, displacement of land races by modern cultivars, changes in land use systems by mono–cropping and commercialization. Growing population, over exploitation and recent phenomenon of climate change are also considered as threats, contributing towards the loss of genetic resources of medicinal plants in Bangladesh. Although some efforts have been made to protect forest resources by declaring them as Reserve Forests, Protected areas, Sanctuaries, etc., there is no effective conservation effort for conserving the endangered species of medicinal plants and associated knowledge and practices . The CHT comprises of three hilly districts namely; Bandarban, Rangamati and Khagrachari covering about 10 percent of the total land area of Bangladesh. This land area harbors one-third of the flowering plants of the country and is endowed with floral biodiversity. There are about thirteen ethnic communities inhabiting in this hilly region of the country (IUCN, 2011).

The ethnic communities of the hill districts are in continuous search of indigenous plants for various uses, and in course of time they have accumulated important knowledge of the use wild plants. This TK of plants is intertwined with their culture , geographical environment and heritage. It is the cultural heritage of the ethnic women to collect the wild plants from the surrounding forests every day, to meet their daily needs and without destroying their natural habitats. The indigenous knowledge of plant use is transmitted orally from generation without any written documentation or record.

Medicinal plants are the gift to mankind because they cure diseases without any side effects. Herbs have been playing a major role in curing various ailments and diseases from antiquity.

Herbal medicines used widely by the tribals and rural people, as they are available in the vicinity of their homes. Herbs contain a large number of naturally occurring substances that work to alter the body's chemistry in order to return it to its natural state of health. In recent years, due to fast and busy life style, mental tension, low physical activity, many diseases and disorders are increasing (Sahu, 2010). One of the most common musculoskeletal disease and disorder is rheumatism, which is more frequent in women at the age of forty and above. The cause of rheumatism is due to deposition of uric acid in cartilage of joints. Recurrent attacks, pains and swelling of joints, with crippling effects in some cases, have also been observed in various joint diseases. Herbs have been used for centuries in the treatment of many diseases and it has been demonstrated that some of them can have an incredible effect as an herbal treatment for rheumatism. In modern allopathic system many medicines are also prescribed for this disorder, but they have many side effects. Therefore to avoid their side effects, now days, people are much inclined to use herbs based medicines rather than modern allopathic (Samvatsar and Diwanji 1999). Keeping this in view, present paper highlights the ethnomedicinal plants which are used traditionally for treatment of rheumatism in Satna district. These herbs have properties that can significantly reduce joint pain or swelling and have no side effects. (Lipika Devi Bal & Ravindra Singh, 2013).

Traditional knowledge of medicine has long been used since ages for curing various human ailments. About 60-80% of world populations still rely on plant based medicines [Santhi ei, al, 2011]. Though the traditional Indian system of medicine has a long history of use, yet they lack adequate scientific documentation, particularly in light of modern scientific knowledge [Shrivastava S, 2010]. The medicinal value of plant lies in the bioactive hytochemical constituents of the plant and which shows various physiological effects on human body (Nilofer Sheik, 2013)

Traditional medicine is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illness. Traditional medicine that has been adopted by other populations (outside its indigenous culture) is often termed alternative or complementary medicine. Herbal medicines include herbs, herbal materials, herbal preparations, and finished herbal products that contain parts of plants or other plant materials as active ingredients.

A considerable number of definitions have been proposed for medicinal plants. According to the WHO, "A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis." When a plant is designated as 'medicinal', it is implied that the said plant is useful as a drug or therapeutic agent or an active ingredient of a medicinal preparation. Medicinal plants may therefore be defined as a group of plants that possess some special properties or virtues that qualify them as articles of drugs and therapeutic agents, and are used for medicinal purposes. A total of 2, 50,000 species of flowering plants are referred to as medicinal plants. The World Health Organizations (WHO) enlisted some 21,000 medicinal plant species.In some Asian and African countries, 80% of the population depends on traditional medicine for primary health care. In many developed countries, 70% to 80% of the population has used some form of alternative or complementary medicine (e.g. acupuncture). According to WHO About 25% of modern medicines are descended from plants that were first used traditionally. Likewise, almost 70% of modern medicines in India are derived from natural products.

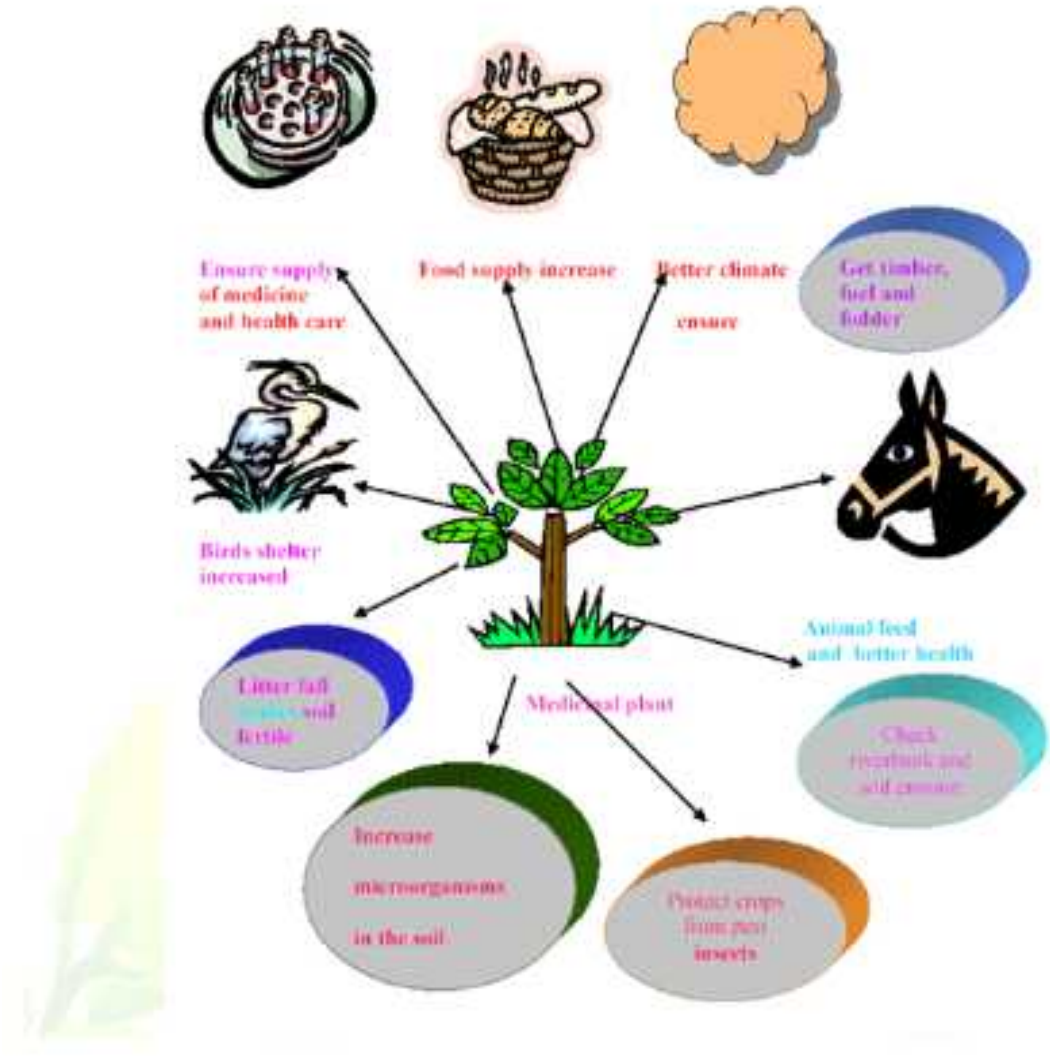


Figure: 1.1 pictorial view of Medicinal Plant for Biodiversity conservation[7]

Plant could be medicinal that contain substances which can be apply for the production of useful drugs (Junaid *et al*, 2006).The medicinal properties of plants are due to some chemical substances that produce certain definite physiological action on the human body (R K Shah & R.N.S. Yadav, 2015).

A major part of the population in developing countries still uses traditional folk medicines obtained from plant resources (Farnsworth, 1994).WHO estimated that as many as 80% of world's population living in rural areas rely on herbal traditional medicines as their primary health care. In recent years, interest has been raised to evaluate plants possessing antibacterial activity for various diseases (Clark and Hufford, 1993). Before the early 20th century, treatment for infections was based primary on medicinal folklore. Plant extracts with antimicrobial properties that were used in treatments of infections were described over 2000 years ago (Lindbland**,** 2008). Many ancient cultures, including the ancient Egyptians and ancient Greeks, used specially selected molds and plant materials for the discovery of natural antimicrobials produced by micro-organisms. Louis Pasteur observed, if we could intervene in

[7] www.bpc.org.bd/mphpbpc_sector_profile.php

the antagonism observed between some bacteria, it would offer perhaps the greatest hopes for therapeutics (Kingston,2008).

Indigenous and ethnic plants that are widely used in folk-medicines are numerous and diverse. In Bangladesh, 787 plants species have been identified as medicinal plant having therapeutic properties (Yusuf *et al*., 2009). Despite development of modern medicine, many rural people of Bangladesh still depend on plant products and herbal remedies for treatment of their ailments. Although reports of antibacterial activity of indigenous plants have been published from many regions (Nadkarni, 1908; Dhar *et al*., 1968), they have not been systematically conducted, except in a few cases, thereby leading to confusion in drawing a meaningful conclusions (Padmaja *et al.,* 1993; Ndamba*et al*., 1994; Vijaya *et al.,* 1995). Researchers are increasingly turning their attention to folk medicine looking for new leads to develop better drugs against viral and microbial infections (Hoffmann *et al.,* 1993). Although hundreds of plant species have been tested for antimicrobial properties, the vast majority have not yet been adequately evaluated (Tshikalange*et al*., 2005; Dalmarco *et al*., 2010).

Traditional knowledge of medicine has long been used since ages for curing various human ailments. About 60-80% of world populations still rely on plant based medicines (Shanthi, R *et al*, 2011) Though the traditional Indian system of medicine has a long history of use, yet they lack adequate scientific documentation, particularly in light of modern scientific knowledge (Shrivasta S, 2010). The medicinal value of plant lies in the bioactive phytochemical constituents of the plant and which shows various physiological effects on human body.

The people and even the crops of a country like Bangladesh, with moderately high temperature and relatively high humidity, are vulnerable to frequent bacterial attack and in every year a large number of people die suffering from various infectious diseases.

From time immemorial people have been using plant materials to combat all types of ailments, even until the invention and production of antibiotic medicines the practice was continued all over the world. After the invention and large scale production of antibiotics physicians of the developed countries of the world started prescribing antibiotics against diseases caused by pathogenic bacteria. The practice then gradually spread throughout the globe.

However, due to indiscriminate use of antibiotic drugs, the bacteria have developed resistance to many antibiotics. This has created immense clinical problem in the treatment of infectious diseases (Marta *et al.,* 2005). In addition to this problem most antibiotics are associated with adverse effects on the patient, even damaging vital organs. Therefore, there is an urgent need to develop alternative antibacterial drugs with no or very little side effects on the host for the treatment of infectious diseases.

Screening of local plants is one of the approaches to find out alternative way to treat infectious diseases with little or no adverse effect on the host. Therefore, the researchers are now turning their attention to local plants, especially to folk medicine looking for new leads to develop better drugs against infectious bacteria.

Like other parts of the world, scientists in Bangladesh are also working in this line. First paper on the antibacterial activities of plant materials in Bangladesh was published by Hoque *et al*. (1986). Since then works on the antibacterial and antimicrobial activities of plant materials are on gradual increase (Hoque *et al*., 1989; Islam *et al*., 2008; Hasan *et al*., 2009; Rahman *et al.,* 2009; Hassan *et al.,* 2011). However, although hundreds of plant species have been tested for antimicrobial properties the vast majority have not yet been adequately evaluated.

Many developing countries use traditional medicine, in particular herbal medicine, because it is an affordable source for healthcare (Bhattarai, 1993; Manandhar, 1995), and are now using herbal medicine for chronic diseases (WHO, 2002). The use of ethnobotany has a long folkloric history for the treatment of blood glucose abnormalities (Sharma *et al.,* 2007). Plants

or herbal drugs are widely prescribed because of their effectiveness, less side effects and relatively low cost. This is why the use of herbs has become triple over the last 10 years (Eisenberg, 1998). Some nutraceuticals have been reported to have hypoglycaemic properties (Alarcon *et al.*, 1998).

