CYTOTAXONOMY OF MEDICINALLY IMPORTANT *PHYLLANTHUS* SPP. IN BANGLADESH

PH.D. THESIS

BY

MD. SHAHIDUR RAHMAN

REGISTRATION NO. 26/2016-17
CYTOGENETICS LABORATORY
DEPARTMENT OF BOTANY
UNIVERSITY OF DHAKA
DHAKA 1000
BANGLADESH

DECEMBER 2019

CYTOTAXONOMY OF MEDICINALLY IMPORTANT *PHYLLANTHUS* SPP. IN BANGLADESH

A DISSERTATION
SUBMITTED TO THE UNIVERSITY OF DHAKA
IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
BOTANY
(CYTOTAXONOMY)

BY
MD. SHAHIDUR RAHMAN

REGISTRATION NO. 26/2016-17
CYTOGENETICS LABORATORY
DEPARTMENT OF BOTANY
UNIVERSITY OF DHAKA
DHAKA 1000
BANGLADESH

DECEMBER 2019

Dedicated

to

my respected teacher

Dr. Sheikh Shamimul Alam

ACKNOWLEDGEMENT

At first my heartiest thanks to the most gracious and merciful almighty Allah for providing me patience, strength, energy and courage to complete my research work within due time.

Foremost, I feel pleasure to express my immense respect and heartfelt gratitude to Late Professor Dr. Sheikh Shamimul Alam, Cytogenetics Laboratory, Department of Botany, University of Dhaka, Bangladesh who died on 18 December in 2017, for his intellectual guidance, constructive criticism, and untiring supervision during my Ph.D. research work and inspiration to work further in Plant Cytogenetics. I am always grateful to him for giving me a chance to work under his generous supervision. I am lucky that I got the opportunity to work with my favourite teacher.

Sincere and heartiest gratitude to Professor Dr. Md. Abul Hassan, Department of Botany, University of Dhaka, for the continuous support, patience, motivation, enthusiasm, immense knowledge and encouragement to complete the research within limited time frame. I express my sincere and heartiest gratitude to Dr. Syeda Sharmeen Sultana, Associate Professor, Cytogenetics Laboratory, Department of Botany, University of Dhaka, for her help, invaluable suggestions, proper guidance and warm encouragement to complete the study. I am also grateful to them for giving me a chance to work under their generous supervision.

I am thankful to University Grants Commission (UGC) of Bangladesh for financial assistance under the Ph.D. programme.

I like to express my sincere thanks to the Bangladesh Open University, Gazipur for approving study leave during the research period.

I would like to express my sincere and heartiest gratitude to present chairman Professor Dr. Rakha Hari Sarker, former chairman Professor Dr. Abdul Bashar, Department of Botany, University of Dhaka, for providing the necessary facilities of the department. I am also thankful to Professor Dr. M. Imdadul Hoque, Dean, Faculty of Biological Sciences, University of Dhaka for his encouragement during the course of my research work.

I am grateful to Professor Dr. Masuda Khatun and Professor Dr. Rokeya Begum, Department of Botany, University of Dhaka, Sheikh Tahmina Awal, Assistant Professor, Institute of Education and Research, University of Dhaka and Dr. Mohammad Moniruzzaman, Principle Scientific Officer, Bangladesh Council of Scientific and Industrial Research, Dhanmondi, Dhaka, for their generous support, help, invaluable suggestions and continuous encouragement during the course of my research work.

I express my sincere and heartiest gratitude to all of my respected teachers, Department of Botany, University of Dhaka, for their valuable suggestions, support and encouragement during my research work.

I express my heartiest gratitude to Mahin Afroz, Assistant Professor, Department of Botany, University of Barisal, Chandan Kumar Dash, Lecturer, Department of Botany, University of Dhaka, for their help, inspiration, immense assistance to my work and the warm encouragement to complete the study.

Special thanks are due to all of the students of the Cytogenetics Laboratory, Department of Botany, University of Dhaka for their generous co-operation.

Sincere thanks to all the staffs of Department of Botany, University of Dhaka specially Mr. Azam, former Laboratory assistant, Mr. Shawkat Hossain, Laboratory attendent and Mr. Shuvas Roy, gardener, Department of Botany, University of Dhaka for providing all the garden facilities and assistance.

During the present investigation, I met many generous peoples who were very friendly and supportive. I like to thank them from the core of my heart.

I would like to express my heartfelt gratitude to my respected father Md. Mazibar Rahman, mother Mrs. Saleha Begum and beloved brother Md. Habibur Rahman to keep me in their prayer and for co-operation during my research. Special thanks are due to my beloved wife Khadiza Akter Parvin and my beautiful daughters Shreoshi Rahman and Preoshi Rahman.

The Author

CONTENTS

ABSTRACT

Abstract

A combination of taxonomical analysis, cytogenetical method and molecular technique had done for authentic characterization of medicinally important five *Phyllanthus* L. species *viz*. *P. acidus* (L.) Skeels, *P. emblica* L. (small fruit form), *P. emblica* L. (large fruit form), *P. niruri* L., *P. reticulatus* Poir. and *P. urinaria* L. They were analyzed with several taxonomical parameters such as branching pattern, morphology of bark, leaves, flowers, fruits, seeds, flowering and fruiting time. Interphase nuclei and prophase chromosomes of these species showed notable variation and displayed the presence and distribution of both constitutive and facultative heterochromatin. *Phyllanthus* species, as studied here, showed variation in somatic chromosome counts such as 2n=2x=26 in *P. acidus*, *P. niruri* and *P. reticulatus*, 2n=6x=48 in *P. urinaria* and 2n=10x=100 in *P. emblica* (both small and large fruit forms), represented with multi-basic chromosome number. The karyomorphology of these *Phyllanthus* species showed that these species were more or less primitive in nature of which *P. acidus*, *P. niruri* and *P. reticulatus* were comparatively advanced than the others in evolutionary point of view. Formation of bivalent in addition with several multivalent at diakinesis and metaphase-I of *P. emblica* (both forms) indicated that this species has the possibility of being auto-allopolyploid or segmental-allopolyploid that are going through the process of diplodization. These five *Phyllanthus* species showed distinctiveness in their number, position, distribution and the percentage of GC- and AT-rich repetitive segments that allowed to analyze the reshuffled banded regions with the help of CMA and DAPI-banding method. RAPD analysis of five *Phyllanthus* spp. displayed that *P. emblica* and *P. reticulatus* were closely related whereas *P. acidus* and *P. emblica* were genetically distantly related. The two forms (small and large fruit forms) of *P. emblica* showed similarity in most important taxonomic parameters and somatic chromosome numbers and dissimilarity in some morphological characters.

CHAPTER ONE: INTRODUCTION

CHAPTER ONE: INTRODUCTION

The genus *Phyllanthus* L. commonly called "carry me seed", "stonebreaker", "windbreaker", "gulf leaf flower" or "gala of wind", belongs to Euphorbiaceae family (Bharatiya 1992). This tremendous medicinally important genus is distributed in almost all tropical and subtropical countries of Asia, Africa and America (Burkill 1996, Abdulla *et al.* 2010). The members of this genus have a remarkable diversity of growth forms including annual and perennial herbs, shrubs, trees, climbers, floating aquatics and succulents (Abdulla *et al*. 2010).

Phyllanthus L. comprises of about 1200 species of which eleven species reported from Bangladesh *viz. P. acidus* (L.) Skeels, *P. boeobatryoides* Wall., *P. emblica* L., *P. maderaspatensis* L., *P. niruri* L., *P. pendulus* Roxb., *P. reticulatus* Poir., *P. roxburghii* Muell. -Arg., *P. sikkimensis* Muell. -Arg., *P. urinaria* L. and *P. virgatus* Forst. f. (Ahmed *et al*. 2008). Among these, five medicinally important species *viz. P. acidus* (L.) Skeels*, P. emblica* L.*, P. niruri* L.*, P. reticulatus* Poir. and *P. urinaria* L. are most commonly available in Bangladesh (Abdulla *et al.* 2010).

This genus has a long history in herbal medicine systems worldwide. The genus is of medicinal importance for numerous ailments (Tables 1-5). Moreover, these plants have a vast range of medicinal uses because of containing different types of secondary metabolites and chemical constituents (Table 6).

However, Euphorbiaceae is recognized as one of the largest family among the dicot with about 8,000 species under 300 genera (Perry 1978). This family represents such a vegetative and floral diversity that morphometric investigation of this group has always been controversial because the taxonomic data of these plants have not been made systematically (Perry 1943, Raghavan 1957, Janaki Ammal and Raghavan 1958, Raghavan and Arora 1958, Thombre 1959). In contrast, such large families like Asteraceae, Fabaceae and Poaceae, the Euphorbiaceae have been relatively little studied cytologically. Most of the studies have been focused in taxonomical and pharmacological aspects of different species of *Phyllanthus* L. (Tables 1-6). Despite these contributions, the chromosomal information is still scarce. Report on multiple basic chromosome numbers, different ploidy level or cytotypes in the genus *Phyllanthus* L. also created a topic of interest to plant cytogeneticists (Table 7).

Table 1. Traditional and pharmacological uses of *Phyllanthus acidus* (L.) Skeels

Medicinal properties	References
Astringent, appetite, tonic to liver to enrich the blood, thirst, constipation, biliousness, vomiting, emetic, purgative and piles, bronchitis, urinary concretions, analgesic, antipyretic and antirheumatic, small pox, jaundice, utricaria, gum infection, liver disease, blood purifier	Habib *et al*. 2011, Jain and Singhai 2011
Fever, respiratory disorders, inflammation, diabetes, several pains etc. and also helpful to cure cough, psoriasis, sudorific, to improve eyesight and memory	Habib *et al*. 2011
Cathartic, diaphoretic, sudorific, demulcent in cases of gonorrhea, stomachic, asthma, drastic purgative, coughs and headache, cure skin diseases itching	Mackeen *et al*. 1997, Ghosh *et al*. 2015
Antioxidant activity	Jain and Singhai 2011, Moniruzzaman *et al*. 2015
Hepatoprotective activity	Jain *et al*. 2011, Jain and Singhai 2011
Antidiabetic activity	Ghosh *et al*. 2015
Anticancer activity	Vongvanich *et al*. 2000
Anticystic fibrosis activity	Sousa *et al*. 2007
Hypotensive and hypolipidemic activity	Leeya *et al*. 2010, Maruthappan and Shree 2010
Antimicrobial activity	Melendez and Capriles 2006, Jagessar *et al*. 2008
Antiplasmodial	Bagavan *et al*. 2011

Table 2. Traditional and pharmacological uses of *Phyllanthus emblica* L.

Medicinal properties	References
Inflammation, lithiasis, fever, malaria, hepatitis and gonorrhea, liver diseases	Omulokoli *et al*. 1997, Tona *et al*. 1999, Adil *et al.* 2010, Nain *et al*. 2012
Astringent, cough, gastrointestinal chronic diseases, gastropathy, acrid, aperient, antacid, carminative, cooling, diuretic, refrigerant, sour, stomachic, tonic, laxative, asthma, bronchitis, biliousness, bacillary dysentery, dyspepsia, heart diseases, jaundice, thirst and piles and anemia	Kirtikar and Basu 1975, Baliga 2010
Human scurvy	Chopra *et al*. 1956
Antioxidant	Kumaran and Karunakaran 2006, Kumar *et al*. 2006, Pozharitskaya *et al*. 2007, Nampoothiri *et al*. 2011, Reddy *et al*. 2011
Anticancer	Ngamkitidechakul *et al*. 2010, Baliga and Dsouza 2011, Ismail *et al*. 2012, Mahata *et al*. 2013, Tang *et al*. 2013, Ferlay *et al*. 2015, Zhao *et al*. 2015
Antibacterial	Saeed and Tariq 2007, Srikumar *et al*. 2007, Saini *et al*. 2008, Rahman *et al*. 2009
Antiviral	El-Mekkawys *et al*. 1995, Unander 1995, Xiang *et al.* 2011, Jiang *et al*. 2014
Antifungal	Rao and Siddique 1964
Antitumor	Praveenkumar *et al*. 1994a, Praveenkumar *et al*. 1994b, Rekha *et al*. 2001
Anti-inflammatory	Asmawi *et al*. 1993, Ihantola-Vormisto*et al*. 1997, Muthuraman *et al*. 2010, Nicolis *et al*. 2008
Antidiabetic	Sabu and Kuttan 2002, Suryanarayana *et al*. 2004, Suryanarayana *et al*. 2007, Mehta *et al*. 2009, Chen *et al*. 2011, Nampoothiri *et al*. 2011, Tiwari *et al*. 2011, Nain *et al*. 2012, Kalekar *et al*. 2013
Antiulcer	Bandyopadhyay *et al*. 2000, Bafna and Balaraman 2005, Bhattacharya *et al*. 2007, Chatterjee *et al*. 2011, Chatterjee *et al.* 2012
Cytotoxic	Sumantran *et al*. 2007, Pinmai *et al*. 2008, Pinmai *et al*. 2010
Wound healing activities	Sumitra *et al*. 2009, Bandyopadhyay *et al*. 2011
Antitussive activity	Nosál'ová *et al*. 2003

Table 3. Traditional and pharmacological uses of *Phyllanthus niruri* L.

Medicinal properties	References
Diuretic in gonorrhea and other ailments of genito-urinary tract, jaundice, dysentery, bruises and wounds, scabby infections, tonic, sores, purgative	Kirtikar *et al*. 1935
Astringent, cooling, deobstruent, stomachic, febrifuge and antiseptic and also used for the treatment of leucorrhoea, menorrhagia, dyspepsia, colic, diarrhea, dysentery, dropsy, diabetes, otitis, swelling, skin ulcers, gastrointestinal disturbances, ringworms	Oluwafemi and Debiri 2008
Anticancer activity	Hari *et al*. 2006, Lee *et al*. 2011
Antioxidant activity	Lim and Murtijaya 2007, Patel *et al*. 2011
Hepatoprotective effect	Syamasundar *et al*. 1985, Thyagarajan *et al*. 1988, Chatterjee *et al*. 2006
HIV replication inhibition	Naik *et al*. 2003
Lipid lowering activity	Chandra 2000, Khanna *et al*. 2002
Antidiabetic activity	Srividya and Periwal 1995, Raphael and Sabu 2000
Antimalarial activity	Neraliya and Gaur 2004, Subeki *et al*. 2005
Antispasmodic activity	Grewal 1984
Analgesic activity	Santos 1994
Chromosome aberration Inhibition	Holdsworth and Wamoi 1982

Table 4. Traditional and pharmacological uses of *Phyllanthus reticulatus* Poir.

Medicinal properties	References
Oral disorders, astringent and inflammation, blood diseases, alterative, attenuate, diuretic, cooling, bleeding gums	Kirtikar *et al*. 1935
Smallpox, syphilis, bleeding gums, dysmenorrheal, gonorrhea, intestinal hemorrhage and anemia, muscle spasms, dysmenorrhea, diarrhea with anal bleeding, infertility, sore eyes	Kirtikar *et al*. 1935, Rao and Henry 1996
Malaria	Ghani 2003
Antidiabetic activity	Kumar *et al*. 2008
Immunomodulatory activity	Agarwal and Singh 1999
Antiplasmodial activity	Omulokoli *et al*. 1997
Hypocholesterolemic activity	Anuradha and Ravikumar 2001,
Antimicrobial activity	Begum *et al*. 2006
Cytotoxic activity	Das *et al*. 2006
Hepatoprotective activity	Shruthi *et al*. 2010,
Antibacterial activity	Rahmatullah *et al*. 2010
Antinociceptive and antihyperglycemic activity	Saha *et al.* 2007
Antioxidant activity	Ram *et al*. 2008, Shruthi and Ramachandra 2011
Antiviral activity	Rahmatullah *et al*. 2010

Table 5. Traditional and pharmacological uses of *Phyllanthus urinaria* L.

Medicinal properties	References
Diuretic, sudorific, depurative, emmenagogue, dropsical affections, gonorrhea and other genito-urinary problems, acrid, sour and cooling, thirst, bronchitis, leprosy, anemia, urinary discharges, anuria, hiccup, biliousness, asthma, fish poison	Chopra *et al*. 1956
Astringent, tonic, febrifuge, liver complaint, diarrhea, insomniac children	Kirtikar *et al*. 1935
Kidney and urinary bladder disturbances, intestinal infections, diabetes and hepatitis B	Calixto *et al*. 1998
Painful disorder, jaundice, enteritis, diarrhea, dropsy and inflammation, jaundice and diabetes	Huang *et al*. 2003, Fang *et al*. 2008, Lin *et al*. 2008, Poompachece and Chudapongse 2011
Antioxidant activity	Lin *et al*. 2008
Anticancer activity	Huang *et al*. 2004, Rajkapoor *et al*. 2007, Lai *et al*. 2008, Huang *et al*. 2010, Ngamkitidechakul *et al*. 2010, Tang *et al*. 2010, Bagalkotkar *et al*. 2011, Lee *et al*. 2011, Ismail *et al*. 2012, Ramasamy *et al*. 2012, Tang *et al*. 2013, Huang *et al*. 2014, Zhao *et al*. 2015
Immunomodulatory activity	Jantan *et al*. 2014
Antibacterial activity	Marshall and Warren 1984, Marshall *et al*. 1985, Leunk *et al*. 1988, Nomura *et al*. 1991, Catrenich and Chestnut 1992, Blaser 1993, Parsonnet *et al*. 1994, Telford *et al*. 1994, Censini *et al*. 1996, Cover and Blaser 1996, Lai *et al*. 2008
Antiviral activity	Higashino *et al*. 1992, Dias *et al*. 1995, Paulino *et al*. 1996, Zhou *et al*. 1997, Cooper 2001, Giridharan *et al*. 2002, Lee *et al*. 2006, Saddi *et al*. 2007, Fang *et al*. 2008, Mukhtar *et al*. 2008, Shen *et al*. 2008, Zhang *et al*. 2008, Rajbhandari *et al*. 2009
Antimalarial activity	Bruce-Chwatt 1985, Oduola *et al*. 1992, Wongsrichanalai *et al*. 1992
Antiangiogenic activity	Ingber *et al*. 1990, Hanahan and Folkman 1996, Itoh *et al*. 1998, Carmeliet and Jain 2000, Seftor *et al*. 2001, Silvestre *et al*. 2001, Huang *et al*. 2006, 2011
Antitumor activity	Huang *et al*. 2006
Antiinflammatory activity	Fang *et al*. 2008
Antidiabetic activity	Lans 2006

Table 6. Phytochemicals obtained from five *Phyllanthus* L. species

Name of species	Name of Phytochemicals	References
P. acidus	Cyclohexanone, terpenoids, phenols alchohol, aldehide, fatty acid, pyrane	Sengupta and Mukhopad 1966, Vongvanich *et al*. 2000
P. emblica	Alkaloids, benzenoids, Carbohydrates, flavonoids furanlactone, phenylpropanoid, lignans, sterols, terpene, tannins, hydrolysable tannins, phenols and some other derivatives	Laumas and Seshardi 1958, Theresa *et al*. 1967, Hui and Sung 1968, Shah and Hamid 1968, Subramanian *et al*. 1971, Desai *et al*. 1977, Basa and Srinivasulu 1987, Zhang *et al*. 2004, Yang *et al*. 2007, Nicolis *et al*. 2008, Luo *et al*. 2009, Luo *et al*. 2011, Liu *et al*. 2012, She *et al*. 2013, Zhang *et al*. 2014
P. niruri	Alkaloids, phenylpropanoid, terpenoids, tannins, phenols	Wei and Pan 2002, Ahmad and Alam 2003, Wei *et al*. 2004, Hossain and Salehuddin 2006, Than *et al*. 2006, Boeira *et al*. 2011, Gambari *et al*. 2012, Sun 2012, Zhou *et al*. 2012, Liu *et al*. 2014, Ooi *et al*. 2015
P. reticulatus	Alkaloid, flavonoid, phenol, phytallate, sterol, phenylpropanoid, terpenoids, tannins	Joshi *et al*. 1981, Ghani 2003, Lam *et al*. 2007, Lan *et al*. 2010, Ma *et al*. 2012
P. urinaria	Acid, alkanol, coumarin, flavonoid, lignins, phenol, phytallate, sterol, tannins, terpenoids, simple, phenylpropanoids	Miyoshi *et al.* 1987, Satyan *et al*. 1995, Chen *et al*. 1999, Chang *et al*. 2003, Wang and Lee 2005, Tran *et al*. 2007, Fang *et al*. 2008, Cheng *et al.* 2011, Pang *et al*. 2011, Shanker *et al*. 2011, Hu *et al*. 2014, Yeo *et al*. 2015

Table 7. Previous chromosomal count database of five *Phyllanthus* L. species

Name of species	Chromosome count database
P. acidus	2n=26 (Raghavan 1959, Webster and Ellis 1962, Bancilhon 1971, Sarkar *et al*. 1976, Krishnappa and Reshme 1980, Kothari *et al*. 1981) 2n=28 (Thombre 1959)
P. emblica	2n=28 (Perry 1943) 2n=52 (Chatha and Bir 1987) 2n=98 (Raghavan 1957, Janaki Ammal and Raghavan 1958, Mehra 1972, Mehra and Hans 1972, Hans 1973, Mehra 1976, Ohri and Kumar 1986) 2n=104 (Bancilhon 1971, Sarkar and Datta 1980, Gill *et al*. 1981) 2n=98-104 (Janaki Ammal and Raghavan 1958)
P. niruri	2n=14 (Hsu 1967, Bancilhon 1971) 2n=26 (Webster and Ellis 1962, Chuang *et al*. 1963, Champault 1970, Krishnappa and Reshme 1980, Kothari *et al*. 1981, Trivedi *et al*. 1986) 2n=28 (Sharma and Jash 1958, Bancilhon 1971) 2n= 36 (Bancilhon 1971)
P. reticulatus	2n=26 (Webster and Ellis 1962, Mehra and Hans 1969, Bancilhon 1971, Brunel 1976, Bedi *et al*. 1980, Krishnappa and Reshme 1980, Sarkar and Datta 1980 2n=28 (Sharma and Jash 1958)
P. urinaria	2n=14 (Chuang *et al*. 1963) 2n=24 (Kothari *et al*. 1981) 2n=26 (Huang *et al*. 1988) 2n=28 (Sharma and Jash 1958) 2n=48 (Bancilhon 1971) 2n=50 (Rossignol *et al*. 1987) 2n=52 (Janaki Ammal and Raghavan 1958, Raghavan and Arora 1958, Webster and Ellis 1962, Datta 1967, Champault 1970, Bancilhon 1971, Gill *et al*. 1973, Sarkar and Datta 1980, Chatha and Bir 1987)

Morphological characters are the features of external forms or appearance which generally provide the character used for hypothesizing phylogenetic relationships. These features have been used for a longer time than the cytogenetical and molecular evidence in the beginning of plant systematic. Morphological characters such as size, shape and colour of different parts of a species could be easily observed and used in the preparation of taxonomic key. However, most of the plants are classified based on external morphological structures such as flowers and fruits. These structures are not always observable on plants because of being seasonal in nature.

Classical taxonomy often failed to identify some species because of high morphological similarity and phenotypic plasticity for adaptation in different environmental conditions. These species must be characterized by alternative methods, such as morphometric data, cytogenetics and molecular analyses. In this context, cytotaxonomy could be considered as a powerful and informative tool to identify morphologically similar populations on the basis cytogenetical and taxonomical status (King 1993, Taylor 2000).

According to available literature, a number of cytological works have been reported and those were mostly confined to chromosome count only (Table 7). The chromosomal studies of these species are difficult because of having small sized and sticky chromosomes. As a result, comparative study of chromosome morphology and detail karyotypic features among different species of *Phyllanthus* L. has not been well documented and most of the earlier researches were confined to count the diploid chromosome numbers. Consequently, information about detailed karyotypic features of *Phyllanthus* L. species is unavailable.

Karyotype analysis is one of the informative parameters for proper characterization since it is a stable character and specific for each species. However, conventional karyotype analysis is unable to express critically the differences among different species of a genus since the species of a genus sometimes possess similar 2n chromosome numbers and even other karyotypic parameters (Khatun and Alam 2010, Khatun *et al.* 2011). Moreover, under the consideration of chromosome length, arm ratio, position and number of secondary constrictions are not always sufficient

to differentiate individual chromosome. Minute deletion, inversion, tandem duplication, etc. could not be possible to detect by conventional karyotype analysis. In such case, molecular cytogenetic approaches should be undertaken for comparative study among different species of a genus which enables to find out minute differences in their genomes.

Using fluorescent banding with chromomycin A_3 (CMA) and 4′, 6-diamidino-2-phenylindole (DAPI), it is possible to determine the arrangement of GC- and AT-rich repeats in the genome, respectively (Alam and Kondo 1995, Akter and Alam 2005). Schweizer (1976) for the first time initiated this technique. CMA binds with GC (Guanine-Cytosine)-rich repetitive sequences of the genome and gives characteristics yellow colour bands. On the other hand, DAPI binds to AT (Adenine-Thymine)-rich repeats showing characteristic blue colour bands (Schweizer 1976, Alam and Kondo 1995, Kondo and Hizume 1982, Akhter and Alam 2005, Jessy *et al.* 2005, Islam and Alam 2011, Sultana *et al.* 2011). With the help of fluorescent banding, it was possible to identify even a particular chromosome (Schweizer 1976, Kondo and Hizume 1982, Hizume *et al.* 1988, Alam and Kondo 1995).

Moreover, the staining property of interphase nuclei and prophase chromosomes add another dimension for comparative karyological study. Tanaka (1971) classified the different types of interphase nuclei and prophase chromosomes based on staining property. The outcome of this study showed that various species could be distinguished by their staining properties of interphase nuclei and prophase chromosomes.

DNA fingerprinting by RAPD is one of the molecular methods for characterizing species. Information on genetic diversity would provide advanced information for understanding the genetic diversity of different *Phyllanthus* L. species for appropriate management of this species. Extent of polymorphism among *Phyllanthus* L. species can be determined using the banding pattern obtained through PCR with RAPD markers. Cluster analysis of these species can be done and genetic distance can be estimated.

Though some conventional karyotype and PCR based marker analysis of *Phyllanthus* L. species have been undertaken, those were scattered and not exactly used for

characterization (Dnyaneshwar *et al.* 2006, Manissorn *et al.* 2010, Bandyopadhyay and Raychaudhuri 2013). There was no report on fluorescent banding for comparative karyotype analysis of *Phyllanthus* L. species. Any attempt has neither been taken so far nor being initiated to combine taxonomical, cytogenetical and molecular data for characterizing *Phyllanthus* L. species.

Considering the importance of studying the genetic variability of this genus in the present study, a combination of taxonomical, conventional and advanced cytogenetical methods and molecular markers like RAPD will be used for an authentic characterization of *Phyllanthus* L. species with the following aims:

a. To compare the taxonomical characteristics of different medicinally important *Phyllanthus* L. species.
b. To prepare a key to the species based on the external morphology and ploidy level.
c. To compare the staining property of the interphase nuclei and prophase chromosomes.
d. To make a comparative account among five *Phyllanthus* L. species based on karyomorphological parameters.
e. To compare the fluorescent banding pattern with CMA- and DAPI- stain.
f. To elucidate the three dimensional aspects of the genus *Phyllanthus* L. assembled with taxonomic, cytogenetic and molecular point.

CHAPTER TWO:
MATERIALS
AND
METHODS

CHAPTER TWO: MATERIALS AND METHODS

2.1. Materials

Five *Phyllanthus* L. species of Bangladesh were used as plant materials in the present investigation. These were:

1. *P. acidus* (L.) Skeels, U.S. Dept. Agric. Bur. Pl. Ind. Bull. 148: 17 (1909).
2. *P. emblica* L., Sp. Pl.: 982 (1753).

   It included two distinct forms, one bearing smaller fruits and the other bearing larger fruits.
3. *P. niruri* L., Sp. Pl.: 981 (1753).
4. *P. reticulatus* Poir., Encycl. Meth. 5: 298 (1804).
5. *P. urinaria* L., Sp. Pl.: 982 (1753).

2.2. Methods

2.2.1. Methods for taxonomical studies

(a) Sample collection

Five *Phyllanthus* L. species had been collected from different parts of Bangladesh and maintained in the Botanical Garden, Department of Botany, University of Dhaka, Bangladesh (Table 8).

(b) Sample identification

The studied species were identified by Professor Dr. Md. Abul Hassan, Department of Botany, University of Dhaka, Bangladesh (Personal Communication), reputed scientist in the field of Plant Taxonomy.

Table 8. Scientific name, distribution and sources in Bangladesh of medicinally important five species of *Phyllanthus* L.

Serial no.	Scientific name	Collected area in Bangladesh	Distribution in Bangladesh
1.	*Phyllanthus acidus** (L.) Skeels, U.S. Dept. Agric. Bur. Pl. Ind. Bull. 148: 17 (1909).	National Botanical Garden of Bangladesh, BRAC (Bangladesh Rural Advancement Committee) Nursery of Bangladesh	
2.	*Phyllanthus emblica* L., Sp. Pl.: 982 (1753). (small fruit form)	National Botanical Garden of Bangladesh, BRAC	
	Phyllanthus emblica L., Sp. Pl.: 982 (1753). (large fruit form)	Botanical Garden, Department of Botany, University of Dhaka	Throughout Bangladesh
3.	*Phyllanthus niruri* L., Sp. Pl.: 981 (1753).	Botanical Garden, Department of Botany, University of Dhaka.	
4.	*Phyllanthus reticulatus* Poir., Encycl. Meth. 5: 298 (1804).	National Botanical Garden of Bangladesh and Botanical Garden, Department of Botany, University of Dhaka	
5.	*Phyllanthus urinaria* L., Sp. Pl.: 982 (1753).	Botanical Garden, Department of Botany, University of Dhaka	In Bangladesh, this species was found in Chattogram, Cox's Bazar, Dhaka, Jashore, Rajshahi and Rangamati districts

*Species name arranged alphabetically.

(c) Herbarium sheet preparation

For Herbarium sheet preparation, plant specimens were dried, pressed and mounted on mounting sheets. The main objectives of making herbarium sheets are to preserve plant specimens for reference and to identify plants based on herbarium specimens.

The following equipment's were used in the preparation of herbarium sheets:

1. Plant press
2. Vasculum
3. Scissors
4. One sharp knife
5. One pair of forceps
6. Hand lens and pocket lens
7. Scotch tape
8. Threads
9. Gum and
10. Note Pad

The main steps to prepare herbarium sheet are:

(i) Collection and pressing of specimens

Fresh materials of two specimens of the same plant were pressed in plant press. Underground parts were made dust free before preserving.

(ii) Drying of specimens

For drying of specimens, plants were kept in herbarium press for 24 hours. Blotting paper will absorb moisture. Again, this process was repeated with fresh blotting pater for 24 to 72 hours. Specimens become dry after such process.

(iii) Mounting of specimens on herbarium sheets

Well–pressed and well dried specimens were mounted on 11.5''×16.5" herbariums sheets with the help of gum.

(iv) Labeling of specimens

Labeling was done after mounting. After fixing specimens on herbarium sheet, the following information was entered at right side of the lower corner of herbarium sheet according to Dhaka University Salar Khan Herbarium (Flora of Bangladesh):

Bot. Name	:	_____
Local Name	:	_____
Family	:	_____
Habit and Habitat	:	_____
Locality	:	_____
Collector	:	_____
Date of Collection	:	_____
Habit	:	_____
Date of collection	:	_____
Identified by	:	_____
Collection Number	:	_____

(v) Storage and making of herbarium sheets

Properly mounted and labeled herbarium sheets were preserved for future records. Specimens were arranged according to recognized system of classification (Cronquist 1981).

(d) Plantation in field

The live specimens of different *Phyllanthus* L. species under studied were planted and maintained in the Botanical Garden, Department of Botany, University of Dhaka, Bangladesh for further studies.

(e) Taxonomical observation

The flowering and fruiting time were carefully observed and recorded in Table (13). Morphological analysis of habits, barks, branches, leaves, flowers, fruits and seeds were also performed.

(f) Preparation of key to species

A taxonomic key to species was made for quick and authentic identification. A taxonomic key to species is consisting of a series of contrasting statements required for the identification and to make comparisons.

2.2.2. Methods for cytogenetical studies

2.2.2.1. Preparation of reagents

(a) Pretreating agent: (0.002 M) 8-hydroxyquinoline

0.29 gm of 8-hydroxyquinoline crystals were thoroughly dissolved in a liter of distilled water (dH_2O) by constant stirring and kept at below 20 ˚C for future use.

(b) (45%) Fixative: Acetic acid

45 ml of glacial acetic acid was mixed with 55 ml of distilled water (dH_2O).

(c) (70%) Preservative: Alcohol

70 ml of absolute alcohol was mixed with 30 ml of distilled water (dH_2O).

(d) Hydrolyzing agent: 1 N HCl

36.5 ml of HCl were thoroughly dissolved in a liter of distilled water by constant stirring and kept for future use at normal temperature.

(e) Hydrolyzing solution:

Two parts of 1 N HCl was mixed with one part of 45% acetic acid.

(f) Stains

(i) (1%) Aceto-orcein

1 gm of orcein dye (Sigma) was added to a flask containing 45 ml acetic acid (100%). These were heated (not boiling) for about 20 h and messed up to 100 ml by adding distilled water (dH_2O). It was then filtered quickly and stored at room temperature for future use.

(ii) (0.1 mg/ml) Chromomycin A_3 (CMA)

1 mg of Chromomycin A_3 (CMA) (Sigma) was thoroughly dissolved in 10 ml of McIlvaine's buffer supplemented with Mg^{2+} by stirring and kept at –20 ˚C for future use.

(iii) (0.01 mg/ml) 4', 6-Diamidino-2-phenylindole (DAPI)

1 mg 4', 6-Diamidino-2-phenylindole (DAPI) (Carl Roth) was thoroughly dissolved in 100 ml McIlvaine's buffer (without Mg^{2+}) by stirring and kept at –20 ˚C for future use.

(g) Buffer

(i) McIlvaine's buffer (pH 7.0)

About 59 (58.99) gm of $Na_2HPO_4.12H_2O$ (FW 358.14) and 3.71 gm citric acid were measured and mixed in one liter distilled water. It was then autoclaved for 10 m and kept at 4 ˚C for future use.

(ii) McIlvaine's buffer with Mg^{2+} (pH 7.0)

About 59 (58.99) gm of $Na_2HPO_4.12H_2O$ (FW 358.14) and 3.71 gm of citric acid were mixed in one liter of distilled water. After autoclaving for 10 min, 2.46 gm of $MgSO_4$ was added to it and kept at 4 ˚C for future use.

2.2.2.2. Procedure for studying mitosis

(a) Collection of root-tips (RTs)

Roots were collected from the Botanic garden of the Department of Botany, University of Dhaka. The young healthy roots were cut 0.5 cm away from the tip by a clean blade.

(b) Pre-treatment

The collected RTs were soaked on a filter paper to remove surface water and pretreated with 8-hydroxyquinoline (0.002 M) for 20 min.

(c) Fixation

RTs were fixed in 45% acetic acid for 15 min at 4 ˚C.

(d) Preparation of slide for orcein staining

The pretreated roots of *Phyllanthus* L. species were hydrolyzed for 2 min 30 sec at 64 ˚C in a mixture of 1 N HCl and 45% acetic acid (2:1). Then the hydrolyzed root tips were soaked on a filter paper and taken on a clean slide. The meristematic region was cut with a fine blade. A drop of 1% aceto-orcein was added to the material and kept in an acetic acid chamber for 2 h. A clean cover glass was placed on the material. At first, the materials were tapped gently by a tooth pick and then squashed by placing thumbs. During tapping and squashing care was taken so that the cover glass should not be moved because a minute displacement of it could damage the entire preparation. The slides were observed under Micros microscope.

(e) Preparation of slide for fluorescent staining

(i) Preparation of air-dried slides

After hydrolyzing and dissecting, the materials were tapped and squashed with 45% acetic-acid. The cover glasses were removed quickly and dried in air for at least 24 h before study.

(ii) CMA-staining

Method proposed by Alam and Kondo (1995) was followed with slight modifications. After 24 h of air drying, the slide was first pre-incubated in McIlvaine's buffer (pH 7.0)

for 30 min. Then the slide was rinsed mildly in McIlvaine's buffer supplemented with $MgSO_4$ for 15 min. Then one drop of chromomycin A_3 (0.1 mg/ml) was added to the materials of slide and a clean cover glass was placed on it. The slide was kept in a humid chamber for 3 h. The slide was washed with distilled water in such a way that the cover glass was removed. The slide was treated again for 10 min in McIlvaine's buffer with $MgSO_4$ and 10 min McIlvaine's buffer without $MgSO_4$. Slides were mounted in 50% glycerol and kept at 4 ˚C for overnight before observation. These were observed under Nikon (Eclipse 50i) fluorescent microscope with blue violet (BV) filter cassette.

(iii) DAPI-staining

For DAPI-staining method proposed by Alam and Kondo (1995) was followed after slight modifications. For direct DAPI-staining, after 24 h of air drying, the slide was first pre-incubated in McIlvaine's buffer (pH 7.0) for 25 min. The slide was immersed again in McIlvaine's buffer (pH 7.0) for 15 minutes followed by treating in DAPI solution (0.1mg/ml) for 3 h. After rinsing in McIlvaine's buffer (pH 7.0) for 10 min, the slide was mounted with 50% glycerol and kept at 4 ˚C. These were observed under a Nikon (Eclipse 50i) fluorescent microscope with ultra violet (UV) filter cassette.

2.2.2.3. Determination of centromeric type, relative length and centromeric index

(a) Centromeric type

A procedure proposed by Levan *et al.* (1964) for determining centromeric type of chromosomes was followed with slight modification. In this study, the l/s arm ratio (l=length of the longer arm and s=length of the shorter arm) was considered in the following manner:

	Chromosome type (ct)	l/s ratio
(a)	Metacentric (m)	1.00- 1.50
(b)	Sub-metacentric (sm)	1.51- 3.00
(c)	Acrocentric (ac)	3.01- 7.00
(d)	Telocentric (t)	above 7.00

(b) Relative length (RL) of chromosomes

The measurement of relative length was done by dividing the length of a particular chromosome with the total length of the diploid complements. It is represented as follows:

$$\text{Relative length (RL)} = \frac{\text{length of a particular chromosome}}{\text{total length of the diploid complements}}$$

(c) Centromeric index (CI)

It was measured by the ratio of short arm to total length of that chromosome, expressed as percent. It can be shown as below:

$$\text{Centromeric index (CI)} = \frac{\text{length of short arm}}{\text{total length of that chromosome}} \times 100$$

(d) Classification of interphase nuclei and prophase chromosomes in orcein-staining

The classification proposed by Tanaka (1971) was followed:

(i) Interphase nuclei

(1) Diffuse type

(2) Simple chromocenter type

(3) Complex chromocenter type

(4) Rod pro-chromosome type

(5) Round pro-chromosome type

(ii) Prophase chromosomes

(1) Continuous type

(2) Interstitial type

(3) Gradient type

(4) Proximal type

(5) Tenuous type

2.2.2.4. Classification of fluorescent bands

According to the different fluorescent banding patterns observed in this study, chromosomes were classified as follows:

α=Band in centromeric region

β=Band in terminal region on the short arm

δ=Band in terminal region on the long arm

γ=Band in whole chromosome

θ=No band

2.2.2.5. Procedure for studying meiosis

The young unopen buds of *Phyllanthus emblica* L. (small fruit form), *P. emblica* L. (large fruit form) and *P. urinaria* L. were collected from the Botanic Garden, University of Dhaka at 9 a.m. and kept in a watch glass with distilled water. The bud was opened by a pair of forceps. Two - three anthers were taken on a clean slide. A drop of orcein was added to it and kept for one minute. The anthers were gently tapped with a plastic taper. The debris was discarded. A clean cover slip was placed carefully on the materials. The slide was wrapped with a filter paper and very gently pressed by thumbs. A drop of orcein was added to the corner of the cover slip and observed under light microscope.

2.2.2.6. Procedure for studying pollen viability

Mature flowers of the selected plants with yellow anthers were collected from the Botanic garden, University of Dhaka. One drop of orcein was placed on a clean slide. The mature anthers were touched to the orcein dye in such a way so that the pollen grains came in contact with orcein. A clean cover slip was placed on it and observed under microscope.

2.2.2.7. Magnification

The photography was taken by using 8 mega pixels canon power shoot A720 model with the magnification of 7xs at Auto mode. For measuring the magnification, at first the magnification was calculated by multiplying the magnification of objective (40xs), tube length (1.25xs) and camera lens (5xs, 3.5xs). From print, the final magnification was calculated.

2.2.3. Method for molecular studies (RAPD)

Methods used in RAPD and SSR marker analysis have been presented below:

2.2.3.1 Isolation of genomic DNA

To extract genomic DNA, young and actively growing fresh leaves were collected from each of the ten germplasms of *Crotalaria* spp. These leaves were cut apart with sterilized scissors and washed well initially in distilled water and then ethanol. These were dried on fresh tissue paper to remove spore of microorganisms and other sources of foreign DNA.

Preparation of different stock solutions and working solutions used for DNA isolation

For conducting the isolation procedures, the following stock solutions and working solutions were prepared:

β-Mercaptoethanol

β-Mercaptoethanol (Merck, Germany) was obtained as a 14.4 M solution from company and it was stored in a dark bottle at room temperature.

Ribonuclease-A stock solution

10 mg RNase-A (Merck, Germany) was dissolved in 1 ml of deionized distilled water and stored in -20 ˚C.

70% Ethanol (100 ml)

30 ml double distilled water (ddH_2O) was added in 70 ml absolute ethanol.

Required equipment's

1. Plant tissues (leaf tissues in this experiment)
2. Autoclave machine
3. Digital balance
4. Beaker
5. Centrifuge machine with 10,000-14,000 rpm
6. Conical flasks
7. Eppendorf tubes
8. Gloves
9. Ice machine
10. Water bath capable of maintaining 65 ˚C

11. Micropipettes and nuclease free micropipette tips
12. Mortar and pestle
13. pH meter
14. Refrigerator
15. Water de-ionizer
16. Water distillation plant
17. Fume hood

Required Chemicals

1. 100% ethanol
2. CTAB (Cetyl Trimethyl Ammonium Bromide)
3. β-Mercaptoethanol
4. 70% ethanol
5. DNA isolation Kit (Roti®-Prep Genomic DNA MINI, Carl Roth, Germany; Art.-Nr. 8472.2)

(a) DNA isolation procedure

(i) Collection of leaf sample

To extract genomic DNA, young and actively growing fresh leaves of five *Phyllanthus* L. species were collected from Botanical Garden, Department of Botany, University of Dhaka. These leaves were cut apart with sterilized scissors and washed well initially in distilled water and then in ethanol. These were dried on fresh tissue paper to remove spore of microorganisms and any other sources of foreign DNA.

(ii) Tissue lysis

Freshly harvested 40 mg leaf tissue sample into small pieces and placed the tissue into a 1.5 ml reaction tube. Added 400 µl Lysis Buffer LSA, 25 µl Proteinase K solution, and 3 µl RNase A (Stock solution 100 mg/ml; not included in the kit). Mixed vigorously by pulsed vortexing for 5 sec. Incubated at 50 ˚C under constant agitation until the sample is completely lysed, approximately 0.5-2 h for tissue sample. After lysis has been completed, centrifuged the lysed sample at 12000 rpm for 30 sec to spin down unlysed material. Transferred the supernatant to a fresh 1.5 ml reaction tube. Add 200 µl Binding Buffer BSN to the supernatant. Mixed it by vortexing until a homogenous solution was achieved.

(iii) Column loading

Placed each Spin Column into a 2 ml collection tube. Applied the mix of supernatant (Binding Buffer BSN) to the spin column. Centrifuge at 12000 rpm for 2 min at room temperature and discarded the flow-through.

(iv) Column washing

Added 700 µl of Washing Buffer WST to the Spin Column. Centrifuge at 12000 rpm for 1 min at room temperature and discarded the flow-through. Added 700 µl of Washing Buffer WST to the Spin Column. Centrifuged at 12000 rpm for 1 min at room temperature and discarded the flow-through. Again, centrifuge columns at full speed for 2 mins at room temperature in order to remove residual ethanol.

(v) Elution

Placed the Spin Column into a clean 1.5 ml elution tube. Added 200 µl Elution Buffer EB to the centre of the membrane. Incubated it for 1 min at room temperature. Centrifuged at 8000 rpm for 1 min at room temperature to elute DNA.

(b) Qualification and quantification of isolated DNA

Before PCR amplification, it is important to know the concentration of genomic DNA because different DNA extraction methods produced DNA of widely different purity. It is necessary to optimize the amount of DNA for reproducibility and strong signal in PCR assay. Excessive genomic DNA may result smears lack of clearly defined bands in the gel. On the other hand, too little DNA will give non-reproducible patterns (Williams *et al*. 1993). Measurement of isolated DNA concentration can be done by comparing DNA with the standard DNA on agarose gel electrophoresis or by estimating the absorbance of DNA by spectrophotometer at 260 nm wavelengths. Both the methods were carried out in this experiment.

(c) Measurement of DNA concentration and quality by agarose gel electrophoresis

The following equipments and chemicals were used to conduct agarose gel electrophoresis:

1. A horizontal electrophoresis chamber and power supply
2. Gel casting tray and combs
3. Gel Documentation System (BioSciTec, Gelscan 6.0 Professional, German)
4. Gloves
5. Pipette and tips

6. 1 kb DNA ladder
7. Electrophoresis buffer (TAE)
8. 6x sample loading buffer
9. Agarose
10. DNA stain (Ethidium bromide)

(d) Preparation of stock solutions used for gel electrophoresis

For conducting the gel electrophoresis, the following stock solutions and other solutions were prepared:

(i) 50x TAE Buffer (pH 8.3) (1 liter)

242 g Trizma base (MW=121.14) was dissolved in 900 ml of sterile de-ionized distilled water. Then 57 ml glacial acetic acid was added to the solution. Finally, 100 ml 0.5 EDTA (pH 8.0) was added in it. These were mixed well. The pH of the solution was adjusted by mixing concentrated HCl to pH 8.3. The final volume of the solution was adjusted to 1000 ml.

(ii) 10x loading dye

For making 10 ml of 10x loading dye, 40 mg of Bromophenol blue (*i.e*., the final concentration was 0.4%), 40 mg of Xylene cyanol FF (*i.e*. the final concentration was 0.4%) and 5 ml of 98% glycerol (*i.e*. the final concentration was 50%) were added to 4 ml of sterile de-ionized distilled water mixed properly. The final volume was adjusted to 10 ml with sterile de-ionized distilled water and stored at 4˚C.