Currently several therapeutic strategies are being used for the treatment of Type II diabetes, but still it remains as a global major health problem. In addition to using different therapeutics such as biguanides and sulfonylureas, Type I DM is treated with exogenous insulin and type II with synthetic oral hypoglycaemic agents and/or insulin (Pepato, 2005). However, still then, no satisfactory effective therapy is available for the management of diabetes mellitus (Sumana and Suryawashi, 2001). The oral drugs (hypoglycaemic) and insulin have limitations of their own in treating non-insulin dependent diabetes mellitus (Berger, 1985; Hupponen, 1978). Thus searching for a newer class of compounds is essential to overcome these problems (Kamaeswara *et al.,* 2001).

Diabetes represents a group of metabolic disorders in which there is impaired glucose utilization (Robbin, 1994). It is a widely spread and complex endocrine disorder. Diabetic is a clinical condition characterized by high level of blood glucose due to defects in insulin production, insulin action or both. It is a complex and multifarious group of disorders characterized by hyperglycemia that has reached epidemic proportion in the present century. In 2000, it was estimated that approximately 151 million or about 4.6% people of age 20-79 years have diabetic mellitus worldwide. It also estimated that 4.9 million people in all age group have Type I diabetes and 0.91 million in South East Asian region. The incidence of Type I diabetes in IDF (International Diabetic Federation) member countries is 77,000 persons per year in 0-14 years age group and 1,19,000 persons per year in the 15^{+} age group (IDF report, 2000).

1.3 Rationale of the study

The ethnic communities of the hill districts are in continuous search of indigenous plants for various uses, and in course of time they have accumulated important knowledge of the use wild plants. This traditional knowledge (TK) of plants is intertwined with their culture, geographical environment and heritage. It is the cultural heritage of the ethnic women to collect the wild plants from the surrounding forests every day, to meet their daily needs and without destroying their natural habitats. The indigenous knowledge of plant use is transmitted orally from generation without any written documentation or record.

The documentation of TK can ensure local people's rights in the light of intellectual property rights and help avoid adverse impacts on local people and the environment. The use of the TK in sustainable forest management can significantly contribute to the research and development of medicinal plants and reduce associated costs. TK on habitat, habit and use patterns of the wild plants by the ethnic communities are essential for a sustainable forest management plan, which is essential for restoration and conservation of wild biodiversity

Comprehensive food consumption survey (CFCS) and focous group discussions (FGDs) data document that ethnic people uses a variety of their foods in treatment of many ailments (Islam *et al*, 2010). By far, none of these ethnic foods has been explored for their medicinal principles.Medicinal plant foods provide this opportunity to improve the health status, reduces health care costs and support economic development in rural communities.

Interest in the exploration of the medicinal, aromatic plants as pharmaceuticals, herbal remedies, flavouring, perfumes and cosmetics and other natural products has greatly increased in the recent years(Anon, 1994; Ayensu, 1996; Sallah *et al*, 1997).

1.4 Objective of the study

The objective of the present study was to learn more about medicinal plants that have been utilized for hundreds of years and so have demonstrated their potential efficacies, even though such efficacies may not have been thus far validated through modern scientific methods. We therefore undertook an ethnomedicinal survey of traditional medicinal healers (known generally as kavirajes or vaidyas by the mainstream community) in several districts and tribal groups to sample information on medicinal plants.

This study aimed to analyze nutrient composition and medicinal properties of some selected ethnic plant foods. In line of its objective, it has been designed to

Screening of medicinal properties of selected ethnic plant foods, that included

a) **Antibacterial property - sensitivity test screening**
b) **Hypoglycaemic property in animal models**

CHAPTER 2

Materials and Methods

2.1 Materials and Methods

Many plants, due to some chemical constituents they posses, show mild to remarkable activities against some bacteria, specially pathogenic bacteria. These activities against some bacteria are known as “antibacterial activities”. In fact, they inhibit bacterial growth or kill them. In broad sense this may be called antimicrobial agent, as bacteria are microbs and the plant extract inhibit their growth. Other microbs are fungi, nematodes etc.

Collection and processing of plant foods

Nearly one thousand species of plants with edible leaves are known (Jacob and Shenbagaraman, 2011). Leafy vegetables most often come from short lived herbaceous plants such as lettuce and spinach. Woody plants whose leaves and fruits can also be eaten as leafy vegetables. In the present study medicinal plants were selected on the basis of their medicinal importance in literature and to people, especially live at CHTs in Bangladesh. The plant food samples were categorically identified and certified by personnel of Department of Agricultural Extension (DAE) and the taxonomist of the Department of Botany, Dhaka University.

Key informants: Smritibindu Chakma in Bandarban district; Bigganto talukdar in Rangamati;Gopal barman in Rajendrepur (Barman para) Gazipur; Dr. Maksuda Khatun (Taxonomist) were the major informants.

Image: 2.1

Most of the ethnic foods were collected during the month of April to May. A few was collected during September to December. Ethnic food samples were purchased from the weekly local marketsof CHTs with the help of local ethnic DAE staff, who confirmed its identity. After taking the food sample to the lab, the taxonomic expert further identified it for its scientific and English name.

Table 2.1: List of selected ethnic plants tested for medicinal properties.

Local name	Scientific name	Edible portion used	Diseased to be treated	Reference
Khoro pata	*Cissus repens Lam.*	Leaves	Perineal healing,rectraction of the uterus.	Lamxay *et al*. 2011
Kamino	*Caesalpinia digyna*	Leaves	Hypoglycemic, snakebite, rheumatism.	Das *et al*.,2007
Moroi	*Foeniculum vulgare*	Leaves	Memory enhancing, antimicrobial, anti-inflammatory, cyto toxic, hypotensive etc.	Rojahahimi *et al*, 2013.
Yangfo	*Feics benghalensis*	Leaves	Diabetic, constipation, Antibacterial, costharoag.	Useful plant of Bangladesh-Dr. Tapan qumar Day.
Ozon shak	*Spilanthes calva*	Leaves flowers	inflammation, toothache, skin diseases, purgative, diuretic, lithotripter and dysentery.	(Krishnaswami *et al*. 1975, Mukharya *et al*. 1986).
Mimini	*Centella assiatica*	Leaves	Management of central nervous system, skin and GITs disorder.	D. Arora *et al*, 2002.
Chikipung	*Rumex vesicarius L.*	Leaves	Cooling agent, curing stomach heat, toothache and to check nausea diabetes fever.	Palani samy Hariprashad and Ramakrishnan; 2011.
Tak bagun	*Solanum virginianum*	Fruit	Epilepsy, pain relieving, head ache, migraine, hair fall, bronchial asthma, skin problem, cough &other diseases.	www.bimbima.com/health/post/2012/11/09/medicinal-use-of-kantkari-or-solanum-virginianum-linn.aspx
Aam pata	*Mangifera indica*	Leaves	Mouth infections, GITs disorder, diabetes, diarrhea, scurvy ,typhoid fever, sore throat, dysentery etc.	Fowler 2006; Campbell *et al*., 2002
Bala pata	*Pouzolzia hirta*	Leaves	Ulcer, syphilis, gonorhoea, sores, stomachache.	Yusuf *et al*.2009, medicinal value of \bangladesh.

2.2 Introducing selected medicinal plant food samples:

Most people in Bangladesh and especially in the tribal communities rely on traditional medicinal healers for treatment of their ailments. This is true for diseases like of modern medicines. Moreover, the rural people of Bangladesh lack access to modern medicinal facilities. The traditional healers use medicinal plants for treatment and are considered experts in their knowledge of plants and their preparation

in disease-treating formulations. This knowledge is on the verge of disappearing because of loss of forest regions and consequent endangerment of medicinal plants. The practice of traditional healers keeping their knowledge confined to their immediate family may also contribute to this disappearance. Recent years have witnessed a gradual migration of traditional medicinal healers to other jobs, which has been more pronounced in their sons and daughters, who after receiving formal education are more inclined to give up traditional medicinal practices and migrate to jobs in the bigger cities. As a consequence, the age-old medicinal knowledge is fast disappearing. This is unfortunate because the medicinal plants utilized by the healers are under-studied and can be a potential source of new and effective drugs (Hossan *et al*.2010)

1. Khoro pata

Scientific name: *Cissus repens* Lam.

Medicinal use: *Cissus repens (CR)* is a genus of about 200 species found in tropical and subtropical regions. *CR* has been evaluated for potential medical uses. As a source of carotenoids, triterpenoids and ascorbic acid the extracts may have potential for medical effects, including "gastroprotective activity[1] and benefits in terms of "lipid metabolism and oxidative stress". *Cissus quinquangularis* was used by the Maasai people of Kenya to relieve some of the symptoms of malaria. In Taiwan, CR is used for the treatment of many diseases, such as epilepsy, stroke, abscess, and diabetes. In addition, it also has anti-inflammatory and antirheumatic activity (Araújo,*et al*,1996).

Image: 2.3

. Kamino

Local name: Kamino(marma)

Scientific name: ***Caesalpinia digyna Rottler***

Medical use: Leaves are use to treat Hypoglycemic, snakebite, rheumatism. (Das *et al*.,2007) . Roots are astringent; used internally in phthisis, scrofula and diabetes. The powder of the root is useful in diarrhoea and other chronic fluxes. Root pounded and mixed with water is drunk as a febrifuge. EtOH (50%) extract is active *in vitro* against *Mycobacterium tuberculosis* in guinea pigs (Asolkar *et al*., 1992).

Image: 2.3

3. Moroi skak

Scientific name*:* *Foeniculum vulgare.*

Medical use: Foeniculum vulgare. (F. vulgare), commonly known as Fennel, is a popular medicinal plant with various pharmacological activities mentioned in traditional Iranian medicine (TIM) and modern phytotherapy such as antioxidant, cytotoxic, anti-inflammatory, antimicrobial, bronchodilatory, estrogenic, diuretic, lithontripic, galactogogue, emmenagogue, antithrombotic, hypotensive, gastroprotective, hepatoprotective, memory enhancing, and antimutagenic activities. No serious adverse events were recorded after ingestion of F. vulgare except some cases of allergic reactions. The estrogenic activity of F. vulgare brings some side effects such as decrease in protein concentration and acid and alkaline phosphatase in male genital organs, increase in weight of mammary glands and reproductive organs in women and premature thelarche in girls(Roja Rohimi and Mohammad Reza Shams, 2013)

Image: 2.4

4. Yangfu

Scientific name: *Feics benghalensis*

Medical use: Aqueous extract of leaf buds of *Ficus bengalensis* (*Fb*) mixed with sugar and honey for checking diarrhoea; milk processed with the aerial roots or leaf buds of *Fb* in hemorrhages and bleeding piles; a decoction of leaf buds and aerial roots of *Fb*, mixed with honey, was given for checking vomiting and thirst; also during fevers with burning sensation (Astaanga Hridaya, Vrindamaadhava, vaidyamanorama) (C.P. Khare, 2004). The aerial roots are useful in obstinate vomiting and leucorrhoea and are said to be used in ostyeomalacia of the limbs. The bark is useful in burning sensation, haemoptysis, haemorrhages, diarrhoea, dysentery, diabetes, enuresis, ulcers, skin diseases, gonorrhea, leucorrhoea, and hyperpiesia.

Image: 2.5

The leaves are good for ulcers, leprosy, allergic onditions of skin, burning sensations and abscesses. The buds are useful in diarrhoea and dysentery. The fruits are refrigerant and tonic and are useful in vitiated condition of *pitta*. The latex is useful in neuralgia, rheumatism and lumbago bruises, nasitis, ulorrhagia, ulitis, odontopathy, hemorrhoids, gonorrhea, inflammations, cracks of the sole and skin diseases(Prajapai, Kumar Agro's, 2003). Milky juice and seeds are beneficial as local application to sores and ulcers, soles of the feet when cracked or inflamed and in rheumatism. Leave s are heated and applied as a poultice to abscesses; tender leaves pasted with honey beneficial in *raktapitta*. Tender ends of the hanging (aerial roots) are antiemetic. Seeds are cooling and tonic (The Wealth of India, 1999 Parrotta John A, 2001; A. Chatterjee, 1997).

5. Ozon shak

Scientific name:*Spilanthes calva*

Medical use Flowers head is stimulant and sialagogue; given in toothache, affections of throat and gums and paralysis of the tongue. It is a powerful mosquito larvicide. The decoction of the plant is diuretic and lithontriptic; useful in dysentery and is employed as a bath for rheumatism and as a lotion in scabies and psoriasis. Crushed plant is used as fish poison. It is prescribed for few days to cure glossitis. Juice of the plant and flower head is rubbed in scabies to cure. Roots are used as a purgative.
http://herbalinformation.awardspace.com/?cm=c&fn= Spilanthes_calva*.*

Image: 2.6

6. Mimini

Scientific name:*Centella assiatica*

Medical use: Plant alkaloids possess effective anticancer properties and are used successfully in children and Hodgkin's disease. Leaves use for management of central nervous system, skin and GITs disorder (D. Arora *et al*, 2002.)

Treatment of wounds, burns, and ulcerous skin ailments, and prevention of keloid and hypertrophic scars. Extracts of the plant have been employed to treat second- and third-degree burns. Extracts have been used topically to accelerate healing, particularly in cases of chronic postsurgical and post-trauma wounds. Extracts have been administered orally to treat stressinduced stomach and duodenal ulcers.
http://herbalinformation.awardspace.com/?cm=c&fn=centella_asiatica.

Image: 2.7

7.Chikipung

Scientific name: *Rumex vesicarius* L.

Medicinal use: Cooling agent, curing stomach heat, toothache and to check nausea diabetes & fever (Palani samy Hariprashad and Ramakrishnan; 2011). The plant has many important medicinal uses. It is a stimulant, tonic, and acts as an aphrodisiac agent (Gopal *et al*.2008).It is a wild edible plant used as a sorrel and is collected in spring time and eaten fresh (Batanouny, 1999), or cooked (Al-Quran,2009). It was considered a dietary complementary plant, since it is a rich source of β carotenes (Bélanger *et al*.2010).

Image : 2.8

8.Tak begun

Scientific name: *Solanum virginianum*

Medicinal use: Epilepsy, pain relieving, head ache, migraine, hair fall, bronchial asthma, skin problem, cough &other diseases.The plant possesses a steroidal alkaloid solasodine as the principal alkaloid along with olasonine, solamargine, beta-solamargine, solanocapine, and solanocarpidine. Dry fruit contains traces of isochlorogenenic, neochronogenic, chronogenic and caffeic acid. Crude plant extract is beneficial in bronchial asthma and non-specific cough, influenza, painful and difficult urination, bladder stones and rheumatism.Plant possesses antiurolithiatic and natriuretic, tumoricidal, anti-

allergic and anticancerousactivity (Dahake P.R.& Kohar P.Y)

Image: 2.9

9. Kochi aam pata

Scientific name:

Medicinal use: Mouth infections, GITs disorder, diabetes, diarrhea, scurvy, typhoid fever, sore throat, dysentery etc. A tea of the leaves are used to stop diarrhea, fever, and to prevent insomnia and hypertension. It is seen as a common folk remedy for diabetes. The use of leaves as medicinal herbs and as alternative medicine. It is an alternative remedy for all kinds of coughs, especially whooping cough and it is also useful for asthma, bronchitis, as well as colds.It is a good aid in every respiratory condition. The tea also makes an excellent herbal mouthwash for various gum problems. It will alleviate the pain and will bring relief to the mouth. Mango leaves tea is also good to lower high blood pressure, as well as treat restlessness in an individual. Two to three cups of tea can be added to bath water and used as a herbal bath for that invigorating and cool spirit. (http://www.medicinalherbs-4u.com/mango-leaves.html)

Image: 2.10

. Bala pata

Scientific name: Pouzolzia hirta

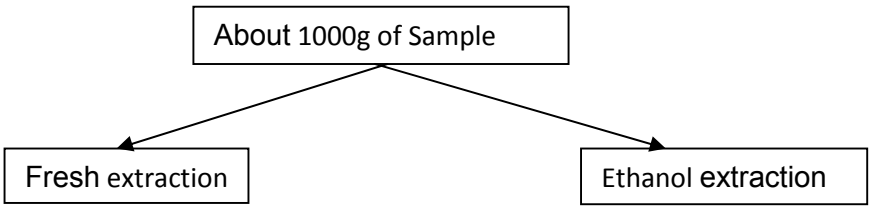
Medicinal use:
Ulcer,syphilis,gonorhoea,sores, stomachache (Yusuf *et al*.2009,medicinal value of \bangladesh). Stem and leaves are used as vegetable. It is considered by Adi tribes to increase lactation in women (R C Srivastava & Adi community, 2009).The roots, the leaves or the whole plant can be used. They are used to treat boils and abscesses, abdominal cramps in females and leucorrhoea. They are also used to treat bone dislocations and fractures. The leaves are rubbed on the throat giving a numbing sensation, and are eaten when a person has a severe cough or sore throat. The crushed leaves are applied as a poultice on ulcers (Ken Fern, 2014).

Image: 2.11

2.3 Preparation of plant extracts:

Significance of using two extraction methods (Fresh vs. ethanolic extraction) is to determine whether the substance that has the anti-bacterial and hypoglycemic effect is water- or lipid-soluble. Further experimental methods such as determining the specific water- and lipid-soluble substances could help identify which of them was able to exhibit the anti-bacterial and hypoglycemic effect

For medicinal property analysis ethnic food samples were extracted into two ways:



Fresh extraction is used to obtain water-soluble substances from organic materials.

(**i) Fresh extraction:**

- About 1000g(1Kg) of fresh sample were washed & rinsed with distilled water.
- Wiped out water using tissue paper then kept open for natural air drying.
- The samples were then cut into small pieces & blended with mortar & pastel.
- The fresh juice from the blended samples were collected by filtering them through muslin clothe then re-filtered by filter paper.
- It was then stored in to a screw cap vial and refrigerated.

Ethanolic extraction is used to obtain lipid-sobule substances from organic materials. The solvent used in ethanolic extraction is ethanol.

(ii) Ethanolic extraction:

- About 1000g (1Kg) of fresh sample was washed & rinsed with distilled water.
- The waters were wiped out using tissue paper then kept open for natural air drying.
- The samples were then cut into small pieces & blended with mortar & pastel.
- The blended samples were then soaked into 98% ethanol (w:v) 1:2 for 48 hours.
- The juice was extracted by filtering through muslin clothe then re-filtered by filter paper.
- The volume of the extracts was reduced at low temperature under vacuum with a rotator evaporator.
- It was then stored in to a screw cap vial and refrigerated.

2.4 Screening of antibacterial activity:

In tropical countries, one of main causes of approximately one-half of all deaths is infectious disease. Perhaps it is not surprising to see these statistics in developing nations, but in developed countries, infectious disease mortality rates are actually increasing, such as the United States. In 1981, death from infectious disease ranked 5th that has become the 3rd leading cause of death in 1992 which indicates an increase of 58% .It is estimated that about 8% of the deaths is caused by infectious disease (Pinner *et al*., 1996). An increase in antibiotic resistance in nosocomial and community acquired infections are other contributing factors. Furthermore, the 25–44 year old age group is most susceptible (Pinner *et al*., 1996).

These negative health trends call for a renewed interest in infectious disease in the medical and public health communities and renewed strategies on treatment and prevention. It is this last solution that would encompass the development of new antimicrobials (Fauci, 1998).

Plants based antimicrobials have enormous therapeutic potential. They are effective in the treatment of infectious diseases and mitigating many of the side effects that are often associated with synthetic antimicrobials (Murray, 1995).

Traditionally many plants are being used to prevent or cure infectious disease. Many of these plants have been investigated scientifically for antimicrobial activity and a large number of plant products have been shown to inhibit the growth of pathogenic microorganisms. The structures and modes of action of a number of these agents are distinct from those of the antibiotics in current use, indicating that cross-resistance with agents already in use may be minimal (Wikipedia, 2008)

The susceptibility of various fungi and bacteria to any agent can be ascertained by antimicrobial screening which is the first stage of antimicrobial drug research. The ability of each test sample to inhibit the *in vitro* fungal and bacterial growth can be measured by this test. This ability may be estimated by any of the following three methods (Ayafor *et al*., 1982).

- ✓ Disc diffusion method
- ✓ Serial dilution method
- ✓ Bioautographic method

But there is no standardized method for expressing the results of antimicrobial screening (Ayafor *et al*., 1982). The diameter of zone of inhibition and/or the minimum weight of extract to inhibit the growth of microorganisms are used by some investigators. However, the results can be influenced by a great number of factors viz., the extraction methods, inoculums volume, culture medium composition (Bayer *et al*., 1966), p^{H}, and incubation temperature etc.

Among the above mentioned techniques the disc diffusion (Bayer *et al*., 1966) is a widely accepted in vitro investigation for preliminary screening of test agents which may possess antimicrobial activity. The sensitivity or resistance of the microorganisms to the test materials is indicated by this quantitative or qualitative test. However, this method cannot distinct between bacteriostatic and bactericidal activity (Roland R, 1982).

Principle of Disc Diffusion Method

In this classical method, antibiotics diffuse from a confined source through the nutrient agar gel and create a concentration gradient. Dried and sterilized filter paper discs (6 mm diameter) containing the test samples of known amounts are placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic (ciprofloxacin) discs and blank discs are used as positive and negative control. These plates are kept at low temperature (4°C) for 24 hours to allow maximum diffusion of the test materials to the surrounding media (Barry, 1976). The plates are then inverted and incubated at 37°C for 24 hours for optimum growth of the organisms. The test materials having antimicrobial property inhibit microbial growth in the media surrounding the discs and thereby yield a clear, distinct area defined as zone of

inhibition. The antimicrobial activity of the test agent is then determined by measuring the diameter of zone of inhibition expressed in millimeter (Barry, 1976; Bayer *et al.,* 1966.)

In the present study the crude extracts, fractions of leaf and stem bark of *Acanthus ilicifolius* Linn. were tested for antimicrobial activity by disc diffusion method. The experiment is carried out more than once and the mean of the readings is recorded (Bayer *et al.,* 1966).

Apparatus and reagent

Whatman no. 3 filter paper discs
Nutrient Agar media
Petri-dishes
Sterile cotton
Micro pipette
Inoculating loop
Sterile forceps
Nose mask and Hand gloves
Screw cap vials
Screw cap test tubes
Autoclave
Laminar air flow hood
Spirit burner
Refrigerator
Incubator Chloroform
Ethanol
Sterile cotton

Test organisms

The bacterial strains used for the experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka. Both gram positive and gram-negative organisms were taken for the test and they are listed in table: 2.2.

Table 2.2: List of tested bacteria

Sl	Gram positive Bacteria	Strain number	Sl	Gram negetitive Bacteria	Strain number
1	*Bacillus subtilis*	BTCC 17	1	*Shigella flexneri*	BTCC 499
2	*Bacillus megateri*	BTCC 18	2	*Shigella boydii*	BTCC 498
3	*Bacillus cereus*	BTCC 19	3	*Escherichia.coli*	BTCC 482
4	*staphylococcus aureus*	BTCC 43	4	*Shigella dysenteriae type-1*	BTCC 500
5	*Sarcina lutea*	BTCC 484	5	*Salmonella B*	BTCC 495
6	*Bacillus polymyxa*	BTCC 16	6	*klebsiella psecies*	BTCC 13
			7	*Shigella sonnei*	BTCC 497
			8	*Serratia species*	BTCC 198

Culture Medium and Their Composition

The following media were used to demonstrate the antimicrobial activity and to make subculture of the test organisms.

Composition of Nutrient Agar Medium

Ingredients	Amounts
Bacto peptone	0.5 gm
Sodium chloride	0.5 gm
Bacto yeast extract	1.0 gm
Bacto agar	2.0 gm
Distilled water q.s.	100 ml
p^H	7.2 + 0.1 at 25^0C

Composition Of Nutrient Broth Medium

Ingredients	Amounts
Bacto beef extract	0.3 gm
Bacto peptone	0.5 gm
Distilled water q.s.	100 ml
p^H	7.2 $\pm$0.1 at 25^0C

Nutrient agar medium is the most frequently used and also used in the present study for testing the sensitivity of the organisms to the test materials and to prepare fresh cultures.

Preparation of the Medium

To prepare required volume of this medium, calculated amount of each of the constituents was taken in a conical flask and distilled water was added to it to make the required volume. The contents were then heated in a water bath to make a clear solution. The pH (at 25^0C) was adjusted at 7.2-7.6 using NaOH or HCl. 10 ml and 5 ml of the medium was then transferred in screw cap test tubes to prepare plates and slants respectively. The test tubes were then capped and sterilized by autoclaving at 15-lbs. pressure at 121^0C for 20 minutes. The slants were used for making fresh culture of bacteria that were in turn used for sensitivity study.