(iii) Ethidium bromide solution

For preparing 1 ml solution, Ethidium Bromide (05 mg) was added to 1 ml of sterile de-ionized distilled water. It was then mixed by hand shaking. The solution was then transferred to a dark bottle and stored at room temperature. Stock solution of 10 mg/ml can be purchased directly from companies.

(iv) Preparation of 1% agarose gel

For agarose gel electrophoresis, 100 ml of 1% agarose gel was prepared (100 ml was required for 15×15 cm tray with 0.5 cm thickness). The following steps were followed during casting the gel-

1.0 gm agarose powder were measured and put in a 250 ml conical flask. 100 ml of 1x TAE buffer was added to the flask. The agarose was melted in a microwave oven for several short intervals until the solution became clear. The solution did not allow

boiling for long periods. When the agarose solution was cooled to about 50 ˚C (the flask was cooled enough to hold comfortably with bare hand), 5 µl (from 10 mg/ml solution) of ethidium bromide was added so that in the gel the concentration of ethidium bromide maintained 0.8 µg/ml (the final concentration of ethidium bromide in the melted agarose solution ranged from 0.5-1.0 µg/ml) and mixed well by gentle shaking.

(e) Comb set-up

The ends of the gel casting tray was sealed and fitted with casting dams and the combs placed in the gel casting tray. The melted agarose solution was poured into the casting tray and let it to cool until solid. The comb was removed carefully from the solidified gel. The casting dams or gates were removed from the edges of the gel tray carefully so that the gel does not slide of the tray.

(f) Preparation of DNA sample for electrophoresis

At first 2 µl 6x loading dye was placed on a piece of parafilm by a micropipette. 10 µl DNA sample (after thawing from frozen stock) was added to it and mixed 4x with the adjustable micropipette.

(g) Electrophoresis

The gel was placed in the electrophoresis chamber in such a way that the sample wells remained near to the cathode (negative end generally marked as black). DNA sample would migrate towards the anode (positive end generally marked as red) during electrophoresis. The gel was covered by sufficient amount of 1x TAE buffer. The volume of the electrophoresis buffer should not above the maximum buffer mark on electrophoresis system. The prepared samples were loaded slowly and allowed to sink to the bottom of the wells.10 µl of 1 kb DNA ladder (marker) was also loaded at least in one well (generally the first one). Electrophoresis was carried out at 50 volts and 100 mA for 1.5 h. The separation process was monitored by the migration of the dye on the gel. When the first dye (bromophenol blue) had reached about three-fourths of the gel length, the electrophoresis was completed and stopped.

(h) Documentation of the DNA sample

After electrophoresis, the gel was taken out carefully from the electrophoresis chamber and placed in Gel Documentation System (BioSciTec, Gelscan 6.0 Professional, Germany) for observing the DNA bands and the DNA was observed as

band and photographed using Gel Documentation system. The electrophoregram of DNA samples of five species *Phyllanthus* L. are shown in figure (Fig. 115).

(i) Quantification and qualification of DNA by spectrophotometer

For more confirmation, DNA was also quantified through spectrophotometer. Spectrophotometer is commonly used in laboratories for the measurement of DNA concentration and purity. The DNA concentration was obtained by multiplying the absorbance at 260 nm wave length by a constant. The DNA purity was measured by dividing the absorbance at 260 nm wave length by the absorbance at 280 nm wave length. Good quality DNA should give the ratio (A260/A280) ranging from 1.8–2.0. The A260/A280 ratio higher than 2.0 and lower than 1.8 generally indicates RNA and protein contamination respectively, during extraction process. Following Sambrook *et al.* (1989), the DNA concentration was calculated (1 O. D.) at 260 nm corresponds to 50 μl/ml of double stranded DNA.

(j) Set-up the spectrophotometer

To estimate genomic DNA concentration, absorbance readings of the DNA samples were recorded at 260 nm using a spectrophotometer (Analylikjena, Specord 50, Germany).

At first, the spectrophotometer UV-lamp was turned on. After warming up the wave length was set at 260 nm. After washing the cuvette (the 'zero' or 'blank' cuvette) was filled with 2 ml sterile distilled water and placed on cuvette chamber. The absorbance reading was adjusted to zero for standardization.

(k) Preparation of the DNA sample for spectrophotometry

The test samples were prepared by taking 2μl of each DNA sample in the cuvette containing 1,998 μl sterile distilled water. The samples were mixed well by using an adjustable micropipette. The reading was taken by viewing the monitor of spectrophotometer at 260 nm and 280 nm wave length. After recording the absorbance readings, the cuvette was rinsed out with sterile distilled water, tamped out on a paper and wiped. The absorbance reading for each sample was recorded in the same way. The ratios of spectrophotometric absorption readings at 260 nm of different samples for quality determination of DNA are shown in the Table 9.

(l) Calculation for the concentration of DNA

Before PCR, DNA concentrations were determined according to the following formula:

$$\text{DNA concentration} = A260 \times \text{Dilution factor} \times \text{Conversion factor (50 µg/ml)}$$

$$= A260 \times \frac{\text{Volume of distilled water (µl)}}{\text{Amount of DNA sample (µl)}} \times \text{Conversion factor (50 µg/ml)}$$

$$= (\mu g/ml) \quad [\text{since } 1\ \mu g = 10^{-3}\ ng, \textit{i.e.,}\ \mu g/ml = ng/\mu l]$$

A260=spectrophotometric absorbance reading at 260 nm of DNA sample.

Dilution factor=the ratio of distilled water (µl) to amount of DNA sample (µl).

Conversion factor 50=the 50 µg/ml of DNA contained in a solution which gives the spectrophotometric absorbance reading at 260 nm equal to 1.

Note: The calculated DNA concentration expressed in µg/ml gave a fraction. To avoid fraction it was converted into ng/µl (1 $\mu g = 10^{-3}$ ng) and therefore, multiplied with 1000.

Table 9. Spectrophotometric absorbance readings at 260 nm wave length and concentration of DNA of five species of *Phyllanthus* L.

Species	Absorbance reading at 260 nm	Concentration of DNA (ng/µl)
P. acidus	0.0304	1520
P. emblica (small fruit form)	0.0030	0141
P. emblica (large fruit form)	0.0072	0362
P. niruri	0.0483	2405
P. reticulatus	0.0430	2149
P. urinaria	0.0120	0588

(m) Amplification of DNA by polymerase chain reaction (PCR) using RAPD markers

To perform the amplification of RAPD, a single oligonucleotide of arbitrary DNA sequence is mixed with genomic DNA in the presence of a thermostable DNA

polymerase with suitable buffer and subjected to temperature cycling conditions typical for the polymerase chain reaction (PCR).

(n) Preparation of working solution (25 ng/µl) of DNA samples for PCR reaction

Original stock solution concentration of each DNA sample was adjusted to a unique concentration (25 ng/µl) using the following formula:

$S_1 \times V_1 = S_2 \times V_2$

$V_1 = S_2 \times V_2 / S_1$

Where,

S_1=stock DNA concentration (ng/µl)

V_1=volume require (µl)

S_2=working DNA concentration (ng/µl)

V_2=working volume of DNA solution (µl)

Original stock DNA (2 µl) was taken in a 2 ml eppendorf tube and required amount of TE buffer calculated from the above formula added to it. Calculated required volume of TE buffer for each sample was shown in Table 10.

Table 10. Preparation of working DNA solution (25 ng/µl) for PCR reaction

Species	Working solution (25 ng/µl) for PCR	
	TE buffer/ de-ionized water (µl) required	DNA (µl) required
P. acidus	59	2
P. emblica (small fruit form)	03	2
P. emblica (large fruit form)	13	2
P. niruri	95	2
P. reticulatus	83	2
P. urinaria	21	2

(o) Primer test

Primarily 24 decamer primers were tested for RAPD amplification of which 12 primers exhibited good quality banding patterns and sufficient variability. These 12 primers were selected for further analysis. The details of the 12 primers were given in Table 11.

Table 11. Twelve random primers used in the present study for screening

Primer code	Sequence (5'—3')	Annealing temperature (˚C)	G+C content (%)
OPA-18	AGG TGA CCG T	32	60.00
OPB-19	ACC CCC GAA G	34	70.00
OPAB-5	CCC GAA GCG A	34	70.00
OPAB-6	GTG GCT TGG A	32	60.00
OPC-13	AAG CCT CGT C	32	60.00
OPC-15	GAC GGA TCA G	32	60.00
OPC-96	ACC AAG AAA GGG	36	50.00
OPD-69	CGC TCC AAA TCA	36	50.00
OPF-22	AAG ATC AAA GAC	32	33.33
OPH-12	ACG CGC ATG T	32	60.00
OPG-5	AGT CGT CCC C	34	70.00
OPG-7	GAA CCT GCG G	34	70.00

(p) Preparation of primers

The supplied primers were diluted to 100 µM. To make 100 µM of each primer, the following mathematical deduction was followed.

We know,

n=cv (Where n=Number of mole)

Here, c=Molarity *i.e.* concentration in molarity and v=Volume.

Generally, 100 µM concentration of primer has to prepare as main stock solution. In this case, c=100 µM since 53.4 nM of primer was present in the vial used in the investigation, supplied from the company (Neer Biotech concern), n=53.4 nM

And v=Required volume of TE buffer has to add in the supplied vial to make 100 µM main stocks. Using the formula-

$$v = \frac{n}{c}$$

$$v = \frac{53.4 \text{ nmol}}{100 \text{ µM}}$$

$$v = \frac{53.4\times10^{-9} \text{ mol}}{100\times10^{-6} \text{ M}}$$

$$v = \frac{53.4\times10^{-5} \text{ mol}}{\text{M}}$$

$$v = \frac{53.4\times10^{-5} \text{ mol}}{\text{moll}^{-1}}$$

$$v = \frac{53.4\times10^{-5}}{\text{L}^{-1}}$$

$$v = 53.4\times10^{-5} \text{ L}$$

$$v = 53.4\times10^{-5}\times1000 \text{ ml}$$

$$v = 53.4\times10^{-2} \text{ ml}$$

$$v = 53.4\times10^{-2}\times100 \text{ µl}$$

$$v = 53.4 \text{ µl}$$

Therefore, 534 µl of TE buffer was added to the vial to make 100 µM main stocks. Using the above deduction method, all primer pairs were diluted to 100 µM main stock. All primers were diluted to 50 times *i.e.* 2 µM to make working solution for use.

(q) Preparation of PCR reaction mixture (PCR cocktail)

The following components were used to prepare PCR cocktail (Table 12). The total volume of PCR cocktail was 175 µl per sample.

Table 12. Component of PCR cocktail (for 7 reactions)

Sl. no.	Reagents	Amount per sample	Total
1	Sterile de-ionized distilled water	17.3 µl	121.1 µl
2	*Taq* Buffer A 10x (Tris without 15 mM $MgCl_2$)	2.5 µl	17.5 µl
3	$MgCl_2$	1.5 µl	10.5 µl
4	Primer	1.0 µl	07.0 µl
5	dNTPs (2.5 mM)	0.5 µl	03.5 µl
6	*Taq* DNA Polymerase (5 U/µl)	0.2 µl	1.4 µl
7	Template DNA (25 ng/µl)	2.0 µl	14.0 µl
	Total	**25.0 µl**	**175.0 µl**

During the experiment, PCR buffer, dNTPs, primers and DNA sample solution were thawed from frozen stocks, mixed well by vortexing and kept on ice. Template DNA (25 ng/µl) were pipetted (2.0 µl) first into PCR tubes (0.5 ml) compatible with the thermocycler. For each DNA sample being tested, a pre-mix was prepared in the following order- buffer, dNTPs, DNA template and sterile distilled water. Taq DNA polymerase enzyme was added to the pre-mix. The pre-mix was then mixed well and aliquoted into the tubes containing primers. The tubes were then sealed and placed in a thermocycler and the cycling started immediately.

(r) PCR amplification

PCR amplification was done in an oil free thermal cycler (Biometra UNOII, Germany). The optimum amplification cycle was as follows:

Denaturation/Annealing/Extension		**Temperature (˚C)**	**Time**
	Initial denaturation	94	5 min
45 cycles	Denaturation at	94	1 min
	Annealing at	34–36	30 sec
	Extension at	72	3 min
	Final extension at	72	5 min

After completion of cycling program, the reactions were held at 4 ˚C.

(s) Electrophoresis of the amplified products and documentation

The amplified products were separated electrophoretically on 1% agarose gel. The gel was prepared using 1.0 gm agarose powder containing ethidium bromide 8 µl and 100 ml 1x TAE buffer. Agarose gel electrophoresis was conducted in 1x TAE buffer at 50 Volts and 100 mA for 1.5 h. 1 kb DNA ladder was electrophoresed alongside the RAPD reactions as marker. DNA bands were observed on UV-transilluminator and photographed by a Gel Documentation system as before.

(t) RAPD data analysis

The PCR products were analyzed after gel electrophoresis. The photographs were critically discussed on the basis of presence (1), absence (0) and size of bands and overall polymorphism of bands. The scores obtained using all primers in the RAPD analysis were then pooled for constructing a single data matrix. This was used for estimating polymorphic loci, Nei (1972) gene diversity, genetic distance (D) and constructing a UPGMA (Unweighted Pair Group Method of Arithmetic Means) Dendrogram among the specimen using computer program "POPGENE" (Version 32).

CHAPTER THREE:
RESULTS

CHAPTER THREE: RESULTS

3.1. Taxonomical features of five species of *Phyllanthus* L.

3.1.1. *Phyllanthus acidus* (L.) Skeels, U.S. Dept. Agric. Bur. Pl. Ind. Bull. 148: 17 (1909)

Synonyms: *Averrhoa acida* L., Sp. Pl. 428. 1753., *Phyllanthus acidissimus* (Blanco) Muell.-Arg. 1863, *Cicca acida* (L.) Merrill, Interpr. Herb. Amboin. 314. 1917., *Cicca acidissima* Blanco. 1837., *Cicca disticha* L., Mant. Pl. 124. 1767., *Phyllanthus cicca* Müller Argoviensis, Linnaea 32: 50. 1863., *Diasperus distichus* (L.) Kuntze, Revis. Gen. Pl. 2: 599. 1891., Nom. Illegit., *Phyllanthus distichus* (L.) Muell.-Arg. 1866.

English name: Country Gooseberry, Malay Gooseberry, Otaheite Gooseberry, Star Gooseberry, Starberry, Tahitian Gooseberry, West India Gooseberry, Plum.

Local name: Amla, Harbari, Hariphul, Harfarauri, Loda, Orbori, Noari, Neur.

Description: *Phyllanthus acidus* (L.) Skeels is a small glabrous medium tree with phyllanthoid branching, branches robust and stout, leafless, with slender deciduous leafy branchlets toward the ends, the bark very rough, hard and whitish-gray. The branchlets bear alternate, simple and distichous leaves that are green and smooth on the upper side and blue-green on the underside, stipules lanceolate, c 1 mm long, petiolate, petioles 2-3 mm long, pinnately distichous, leaf blade ovate to ovate-lanceolate, 3-8×1-4 cm, acute or acuminate at the apex, rounded or broadly cuneate at the base, glabrous, pale-coloured beneath. Flowers are formed at leafless parts of the main branches, at the upper part of the tree. Annular floral disk is bearing male, female or hermaphrodite flowers. Flowers minute, monoecious, red, most densely clustered forming slender, clusters axillary or glabrous, interrupted slender racemes in the axils of the leaves or more usually arising along the stem and branches from reduced tubercle-shaped branchlets. Male flowers pedicellate, pedicels slender, 1-3 mm long, sepals 4, orbicular, c 1 mm long, disc glands usually 4, stamens 4, free, 2 shorter. Female flowers few, sepals 4, c 1.5 mm long, disc glands 4, kidney-shaped, staminodes usually present, ovary 3-4 celled, glabrous, styles 4, free, bifid, recurved, ovary superior. Fruits numerous, oblate, densely clustered, waxy, crisp and juicy. It is drupaceous, depressed-

globose, fleshy, flesh firm, very sour, 1-1.5×1.5-2.2 cm, 6-8 lobed, greenish-yellow to whitish. Fruit contains 6-8 smooth trigonous or convex light brown seeds. The seed apex and base is truncate, margin crenate, dorsal surface with ridges and furrows.

Flowering and fruiting time: May-June (in Bangladesh).

Distribution: India, Indonesia, Laos, Madagascar, Malaysia, Myanmar, the Philippines, Pakistan, Thailand, America, Vietnam and Zanzibar. In Bangladesh, this species is cultivated throughout the country.

Origin: *Phyllanthus acidus* (L.) Skeels is probably native to Brazil and Colombia (Orwa *et al*. 2009).

Specimens examined: National Botanical Garden Mirpur, Dhaka, 12.5.2017, Md. Shahidur Rahman, Coll. No. 3 (DUSH), Accession no. 54668 (DACB); Chittagong, St. Maartin`s island, 10.2.1980, Khan and Rahman, Accession no. 12046 (DACB); Botanical Garden, Department of Botany, University of Dhaka, Bangladesh, 22.12.1963, A. F. Muhammad, Coll. No. 65 (DUSH); Botanical Garden, Department of Botany, University of Dhaka, Bangladesh, 24.3.1968, Paritosh, Coll. No. 45 (DUSH); Sahjahanpur, Dhaka, 29.5.1970, Sufia, Coll. no. 13 (DUSH).

3.1.2. *Phyllanthus emblica* L., Sp. Pl.: 982 (1753) (small fruit form)

Synonyms: *Emblica officinalis* Gaertn. (1790), *Dichelactina nodicaulis* Hance (1852). The genus name *Phyllanthus* is derived from Greek words meaning leaf-flower, an allusion to the apparent bearing of flowers on the leaves.

English name: Myrobalan, Emblic Myrobalan, Indian Gooseberry, Gooseberry, Malacca Tree.

Local name: Amloki, Amla, Ambolati, Awla, Aonla, Emblic.

Description: *Phyllanthus emblica* L. is a glabrous, deciduous tree. The tree has crooked trunk, spreading and tawny-pubescent branches. The bark is thick to 10 mm, smooth, hard and blackish, irregularly flaking; blaze pink-red, up to 35 cm in diameter with

numerous knobs. The leaf simple, alternate, sessile, nearly stalkless and closely set along the slender branchlets. The leaves are often mistaken for leaflets of pinnate leaves. It is stipulate, stipules triangular, 0.8-1.6 mm long, attenuate to acuminate, ciliolate, brown, petiolate, petioles 0.3-0.7 mm long, distichous, densely crowded along lateral twigs, leaf blade oblong or linear-oblong, 8-20×2-6 mm, obtuse, shallowly cordate and slightly oblique at the base, margin thickened and enrolled, firmly chartaceous, lateral veins 4-9 pairs, sometimes indistinct, upper side green and under side blue green. Flowers are small and monoecious, greenish white. The flowers have six segments, but no real petals. Flowers in axillary cymes, all male flowers or many male flowers and a single female flower per cyme. Male and female flowers are carried separately on the same branch. Male flowers pedicellate, pedicels 1-3 mm long, slender, sepals 6, membranous, 1.2-2.5×0.5-1.0 mm, oblong-obovate or spathulate, subequal, obtuse or rounded, entire, with a pale hyaline margin, stamens 3, filaments coherent into a central column, 0.3-0.7 mm long, anthers erect, oblong, 0.5-0.9 mm long, longitudinally dehiscent, disk glands 6, smooth, 0.3-0.5 mm in diameter. Female flowers also pedicellate, pedicels c 0.8 mm long, sepals 6, oblong or spathulate, 1.5-2.5×0.7-1.3 mm, obtuse or rounded, entire, disc c 1 mm in diameter, ovary ovoid, smooth, 3-celled, styles 3, stout, fleshy, 1.5-4 mm long, connate at the base, apex deeply bifid. Fruits drupaceous, subglobose, c 2.0-2.2 cm in diameter, smooth and hard on appearance, succulent, greenish or yellowish-white 6 vertical stripes or furrows, c 5.3 gm. tightly embedded in the center of the flesh is a slightly hexagonal stone containing 6 small seeds. Seeds smooth, trigonous or planoconvex, 3-6×2-3 mm, smooth, chestnut-brown.

Flowering and fruiting time: March-September (in Bangladesh).

Distribution: Cambodia, China, Hong Kong, India, Indonesia, Laos, Malaysia, The Philippines, Sri Lanka, Southern America. In Bangladesh, this species is found throughout the country.

Origin: *Phyllanthus emblica* L. is native to tropical southeastern Asia specifically central and southern India (Firminger 1947).

Specimens examined: National Botanical Garden Mirpur, Dhaka, 24.4.2017, Md. Shahidur Rahman, Coll. no. 1, Accession no. 54666 (DACB); Chandranath, Sitakundo, Chittagong, 11.09.2017, Md. Monirujjaman, Al-Amin and Kawsar, Accession no. 53429 (DACB); Chittagong district, Hill Forest, 22.2.1966, M. S. Khan, Coll. No. 1379 (DUSH); Highcourt compound, Ramna, Dacca, 13.3.1964, A. F. Muhammad, Coll. no. 117 (DUSH).

3.1.3. *Phyllanthus emblica* L., Sp. Pl.: 982 (1753) (large fruit form)

Description: The bark is comparatively thin, thick to 5 mm, grayish, upper side yellowish but under side purplish green of the leaves. The flower c 3.5 mm, greenish red in colour. Fruit 3.0-3.3 cm in diameter, average weight c 25.18 gm. This form mainly differs in fruit size which are much bigger than the fruits of small fruit form (2.0-2.2 cm in diameter). This form has been introduced in Bangladesh very recently, now its cultivation is gradually increasing.

Flowering and fruiting time: January-March (in Bangladesh).

Specimens examined: Botanical Garden, Department of Botany, University of Dhaka, Bangladesh, 14.4.2019, Md. Shahidur Rahman, Coll. No. 11, Accession no. 54666 (DACB).

3.1.4. *Phyllanthus niruri* L., Sp. Pl.: 981 (1753)

Synonyms: *Phyllanthus carolinianus* Blanco, Fl. Filip. 691. 1837., *Nymphanthus niruri* (L.) Lour., Fl. Cochinch. 545. 1790., *Diasperus niruri* (L.) Kuntze: In: Revis. Gen. Pl. 2: 600. 1891., *Niruris annua* Raf., Sylva Tellur. 91. 1838., *Niruris indica* Raf., Sylva Tellur. 91. 1838., *Phyllanthus filiformis* Pav. ex Baill.:, Recueil Observ. Bot. 1: 29. 1860., *Phyllanthus niruri* var. genuinus Müll.Arg., nom. inval.: In: 195 Prodr. 15(2): 406. 1866., *Phyllanthus lathyroides* var. *commutatus* Müll.Arg., Linnaea 32: 41. 1863.

English name: Niruri, Gulf Leafflower.

Local name: Bhuiamla.

Description: A monoecious, erect annual herb, branches angular. Leaves simple, alternate, stipulate, stipules 1.0-1.2 mm long, lanceolate, scarious, acute, petiolate, petioles very short, leaf blade elliptic-oblong to elliptic-oblanceolate, 5-12×2-5 mm, obtuse or rounded at the apex and base or sometimes tapering to the base, membranous, lateral nerves 4-7 pairs, indistinct, dark green above, paler and grayish beneath. Flowers yellowish, very numerous, axillary, the males 1-3, the female solitary. Male flowers pedicellate, pedicels 1 mm long, sepals 6, suborbicular-obovate, c 0.5×0.05 mm long, rounded, midrib yellow, disc glands 6, verruculose, lobulate, stamens 3, filaments united into a short column, anthers more or less horizontal, dehiscing transversely. Female flowers with pedicels 1.4-1.9 mm long, sepals 6, unequal, 1.0-1.5×0.4-0.5 mm, oblong-oblanceolate, rounded, white, disk thin, flat, irregular deeply lobed into 6-10 segments, some crenate and broad, some triangular and bifid, some others linear and entire, ovary subglobose, c 1 mm in diameter, smooth, styles minute, free, adpressed or ascending, the lobes recurved. Fruits trilobate-subglobose, 1.5-2.5 mm in diameter, smooth, olivaceous or stramineous. Seeds 1.0×0.6 mm, longitudinally 7-8 ridged on the back, ochreous-fulvous.

Flowering and fruiting time: August-October (in Bangladesh).

Distribution: Africa, India, Pakistan, Saudi Arabia, West Indies. In Bangladesh, this species is found throughout the country.

Origin: *Phyllanthus niruri* L. originated in India, usually occurring as a winter weed throughout the hotter parts (Paithankar *et al*. 2011).

Specimens examined: Botanical Garden, Department of Botany, University of Dhaka, Bangladesh, 12.5.2017, Md. Shahidur Rahman, Coll. No. 2, Accession no. 54667 (DACB); Madhabkundo Ecopark, Borolekha, Moulovibazar, 21.9.2014, Sarder Nasir Uddin, Accession no. 42487 (DACB); Dhaka Mirpur, 12.3.1964, M. A. Baqui, Coll. No. 110 (DUSH); Dhaka university area, 24.4.1960, Almas Uddin, Coll. No. 19 (DUSH); Sonargaon, Narayengonj, 8.8.1995, Md. Abu Rahat, Coll. No. 2 (DUSH).

3.1.5. *Phyllanthus reticulatus* Poir., Encycl. Meth. 5: 298 (1804)

Synonyms: *Anisonema multiflora* (Wild.) Wight. 1852., *Phyllanthus multiflorus* Wild. 1805., *Cicca reticulate* (Poir.) Kurz: In: Forest Fl. Burma 2: 354. 1877., *Anisonema reticulatum* (Poir.) A. Juss.: In: Euphorb. Gen.: 4. 1824., *Anisonema jamaicense* (Griseb.) Griseb.: In: Fl. Brit. W. I.: 716. 1864., *Diasperus reticulatus* (Poir.) Kuntze: In: Revis. Gen. Pl. 2: 600. 1891., *Kirganelia reticulata* (Poir.) Baill.: In: Étude Euphorb.: 613. 1858., *Anisonema dubium* Blume: In: Bijdr. Fl. Ned. Ind.: 589. 1826.

English name: Reticulated Leaf-flaver, Potato plant, Potato smell, Seaside laurel.

Local name: Chitki, Pankushi, Panjuli, Panseuli.

Description: A much branched shrub, bark peeling or flaking, grayish-brown, young branchlets, leaves and pedicels puberulous or glabrous, yellowish. Leaves alternate, simple, stipulate, stipules subulate-lanceolate, 0.8-1.5 mm long, acuminate, truncate at the base, brownish, petiolate, petioles 1.4-3.5 mm long, chartaceous, leaf blade varying in shape, mostly elliptic to ovate, 15-30×6-12 mm, obtuse to rounded at the apex, cuneate at the base, lateral veins 5-9 pairs, slightly raised beneath, tertiary veins reticulate, glabrous. Flowers with 1-2 female and several male flowers per node. Male flowers pedicellate, pedicels 5-10 mm long, delicate, sepals 5-6, unequal, 0.7-1.5×0.5-1.2 mm, elliptic to suborbicular-obovate, entire, disc glands 5, scale like, 0.5 mm in diameter, stamens 5, unequal, outer 2 free, short, inner 3 usually united at least at the base, longer, filaments stout, anthers 0.3-0.5 mm long, longitudinally dehiscent. Female flowers with pedicels 3-7 mm long, slender, sepals 5-6, in 2 or 3 series, unequal, 1.5-1.8×0.8-1.2 mm, oblong-elliptic or suborbicular, disc glands 5-6, free, oblong or obovate, flattened, ovary 4-12 celled, smooth, c 1 mm across, styles free, bifid at the apex, lobes linear, revolute and connivent over the top of the ovary. Fruits globose to oblate, c 4-6 mm in diameter, smooth, baccate, ripe fruit black. Seeds trigonous, 1.5-2.0 mm long, faintly reticulate, brown.

Flowering and fruiting time: March-October (in Bangladesh).

Distribution: Africa, Cambodia, China, India, Indonesia, Laos, Malaysia, Australia, The Philippines, Sri Lanka, Thailand and Vietnam. In Bangladesh, this species is found throughout the country.

Origin: It is widespread in the Old World tropics, from tropical Africa to Pakistan, India, Sri Lanka, Burma (Myanmar), Indo-China, southern China, and Thailand and throughout the Malesian region towards northern Australia (Queensland) (Maroyi 2008).

Specimens examined: National Botanical Garden Mirpur, Dhaka, 25.5.2017, Md. Shahidur Rahman, Coll. no. 5, Accession no. 54670 (DACB); CRB and Tiger pass, Chittagong, 14.9.2016, Tajul, Owahid and Kawsar, Accession no. 50336 (DACB); Chittagong Hill Tracts, Kaptai, Sitapahad West Research Forest, 5.6.1969, M. S. Khan, Coll. no. 1679 (DUSH); Chittagong, St. Martin`s islands, 30.10.1963, M. S. Khan, Coll. no. 677 (DUSH); Dhaka Munshigonj, 10.5.1994, M. RahmanColl. no. 4 (DUSH).

3.1.6. *Phyllanthus urinaria* L., Sp. Pl.: 982 (1753)

Synonyms: *Phyllanthus leprocarpus* Wight: In: Icon. Pl. Ind. Orient. 5: t. 1895. 1852., *Diasperus urinaria* (L.) Kuntze: In: Revis. Gen. Pl. 2: 601. 1851., *Phyllanthus croizatii* Steyerm.: In: Fieldiana, Bot. 28: 317. 1952., *Phyllanthus verrucosus* Elmer, nom. illeg.: In: Leafl. Philipp. Bot. 7: 2649. 1915., *Phyllanthus urinaria* var. *laevis* Haines: In: Bot. Bihar Orissa 2: 125. 1921.

English name: Chamber Bitter, Gripeweed, Shatterstone, Stonebreaker.

Local name: Hazarmani, Kalochitki.

Description: This plant is an annual or sometimes perennial, erect or decumbent, glabrous or puberulous herb. Stem much branched at the base, branchlets 3-10 cm long, flattened, winged. Leaves simple, alternate, stipulate, stipules ovate-lanceolate, c 1.5 mm long, acuminate, base conspicuously auriculate, petiolate, petioles very short, c 0.5 mm long, lamina linear or oblong to oblong-obovate, 5-20×2-9 mm, sometimes slightly falcate, obtuse or acute and mucronulate at the apex, obtuse and sometimes conspicuously asymmetrical at the base, often with tooth-like hairs around the margin,

bright or dark green above, lateral veins 4-6 pairs, conspicuous, paler and grayish beneath. Flowers minute, grayish-yellow to reddish blotches, in axillary glomerules on deciduous branchlets. Male flowers pedicellate, pedicels c 0.5 mm long, auriculate above the middle, sepals 6, grayish-yellow, 0.3-0.6×0.2-0.4 mm, elliptic to oblong-obovate, obtuse at the apex, glabrous, disc glands 6, green, papillose, alternate with the sepals, stamens 3, filaments completely united into a slender column, anthers sessile but not fused together, erect, longitudinally dehiscent. Female flower solitary, in axillary on middle and lower part of branchlets, pedicellate, pedicels c 0.5 mm long, sepals 6, sub equal, 0.7-1.1×0.2-0.4 mm, oblong-lanceolate, obtuse or subacute, subglabrous, yellowish with a reddish-olive midrib, disc orbicular, flat, entire, ovary subglobose, 1 mm across, styles 3, free, appressed to the top of the ovary, bifid, lobes recurved. Fruit a capsule, globose, smooth, c 4 mm in diameter, with yellowish and scurfy-tuberculate, fruits sepals. Seeds triangular, 1.0-1.2×0.8-1.0 mm, transversely ridged, with 12-15 ridges on the back and sides, light grayish-brown.

Flowering and fruiting time: March-October (in Bangladesh).

Distribution: Cambodia, China, India, Laos, Indonesia, Japan, Malaysia, Sri Lanka and Vietnam. In Bangladesh, this species is found in Chattogram, Cox`s Bazar, Dhaka, Jashore, Rajshahi and Rangamati districts.

Origin: *P. urinaria* L. is native to tropical Asia but has spread and naturalized in the warm tropics and subtropics and is now prevalent worldwide (Pancho and Obien 1995).

Specimens examined: Botanical Garden, Department of Botany, University of Dhaka, Bangladesh, 25.5.2017, Md. Shahidur Rahman, Coll. no. 4, Accession no. 54669 (DACB); Dinajpur district, Singra forest, 12.10.1980, Huq, Rahman, Mia and Mahbuba H, Accession no. 12303 (DACB); Chittagong Hill Tracts Kathaltali, 15.6.1969, Alo Rani, Coll. no. 249 (DUSH); Chittagong Hill Tracts Rangapani, M. S. Khan, Coll. no. 1758 (DUSH).

Table 13. Comparative morphological analysis of five *Phyllanthus* L.

Morphology	*P. acidus*	*P. emblica* (small fruit form)	*P. emblica* (large fruit form)	*P. niruri*	*P. reticulatus*	*P. urinaria*
Habit	Tree	Tree	Tree	Herb	Shrub	Herb
Branching pattern	Robust, stout, leafless	Tawny pubescent	Tawny pubescent	Angular, less branched	Stout, much branched	Stem much branched
Morphology of bark	Very rough, hard, whitish gray	Smooth, thick, hard, blackish	Smooth, thin, hard, grayish	Smooth, thin, soft, light green	Rough, hard, thick, peeling or flaking, grayish-brown	Smooth, thin, soft, deep green
Morphology of leaves	Simple, distichous, alternate, quite entire, pinnate, petiolate, stipulate, upper side green and under side blue green, ovate to ovate lanceolate	Simple, distichous, alternate, quite entire, pinnate, sessile, petiolate, stipulate, upper side green and under side blue green, oblong or linear oblong	Simple, distichous, alternate, quite entire, pinnate, sessile, petiolate, stipulate, upper side yellowish and under side purplish green, oblong or linear oblong	Simple, alternate, quite entire, pinnate, petiolate, stipulate, dark green above, paler and grayish beneath, elliptic-oblong to elliptic-lanceolate	Simple, alternate, quite entire, pinnate, petiolate, stipulate, apex yellowish but base greenish	Simple, alternate, quite entire, pinnate, petiolate, stipulate, dark green above, paler and grayish beneath, linear or oblong to oblong-obovate
Morphology of flower	Minute, axillary, monoecious, reddish, male pedicellate, female flowers few and also pedicellate, red, stamens 4, free	Minute, axillary, monoecious, greenish white, male and female both pedicellate, stamens 3, filaments coherent into a central column	Minute, axillary, monoecious, greenish red, male and female both pedicellate, stamens 3, filaments coherent into a central column	Minute, axillary, monoecious, light yellowish, male pedicellate, female solitary	Minute, axillary, monoecious, yellowish red, male pedicellate, female solitary, stamens 5, ripe fruit black	Minute, axillary, monoecious, grayish-yellow to reddish blotches, male pedicellate, female solitary
Morphology of fruits	Drupe, fleshy, acidic, depressed-globose, smooth, 6-8 lobed, greenish-yellow to whitish	Drupe, fleshy, sub globose, smooth, greenish, A fruit size average 2.2 cm and weight 5.33 gm	Drupe, fleshy, sub globose, smooth, greenish or yellowish to whitish, A fruit size average 3.2 cm and weight 18.8 gm	Capsule of 3 crustaceous, trilobate-sub globose, smooth, greenish, fruiting sepals, fruit trilobate-subglobose	Berry with 4-8 cells, globose to oblate, smooth, baccate black with dark purplish at maturity	Capsule, globose, smooth, with reddish blotches and tuberculate, yellowish and fruiting sepals, fruit globose
Morphology of seeds	Smooth, trigonous or convex, apex and base truncate, light brown	Smooth, trigonous or plano-convex, chestnut-brown	Smooth, trigonous or plano -convex, light-brown	Ochreous-fulvous, light grayish-brown	Seed trigonous, faintly reticulate, brown	Triangular, light grayish-brown
Flowering and fruiting time	May-June	March-September	January-March	August-October	March-October	March-October

3.1.7. Key to species the genus *Phyllanthus* L. (for five species under study)

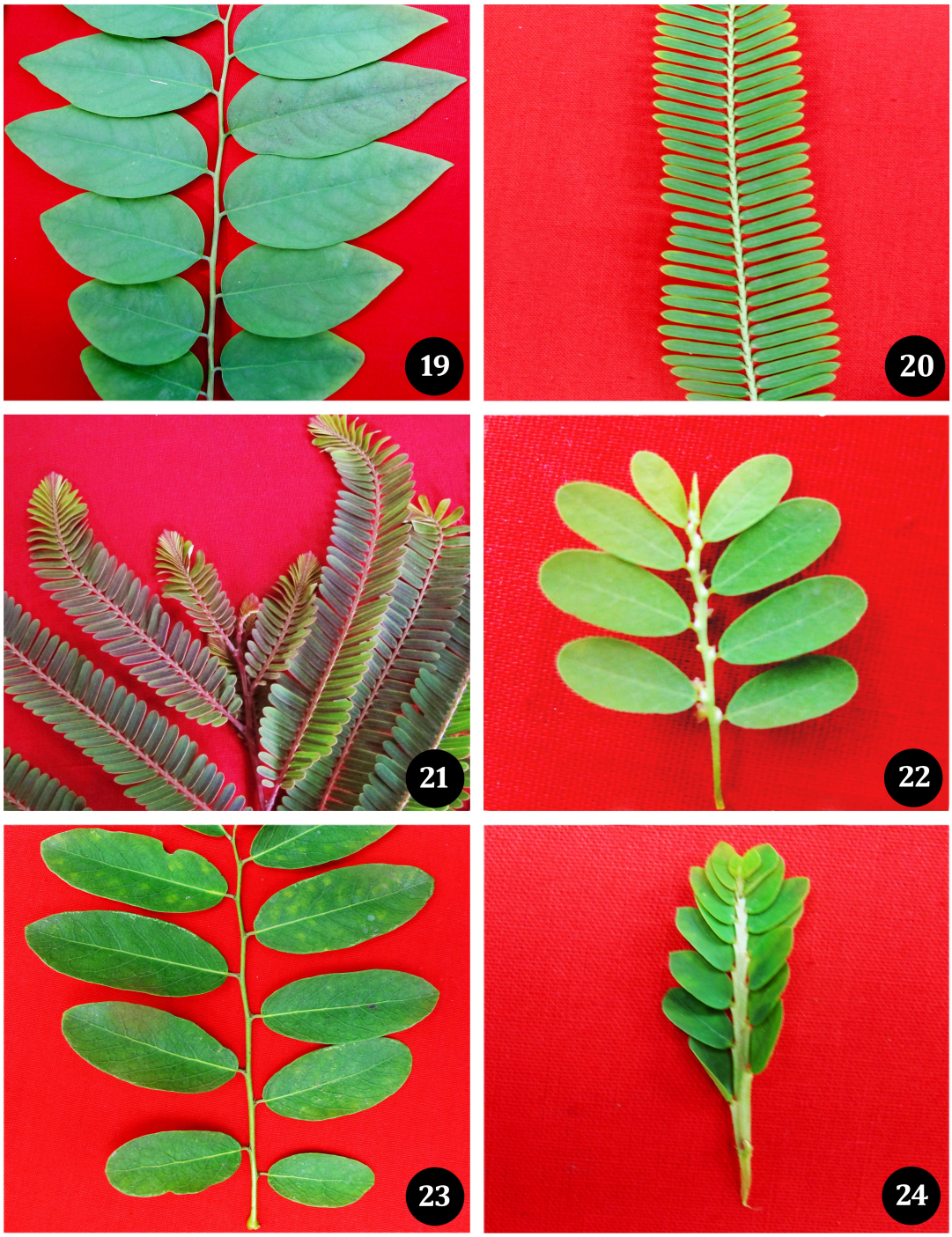
A key to species was prepared based on the external morphology and ploidy level-

1. Plants tree..... 2
- Plants herbs or shrubs..... 3
2. Lamina ovate to ovate-lanceolate; flowers red, stamens 4, free; fruits drupe, depressed-globose. Plant diploid..... *P. acidus*
- Lamina oblong or linear-oblong; greenish white or greenish red; stamens 3, filaments coherent into a central column. Plant polyploid ..... *P. emblica*
3. Plants shrubs; stamens 5; ripe fruit black. Plant diploid..... *P. reticulatus*
- Plant herbs; stamens 3; fruit greenish or yellowish..... 4
4. Lamina elliptic-oblong to elliptic-lanceolate; fruit trilobate-subglobose. Plant diploid..... *P. niruri*
- Lamina linear or oblong to oblong-obovate; fruit globose. Plant polyploid..... *P. urinaria*

Figs. 1-6. Habit of five species of *Phyllanthus* L. 1. *P. acidus* (L.) Skeels, 2. *P. emblica* L. (small fruit form), 3. *P. emblica* L. (large fruit form), 4. *P. niruri* L., 5. *P. reticulatus* Poir. and 6. *P. urinaria* L.

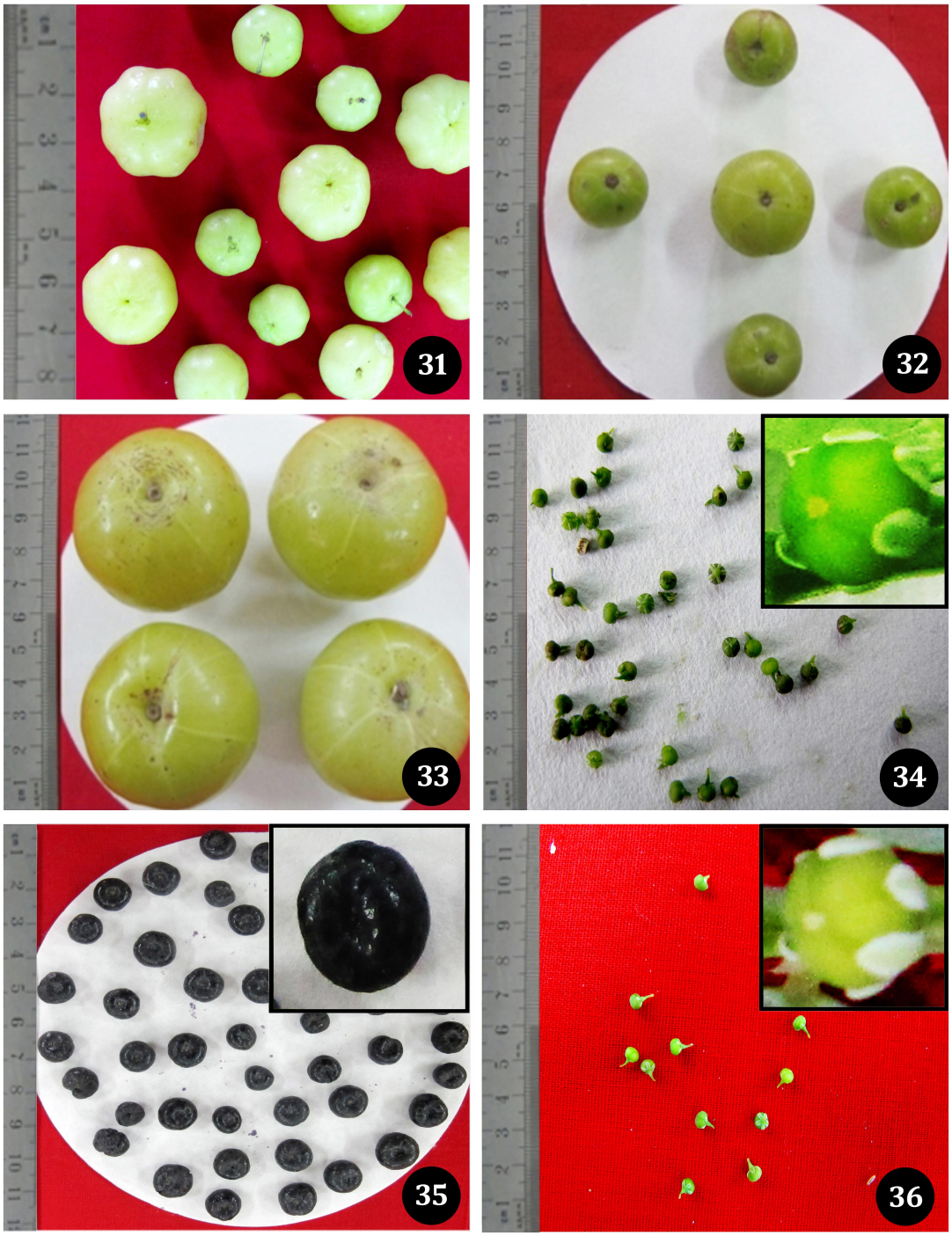
Figs. 7-12. Branching pattern of five species of *Phyllanthus* L. 7. *P. acidus* (L.) Skeels, 8. *P. emblica* L. (small fruit form), 9. *P. emblica* L. (large fruit form), 10. *P. niruri* L., 11. *P. reticulatus* Poir. and 12. *P. urinaria* L.

Figs. 13-18. Morphology of bark of five species of *Phyllanthus* L. 13. *P. acidus* (L.) Skeels, 14. *P. emblica* L. (small fruit form), 15. *P. emblica* L. (large fruit form), 16. *P. niruri* L., 17. *P. reticulatus* Poir. and 18. *P. urinaria* L.

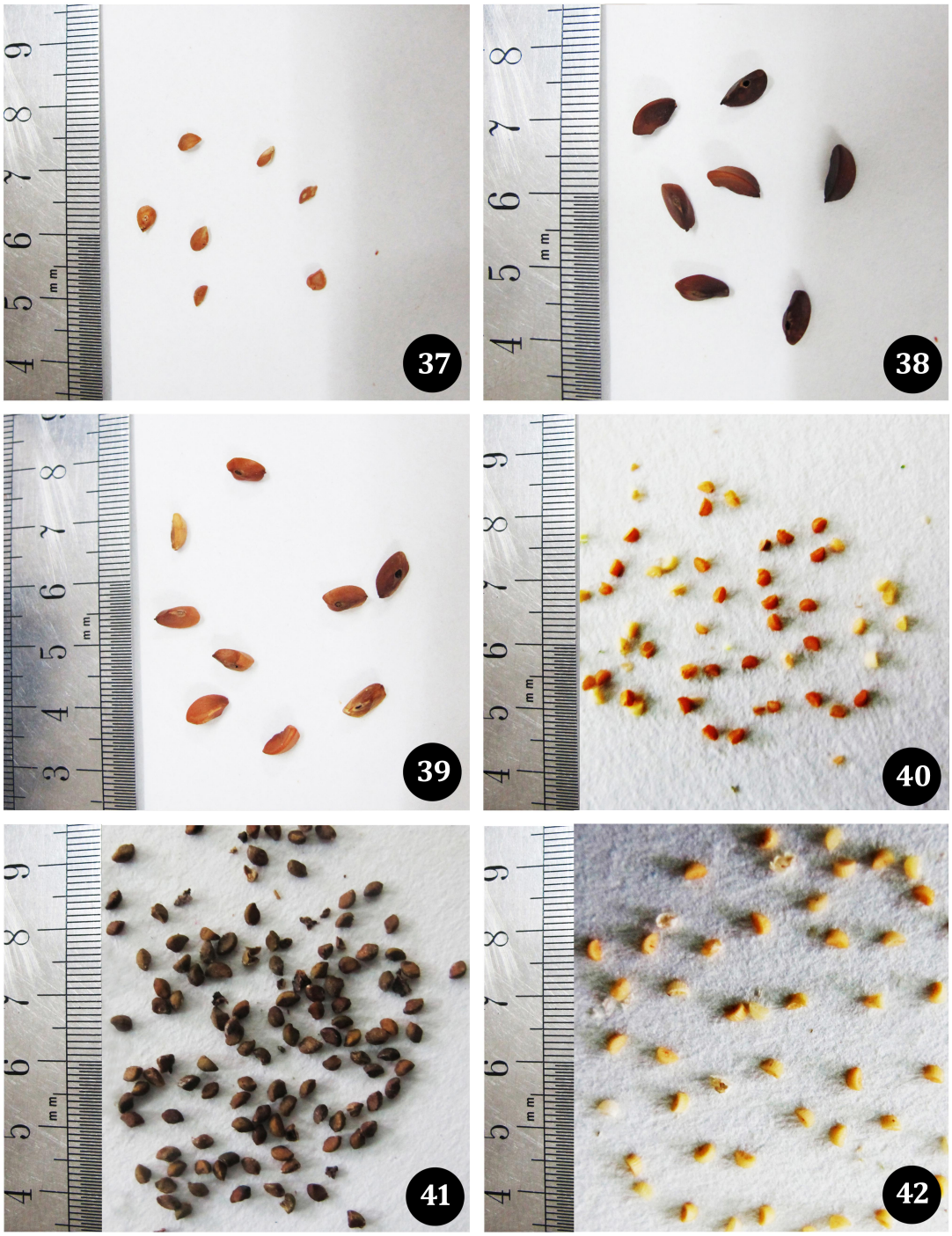


Figs. 19-24. Morphology of leaves of five species of *Phyllanthus* L. 19. *P. acidus* (L.) Skeels, 20. *P. emblica* L. (small fruit form), 21. *P. emblica* L. (large fruit form), 22. *P. niruri* L., 23. *P. reticulatus* Poir. and 24. *P. urinaria* L.

Figs. 31-36. Morphology of fruits of five species of *Phyllanthus* L. 31. *P. acidus* (L.) Skeels, 32. *P. emblica* L. (small fruit form), 33. *P. emblica* L. (large fruit form), 34. *P. niruri* L. (Larger image in inset), 35. *P. reticulatus* Poir. (Larger image in inset) and 36. *P. urinaria* L. (Larger image in inset).



Figs. 31-36. Morphology of fruits of five species of *Phyllanthus* L. 31. *P. acidus* (L.) Skeels, 32. *P. emblica* L. (small fruit form), 33. *P. emblica* L. (large fruit form), 34. *P. niruri* L. (Larger image in inset), 35. *P. reticulatus* Poir. (Larger image in inset) and 36. *P. urinaria* L. (Larger image in inset).



Figs. 37-42. Morphology of seeds of five species of *Phyllanthus* L. 37. *P. acidus* (L.) Skeels, 38. *P. emblica* L. (small fruit form), 39. *P. emblica* L. (large fruit form), 40. *P. niruri* L., 41. *P. reticulatus* Poir. and 42. *P. urinaria* L.

3.2. Cytogenetical features of five species of *Phyllanthus* L.

3.2.1. Appropriate season for obtaining maximum number of dividing cells from the root-tips

Although a few dividing cells was observed throughout the year, maximum number of dividing cells were found in the root tip during May to October (about 60%). The number of dividing cells was very poor in extreme high or low temperature.

3.2.2. Appropriate time for obtaining maximum number of dividing cells from the root tips

Root tips were collected from 8.30 a.m. with 30 min interval *viz*. 9.00, 9.30, 10.00, 10.30, 11.00 and 11.30 a.m. It was found that 10.00 a.m. to 11.00 a.m. was the optimum time for obtaining maximum number of dividing cells (about 60%).

3.2.3. Appropriate chemical for pretreatment

Different chemicals like 8-hydroxyquinoline, colchicine and para dichloro benzene (PDB) were tried for pretreatment to find out proper contraction and intact morphology of chromosomes. It was found that only 8-hydroxyquinoline solution (0.002 M) for 20 min and 45 min gave the best result for orcein staining and fluorescent banding, respectively.

3.2.4. Karyotypes

On the basis of overall length and centromeric position, somatic chromosomes of *P. acidus, P. niruri* and *P. reticulatus* could be assembled in 13 pairs (Figs. 97, 100, 101, Tables 14, 17, 18). In contrast, somatic chromosomes of *P. emblica* (both small fruit form and large fruit form) and *P. urinaria* could be assembled in 50 and 24 pairs, respectively (Figs. 98, 99, 102, Tables 15, 16, 19).

3.3. Mitotic interphase nuclei

For every staining, at least 50 interphase nuclei were observed in each species of *Phyllanthus* L.

3.3.1. Orcein staining

The studied species of *Phyllanthus* L. species were found to possess a prominent nucleolus in the interphase nuclei after orcein-staining except *P. niruri* (Figs. 43-45, 47, 48, arrow). Few darkly stained large heterochromatic blocks were observed in the nucleus of *P. acidus* (Fig. 43). On the other hand, *P. emblica* (both small and large fruit form) and *P. reticulatus* were found to possess several darkly stained small heterochromatic regions in the nucleus (Figs. 44, 45, 47). In contrast, some faintly stained heterochromatic regions were present in interphase nuclei of *P. niruri* and *P. urinaria* (Figs. 46, 48).

3.3.2. CMA-staining

A conspicuous non-staining region was observed in the interphase nuclei of the studied species of *Phyllanthus* L. species after CMA-staining except *P. niruri* (Figs. 49, 50, 51, 53, 54, arrow). Numerous CMA-positive bands were scatteredly located in the interphase nuclei of *P. acidus.* Three prominent large CMA-bands were found in interphase nuclei of *P. emblica* (small fruit form). Several CMA-bands were located at the peripheral region of the nucleus of *P. emblica* (large fruit form) of which two bands were relatively bigger. The number of CMA-bands in the interphase nuclei of *P. niruri* are more or less corresponds to the somatic chromosome number (2n=26). In *P. reticulatus*, three bigger CMA-stained blocks were present in the interphase nuclei. Interphase nuclei of *P. urinaria* were homogenously stained with CMA. However, the peripheral regions of interphase nuclei were brightly fluoresced in *P. urinaria* (Fig. 54).

3.3.3. DAPI-staining

A conspicuous non-staining region was observed in the interphase nuclei of the studied species of *Phyllanthus* L. species after DAPI-staining except *P. niruri* and *P. emblica*

(large fruit form) (Figs. 55, 56, 59, 60, arrow). Several DAPI-bands were located at the peripheral region of the nucleus of *P. acidus* of which three bands were relatively bigger. Numerous smaller and brightly stained regions was scattered around the interphase nuclei of *P. emblica* (both small and large fruit form) (Figs. 56, 57). The number of DAPI-bands in the interphase nuclei of *P. niruri* are more or less corresponds to the somatic chromosome number (2n=26). Few bigger brightly DAPI-fluoresced areas was found at the peripheral regions of interphase nuclei of *P. reticulatus* (Fig. 59). Several small bands were located in the interphase nuclei of *P. urinaria* after DAPI-banding (Fig. 60).

3.4. Mitotic prophase chromosomes

For every staining, at least 50 prophase stages were observed for each *Phyllanthus* L. species.

3.4.1. Orcein staining

In *P. acidus* and *P. niruri,* most of the prophase chromosomes were stained homogenously along the entire length (Figs. 61, 64). The prophase chromosomes of *P. emblica* (both small and large fruit form), *P. reticulatus* and *P. urinaria* stained only at the interstitial regions (Figs. 62, 63, 65, 66). A nucleolus was found in almost every prophase stage of these studied species (Figs. 61-66, arrow).

3.4.2. CMA-staining

Terminal portions of some chromosomes of *P. acidus* were brightly fluoresced whereas the rest of the chromosomes were homogeneously stained with CMA. In addition, 2 dot like prominent CMA-positive bands were also observed in this species (Fig. 67). Numerous dot like CMA-positive bands were scatteredly located in the interphase nuclei of *P. emblica* (both small and large fruit form) and *P. urinaria* (Figs. 68, 69, 72). In *P. niruri*, most of the chromosomes were homogeneously stained with CMA (Fig. 70). In contrast interstitial portions of prophase chromosomes were brightly fluoresced with CMA in *P. reticulatus* (Fig. 71).

3.4.3. DAPI-staining

In *P. acidus* and *P. niruri*, most of the chromosomes were homogeneously stained with DAPI (Figs. 73, 76). Numerous dot like DAPI-positive bands were scatteredly located in the interphase nuclei of *P. emblica* (small fruit form) (Fig. 74). A number of DAPI-positive bands were found at different location of prophase chromosomes in *P. emblica* (large fruit form) (Fig. 75). On the other hand, interstitial portions of prophase chromosomes were brightly fluoresced with DAPI in *P. reticulatus* (Fig. 77). However, no prominent DAPI-positive bands were observed in the prophase chromosomes of *P. urinaria* (Fig. 78).

3.5. Mitotic metaphase chromosomes

For every staining, at least 50 metaphase cells were observed for each *Phyllanthus* L. species.

3.5.1. Orcein-staining

Total length, arm ratio, centromeric index, relative length and centromeric type of each chromosome of *Phyllanthus* L. species were tabulated (Tables 14-19).

The six species of *Phyllanthus* L. species were found to possess different somatic chromosome numbers and other karyomorphological features (Figs. 79-84, Table 20). However, no satellite was found after orcein staining in the studied species except *P. acidus*. The conventional karyotype features of each species were described below:

In this study, 2n=26 chromosomes were found in the somatic cells of *P. acidus*. The total length of the chromosome complements was 129.45(±1.19) µm. The relative length of chromosome and individual chromosomal length were ranging from 0.03 to 0.05 and 3.86(±0.11) µm to 6.33(±0.19) µm, respectively (Table 14). The value of centromeric index was varied from 26.92 to 47.67. The centromeric formula of this species is 12m+14sm (Figs. 79, 97, Table 20). A pair of satellite (about 0.1 µm) was observed on the short arm of both member of chromosome pair 13 (Figs. 79, 97, arrow).

Phyllanthus emblica (small fruit form) was found to possess 2n=100 (in 90% cell) somatic chromosomes. Besides 2n=100 somatic chromosome numbers, 2n=78 (in 6% cell) and 2n=104 (in 4% cell) were also found in few mitotic metaphase stage which was not significant. The total length of diploid complements in this species was 140.03(±2.20) μm (Table 15). The relative length of individual chromosome ranged from 0.01 to 0.02 whereas individual chromosomal length was ranging from 0.99(±0.06) μm to 2.30(±0.12) μm (Tables 15, 20). The centromeric position was so indistinct due to smaller chromosome size. As a result, the length of short arm, length of long arm, centromeric index and centromeric formulae could not be determined (Figs. 80, 98, Table 15).

2n=100 (in 95.56% cell) chromosomes were also observed in the somatic cells of *P. emblica* (large fruit form). Besides 2n=100 somatic chromosome numbers, 2n=90 (in 4.44% cell) was also found in few mitotic metaphase stage which was not significant. The total length of 2n chromosome complements of this species was 149.02(±0.88) μm (Tables 16, 20). This length was the largest among the five species. The relative length of the individual chromosomes was 0.01 and individual chromosome length from 0.95(±0.09) μm to 2.19(±0.16) μm (Tables 16, 20). The centromeric position could not be detected due to its smaller chromosome size. Therefore, the length of short arm, length of long arm, centromeric index and centromeric formulae could not be prepared (Figs. 81, 99, Table 16).

The somatic cells of *P. niruri* had 2n=26 chromosomes. The total length of the chromosome complements was 50.26(±0.45) μm. This length was the shortest among the five species. The relative length of chromosome and individual chromosomal length were ranging from 0.03 to 0.05 and 1.27(±0.15) μm to 2.58(±0.16) μm, respectively (Tables 17, 20). The value of centromeric index was varied from 26.59 to 50.00. The centromeric formula of this species is 20m+6sm (Figs. 82, 100, Table 20).

Phyllanthus reticulatus was found to possess 2n=26 somatic chromosomes. The total length of diploid complements in this species was 107.61(±0.29) μm (Tables 18, 20). The relative length of individual chromosome ranged from 0.03 to 0.05 whereas

individual chromosomal length was ranging from 2.93(±0.20) µm to 5.29(±0.33) µm (Table 18). The value of centromeric index was varied from 27.84 to 44.77. The centromeric formula of this species is 4m+22sm (Figs. 83, 101, Table 20).

2n=48 chromosomes (in 90% cell) were also observed in the somatic cells of *P. urinaria.* Besides 2n=48 somatic chromosome numbers, 2n=50 (in 5% cell) and 2n=52 (in 5% cell) were also found in few mitotic metaphase stage. The total length of 2n chromosome complements of this species was 74.98(±0.74) µm (Tables 19, 20). The range of relative length of the individual chromosomes was 0.01-0.03 and individual chromosome length from 1.01(±0.08) µm to 2.51(±0.17) µm (Table 19). The centromeric position could not be detected due to its smaller chromosome size. Therefore, the length of short arm, length of long arm, centromeric index and centromeric formulae could not be determined (Figs. 84, 102, Tables 19, 20).

3.7.2. CMA-staining

In *P. acidus*, three CMA-positive bands were observed. Two CMA-bands were found at terminal portion on short arm in both the members of chromosome pair-5 (Figs. 85, 103). However, heteromorphicity in respect of banding pattern was found in pair-7 where a member did not show any CMA-bands while its homologue member had terminal CMA-positive bands on short arm. The total length of CMA banded region was 3.13 µm, which occupied about 3.69% of the total chromatin length. The CMA banded karyotype formula of this species was determined as 3β+23θ (Table 22).

No CMA-positive bright bands were found in *P. emblica* (small fruit form) after CMA-staining (Figs. 86, 104, Table 22). Whole chromosomal portion of all the chromosomes were fluoresced homogeneously with CMA fluorochrome. The CMA-banded karyotype formula of this form was 100θ (Table 22).

Highest number of CMA-positive bands (15) were found in *P. emblica* L. (large fruit form) (Figs. 87, 105, Table 22) and the CMA-banded region was 24.31 µm, which covered about 12.19% of the total chromatin length. Ten centromeric CMA-bands were observed in both chromosomes on the short arm of pair-2, 15, 34, 43 and 47. In

contrast, four terminal CMA-positive bands were found in the chromosome pair-17 and 23 (Fig. 105). Heteromorphicity in respect of banding pattern was observed in chromosome pair-4. In pair-4, whole chromosomal portion of one homologue was fluoresced with CMA whereas the other homologue did not show any CMA-band (Fig. 105). The CMA-banded karyotype formula of this form of *P. emblica* was 10α+4β+1γ+85θ (Table 22).

Three CMA-positive bands were observed *P. niruri*. Two CMA-bands were found adjacent to the centromeric portion in both the members of chromosome pair-5 (Figs. 88, 106). However, heteromorphicity in respect of banding pattern was found in pair-11 where a member did not show any CMA-bands while its homologue member had terminal CMA-positive bands on short arm. The total length of CMA banded region was 1.73 µm, which occupied about 2.71% of the total chromatin length. The CMA banded karyotype formula of this species was determined as 2α+1β+23θ (Table 22).

In *P. reticulatus*, four CMA-positive bright bands were observed of which two were at the terminal portions on short arm of both the members of chromosome pair-1. Centromeric portions of both the homologue member of chromosome pair-5 showed CMA-band (Figs. 89, 107, Table 22). The total length of CMA banded region was 2.69 µm which occupied about 3.21% of the total chromatin length. The CMA banded karyotype formula of this species was 2α+2β+22θ (Table 22).

In total 13 CMA-positive bands were observed in *P. urinaria.* The entire chromosomal portion of both members of pair-4, 6, 17 and 20 were fluoresced with CMA fluorochrome (Figs. 90, 108). Two CMA-positive terminal bands were found on short arm of both the members of chromosome pair-7 (Fig. 108). Both the members of pair-21 had CMA-positive banded region in centromeric portion. In pair-23, heteromorphicity regarding banding pattern was observed where one member had terminal CMA-bands on short arm and the other one did not show any band (Fig. 108). The total length of CMA-banded region was 17.62 µm that covered about 19.09% of total chromatin length. The CMA- banded karyotype formula of this species was 2α+3β+8γ+35θ (Table 22).

3.7.3. DAPI-staining

In *P. acidus*, two DAPI-positive bands were observed at centromeric portion in both the members of chromosome pair 9 (Figs. 91, 109). The total length of DAPI banded region was 1.91 μm, which occupied about 1.48% of the total chromatin length. The CMA banded karyotype formula of this species was determined as 2α+24θ (Table 23).

Six DAPI-positive bright bands were found in *P. emblica* (small fruit form) after DAPI-staining (Fig. 110, Table 23). The entire chromosomal portion of both members of pair-23 and 27 were fluoresced with DAPI fluorochrome (Fig. 110). Two DAPI-positive terminal bands were found on short arm of both the members of chromosome pair-1 (Figs. 92, 110). The total length of DAPI banded region was 8.37 μm, which occupied about 4.16% of the total chromatin length. The DAPI-banded karyotype formula of this form of *P. emblica* was 2β+4γ+94θ (Table 23).

Seven DAPI-positive bands were present in *P. emblica* (large fruit form). Both the members of pair-21, 33, 45 and one member of pair 50 were found to possess centromeric DAPI-positive bands (Figs. 93, 111, Table 23). Heteromorphicity in respect of banding pattern was observed in chromosome pair 50 where one member did not show any band and other member possessed centromeric band. The DAPI-banded region was 1.17 μm, which covered about 2.27% of the total chromatin. The DAPI-banded karyotype formula of this form was 7α+93θ (Table 23).

In *P. niruri*, the highest number of DAPI-positive bands (26) was observed. All the 26 somatic chromosomes of this species showed centromeric DAPI-positive bands (Figs. 94, 112). The total length of DAPI banded region was the highest in this species *i.e.* 18.84 μm, which occupied about 28.5% of the total chromatin length. The DAPI banded karyotype formula of this species was determined as 26α (Table 23).

In *P. reticulatus*, four DAPI-positive bright bands were observed at the terminal portions on short arm of both the members of chromosome pair 1 and 4 (Figs. 95, 113, Table 23). The total length of DAPI banded region was 3.10 μm which occupied about

4.05% of the total chromatin length. The DAPI banded karyotype formula of this species was 4β+22θ (Table 23).

In total 7 DAPI-positive bands were observed in *P. urinaria*. Both the members of pair-2 had CMA-positive banded region in centromeric portion. Two DAPI-positive terminal bands were found on short arm of both the members of chromosome pair-3 (Figs. 96, 114). Both the members of pair-8 were found to possess DAPI-positive banded region in two-third portion in the terminal region of the long arm. In pair-22, heteromorphicity regarding banding pattern was observed where one member had terminal CMA-bands on short arm and the other one did not show any band (Fig. 114). The total length of DAPI-banded region was 24.50 μm that covered about 21.05% of total chromatin length. The DAPI- banded karyotype formula of this species was 2α+3β+2δ+41θ (Table 23).

3.8. Meiotic behavior of *P. emblica* L. (both small and large fruit form)

Phyllanthus emblica (both small and large fruit form) showed peculiar behavior at meiotic cell division. Some meiotic metaphase stage showed all bivalents (IIs), some possess both bivalents and irregular multivalent while other had only multivalent (Figs. 115-120, arrows). However, these chromosomes were segregated regularly during meiotic Anaphase-I (Figs. 121-123).

3.9. Pollen viability

Pollens were full and well-stained with orcein and showed about 98% viability in both small and large fruited species of *P. emblica* and *P. urinaria* (Figs. 124-126).

Table 14. Length (µm), arm ratio, relative length, centromeric index and centromeric type of metaphase chromosomes of *Phyllanthus acidus* (L.) Skeels

Chrom-osome pair	Long arm (l) µm(±SD)	Short arm (s) µm(±SD)	Total length (T) µm(±SD)	Arm ratio l/s	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
I	4.60(±0.60)	1.73(±0.12)	6.33(±0.19)	2.66	0.05	27.33	sm
	4.37(±0.70)	1.61(±0.16)	5.98(±0.16)	2.71	0.04	26.92	sm
II	3.68(±0.26)	2.30(±0.15)	5.98(±0.33)	1.60	0.04	38.46	sm
	4.00(±0.12)	1.75(±0.17)	5.75(±0.25)	2.29	0.04	30.43	sm
III	3.35(±0.90)	2.29(±0.13)	5.64(±0.60)	1.46	0.04	40.40	m
	2.88(±0.81)	2.65(±0.36)	5.53(±0.61)	1.09	0.04	47.92	m
IV	3.53(±0.50)	2.00(±0.25)	5.53(±0.47)	1.76	0.04	36.17	sm
	3.91(±0.60)	1.61(±0.14)	5.52(±0.11)	2.43	0.04	29.17	sm
V	3.68(±0.35)	1.61(±0.21)	5.29(±0.15)	2.29	0.04	30.43	sm
	3.18(±0.35)	2.00(±0.22)	5.18(±0.41)	1.59	0.04	38.61	sm
VI	3.68(±0.81)	1.38(±0.14)	5.06(±0.40)	2.67	0.04	27.27	sm
	3.17(±0.61)	1.79(±0.36)	4.96(±0.37)	1.77	0.04	36.09	sm
VII	2.99(±0.60)	1.96(±0.14)	4.95(±0.85)	1.53	0.04	39.60	sm
	3.45(±0.32)	1.50(±0.64)	4.95(±0.33)	2.30	0.04	30.30	sm
VIII	2.90(±0.28)	1.93(±0.17)	4.83(±0.72)	1.50	0.04	39.96	m
	2.42(±0.32)	2.19(±0.18)	4.61(±0.81)	1.11	0.04	47.51	m
IX	2.52(±0.24)	2.08(±0.40)	4.60(±0.63)	1.21	0.04	45.22	m
	2.52(±0.24)	2.08(±0.40)	4.60(±0.63)	1.21	0.04	45.22	m
X	2.52(±0.24)	2.08(±0.40)	4.60(±0.63)	1.21	0.04	45.22	m
	2.38(±0.18)	2.11(±0.80)	4.49(±0.52)	1.28	0.03	47.00	m
XI	2.53(±0.67)	1.84(±0.09)	4.37(±0.41)	1.38	0.03	42.10	m
	2.53(±0.64)	1.84(±0.25)	4.37(±0.62)	1.38	0.03	42.10	m
XII	3.03(±0.72)	1.34(±0.06)	4.37(±0.63)	2.26	0.03	30.66	sm
	2.80(±0.20)	1.39(±0.62)	4.19(±0.23)	2.01	0.03	33.17	sm
XIII	2.33(±0.16)	1.68(±0.60) *1.00(±0.36)	3.91(±0.55)	1.38	0.03	42.97	m
	2.02(±0.74)	1.84(±0.25) *1.00(±0.25)	3.86(±0.11)	1.10	0.03	47.67	m
GT=129.45(±1.19)							

m=metacentric chromosome, sm=submetacentric chromosome

* Length of satellite

Table 15. Total Length (µm) and relative length of metaphase chromosomes of *Phyllanthus emblica* L. (small fruit form)

Chromosome pair	Total length (T) µm(±SD)	Relative length (RL)
I	2.30(±0.12)	0.02
	2.07(±0.13)	0.01
II	2.07(±0.14)	0.01
	2.07(±0.13)	0.01
III	2.05(±0.21)	0.01
	1.84(±0.22)	0.01
IV	1.84(±0.23)	0.01
	1.66(±0.24)	0.01
V	1.66(±0.19)	0.01
	1.66(±0.20)	0.01
VI	1.66(±0.25)	0.01
	1.66(±0.26)	0.01
VII	1.62(±0.28)	0.01
	1.62(±0.29)	0.01
VIII	1.62(±0.27)	0.01
	1.62(±0.25)	0.01
IX	1.62(±0.21)	0.01
	1.62(±0.31)	0.01
X	1.62(±0.24)	0.01
	1.62(±0.23)	0.01
XI	1.61(±0.13)	0.01
	1.61(±0.28)	0.01
XII	1.61(±0.31)	0.01
	1.56(±0.38)	0.01
XIII	1.56(±0.25)	0.01
	1.56(±0.32)	0.01

Table continued

Chromosome pair	Total length (T) µm(±SD)	Relative length (RL)
XIV	1.56(±0.27)	0.01
	1.50(±0.24)	0.01
XV	1.50(±0.27)	0.01
	1.50(±0.26)	0.01
XVI	1.50(±0.24)	0.01
	1.50(±0.21)	0.01
XVII	1.50(±0.18)	0.01
	1.50(±0.17)	0.01
XXVIII	1.50(±0.15)	0.01
	1.50(±0.16)	0.01
XIX	1.50(±0.13)	0.01
	1.43(±0.12)	0.01
XX	1.43(±0.19)	0.01
	1.43(±0.08)	0.01
XXI	1.43(±0.31)	0.01
	1.43(±0.14)	0.01
XXII	1.41(±0.07)	0.01
	1.41(±0.06)	0.01
XXIII	1.41(±0.08)	0.01
	1.41(±0.07)	0.01
XXIV	1.41(±0.04)	0.01
	1.41(±0.32)	0.01
XXV	1.41(±0.24)	0.01
	1.41(±0.23)	0.01
XXVI	1.41(±0.25)	0.01
	1.40(±0.27)	0.01
XXVII	1.40(±0.31)	0.01
	1.34(±0.41)	0.01

Table continued

Chromosome pair	Total length (T) µm(±SD)	Relative length (RL)
XXVIII	1.32(±0.22)	0.01
	1.32(±0.23)	0.01
XXIX	1.32(±0.24)	0.01
	1.32(±0.29)	0.01
XXX	1.27(±0.31)	0.01
	1.27(±0.41)	0.01
XXXI	1.22(±0.16)	0.01
	1.22(±0.22)	0.01
XXXII	1.22(±0.24)	0.01
	1.22(±0.25)	0.01
XXXIII	1.22(±0.34)	0.01
	1.22(±0.27)	0.01
XXXIV	1.22(±0.29)	0.01
	1.22(±0.26)	0.01
XXXV	1.22(±0.37)	0.01
	1.22(±0.61)	0.01
XXXVI	1.22(±0.55)	0.01
	1.22(±0.34)	0.01
XXXVII	1.22(±0.16)	0.01
	1.22(±0.25)	0.01
XXXVIII	1.22(±0.25)	0.01
	1.22(±0.13)	0.01
XXXIX	1.22(±0.09)	0.01
	1.22(±0.12)	0.01
XL	1.22(±0.04)	0.01
	1.22(±0.06)	0.01

Table continued

Chromosome pair	Total length (T) µm(±SD)	Relative length (RL)
XLI	1.22(±0.02)	0.01
	1.15(±0.03)	0.01
XLII	1.15(±0.01)	0.01
	1.15(±0.05)	0.01
XLIII	1.15(±0.03)	0.01
	1.15(±0.04)	0.01
XLIV	1.15(±0.07)	0.01
	1.15(±0.06)	0.01
XLV	1.15(±0.04)	0.01
	1.15(±0.04)	0.01
XLVI	1.15(±0.05)	0.01
	1.16(±0.07)	0.01
XLVII	1.09(±0.03)	0.01
	1.09(±0.05)	0.01
XLVIII	1.09(±0.09)	0.01
	1.04(±0.04)	0.01
XLIX	1.04(±0.07)	0.01
	1.02(±0.06)	0.01
L	1.02(±0.08)	0.01
	0.99(±0.06)	0.01
GT=140.03(±2.20)		

Table 16. Total Length (µm) and relative length of metaphase chromosomes of *Phyllanthus emblica* L. (large fruit form)

Chromosome pair	Total length (T) µm(±SD)	Relative length (RL)
I	2.19(±0.16)	0.01
	2.19(±0.13)	0.01
II	2.19(±0.57)	0.01
	1.84(±0.96)	0.01
III	1.84(±0.14)	0.01
	1.84(±0.15)	0.01
IV	1.84(±0.81)	0.01
	1.84(±0.77)	0.01
V	1.84(±0.13)	0.01
	1.84(±0.32)	0.01
VI	1.84(±0.22)	0.01
	1.84(±0.16)	0.01
VII	1.84(±0.33)	0.01
	1.84(±0.61)	0.01
VIII	1.84(±0.19)	0.01
	1.79(±0.70)	0.01
IX	1.79(±0.56)	0.01
	1.61(±0.61)	0.01
X	1.61(±0.82)	0.01
	1.61(±0.20)	0.01
XI	1.61(±0.50)	0.01
	1.61(±0.49)	0.01
XII	1.61(±0.44)	0.01
	1.61(±0.10)	0.01
XIII	1.61(±0.60)	0.01
	1.61(±0.99)	0.01

Table continued

Chromosome pair	Total length (T) µm(±SD)	Relative length (RL)
XIV	1.61(±0.77)	0.01
	1.61(±0.19)	0.01
XV	1.61(±0.17)	0.01
	1.61(±0.44)	0.01
XVI	1.61(±0.44)	0.01
	1.59(±0.16)	0.01
XVII	1.56(±0.77)	0.01
	1.56(±0.33)	0.01
XXVIII	1.56(±0.45)	0.01
	1.56(±0.66)	0.01
XIX	1.56(±0.16)	0.01
	1.56(±0.31)	0.01
XX	1.56(±0.41)	0.01
	1.56(±0.51)	0.01
XXI	1.56(±0.85)	0.01
	1.56(±0.61)	0.01
XXII	1.56(±0.61)	0.01
	1.56(±0.13)	0.01
XXIII	1.56(±0.61)	0.01
	1.56(±0.71)	0.01
XXIV	1.56(±0.31)	0.01
	1.56(±0.90)	0.01
XXV	1.56(±0.51)	0.01
	1.56(±0.17)	0.01
XXVI	1.56(±0.60)	0.01
	1.45(±0.16)	0.01
XXVII	1.40(±0.19)	0.01
	1.40(±0.71)	0.01

Table continued

Chromosome pair	Total length (T) µm(±SD)	Relative length (RL)
XXVIII	1.40(±0.81)	0.01
	1.40(±0.61)	0.01
XXIX	1.40(±0.51)	0.01
	1.40(±0.25)	0.01
XXX	1.40(±0.19)	0.01
	1.40(±0.13)	0.01
XXXI	1.40(±0.17)	0.01
	1.40(±0.12)	0.01
XXXII	1.40(±0.17)	0.01
	1.40(±0.18)	0.01
XXXIII	1.40(±0.11)	0.01
	1.40(±0.91)	0.01
XXXIV	1.38(±0.61)	0.01
	1.38(±0.35)	0.01
XXXV	1.38(±0.18)	0.01
	1.38(±0.17)	0.01
XXXVI	1.38(±0.18)	0.01
	1.38(±0.16)	0.01
XXXVII	1.38(±0.31)	0.01
	1.27(±0.21)	0.01
XXXVIII	1.27(±0.19)	0.01
	1.27(±0.16)	0.01
XXXIX	1.27(±0.17)	0.01
	1.27(±0.14)	0.01
XL	1.27(±0.17)	0.01
	1.27(±0.18)	0.01

Table continued

Chromosome pair	Total length (T) µm(±SD)	Relative length (RL)
XLI	1.27(±0.19)	0.01
	1.27(±0.16)	0.01
XLII	1.27(±0.21)	0.01
	1.27(±0.13)	0.01
XLIII	1.27(±0.18)	0.01
	1.27(±0.70)	0.01
XLIV	1.20(±0.12)	0.01
	1.20(±0.05)	0.01
XLV	1.16(±0.18)	0.01
	1.16(±0.70)	0.01
XLVI	1.16(±0.31)	0.01
	1.16(±0.16)	0.01
XLVII	1.16(±0.13)	0.01
	1.15(±0.03)	0.01
XLVIII	1.15(±0.07)	0.01
	1.15(±0.08)	0.01
XLIX	1.15(±0.11)	0.01
	1.06(±0.06)	0.01
L	0.99(±0.02)	0.01
	0.95(±0.09)	0.01
GT=149.02(±0.88)		

Table 17. Length (µm), arm ratio, relative length, centromeric index and centromeric type of metaphase chromosomes of *Phyllanthus niruri* L.

Chrom-osome pair	Long arm (l) µm(±SD)	Short arm (s) µm(±SD)	Total length (T) µm(±SD)	Arm ratio l/s	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
I	1.40(±0.03)	1.17(±0.09)	2.58(±0.16)	1.20	0.05	45.35	m
	1.36(±0.11)	1.04(±0.12)	2.39(±0.21)	1.31	0.05	43.51	m
II	1.15(±0.03)	1.15(±0.01)	2.30(±0.11)	1.00	0.05	50.00	m
	1.30(±0.08)	0.95(±0.05)	2.25(±0.12)	1.37	0.04	42.22	m
III	1.33(±0.07)	0.92(±0.05)	2.25(±0.13)	1.45	0.04	40.89	m
	1.33(±0.06)	0.92(±0.07)	2.25(±0.16)	1.45	0.04	40.89	m
IV	1.33(±0.06)	0.92(±0.04)	2.25(±0.16)	1.45	0.04	40.89	m
	1.27(±0.08)	0.92(±0.06)	2.19(±0.15)	1.38	0.04	42.01	m
V	1.33(±0.09)	0.81(±0.05)	2.14(±0.18)	1.66	0.04	37.85	sm
	1.38(±0.02)	0.69(±0.03)	2.07(±0.14)	2.00	0.04	33.33	sm
VI	1.27(±0.01)	0.81(±0.05)	2.07(±0.11)	1.57	0.04	39.13	sm
	1.27(±0.06)	0.81(±0.02)	2.07(±0.14)	1.57	0.04	39.13	sm
VII	1.15(±0.08)	0.81(±0.03)	1.96(±0.17)	1.43	0.04	41.33	m
	1.15(±0.05)	0.81(±0.06)	1.96(±0.15)	1.43	0.04	41.33	m
VIII	1.27(±0.02)	0.67(±0.04)	1.93(±0.12)	1.90	0.04	34.72	sm
	1.17(±0.01)	0.71(±0.06)	1.89(±0.13)	1.65	0.04	37.57	sm
IX	1.15(±0.04)	0.69(±0.03)	1.84(±0.17)	1.67	0.04	37.50	sm
	1.27(±0.02)	0.46(±0.06)	1.73(±0.12)	2.75	0.03	26.59	sm
X	0.90(±0.02)	0.81(±0.02)	1.70(±0.13)	1.11	0.03	47.65	m
	1.00(±0.01)	0.68(±0.05)	1.68(±0.16)	1.47	0.03	40.48	m
XI	0.97(±0.01)	0.64(±0.02)	1.61(±0.17)	1.50	0.03	39.75	m
	0.90(±0.01)	0.69(±0.01)	1.59(±0.15)	1.30	0.03	43.40	m
XII	0.92(±0.01)	0.53(±0.02)	1.45(±0.16)	1.74	0.03	36.55	sm
	0.92(±0.02)	0.53(±0.02)	1.45(±0.14)	1.74	0.03	36.55	sm
XIII	0.80(±0.09)	0.61(±0.03)	1.40(±0.15)	1.31	0.03	43.57	m
	0.74(±0.05)	0.53(±0.02)	1.27(±0.15)	1.39	0.03	41.73	m
GT=50.26(±0.45)							

m=metacentric chromosome, sm=submetacentric chromosome

Table 18. Length (µm), arm ratio, relative length, centromeric index and centromeric type of metaphase chromosomes of *Phyllanthus reticulatus* Poir.

Chrom-osome pair	Long arm (l) µm(±SD)	Short arm (s) µm(±SD)	Total length (T) µm(±SD)	Arm ratio l/s	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
I	3.68(±0.11)	1.61(±0.12)	5.29(±0.33)	2.29	0.05	30.43	sm
	3.21(±0.13)	1.92(±0.14)	5.13(±0.18)	1.67	0.05	37.43	sm
II	3.00(±0.12)	2.08(±0.09)	5.08(±0.17)	1.44	0.05	40.94	m
	2.88(±0.15)	2.07(±0.11)	4.95(±0.16)	1.39	0.05	41.82	m
III	2.98(±0.12)	1.97(±0.13)	4.95(±0.14)	1.51	0.05	39.80	sm
	2.88(±0.01)	1.86(±0.01)	4.74(±0.17)	1.55	0.04	39.24	sm
IV	2.80(±0.03)	1.92(±0.05)	4.72(±0.15)	1.46	0.04	40.68	m
	2.48(±0.03)	2.01(±0.06)	4.49(±0.07)	1.23	0.04	44.77	m
V	2.88(±0.05)	1.61(±0.08)	4.49(±0.19)	1.79	0.04	35.86	sm
	2.81(±0.07)	1.40(±0.02)	4.21(±0.13)	2.01	0.04	33.25	sm
VI	2.76(±0.09)	1.40(±0.05)	4.16(±0.18)	1.97	0.04	33.65	sm
	2.88(±0.05)	1.27(±0.07)	4.15(±0.08)	2.27	0.04	30.60	sm
VII	2.88(±0.09)	1.27(±0.08)	4.15(±0.17)	2.27	0.04	30.60	sm
	2.76(±0.04)	1.33(±0.05)	4.09(±0.19)	2.08	0.04	32.52	sm
VIII	2.65(±0.02)	1.38(±0.03)	4.03(±0.09)	1.92	0.04	34.24	sm
	2.67(±0.03)	1.33(±0.01)	4.00(±0.19)	2.01	0.04	33.25	sm
IX	2.42(±0.07)	1.45(±0.08)	3.87(±0.15)	1.67	0.04	37.47	sm
	2.42(±0.03)	1.45(±0.04)	3.87(±0.15)	1.67	0.04	37.47	sm
X	2.53(±0.07)	1.33(±0.05)	3.86(±0.19)	1.90	0.04	34.46	sm
	2.53(±0.08)	1.33(±0.07)	3.86(±0.18)	1.90	0.04	34.46	sm
XI	2.25(±0.05)	1.40(±0.06)	3.65(±0.16)	1.61	0.03	38.36	sm
	2.35(±0.06)	1.10(±0.03)	3.45(±0.14)	2.14	0.03	31.88	sm
XII	2.35(±0.06)	1.10(±0.08)	3.45(±0.16)	2.14	0.03	31.88	sm
	1.90(±0.03)	1.21(±0.01)	3.11(±0.13)	1.57	0.03	38.91	sm
XIII	1.93(±0.08)	1.00(±0.08)	2.93(±0.15)	1.93	0.03	34.13	sm
	2.12(±0.02)	0.81(±0.01)	2.93(±0.20)	2.62	0.03	27.84	sm
GT=107.61(±0.29)							

m=metacentric chromosome, sm=submetacentric chromosome

Table 19. Total Length (µm) and relative length of metaphase chromosomes of *Phyllanthus urinaria* L.

Chromosome pair	Total length (T) µm(±SD)	Relative length (RL)
I	2.51(±0.17)	0.03
	2.30(±0.15)	0.03
II	2.19(±0.09)	0.03
	2.19(±0.18)	0.03
III	2.07(±0.17)	0.03
	2.07(±0.16)	0.03
IV	2.07(±0.13)	0.03
	2.07(±0.12)	0.03
V	2.07(±0.14)	0.03
	2.07(±0.16)	0.03
VI	1.96(±0.18)	0.03
	1.77(±0.17)	0.02
VII	1.77(±0.16)	0.02
	1.77(±0.12)	0.02
VIII	1.66(±0.17)	0.02
	1.66(±0.12)	0.02
IX	1.66(±0.13)	0.02
	1.66(±0.21)	0.02
X	1.66(±0.20)	0.02
	1.66(±0.20)	0.02
XI	1.66(±0.10)	0.02
	1.66(±0.16)	0.02
XII	1.47(±0.18)	0.02
	1.47(±0.17)	0.02
XIII	1.47(±0.17)	0.02
	1.47(±0.15)	0.02

Table continued

Chromosome pair	Total length (T) µm(±SD)	Relative length (RL)
XIV	1.47(±0.17)	0.02
	1.31(±0.09)	0.02
XV	1.31(±0.16)	0.02
	1.31(±0.13)	0.02
XVI	1.31(±0.17)	0.02
	1.31(±0.16)	0.02
XVII	1.31(±0.19)	0.02
	1.31(±0.16)	0.02
XXVIII	1.31(±0.12)	0.02
	1.27(±0.09)	0.02
XIX	1.27(±0.17)	0.02
	1.27(±0.80)	0.02
XX	1.24(±0.16)	0.02
	1.24(±0.19)	0.02
XXI	1.15(±0.12)	0.02
	1.15(±0.07)	0.02
XXII	1.15(±0.06)	0.02
	1.15(±0.04)	0.02
XXIII	1.15(±0.06)	0.02
	1.15(±0.07)	0.02
XXIV	1.15(±0.05)	0.02
	1.15(±0.05)	0.02
XIV	1.15(±0.19)	0.02
	1.01(±0.08)	0.01
GT=74.98(±0.74)		

Table 20. Comparative orcein-stained karyotype analysis of five species of *Phyllanthus* L.

Species	2n	Range of chromosomal length (µm) (SD)	Total length of 2n chromosome complements (µm)(±SD)	Range of Relative length (RL)	Centromeric formulae	Karyotype (Stebbins 1971)
P. acidus	26	3.86(±0.11) - 6.33(±0.19)	129.45(±1.19)	0.03-0.05	12m+14sm	Relatively Asymmetric
P. emblica (small fruit form)	100	0.99(±0.06) - 2.30(±0.12)	140.03(±2.20)	0.01-0.02	*	Symmetric
P. emblica (large fruit form)	100	0.95(±0.09) - 2.19(±0.16)	149.02(±0.88)	0.01	*	Symmetric
P. niruri	26	1.27(±0.15) - 2.58(±0.16)	50.26(±0.45)	0.03-0.05	20m+6sm	Relatively Asymmetric
P. reticulatus	26	2.93(±0.20) - 5.29(±0.33)	107.61(±0.29)	0.03-0.05	4m+22sm	Relatively Asymmetric
P. urinaria	48	1.01(±0.08) - 2.51(±0.17)	74.98(±0.74)	0.01-0.03	*	Symmetric

m=metacentric chromosome, sm=submetacentric chromosome

*=The centromeric position is indistinct due to the smaller chromosome size (0.95-2.51 µm) of chromosome. As a result, the centromeric type could not be determined.

Table 21. Number and frequency of variation of somatic chromosome number of two species of *Phyllanthus* L.

Plant species studied	No. of somatic chromosome	Total number of cell count	% of cells
P. emblica	2n=100	135 (Out of 150 cells)	90.00
(small fruit form)	2n=78	9 (Out of 150 cells)	06.00
	2n=104	6 (Out of 150 cells)	04.00
P. emblica	2n=100	86 (Out of 90 cells)	95.56
(large fruit form)	2n=90	4 (Out of 90 cells)	04.44
P. urinaria	2n=48	108 (Out of 120 cells)	90.00
	2n=50	6 (Out of 120 cells)	05.00
	2n=52	6 (Out of 120 cells)	05.00

Table 22. Comparative CMA-banding analysis of five species of *Phyllanthus* L.

Species	No. of CMA-bands	Total length of CMA- banded region (µm)	CMA- banded karyotypic formulae	% of CMA-banded region
P. acidus	03	3.13	3β+23θ	3.69
P. emblica (small fruit form)	-	-	100θ	-
P. emblica L. (large fruit form)	15	24.31	10α+4β+1γ+85θ	12.19
P. niruri	03	01.73	2α+1β+23θ	2.71
P. reticulatus	04	2.69	2α+2β+22θ	3.21
P. urinaria	13	17.62	2α+3β+8γ+35θ	19.09

Classification of CMA positive bands

α=Band in centromeric region

β=Band in terminal region on the short arm

δ= Band in terminal region on the long arm

γ=Band in whole chromosome

θ=No band

Table 23. Comparative DAPI-banding analysis of five species of *Phyllanthus* L.