Sterilization Procedure

In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood and all types of precautions were highly maintained. UV light was switched on one hour before working in the Laminar Hood. Petri dishes and other glassware's were sterilized by autoclaving at a temperature of 121^0C and a pressure of 15-lbs/sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized.

Preparation of Subculture

In an aseptic condition under laminar air cabinet, the test organisms were transferred from the pure cultures to the agar slants with the help of a transfer loop to have fresh pure cultures. The inoculated strains were then incubated for 24 hours at 37^0C for their optimum growth. These fresh cultures were used for the sensitivity test.

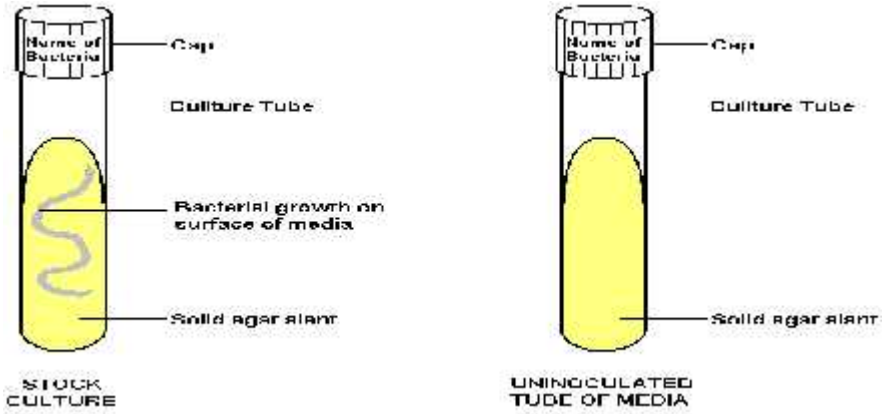
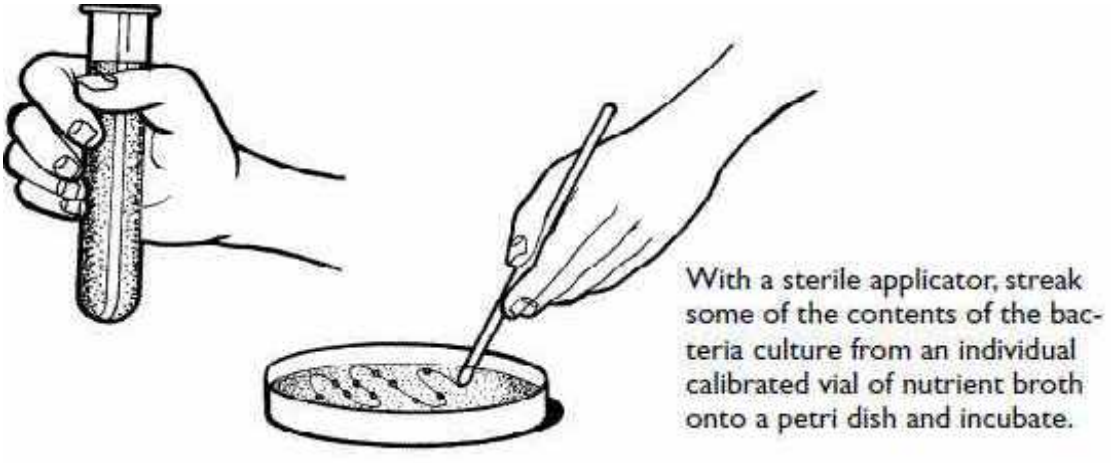


Image: 2.12 Preparation of subculture

Preparation of the Test Plate

The test organisms were transferred from the subculture to the test tubes containing about 10 ml of melted and sterilized agar medium with the help of a sterilized transfer loop in an aseptic area. The test tubes were shaken by rotation to get a uniform suspension of the organisms. The bacterial suspension was immediately transferred to the sterilized Petri dishes. The Petri dishes were rotated several times clockwise and anticlockwise to assure homogenous distribution of the test organisms in the media.

(a) (b)



(c)

Image: 2.13 Preparation of the test plates: (a) Freshly prepared culture medium, (b) Pouring culture medium to petridishes and (c) Transfer of bacterial suspension to the petridishes.

Standard Discs: These were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of the test sample. In this investigation, Ciprofloxacin (30μg/disc) standard disc was used as the reference.

Blank Discs: These were used as negative controls which ensure that the residual solvents (left over the discs even after air-drying) and the filter paper were not active themselves.

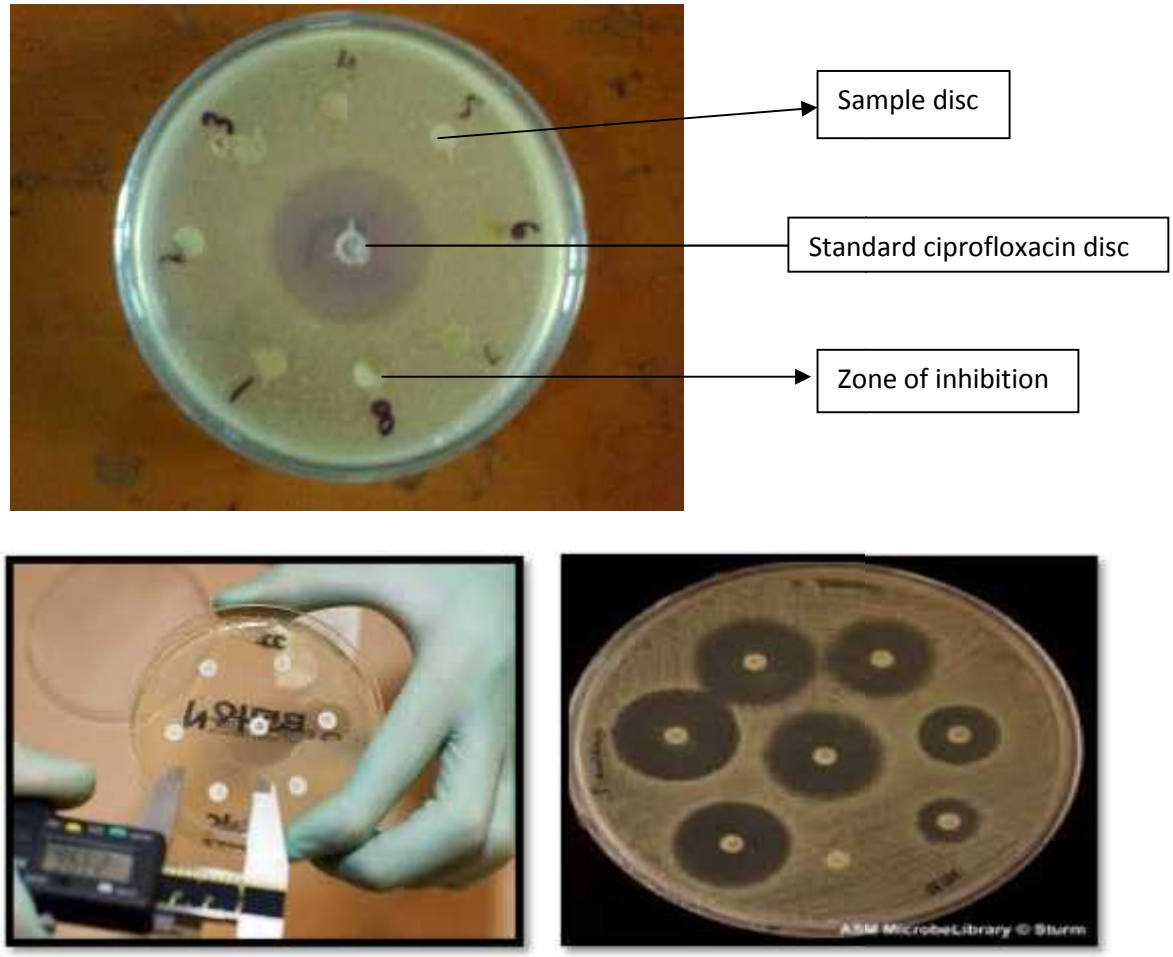
Preparation of Sample Discs with Test Sample: At first, metrical (BBL, Cocksville, USA) filter paper discs were made and taken in a blank wide mouth screw cap vial for sterilization. These discs were then sterilized properly. Measured amounts of each test sample were dissolved in specific volume of solvent to obtain the desired concentrations in an aseptic condition. Then the discs were soaked with solutions of test samples and dried.

Diffusion and Incubation

The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked spots in the agar plates pre-inoculated with test bacteria. The plates were then kept in a refrigerator at 4^0C for about 24 hours upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 37^0C for 24 hours.

Determination of antimicrobial activity by measuring the zone of inhibition

The antimicrobial potency of the test agents were measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of inhibition. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale.



Imge: 2.14 Measurement of zone of inhibition

2.5 Screening of hypoglycemic activity:

The existence of experimental animal model of a disease aids not only the understanding of the path physiology of such disease, but also the development of drugs for its treatment. According to the WHO, there are approximately 160,000 diabetics world wide, the number of diabetics has double in the last few years and is expected to double once again in the year 2025(Beretta,2001). Due to its high prevalence and potential deleterious effect on a patient physical and physiological state, diabetes is a major medical concern (Macedo *et al*, 2002). The disease remains incurable and can only be controlled with drugs(Etuk, E.U, 2010).

Chemical diabetic agent

Alloxan was purchased from Sigma Chemicals (St. Louis, Mo, USA). Oral hypoglycaemic drug- Dimerol BP 80 m tablet of Square Pharmaceutical Ltd, Bangladesh was purchased from a drug store.

Animal experimentation:

Imge: 2.15 white albino mice

Eighty four white albino mice (25-35 gm) each of both sexes were procured from the animal house of Jahangir Nagar University. They were housed at standard environmental conditions of temperature and dark/light cycle, and were fed with commercial pellet diet and drinking water. The animal were fasted for 12 hours before the experiments, but had free access to water. Ethical guidelines in animal handling and use were adequately maintained during the study.

Table 2.3: Hypoglycemic experimental groups of albino mice (n=84)

Group no	Name of plant
Groups=1	Positive control (Dimerol + normal food)
Groups=2	Normal mice (no medication + normal food)
Groups=3	Fresh extract of kochi aam pata
Groups=4	Ethanolic extract of kochi aam pata
Groups=5	Fresh extract of ozon shak
Groups=6	Ethanolic extract of ozon shak
Groups=7	Fresh extract of khoro pata
Groups=8	Ethanolic extract of khoro pata
Groups=9	Ethanolic extract of cikipung
Groups=10	Ethanolic extract of kamino
Groups=11	Ethanolic extract of mimioni
Groups=12	Ethanolic extract of yangfo

Hypoglycemic experimental procedure

Diabetes was induced in 84 fasting mice with a single dose of intraperitoneal injection of alloxan (120 mg/kg body weight). The diabetes was confirmed by estimating fasting blood glucose level of the alloxan injected mice. Fasting blood glucose level was 3.5-5.5 mmol/L. Diabetes mellitus is characterized by fasting blood glucose level ≥7.0 mmol/L.

The diabetes mice were divided into 12 groups each comprising 5 mice. Group I-X were used as experimental sets receiving respective plant extracts, group XI was used as positive control receiving Dimerol and group XII was used as negative control taking only normal diet.

The diabetes mice in experimental groups orally received each of the respective 10 plant extracts with 300 mg extract per kg body weight daily for 28 days. The mice of group XI received Dimerol (2.5mg/kg body weight) daily for the same period.

Estimation of serum glucose level

Diabetes mellitus is the most common endocrine disease which causes about 5% of all deaths globally each year and 80% of the people with diabetes live in low and middle income countries. Diabetes deaths are likely to increase by >50% in the next 10 years unless urgent action is taken (WHO, 2008). Most prevalent form of diabetes is non-insulin dependent diabetes mellitus (NIDDM/ Type II). Diabetes mellitus (DM) is a syndrome resulting from a variable interaction of hereditary and environmental factors, characterized by damaged β-cells of the pancreas and an increased risk of complications of vascular disease (Vinuthan, 2007). According to World Health Organization (WHO) projections, the prevalence of diabetes is likely to increase by 35%. Currently there are over 150 million diabetics worldwide and this is likely to increase 300 million by the year 2025 (Satyanarayana *et al.,* 2006).