Species	No. of DAPI-bands	Total length of DAPI- banded region (µm)	CMA- banded karyotypic formulae	% of DAPI-banded region
P. acidus	02	1.91	2α+24θ	1.48
P. emblica (small fruit form)	06	8.37	2β+4γ+94θ	4.16
P. emblica (large fruit form)	07	1.17	7α+93θ	2.27
P. niruri	26	18.84	26α	28.5
P. reticulatus	04	3.10	4β+22θ	4.05
P. urinaria	07	24.50	2α+3β+2δ+41θ	21.05

Classification of DAPI positive bands-

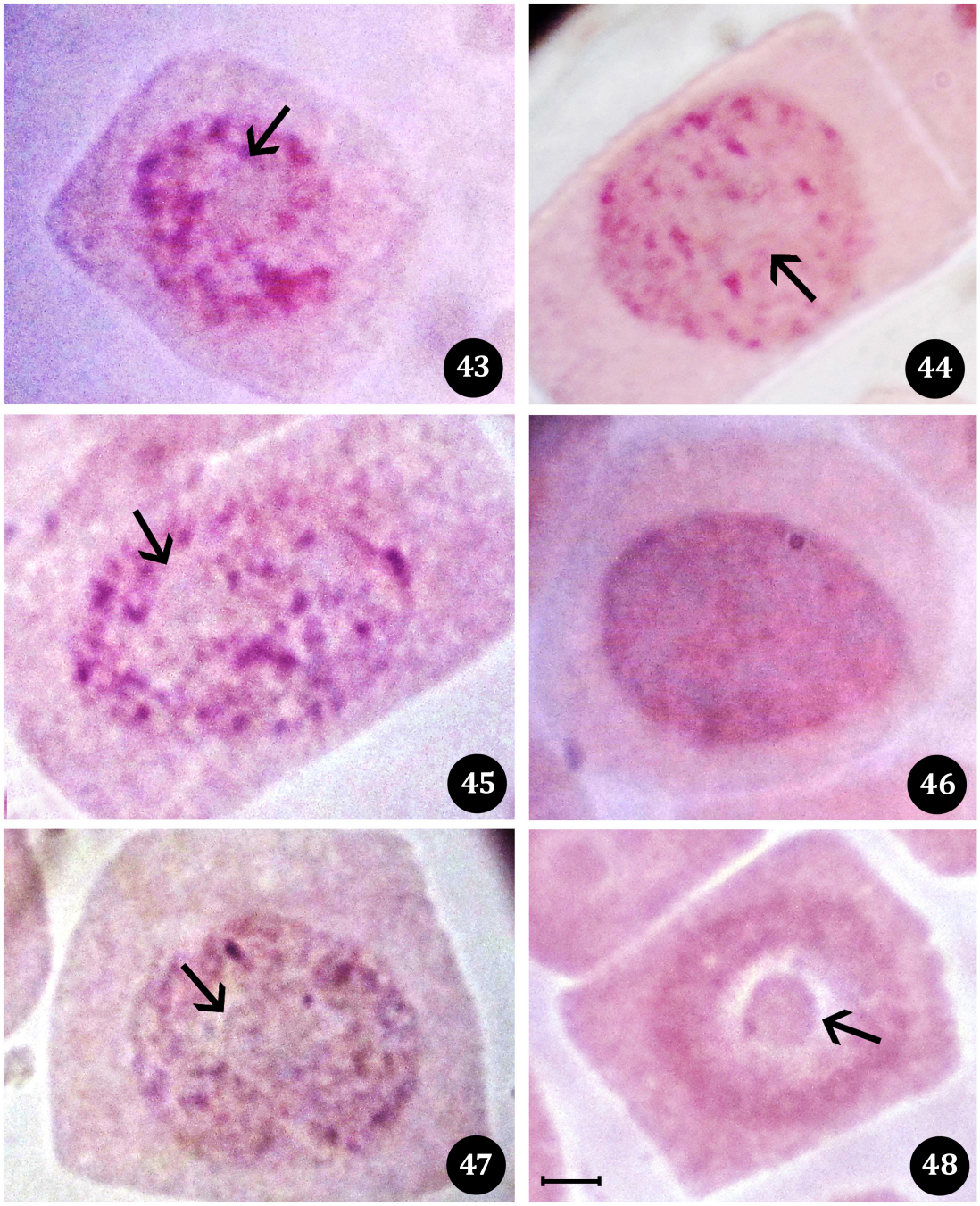
α=Band in centromeric region

β=Band in terminal region on the short arm

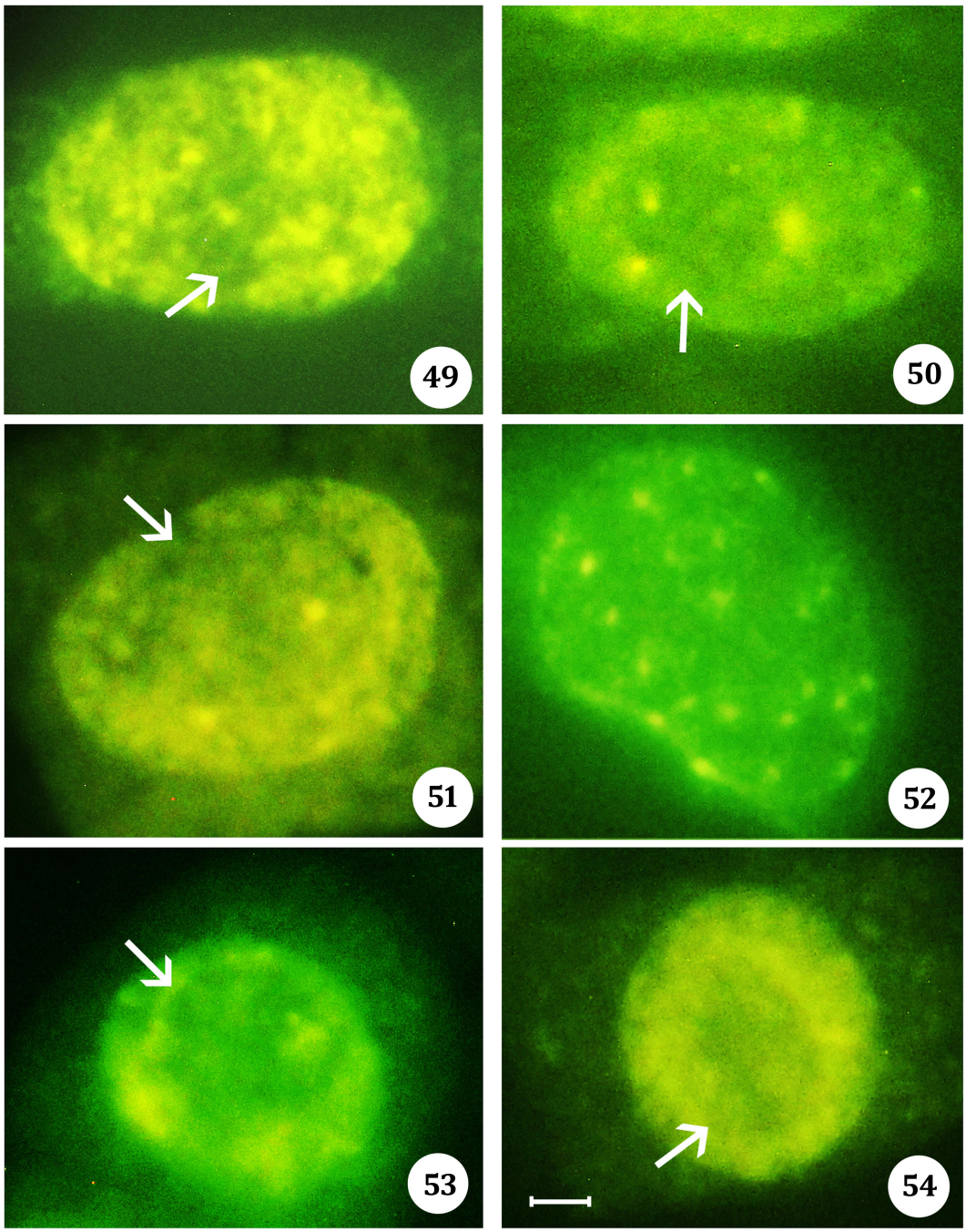
δ= Band in terminal region on the long arm

γ=Band in whole chromosome

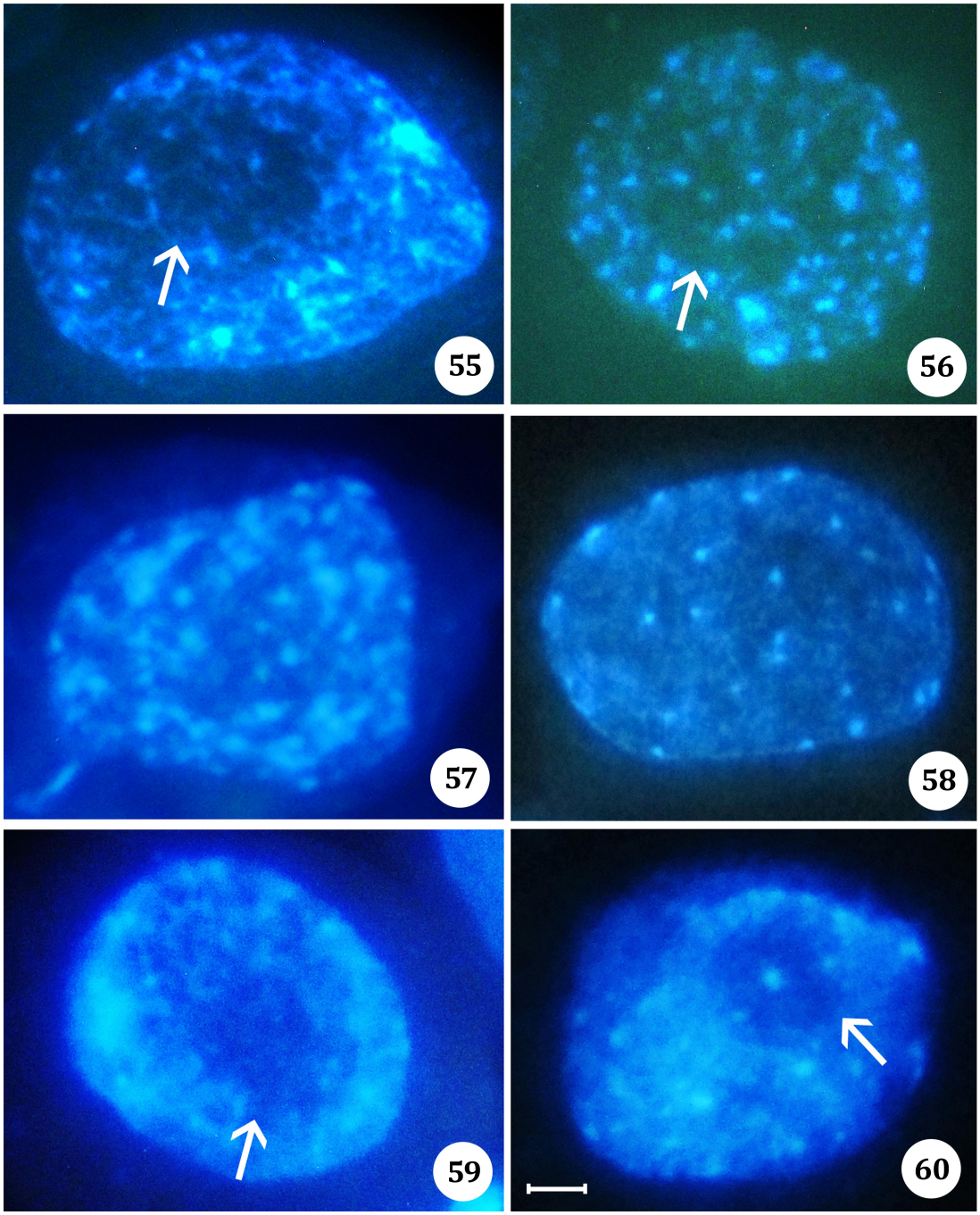
θ=No band



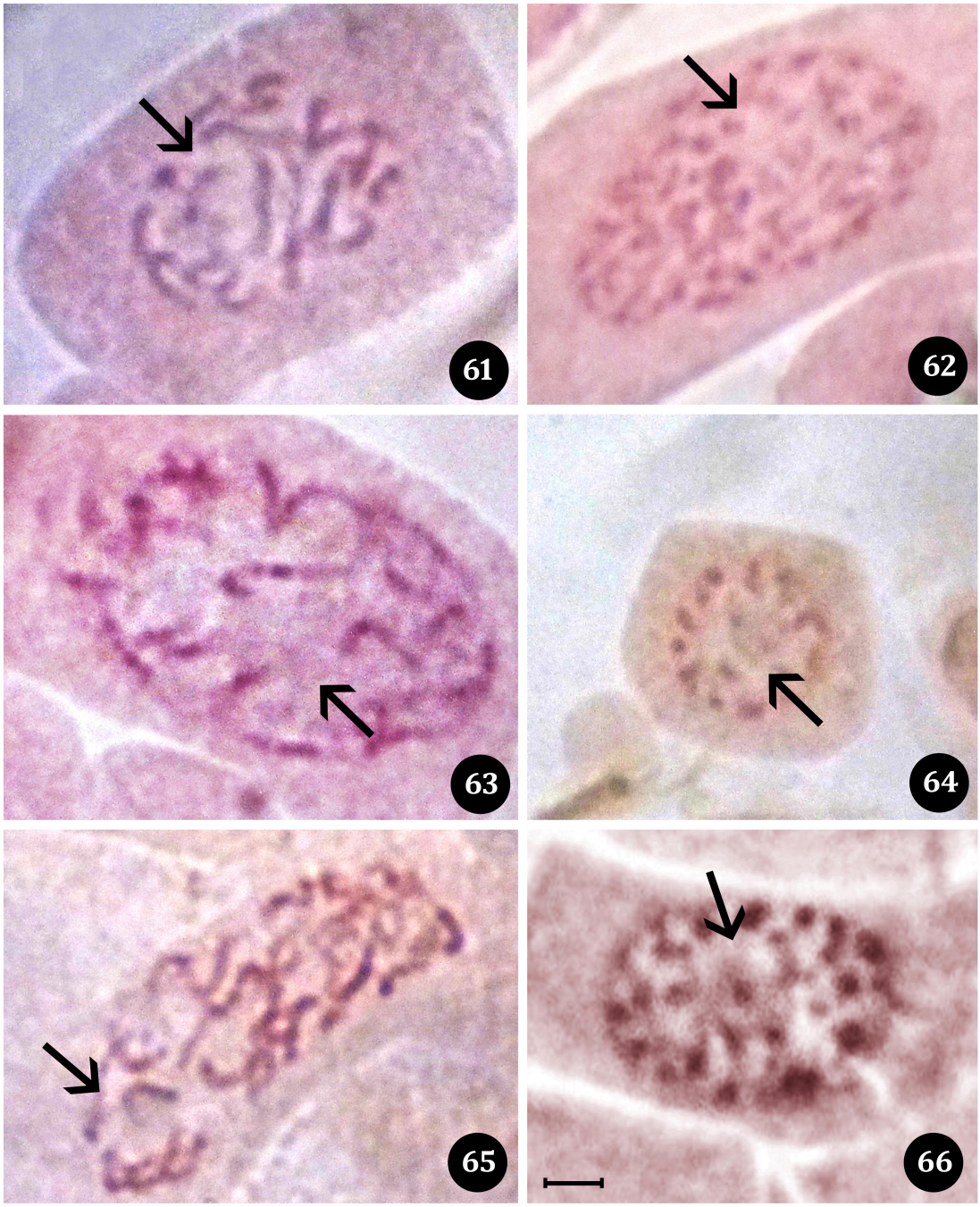
Figs. 43-48. Orcein-stained mitotic interphase nuclei of five species of *Phyllanthus* L. 43. *P. acidus* (L.) Skeels, 44. *P. emblica* L. (small fruit form), 45. *P. emblica* L. (large fruit form), 46. *P. niruri* L., 47. *P. reticulatus* Poir. and 48. *P. urinaria* L. Bar=5µm, arrow (→) indicates nucleolus.



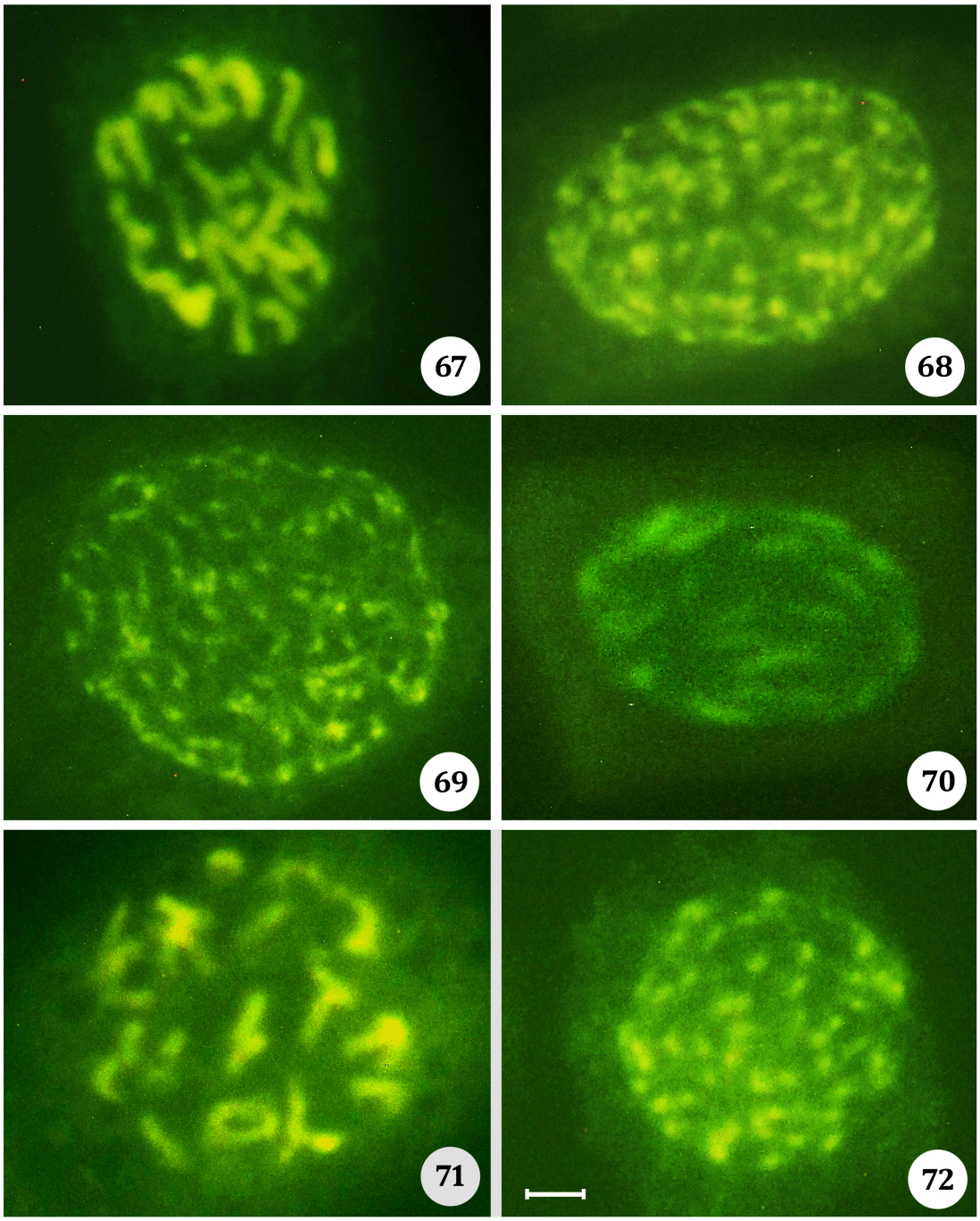
Figs. 49-54. CMA-stained mitotic interphase nuclei of five species of *Phyllanthus* L. 49. *P. acidus* (L.) Skeels, 50. *P. emblica* L. (small fruit form), 51. *P. emblica* L. (large fruit form), 52. *P. niruri* L., 53. *P. reticulatus* Poir. and 54. *P. urinaria* L. Bar=5µm, arrow (→) indicates nucleolus.



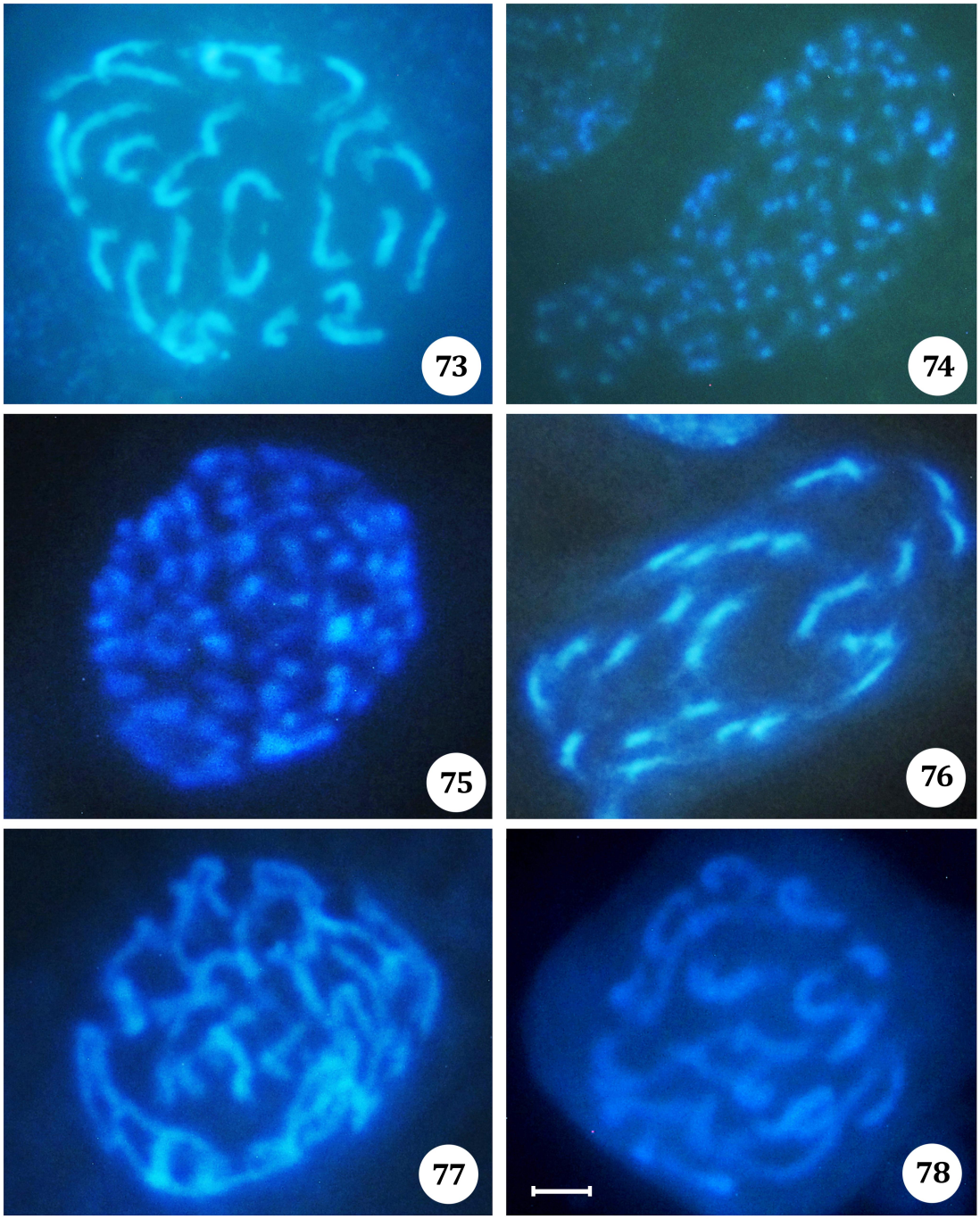
Figs. 55-60. DAPI-stained mitotic interphase nuclei of five species of *Phyllanthus* L. 55. *P. acidus* (L.) Skeels, 56. *P. emblica* L. (small fruit form), 57. *P. emblica* L. (large fruit form), 58. *P. niruri* L., 59. *P. reticulatus* Poir. and 60. *P. urinaria* L. Bar=5µm, arrow (→) indicates nucleolus.



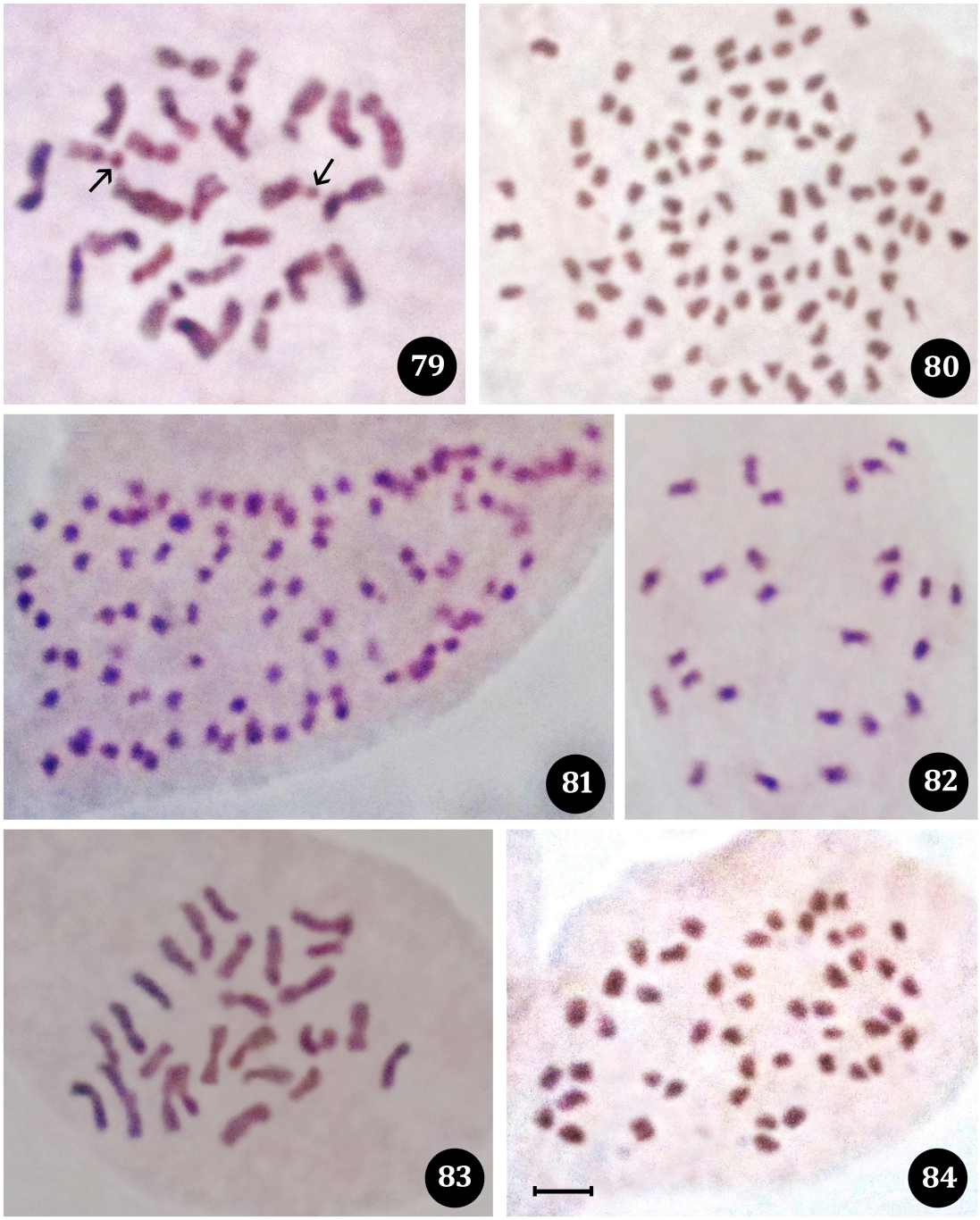
Figs. 61-66. Orcein-stained mitotic prophase chromosomes of five species of *Phyllanthus* L. 61. *P. acidus* (L.) Skeels, 62. *P. emblica* L. (small fruit form), 63. *P. emblica* L. (large fruit form), 64. *P. niruri* L., 65. *P. reticulatus* Poir. and 66. *P. urinaria* L. Bar=5µm, arrow (→) indicates nucleolus.



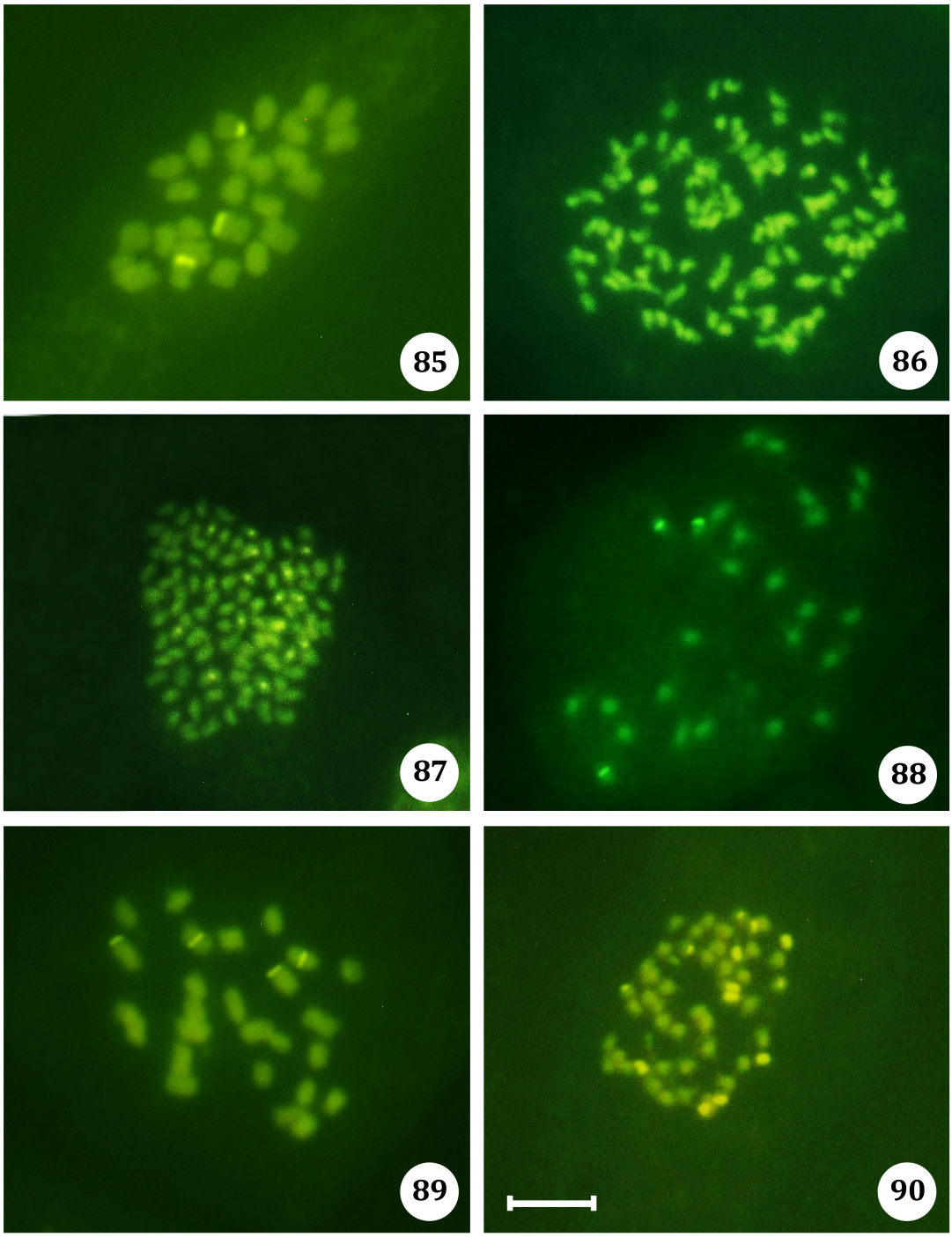
Figs. 67-72. CMA-stained mitotic prophase chromosomes of five species of *Phyllanthus* L. 67. *P. acidus* (L.) Skeels, 68. *P. emblica* L. (small fruit form), 69. *P. emblica* L. (large fruit form), 70. *P. niruri* L., 71. *P. reticulatus* Poir. and 72. *P. urinaria* L. Bar=5µm.



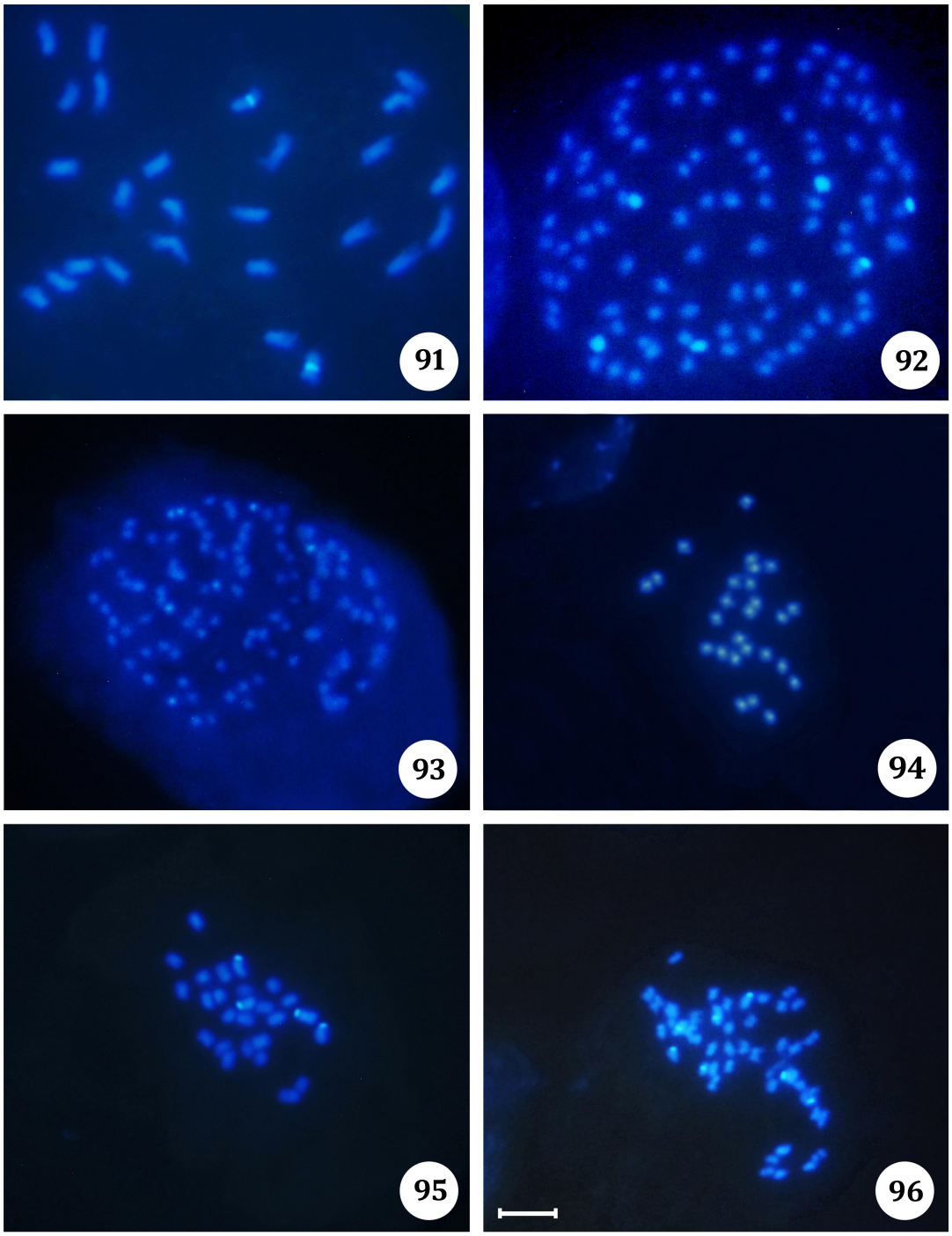
Figs. 73-78. DAPI-stained mitotic prophase chromosomes of five species of *Phyllanthus* L. 73. *P. acidus* (L.) Skeels, 74. *P. emblica* L. (small fruit form), 75. *P. emblica* L. (large fruit form), 76. *P. niruri* L., 77. *P. reticulatus* Poir. and 78. *P. urinaria* L. Bar=5µm.



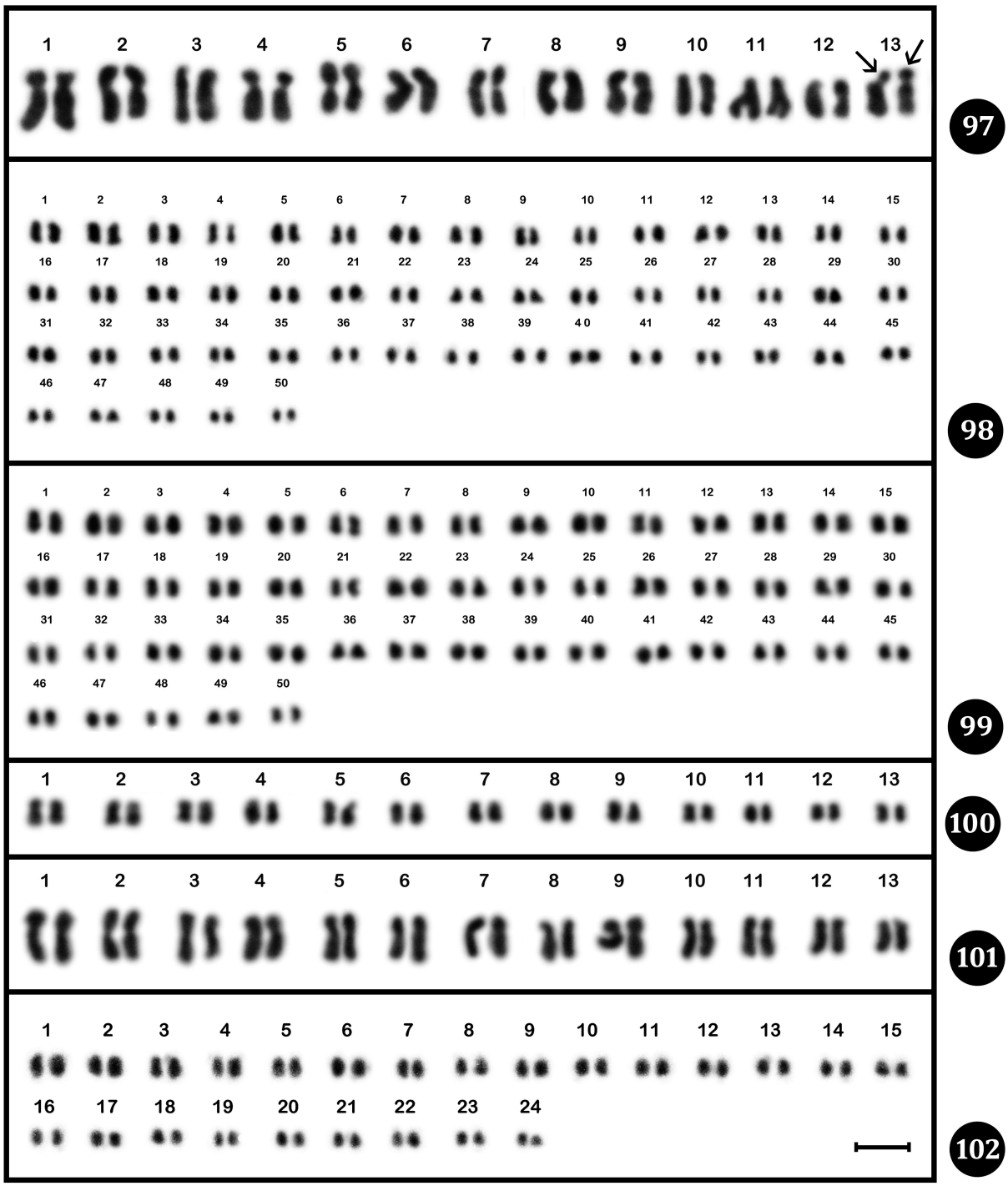
Figs. 79-84. Orcein-stained mitotic metaphase chromosomes of five species of *Phyllanthus* L. 79. *P. acidus* (L.) Skeels, 80. *P. emblica* L. (small fruit form), 81. *P. emblica* L. (large fruit form), 82. *P. niruri* L.,83. *P. reticulatus* Poir. and 84. *P. urinaria* L.Bar=5µm. Arrow(→) indicates satellite.



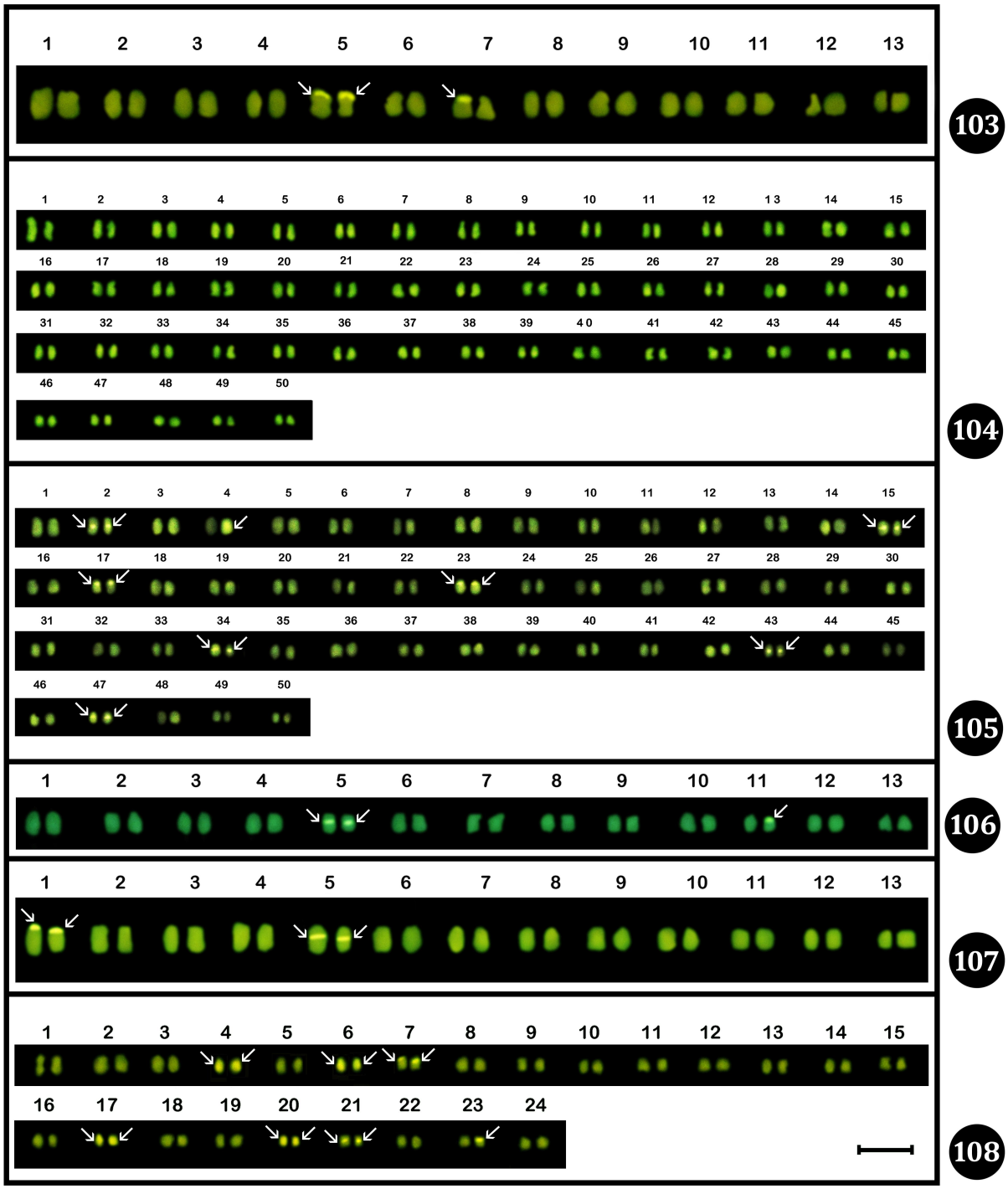
Figs. 85-90. CMA-stained mitotic metaphase chromosomes of five species of *Phyllanthus* L. 85. *P. acidus* (L.) Skeels, 86. *P. emblica* L. (small fruit form), 87. *P. emblica* L. (large fruit form), 88. *P. niruri* L., 89. *P. reticulatus* Poir. and 90. *P. urinaria* L. Bar=5µm.



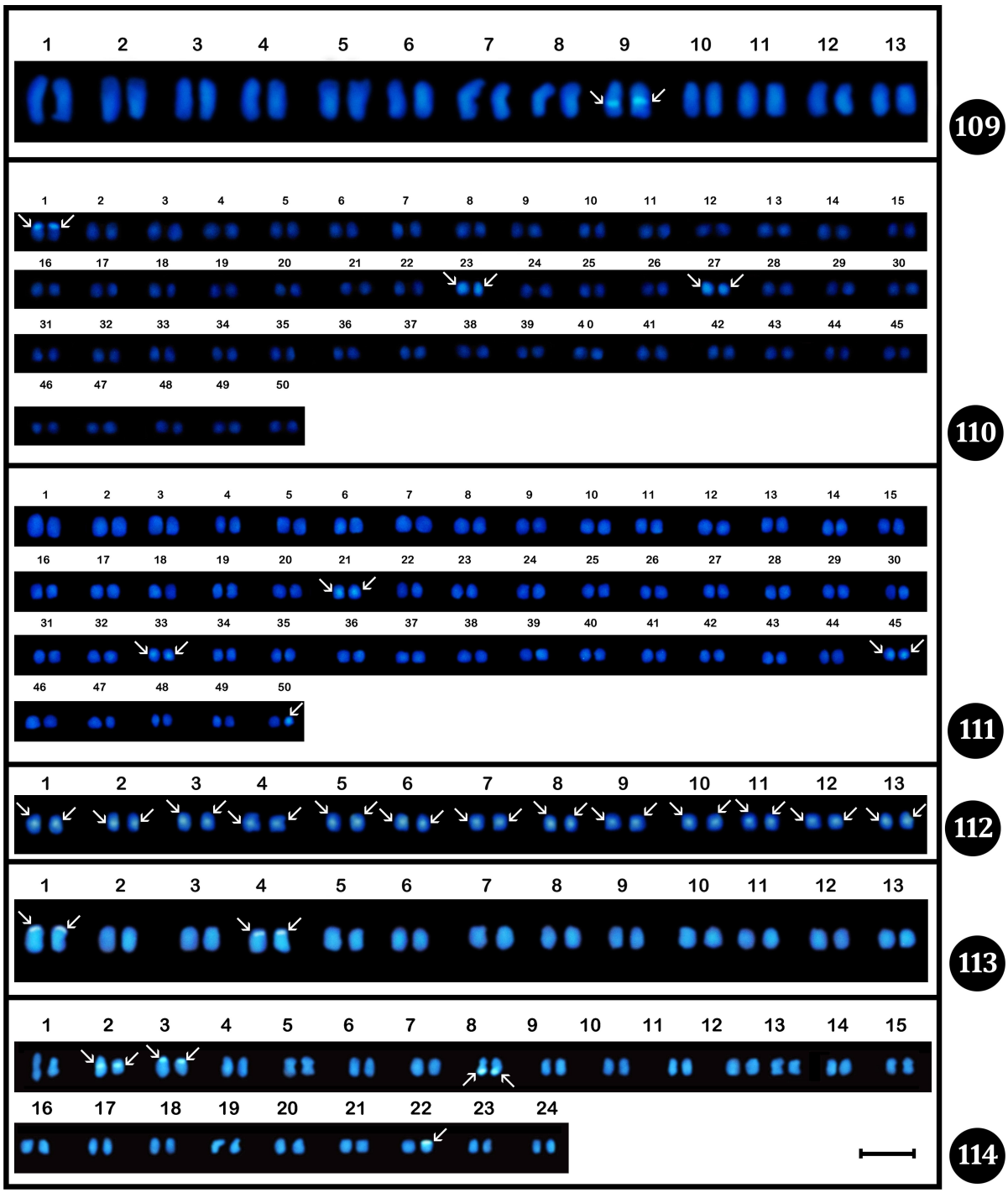
Figs. 91-96. DAPI-stained mitotic metaphase chromosomes of five species of *Phyllanthus* L. 91. *P. acidus* (L.) Skeels, 92. *P. emblica* L. (small fruit form), 93. *P. emblica* L. (large fruit form), 94. *P. niruri* L., 95. *P. reticulatus* Poir. and 96. *P. urinaria* L. Bar=5µm.



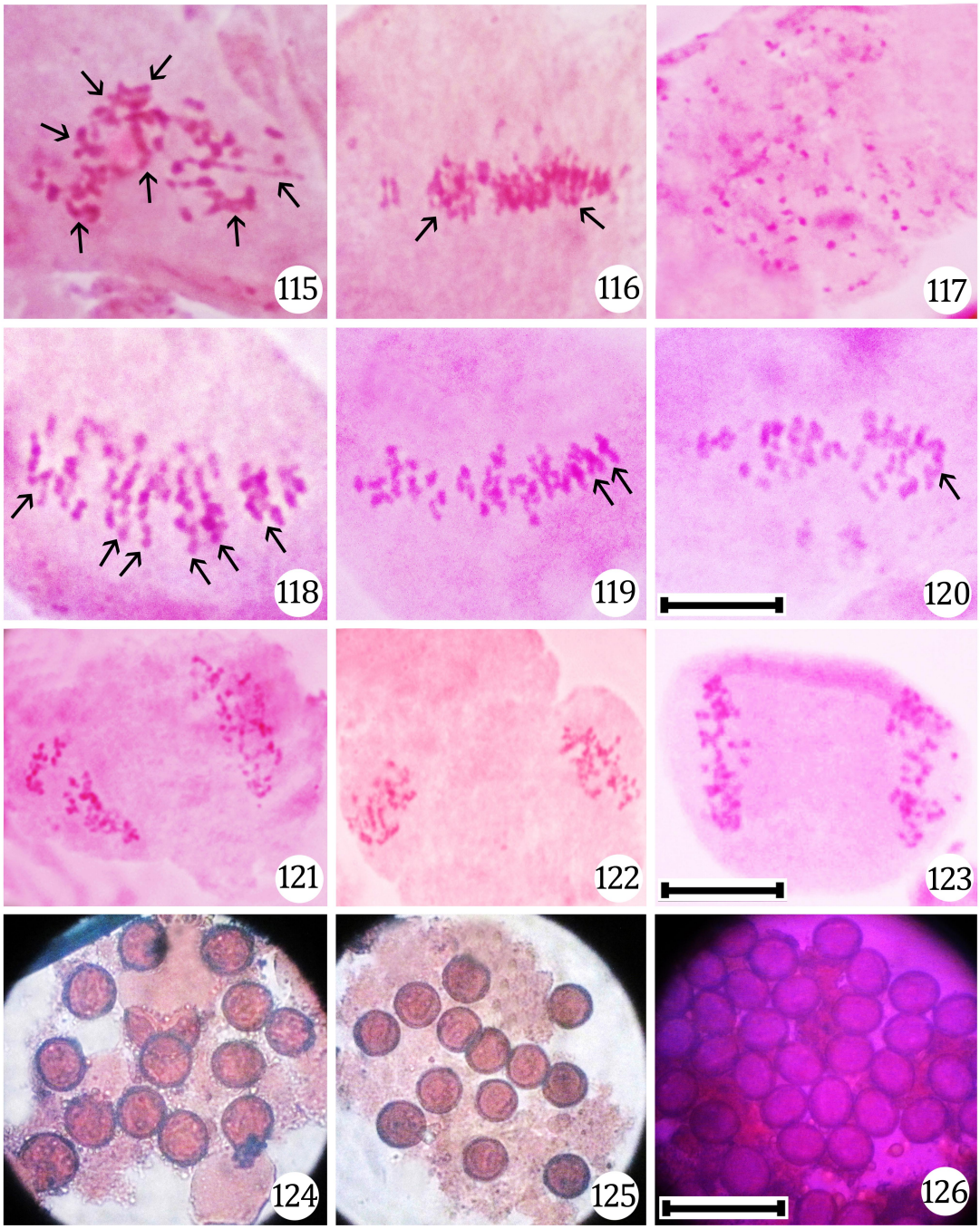
Figs. 97-102. Karyotype prepared from orcein-stained mitotic metaphase chromosomes of five species of *Phyllanthus* L. 97. *P. acidus* (L.) Skeels, 98. *P. emblica* L. (small fruit form), 99. *P. emblica* L. (large fruit form), 100. *P. niruri* L., 101. *P. reticulatus* Poir. and 102. *P. urinaria* L. Bar=5µm. Arrow (→) indicates satellite.



Figs. 103-108. Karyotype prepared from CMA-stained mitotic metaphase chromosomes of five species of *Phyllanthus* L. 103. *P. acidus* (L.) Skeels, 104. *P. emblica* L. (small fruit form), 105. *P. emblica* L. (large fruit form), 106. *P. niruri* L., 107. *P. reticulatus* Poir. and 108. *P. urinaria* L. Arrow (→) indicates CMA-positive bands. Bar=5µm.



Figs. 109-114. Karyotype prepared from DAPI-stained mitotic metaphase chromosomes of five species of *Phyllanthus* L. 109. *P. acidus* (L.) Skeels, 110. *P. emblica* L. (small fruit form), 111. *P. emblica* L. (large fruit form), 112. *P. niruri* L., 113. *P. reticulatus* Poir. and 114. *P. urinaria* L. Arrow (→) indicates DAPI-positive bands. Bar=5µm.



Figs. 115-126. Orcein-stained meiotic stages and pollen grains of polyploid species of *Phyllanthus* L. 115-117. Metaphase-I of *P. emblica* L. (small fruit form), 118-120. Metaphase-I of *P. emblica* L. (large fruit form), 121-122. Anaphase-I of *P. emblica* L. (small fruit form), 123. Anaphase-I of *P. emblica* L. (large fruit form), 124. Pollen grains of *P. emblica* L. (small fruit form), 125. Pollen grains of *P. emblica* L. (large fruit form), 126. Pollen grains of *P. urinaria* L., Bar=10µm. Arrow (→) indicates multivalent.

3.6. RAPD analysis

Twelve oligonucleotide primers *viz.* i) OPA-18 (5'-AGG TGA CCG T-3'), ii) OPB-19 (5'-CC CCC GAA G-3'), iii) OPAB-5 (5'-CCC GAA GCG A-3'), iv) OPAB-6(5'-GTG GCT TGG A-3'), v) OPC-13 (5'-AAG CCT CGT C-3'), vi) OPC-15 (5'-GAC GGA TCA G-3'), vii) OPC-96 (5'-ACC AAG AAA GGG-3'), viii) OPD-69 (5'-CGC TCC AAA TCA-3'), ix) OPF-22 (5'-AAG ATC AAA GAC-3'), x) OPH-12 (5'-ACG CGC ATG T-3'), xi) OPG-5 (5'-AGT CGT CCC C-3') and xii) OPG-7 (5'-GAA CCT GCG G-3') were used for RAPD analysis of five species of *Phyllanthus* L. Each primer showed different banding patterns. The RAPD analyses of five species of *Phyllanthus* L. were described below:

3.6.1. OPA-18 (5'-AGG TGA CCG T-3')

With primer OPA-18, a number of different sized bands were found in *Phyllanthus acidus* and *P. niruri*. However, no band was observed in any form of *P. emblica*, *P. reticulatus* and *P. urinaria*. *P. acidus* showed 14 RAPD bands (3000, 2100, 1900, 1700, 1500, 1300, 1100, 800, 750, 700, 650, 550, 350 and 300 bp). Among these 13 bands of *P. acidus*, 10 were unique (3000, 2100, 1700, 1500, 750, 700, 650, 550, 350 and 300 bp) since absent in other species. A total of 8 RAPD bands (1900, 1600, 1300, 1200, 1100, 1000, 800 and 600 bp) were observed in *P. niruri* of which 4 were unique (1600, 1200, 1000 and 600 bp). No common band was observed among these five species of *Phyllanthus* L. However, *P. acidus* and *P. niruri* shared 4 RAPD fragments (1900, 1300, 1100 and 800 bp) (Fig. 128, Table 24).

3.6.2. OPB-19 (5'-ACC CCC GAA G-3')

With this primer, only *Phyllanthus acidus* showed 11 RAPD bands (4000, 2800, 2400, 2200, 1400, 1300, 850, 700, 650, 600 and 400 bp). All of these bands in *P. acidus* could be considered as unique bands since the other four species did not possess any band with this primer OPB-19. Moreover, no common band was observed among these five species of *Phyllanthus* L. (Fig. 129, Table 25).

3.6.3. OPAB-5 (5'-CCC GAA GCG A-3')

Phyllanthus acidus showed 9 RAPD bands (2100, 1500, 1200, 1100, 900, 750, 700, 600 and 400 bp) of which 5 were unique (2100, 1200, 750, 600 and 400 bp) since these were absent in other species. Twelve different sized bands (2800, 2400, 1800, 1700, 1500, 1300, 1100, 900, 800, 700, 650 and 550 bp) were found in *P. niruri* with primer OPAB-5 of which 8 were unique (2800, 2400, 1800, 1700, 1300, 800, 650 and 550 bp). However, no band was observed in any form of *P. emblica*, *P. reticulatus* and *P. urinaria*. These five species of *Phyllanthus* L. did not share any common band. However, *P. acidus* and *P. niruri* shared 4 RAPD fragments (1500, 1100, 900 and 700 bp) (Fig. 130, Table 26).

3.6.4. OPAB-6 (5'-GTG GCT TGG A-3')

With primer OPAB-6, no band was observed in *Phyllanthus emblica* (both forms), *P. reticulatus* and *P. urinaria*. *P. acidus* showed 4 RAPD bands (1600, 1400, 1050 and 700 bp). Among these 4 bands of *P. acidus*, 2 were unique (1600 and 700 bp) since absent in other species. Four RAPD bands (2500, 1950, 1400 and 1050 bp) were observed in *P. niruri* of which 2 were unique (2500 and 1950 bp). *P. acidus* and *P. niruri* showed 2 common RAPD fragments (1400 and 1050 bp). However, these five species of *Phyllanthus* L. did not share any common band (Fig. 131, Table 27).

3.6.5. OPC-13 (5'-AAG CCT CGT C-3')

Only *Phyllanthus acidus* showed 7 RAPD bands (1500, 1050, 900, 700, 600, 400 and 250 bp) in case of the primer OPC-13. All of these bands in *P. acidus* could be considered as unique bands since the other four species did not possess any band with this primer. Moreover, no common band was observed among these five species of *Phyllanthus* L. (Fig. 132, Table 28).

3.6.6. OPC-15 (5'-GAC GGA TCA G-3')

With this primer, only *Phyllanthus urinaria* showed 9 RAPD bands (1200, 1000, 800, 700, 550, 500, 450, 400 and 300 bp). The other four species did not possess any band

with primer OPC-15. Therefore, all of these bands in *P. acidus* could be regarded as unique bands. These five species of *Phyllanthus* L. did not share any common band (Fig. 133, Table 29).

3.6.7. OPC-96 (5'-ACC AAG AAA GGG-3')

With primer OPC-96, a number of different sized bands were found in *Phyllanthus acidus* and *P. niruri*. *P. acidus* showed 7 RAPD bands (1400, 1000, 800, 750, 600, 500 and 450 bp) of which 5 were unique (1400, 1000, 600, 500 and 450 bp) since these bands were absent in other species. On the other hand, *P. niruri* showed 2 RAPD band (800 and 750 bp) which were common to *P. acidus*. However, no band was observed in *P. emblica* (both forms), *P. reticulatus* and *P. urinaria*. (Fig. 134, Table 30).

3.6.8. OPD-69 (5'-CGC TCC AAA TCA-3')

Phyllanthus acidus showed 5 RAPD bands (1100, 800, 700, 450 and 400 bp) of which 2 were unique (1100 and 450 bp) since these were absent in other species. Six different sized bands (1000, 800, 750, 700, 550 and 400 bp) were found in *P. niruri* with primer OPD-69 of which 3 were unique (1000, 750 and 550 bp). No band was observed in *P. emblica* (both forms), *P. reticulatus* and *P. urinaria*. These five species of *Phyllanthus* L. did not share any common band. However, *P. acidus* and *P. niruri* shared 3 RAPD fragments (800, 700 and 400 bp) (Fig. 135, Table 31).

3.6.9. OPF-22 (5'-AAG ATC AAA GAC-3')

With primer OPF-22, *Phyllanthus niruri* and *P. reticulatus* showed 6 bands (1100, 850, 750, 700, 600 and 500 bp) and 2 bands (900 and 450 bp), respectively. All of these bands were unique since these were absent in other species. No band was observed in any form of *P. emblica*, *P. acidus* and *P. urinaria*. These five species of *Phyllanthus* L. did not share any common band (Fig. 136, Table 32).

3.6.10. OPH-12 (5'-ACG CGC ATG T-3')

With this primer, *Phyllanthus acidus* showed 7 RAPD bands (1300, 1050, 850, 700, 550, 500 and 400 bp) of which 4 were unique (850, 700, 500 and 400 bp) since these bands

were absent in other species. Moreover, *P. niruri* showed 3 RAPD fragments (1300, 1050 and 550 bp) which were also found in *P. acidus*. No band was observed in any form of *P. emblica*, *P. reticulatus* and *P. urinaria* (Fig. 137, Table 33).

3.6.11. OPG-5 (5'-AGT CGT CCC C-3')

Phyllanthus acidus and *P. niruri* showed 7 bands (1000, 800, 750, 700, 600, 500 and 200 bp) and 6 bands (2000, 1600, 1300, 1000, 900 and 700 bp), respectively. Among these 5 bands (800, 750, 600, 500 and 200 bp) and 4 bands (2000, 1600, 1300 and 900 bp) were unique in *P. acidus* and *P. niruri* respectively. No band was observed in any form of *P. emblica*, *P. acidus* and *P. urinaria*. These five species of *Phyllanthus* L. did not share any common band. However, *P. acidus* and *P. niruri* showed 2 common fragments (1000 and 700 bp) (Fig. 138, Table 34).

3.6.12. OPG-7 (5'-GAA CCT GCG G-3')

No bands was observed in *Phyllanthus emblica* (both forms), *P. niruri* and *P. reticulatus* with primer OPG-7. Moreover, *P. acidus* and *P. urinaria* showed 8 (2400, 1900, 1700, 1500, 1100, 650, 350 and 150bp) and 4 (1700, 1100, 750, 550 bp) RAPD bands, respectively. All of these bands were unique except 1700 and 1100 bp which were common in *P. acidus* and *P. urinaria*. Moreover, no common band was observed among these five species of *Phyllanthus* L. (Fig. 139, Table 35).

3.7. Genetic distances

The values of pair-wise Nei's (1972) genetic distances analyzed by using computer software "popgene32" among five species of *Phyllanthus* were computed from combined data for the twelve RAPD primers. The highest genetic distance (1.6011) was found between *P. acidus* with rest 4 species *i.e. P. emblica*, *P. niruri, P. reticulatus* and *P. urinaria*. The lowest (0.0169) genetic distance was observed between *P. reticulatus* and *P. emblica*. (Fig. 140, Table 37).

3.8. Cluster analysis (Tree Diagram)

A cluster analysis on the basis of DNA fingerprinting by RAPD was carried out. Dendrogram based on Nei's (1972) genetic distance using UPGMA (Unweighted Pair Group Method of Arithmetic Means) segregated five *Phyllanthus* L. species into two major clusters C_1 and C_2 (Fig. 128). According to dendrogram, *Phyllanthus acidus* is separated from other 4 species with high genetic distance 1.6011 and placed in a separate cluster C_1 (Fig. 128, Table 37). On the other hand, other 4 species were placed in cluster C_2. The lowest genetic distance 0.0169 was found between *P. reticulatus* and *P. emblica* whereas the highest genetic distance 0.5025 was found between *P. niruri* and *P. emblica* within cluster C_2. So among five *Phyllanthus* L. species, *P. emblica* and *P. reticulatus* were closely related whereas *P. acidus* and *P. emblica* were genetically distantly related.

Table 24. RAPD analysis with primer OPA-18 of five species of *Phyllanthus* L.

Species	Total No. of bands with molecular weight (bp)	No. of unique bands with molecular weight (bp)	No. of common bands with molecular weight (bp)	No. of polymorphic bands with molecular weight (bp)
P. acidus	14 (3000, 2100, 1900, 1700, 1500, 1300, 1100, 800, 750, 700, 650, 550, 350, 300)	10 (3000, 2100, 1700, 1500, 750, 700, 650, 550, 350, 300)		10 (3000, 2100, 1700, 1500, 750, 700, 650, 550, 350, 300)
P. emblica (small fruit form)	-	-	4 (1900, 1300, 1100, 800) except *P. emblica* (both forms), *P. reticulatus* and *P. urinaria*	-
P. emblica (large fruit form)	-	-		-
P. niruri	8 (1900, 1600, 1300, 1200, 1100, 1000, 800, 600)	4 (1600, 1200, 1000, 600)		4 (1600, 1200, 1000, 600)
P. reticulatus	-	-		-
P. urinaria	-	-		-

Table 25. RAPD analysis with primer OPB-19 of five species of *Phyllanthus* L.

Species	Total No. of bands with molecular weight (bp)	No. of unique bands with molecular weight (bp)	No. of common bands with molecular weight (bp)	No. of polymorphic bands with molecular weight (bp)
P. acidus	11 (4000, 2800, 2400, 2200, 1400, 1300, 850, 700, 650, 600, 400)	11 (4000, 2800, 2400, 2200, 1400, 1300, 850, 700, 650, 600, 400)		11 (4000, 2800, 2400, 2200, 1400, 1300, 850, 700, 650, 600, 400)
P. emblica (small fruit form)	-	-		-
P. emblica (large fruit form)	-	-	-	-
P. niruri	-	-		-
P. reticulatus	-			
P. urinaria	-	-		-

Table 26. RAPD analysis with primer OPAB-5 of five species of *Phyllanthus* L.

Species	Total No. of bands with molecular weight (bp)	No. of unique bands with molecular weight (bp)	No. of common bands with molecular weight (bp)	No. of polymorphic bands with molecular weight (bp)
P. acidus	9 (2100, 1500, 1200, 1100, 900, 750, 700, 600, 400)	5 (2100, 1200, 750, 600, 400)		5 (2100, 1200, 750, 600, 400)
P. emblica (small fruit form)	-	-		-
P. emblica (large fruit form)	-	-	4 (1500, 1100, 900, 700) except *P. emblica* (both forms), *P. reticulatus* and *P. urinaria*	-
P. niruri	12 (2800, 2400, 1800, 1700, 1500, 1300, 1100, 900, 800, 700, 650, 550)	8 (2800, 2400, 1800, 1700, 1300, 800, 650, 550)		8 (2800, 2400, 1800, 1700, 1300, 800, 650, 550)
P. reticulatus	-	-		-
P. urinaria	-	-		-

Table 27. RAPD analysis with primer OPAB-6 of five species of *Phyllanthus* L.

Species	Total No. of bands with molecular weight (bp)	No. of unique bands with molecular weight (bp)	No. of common bands with molecular weight (bp)	No. of polymorphic bands with molecular weight (bp)
P. acidus	4 (1600, 1400, 1050, 700)	2 (1600, 700)	2 (1400, 1050) except *P. emblica* (both forms), *P. reticulatus* and *P. urinaria*	2 (1600, 700)
P. emblica (small fruit form)	-	-		-
P. emblica (large fruit form)	-	-		-
P. niruri	4 (2500, 1950, 1400, 1050)	2 (2500, 1950)		2 (2500, 1950)
P. reticulatus	-	-		-
P. urinaria	-	-		-

Table 28. RAPD analysis with primer OPC-13 of five species of *Phyllanthus* L.

Species	Total No. of bands with molecular weight (bp)	No. of unique bands with molecular weight (bp)	No. of common bands with molecular weight (bp)	No. of polymorphic bands with molecular weight (bp)
P. acidus	7 (1500, 1050, 900, 700, 600, 400, 250)	7 (1500, 1050, 900, 700, 600, 400, 250)		7 (1500, 1050, 900, 700, 600, 400, 250)
P. emblica (small fruit form)	-	-		-
P. emblica (large fruit form)	-	-	-	-
P. niruri	-	-		-
P. reticulatus	-	-		-
P. urinaria	-	-		-

Table 29. RAPD analysis with primer OPC-15 of five species of *Phyllanthus* L.

Species	Total No. of bands with molecular weight (bp)	No. of unique bands with molecular weight (bp)	No. of common bands with molecular weight (bp)	No. of polymorphic bands with molecular weight (bp)
P. acidus	-	-		-
P. emblica (small fruit form)	-	-		-
P. emblica (large fruit form)	-	-		-
P. niruri	-	-	-	-
P. reticulatus	-	-		-
P. urinaria	9 (1200, 1000, 800, 700, 550, 500, 450, 400, 300)	9 (1200, 1000, 800, 700, 550, 500, 450, 400, 300)		9 (1200, 1000, 800, 700, 550, 500, 450, 400, 300)

Table 30. RAPD analysis with primer OPC-96 of five species of *Phyllanthus* L.

Species	Total No. of bands with molecular weight (bp)	No. of unique bands with molecular weight (bp)	No. of common bands with molecular weight (bp)	No. of polymorphic bands with molecular weight (bp)
P. acidus	7 (1400, 1000, 800, 750, 600, 500, 450)	5 (1400, 1000, 600, 500, 450)		5 (1400, 1000, 600, 500, 450)
P. emblica (small fruit form)	-	-	2 (800, 750) except *P. emblica* (both forms), *P. reticulatus* and *P. urinaria*	-
P. emblica (large fruit form)	-	-		-
P. niruri	2 (800, 750)	-		-
P. reticulatus	-	-		-
P. urinaria	-	-		-

Table 31. RAPD analysis with primer OPD-69 of five species of *Phyllanthus* L.

Species	Total No. of bands with molecular weight (bp)	No. of unique bands with molecular weight (bp)	No. of common bands with molecular weight (bp)	No. of polymorphic bands with molecular weight (bp)
P. acidus	5 (1100, 800, 700, 450, 400)	2 (1100, 450)		2 (1100, 450)
P. emblica (small fruit form)	-	-	3 (800, 700, 400) except *P. emblica* (both forms), *P. reticulatus* and *P. urinaria*	-
P. emblica (large fruit form)	-	-		-
P. niruri	6 (1000, 800, 750, 700, 550, 400)	3 (1000, 750, 550)		3 (1000, 750, 550)
P. reticulatus	-	-		-
P. urinaria	-	-		-

Table 32. RAPD analysis with primer OPF-22 of five species of *Phyllanthus* L.

Species	Total No. of bands with molecular weight (bp)	No. of unique bands with molecular weight (bp)	No. of common bands with molecular weight (bp)	No. of polymorphic bands with molecular weight (bp)
P. acidus	-	-		-
P. emblica (small fruit form)	-	-		-
P. emblica (large fruit form)	-	-		-
P. niruri	6 (1100, 850, 750, 700, 600, 500)	6 (1100, 850, 750, 700, 600, 500)	-	6 (1100, 850, 750, 700, 600, 500)
P. reticulatus	2 (900, 450)	2 (900, 450)		2 (900, 450)
P. urinaria	-	-		-

Table 33. RAPD analysis with primer OPH-12 of five species of *Phyllanthus* L.

Species	Total No. of bands with molecular weight (bp)	No. of unique bands with molecular weight (bp)	No. of common bands with molecular weight (bp)	No. of polymorphic bands with molecular weight (bp)
P. acidus	7 (1300, 1050, 850, 700, 550, 500, 400)	4 (850, 700, 500, 400)		4 (850, 700, 500, 400)
P. emblica (small fruit form)	-	-	3 (1300, 1050, 550) except *P. emblica* (both forms), *P. reticulatus* and *P. urinaria*	-
P. emblica (large fruit form)	-	-		-
P. niruri	3 (1300, 1050, 550)	-		-
P. reticulatus	-	-		-
P. urinaria	-	-		-

Table 34. RAPD analysis with primer OPG-5 of five species of *Phyllanthus* L.

Species	Total No. of bands with molecular weight (bp)	No. of unique bands with molecular weight (bp)	No. of common bands with molecular weight (bp)	No. of polymorphic bands with molecular weight (bp)
P. acidus	7 (1000, 800, 750, 700, 600, 500, 200)	5 (800, 750, 600, 500, 200)		4 (800, 750, 600, 500, 200)
P. emblica (small fruit form)	-	-		-
P. emblica (large fruit form)	-	-	1 (1000) except *P. emblica* (both forms), *P. reticulatus* and *P. urinaria*	-
P. niruri	6 (2000, 1600, 1300, 1000, 900, 700)	5 (2000, 1600, 1300, 900, 700)		5 (2000, 1600, 1300, 900, 700)
P. reticulatus	-	-		-
P. urinaria	-	-		-

Table 35. RAPD analysis with primer OPG-7 of five species of *Phyllanthus* L.

Species	Total No. of bands with molecular weight (bp)	No. of unique bands with molecular weight (bp)	No. of common bands with molecular weight (bp)	No. of polymorphic bands with molecular weight (bp)
P. acidus	8 (2400, 1900, 1700, 1500, 1100, 650, 350, 150)	6 (2400, 1900, 1500, 650, 350, 150)		6 (2400, 1900, 1500, 650, 350, 150)
P. emblica (small fruit form)	-	-	2 (1700, 1100) except *P. emblica* (both forms), p. *niruri* and *P. reticulatus*	-
P. emblica (large fruit form)	-	-		-
P. niruri	-	-		-
P. reticulatus	-	-		-
P. urinaria	4 (1700, 1100, 750, 550)	2 (750, 550)		2 (750, 550)

Table 36. Compilation of RAPD analysis in five species of *Phyllanthus* L.

Primer codes	Total bands	Size ranges (bp)	Number of polymorphic bands	Number of species specific unique bands	Polymorphisms (%)
OPA-18	22	300-3000	14	10 in *P. acidus* and 4 in *P. niruri*	63.64
OPB-19	11	400-4000	11	11 in *P. acidus*	100.00
OPAB-5	21	400-2800	13	5 in *P. acidus* and 8 in *P. niruri*	61.90
OPAB-6	8	700-2500	4	2 in *P. acidus* and 2 in *P. niruri*	50.00
OPC-13	7	250-1500	7	7 in *P. acidus*	100.00
OPC-15	9	300-1200	9	9 in *P. urinaria*	100.00
OPC-96	9	450-1400	5	5 in *P. acidus*	55.56
OPD-69	11	400-1100	5	2 in *P. acidus* and 3 in *P. niruri*	45.45
OPF-22	8	450-1100	8	6 in *P. acidus* and 2 in *P. reticulatus*	100.00
OPH-12	10	400-1300	4	4 in *P. acidus*	40.00
OPG-5	13	200-2000	9	5 in *P. acidus* and 4 in *P. niruri*	76.92
OPG-7	12	150-2400	8	6 in *P. acidus* and 2 in *P. urinaria*	66.67
Grand Total	**141**	**150-4000**	**97**	**97**	**71.68**

Table 37. Summary of Nei's (1972) genetic distances of five species of *Phyllanthus* L., according to RAPD analysis

pop ID	I	II	III	IV	V	VI
I	****					
II	1.3451	****				
III	1.3451	0.0000	****			
IV	1.6011	0.5025	0.5025	****		
V	1.4118	0.0169	0.0169	0.5306	****	
VI	1.3451	0.0342	0.0342	0.5596	0.0517	****

I. *P. acidus*
II. *P. emblica* (small fruit form)
III. *P. emblica* (large fruit form)
IV. *P. niruri*
V. *P. reticulatus*
VI. *P. urinaria*

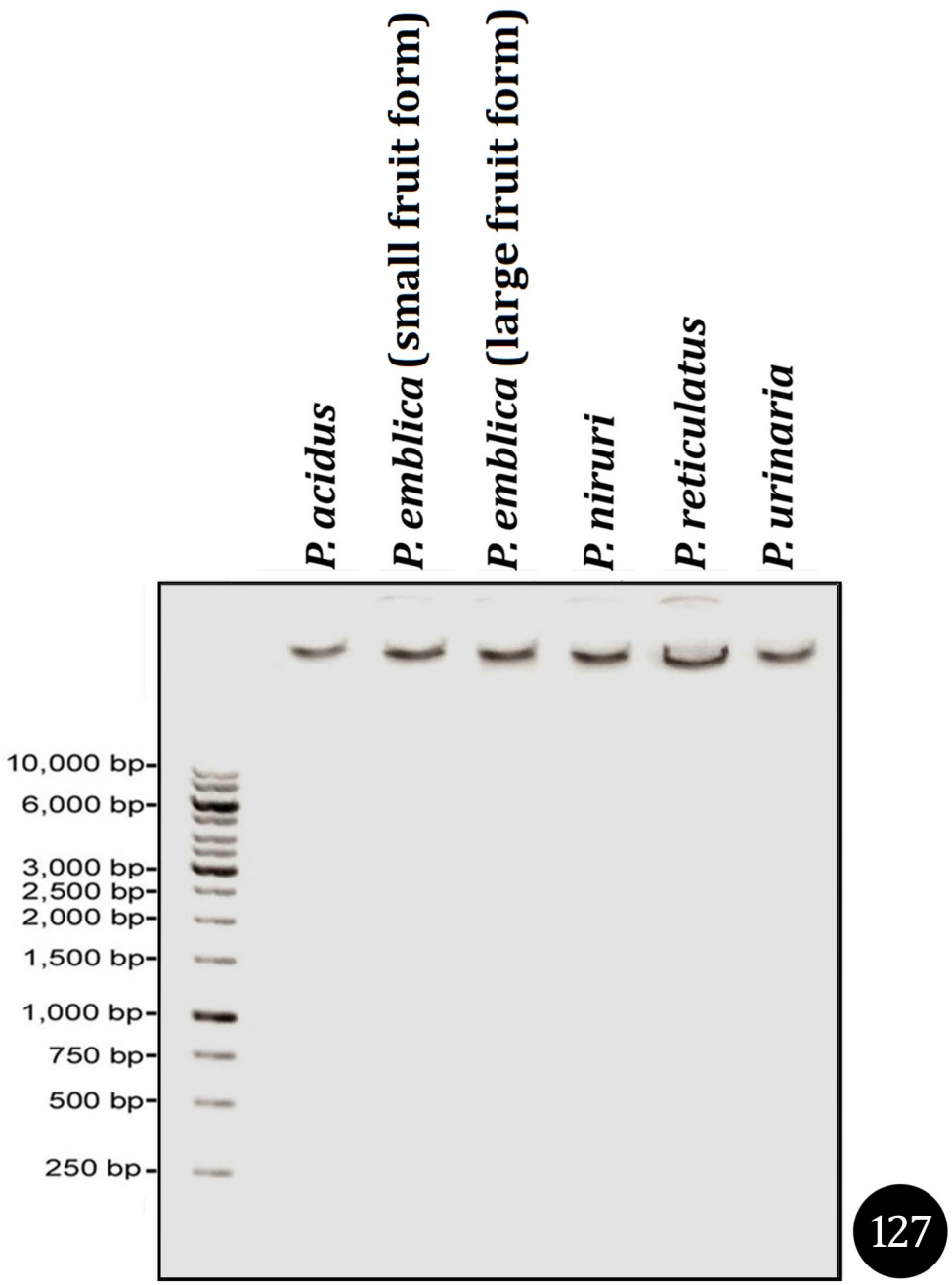


Fig. 127. Electrophoretogram of ethidium bromide stained genomic DNA samples of five species of *Phyllanthus* L.

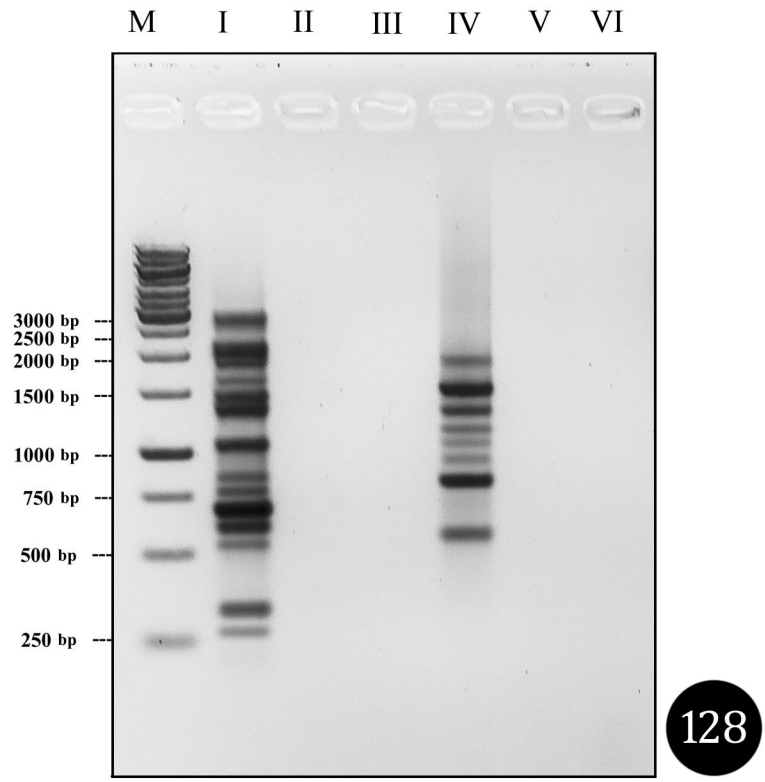


Fig. 128. RAPD profiles of five species of *Phyllanthus* L. showing amplification of bands with OPA-18 primer. Here, M=1 Kb DNA ladder, I=*P. acidus* (L.) Skeels, II=*P. emblica* L. (small fruit form), III=*P. emblica* L. (large fruit form), IV=*P. niruri* L., V=*P. reticulatus* Poir., VI=*P. urinaria* L.

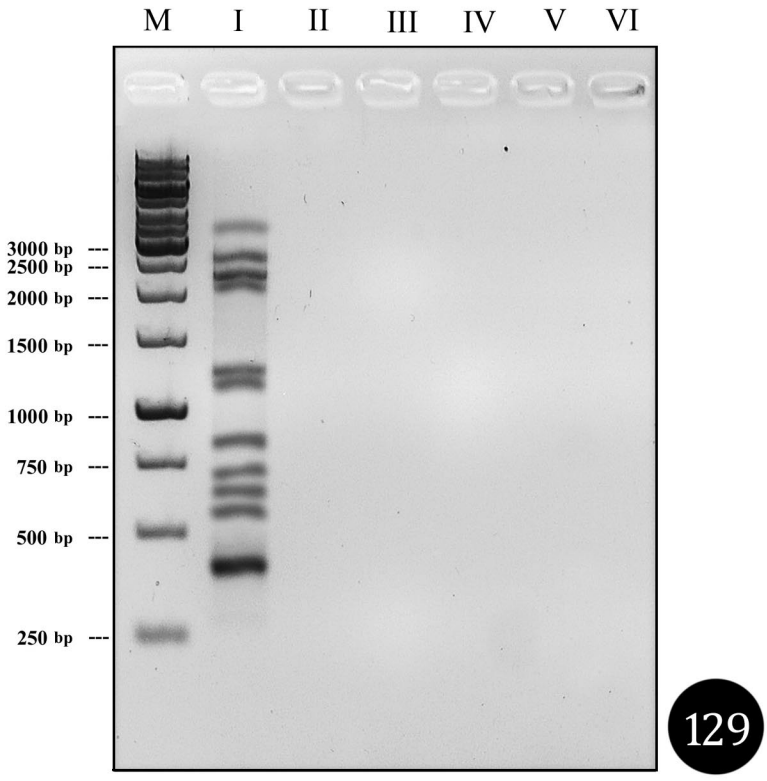


Fig. 129. RAPD profiles of five species of *Phyllanthus* L. showing amplification of bands with OPB-19 primer. Here, M=1 Kb DNA ladder, I=*P. acidus* (L.) Skeels, II=*P. emblica* L. (small fruit form), III=*P. emblica* L. (large fruit form), IV=*P. niruri* L., V=*P. reticulatus* Poir., VI=*P. urinaria* L.

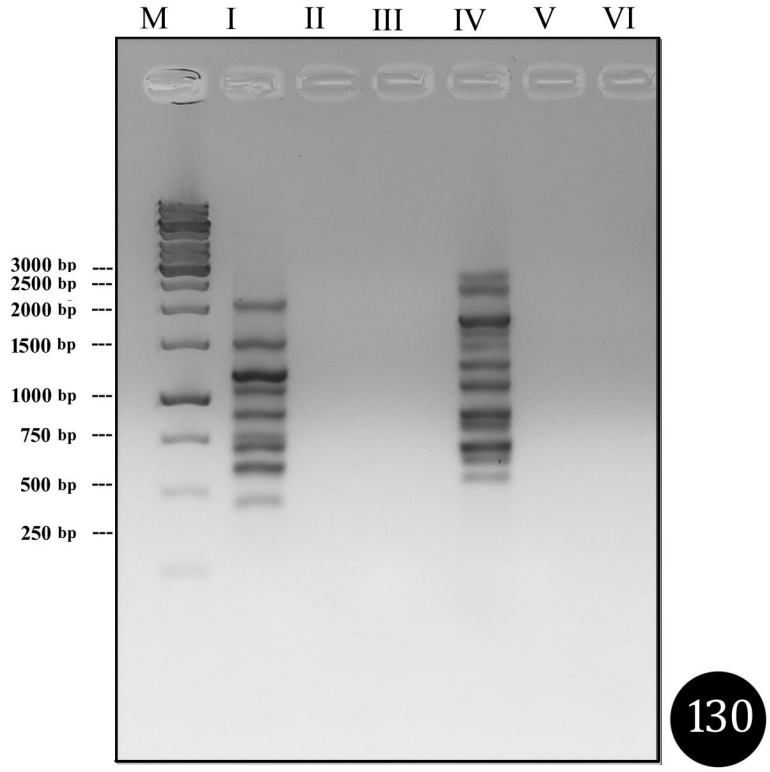


Fig. 130. RAPD profiles of five species of *Phyllanthus* L. showing amplification of bands with OPAB-5 primer. Here, M=1 Kb DNA ladder, I=*P. acidus* (L.) Skeels, II=*P. emblica* L. (small fruit form), III=*P. emblica* L. (large fruit form), IV=*P. niruri* L., V=*P. reticulatus* Poir., VI=*P. urinaria* L.

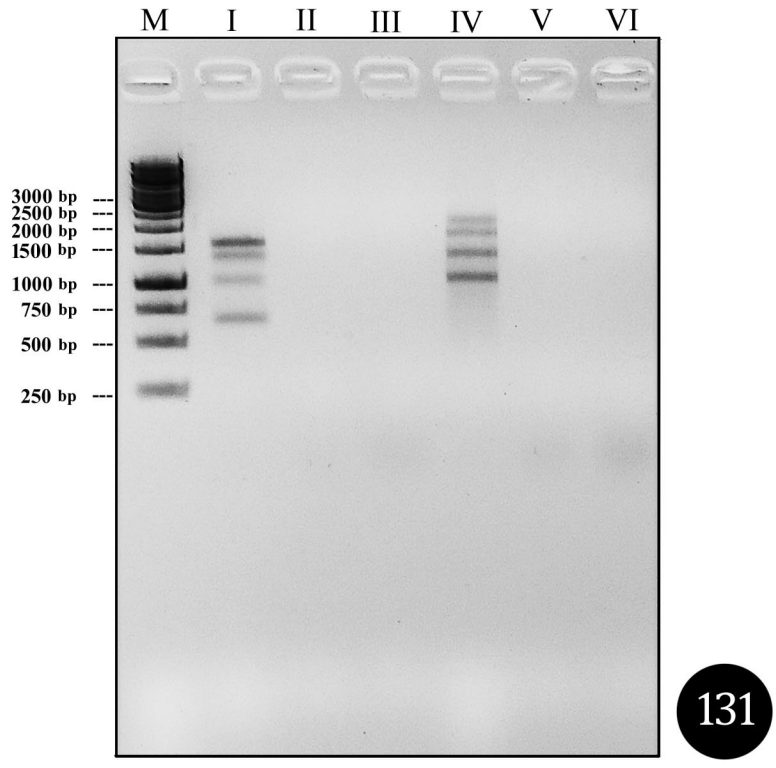


Fig. 131. RAPD profiles of five species of *Phyllanthus* L. showing amplification of bands with OPAB-6 primer. Here, M=1 Kb DNA ladder, I=*P. acidus* (L.) Skeels, II=*P. emblica* L. (small fruit form), III=*P. emblica* L. (large fruit form), IV=*P. niruri* L., V=*P. reticulatus* Poir., VI=*P. urinaria* L.

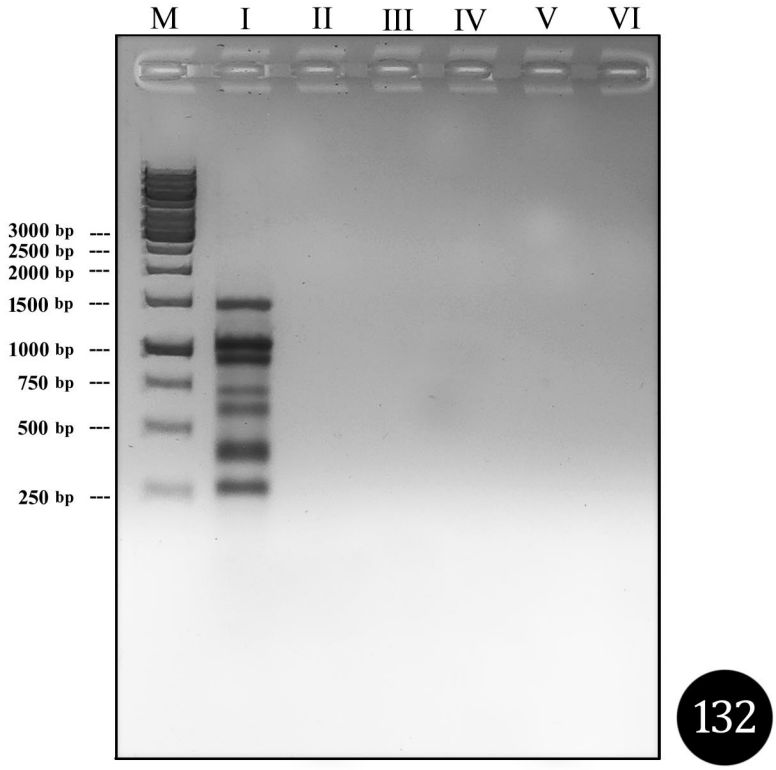


Fig. 132. RAPD profiles of five species of *Phyllanthus* L. showing amplification of bands with OPC-13 primer. Here, M=1 Kb DNA ladder, I=*P. acidus* (L.) Skeels, II=*P. emblica* L. (small fruit form), III=*P. emblica* L. (large fruit form), IV=*P. niruri* L., V=*P. reticulatus* Poir., VI=*P. urinaria* L.

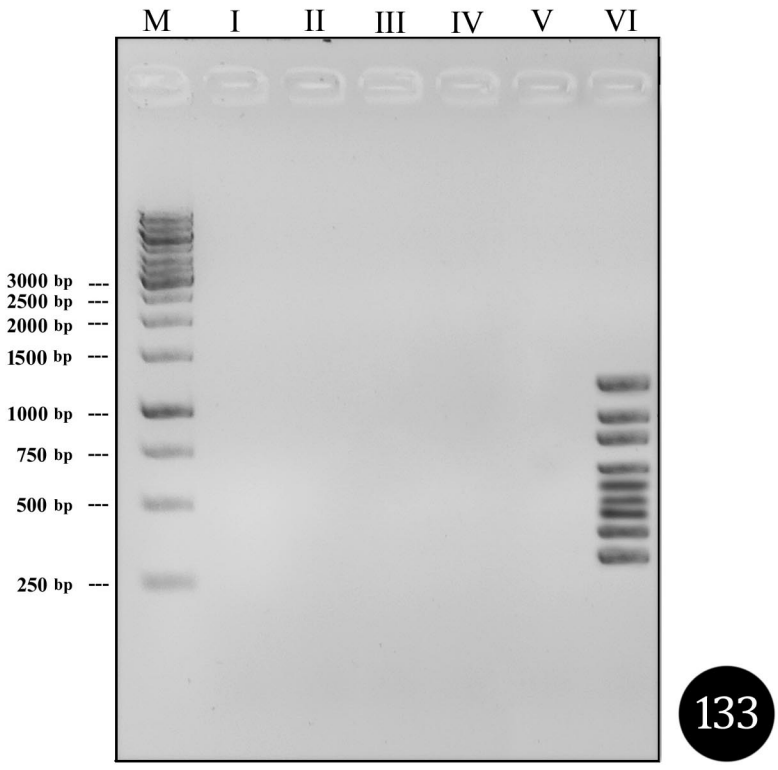


Fig. 133. RAPD profiles of five species of *Phyllanthus* L. showing amplification of bands with OPC-15 primer. Here, M=1 Kb DNA ladder, I=*P. acidus* (L.) Skeels, II=*P. emblica* L. (small fruit form), III=*P. emblica* L. (large fruit form), IV=*P. niruri* L., V=*P. reticulatus* Poir., VI=*P. urinaria* L.