Fasting blood samples of normal and diabetes mice were drawn by tail bleeding at different time intervals at 0 day, 7th day, 14th day, 21st day and 28th day for estimation of serum glucose level. After taking the blood sample into eppendorf tube, it was centrifuged at 3000 rpm for 10-15 minutes. Serum glucose level was estimated by Glucose oxidase method (Trinder, 1969; Glynn, 1991) using commercial kit [Human, Germany with the help of ELISA plate reader (Labsystem, Finland)].

Imge: 2.16

Principle for estimation of serum glucose level

The aldehyde group of glucose is oxidized by glucose oxidase to give gluconic acid and hydrogen peroxide. Hydrogen peroxide reacts under catalysis of peroxidase with phenol and 4-aminophenazone to produce a red violet quinoneimine dye as indicator.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{glucose oxidase (GOD)}} \text{gluconic acid} + H_2O_2$$

$$2\,H_2O_2 + \text{4–aminophenazone} + \text{phenol} \xrightarrow[\text{Peroxidase (POD)}]{} \text{quinoneimine} + 4\,H_2O$$

Procedure

An amount of 10 µl standard glucose solution (1mg/ml) was taken in each of 16 wells of a 96 well ELISA plate. Then 10 µl mice serum was pipette to the other pre-marked wells. It was followed by pipetting of 250 µl GOD-PAP reagent to the wells of glucose and serum. The plate containing standard glucose, mice serum with GOD-PAP reagent in the wells was incubated for 15 minutes at 37^0C. Absorbance was read at 490 nm in a ELISA plate reader. Three wells were taken for each serum sample.

Calculation

Concentration of serum glucose was calculated as follows:

$$\text{Concentration of blood glucose C} = 5.55 \times \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{ mmol/lL}$$

Statistical analysis

SPSS software package (version 17 Inc. Chicago USA) was used to analyse the data. Descriptive statistics were calculated for all variables. Values were expressed as mean ± SD. Comparison of the serum glucose levels in the seven day interval was performed by independent sample t-test (i.e. unpaired 't' test) and p values less than 0.05 were considered significant. Repeated measures of ANOVA were then used to assess for significant differences between the various time points in the subjects of both groups independently. The significance level was set at $p<0.05$.

CHAPTER 3

Results and Discusion

3.1 Antibacterial Activity Based results

Table 3.1 depicts that among 14 gram positive and gram negative bacteria, 13 of those bacterial activity remarkably inhibited by fresh extract of kamino shak. Kochi amm pata and khoropata shak also showed pretty good inhibition activity on those bacteria. However, yangfoo, ozon shak and tak bagun exhibited moderate antimicrobial activity against *on Bacillus subtilis*, *Bacillus megateri* and *Bacillus cerecus* etc. Only kamino shak, moroi shak and tak bagun showed good activity against *Shigella sonni.* Fresh extract of *moroi shak* exerted good and very little activity against *Shigella sonni and Bacillus subtilis* respectively.

Table 3.1: Antibacterial activity of selected ethnic vegetables in fresh extract

Sl no	Food sample	1	2	3	4	5	6	7	8	9	Ciprofloxacin
	Gram (+)ve	**Zone of inhibition (mm)**									
1	Bacillus subtilis	12	24	7	8	13	11	14	9	18	39
2	Bacillus megateri	11	23	-	16	17	7	13	7	22	28
3	Bacillus cereus	12	17	-	8	12	8	12	8	26	42
4	Staphylococcus aureus	7	29	-	13	7	8	9	-	17	36
5	Sercina lutea	13	32	-			11	13	-	18	33
6	Bacillus polymyxa	11	28	-	-	7	9	9	-	17	43
	Gram (-)ve										
7	Bacillus polymyxa	9	17	-	8	8	-	10	-	18	39
8	Shigella Flexneri	10	21	-	-		-	8	-	15	26
9	Shigella boydii	9	20	-	-	-	-	13	-	12	50
10	E. Coli	9	24	-	8	-		10	-	15	60
11	Shigella dysenteriae type-1	10	21	-	-	7	-	11	-	16	36
12	Selmonella B	12	16	-	-	-	-	8	-	17	14
13	Klabsiella S	10	13	-	-	-	7	14	-	18	37
14	Shigella sonni		12	15	-	-	-	-	11	-	51

Note: (-) Zone of inhibition (mm)
code of plant spacis 1=Khoropata shak *(Cissus repens Lam.),* 2=Kamino shak (*Caesalpinia digyna)*
, 3=Moroi shak(*Foeniculum vulgare*); 4=Yangfoo (*Feics benghalensis*);5=Ozon(*Spilanthes calva*) shak; 6=Mimini (*Centella assiatica*); 7=Chikipung (*Rumex vesicarius L.*); 8=Tak bagun (*Solanum virginianum*) ; 9=Kochi amm pata (*Mangifera indica)*; 10=Balapata shak(*Pouzolzia hirta*).

Table 3.2 postulated that ethanolic extract of 'kamino shak' had pretty good microbial inhibitiory activity against *Bacillus subtilis gram (+) ve* and klabsiella *S*, *Shigella sonni* gram (-) ve bacteria except E.Coli other gram (+) ve and gram (-) ve bacteria inhibited moderately by ethanolic extract of kamino shak. 'Khoropata shak' exhibited moderate activity against *Bacillus subtulis, Bacillus cereus*, *Bacillus polymyxa, Selmonella B and Klabsiella. This plant* also showed little activity against *Becillus megateri, Sercina lutea, Sercina lutea, Shigella boydii, E. Coli, Shigella dysenteriae type-1 and Shigella sonni. '*Khoropata shak' and 'Chikipung' didn't show any activity on *Staphylococcus aureus* and *Shigella dysenteriae*. Chikipung exhibited moderate activity against *Bacillus cereus*, *Bacillus megateri, Sercina lutea, Bacillus polymyxa, Shigella boydii, Shigella dysenteriae type-1* and *Shigella sonni.*

Table 3.2: Antibacterial activity of selected ethnic plant foods in ethanolic extract

Sl no	Food sample	1	2	7	Ciprofloxacin
	Gram (+)ve	**Zone of inhibition (mm)**			
1	*Bacillus subtilis*	10	15	-	39
2	*Bacillus megateri*	9	13	8	28
3	*Bacillus cereus*	12	13	11	42
4	*Staphylococcus ureus*	-	11	-	36
5	*Sercina lutea*	8	12	9	33
6	*Bacillus subtilis*	9	15	7	43
	Gram (-)ve				
7	*Bacillus polymyxa*	10	15	8	39
8	*Shigella Flexneri*	-	10	-	26
9	*Shigella boydii*	7	10	9	50
10	*E. Coli*	8	-	-	60
11	*Shigella dysenteriae type-1*	7	10	8	36
12	*Selmonella B*	11	10	-	14
13	*Klabsiella S*	10	14	8	37
14	*Shigella sonni*	8	14	-	51

Note: (-) Zone of inhibition (mm)
code of plant spacis 1=Khoropata shak *(Cissus repens Lam.),* 2=Kamino shak (*Caesalpinia digyna)* , 3=Moroi shak(*Foeniculum vulgare*); 4=Yangfoo (*Feics benghalensis*);5=Ozon(*Spilanthes calva*) shak; 6=Mimini (*Centella assiatica*); 7=Chikipung (*Rumex vesicarius L.*); 8=Tak bagun (*Solanum virginianum*) ; 9=Kochi amm pata (*Mangifera indica)*; 10=Balapata shak(*Pouzolzia hirta*).

3. 3 hypoglycemic activity based result

Glucose lowering effect of 'ethnic plant food extract' (both fresh and ethanolic) on the alloxan induced 'diabetic mice model' is outlined in Table 3.3. It was observed that all the fresh extracts (n=07) of ethnic plant foods were significantly ($P<0.05$) more active than ethanolic extract. It was also noted that all the ethnic plant food extracts (either fresh or ethanolic) were significantly ($P<0.05$) effective in lowering "blood glucose in diabetic mice" during seven day interval except 'normal mice with normal diet' and 'ethanolic extract from mimini plant'. Moreover, it was found that within 28 days, plant extracts were excelently worked on reducing the glucose level back to normal.

The rate of 'glucose level' lowering effect was found to vary with the selected plant extract.The serum glucose levels of diabetic mice for the effect of kochi aam pata (fresh extract) were 9.17±0.51 mmol/L for 0 days, 7.74±0.53 mmol/L for 7 days, 6.74±0.52 mmol/L for 14 days, 5.10±0.57 mmol/L for 21 days and 4.28±0.32 for 28 days. Similarly it was 9.69±0.49, 9.17±0.51, and 8.75±0.47, 6.56±0.42 and 5.08±0.57 mmol/L for o days, 7 days, 14 days, 21 days and 28 days respectively. Like 'fresh' and 'ethanolic extract' of 'kochi aam pata', both 'Ozon leaves' and 'khoro pata', eventually lowered the glucose level of diabetic mice. However, chilkipung, tak begun and Yangfu lowered the glucose level merginally. The rate of glucose lowering effect was found faster for 'ethanolic extract' of 'Kamino shak' (figure-10) as compared to chikipung (5.39 mmol/L), yangfu and khao pata (5.85 mmol/L). Blood glucose lowering effect of Kamino shak' (figure-10) showed normal (4.76 mmol/L) blood glucose level within 14 days and then it maintained it, as compared to chikipung (5.39 mmol/L), yangfu (7.10 mmol/L) and khao pata (5.85 mmol/L).

Table 3.3: Hypoglycemic activity of selected ethnic plants extracts (fresh and ethanolic) on alloxen induced diabetic mice model.

Medicinal plants	Serum glucose level (mmol/L) Mean±SD					
	Dose µl/day	0 day[a]	07th day[b]	14th day[c]	21st day[d]	28th day[e]
Positive control[1]	200µl	8.85±0.47	7.13±0.32	6.07±0.34	5.19±0.24	4.39±0.32**
Normal mice[2]	Normal diet	9.32±023	9.61±0.35	10.18±0.18	10.57±0.25	11.29±0.41
Kochi aam pata Fresh extract[3]	300µl	9.17±0.51	7.74±0.53	6.74±0.52	5.10±0.57	4.28±0.32**
Kochi aam pata Ethanolic extract[4]	300µl	9.69±0.49	9.17±0.51	8.75±0.47	6.56±0.42	5.08±0.57**
Ozon leaves Fresh extract[5]	300µl	9.74±0.55	8.99±0.63	7.24*±0.55	6.32*±0.34	4.20*±o.28**
Ozon leaves Ethanolic extract[6]	300µl	10.34±0.40	9.32±0.32	8.52±0.48	7.63±0.27	6.04±0.61**
Khoro pata Fresh extract[7]	300µl	9.88±0.56	8.28*±0.27	7.85*±0.25	6.76*±0.18	4.49*±o.11**
Khoro pata Ethanolic extract[8]	300µl	7.88±0.56	6.78±0.27	5.85±0.25	5.76±0.18	5.79±o.11**
Chikipung Ethanolic extract[9]	300µl	9.42±0.87	7. 37±0.16	5.39±4.17	5.21±4.04	5.42±4.20**
Kamino Ethanolic extract[10]	300µl	8.19±0.48	5.97±2.96	4.76±2.38	4.76±2.35	4.55±2.26**
Mimini Ethanolic xtract[11]	300µl	10.6±0.27	9.82±0.88	10.24±0.62	11.35±0.81	11.24±0.57
Yangfu Ethanol extic ract[12]	300µl	8.03±0.84	7.73±3.70	7.10±3.11	6.67±2.85	5.47±2.69**