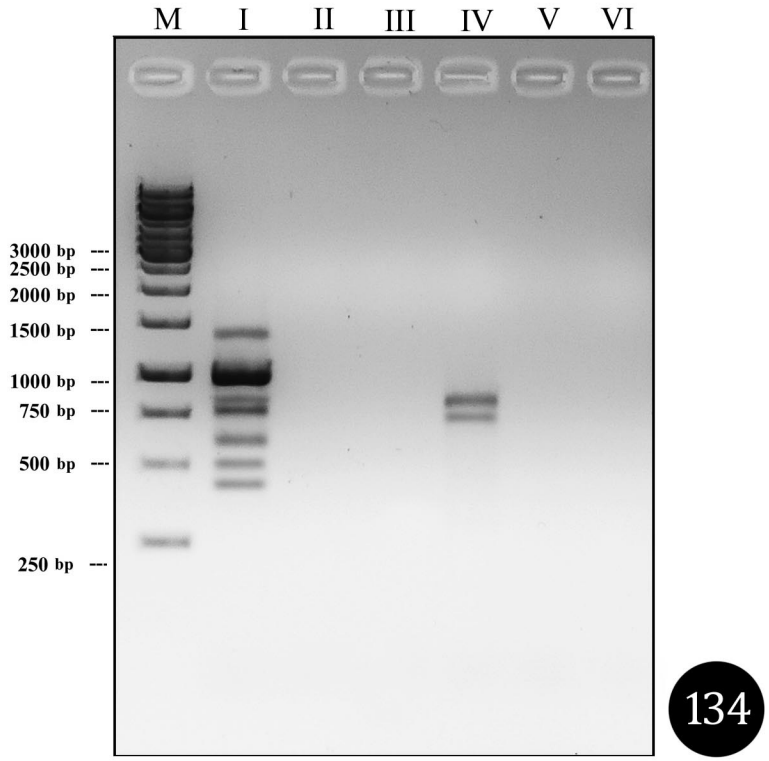


Fig. 134. RAPD profiles of five species of *Phyllanthus* L. showing amplification of bands with OPC-96 primer. Here, M=1 Kb DNA ladder, I=*P. acidus* (L.) Skeels, II=*P. emblica* L. (small fruit form), III=*P. emblica* L. (large fruit form), IV=*P. niruri* L., V=*P. reticulatus* Poir., VI=*P. urinaria* L.

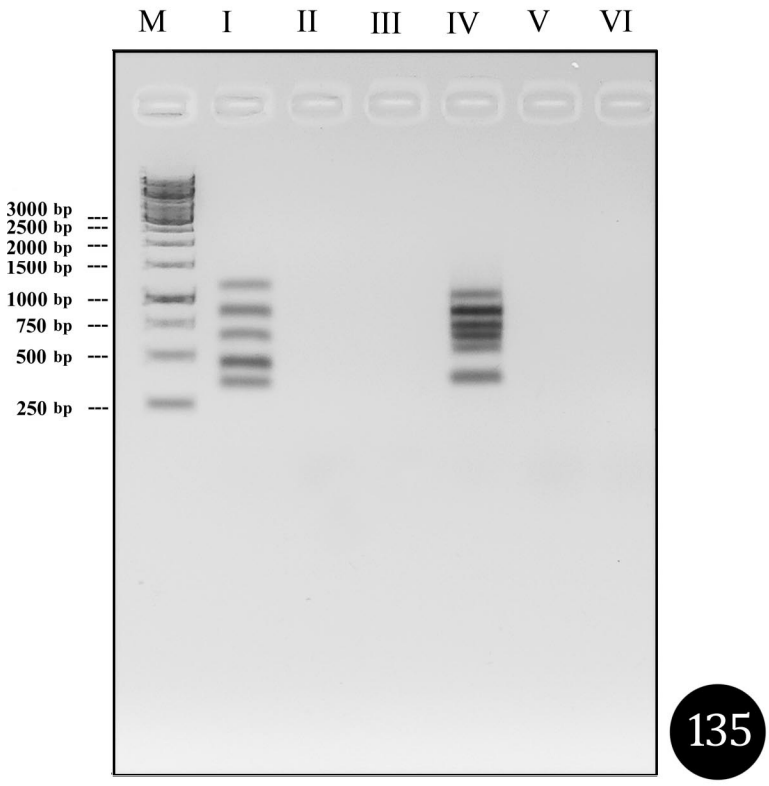


Fig. 135. RAPD profiles of five species of *Phyllanthus* L. showing amplification of bands with OPD-69 primer. Here, M=1 Kb DNA ladder, I=*P. acidus* (L.) Skeels, II=*P. emblica* L. (small fruit form), III=*P. emblica* L. (large fruit form), IV=*P. niruri* L., V=*P. reticulatus* Poir., VI=*P. urinaria* L.

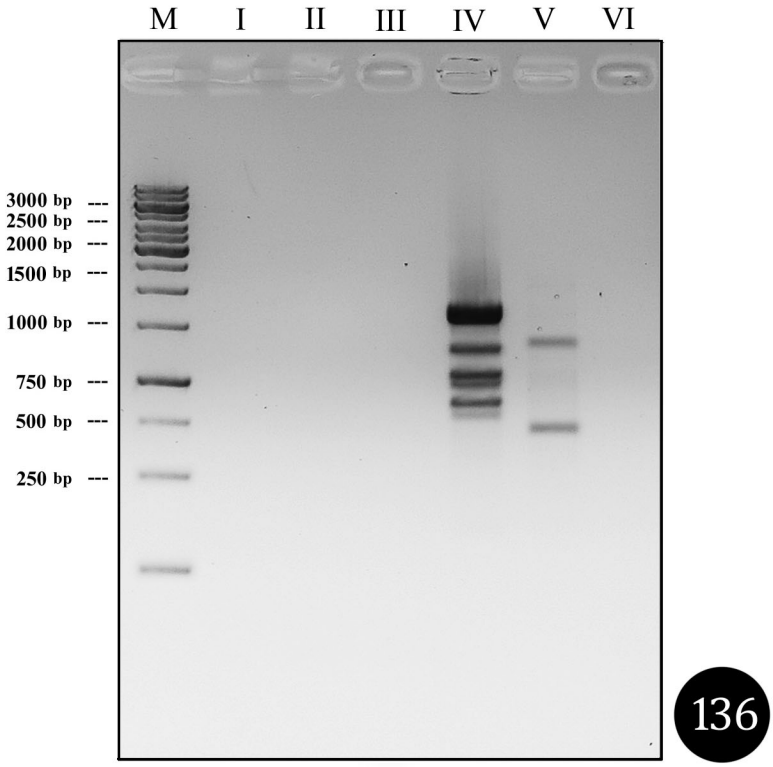


Fig. 136. RAPD profiles of five species of *Phyllanthus* L. showing amplification of bands with OPF-22 primer. Here, M=1 Kb DNA ladder, I=*P. acidus* (L.) Skeels, II=*P. emblica* L. (small fruit form), III=*P. emblica* L. (large fruit form), IV=*P. niruri* L., V=*P. reticulatus* Poir., VI=*P. urinaria* L.

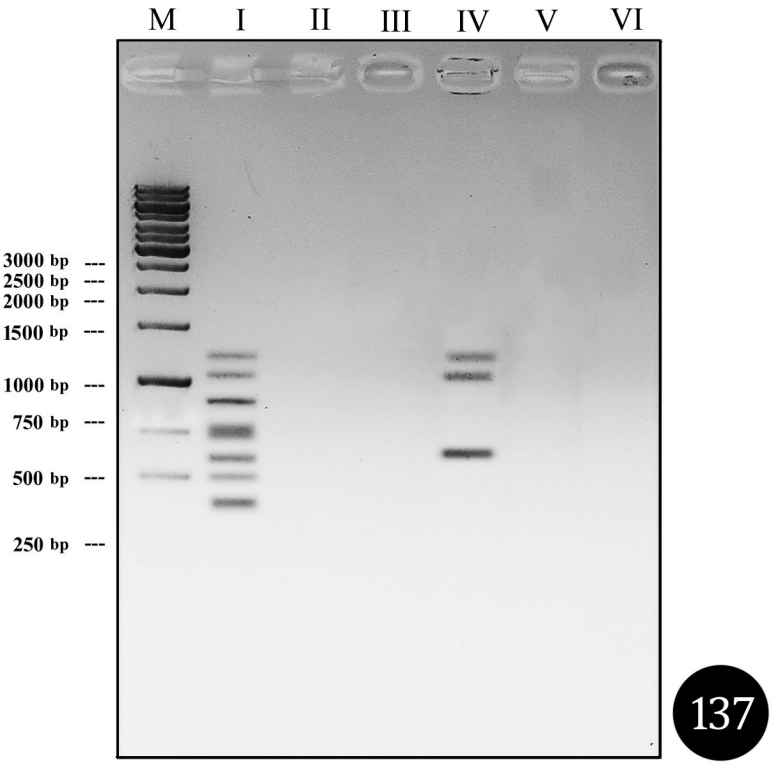


Fig. 137. RAPD profiles of five species of *Phyllanthus* L. showing amplification of bands with OPH-12 primer. Here, M=1 Kb DNA ladder, I=*P. acidus* (L.) Skeels, II=*P. emblica* L. (small fruit form), III=*P. emblica* L. (large fruit form), IV=*P. niruri* L., V=*P. reticulatus* Poir., VI=*P. urinaria* L.

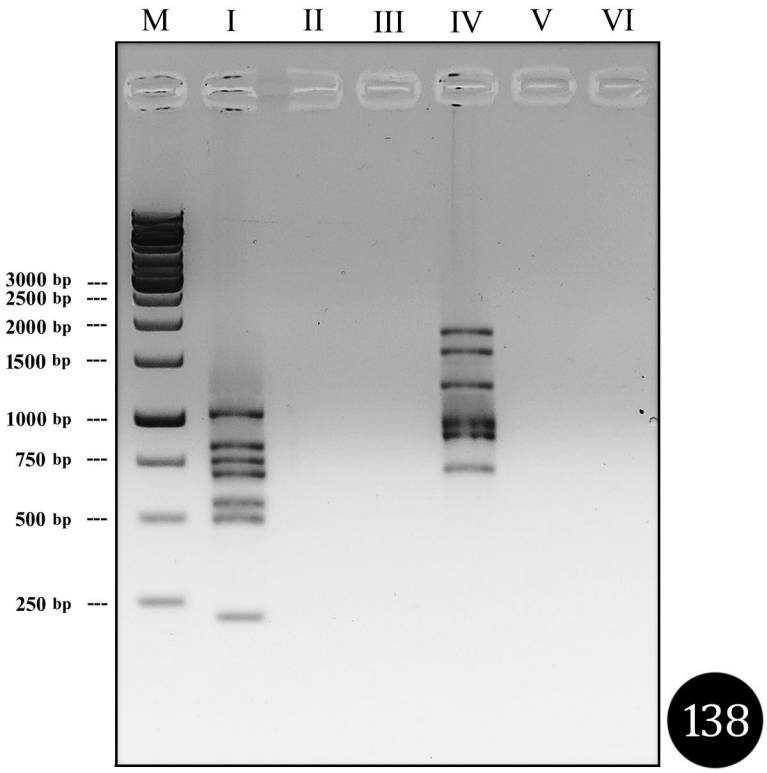


Fig. 138. RAPD profiles of five species of *Phyllanthus* L. showing amplification of bands with OPG-5 primer. Here, M=1 Kb DNA ladder, I=*P. acidus* (L.) Skeels, II=*P. emblica* L. (small fruit form), III=*P. emblica* L. (large fruit form), IV=*P. niruri* L., V=*P. reticulatus* Poir., VI=*P. urinaria* L.

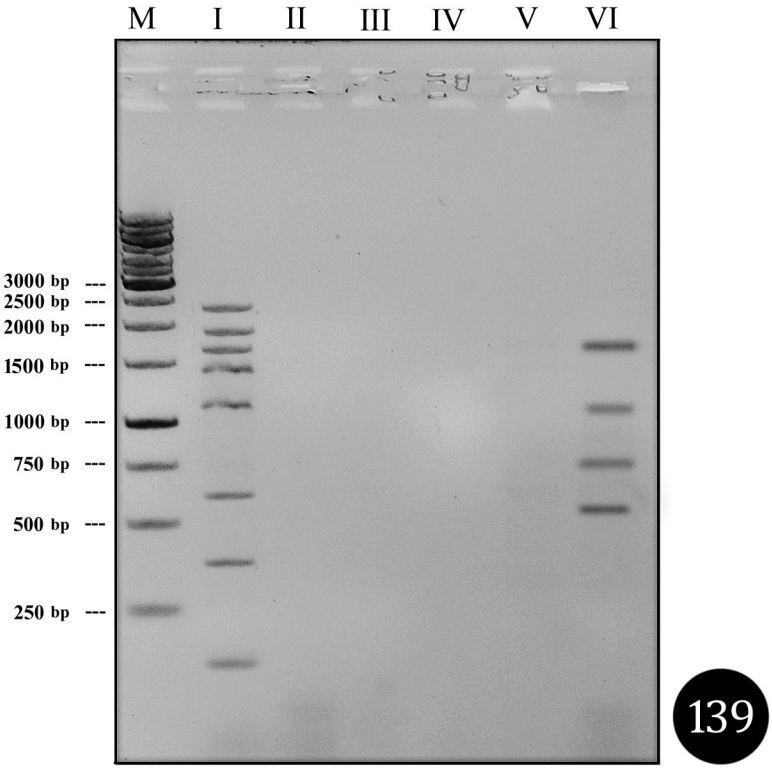


Fig. 139. RAPD profiles of five species of *Phyllanthus* L. showing amplification of bands with OPG-7 primer. Here, M=1 Kb DNA ladder, I=*P. acidus* (L.) Skeels, II=*P. emblica* L. (small fruit form), III=*P. emblica* L. (large fruit form), IV=*P. niruri* L., V=*P. reticulatus* Poir., VI=*P. urinaria* L.

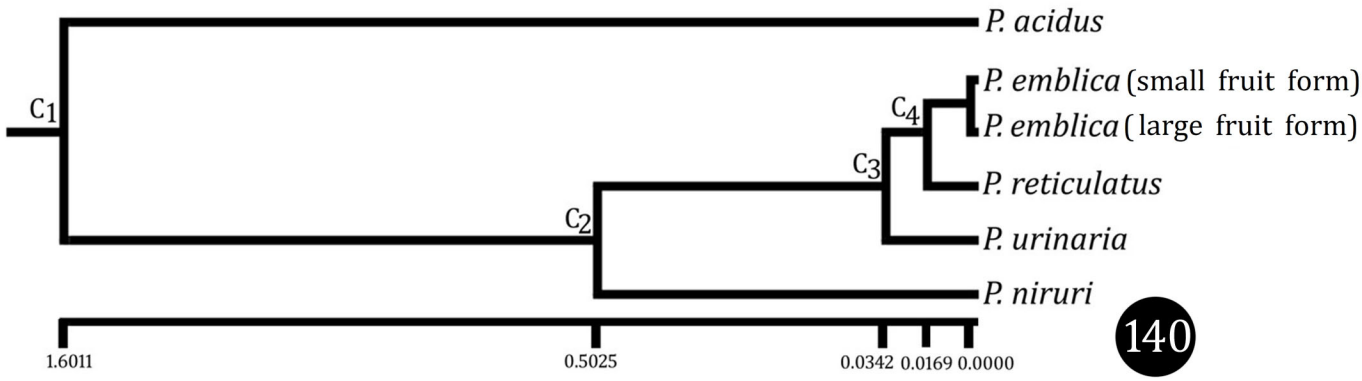


Fig. 140. UPGMA dendrogram based on Nei's (1972) genetic distance summarizing the data on differentiation among five species of *Phyllanthus* L. according to RAPD analysis.

CHAPTER FOUR:
DISCUSSION

CHAPTER FOUR: DISCUSSION

A combination of taxonomical, cytogenetical and molecular investigations of five *Phyllanthus* L. species *viz. P. acidus*, *P. emblica* (small fruit form), *P. emblica* (large fruit form), *P. niruri*, *P. reticulatus* and *P. urinaria* were discussed for the first time in Bangladesh.

4.1. Taxonomical diversification in five species of *Phyllanthus* L.

In the present investigation, five species of *Phyllanthus* L. differed in several taxonomical parameters such as branching pattern, morphology of bark, leaves, flower, fruits, seeds, flowering and fruiting time (Figs. 1-42, Table 13). *Phyllanthus acidus* and *P. emblica* are tree, these two species differed in respect of some other characteristic features such as lamina ovate to ovate-lanceolate, flowers red, stamens 4, free, fruits drupe, depressed-globose in *P. acidus* and lamina oblong or linear-oblong, flowers greenish white or greenish red, stamens 3, filaments connate in a column in *P. emblica.* These two species also differ in cytogenetical character such as their ploidy (*P. acidus* diploid but *P. emblica* polyploid), centromeric formula (*P. acidus* 12m+14sm but in case of *P. emblica* this is indistinct), DAPI band (in *P. acidus* there are 2 bands, where as in *P. emblica* there are 6-7 bands). *Phyllanthus reticulatus* showed several distinct characteristics such as habit shrubs, stamens 5 and black coloured ripe fruit. This species is also distinct in centromeric formula, number of CMA and DAPI bands. *Phyllanthus niruri* and *P. urinaria* both are herb but differed regarding some features such as lamina shape, fruit shape. They also differ in somatic chromosome number, ploidy (diploid in *P. niruri* and polyploid in *P. urinaria*), number of CMA (3 and 13, respectively) and DAPI bands (26 and 7, respectively) (Tables 20-23).

4.2. Cytogenetical characterization of five species of *Phyllanthus* L.

4.2.1. Orcein-staining properties of interphase nuclei and prophase chromosomes

The staining properties of interphase nuclei and prophase chromosomes usually provide karyomorphological features that help to characterize different species.

Tanaka (1971) was the pioneer of proposing these criteria for karyomorphological investigation. He found that the nature of staining of heterochromatins present in the interphase nuclei and prophase chromosomes were different in different species. On the basis of the staining property he classified interphase nuclei and prophase chromosomes in five different categories. Later different investigators applied these criteria in characterizing different plant materials of diverge nature (Alam *et al.* 1993, Begum and Alam 2004, Hossain *et al.* 2016, Sultana and Alam 2016a, Bonna *et al*. 2018).

In this study, interphase nuclei of five species of *Phyllanthus* L. were grouped into three categories such as "Diffuse type", "Simple chromocenter type" and "Complex chromocenter type" on the basis of nature of staining properties of heterochromatin after orcein staining.

Interphase nuclei of *P. niruri* and *P. urinaria* were homogenously stained with orcein (Figs. 46, 48, Table 38). According to Tanaka (1971), these could be regarded as "Diffuse type". In contrast, several number of small heterochromatic regions were scatterdly distributed around the nucleus of *P. emblica* (both forms) and *P. reticulatus* which could be considered as "Simple chromocenter type" (Figs. 44, 45, 47, Table 38). On the other hand, few large heterochromatic regions were observed in the interphase nuclei of *P. acidus* (Fig. 43, Table 38). This type of interphase nuclei were considered as "Complex chromocenter type".

The prophase chromosome of *P. emblica* (both forms), *P. reticulatus* and *P. urinaria* were darkly stained at different interstitial portions of (Figs. 62, 63, 65, 66, Table 38). According to Tanaka 1971, this type of prophase chromosome could be regarded as "Interstitial Type". However, "Continuous type" of prophase chromosomes were found in *P. acidus* and *P. niruri* where prophase chromosomes were uniformly stained along the length with orcein (Figs. 61, 64, Table 38).

Usually, species with "Diffuse type" of interphase nuclei showed "Continuous type" of prophase chromosome and "Simple or Complex chromocenter type" of interphase nuclei showed "Gradient or Interstitial type" of prophase chromosomes. In this study, *P. emblica* (both forms), *P. reticulatus* followed the general rule showing "Simple chromocenter type" of interphase nuclei and "Interstitial type" of prophase

chromosomes. *Phyllanthus niruri* also followed the general rule possessing "Diffuse type" of interphase nuclei and "Continuous type" of prophase chromosome. However, *P. acidus* and *P. urinaria* did not follow the usual regulation of heterochromatin. In *P. acidus,* heterochromatins are condensed to form bigger block whether these are homogeneously distributed along the chromosomal length. On the other hand, heterochromatins are homogeneously distributed in the interphase nuclei but distributed at the interstitial portions of prophase chromosomes in *P. urinaria*. Presence of facultative heterochromatin might be one of the reasons for this type of observation. Whatever the reason is, the five *Phyllanthus* L. species could be characterized on the basis of these characters of interphase nuclei and prophase chromosomes (Figs. 43-54, 61-66, Table 38).

A nucleolus was observed in interphase stages in four species of *Phyllanthus* L. except *P. niruri*. However, although nucleolus was not found in interphase nuclei but was appeared in prophase stage in *P. niruri* suggesting the late transcription of rDNA to rRNA and late transportation of rRNA from the nucleus to the cytoplasm as compared to other studied species. On the other hand, in *P. emblica* (large fruit form) and *P. urinaria,* a nucleolus was found in interphase nuclei but absent in prophase stage (Figs. 45, 48). These observations suggested the early transcription of rDNA to rRNA and early transportation of rRNA from the nucleus to the cytoplasm.

Table 38. Types of interphase nuclei and prophase chromosomes of five species of *Phyllanthus* L. after staining with orcein

Species	Type of chromocentric interphase nuclei	Type of prophase chromosomes
P. acidus	Complex Chromocenter	Continuous
P. emblica (small fruit form)	Simple Chromocenter	Interstitial
P. emblica (large fruit form)	Simple Chromocenter	Interstitial
P. niruri	Diffuse	Continuous
P. reticulatus	Simple Chromocenter	Interstitial
P. urinaria	Diffuse	Interstitial

4.2.2. Probable ways of origin of multiple basic and somatic chromosome number in different *Phyllanthus* L. species

In this investigation, *Phyllanthus* L. species showed variation in somatic chromosome counts such as 2n=26 in *P. acidus*, *P. niruri* and *P. reticulatus*, 2n=48 in *P. urinaria* and 2n=100 in *P. emblica* (both forms) with different basic chromosome number (Figs. 79-84, Table 39). In addition it was observed that, aneuploidy and polyploidy played important role in the evolution of a series of new basic chromosome numbers (x=6, 7, 8, 10, 13, 14 and 25), accompanied with the diversification of species within the genus *Phyllanthus* L.

According to the previous chromosome number records, 2n=26 and 2n=28 chromosomes were reported for *P. acidus* (Tables 7, 39). The diploid chromosome number of 2n=26 for *P. acidus* was determined in the present investigation which correlates with the previous reports of different scientists and indicate basic chromosome number x=13 (Tables 7, 39). However, 2n=28 (Thombre 1959) for this species might possess different basic chromosome number x=14.

On the basis of previous literature, *P. emblica* had different 2n chromosome number such as 2n=28, 52, 98, 104 and 98-104 (Tables 7, 39). If the basic chromosome number was x=14, *P. emblica* with 2n=2x=28 and 2n=7x=98 could be regarded as diploid and heptaploid, respectively. In contrast, if the basic chromosome number was x=13, *P. emblica* with 2n=4x=52 and 2n=8x=104 might be considered as tetraploid and octaploid, respectively. On the other hand, 2n=99-103 (Janaki Ammal and Raghavan 1958) could be originated through different ways such as:

i. Secondary modification (both hyper or hypo-aneuploidy) of polyploidy as proposed by Stebbins (1971).
ii. Presence of B-chromosome as found in other species belonging to the genus *Phyllanthus* L. (6-10B in *P. pulcher,* 1B in *P. parvifolius*) (Table 39).
iii. Numerical chromosomal aberration in the polyploidy generation because the effect of addition or deletion of few chromosomes could not be lethal in a polyploidy species due to possessing multiple copies of a basic set of chromosomes.

In the present study, *P. emblica* (small fruit form) was found to possess 2n=100 chromosomes (in 90% cells) (Fig. 80). Besides 2n=100 somatic chromosome numbers, 2n=78 (in 6% cell) and 2n=104 (in 4% cell) were also found in this study in few mitotic metaphase stage of *P. emblica* (small fruit form) which was not significant. On the other hand, *P. emblica* (large fruit form) had 2n=100 chromosomes (in 95.56% cells) in present study. In addition, few mitotic metaphase stage of this form showed 2n=90 (in 4.44% cells) besides 2n=100 which was also non-significant. Thus, the present findings (2n=100 and 104) supports the previous report 2n=98-104 (Table 39). In the available internet sources and relevant literature 2n=78 (small fruit form) and 2n=90 (large fruit form) was not reported earlier for *P. emblica* (small fruit form) thus could be regarded as first report for *P. emblica* (Figs. 80, 81, Table 21). The above data indicated that *P. emblica* (small fruit form) examined in this study had x=13 and might be considered as (2n=6x=78) hexaploid and (2n=8x=104) octaploid. However, 2n=10x=100 (in both forms) and 2n=9x=90 (in large fruit form) represent a new basic chromosome number x=10, which was not recorded previously for this species and could be considered as decaploid and nanoploid, respectively.

In *P. niruri*, 2n=26 chromosomes were observed in this research work (Fig. 82). Moreover, different 2n chromosome number of this species were reported earlier such as 2n=14, 26, 28 and 36 (Table 39). Therefore, the diploid chromosome number of 2n=26 for *P. niruri* was determined in the present investigation which correlates with the previous reports of different scientists (Table 39). If the basic chromosome number was x=7, species with 2n=2x=14, and 2n=4x=28 might be diploid and tetraploid, respectively. Species with 2n=2x=26 and 2n=6x=36 for this species might possess different basic chromosome number such as x=13 and x=6, respectively. However, according to previous literature and present investigation, x=7 and 13 were more frequent.

In case of the previous chromosome number records, 2n=26 and 28 chromosomes were reported for *P. reticulatus* (Table 39). The diploid chromosome number of 2n=2x=26 with x=13 for *P. reticulatus* was determined in the present investigation which correlates with the previous reports of different scientists. However, 2n=28

(Sharma and Jash 1958) for this species might be a case of hyper-aneuploidy which was not found in this research.

In the present study, 2n=6x=48 (hexaploid) with x=8 were observed in 90% somatic cells of *P. urinaria.* In addition, 2n=50 (in 5% cell) and 2n=52 (in 5% cell) were also found in few mitotic metaphase stage which might be due to the presence of B-chromosomes. Moreover, different 2n chromosome number of this species were reported earlier such as 2n=14, 24, 26, 28, 48, 50 and 52 (Table 39). Therefore, the somatic chromosome number of 2n=48 for *P. urinaria* was determined in the present investigation correlates with the previous report of Bancilhon (1971). However, *P. urinaria* have found to be reported multiple basic chromosome number such as x=6, 7, 8, 10, 13, 14 and 25, among these, x=8 and 13 are more frequent than others, showing predominantly retain these two basic number linage. According to Haicour *et al.* (1994), the variable base number could be originated from the ancestral one *i.e.* x=13 where x=6, 7, 8, 10 formed by decreasing aneuploidy and x=14, 25 by upward disploidy. Polyploid complex with the presence of hexaploid (2n=6x=48) cytotypes of x=8 and diploid (2n=2x=26) and tetraploid (2n=4x=52) cytotypes of x=13 indicated the existence of two dominant polyploid complex in nature.

Perry (1943) and Janaki Ammal and Raghavan (1958) hypothesized that the herbaceous species of *Phyllanthus*, with x=13, have a different base number from the woody ones, with x=7. According to the present investigation, Perry's and Janaki Ammal and Raghavan's observations substantiate to be incorrect and seem to have no correlation among basic chromosome number with the plant habit which supports the Webster and Ellis's (1962) findings.

Inconstancy in the somatic chromosome complement reported in the genus *Phyllanthus* L.*,* has revealed a number of interesting correlation regarding the basic set of chromosomes. Therefore, the diversity in basic chromosome number can be explained either to non-disjunction or possible hybridization within different cytotypes among these species or numerical chromosomal aberration in the polyploid generation.

4.2.3. Karyotype diversity among different *Phyllanthus* L. species

Five species of *Phyllanthus* L. differed in respect of somatic chromosome numbers and other karyomorphological features (Figs. 97-102, Table 20). Among these, comparatively bigger sized chromosomes was found in *P. acidus* [3.86(±0.11)-6.33(±0.19) µm] and *P. reticulatus* [2.93(±0.20)-5.29(±0.33) µm] than the rest species. Total length of 2n chromosome complements was the highest in *P. emblica* (large fruit form) 149.02(±0.88) µm which corresponds with its maximum number of somatic chromosome (2n=100) while it was the lowest in *P. niruri* 50.26(±0.45) µm (Table 20). The centromeric formulae was recorded as 12m+14sm for *P. acidus*, 20m+6sm for *P. niruri* and 4m+22sm for *P. reticulatus* whereas the centromeric position is visibly indistinct in *P. emblica* (both forms) and *P. urinaria* – two polyploid species, due to the smaller size of chromosome. However, in two polyploid species, the range of chromosomal length was almost negligible *i.e.* distance between small and large chromosomes was about 1.5 µm (Table 20). Presence of similar sized chromosomes is a feature of symmetric karyotype (Stebbins 1971). In contrast, few submetacentric chromosomes were observed in *P. acidus* (12m+14sm), *P. niruri* (20m+6sm) and *P. reticulatus* (4m+22sm) which indicated relatively asymmetric nature of their karyotype (Figs. 97, 100, 101, Table 20). Stebbins (1971) mentioned that the symmetric karyotypes were primitive character. Therefore, the five species of *Phyllanthus* L. were more or less primitive of which *P. acidus, P. niruri* and *P. reticulatus* were comparatively advanced than the rest.

4.2.4. Meiotic behavior and pollen fertility

Meiotic behavior of polyploid species of *Phyllanthus* L. varies sharply due to unstable somatic chromosome number such as 2n=100 (about 90% cells of small and large fruit forms of *P. emblica*), 2n=90 (in large fruit form of *P. emblica*) and 2n=78, 104 (in small fruit form of *P. emblica*) with probable two different basic chromosome number x=10 and 13 (Table 39). During meiosis, some meiotic metaphase-I stage showed all bivalents (IIs) while some metaphase-I stage had both bivalents (IIs) and multivalent in both small and large fruit forms of *P. emblica* (Figs. 115-120). Formation of bivalent suggested this plant as an allo-polyploid. In addition, at

diakinesis and metaphase-I several multivalent were also observed. This features indicated that chromosomes involved in the formation of multivalent might possess segmental homology and it can be pointed out the possibility of their being auto-allopolyploid or segmental-allopolyploid. However, multivalent number varies from cell to cell because it depends on some factors such as asynapsis and de-synapsis, number and position of chiasma, degree of terminalization of chiasma, etc. This cytogenetic observations such as formation of bivalents and some irregular multivalent in meiotic chromosomes indicated the presently examined polyploid species as interspecific hybrid which should be partly fertile. However, about 98% pollens were viable in both forms of *P. emblica* since chromosomes were segregated regularly at anaphase during meiosis (Figs. 121-126). Stebbins (1971) reported diplodization of polyploid in many ancient polyploid species. From the above features it can be suggested that these species are going through the process of diplodization.

4.2.5. Fluorescence banding pattern

Chromomycin A_3 (CMA) and 4´, 6-Diamidino-2-Phenylindole (DAPI) are two fluorochrome specific to GC- and AT-rich repetitive sequence, respectively (Schweizer 1976). Fluorescent banding gives decisive analysis of karyotype, even chromosome having similar morphology and other conventional karyotypic features. In this study, these two fluorochrome were used for making a comparative account of five *Phyllanthus* L. species. Characterization of *Phyllanthus* L. species through CMA and DAPI-banding was the pioneer attempt in Bangladesh.

4.2.5.1. CMA-banding pattern

In CMA-staining, bands were found in all species of *Phyllanthus* L. except *P. emblica* (small fruit form). The number, location and distribution of CMA-bands varied in different species. The numbers of CMA-bands are 3 in *P. acidus* and *P. niruri*, 15 in *P. emblica* (large fruit form), 4 in *P. reticulatus* and 13 in *P. urinaria* (Figs. 103-108). Most of the CMA-bands were located at the terminal or centromeric portions of chromosome which indicated a tendency of accumulating GC-rich sequences at centromeric regions or chromosomal ends. In addition, few chromosomes were

entirely fluoresced with CMA where GC-rich repeats were not confined to the terminal or centromeric region rather distributed along the entire length of chromosomes (Figs. 103-108). The possible reason for these entirely fluoresced chromosomes was tandem duplication of GC-rich repeats (Sultana and Alam 2016b, Khatun and Alam 2010, Sultana and Alam 2007, Hiron *et al*. 2006, Mahbub *et al*. 2007). Total length of CMA-positive banded region ranging from 1.73 µm (*P. niruri*) to 24.31 µm (large fruit form of *P. emblica*). The percentage of GC-rich repeats was the lowest in 2.71 (*P. niruri*) and the highest in 19.09 (*P. urinaria*) (Table 22). On the basis of number and location of CMA-bands, karyotypes formulae were prepared for each species. It has seen that each species has distinct CMA-banded karyotype formula (Table 22).

In case of *P. acidus, P. niruri* and *P. urinaria*, heteromorphicity was found in chromosome pair 7, 11 and 23, respectively where one homologue showed terminal band while no band was observed in their homologue suggesting probable deletion of the banded region from the respective chromosomes. In contrast, in pair 4 of *P. emblica* (large fruit form), one chromosome fluoresced entirely while its homologue member lacks CMA-positive band. In this case, GC-rich repeats of one member may tandemly duplicated along the length of the respective chromosomes (Figs. 103, 106, 108). With the help of CMA-banding, a comparative analysis of banding pattern revealed the occurrence of structural aberration such as deletion and tandem duplication in the *Phyllanthus* L. species.

4.2.5.2. DAPI-banding pattern

In the present study, five *Phyllanthus* L. species possessed distinct DAPI-banding pattern. *Phyllanthus acidus, P. emblica* (small fruit form), *P. emblica* (large fruit form), *P. niruri*, *P. reticulatus* and *P. urinaria* were found to possess 2, 6, 7, 26, 4 and 7 DAPI-positive bands, respectively. Among these 52 bands, 37 were located at the centromeric portions and 11 were observed in terminal portions of chromosome which indicated a tendency of accumulating AT-rich sequences at centromeric regions or chromosomal ends. In addition, 4 chromosomes were entirely fluoresced with DAPI where AT-rich repeats were not confined to the terminal or centromeric region rather distributed along the chromosomes (Figs. 109-114). The possible

reason for these entirely fluoresced chromosomes was tandem duplication of AT-rich repeats (Hiron *et al*. 2006, Mahbub *et al*. 2007, Khatun and Alam 2010). Total length of DAPI-positive banded region ranging from 1.17μm (large fruit form of *P. emblica*) to 24.5 μm (*P. urinaria*). The percentage of AT-rich repeats was the lowest in 1.48 (*P. acidus*) and the highest in 28.5 (*P. niruri*) (Table 23). On the basis of number and location of DAPI-bands, all these species showed diversity in their karyotype (Table 23).

In case of *P. emblica* (large fruit form) and *P. urinaria*, heteromorphicity was found in chromosome pair 50 and 22, respectively. In *P. emblica* (large fruit form) one chromosome of pair 50 showed centromeric band while no band was observed in its homologue. In contrast, in pair 22, one chromosome showed terminal DAPI-bands while the homologue member lacks DAPI-positive band. The above observations suggested the probable deletion of the banded region from those chromosomes (Figs. 111, 114). Therefore, few structural aberrations such as deletion and tandem duplication were observed after DAPI-banding in studied *Phyllanthus* L. species.

Table 39. Multiple basic and somatic chromosome number in different *Phyllanthus* L. species

Basic chrom-osome number (x)	Somatic chrom-osome number (2n)	Probable ploidy status	Species belonging to the genus *Phyllanthus* L.	References
6	2n=2x=12	Diploid	*P. parvifolius* *P. odontadenius*	Malla *et al.* 1975 Haicour *et al.* 1994
	2n=4x=24	Tetraploid	*P. debilis* *P. urinaria* *P. odontadenius*	Bancilhon1971 Kothari *et al.* 1981 Haicour *et al.* 1994
	2n=6x=36	Hexaploid	*P. caroliniensis* subsp. *guianensis,* *P. niruri* subsp. *lathyroides*	Webster and Ellis 1962 Bancilhon 1971
7	2n=2x=14	Diploid	*P. niruri* *P. urinaria* *P. debilis*	Chuang *et al.* 1963 Hsu 1967, Bancilhon 1971 Krishnappa and Reshme1980
	2n=4x=28	Tetraploid	*P. niruri*	Webster and Ellis 1962
	2n=8x=56	Octaploid	*P. odontadenius* *P. bancilhonae*	Bancilhon 1971 Haicour *et al.* 1994
8	2n=2x=16	Diploid	*P. polygonoides*	Webster and Ellis 1962
	2n=6x=48	Hexaploid	*P. urinaria*	Bancilhon 1971
10	2n=4x=40	Tetraploid	*P. debilis* *P. magnificens*	Trivedi *et al.* 1986 Haicour *et al.* 1994
	50=(2n=5x)+(6-10B)	Pentaploid and presence of B-chromosome	*P. pulcher*	Webster and Ellis 1962
	2n=10x=100	Decaploid	*P. embergeri*	Rossignol *et al.* 1987
13	2n=2x=26	Diploid	*P. acidus* *P. maderaspatensis* *P. niruri* *P. reticulatus* *P. urinaria*	Raghavan 1957, Raghavan 1959, Thombre 1959, Webster and Ellis 1962, Chuang *et al.* 1963, Raman and Kesavan 1963, Mehra and Hans 1969, Champault 1970, Bancilhon 1971, Brunel 1976, Sarkar *et al.* 1976, Bedi *et al*. 1980, Krishnappa and Reshme 1980, Sarkar and Datta 1980, Kothari *et al.* 1981, Trivedi *et al*. 1986, Huang *et al.* 1988

Table Continued

Basic chrom-osome number (x)	**Somatic chrom-osome number (2n)**	**Probable ploidy status**	**Species belonging to the genus *Phyllanthus***	**References**
13	2n=4x=52	Tetraploid	*P. acuminatus* *P. amarus* *P. axillaris* *P. maderaspatensis* *P. mimosoides* *P. nutans* *P. ovatas* *P. pentaphyllus* *P. subglomeratus* *P. urinaria* *P. emblica*	Janaki Ammal and Raghavan 1958, Raghavan and Arora 1958, Webster and Ellis 1962, Datta 1967, Champault 1970, Bancilhon 1971, Gill *et al.* 1973, Sarkar and Datta 1980, Chatha and Bir 1987
	(2n=4x)52+1B	Tetraploid and presence of B-chromosome	*P. parvifolius*	Sandhu and Mann 1989
	2n=6x=78	Hexaploid	*P. pulcher*	Webster and Ellis 1962
	98-104 =(2n=8x)104-(1-6)	Octaploid with hypo aneuploidy	*P. emblica*	Janaki Ammal and Raghavan 1958
	2n=8x=104	Octaploid	*P. emblica* *P. lawii*	Sarkar and Datta 1980, Bancilhon 1971, Gill *et al*. 1981, Kothari *et al*. 1981
	2n=12x=156	Dodecaploid	*P. grandifolius*	Miller and Webster 1966
	2n=12x=c.156	Dodecaploid	*P. juglandifolius*	Webster and Ellis 1962
14	2n=2x=28	Diploid	*P. acidus* *P. carolinensis* *P. emblica* *P. urinaria* *P. reticulatus* *P. odontadenius*	Perry 1943, Thombre 1959, Sharma and Jash 1958, Borgmann 1964, Bancilhon 1971,
	2n=7x=98	Heptaploid	*P. emblica*	Raghavan 1957, Janaki Ammal and Raghavan 1958, Mehra 1972, Mehra and Hans 1972, Hans 1973, Mehra 1976, Ohri and Kumar 1986
25	2n=2x=50	Diploid	*P. urinaria* Complex	Rossignol *et al.* 1987, Haicour *et al.* 1994

4.3. DNA fingerprinting by Random Amplified of Polymorphic DNA (RAPD)

RAPD is a PCR based marker technique that has been used for estimation of genetic diversity of populations and for studying the genetic relationships among different genotypes (Esmail *et al.* 2008). In this investigation, 12 oligonucleotide primers for RAPD were utilized to study the genetic relationship among the five *Phyllanthus* L. species. The data obtained following RAPD techniques were analyzed using "popgene32" computer package. The data were used to generate dissimilarity matrix for RAPD bands obtained through gel electrophoresis. The distance matrix between each pair of species was used to construct dendrogram using unweighted pair group method of arithmetical means (UPGMA). Five *Phyllanthus* L. species represented a broad spectrum of variation in RAPD banding pattern.

The primer sequence, band size and banding pattern of five of *Phyllanthus* L. species were shown in Tables 7 and 26-40. The 12 primers generated 141 distinct bands of which 97 were considered as polymorphic. Band size ranging from 150-4000 bp of PCR amplification products scored for all primers. A diverse level of polymorphism in different crops have been reported earlier such as Chickpea 98.14% (Rasool 2013), 87.00% (Datta *et al*. 2010), 14.56% (Sant *et al*. 1999) and 25.5% (Sonnante *et al*. 1997), *Brassica* 98.03% (Ghosh *et al.* 2009), Eggplant 57.89% (Biswas *et al*. 2009), Chilli 90% (Paran *et al.* 1998), Cotton 84.95% (Esmail *et al.* 2008), 90.96% (Maleia *et al.* 2010), 69.37% (Saravanan *et al.* 2006), 63.20% (Hussain *et al.* 2007) and 100% (Sultana and Alam 2016c), peanut 96% (Lang and Hang 2007), 42.7% (Raina *et al.* 2001) and 21% (He and Prakash 1997), Groundnut 6.68% (Subramanian *et al.* 2000), *Crotalaria* 48% (Wang *et al.* 2006). The results of the present investigation showed about 71.68% polymorphism among five species of *Phyllanthus* L. The broad range of polymorphism revealed wide diversity in *Phyllanthus* L. species.

4.3.1. Unique RAPD markers

A number of species specific unique bands were observed in *Phyllanthus* L. species such as 10 in *P. acidus* and 4 in *P. niruri* (OPA-18), 11 in *P. acidus* (OPB-19), 5 in *P. acidus* and 8 in *P. niruri* (OPAB-5), 2 in *P. acidus* and 2 in *P. niruri* (OPAB-6), 7 in *P. acidus* (OPC-13), 9 in *P. urinaria* (OPC-15), 5 in *P. acidus* (OPC-96), 2 in *P. acidus* and

3 in *P. niruri* (OPD-69), 6 in *P. acidus* and 2 in *P. reticulatus* (OPF-22), 4 in *P. acidus* (OPH-12), 5 in *P. acidus* and 4 in *P. niruri* (OPG-5), 6 in *P. acidus* and 2 in *P. urinaria* (OPG-7). The term unique sequence means that the sequence found in a species with a certain primer was absent in other species (Figs. 128-139, Tables 24-35). In the earlier literature, there was no information about unique bands of *Phyllanthus* L. species (Wang *et al.* 2006). The unique bands were stable and specific for the respective species and thus could be used as a tool for characterization.

4.4. Genetic relationships and Cluster analysis of studied *Phyllanthus* L. species

The values of pair-wise Nei's (1972) genetic distances analyzed by using computer software "popgene32" among five *Phyllanthus* L. species were computed from combined data for the twelve RAPD primers. The highest genetic distance (1.6011) was found between *P. acidus* with rest 4 species *i.e. P. emblica* (both forms), *P. niruri, P. reticulatus* and *P. urinaria*. The lowest (0.0169) genetic distance was observed between *P. reticulatus* and *P. emblica*. (Fig. 140, Table 37).

A cluster analysis on the basis of DNA fingerprinting by RAPD was carried out. Dendrogram based on Nei's (1972) genetic distance using UPGMA (Unweighted Pair Group Method of Arithmetic Means) segregated five *Phyllanthus* L. species into two major clusters C_1 and C_2 (Fig. 140). According to dendrogram, *P. acidus* is separated from other 4 species with high genetic distance 1.6011 and placed in a separate cluster C_1 (Fig. 140, Table 37). On the other hand, other 4 species were placed in cluster C_2. The lowest genetic distance 0.0169 was found between *P. reticulatus* and *P. emblica* whereas the highest genetic distance 0.5025 was found between *P. niruri* and *P. emblica* within cluster C_2. So among five *Phyllanthus* L., *P. emblica* and *P. reticulatus* were closely related whereas *P. acidus* and *P. emblica* were genetically distantly related.

4.5. The three dimensional aspects of the genus *Phyllanthus* L. assembled with taxonomic, cytogenetic and molecular point

A cyto-taxonomic study with RAPD analysis in the *Phyllanthus* L. has been done in present investigation. In presenting the findings of this study, we have tried to provide a comparative discussion of our results with the previous experimental studies of taxonomical and cytogenetical analysis of *Phyllanthus* L. species.

The wide numerical chromosome variation was observed and among these species *P. acidus, P. reticulatus* and *P. niruri* were diploid while *P. emblica* (both forms) and *P. urinaria* were segmental allopolyploid. It was well known that the phenomenon "Polyploidy" was more frequent in herbs and rarely found in trees. However, present investigation displayed fluctuation from that hypothesis, having inconsistent polyploid progenies of *P. emblica* (Tree). Otherwise, the rest correlates with that hypothesis.

Based on dendrogram prepared from RAPD analysis, *P. emblica* (both forms), *P. reticulatus* and *P. urinaria* were closely associated. Since *P. emblica* and *P. urinaria* were polyploid and *P. reticulatus* was the only diploid species among these three, it could be suggested that they might have shared some common genome that passing through the polyploid complex of *P. emblica* and *P. urinaria.*

There was a positive correlation between nature of heterochromatin condensation and RAPD dendrogram. *Phyllanthus acidus*, which showed complex chromocenter type of interphase nuclei to continuous type of prophase chromosomes, lied separately from other four species. The nature of heterochromatin condensation is also different to that of the rest species. Two species, *P. emblica* and *P. reticulatus* are closely related and they also showed similarity in heterochromatin condensation nature *i.e.* simple chromocenter type of interphase nuclei to interstitial type of prophase chromosomes. *Phyllanthus urinaria* had shown diffuse type of interphase and continuous type of prophase reside in a separate cluster next to the cluster of the *P. emblica*, *P. reticulatus* and *P. urinaria*.

The two forms (small and large fruit forms) of *P. emblica* displayed similarity in most important taxonomic parameters and somatic chromosome numbers. These

two forms also stayed closely in the dendrogram of RAPD. However, these two forms showed some dissimilarity. These were:

I. The bark colour was blackish in small fruit type and grayish in large fruit type.
II. The young leaves colour was green in small fruit type and purplish green in large fruit type.
III. The flower colour was greenish white in small fruit type and greenish red in large fruit type.
IV. The fruits were sharply different in respect of their sizes.
V. Flowering time was March to September for in small fruit form and January to March for large fruit form.

Plants belonging to *P. emblica* may be divided into two groups. Group-I containing plants yielding smaller fruits (size 2.0-2.2 cm and average weight 5.30 gm), may be regarded as small fruit form. Group-II containing plants yielding larger fruits (size 3.0-3.3 cm and average weight 25.18 gm), may be regarded as large fruit form. The size of fruits is a constant and good taxonomic character. This also a heritable character and the distinction is discontinuous. Therefore, these two forms may be given distinct varietal rank. However all cytogenetical characters as revealed from the present study are similar.

Thus, a subtle revision is necessary in the taxonomical point of view to update their taxa. Currently in Bangladesh, there is need to employ the system of cyto-taxonomical and molecular identification of every plant in order to establish a standard genetic library. This will help to curtail the confusion created by ambiguous morphological identification. Moreover, it will also aid to review obsolete literature in the field of taxonomy because recent discoveries will eradicate wrong information on overlapping species identification, nomenclature and classification.

Phyllanthus L. remains as an interesting and challenging genus. This study has proved to us that while we try and fit all species in its own containers, nature has its own way and is constantly developing and changing. However, it could be said that, *Phyllanthus* is a gift from nature to human civilization -that is there for us to enjoy and study.

CHAPTER FOUR: DISCUSSION

A combination of taxonomical, cytogenetical and molecular investigations of five *Phyllanthus* L. species *viz. P. acidus*, *P. emblica* (small fruit form), *P. emblica* (large fruit form), *P. niruri*, *P. reticulatus* and *P. urinaria* were discussed for the first time in Bangladesh.

4.1. Taxonomical diversification in five species of *Phyllanthus* L.

In the present investigation, five species of *Phyllanthus* L. differed in several taxonomical parameters such as branching pattern, morphology of bark, leaves, flower, fruits, seeds, flowering and fruiting time (Figs. 1-42, Table 13). *Phyllanthus acidus* and *P. emblica* are tree, these two species differed in respect of some other characteristic features such as lamina ovate to ovate-lanceolate, flowers red, stamens 4, free, fruits drupe, depressed-globose in *P. acidus* and lamina oblong or linear-oblong, flowers greenish white or greenish red, stamens 3, filaments connate in a column in *P. emblica.* These two species also differ in cytogenetical character such as their ploidy (*P. acidus* diploid but *P. emblica* polyploid), centromeric formula (*P. acidus* 12m+14sm but in case of *P. emblica* this is indistinct), DAPI band (in *P. acidus* there are 2 bands, where as in *P. emblica* there are 6-7 bands). *Phyllanthus reticulatus* showed several distinct characteristics such as habit shrubs, stamens 5 and black coloured ripe fruit. This species is also distinct in centromeric formula, number of CMA and DAPI bands. *Phyllanthus niruri* and *P. urinaria* both are herb but differed regarding some features such as lamina shape, fruit shape. They also differ in somatic chromosome number, ploidy (diploid in *P. niruri* and polyploid in *P. urinaria*), number of CMA (3 and 13, respectively) and DAPI bands (26 and 7, respectively) (Tables 20-23).

4.2. Cytogenetical characterization of five species of *Phyllanthus* L.

4.2.1. Orcein-staining properties of interphase nuclei and prophase chromosomes

The staining properties of interphase nuclei and prophase chromosomes usually provide karyomorphological features that help to characterize different species.

Tanaka (1971) was the pioneer of proposing these criteria for karyomorphological investigation. He found that the nature of staining of heterochromatins present in the interphase nuclei and prophase chromosomes were different in different species. On the basis of the staining property he classified interphase nuclei and prophase chromosomes in five different categories. Later different investigators applied these criteria in characterizing different plant materials of diverge nature (Alam *et al.* 1993, Begum and Alam 2004, Hossain *et al.* 2016, Sultana and Alam 2016a, Bonna *et al*. 2018).

In this study, interphase nuclei of five species of *Phyllanthus* L. were grouped into three categories such as "Diffuse type", "Simple chromocenter type" and "Complex chromocenter type" on the basis of nature of staining properties of heterochromatin after orcein staining.

Interphase nuclei of *P. niruri* and *P. urinaria* were homogenously stained with orcein (Figs. 46, 48, Table 38). According to Tanaka (1971), these could be regarded as "Diffuse type". In contrast, several number of small heterochromatic regions were scatterdly distributed around the nucleus of *P. emblica* (both forms) and *P. reticulatus* which could be considered as "Simple chromocenter type" (Figs. 44, 45, 47, Table 38). On the other hand, few large heterochromatic regions were observed in the interphase nuclei of *P. acidus* (Fig. 43, Table 38). This type of interphase nuclei were considered as "Complex chromocenter type".

The prophase chromosome of *P. emblica* (both forms), *P. reticulatus* and *P. urinaria* were darkly stained at different interstitial portions of (Figs. 62, 63, 65, 66, Table 38). According to Tanaka 1971, this type of prophase chromosome could be regarded as "Interstitial Type". However, "Continuous type" of prophase chromosomes were found in *P. acidus* and *P. niruri* where prophase chromosomes were uniformly stained along the length with orcein (Figs. 61, 64, Table 38).

Usually, species with "Diffuse type" of interphase nuclei showed "Continuous type" of prophase chromosome and "Simple or Complex chromocenter type" of interphase nuclei showed "Gradient or Interstitial type" of prophase chromosomes. In this study, *P. emblica* (both forms), *P. reticulatus* followed the general rule showing "Simple chromocenter type" of interphase nuclei and "Interstitial type" of prophase

chromosomes. *Phyllanthus niruri* also followed the general rule possessing "Diffuse type" of interphase nuclei and "Continuous type" of prophase chromosome. However, *P. acidus* and *P. urinaria* did not follow the usual regulation of heterochromatin. In *P. acidus,* heterochromatins are condensed to form bigger block whether these are homogeneously distributed along the chromosomal length. On the other hand, heterochromatins are homogeneously distributed in the interphase nuclei but distributed at the interstitial portions of prophase chromosomes in *P. urinaria*. Presence of facultative heterochromatin might be one of the reasons for this type of observation. Whatever the reason is, the five *Phyllanthus* L. species could be characterized on the basis of these characters of interphase nuclei and prophase chromosomes (Figs. 43-54, 61-66, Table 38).

A nucleolus was observed in interphase stages in four species of *Phyllanthus* L. except *P. niruri*. However, although nucleolus was not found in interphase nuclei but was appeared in prophase stage in *P. niruri* suggesting the late transcription of rDNA to rRNA and late transportation of rRNA from the nucleus to the cytoplasm as compared to other studied species. On the other hand, in *P. emblica* (large fruit form) and *P. urinaria,* a nucleolus was found in interphase nuclei but absent in prophase stage (Figs. 45, 48). These observations suggested the early transcription of rDNA to rRNA and early transportation of rRNA from the nucleus to the cytoplasm.

Table 38. Types of interphase nuclei and prophase chromosomes of five species of *Phyllanthus* L. after staining with orcein

Species	Type of chromocentric interphase nuclei	Type of prophase chromosomes
P. acidus	Complex Chromocenter	Continuous
P. emblica (small fruit form)	Simple Chromocenter	Interstitial
P. emblica (large fruit form)	Simple Chromocenter	Interstitial
P. niruri	Diffuse	Continuous
P. reticulatus	Simple Chromocenter	Interstitial
P. urinaria	Diffuse	Interstitial

4.2.2. Probable ways of origin of multiple basic and somatic chromosome number in different *Phyllanthus* L. species

In this investigation, *Phyllanthus* L. species showed variation in somatic chromosome counts such as 2n=26 in *P. acidus*, *P. niruri* and *P. reticulatus*, 2n=48 in *P. urinaria* and 2n=100 in *P. emblica* (both forms) with different basic chromosome number (Figs. 79-84, Table 39). In addition it was observed that, aneuploidy and polyploidy played important role in the evolution of a series of new basic chromosome numbers (x=6, 7, 8, 10, 13, 14 and 25), accompanied with the diversification of species within the genus *Phyllanthus* L.

According to the previous chromosome number records, 2n=26 and 2n=28 chromosomes were reported for *P. acidus* (Tables 7, 39). The diploid chromosome number of 2n=26 for *P. acidus* was determined in the present investigation which correlates with the previous reports of different scientists and indicate basic chromosome number x=13 (Tables 7, 39). However, 2n=28 (Thombre 1959) for this species might possess different basic chromosome number x=14.

On the basis of previous literature, *P. emblica* had different 2n chromosome number such as 2n=28, 52, 98, 104 and 98-104 (Tables 7, 39). If the basic chromosome number was x=14, *P. emblica* with 2n=2x=28 and 2n=7x=98 could be regarded as diploid and heptaploid, respectively. In contrast, if the basic chromosome number was x=13, *P. emblica* with 2n=4x=52 and 2n=8x=104 might be considered as tetraploid and octaploid, respectively. On the other hand, 2n=99-103 (Janaki Ammal and Raghavan 1958) could be originated through different ways such as:

i. Secondary modification (both hyper or hypo-aneuploidy) of polyploidy as proposed by Stebbins (1971).
ii. Presence of B-chromosome as found in other species belonging to the genus *Phyllanthus* L. (6-10B in *P. pulcher,* 1B in *P. parvifolius*) (Table 39).
iii. Numerical chromosomal aberration in the polyploidy generation because the effect of addition or deletion of few chromosomes could not be lethal in a polyploidy species due to possessing multiple copies of a basic set of chromosomes.

In the present study, *P. emblica* (small fruit form) was found to possess 2n=100 chromosomes (in 90% cells) (Fig. 80). Besides 2n=100 somatic chromosome numbers, 2n=78 (in 6% cell) and 2n=104 (in 4% cell) were also found in this study in few mitotic metaphase stage of *P. emblica* (small fruit form) which was not significant. On the other hand, *P. emblica* (large fruit form) had 2n=100 chromosomes (in 95.56% cells) in present study. In addition, few mitotic metaphase stage of this form showed 2n=90 (in 4.44% cells) besides 2n=100 which was also non-significant. Thus, the present findings (2n=100 and 104) supports the previous report 2n=98-104 (Table 39). In the available internet sources and relevant literature 2n=78 (small fruit form) and 2n=90 (large fruit form) was not reported earlier for *P. emblica* (small fruit form) thus could be regarded as first report for *P. emblica* (Figs. 80, 81, Table 21). The above data indicated that *P. emblica* (small fruit form) examined in this study had x=13 and might be considered as (2n=6x=78) hexaploid and (2n=8x=104) octaploid. However, 2n=10x=100 (in both forms) and 2n=9x=90 (in large fruit form) represent a new basic chromosome number x=10, which was not recorded previously for this species and could be considered as decaploid and nanoploid, respectively.

In *P. niruri*, 2n=26 chromosomes were observed in this research work (Fig. 82). Moreover, different 2n chromosome number of this species were reported earlier such as 2n=14, 26, 28 and 36 (Table 39). Therefore, the diploid chromosome number of 2n=26 for *P. niruri* was determined in the present investigation which correlates with the previous reports of different scientists (Table 39). If the basic chromosome number was x=7, species with 2n=2x=14, and 2n=4x=28 might be diploid and tetraploid, respectively. Species with 2n=2x=26 and 2n=6x=36 for this species might possess different basic chromosome number such as x=13 and x=6, respectively. However, according to previous literature and present investigation, x=7 and 13 were more frequent.

In case of the previous chromosome number records, 2n=26 and 28 chromosomes were reported for *P. reticulatus* (Table 39). The diploid chromosome number of 2n=2x=26 with x=13 for *P. reticulatus* was determined in the present investigation which correlates with the previous reports of different scientists. However, 2n=28

(Sharma and Jash 1958) for this species might be a case of hyper-aneuploidy which was not found in this research.

In the present study, 2n=6x=48 (hexaploid) with x=8 were observed in 90% somatic cells of *P. urinaria.* In addition, 2n=50 (in 5% cell) and 2n=52 (in 5% cell) were also found in few mitotic metaphase stage which might be due to the presence of B-chromosomes. Moreover, different 2n chromosome number of this species were reported earlier such as 2n=14, 24, 26, 28, 48, 50 and 52 (Table 39). Therefore, the somatic chromosome number of 2n=48 for *P. urinaria* was determined in the present investigation correlates with the previous report of Bancilhon (1971). However, *P. urinaria* have found to be reported multiple basic chromosome number such as x=6, 7, 8, 10, 13, 14 and 25, among these, x=8 and 13 are more frequent than others, showing predominantly retain these two basic number linage. According to Haicour *et al.* (1994), the variable base number could be originated from the ancestral one *i.e.* x=13 where x=6, 7, 8, 10 formed by decreasing aneuploidy and x=14, 25 by upward disploidy. Polyploid complex with the presence of hexaploid (2n=6x=48) cytotypes of x=8 and diploid (2n=2x=26) and tetraploid (2n=4x=52) cytotypes of x=13 indicated the existence of two dominant polyploid complex in nature.

Perry (1943) and Janaki Ammal and Raghavan (1958) hypothesized that the herbaceous species of *Phyllanthus*, with x=13, have a different base number from the woody ones, with x=7. According to the present investigation, Perry's and Janaki Ammal and Raghavan's observations substantiate to be incorrect and seem to have no correlation among basic chromosome number with the plant habit which supports the Webster and Ellis's (1962) findings.

Inconstancy in the somatic chromosome complement reported in the genus *Phyllanthus* L.*,* has revealed a number of interesting correlation regarding the basic set of chromosomes. Therefore, the diversity in basic chromosome number can be explained either to non-disjunction or possible hybridization within different cytotypes among these species or numerical chromosomal aberration in the polyploid generation.

4.2.3. Karyotype diversity among different *Phyllanthus* L. species

Five species of *Phyllanthus* L. differed in respect of somatic chromosome numbers and other karyomorphological features (Figs. 97-102, Table 20). Among these, comparatively bigger sized chromosomes was found in *P. acidus* [3.86(±0.11)-6.33(±0.19) µm] and *P. reticulatus* [2.93(±0.20)-5.29(±0.33) µm] than the rest species. Total length of 2n chromosome complements was the highest in *P. emblica* (large fruit form) 149.02(±0.88) µm which corresponds with its maximum number of somatic chromosome (2n=100) while it was the lowest in *P. niruri* 50.26(±0.45) µm (Table 20). The centromeric formulae was recorded as 12m+14sm for *P. acidus*, 20m+6sm for *P. niruri* and 4m+22sm for *P. reticulatus* whereas the centromeric position is visibly indistinct in *P. emblica* (both forms) and *P. urinaria* – two polyploid species, due to the smaller size of chromosome. However, in two polyploid species, the range of chromosomal length was almost negligible *i.e.* distance between small and large chromosomes was about 1.5 µm (Table 20). Presence of similar sized chromosomes is a feature of symmetric karyotype (Stebbins 1971). In contrast, few submetacentric chromosomes were observed in *P. acidus* (12m+14sm), *P. niruri* (20m+6sm) and *P. reticulatus* (4m+22sm) which indicated relatively asymmetric nature of their karyotype (Figs. 97, 100, 101, Table 20). Stebbins (1971) mentioned that the symmetric karyotypes were primitive character. Therefore, the five species of *Phyllanthus* L. were more or less primitive of which *P. acidus, P. niruri* and *P. reticulatus* were comparatively advanced than the rest.

4.2.4. Meiotic behavior and pollen fertility

Meiotic behavior of polyploid species of *Phyllanthus* L. varies sharply due to unstable somatic chromosome number such as 2n=100 (about 90% cells of small and large fruit forms of *P. emblica*), 2n=90 (in large fruit form of *P. emblica*) and 2n=78, 104 (in small fruit form of *P. emblica*) with probable two different basic chromosome number x=10 and 13 (Table 39). During meiosis, some meiotic metaphase-I stage showed all bivalents (IIs) while some metaphase-I stage had both bivalents (IIs) and multivalent in both small and large fruit forms of *P. emblica* (Figs. 115-120). Formation of bivalent suggested this plant as an allo-polyploid. In addition, at

diakinesis and metaphase-I several multivalent were also observed. This features indicated that chromosomes involved in the formation of multivalent might possess segmental homology and it can be pointed out the possibility of their being auto-allopolyploid or segmental-allopolyploid. However, multivalent number varies from cell to cell because it depends on some factors such as asynapsis and de-synapsis, number and position of chiasma, degree of terminalization of chiasma, etc. This cytogenetic observations such as formation of bivalents and some irregular multivalent in meiotic chromosomes indicated the presently examined polyploid species as interspecific hybrid which should be partly fertile. However, about 98% pollens were viable in both forms of *P. emblica* since chromosomes were segregated regularly at anaphase during meiosis (Figs. 121-126). Stebbins (1971) reported diplodization of polyploid in many ancient polyploid species. From the above features it can be suggested that these species are going through the process of diplodization.

4.2.5. Fluorescence banding pattern

Chromomycin A_3 (CMA) and 4´, 6-Diamidino-2-Phenylindole (DAPI) are two fluorochrome specific to GC- and AT-rich repetitive sequence, respectively (Schweizer 1976). Fluorescent banding gives decisive analysis of karyotype, even chromosome having similar morphology and other conventional karyotypic features. In this study, these two fluorochrome were used for making a comparative account of five *Phyllanthus* L. species. Characterization of *Phyllanthus* L. species through CMA and DAPI-banding was the pioneer attempt in Bangladesh.

4.2.5.1. CMA-banding pattern

In CMA-staining, bands were found in all species of *Phyllanthus* L. except *P. emblica* (small fruit form). The number, location and distribution of CMA-bands varied in different species. The numbers of CMA-bands are 3 in *P. acidus* and *P. niruri*, 15 in *P. emblica* (large fruit form), 4 in *P. reticulatus* and 13 in *P. urinaria* (Figs. 103-108). Most of the CMA-bands were located at the terminal or centromeric portions of chromosome which indicated a tendency of accumulating GC-rich sequences at centromeric regions or chromosomal ends. In addition, few chromosomes were

entirely fluoresced with CMA where GC-rich repeats were not confined to the terminal or centromeric region rather distributed along the entire length of chromosomes (Figs. 103-108). The possible reason for these entirely fluoresced chromosomes was tandem duplication of GC-rich repeats (Sultana and Alam 2016b, Khatun and Alam 2010, Sultana and Alam 2007, Hiron *et al*. 2006, Mahbub *et al*. 2007). Total length of CMA-positive banded region ranging from 1.73 µm (*P. niruri*) to 24.31 µm (large fruit form of *P. emblica*). The percentage of GC-rich repeats was the lowest in 2.71 (*P. niruri*) and the highest in 19.09 (*P. urinaria*) (Table 22). On the basis of number and location of CMA-bands, karyotypes formulae were prepared for each species. It has seen that each species has distinct CMA-banded karyotype formula (Table 22).

In case of *P. acidus, P. niruri* and *P. urinaria*, heteromorphicity was found in chromosome pair 7, 11 and 23, respectively where one homologue showed terminal band while no band was observed in their homologue suggesting probable deletion of the banded region from the respective chromosomes. In contrast, in pair 4 of *P. emblica* (large fruit form), one chromosome fluoresced entirely while its homologue member lacks CMA-positive band. In this case, GC-rich repeats of one member may tandemly duplicated along the length of the respective chromosomes (Figs. 103, 106, 108). With the help of CMA-banding, a comparative analysis of banding pattern revealed the occurrence of structural aberration such as deletion and tandem duplication in the *Phyllanthus* L. species.

4.2.5.2. DAPI-banding pattern

In the present study, five *Phyllanthus* L. species possessed distinct DAPI-banding pattern. *Phyllanthus acidus, P. emblica* (small fruit form), *P. emblica* (large fruit form), *P. niruri*, *P. reticulatus* and *P. urinaria* were found to possess 2, 6, 7, 26, 4 and 7 DAPI-positive bands, respectively. Among these 52 bands, 37 were located at the centromeric portions and 11 were observed in terminal portions of chromosome which indicated a tendency of accumulating AT-rich sequences at centromeric regions or chromosomal ends. In addition, 4 chromosomes were entirely fluoresced with DAPI where AT-rich repeats were not confined to the terminal or centromeric region rather distributed along the chromosomes (Figs. 109-114). The possible

reason for these entirely fluoresced chromosomes was tandem duplication of AT-rich repeats (Hiron *et al*. 2006, Mahbub *et al*. 2007, Khatun and Alam 2010). Total length of DAPI-positive banded region ranging from 1.17μm (large fruit form of *P. emblica*) to 24.5 μm (*P. urinaria*). The percentage of AT-rich repeats was the lowest in 1.48 (*P. acidus*) and the highest in 28.5 (*P. niruri*) (Table 23). On the basis of number and location of DAPI-bands, all these species showed diversity in their karyotype (Table 23).

In case of *P. emblica* (large fruit form) and *P. urinaria*, heteromorphicity was found in chromosome pair 50 and 22, respectively. In *P. emblica* (large fruit form) one chromosome of pair 50 showed centromeric band while no band was observed in its homologue. In contrast, in pair 22, one chromosome showed terminal DAPI-bands while the homologue member lacks DAPI-positive band. The above observations suggested the probable deletion of the banded region from those chromosomes (Figs. 111, 114). Therefore, few structural aberrations such as deletion and tandem duplication were observed after DAPI-banding in studied *Phyllanthus* L. species.

Table 39. Multiple basic and somatic chromosome number in different *Phyllanthus* L. species

Basic chrom-osome number (x)	Somatic chrom-osome number (2n)	Probable ploidy status	Species belonging to the genus *Phyllanthus* L.	References
6	2n=2x=12	Diploid	*P. parvifolius* *P. odontadenius*	Malla *et al.* 1975 Haicour *et al.* 1994
	2n=4x=24	Tetraploid	*P. debilis* *P. urinaria* *P. odontadenius*	Bancilhon1971 Kothari *et al.* 1981 Haicour *et al.* 1994
	2n=6x=36	Hexaploid	*P. caroliniensis* subsp. *guianensis,* *P. niruri* subsp. *lathyroides*	Webster and Ellis 1962 Bancilhon 1971
7	2n=2x=14	Diploid	*P. niruri* *P. urinaria* *P. debilis*	Chuang *et al.* 1963 Hsu 1967, Bancilhon 1971 Krishnappa and Reshme1980
	2n=4x=28	Tetraploid	*P. niruri*	Webster and Ellis 1962
	2n=8x=56	Octaploid	*P. odontadenius* *P. bancilhonae*	Bancilhon 1971 Haicour *et al.* 1994
8	2n=2x=16	Diploid	*P. polygonoides*	Webster and Ellis 1962
	2n=6x=48	Hexaploid	*P. urinaria*	Bancilhon 1971
10	2n=4x=40	Tetraploid	*P. debilis* *P. magnificens*	Trivedi *et al.* 1986 Haicour *et al.* 1994
	50=(2n=5x)+(6-10B)	Pentaploid and presence of B-chromosome	*P. pulcher*	Webster and Ellis 1962
	2n=10x=100	Decaploid	*P. embergeri*	Rossignol *et al.* 1987
13	2n=2x=26	Diploid	*P. acidus* *P. maderaspatensis* *P. niruri* *P. reticulatus* *P. urinaria*	Raghavan 1957, Raghavan 1959, Thombre 1959, Webster and Ellis 1962, Chuang *et al.* 1963, Raman and Kesavan 1963, Mehra and Hans 1969, Champault 1970, Bancilhon 1971, Brunel 1976, Sarkar *et al.* 1976, Bedi *et al*. 1980, Krishnappa and Reshme 1980, Sarkar and Datta 1980, Kothari *et al.* 1981, Trivedi *et al*. 1986, Huang *et al.* 1988

Table Continued

Basic chrom-osome number (x)	**Somatic chrom-osome number (2n)**	**Probable ploidy status**	**Species belonging to the genus *Phyllanthus***	**References**
13	2n=4x=52	Tetraploid	*P. acuminatus* *P. amarus* *P. axillaris* *P. maderaspatensis* *P. mimosoides* *P. nutans* *P. ovatas* *P. pentaphyllus* *P. subglomeratus* *P. urinaria* *P. emblica*	Janaki Ammal and Raghavan 1958, Raghavan and Arora 1958, Webster and Ellis 1962, Datta 1967, Champault 1970, Bancilhon 1971, Gill *et al.* 1973, Sarkar and Datta 1980, Chatha and Bir 1987
	(2n=4x)52+1B	Tetraploid and presence of B-chromosome	*P. parvifolius*	Sandhu and Mann 1989
	2n=6x=78	Hexaploid	*P. pulcher*	Webster and Ellis 1962
	98-104 =(2n=8x)104-(1-6)	Octaploid with hypo aneuploidy	*P. emblica*	Janaki Ammal and Raghavan 1958
	2n=8x=104	Octaploid	*P. emblica* *P. lawii*	Sarkar and Datta 1980, Bancilhon 1971, Gill *et al*. 1981, Kothari *et al*. 1981
	2n=12x=156	Dodecaploid	*P. grandifolius*	Miller and Webster 1966
	2n=12x=c.156	Dodecaploid	*P. juglandifolius*	Webster and Ellis 1962
14	2n=2x=28	Diploid	*P. acidus* *P. carolinensis* *P. emblica* *P. urinaria* *P. reticulatus* *P. odontadenius*	Perry 1943, Thombre 1959, Sharma and Jash 1958, Borgmann 1964, Bancilhon 1971,
	2n=7x=98	Heptaploid	*P. emblica*	Raghavan 1957, Janaki Ammal and Raghavan 1958, Mehra 1972, Mehra and Hans 1972, Hans 1973, Mehra 1976, Ohri and Kumar 1986
25	2n=2x=50	Diploid	*P. urinaria* Complex	Rossignol *et al.* 1987, Haicour *et al.* 1994

4.3. DNA fingerprinting by Random Amplified of Polymorphic DNA (RAPD)

RAPD is a PCR based marker technique that has been used for estimation of genetic diversity of populations and for studying the genetic relationships among different genotypes (Esmail *et al.* 2008). In this investigation, 12 oligonucleotide primers for RAPD were utilized to study the genetic relationship among the five *Phyllanthus* L. species. The data obtained following RAPD techniques were analyzed using "popgene32" computer package. The data were used to generate dissimilarity matrix for RAPD bands obtained through gel electrophoresis. The distance matrix between each pair of species was used to construct dendrogram using unweighted pair group method of arithmetical means (UPGMA). Five *Phyllanthus* L. species represented a broad spectrum of variation in RAPD banding pattern.

The primer sequence, band size and banding pattern of five of *Phyllanthus* L. species were shown in Tables 7 and 26-40. The 12 primers generated 141 distinct bands of which 97 were considered as polymorphic. Band size ranging from 150-4000 bp of PCR amplification products scored for all primers. A diverse level of polymorphism in different crops have been reported earlier such as Chickpea 98.14% (Rasool 2013), 87.00% (Datta *et al*. 2010), 14.56% (Sant *et al*. 1999) and 25.5% (Sonnante *et al*. 1997), *Brassica* 98.03% (Ghosh *et al.* 2009), Eggplant 57.89% (Biswas *et al*. 2009), Chilli 90% (Paran *et al.* 1998), Cotton 84.95% (Esmail *et al.* 2008), 90.96% (Maleia *et al.* 2010), 69.37% (Saravanan *et al.* 2006), 63.20% (Hussain *et al.* 2007) and 100% (Sultana and Alam 2016c), peanut 96% (Lang and Hang 2007), 42.7% (Raina *et al.* 2001) and 21% (He and Prakash 1997), Groundnut 6.68% (Subramanian *et al.* 2000), *Crotalaria* 48% (Wang *et al.* 2006). The results of the present investigation showed about 71.68% polymorphism among five species of *Phyllanthus* L. The broad range of polymorphism revealed wide diversity in *Phyllanthus* L. species.

4.3.1. Unique RAPD markers

A number of species specific unique bands were observed in *Phyllanthus* L. species such as 10 in *P. acidus* and 4 in *P. niruri* (OPA-18), 11 in *P. acidus* (OPB-19), 5 in *P. acidus* and 8 in *P. niruri* (OPAB-5), 2 in *P. acidus* and 2 in *P. niruri* (OPAB-6), 7 in *P. acidus* (OPC-13), 9 in *P. urinaria* (OPC-15), 5 in *P. acidus* (OPC-96), 2 in *P. acidus* and

3 in *P. niruri* (OPD-69), 6 in *P. acidus* and 2 in *P. reticulatus* (OPF-22), 4 in *P. acidus* (OPH-12), 5 in *P. acidus* and 4 in *P. niruri* (OPG-5), 6 in *P. acidus* and 2 in *P. urinaria* (OPG-7). The term unique sequence means that the sequence found in a species with a certain primer was absent in other species (Figs. 128-139, Tables 24-35). In the earlier literature, there was no information about unique bands of *Phyllanthus* L. species (Wang *et al.* 2006). The unique bands were stable and specific for the respective species and thus could be used as a tool for characterization.

4.4. Genetic relationships and Cluster analysis of studied *Phyllanthus* L. species

The values of pair-wise Nei's (1972) genetic distances analyzed by using computer software "popgene32" among five *Phyllanthus* L. species were computed from combined data for the twelve RAPD primers. The highest genetic distance (1.6011) was found between *P. acidus* with rest 4 species *i.e. P. emblica* (both forms), *P. niruri, P. reticulatus* and *P. urinaria*. The lowest (0.0169) genetic distance was observed between *P. reticulatus* and *P. emblica*. (Fig. 140, Table 37).

A cluster analysis on the basis of DNA fingerprinting by RAPD was carried out. Dendrogram based on Nei's (1972) genetic distance using UPGMA (Unweighted Pair Group Method of Arithmetic Means) segregated five *Phyllanthus* L. species into two major clusters C_1 and C_2 (Fig. 140). According to dendrogram, *P. acidus* is separated from other 4 species with high genetic distance 1.6011 and placed in a separate cluster C_1 (Fig. 140, Table 37). On the other hand, other 4 species were placed in cluster C_2. The lowest genetic distance 0.0169 was found between *P. reticulatus* and *P. emblica* whereas the highest genetic distance 0.5025 was found between *P. niruri* and *P. emblica* within cluster C_2. So among five *Phyllanthus* L., *P. emblica* and *P. reticulatus* were closely related whereas *P. acidus* and *P. emblica* were genetically distantly related.

4.5. The three dimensional aspects of the genus *Phyllanthus* L. assembled with taxonomic, cytogenetic and molecular point

A cyto-taxonomic study with RAPD analysis in the *Phyllanthus* L. has been done in present investigation. In presenting the findings of this study, we have tried to provide a comparative discussion of our results with the previous experimental studies of taxonomical and cytogenetical analysis of *Phyllanthus* L. species.

The wide numerical chromosome variation was observed and among these species *P. acidus, P. reticulatus* and *P. niruri* were diploid while *P. emblica* (both forms) and *P. urinaria* were segmental allopolyploid. It was well known that the phenomenon "Polyploidy" was more frequent in herbs and rarely found in trees. However, present investigation displayed fluctuation from that hypothesis, having inconsistent polyploid progenies of *P. emblica* (Tree). Otherwise, the rest correlates with that hypothesis.

Based on dendrogram prepared from RAPD analysis, *P. emblica* (both forms), *P. reticulatus* and *P. urinaria* were closely associated. Since *P. emblica* and *P. urinaria* were polyploid and *P. reticulatus* was the only diploid species among these three, it could be suggested that they might have shared some common genome that passing through the polyploid complex of *P. emblica* and *P. urinaria.*

There was a positive correlation between nature of heterochromatin condensation and RAPD dendrogram. *Phyllanthus acidus*, which showed complex chromocenter type of interphase nuclei to continuous type of prophase chromosomes, lied separately from other four species. The nature of heterochromatin condensation is also different to that of the rest species. Two species, *P. emblica* and *P. reticulatus* are closely related and they also showed similarity in heterochromatin condensation nature *i.e.* simple chromocenter type of interphase nuclei to interstitial type of prophase chromosomes. *Phyllanthus urinaria* had shown diffuse type of interphase and continuous type of prophase reside in a separate cluster next to the cluster of the *P. emblica*, *P. reticulatus* and *P. urinaria*.

The two forms (small and large fruit forms) of *P. emblica* displayed similarity in most important taxonomic parameters and somatic chromosome numbers. These

two forms also stayed closely in the dendrogram of RAPD. However, these two forms showed some dissimilarity. These were:

I. The bark colour was blackish in small fruit type and grayish in large fruit type.
II. The young leaves colour was green in small fruit type and purplish green in large fruit type.
III. The flower colour was greenish white in small fruit type and greenish red in large fruit type.
IV. The fruits were sharply different in respect of their sizes.
V. Flowering time was March to September for in small fruit form and January to March for large fruit form.

Plants belonging to *P. emblica* may be divided into two groups. Group-I containing plants yielding smaller fruits (size 2.0-2.2 cm and average weight 5.30 gm), may be regarded as small fruit form. Group-II containing plants yielding larger fruits (size 3.0-3.3 cm and average weight 25.18 gm), may be regarded as large fruit form. The size of fruits is a constant and good taxonomic character. This also a heritable character and the distinction is discontinuous. Therefore, these two forms may be given distinct varietal rank. However all cytogenetical characters as revealed from the present study are similar.

Thus, a subtle revision is necessary in the taxonomical point of view to update their taxa. Currently in Bangladesh, there is need to employ the system of cyto-taxonomical and molecular identification of every plant in order to establish a standard genetic library. This will help to curtail the confusion created by ambiguous morphological identification. Moreover, it will also aid to review obsolete literature in the field of taxonomy because recent discoveries will eradicate wrong information on overlapping species identification, nomenclature and classification.

Phyllanthus L. remains as an interesting and challenging genus. This study has proved to us that while we try and fit all species in its own containers, nature has its own way and is constantly developing and changing. However, it could be said that, *Phyllanthus* is a gift from nature to human civilization -that is there for us to enjoy and study.

CHAPTER FIVE:
REFERENCES

REFERENCES

Abdulla MA, Ahmed KA, AL-Bayaty FH and Masood Y 2010. Gastroprotective effect of *Phyllanthus niruri* leaf extract against ethanol induced gastric mucosal injury in rats. African J. Phar. Pharmacol. **4**(5): 226-230.

Adil MD, Kaiser P, Satti NK, Zargar AM, Vishwakarma RA and Tasduq SA 2010. Effect of *Emblica officinalis* (fruit) against UVB-induced photo-aging in human skin fibroblasts. J. Ethnopharm. **132**(1): 109-114.

Agarwal SS and Singh VK 1999. Immunomodulators studies on Indian medicinal plants and synthetic part 1: medicinal plants. Proceed. Nation. Acad. Sci. **65**: 179-204.

Ahmad B and Alam T 2003. Components from whole plant of *Phyllanthus amarus* Linn. Indian J. Chem. **42**(7): 1786-1790.

Ahmed ZU, Hassan MA, Begum ZNT, Khondker M, Kabir SMH, Ahmad M, Ahmed ATA, Rahman AKA and Haque EU 2008. Encyclopedia of Flora and Fauna of Bangladesh. Vol. 7. Angiosperms: Dicotyledons (Balsaminaceae-Euphorbiaceae). Asiatic Society of Bangladesh, Dhaka. pp. 463-473.

Akhter S and Alam SS 2005. Differential fluorescent banding pattern in three varieties of *Cicer arietinum* L. (Fabaceae). Cytologia **70**(4): 441-445.

Alam SS and Kondo K 1995. Differential staining with orcein, Giemsa, CMA and DAPI for comparative chromosome study of 12 species of Australian *Drosera* (Droseraceae). American J. Bot. **82**(10): 1278-1286.

Alam SS, Kondo K and Tanaka R 1993. A chromosome study of eight orchid species in Bangladesh. La Kromsomo **11-71-72**: 2456-2464.

Anuradha CV and Ravikumar P 2001. Restoration on tissue antioxidants by fenugreek seeds (*Trigonella foenum-graecum*) in alloxan-diabetic rats. Indian J. Physiol. Pharmacol. **45**: 408-420.

Asmawi MZ, Kankaanranta H, Moilanen E and Vapaatalo H 1993. Antiinflammatory activities of *Emblica officinalis* Gaertn. leaf extracts. J. Pharm. Pharmacol. **45**: 581.

Bafna PA and Balaraman R 2005. Antiulcer and antioxidant activity of pepticare, an herb mineral formulation. Phytomedicine **12**: 264-270.

Bagalkotkar G, Chuan TS and Khalivulla SI 2011. Isolation and cytotoxicity of triterpenes from the roots of *Phyllanthus pulcher* Wall. ex M̈ull . Arg. (Euphorbiaceae). African J. Pharm. Pharmacol. **5**(2): 183-188.

Bagavan A, Rahuman AA, Kamaraj C, Kaushik NK, Mohanakrishnan D and Sahal D 2011. Antiplasmodial activity of botanical extracts against *Plasmodium falciparum*. Parasit. Res. **108**(5): 1099-1109.

Baliga MS 2010. Triphala, ayurvedic formulation for treating and preventing cancer: a review. J. Alt. Comp. Med. **16**(12): 1301-1308.

Baliga MS and Dsouza JJ 2011. Amla (*Emblica officinalis* Gaertn.), a wonder berry in the treatment and prevention of cancer. European J. Canc. Prev. **20**: 225-239.

Bancilhon L 1971. Contribution a!'Etude taxonomique du genre *Phyllanthus* (Euphorbiacees). Boissiera **18**: 1-81.

Bandyopadhyay S and Raychaudhuri SS 2013. Development and comparison of RAPD, SCAR and AFLP markers for distinguishing some medicinally important species of the genus *Phyllanthus.* Plant Biosyst. **147**(1): 12-20.

Bandyopadhyay SK, Chatterjee A and Chattopadhyay A 2011. Biphasic effect of *Phyllanthus emblica* L. extract on NSAID-induced ulcer: An antioxidative trail weaved with immuno-modulatory effect. Evidence-Based Comp. Alt. Med. **2011**: 146808.

Bandyopadhyay SK, Pakrashi SC and Pakrashi A 2000. The role of antioxidant activity of *Phyllanthus emblica* fruits on prevention from indomethacin induced gastric ulcer. J. Ethnopharm. **70**: 171-176.

Basa SC and Shrinivasulu C 1987. Constituents of leaves of *Phyllanthus emblica* Linn. Indian J. Nat. Prod. **3**: 13-14.

Bedi YS, Bir SS and Gill BS 1980. In Löve A(ed) IOPB chromosome number reports LXVII. Taxon **29**: 355-357.

Begum R and Alam SS 2004. Karyomorphological study in two orchid species. Dhaka Univ. J. Biol. Sci. **13**(1): 99-101.

Begum T, Rahman SM and Rashid AM 2006. Phytochemical and biological investigations of *Phyllanthus reticulatus*. Dhaka Univ. J. Pharmaceu. Sci. **5**(1-2): 21-23.

Bharatiya VB 1992. Selected medicinal plants of India. Tafa Press, Bombay. pp. 235-237.

Bhattacharya SK, Chaudhuri SR, Chattopadhyay S and Bandyopadhyay SK 2007. Healing properties of some Indian medicinal plants against indomethacin-induced gastric ulceration of rats. J. Clini. Biochem. Nutr. **41**: 106-114.

Biswas MS, Akhond AAY, Alamin M, Khatun M and Kabir MR 2009. Genetic relationship among ten promising eggplant varieties using RAPD markers. Pl. Tiss. Cul. Biotech. **19**(2): 119-126.

Blaser MJ 1993. *Helicobacter pylori*: microbiology of a 'slow' bacterial infection. Trends Microbiol. **1**: 255-260.

Boeira VT, Leite CE, Santos AAJR, Edelweiss MI, Calixto JB, Campos MM and Morrone FB 2011. Effects of the hydroalcoholic extract of *Phyllanthus niruri* and its isolated compounds on cyclophosphamide-induced hemorrhagic cystitis in mouse. Naunyn-Schmiedeberg's Arch. Pharmacol. **384**(3): 265-275.

Bonna IJ, Alam SS and Sultana SS 2018. Cytogenetical characterization of *Acalypha indica* L. in Bangladesh. Dhaka Univ. J. Biol. Sci. **27**(2): 183-189.

Borgman E 1964. Anteil der polyploiden in der flora des Bismarckgebirges von Ostneuguinea. Zeitschr. Bot. **52**: 118-172.

Bruce-Chwatt LJ 1985. From malaria eradication to malaria control. In: Bruce-Chwatt L.J., ed. Essential malariology. 2nd edition. London, William Heinemann Medical Books. pp. 360-383.

Brunel JF 1976. In IOPB chromosome number reports LI. Taxon **25**: 155-164.

Burkill IH 1996. A dictionary of the economic products of Malay Peninsula. Art Printing Works, Kuala Lumpur. pp. 1748-1749.

Calixto JB, Santos ARS, Filho VC and Yunes RA 1998. A review of the plants of the genus *Phyllanthus*: their chemistry, pharmacology and therapeutic potential. Med. Res. Rev. **18**(4): 225-258.

Carmeliet P and Jain RK 2000. Angiogenesis in cancer and other diseases. Nature **407**(6801): 249-257.

Catrenich CE and Chestnut MH 1992. Character and origin of vacuoles induced in mammalian cells by the cytotoxin of *Helicobacter pylori*. J. Med. Microbiol. **37**: 389-395.

Censini S, Lange C, Xiang Z, Crabtree JE, Ghiara P and Borodovsky M 1996. Cag, a pathogenicity island of *Helicobacter pylori*, encodes type 1- specific and disease associated virulence factors. Proc. Natl. Acad. Sci. USA **93**: 14648-14653.

Champault A 1970. Etude caryosystematique et ecologique de quelques Euphorbiacees herbacees et arbustives africaines. Bull. Seo. Bot. France **117**: 137-168.

Chandra R 2000. Lipid lowering activity of *P. niruri*. J. Med. Aro. Pl. Sci. **22**(1): 29-30.

Chang CC, Lien YC, Liu KCSC and Lee SS 2003. Lignans from *Phyllanthus urinaria.* Phytochemistry **63**(7): 825-833.

Chatha GS and Bir SS 1987. Cytological evaluation of woody taxa of Gamopetalae and Monochlamydeae from south India. Aspects Pl. Sci. **9**: 199-244.

Chatterjee A, Chatterjee S, Biswas A, Bhattacharya S, Chattopadhyay S and Bandyopadhyay SK 2012. Gallic acid enriched fraction of *Phyllanthus emblica* potentiates indomethacin-induced gastric ulcer healing via e-NOS-dependent pathway. Evidence-Based Compl. Alt. Med. **2012**: 1-13.

Chatterjee A, Chattopadhyay S and Bandyopadhyay SK 2011. Biphasic effect of *Phyllanthus emblica* L. extract on NSAID-induced ulcer: an antioxidative trail weaved with immunomodulatory effect. Evidence-based Compl. Alt. Med. **2011**: 1-13.

Chatterjee M and Sil PC 2006. Hepatoprotective effects of aqueous extract of *Phyllanthus niruri* in *in vivo*. Indian J. Biochem. Biophys. **43**: 299-305.

Chen TS, Liou SY, Wu HC, Tsai FJ, Tsai CH, Huang CY and Chang YL 2011. Efficacy of epigallocatechin-3-gallate and Amla (*Emblica officinalis*) extract for the treatment of diabetic-uremic patients. J. Med. Food **14**: 718-723.

Chen YW, Ren LJ, Li KM and Zhang YW 1999. Isolation and identification of novel polyphenolic compound from *Phyllanthus urinaria*. Acta Pharmaceutica Sinica **34**(7): 526-529.

Cheng HY, Yang CM, Lin TC, Lin LT, Chiang LC and Lin CC 2011. Excoecarianin, isolated from *Phyllanthus urinaria* Linnea, inhibits herpes simplex virus type 2 infections through inactivation of viral particles. Evidence-Based Compl. Alt. Med. **2011**: 259103.

Chopra RN, Nayar SL and Chopra IC 1956. Glossary of Indian medicinal plants. Council of Scientific and Industrial Research, New Delhi. pp. **107**.

Chuang TI, Chao CY, Hu WWL and Kwan SC 1963. Chromosome numbers of the vascular plants of Taiwan. I. Taiwanaia **1**: 51-66.

Cooper EL 2001. Drug discovery, CAM and natural products. Evidence-Based Compl. Alt. Med. **1**: 215-217.

Cover TL and Blaser MJ 1996. *Helicobacter pylori* infection, a paradigm for chronic mucosal inflammation: pathogenesis and implications for eradication and prevention. Adv. Int. Med. **41**: 85-117.

Cronquist A 1981. An Integrated system of classification of flowering plants. Columbia University Press. New York.

Das S, Lyla P and Khan SA 2006. Marine microbial diversity and ecology: importance and future perspectives. Curr. Sci. **90**: 1325-1335.

Dash CK, Afroz M, Sultana SS and Alam SS 2017. Conventional and fluorescent karyotype analysis of *Ocimum* spp. Cytologia **82**(4): 429-434.

Datta J, Lal N, Kaashyap M and Gupta PP 2010. Efficiency of three PCR based marker systems for detecting DNA polymorphism in *Cicer arietinum* L. and *Cajanus cajan* L. Millspaugh. Genet. Engin. Biotech. **5**: 1-15.

Datta N 1967. In IOPB chromosome number reports XII. Taxon **16**: 341-350.

Desai HK, Gawad DH, Joshi BS, Parthasthy PC, Ravindranath KR, Saindane MT, Sidhave AR and Viswanathan N 1977. Chemical investigation of Indian plants: Part X. Indian J. Chem. **14B**(3): 291-293.

Dias MA, Campos AH, Cechinel Filho V, Yunes RA and Calixto JB 1995. Analysis of the mechanisms underlying the contractile response induced by the hydroalcoholic extract of *Phyllanthus urinaria* in the guinea-pig urinary bladder *in vitro*. J. Pharm. Pharmacol. **47**: 846-851.

Dnyaneshwar W, Preeti C, Kalpana J and Bhushand P 2006. Development and application of RAPD-SCAR marker for identification of *Phyllanthus emblica* Linn. Biol. Pharm. Bull. **29**(11): 2313-2316.

El-Mekkawy S, Meselhy MR, Kusumoto IT, Kadota S, Haltori M and Namba T 1995. Inhibitor effects of Egyptian folk medicine on human immunodeficiency viruses (HIV) reverse transcriptase. Chem. Pharmaceu. Bull. **43**: 641.

Esmail RM, Zhang JF and Abdel-Hamid AM 2008. Genetic diversity in elite cotton germplasm lines using field performance and RAPD markers. World J. Agri. Sci. **4**(3): 369-375.

Fang SH, Rao YK and Tzeng YM 2008. Antioxidant and inflammatory mediator's growth inhibitory effects of compounds isolated from *Phyllanthus urinaria*. J. Ethnopharm. **116**(2): 333-340.

Ferlay J, Soerjomataram I and Dikshit R 2015. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int. J. Canc. **136**(5): 359-386.

Firminger WK 1947. Firminger`s manual of gardening in India. Thacker Spink, New Delhi. pp. 186-188.