**Significance p<0.05			
1[ab] :t=3.347,p=0.015**	1[ac] t=8.347,p=0.008**	1[ad] t=7.347,p=0.005**	1[ae] t=9.347,p=0.00**
2[ab] :t=0.88,p=0.873	2[ac] :t=0.98,p=0.907	2[ad] :t=0.85,p=0.77	2[ae] :t=0.76,p=1.970
3[ab] :t=7.34,p=0.015**	3[ac] :t=6.34,p=0.005**	3[ad] :t=6.34,p=0.00**	3[ae] :t=3.34,p=0.000**
4[ab] : t=6.64,p=0.015**	4[ac] : t=5.34,p=0.005**	4[ad] t=7.74,p=0.005**	4[ae] : t=9.34,p=0.008**
5[ab] : t=9.34,p=0.006**	5[ac] : t=7.14,p=0.010**	5[ad] : t=7.34,p=0.035**	5[ae] : t=7.34,p=0.005**
6[ab] : t=8.34,p=0.005**	6[ac] : t=12.77,p=0.035**	6[ad] : t=22.14,p=0.008**	6[ae] : t=218.04,p=0.027**
7[ab] :t=9.04,p=0.00**	7[ac] : t=11.34,p=0.02**	7[ad] : t=11.34,p=0.005**	7[ae] : t=8.34,p=0.000**
8[ab] : t=8.34,p=0.005**	8[ac] : t=7.22,p=0.015**	8[ad] : t=10.34,p=0.003**	8[ae] : t=13.30,p=0.005**
9[ab] : t=12.34,p=0.005**	9[ac] : t =28.34,p=0.015**	9[ad] : t=9.34,p=0.025**	9[ae] : t=11.30,p=0.000**
10[ab] : t=18.30,p=0.008**	10[ac] : t=9.33,p=0.050**	10[ad] : t=14.34,p=0.015**	10[ae] : t=8.34,p=0.003**
11[ab] : 0.73,p=2.35	11[ac] : 0.09,p=0.883	11[ad] : 0.79,p=0.973	11[ae] : 0.98,p=1.873
12[ab] : t=8.84,p=0.000**	12[ac] : t=9.34,p=0.015**	12[ad] : t=11.41,p=0.007**	12[ae] : t=9.34,p=0.000**

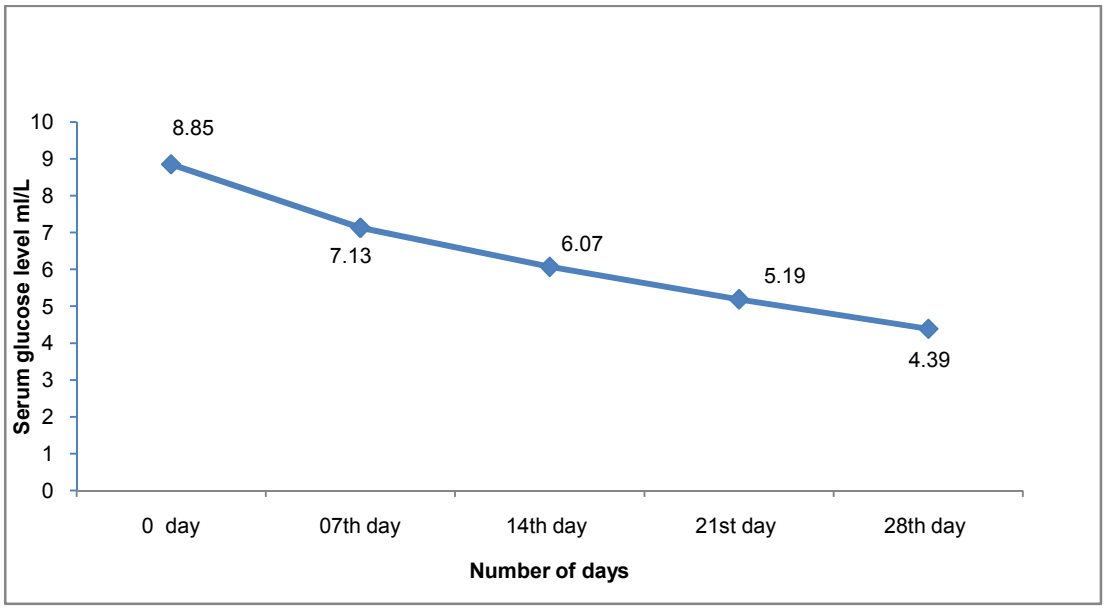


Figure 3.1: Hypoglycaemic activity of Positive control (Diamerol, 200µl/day) on serum glucose level of diabetic mice.

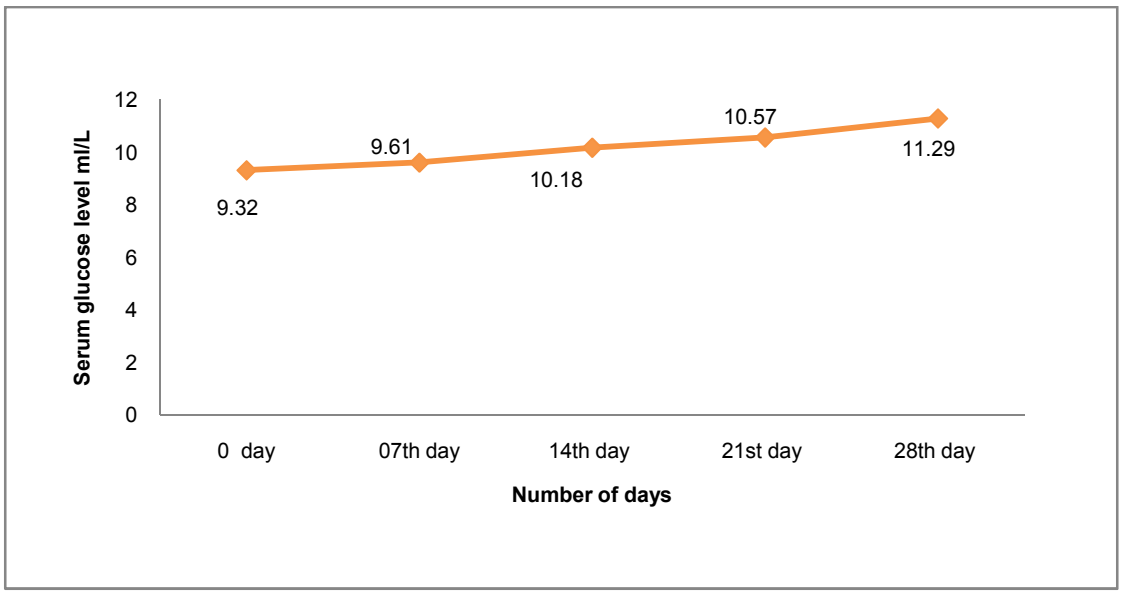


Fig. 3.2 Serum glucose level of diabetic mice giving normal diet no drug.

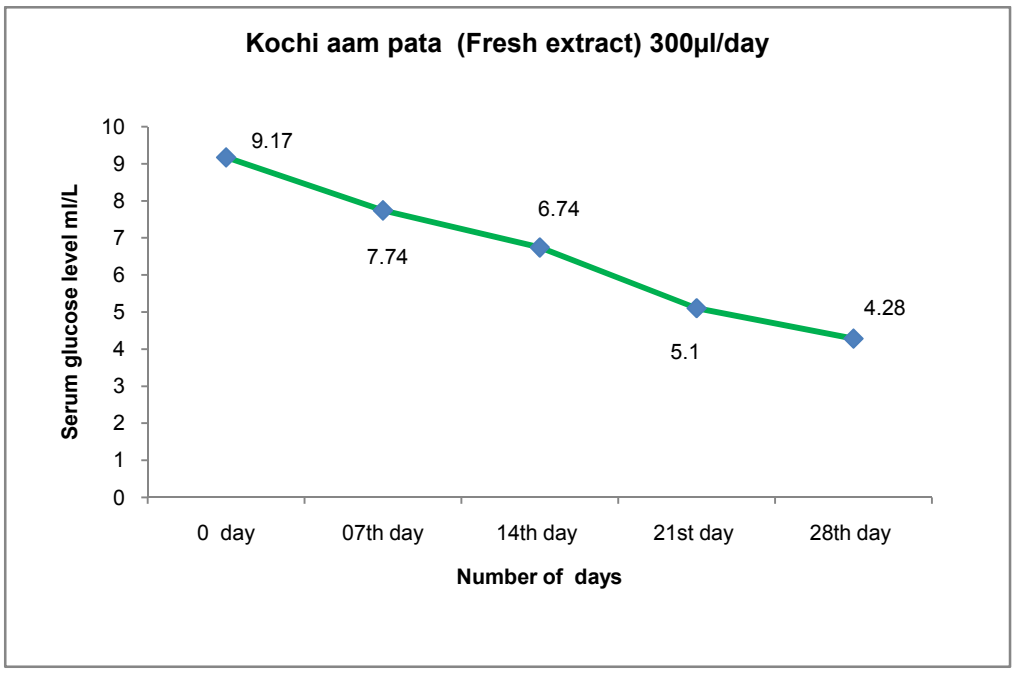


Fig 3.3: Hypoglycaemic activity of Kochi amm pata (fresh extract, 300µl/day) on serum glucose level of diabetic mice.

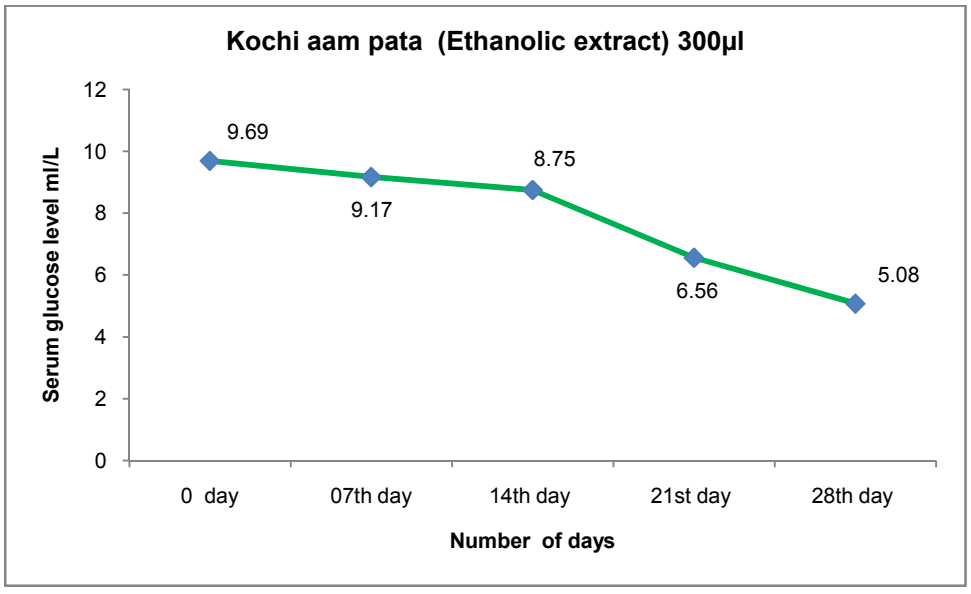


Fig 3. 4: Hypoglycaemic activity of Kochi amm pata (ehanolich extract, 300µl/day) on serum glucose level of diabetic mice.

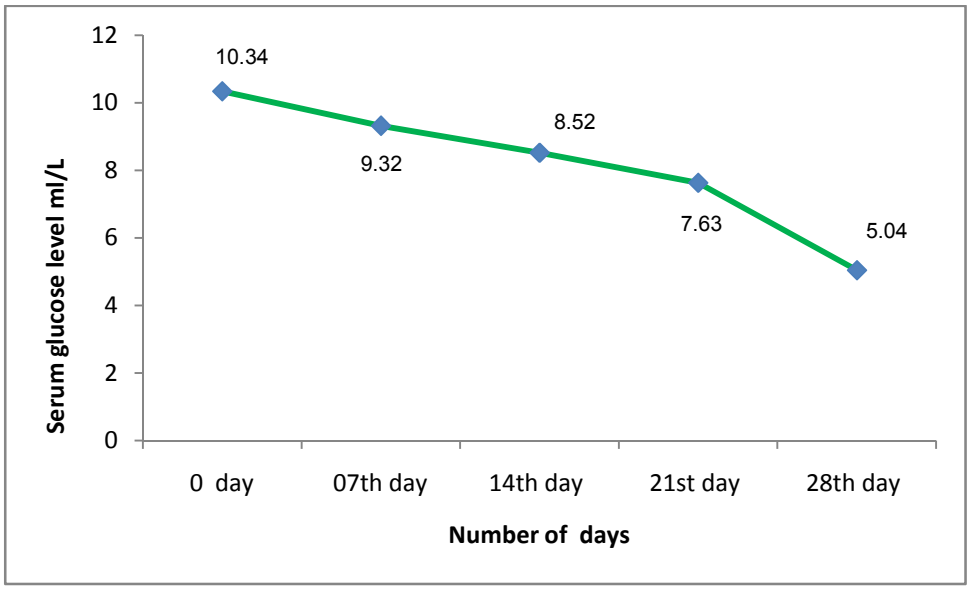


Fig 3.5: Hypoglycaemic activity of Ozon shak (fresh extract, 300µl/day) on serum glucose level of diabetic mice.

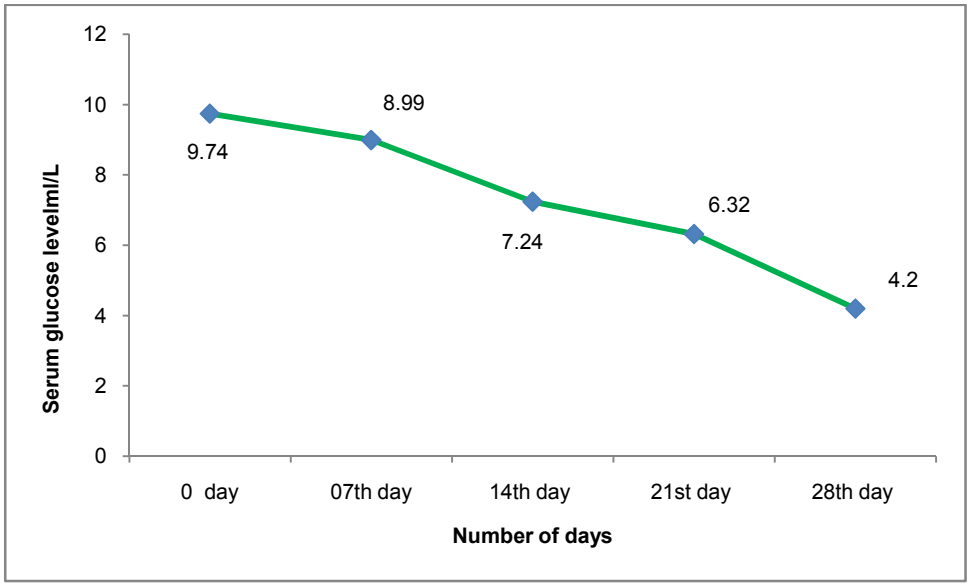


Figure- 3.6: Hypoglycaemic activity of Ozon shak (ethanolic extract 300µl/day) on serum glucose level of diabetic mice.

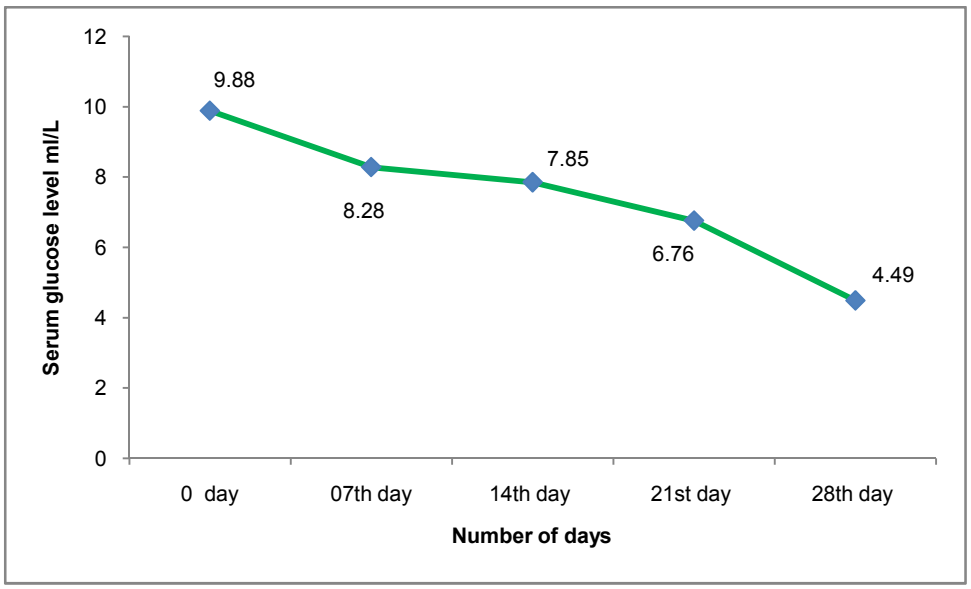


Figure 3.7: Hypoglycaemic activity of Khoro pata (Fresh extract, 300µl/day) on serum glucose level of diabetic mice.

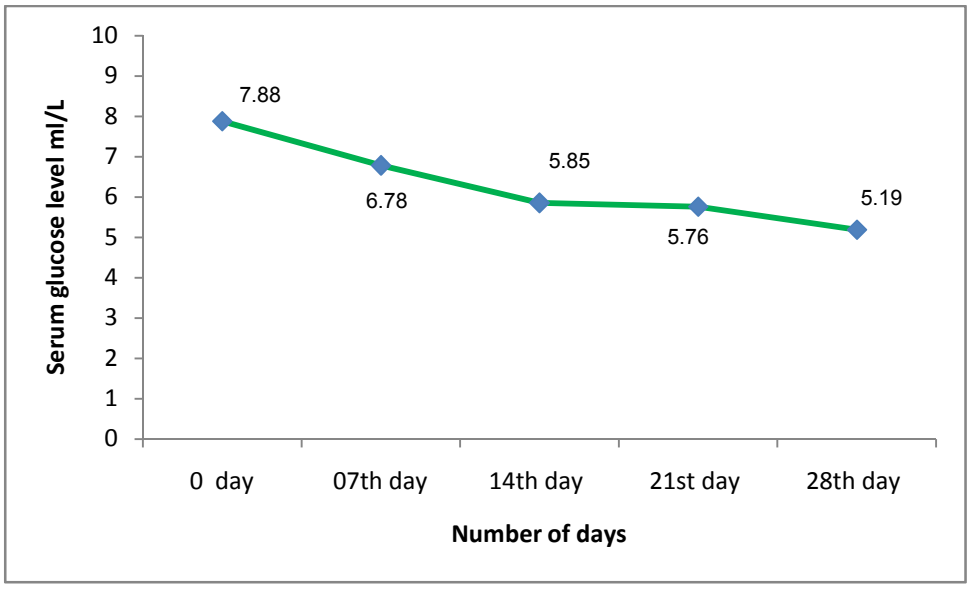


Figure 3.8: Hypoglycaemic activity of Khoro pata (Ethanolic extract, 300µl/day) on serum glucose level of diabetic mice.

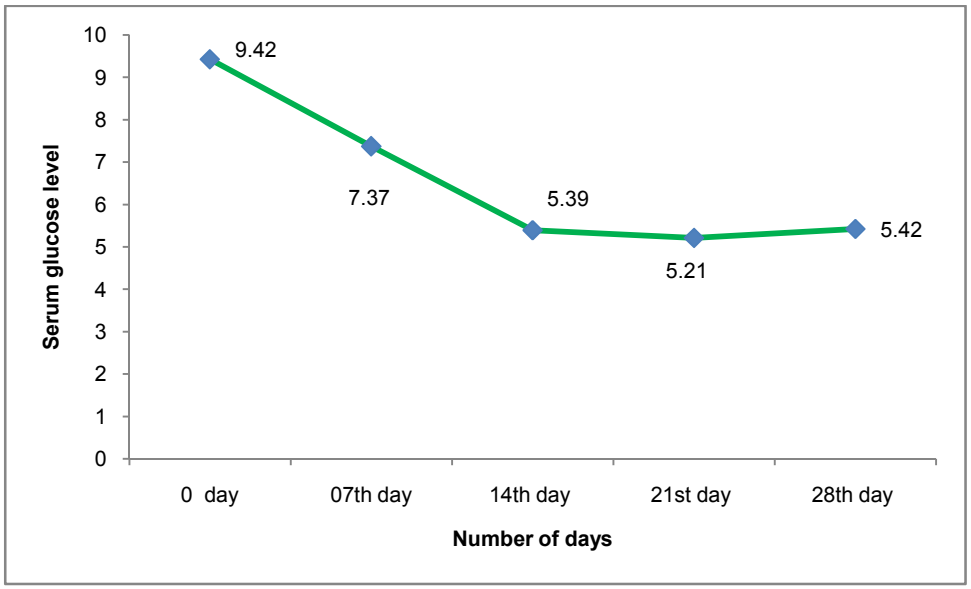


Fig 3.9: Hypoglycaemic activity of Chikipung (Ethanolic extract, 300µl/day) on serum glucose level of diabetic mice.

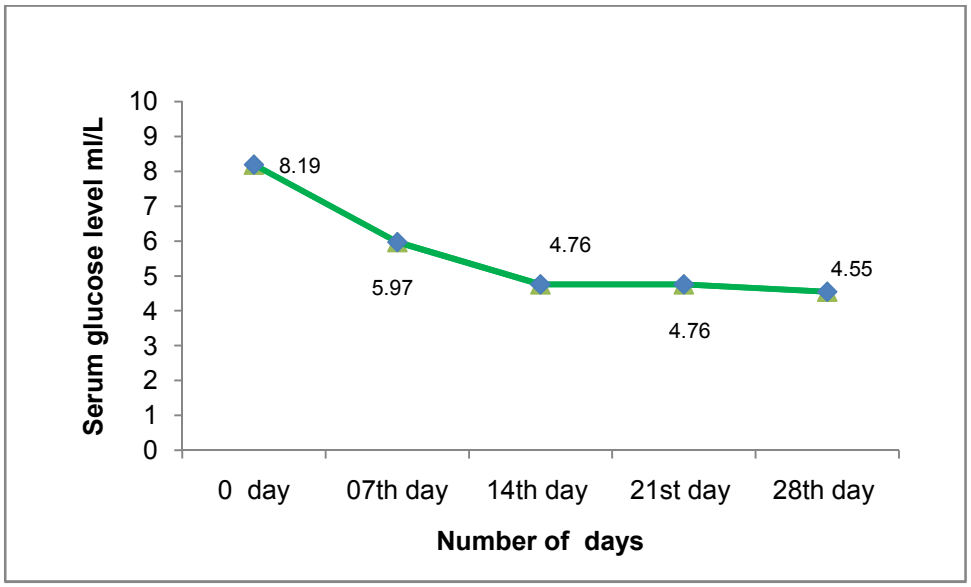


Fig 3.10: Hypoglycaemic activity of Kamino (ethanolic extract, 300µl/day) on serum glucose level of diabetic mice.

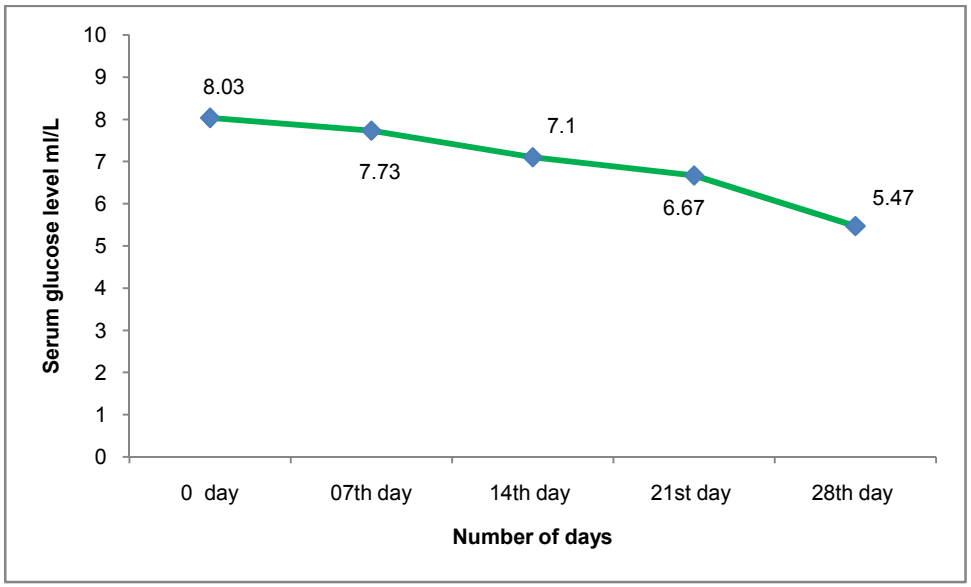


Fig 3.11: Hypoglycaemic activity of Yangfu(ethanolic extract, 300µl/day) on serum glucose level of diabetic mice.