Gambari R, Borgatti M, Lampronti I, Fabbri E, Brognara E, Bianchi N, Piccagli L, Yuen M, Kan C, Hau D, Fong W, Wong W, Wong R and Chui C 2012. Corilagin is a potent inhibitor of NF-kappaB activity and down regulates TNF-alpha induced expression of IL-8 gene in cystic fibrosis IB3-1 cells. Int. Immunopharmacol. **13**(3): 308-315.

Ghani A 2003. Medicinal plants of Bangladesh with chemical constituents and uses. 2nd ed. Asiatic Society of Bangladesh. 5 old Secretariate road, Nimtali, Dhaka, Bangladesh.

Ghosh KK, Huque ME, Parvin MS, Akhter F and Rahim MM 2009. Genetic diversity analysis in *Brassica* varieties through RAPD marker. Bangladesh J. Agri. Res. **34**(3): 493-503.

Ghosh TR, Nath S, Talukdar AD and Choudhury RD 2015. Antidiabetic plants used among the ethnic communities of Unakoti district of Tripura, India. J. Ethnopharm. **160**: 219-226.

Gill BS, Bir SS and Singhal VK 1981. Cytomorphological studies in north Indian pomegranate (*Punica granatum* Linn.). J. Cytol. Genet. **16**: 35-45.

Gill BS, Chawla A and Kanwal R 1973. Cytological studies in North-West Indian Euphorbiaceae. Cytologia **38**: 393-401.

Giridharan P, Somasundaram ST, Perumal K, Vishwakarma RA, Karthikeyan NP, Velmurugan R and Balakrishnan A 2002. Novel substituted methylenedioxy lignan suppresses proliferation of cancer cells by inhibiting telomerase and activation of c-myc and caspases leading to apoptosis. British J. Canc. **87**(1): 98-105.

Grewal RC 1984. Medicinal Plants. Campus Book International: New Delhi, 1st Ed. pp. 298-304.

Habib MR, Sayeed MA, Rahman MM, Hasan MR and Saha A 2011. *In vitro* evaluation of cytotoxic, antibacterial, antioxidant and phytochemical screening of petroleum ether extract of *Phyllanthus acidus*. Int. J. Pharm. Sci. Res. **2**: 875-881.

Haicour R, Rossignol L, Rossignol M and Gaisne C 1994. Patterns of diversity and evolution in *Phyllanthus odontadenius* (Euphorbiaceae). Ann. Missouri Bot. Gard. **81**: 289-301.

Hanahan D and Folkman J 1996. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell **86**(3): 353-364.

Hans AS 1973. Chromosomal conspectus of Euphorbiaceae. Taxon **22**: 591-636.

Hari B, Kurup BM, Varghese JT, Schrama JW and Verdegem MCJ 2006. The effect of carbohydrate addition on water quality and the nitrogen budget in extensive shrimp culture systems. Aquaculture **252**: 248-263.

He G and Prakash CS 1997. Identification of polymorphic DNA markers in cultivated peanut (*Arachis hypogaea* L.). Euphytica **97**: 143-149.

Higashino H, Suzuki A, Tanaka Y and Pootakham K 1992. Hypoglycemic effects of siamese *Momordica charantia* and *Phyllanthus urinaria* extracts in streptozotocin-induced diabetic rats (the first report). Folia Pharmacol. Japonica **100**: 415-421.

Hiron N, Alam N, Ahmed FA, Begum R and Alam SS 2006. Differential fluorescent chromosome banding in *Hibiscus canabinnas* L. and *H. sabdariffa* L. Cytologia **71**(2): 175-180.

Hizume M, Abe KK and Tanaka A 1988. Fluorescent chromosome bandings in the Taxodiaceae. La Kromosomo **11-50**: 1609-1619.

Holdsworth D and Wamoi B 1982. Medicinal plants of the Admiralty Islands, Papau New Guinea. Part 1. Int. J. Crude Drug Res. **20**(4): 169-181.

Hossain MA and Salehuddin SM 2006. Diterpenes from the leaves of *Phyllanthus niruri*. Indian J. Nat. Pro. **22**(2): 18-20.

Hossain MU, Islam M, Afroz M, Sultana SS and Alam SS 2016. Karyotype and RAPD analysis of male and female *Coccinia grandis* L. from Bangladesh. Cytologia **81**(3): 349-355.

Hsu CC I967. Preliminary chromosome studies on the vascular plants of Taiwan I. Taiwania **13**: 117-130.

Hu Z, Lai Y, Zhang J, Wu Y, Luo Z, Yao G, Xue Y and Zhang Y 2014. Phytochemical and chemotaxonomic studies on *Phyllanthus urinaria*. Biochem. Syst. Ecol. **56**: 60-64.

Huang SF, Wang YQ, Chen ZY and Shi XH 1988. Plant chromosome counts (4). Subtrop. Forest Sci. Technol. **16**: 25-30.

Huang ST, Bi KW and Kuo HM 2014. *Phyllanthus urinaria* induces mitochondrial dysfunction in human osteosarcoma 143B cells associated with modulation of mitochondrial fission/fusion proteins. Mitochondrion **17**: 22-33.

Huang ST, Pang JHS and Yang RC 2010. Anti-cancer effects of *Phyllanthus urinaria* and relevant mechanisms. Chang Gung Med. J. **33**(5): 477-487.

Huang ST, Wang CY, Yang RC, Wu HT, Yang SH, Cheng YC and Pang JHS 2011. Ellagic acid, the active compound of *Phyllanthus urinaria*, exerts *in vivo* anti-angiogenic effect and inhibits MMP-2 activity. Evidence-Based Compl. Alt. Med. **2011**: 215035

Huang ST, Yang RC and Lee PN 2006. Anti-tumor and anti-angiogenic effects of *Phyllanthus urinaria* in mice bearing Lewis lung carcinoma. Int. Immunopharmacol. **6**(6): 870-879.

Huang ST, Yang RC, Chen MY and Pang JHS 2004. *Phyllanthus urinaria* induces the Fas receptor/ligand expression and ceramide-mediated apoptosis in HL-60 cells. Life Sci. **75**(3): 339-351.

Huang ST, Yang RC, Yang LJ, Lee PN and Pang JHS 2003. *Phyllanthus urinaria* triggers the apoptosis and Bcl-2 down regulation in Lewis lung carcinoma cells. Life Sci. **72**(15): 1705-1716.

Hui WH and Sung M 1968. An examination of the Euphorbiaceae of Hong Kong. II. The occurrence of epitaraxerol and other triterpenoids. Australian J. Chem. **21**(8): 2137-2140.

Hussain SS, Husnain T and Riazuddin S 2007. Sonication assisted *Agrobacterium* mediated transformation (SAAT): An alternative method for cotton transformation. Pakistan J. Bot. **39**(1): 223-230.

Ihantola-Vormisto A, Summanen J and Kankaanranta H 1997. Antiinflammatory activity of extracts from leaves of *Phyllanthus emblica*. Planta Med. **63**: 518-524.

Ingber DE, Prusty D, Frangioni JV, Cragoe EJ, Lechene C and Schwartz MA 1990. Control of intracellular pH and growth by fibronectin in capillary endothelial cells. J. Cell Biol. **110**(5): 1803-1811.

Islam M and Alam SS 2011. Karyotype characterization with fluorescent banding in one released and two wild germplasms of *Hibiscus cannabinus* L. Cytologia **76**(2): 223-227.

Ismail M, Bagalkotkar G, Iqbal S and Adamu HA 2012. Anticancer properties and phenolic contents of sequentially prepared extracts from different parts of selected medicinal plants indigenous to Malaysia. Molecules **17**(5): 5745-5756.

Itoh T, Tanioka M, Yoshida H, Yoshioka T, Nishimoto H and Itohara S 1998. Reduced angiogenesis and tumor progression in gelatinase A-deficient mice. Can. Res. **58**(5): 1048-1051.

Jagessar RC, Marsa A and Gomesb G 2008. Selective antimicrobial properties of *Phyllanthus acidus* leaf extract against *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus* using stokes disc diffusion, well diffusion, streak plate and a dilution method. Nat. Sci. **6**: 24-38.

Jain NK and Singhai AK 2011. Protective effects of *Phyllanthus acidus* (L.) Skeels leaf extracts on acetaminophen and thioacetamide induced hepatic injuries in Wistar rats. Asian Pac. J. Trop. Med. **4**: 470-474.

Jain NK, Lodhi SA, Jain A, Nahata and Singhai AK 2011. Effects of *Phyllanthus acidus* (L.) Skeels fruit on carbon tetrachloride-induced acute oxidative damage in livers of rats and mice. J. Chin. Integ. Med. **9**(1): 49-56.

Jain R and Jain SK 2010. Traditional medicinal plants as anticancer agents from Chhattishgarh, India: An overview. Int. J. Phytomed. **2**: 186-196.

Janaki Ammal EK and Raghavan RS 1958. Polyploidy and vitamin C in *Emblica officinalis* Gaertn. Proc. Indian Acad. Sci. B. **47**: 312-314.

Jantan I, Ilangkovan M, Yuandani and Mohamad HF 2014. Correlation between the major components of *Phyllanthus amarus* and *Phyllanthus urinaria* and their inhibitory effects on phagocytic activity of human neutrophils. BMC Comp. Alt. Med. **14**: 429.

Jessy NS, Begum R, Khatun M and Alam SS 2005. Differential fluorescent chromosome banding of four species in *Haworthia duval* (Aloaceae). Cytologia **70**(4): 435-440.

Jiang LJ, Wang YF, Zhang JM, Yu S, Zhu HT, Wang D, Cheng RR, Yang CR, Xu M and Zhang YJ 2014. Anti-hepatitis B virus activities and absolute configurations of sesquiterpenoid glycosides from *Phyllanthus emblica*. Org. Biomol. Chem. **12**(43): 8764-8774.

Joshi KC, Singh P and Mehra A 1981. Crystalline components of the roots of *Phyllanthus reticulatus*. J. Indian Chem. Soc. **58**(1): 102-103.

Kalekar SA, Munshi RP, Bhalerao SS and Thatte UM 2013. Insulin sensitizing effect of 3 Indian medicinal plants: An *in vitro* study. Indian J. Pharmacol. **45**: 30-33.

Khanna AK, Rizvi F and Chander R 2002. Lipid lowering activity of *Phyllanthus niruri* in hyperlipaemic rats. J. Ethnopharmacol. **82**: 19-22.

Khatun M and Alam SS 2010. Conformation of species status of *Corchorus trilocularis* by differential chromosome banding and isozyme assay. Cytologia **75**(1): 83-88.

Khatun M, Sultana SS, Ara H, Islam MN and Alam SS 2011. Differential chromosome banding and isozyme assay of three *Corchorus* spp. Cytologia **76**(1): 27-32.

King M 1993. Chromosomal speciation revisited (again). Species evolution. The role of chromosome change. J. Evol. Biol. **7**(6): 578-580.

Kirtikar KR and Basu BD 1975. Indian medicinal plants. International book distributors, Debra Dun, India **3**: 2221.

Kirtikar KR, Basu BD and San IC 1935. Indian medicinal plants. Vols. I-IV, Lalit Mohan Basu, Leader Road, Allahabad, India. pp. 2793.

Kondo T and Hizume M 1982. Banding for the chromosomes of *Cryptomeria japonica* D. Don. Japan J. For. Soc. **64**: 356-358.

Kothari NM, Ninant CA and Kuriachan PI 1981. In Chromosome number reports LXXI. Taxon **30**: 511-512.

Krishnappa DG and Reshme RV 1980. In Chromosome number reports LXVIII. Taxon **29**: 536-537.

Kumar GS, Nayaka H, Dharmesh SM and Slimath PV 2006. Free and bound phenolic antioxidants in amla (*Emblica officinalis*) and turmeric (*Curcuma longa*). J. Food Comp. Anal. **19**: 446-452.

Kumar S, Kumar D, Deshmukh RR, Lokhande PD, More SN and Rangari VD 2008. Antidiabetic potential of *Phyllanthus reticulatus* in alloxan-induced diabetic mice. Fitoterapia **79**(1): 21-23.

Kumaran A and Karunakaran RJ 2006. Nitric oxide radical scavenging active components from *Phyllanthus emblica* L. Pl. Fo. Hum. Nut. **61**: 1-5.

Lai CH, Fang SH and Rao YK 2008. Inhibition of *Helicobacter pylori*-induced inflammation in human gastric epithelial AGS cells by *Phyllanthus urinaria* extracts. J. Ethnopharmacol. **118**(3): 522-526.

Lam SH, Wang CY, Chen CK and Lee SS 2007. Chemical investigation of *Phyllanthus reticulatus* by HPLC-SPE-NMR and conventional methods. Phytochem. Anal. **18**(3): 251-255.

Lan MS, Ma JX, Tan CH, Wei S and Zhu DY 2010. Chemical constituents of *Phyllanthus reticulatus*. Helvetica Chimica Acta **93**(11): 2276-2280.

Lang NT and Hang PTC 2007. Genetic divergence analysis on peanut by RAPDs. Omonrice **15**: 174-178.

Lans CA 2006. Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. J. Ethnobiol. Ethnomed. **2**: 45.

Laumas KR and Seshadri TR 1958. Chemical components of the bark of *Phyllanthus emblica.* J. Sci. Indian Res. **17**(B): 167-168.

Lee CY, Peng WH, Cheng HY, Chen FN, Lai MT and Chiu TH 2006. Hepatoprotective effect of *Phyllanthus* in Taiwan on acute liver damage induced by carbon tetrachloride. American J. Chin. Med. **34**: 471-482.

Lee SH, Jaganath IB, Wang SM and Sekaran SD 2011. Anti-metastatic effects of *Phyllanthus* on human lung (A549) and breast (MCF-7) cancer cell lines, PLoS ONE **6**(6): 20994.

Leeya Y, Mulvany MJ, Queiroz EF, Marston A, Hostettmann K and Jansakul 2010. Hypotensive activity of an n-butanol extract and their purified compounds from leaves of *Phyllanthus acidus* (L.) Skeels in rats. European J. Pharm. **649**(1-3): 301-313.

Leunk R, Johnson P, David B, Kraft W and Morgan D 1988. Cytotoxin activity in broth culture filtrates of *Campylobacter pylori*. J. Med. Microbiol. **26**: 93-99.

Levan A, Fredga K and Sandberg AA 1964. Nomenclature for centromeric position on chromosomes. Hereditas **52**: 201-220.

Lim YY and Murtijaya J 2007. Antioxidant properties of *Phyllanthus amarus* extracts as affected by different drying methods. LWT-Food Sci. Technol. **40**: 1664-1669.

Lin SY, Wang CC, Lu YL, Wu WC and Hou WC 2008. Antioxidant, anti-semicarbazide-sensitive amine oxidase and anti-hypertensive activities of geraniin isolated from *Phyllanthus urinaria*. Fo. Chem. Toxicol. **46**(7): 2485-2492.

Liu S, Wei WX, Li YB, Lin X, Shi K, Cao X and Zhou M 2014. *In vitro* and *in vivo* antihepatitis B virus activities of the lignan nirtetralin B isolated from *Phyllanthus niruri* L. J. Ethnopharmacol. **157**(6): 2-68.

Liu X, Zhao M, Wu K, Chai X, Yu H, Tao Z and Wang J 2012. Immunomodulatory and anti-cancer activities of phenolics from emblica fruit (*Phyllanthus emblica* L.). Food Chem. **131**(2): 685-690.

Luo W, Zhao M, Yang B, Ren J, Shen G and Rao G 2011. Antioxidant and antiproliferative capacities of phenolics purified from *Phyllanthus emblica* L. fruit. Food Chem. **126**(1): 277-282.

Luo W, Zhao M, Yang B, Shen G and Rao G 2009. Identification of bioactive compounds in *Phyllanthus emblica* L. fruit and their free radical scavenging activities. Food Chem. **114**(2): 499-504.

Ma JX, Lan MS, Qu SJ, Tan JJ, Luo HF, Tan CH and Zhu DY 2012. Arylnaphthalene lignan glycosides and other constituents from *Phyllanthus reticulatus*. J. Asian Nat. Prod. Res. **14**(11): 1073-1077.

Mackeen MM, Ali AM, El-Sharkawy SH, Manap MY, Salleh KM, Lajis NH and Kawazu K 1997. Antimicrobial and cytotoxic properties of some Malaysian traditional vegetables (Ulam). Int. J. Pharm. **35**: 174-178.

Mahata S, Pandey A, Shukla S, Tyagi A, Husain SA, Das BC and Bharti AC 2013. Anticancer activity of *Phyllanthus emblica* Linn. (Indian Gooseberry): Inhibition of transcription factor AP-1 and HPV gene expression in cervical cancer cells. Nut. Can. **65**: 88-97.

Mahbub MN, Rubaiyath ANMRB and Alam SS 2007. Development of marker chromosomes in three varieties of *Vigna radiata* (Fabaceae). Cytologia **72**(2): 221-225.

Maleia MP, Filho PSV and Gonçalves-Vidigal MC 2010. Genetic divergence among African and American cotton (*Gossypium hirsutum* L. race *latifolium* H.) cultivars and inbred lines through random amplification of polymorphic DNA (RAPD) markers. African J. Biotec. **9**: 8539-8548.

Malla SB, Bhattarai S, Gorkhali M and Singh MP 1975. In IOPB chromosome number reports XLIX. Taxon **24**: 501-516.

Manissorn J, Ruangrungsi N, Phadungcharoen T and Sukrong S 2010. DNA fingerprinting of selected Thai *Phyllanthus* species by RAPD analysis. J. Health Res. **24**(2): 73-79.

Maroyi A 2008. *Phyllanthus reticulatus* Poir. In: Schmelzer, G.H. and Gurib-Fakim, A. (Editors). PROTA (Plant resources of tropical Africa/Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands.

Marshall BJ and Warren JR 1984. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet **1**: 1311-1315.

Marshall BJ, Armstrong JA, McGechie DB and Glancy RJ 1985. Attempt to fulfill Koch's postulates for pyloric Campylobacter. Med. J. Aust. **142**: 436-439.

Maruthappan V and Shree KS 2010. A report on the antioxidant activity of the powder of the entire plant of *Phyllanthus reticulatus* Poir. Indian J. Pharmacol. **4**(4): 265-269.

Mehra PN 1972. Cytogenetical evolution of hardwoods. Nucleus **15**: 64-83.

Mehra PN 1976. Cytology of Himalayan hardwoods. Sree Saraswaty Press, Calcutta.

Mehra PN and Hans AS 1969. In IOPB chromosome number reports XXI. Taxon **18**: 310-3I5.

Mehra PN and Hans AS 1972. Comparison of diploid and natural polyploid hardwoods. J. Indian Bot. Soc. **51**: 319-325.

Mehta S, Singh RK, Jaiswal D, Rai PK and Watal G 2009. Antidiabetic activity of *Emblica officinalis* in animal models. Pharmaceu. Biol. **47**: 1050-1055.

Melendez PA and Capriles VA 2006. Antibacterial properties of tropical plants from Puerto Rico. Phytomedicine **13**: 272-276.

Miller KI and Webster GL 1966. Chromosome numbers in the Euphorbiaceae. Brittonia **18**: 372-379.

Miyoshi E, Shizuri Y and Yamamura S 1987. Isolation of potassium chelidonate as a bioactive substance concerning with circadian rhythm in nyctinastic plants. Chemistry Lett. **16**(3): 511-514.

Moniruzzaman MD, Rahman MDA and Ferdous A 2015. Evaluation of sedative and hypnotic activity of ethanolic extract of *Scoparia dulcis* Linn. Evidence-Based Compl. Alt. Med. **6**: 873954.

Mukhtar M, Arshad M, Ahmad M, Pomerantz RJ, Wigdahl B and Parveen Z 2008. Antiviral potentials of medicinal plants. Virus Res. **131**(2): 111-120.

Muthuraman A, Sood S and Singla SK 2010. The antiinflammatory potential of phenolic compounds from *Emblica officinalis* L. in rat. Inflammopharmacology **19**(6): 327-334.

Naik AD and Juvekar AR 2003. Effects of alkaloidal extract of *Phyllanthus niruri* on HIV replication. Indian J. Med. Sci. **57**: 387-93.

Nain P, Saini V, Sharma S and Nain J 2012. Antidiabetic and antioxidant potential of *Emblica officinalis* Gaertn. leaves extract in streptozotocin-induced type-2 diabetes mellitus (T2DM) rats. J. Ethnopharmacol. **142**(1): 65-71.

Nampoothiri SV, Prathapan A, Cherian OL, Raghu KG, Venugopalan VV and Sundaresan A 2011. *In vitro* antioxidant and inhibitory potential of *Terminalia bellerica* and *Emblica officinalis* fruits against LDL oxidation and key enzymes linked to type 2 diabetes. Fo. Chem. Toxicol. **49**: 125-131.

Nei M 1972. Genetic distance between populations. American Nat. **106**: 283-292.

Neraliya S and Gaur R 2004. Juvenoid activity in plant extracts against filarial mosquito Culex quinquefasciatus. J. Med. Aro. Pl. Sci. **26**(1): 34-38.

Ngamkitidechakul C, Jaijoy K, Hansakul P, Soonthorn-chareonnon N and Sireeratawong S 2010. Antitumour effects of *Phyllanthus emblica* L.: Induction of cancer cell apoptosis and inhibition of *in vivo* tumor promotion and *in vitro* invasion of human cancer cells. Phyto. Res. **24**(9): 1405-1413.

Nicolis E, Lampronti I and Dechecchi MC 2008. Pyrogallol, an active compound from the medicinal plant *Emblica officinalis*, regulates expression of pro-inflammatory genes in bronchial epithelial cells. Int. Immunopharmacol. **8**: 1672-1680.

Nomura A, Stemmermann GN, Chyou PH, Kato I, Perez-Perez GI and Blaser MJ 1991. *Helicobacter pylori* infection and gastric carcinoma among Japanese Americans in Hawaii. N. Engl. J. Med. **325**: 1132-1136.

Nosál'ová G, Mokr´y J and Hassan KMT 2003. Antitussive activity of the fruit extract of *Emblica officinalis* Gaertn. (Euphorbiaceae). Phytomedicine **10**: 583-589.

Oduola AMJ, Sowunmi A, Milhous WK, Kyle DE, Martin RK, Walker O and Salako LA 1992. Innate resistance to new antimalarial drugs in *Plasmodium falciparum* from Nigeria. Trans. roy. Soc. trop. Med. Hyg. **86**: 123-126.

Ohri D and Kumar A 1986. Nuclear DNA amounts in some tropical hardwoods. Caryologia **39**: 303-307.

Oluwafemi F and Debiri F 2008. Antimicrobial effect of *Phyllantus amarus* and *Parquetina nigrescens* on *Salmonella typhi*. African J. Biomed. Res. **11**: 215-219.

Omulokoli E, Khan B and Chhabra SC 1997. Antiplasmodial activity of four Kenyan medicinal plants. J. Ethnopharmacol. **56**(2): 133-137.

Ooi KL, Loh SI, Sattar MA, Muhammad TST and Sulaiman SF 2015. Cytotoxic, caspase-3 induction and *in vivo* hepatoprotective effects of phyllanthin, a major constituent of *Phyllanthus niruri*. J. Func. Foods **14**: 236-243.

Orwa C, Mutua A, Kindt R, Jamnadass R and Anthony S 2009. Agroforestry database. A tree reference and selection guide. Version 4.0. World Agroforestry Centre, Kenya.

Paithankar VV, Raut KS, Charde RM and Vyas JV 2011. *Phyllanthus niruri*: A magic herb. Res. Pharm. **1**(4): 1-9.

Pancho JV and Obien SR 1995. Manual of rice field weeds in the Philippines. Philippine Rice Research Institute, Munoz, Nueva Ecija, Philippines. pp. 543.

Pang JHS, Huang ST and Wang CY 2011. Ellagic acid, the active compound of *Phyllanthus urinaria*, exerts *in vivo* antiangiogenic effect and inhibits MMP-2 activity. Evidence-Based Compl. Alt. Med. **2011**.

Paran I, Aftergoot E and Shifriss C 1998. Variation in *Capsicum annuum* revealed by RAPD and AFLP markers. Euphytica **99**: 167-173.

Parsonnet J, Hansen S, Rodriguez L, Gelb AB, Warnke RA and Jellum E 1994. *Helicobacter pylori* infection and gastric lymphoma. N. Engl. J. Med. **330**: 1267-1271.

Patel SS and Goyal RK 2011. *Emblica officinalis* Geartn.: A comprehensive review on phytochemistry, pharmacology and ethnomedicinal uses. Res. J. Med. Plants **2011**: 1-11.

Paulino N, Cechinel-Filho V, Yunes RA and Calixto JB 1996. The relaxant effect of extract of *Phyllanthus urinaria* in the guinea-pig isolated trachea. Evidence for involvement of ATP-sensitive potassium channels. J. Pharm. Pharmacol. **48**: 1158-1163.

Perry BA 1943. Chromosome number and phylogenetic relationships in the Euphorbiaceae. American J. Bot. **30**: 527-543.

Perry LM 1978. Medicinal plants of East and Southeast Asia: Attributed properties and uses. The MIT Press, England.

Pinmai K, Chunlaratthanabhorn S, Ngamkitidechakul C, Soonthornchareon N and Hahnvajanawong C 2008. Synergistic growth inhibitory effects of *Phyllanthus emblica* and *Terminalia bellerica* extracts with conventional cytotoxic agents: doxorubicin and cisplatin against human hepatocellular carcinoma and lung cancer cells. W. J. Astroenterol. **14**: 1491-1497.

Pinmai K, Hiriote W, Soonthornchareonnon N, Jongsakul K, Sireeratawong S and Tor-Udom S 2010. *In vitro* and *in vivo* antiplasmodial activity and cytotoxicity of water extracts of *Phyllanthus emblica*, *Terminalia chebula* and *Terminalia bellerica.* J. Med. Asso. Thailand **93**: 120-126.

Poompachee K and Chudapongse N 2011. Comparison of the antioxidant and cytotoxic activities of *Phyllanthus virgatus* and *Phyllanthus amarus* extracts. Med. Prin. Pract. **21**(1): 24-29.

Pozharitskaya ON, Ivanova SA, Shikov AN and Makarov VG 2007. Separation and evaluation of free radical-scavenging activity of phenol components of *Emblica officinalis* extract by using an HPTLC-DPPH* method. J. Sep. Sci. **30**: 1250-1254.

Praveenkumar V, Kuttan R and Kuttan G 1994a. Chemoprotective action of Rasayanas against cyclosphamide toxicity. Tumori **80**: 306-308.

Praveenkumar V, Kuttan R and Kuttan G 1994b. Effect of Rasayanas on normal and tumor bearing animals. J. Exp. Clin. Can. Res. **13**: 67-70.

Raghavan RS 1957. Chromosome numbers in Indian medicinal plants. III. Proc. Indian Acad. Sci. Sect. B. **45**(6): 239-244.

Raghavan RS 1959. Chromosome numbers in Indian medicinal plants. III. Proc. Indian Acad. Sci. Sect. B. **49**(4): 239-244.

Raghavan RS and Arora CM 1958. Chromosome numbers in Indian medicinal plants. II. Proc. Indian Acad. Sci. B. **47**: 352-358.

Rahman S, Akbor MM, Howlader A and Jabbar A 2009. Antimicrobial and cytotoxic activity of the alkaloids of Amlaki (*Emblica officinalis*). Pakistan J. Pharmaceu. Sci. **12**: 1152-1155.

Rahmatullah M, Ghosh KC, Almamun A, Hossain MT and Ahmed S 2010. A pharmacological study on antinociceptive and antihyperglycemic effects of methanol extract of leaves of *Phyllanthus reticulatus* Poir. In swiss albino mice. Adv. Nat. Appl. Sci. **4**(3): 229-232.

Raina SN, Rani V, Kijima T, Ogihara Y, Singh KP and Devarumath RM 2001. RAPD and ISSR fingerprints as useful genetic markers for analysis of genetic diversity, varietal identification and phylogenetic relationships in peanut (*Arachis hypogaea*) cultivar and wild species. Genome **44**(5): 763-772.

Rajbhandari M, Mentel R and Jha PK 2009. Antiviral activity of some plants used in Nepalese traditional medicine. Evidence-Based Compl. Alt. Med. **6**(4): 517-522.

Rajkapoor B, Sankari M and Sumithra M 2007. Antitumor and cytotoxic effects of *Phyllanthus polyphyllus* on ehrlich ascites carcinoma and human cancer cell lines. Biosci. Biotech. Biochem. **71**(9): 2177-2183.

Ram K, Sarin MM and Hegde P 2008. Atmospheric abundances of primary and secondary carbonaceous species at two high altitude sites in India: Sources and temporal variability. Atm. Env. **42**(28): 6785-6796.

Raman VS and Kesavan PC 1963. Chromosome numbers of a few horticultural plants. Sci. Cult. **29**(12): 609-610.

Ramasamy S, Wahab N, Abidin NZ, Manickam S and Zakaria Z 2012. Growth inhibition of human gynecologic and colon cancer cells by *Phyllanthus watsonii* through apoptosis induction. PLoS ONE **7**(4): 34793.

Rao MRR and Siddique HH 1964. Pharmacological studies on *Emblica officinalis* Gaertn. Indian J. Expt. Biol. **2**: 29.

Rao NR and Henry AN 1996. The ethnobotany of eastern ghats in Andhra Pradesh. Indian B.S.I., New Delhi.

Raphael KR and Sabu MC 2000. Antidiabetic activity of *Phyllanthus niruri*. Amala research bulletin **20**: 19-25.

Rasool S 2013. Genetic diversity as revealed by RAPD analysis among chickpea genotypes. Pakistan J. Bot. **45**(3): 829-834.

Reddy VD, Padmavathi P, Kavitha G, Gopi S and Varadacharyulu NCH 2011. *Emblica officinalis* ameliorates alcohol-induced brain mitochondrial dysfunction in rats. J. Med. Food **14**: 62-68.

Rekha PS, Kuttan G and Kuttan R 2001. Antioxidant activity of Brahma Rasayana. Indian J. Exp. Biol. **39**: 447-452.

Rossignol L, Rossignol M and Haicour R 1987. A systematic revision of *Phyllanthus* subsection *urinaria* (Euphorbiaceae). American J. Bot. **74**(12): 1853-1862.

Sabu MC and Kuttan R 2002. Antidiabetic activity of medicinal plants and its relationship with their antioxidant property. J. Ethnopharmacol. **81**: 155-160.

Saddi M, Sanna A, Cottiglia F 2007. Antiherpes virus activity of *Artemisia arborescens* essential oil and inhibition of lateral diffusion in Vero cells. Ann. Cli. Microbiol. Antimic. **6**: 10.

Saeed S and Tariq P 2007. Antibacterial activities of *Emblica officinalis* and *Coriandrum sativum* against gram negative urinary pathogens. Pakistan J. Pharmaceu. Sci. **20**: 32-35.

Saha A, Masud AM, Bachar CS, Kundu KJ, Datta KB, Nahar FL and Sarker DS 2007: The analgesic and anti-inflammatory activities of extracts of *Phyllanthus reticulatus* in mice model. Pharmaceu. Biol. **45**(5): 335-359.

Saini A, Sharma S and Chhibber S 2008. Protective efficacy of *Emblica officinalis* against *Klebsiella pneumoniae* induced pneumonia in mice. Indian J. Med. Res. **128**: 188-193.

Sambrook J, Fritsch EF and Maniatis T 1989. Molecular cloning: a laboratory manual. Cold spring harbor laboratory, cold spring harbor, USA.

Sandhu PS and Mann SK 1989. SOCGI plant chromosome number reports-VIII. J. Cytol. Genet. **24**: 179-183.

Sant VJ, Patankar AG, Sarode ND, Mhase LB, Sainani MN, Deshmukh RB, Ranjekar PK and Gupta VS 1999. Potential of DNA markers in detecting divergence and in analysing heterosis in Indian elite chickpea cultivars. Theor. App. Genet. **98**: 1217-1225.

Santos AR 1994. Analgesic effects of callus culture extracts from selected species of *Phyllanthus* in mice. J. Pharm. Pharmacol. **46**(9): 755-759.

Saravanan S, Arutchendhil P and Raveendran TS 2006. Assessment of genetic divergence among introgressed cultures of *Gossypium hirsutum* L. through RAPD analysis. J. App. Sci. Res. **2**: 1212-1216.

Sarkar AK and Datta N 1980. Cytological assessment of the family Euphorbiaceae. II. Tribe Phyllantheae. Proc. Indian Sci. Congr. Assoc. (III, C) **67**: 48-49.

Sarkar AK, Datta N, Mallick R and Chatterjee U 1976. In IOPB chromosome number reports LIV. Taxon **25**: 631-649.

Satyan KS, Prakash A, Singh RP and Srivastava RS 1995. Phthalic acid bisester and other phytoconstituents of *Phyllanthus urinaria*. Planta Medica **61**(3): 293-294.

Schweizer D 1976. Reverse fluorescent chromosome banding with Chromomycin and DAPI. Chromosoma **58**: 307-324.

Seftor RE, Seftor EA and Koshikawa N 2001. Cooperative interactions of laminin 5 gamma 2 chain, matrix metalloproteinnase-2 and membrane type-1-matrix/metalloproteinase are required for mimicry of embryonic vasculogenesis by aggressive melanoma. Cancer Res. **61**: 6322-6327.

Sengupta P and Mukhopad J 1966. Terpenoids and related compounds 7. Triterpenoids of *Phyllanthus acidus* skeels. Phytochemistry **5**(3): 531-534.

Shah FH and Hamid A 1968. Studies in amla fruit (*Emblica officinalis*). Sci. Ind. (Karachi). **6**: 50-56.

Shanker K, Singh M, Srivastava V, Verma R, Gupta A and Gupta M 2011. Simultaneous analysis of six bioactive lignans in *Phyllanthus* species by reversed phase hyphenated high performance liquid chromatographic technique. Acta Chromatog. **23**(2): 321-337.

Sharma AK and Jash M 1958. Further investigation on the cytology of the Amaryllidaceae. Phyton(Buenos Aires) **11**(2): 103-110.

She G, Cheng R and Sha L 2013. A novel phenolic compound from *Phyllanthus emblica*. Nat. Prod. Commun. **8**(4): 461-462.

Shen B, Yu J and Wang S 2008. *Phyllanthus urinaria* ameliorates the severity of nutritional steatohepatitis both *in vitro* and *in vivo*. Hepatology **47**(2): 473-483.

Shruthi SD and Ramachandra YL 2011. RBP-J as a therapeutic target to rheumatoid arthritis - an insilico study. Int. J. Precl. Pharmaceu. Res. **2**(1): 38-44.

Shruthi SD, Ramachandra YL, Padmalatha RS and Shettty VA 2010. Antibacterial potential of leaf extracts from *Kirganelia reticulate* Baill. Int. J. Pharmaceu. Res. Dev. **2**(6): 0974-9446.

Silvestre JS, Mallat Z, Tamarat R, Duriez M, Tedgui A and Levy BI 2001. Regulation of matrix metalloproteinase activity in ischemic tissue by interleukin-10: role in ischemia-induced angiogenesis. Circul. Res. **89**(3): 259-264.

Sonnante G, Marangi A, Venora G and Pignone D 1997. Using RAPD markers to investigate genetic variation in chickpea. J. Genet. Breed. **51**: 303-307.

Sousa M, Ousingsawat J, Seitz R, Puntheeranurak S, Regalado A, Schmidt A, Grego T, Jansakul C, Amaral MD, Schreiber R and Kunzelmann K 2007. An extract from the

medicinal plant *Phyllanthus acidus* and its isolated compounds induce airway chloride secretion: a potential treatment for cystic fibrosis. Mol. Pharmacol. **71**(1): 366-376.

Srikumar R, Parthasarathy NJ, Shankar EM, Manikandan S, Vijayakumar R, Thangaraj R, Vijayananth K, Devi R and Rao UA 2007. Evaluation of the growth inhibitory activities of Triphala against common bacterial isolates from HIV infected patients. Phytother. Res. **21**: 476-480.

Srividya N and Periwal S 1995: Diuretic, hypotensive and hypoglycaemic effects of *Phyllanthus amarus*. Indian J. Exp. Biol. **33**: 861-864.

Stebbins GL 1971. Chromosomal evolution in higher plants. Addison-Wesley publishing company, California, USA. pp. 208.

Subeki S, Matsuura H, Takahashi K, Yamasaki M, Yamato O, Maede Y, Katakura K, Kobayashi S, Trimurningsih T, Chairul C and Yoshihara T 2005. Anti-babesial and anti-plasmodial compounds from *Phyllanthus niruri*. J. Nat. Prod. **68**(4): 537-539.

Subramanian SS, Nagarajan S and Sulochana N 1971. Euphorbiaceae. Flavonoids of some Euphorbiaceous plants. Phytochemistry **10**: 2548-2549.

Subramanian V, Gurtu S, Rao RC and Nigam SN 2000. Identification of DNA polymorphism in cultivated groundnut using random amplified polymorphic DNA (RAPD) assay. Genome **43**: 656-660.

Sultana SS and Alam SS 2007. Differential fluorescent chromosome banding of *Solanum nigrum* L. and *Solanum villosum* L. from Bangladesh. Cytologia **72**(2): 213-219.

Sultana SS and Alam SS 2016a. Karyomorphology of eleven varieties of *Gossypium hirsutum*. Bangladesh J. Bot. **45**(1): 151-159.

Sultana SS and Alam SS 2016b. Differential fluorescent banding in 11 varieties of *Gossypium hirsutum* L. from Bangladesh. Cytologia **81**(1): 111-117.

Sultana SS and Alam SS 2016c. SSR and RAPD-based genetic diversity in Cotton germplasms. Cytologia **81**(3): 257-262**.**

Sultana SS, Ara H and Alam SS 2011. Karyotype analysis with orcein and CMA in two species of *Alocasia* (Schott) G. Don. Bangladesh J. Bot. **40**(1): 53-56.

Sumantran VN, Boddul S, Koppikar SJ, Dalvi M, Wele A, Gaire V and Wagh UV 2007. Differential growth inhibitory effects of W*. somnifera* root and *E. officinalis* fruits on CHO cells. Phytoth. Res. **21**: 496-499.

Sumitra M, Manikandan P, Gayathri V, Mahendran SP and Suguna L 2009. *Emblica officinalis* exerts wound healing action through up-regulation of collagen and extracellular signal-regulated kinases (ERK1/2). Wound Rep. Regen. **17**(1): 99-107.

Sun W 2012. Chemical constituents of *Phyllanthus amarus*. Chin. Trad. Herb. Drugs **43**(1): 23-26.

Suryanarayana P, Kumar PA, Saraswat M, Petrash JM and Reddy GB 2004. Inhibition of aldose reductase by tannoid principles of *Emblica officinalis*: implications for the prevention of sugar cataract. Mol. Vis. **10**: 148-154.

Suryanarayana P, Saraswat M, Petrash JM and Reddy GB 2007. *Emblica officinalis* and its enriched tannoids delay streptozotocin-induced diabetic cataract in rats. Mol. Vis. **13**: 1291-1297.

Syamasundar KV, Singh B, Thakur RS, Husain A, Kiso Y and Hikino H 1985. Anti-hepatotoxic principles of *Phyllanthus niruri* herb. J. Ethnopharmacol. **14**: 41-44.

Tanaka R 1971. Type of resting nuclei in Orchidaceae. Bot. Mag. Tokyo **84**: 118-122.

Tang YQ, Jaganath I, Manikam R and Sekaran SD 2013. *Phyllanthus* suppresses prostate cancer cell, PC-3, proliferation and induces apoptosis through multiple signaling pathways (MAPKs, PI3K/Akt, NF B, and Hypoxia). Evidence-Based Compl. Alt. Med. **2013**: 609581.

Tang YQ, Jaganath IB and Sekaran SD 2010. *Phyllanthus* spp. induces selective growth inhibition of PC-3 and meow human cancer cells through modulation of cell cycle and induction of apoptosis. PLoS ONE **5**(9): 12644.

Taylor P 2000. Patterns of chromosomal variation in Southern African rodents. J. Mammal. **81**: 317-331.

Telford JL, Ghiara P, Dell'Orco M, Comanducci M, Bur-roni D and Bugnoli M 1994. Gene structure of the *Helicobacter pylori* cytotoxin and evidence of its key role in gastric disease. J. Exp. Med. **179**: 1653-1658.

Than NN, Fotso S, Poeggeler B, Hardeland R and Laatsch H 2006. Niruriflavone, a new antioxidant flavone sulfonic acid from *Phyllanthus niruri*. Zeitschrift fur Naturforschung-Section B: J. Chem. Sci. **61**(1): 57-60.

Theresa YM, Sastry KNS and Nayudamma Y 1967. Studies on biosynthesis of tannins in indigenous plants XIII. Occurrence of a new gallotannin amlaic acid in amla leaves (*Phyllanthus emblica*). Leath. Sci. **14**: 16-17.

Thombre MV 1959. Chromosome numbers in some common flowering plants. Sci. Cult. **25**: 208.

Thyagarajan SP, Subramanian S, Thirunalasundari T, Venkateswaran PS and Blumberg BS 1988. Effect of *Phyllanthus amarus* on chronic carriers of hepatitis B virus. Lancet. **332**(8614): 764-766.

Tiwari V, Kuhad A and Chopra K 2011. *Emblica officinalis* corrects functional, biochemical and molecular deficits in experimental diabetic neuropathy by targeting the oxidonitrosative stress mediated inflammatory cascade. Phytother. Res. **25**: 1527-1536.

Tona T, Ngimbi NP and Tsakala M 1999. Antimalarial activity of 20 crude extracts from nine African medicinal plants used in Kinshasa, Congo. J. Ethnopharmacol. **68**(1-3): 193-203.

Tran DT, Bui QC, Hoang VL and Nguyen XD 2007. Isolation and structural elucidation of some phenolic compounds from *Phyllanthus urinaria* L. in Vietnam. Tap. Chi. Duoc. Hoc. **47**(3): 14-17.

Trivedi RN, Mukherjee S and Trivedi MP 1986. Reproductive behaviour in the genus *Phyllanthus* (Euphorbiaceae). Proc. Indian Sci. Congr. Assoc. **73**(3-VI): 149.

Unander DW, Webster GL and Blumberg BS 1995. Usage and bioassays in *Phyllanthus* (Euphorbiaceae) IV. Clustering of antiviral uses and other effects. J. Ethnopharmacol. **45**: 1-18.

Vongvanich N, Kittakoop P, Kramyu J, Tanticharoen M and Thebtaranonth Y 2000. Phyllanthusols A and B, cytotoxic norbisabolane glycosides from *Phyllanthus acidus* skeels. J. Org. Chem. **65**(17): 5420-5423.

Wang CY and Lee SS 2005. Analysis and identification of lignans in *Phyllanthus urinaria* by HPLC-SPE-NMR. Phytochem. Anal. **16**(2): 120-126.

Wang ML, Mosjidis JA, Morris JB, Dean RE, Jenkins TM and Pederson GA 2006. Genetic diversity of *Crotalaria* germplasm assessed through phylogenetic analysis of EST-SSR markers. Genome **49**(6): 707-715.

Webster GL and Ellis JR 1962. Cytotaxonomic studies in the Euphorbiaceae, subtribe Phyllanthinae. American J. Bot. **49**(1): 14-18.

Wei WX and Pan YJ 2002. The crystal structure of one natural compound cyclo-(1,10-docandiamino-11,20-docanedioic) amide (1,12-diazacyclodocosane-2,11-dione). Bull. Kor. Chem. Soci. **23**(11): 1527-1530.

Wei WX, Pan YJ, Zhang H, Lin CW and Wei TY 2004. Two new compounds from *Phyllanthus niruri*. Chem. Nat. Comp. **40**(5): 460-464.

Williams JGK, Hanafey MK, Rafalski JA and Tingey SV 1993. Genetic analysis using random amplified polymorphic DNA makers. Meth. enzymol. **218**: 705-740.

Wongsrichanalai C, Webster HK, Wimonwattrawatee T, Sookto P, Chuanak N, Thimasarn K and Wernsdorfer WH 1992. Emergence of multidrug-resistant *Plasmodium falciparum* in Thailand: *in vitro* tracking. American J. trop. Med. Hyg. **47**: 112-116.

Xiang Y, Pei Y, Qu C, Lai Z, Ren Z, Yang K, Xiong S, Zhang Y, Yang C, Wang D, Liu Q, Kitazato K and Wang Y 2011. *In vitro* anti-herpes simplex virus activity of 1, 2, 4, 6-tetra-O-galloyl-β-d-glucose from *Phyllanthus emblica* L. (Euphorbiaceae). Phytother. Res. **25**: 975-982.

Yang CB, Zhang F, Deng MC, He GY, Yue JM and Lu RH 2007. A new ellagitannin from the fruit of *Phyllanthus emblica* L. J. Chin. Chem. Soci. **54**(6): 1615-1618.

Yeo SG, Song JH and Hong EH 2015. Antiviral effects of *Phyllanthus urinaria* containing corilagin against human enterovirus 71 and Coxsackievirus A16 *in vitro*. Arch. Pharmacal. Res. **38**(2): 193-202.

Zhang DY, He DW, Wei GH, Liu X, Lin T and Li XL 2008. *Phyllanthus urinaria* extract promotes N-cadherin expression in nitrogen mustard-disrupted testicular tissues *in vivo.* Zhonghua Nan KeXue **14**: 396-400.

Zhang Y, Zhao L, Guo X, Li C, Li H, Lou H and Ren D 2006. Chemical constituents from *Phyllanthus emblica* and the cytoprotective effects on H_2O_2 - induced PC12 cell injuries. Arch. Pharm. Res. **39**: 1202-1211.

Zhang YJ, Nagao T, Tanaka T, Yang CR, Okabe H and Kouno I 2004. Antiproliferative activity of the main constituents from *Phyllanthus emblica*. Bio. Pharmaceu. Bull. **27**(2): 251-255.

Zhao HJ, Liu T and Mao X 2015. Fructus phyllanthi tannin fraction induces apoptosis and inhibits migration and invasion of human lung squamous carcinoma cells *in vitro* via MAPK/MMP pathways. Acta Pharmaco. Sin. **36**(6): 758-768.

Zhou M, Zhu HL, Wang KW, Wei WX and Zhang Y 2012. Isolation and X-ray crystal structure of a securinega-type alkaloid from *Phyllanthus niruri* Linn. Nat. Prod. Res. **26**(8): 762-764.

Zhou S, Xu C, Zhou N, Huang Y, Huang L, Chen X, Hu Y and Liao Y 1997. Mechanism of protective action of *Phyllanthus urinaria* L. against injuries of liver cells. Zhongguo Zhong Yao Za Zhi **22**(2): 109-111.