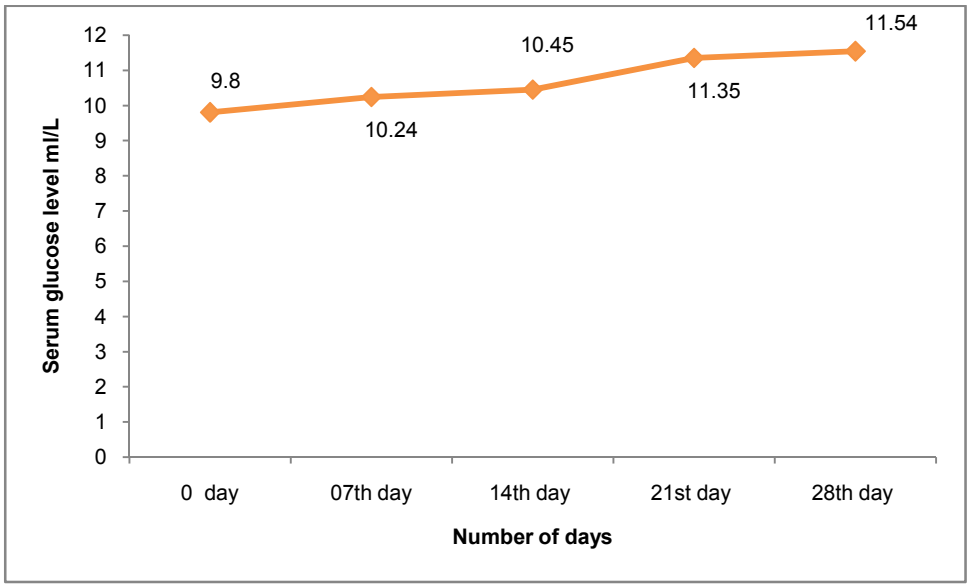


Fig 3.12: Effect of mimini (ethanolic extract, 300µl/day) on serum glucose level of diabetic mice.

3.3 Discussion

Existence and survival of human kind is impossible without plant kingdom, as plants are the primary producers and play important role in sustaining the life forms on eart. This study was designed to evaluate the selected ethnic plant foods to analyze the nutrient composition and screening of medicinal properties. In order to it, this attacheive it, this study was designed to – identify the food items consumed by ethnic groups of CHTs and analyses the selected ethnic plant foods for their nutrient composition.

The foods were identified through comprehensive food consumption survey (CFCS) and focus group discussions (FGDs). Plant foods were randomly selected from Rangamati, Khagrachori and Bandarban. The selected foods comprised 25 foods including 19 leafy and 6 non leafy vegetables.

Among 14 gram positive and gram negative bacteria, bacterial activity of thirteen bacteria remarkably inhibited by fresh extract of kamino shak. Kochi amm pata and khoropata shak also showed pretty good inhibition activity on those bacteria. However, yangfoo, ozon shak and tak bagun exhibited moderate antimicrobial activity against *on Bacillus subtilis*, *Bacillus megateri* and *Bacillus cerecus* etc. Only kamino shak, moroi shak and tak bagun showed good activity against *Shigella sonni.* Fresh extract of *moroi shak* exerted good and very little activity against *Shigella sonni and Bacillus subtilis* respectively.

Ethanolic extract of 'kamino shak' had pretty good microbial inhibitiory activity against *Bacillus subtilis gram (+) ve* and klabsiella *S*, *Shigella sonni* gram (-) ve bacteria except E.Coli other gram (+) ve and gram (-) ve bacteria inhibited moderately by ethanolic extract of kamino shak. 'Khoropata shak' exhibited moderate activity against *Bacillus subtulis, Bacillus cereus*, *Bacillus polymyxa, Selmonella B and Klabsiella.* This plant also showed little activity against *Becillus megateri, Sercina lutea, Sercina lutea, Shigella boydii, E. Coli, Shigella dysenteriae type-1 and Shigella sonni.* 'Khoropata shak' and 'Chikipung' didn't show any activity on *Staphylococcus aureus* and *Shigella dysenteriae*. Chikipung exhibited moderate activity against *Bacillus cereus*, *Bacillus megateri, Sercina lutea, Bacillus polymyxa, Shigella boydii, Shigella dysenteriae type-1* and *Shigella sonni.*

In the present study 'ethanolic extract' and 'fresh extrac't activity of the representative selective samples (n=10) against gram (-) ve and gram (+) ve bacteria was varied significantly ($P<0.05$). Kamino shak (*Caesalpinia digyna Rottler*) showed (table 3.1) high antibacterial activity, at the same time ethanolic extract of kamino shak (table 3.2) was moderately active against selected bacteria.

Earlier, it was found that hydro-alcoholic bark extract of *F. benghalensis* is effective against *Actinomyces viscosus* (Shandavi, 2010). Aerial root aqueous and hexane extracts were found to show anti-microbial activity against *Klebsiella* pneumoniae, *Staphylococcus aureus* and *Escherichia coli* strains (Rakesh and Geeta, 2010). The stem bark was reported to have antimicrobial activity but it varied as geography and environmental conditions changed (15). Methanol extract of bark exhibits good activity compared to chloroform and aqueous extracts against enterotoxigenic *E. coli* isolated from diarrheal patients (Uma, Prabhakar and Rajendran, 2009). Aqueous extracts of its bark exhibited significant antibacterial activity against *S. aureus*, *Pseudomonas aeruginosa* and *K. pneumoniae* (Gayathri and Kannabiran, 2009).

Methanol proved as the most effective solvent for extracting broad spectrum of antimicrobial compounds from plants (Vlacos *et,al.* 1996) In the present study **fresh extract** of selected ethnic foods were more effective against bacterial activity (Table 3.1) specially Khoropata shak, Kamino shak, Chikipung, Kochi aam pata showed hyper activity against most of the bacteria. Moreover, Ozon shak, Yangfoo, Mimini and Tak bagun was observed moderately effective against few of bacteria. Three **ethanolic extract** of those selected plant extracts (Table 3.2) namely Khoropata shak, Kamino shak, and Chikipung showed good activity

against selected bacteria *Bacillus subtilis*, *Bacillus megateri*, *Bacillus cerecus Bacillus polymyxa, Selmonella B and Klabsiella.*etc.

Glucose lowering effect of 'ethnic plant food extract' (both fresh and ethanolic) on the alloxan induced 'diabetic mice model' is outlined in Table 3.3. It was observed that all the fresh extracts (n=07) of ethnic plant foods were significantly ($P<0.05$) more active than ethanolic extract. It was also noted that all the ethnic plant food extracts (either fresh or ethanolic) were significantly ($P<0.05$) effective in lowering "blood glucose in diabetic mice" during seven day interval except 'normal mice with normal diet' and 'ethanolic extract from mimini plant'. Moreover, it was found that within 28 days, plant extracts were excelently worked on reducing the glucose level back to normal.

The rate of 'glucose level' lowering effect was found to vary with the selected plant extract.The serum glucose levels of diabetic mice for the effect of kochi aam pata (fresh extract) were 9.17±0.51 mmol/L for 0 days, 7.74±0.53 mmol/L for 7 days, 6.74±0.52 mmol/L for 14 days, 5.10±0.57 mmol/L for 21 days and 4.28±0.32 for 28 days. Similarly it was 9.69±0.49, 9.17±0.51, and 8.75±0.47, 6.56±0.42 and 5.08±0.57 mmol/L for o days, 7 days, 14 days, 21 days and 28 days respectively. Like 'fresh' and 'ethanolic extract' of 'kochi aam pata', both 'Ozon leaves' and 'khoro pata', eventually lowered the glucose level of diabetic mice. However, chilkipung, tak begun and Yangfu lowered the glucose level merginally. The rate of glucose lowering effect was found faster for 'ethanolic extract' of 'Kamino shak' (figure-10) as compared to chikipung, yangfu and khao pata. Blood glucose lowering effect of Kamino shak' (figure-10) showed normal (4.76 mmol/L) blood glucose level within 14 days (and then it maintained it) as compared to chikipung, yangfu and khao pata.

The hypoglycemic effect of several plants used as anti diabetic remedies has been confirmed, and the mechanisms of hypoglycemic activity of these plants are being studied. Traditional medicines from readily available medicinal plants offer great potential for the discovery of new anti-diabetic drugs (Jung, 2006). This study indicates that except one plant all of those selected plant extracts were potentially effective ($P<0.05$) in lowering of blood glucose level in diabetic mice. It was shown that selected plant extracts tested for lowerring blood glucose level on diabetic mice gradually reduced blood glucose and made it to normal level within 28 days of experimental period. Of those plant extracts ethanolic extract of Kamino, was found to be more effective, it made glucose level of diabetic mice normal (5.97 mmol/L) within 7days (figure-10). Also, both ethanolic extract of Chikipung and Khara pata was effective, made glucose level normal (5.58) within 14 days. Fresh extract of Kochi aam pata decreased the glucose level within 21 days but ethanolic extract needs 28 day to do so. But ethnolic extract of 'mimini' showed totally reverse activity compared to ethanolic extrats of kamino, Kochi aam pata, kharo pata, yangfu, chikipung and Ozon leaves.

Some of ethnic plant (Kamino shak and Kochi amm pata) extract showed high effectiveness against gram (+) ve and (-) ve bacteria. As like as Kamino ethanolic extract showed faster hypoglycemic activity in the present study. Also fresh extracts was effective for anti bacterial activity compared to ethanolic extracts of those same plants. Overall, in this study, both fresh and ethanolic extract of Kamino shak and Kochi amm pata exhibited both anti-bacteriocidal and anti-diabetic activity.

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Conclusions

This study provides a general idea on the ethnic food value that has same exception from local food value. It is revealed that ethnic people are far behind the general population in terms of socioeconomic situation, food security and health care access facilities. Special care should be taken to address these problems. The study indicates that the ethnic people are blessed with marvelous diversity of leafy and non leafy vegetable plants. The phenomena observed in the study area correspond to the severe dependence on their surrounding forest lands. The nutritional content of lesser known ethnic vegetables grown in Bangladesh is appreciable and are the good sources of vitamin C, beta-carotene, minerals and dietary fiber whilst their energy, total fat, protein and calcium contents are generally low. Although ethnic plant foods contain sufficient amount of micronutrients. Present study found that these marvelous non leafy and leafy vegetables and their extracts work as antibacterial and hypoglycemic activity.

This study will help people to know the nutrient content and fulfill the daily requirement by low-price local food items. If regular intake of ethnic vegetables could be improved, then it is possible to alleviate the *hidden hunger*-micronutrient deficiency in Bangladesh.

KEY FINDINGS

Nutrient composition of ethnic plant foods analyzed

- ***Micronutrients:*** Vitamin-B**,** vitamin C was found high in Moroishak, content of carotenoids and carotene profile found good in Mrolaping and missayanu.
- ***Minerals:*** Copper, zinc was high in Forash Dal. Iron, calcium, and phosphorous were high in Kiokokro (sheola).
- ***Proximate nutrients:*** protein, fat, ash, dietary fiber, carbohydrate and energy.
- In over all study of ethnic food composition 'Farash Dal' found as an excilent food because of it's composition. Also other non leafy and leafy vegetables contented good nutritional value compared to local food.

Medicinal Screening findings

- ***Anti bacterial activity:*** Ethnic plants like Kaminoshak and Kochi ammpata both extract showed high effectiveness against gram (+)ve and (-)ve bacteria. As like as Kaminoethanolic extract showed faster hypoglycemic activity in the present study. Also fresh extracts was effective for anti bacterial activity compared to ethanolic extracts of those same plants.
- ***Anti diabetic activity:*** Within selected plant extracts Kamino ethanolic extract, was found to be more effective, it made glucose level normal within 7 days. Also Chikipungethanolic extract and Khaoro pata ethanolic extract both was as well as effective, made glucose level normal within 14 days.

Policy recommendations

Based on the findings, the following policy recommendations have been suggested

- Ethnic foods especially the plant foods need to be popularized through awareness program to the other parts of Bangladesh. Households can get an avenue for income generation through commercialization of these plant species. It may contribute to some extent to achieve food security and food diversity.
- There is need to update the current FCT incorporating nutrient composition of more ethnic foods. Finding of this work would enrich the existing FCT.
- Nutrient composition of ethnic plant foods could help agriculture, food and health policy to improve the supply and demand of ethnic food source.
- The Govt. and NGOs need to take strong initiative for cultivation and conservation of ethnic foods in plane land and also in home gardens.
- The medicinal properties of the ethnic plants need to be explored to introduce into daily food menu to get health benefit.

Future research

The ethnic people, who maintains their identity in the midst of a relatively modern people, needs to enjoy the belongings among the same tribes living in interior areas (Basu, 1993). This has been attributed to better utilization of available natural resources. It is suggested that an in depth research is needed for extensive evaluation of their food habit and analysis of nutrient composition of a wide number of ethnic foods.