Research Paper

Phytochemical Analysis and *In Vitro* Synergistic Anti-Bacterial Effect of Methanolic Extracts of *Phyllanthus* Fruits against Human Pathogens

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The present work focused at antibacterial activity of three different *phyllanthus* species; *phyllanthus acidus*, phyllanthus *emblica* and *phyllanthus niruri*. The antibacterial activities of methanolic extracts of fruits were tested *in vitro* against human pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Salmonella typhi*. Ciprofloxacin (0.02 mg/ml), Gentamycin (0.02 mg/ml), and Tetracycline (0.02 mg/ml) antibiotics were used as standards by disc diffusion method. The synergistic activity of the three *Phyllanthus* species was investigated. The study found that at 100 g/ml, the methanolic extract of Phyllanthus *emblica* had the strongest action against all of the species, whereas *Phyllanthus acidus* and *Phyllanthus niruri* had the lowest activity .The synergistic activity of all the extracts showed potent activity. The Fourier-transform infrared spectroscopy and Ultraviolet-Visible spectrum revealed the existence of functional groups of carboxyl, carbonyl, and aromatic groups.

Key words: *Phyllanthus* sp., methanolic extract, Fourier-transform infrared spectroscopy, ultraviolet-visible spectrum, anti-bacterial activity

Phyllanthus (Euphorbiaceae) is a large genus that can be found in tropical and subtropical countries such as Africa, America and Asia. There are 11 subgenus in this genus, which encompasses approximately 700 species[1]. The most common 550-750 species are largely from the subgenera acidus, emblica and niruri, they have been used for ages by numerous countries[2]. In China, India, Brazil and Southeast Asia, the *Phyllanthus* genus has been used to manufacture herbal treatments for ages. The most common species in Ayurveda are used to treat digestive, genitourinary, respiratory, and skin issues[3-5]. In China, herbs and their prescriptions are used to treat hepatitis B, hypertension, dropsy and sore throat. These natural medications are used to treat jaundice, renal calculus, and malaria, respectively, in Thailand, Latin America (especially Brazil), and Africa[6,7].

Traditional medicine has utilised this plant to treat stomach, intestinal infection, renal and urinary bladder abnormalities, diabetes, and hepatitis B. *Phyllanthus* extracts have been shown to offer a number of pharmacological benefits. It has antibacterial, antiviral, and anti-hepatotoxic properties.

Microorganisms have self-defence systems to protect themselves from free radical attack, including a preventative antioxidant system that delays free radical generation and the production of chain-breaking antioxidants that scavenge and stabilise free radicals. Significant tissue damage happens when the rate of free radical production exceeds the normal capacities of antioxidant defence mechanisms[8]. Continuous exposure to chemicals and contaminants may increase free radicals in the body beyond the body's ability to control them, resulting in irreversible oxidative damage such as biological damage, deoxyribonucleic acid

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damage, diabetes, respiratory tract disorders, carcinogenesis, and cellular degeneration associated with ageing[9]. Natural antioxidants, particularly those produced from plants, have seen a boom in popularity in recent years[10-15]. *P. acidus* and *P. emblica* L fruits are strong in vitamin C and have traditionally been used to enhance eyesight, memory, diabetes prevention, and cough treatment[16]. Another variety of *Phyllanthus* amarus is a popular herbal medication because of its potent antiviral properties, particularly against the hepatitis B virus[17].

Phyllanthus is a genus that has yielded over 510 compounds, primarily lignins, triterpenoids, flavonoids and tannins, many of which possess biological activities. Terpenoids, phenylpropanoids, tannins, flavonoids, sterols, alkaloids, phenols and other compounds are among the most common chemical classes found in this genus. Lignins and tannins are considered to be the most biologically active compounds. Corilagin, geraniin and gallic acid are the three most abundant compounds, while Phyllanthin, niranthin and geraniin have been extensively studied in pharmacological research. These compounds have demonstrated anti-inflammatory, antioxidant, antitumor, antiviral, and immunomodulatory activities[18].

Phyllanthus niruri, also known as gale of the wind, stonebreaker, or seed-under-leaf, is a common tropical plant found in coastal locations. It reaches a height of 50-70 cm (20-28 in) and has rising herbaceous branches[19]. The bark is bright green and smooth. It produces a large number of pale green flowers that are frequently flushed with crimson[20]. The fruits are small, smooth capsules containing seeds that are used in Ayurveda to treat stomach, genitourinary, liver, kidney and spleen issues[21]. The deciduous tree is a type of tree that grows in the fall and from the Sanskrit amalaki, *Phyllanthus emblica* is also known as emblic, embli myrobalan, myrobalan, Indian gooseberry, Malacca tree, or alma[22,23]. The tree grows to a height of 1-8 m (3 ft 3 in to 26 ft 3 in) and is small to medium in size. Simple, sub sessile, and tightly set along branch lets, the leaves are light green and resemble pinnate leaves. The leaves are simple, sub sessile, and densely spaced along branch lets, 10-20 cm (3.9-7.9 in) long, typically deciduous, while the branch lets are not glabrous or finely pubescent, 10-20 cm (3.9-7.9 in) long, usually deciduous. Flowers are with a greenish-yellow coloration. On the outside, the fruit is about spherical, pale greenish yellow, smooth and hard, and on the inside, there are six vertical stripes or furrows. The flavour of Indian emblic is sour, bitter and astringent and it is very fibrous. *Phyllanthus acidus*, also known as the Otaheite gooseberry, Malay gooseberry, Tahitian gooseberry, Country gooseberry, Star gooseberry, Star berry, West India gooseberry, is one of the trees with edible little yellow berries fruit. Except for the acidity of its fruits, the plant bears no resemblance to the gooseberry, despite its name. It has a sour and acidic flavour. *Phyllanthus acidus* is a shrub that grows to be a tree, reaching a height of 2-9 m (612-30 ft)[24].The dense and bushy crown of the tree is made up of thickish, tough main branches with clusters of deciduous, greenish, 15-30 cm long branch lets at the end. The alternate leaves on the branch lets are oval or lanceolate in shape, with short petioles and pointy tips. The leaves are 2-7.5 cm long and thin, with the upper side being green and smooth and the below side being blue-green. Male, female or hermaphrodite flowers are all possible. They're little pinkish and they bloom in clusters in panicles that range in length from 5-12.5 cm. Flowers appear at the highest portion of the tree, on leafless areas of the main branches. The fruits are abundant, oblate, and densely packed, with 6-8 ribs. They're waxy, pale yellow or white, crisp and juicy, and quite sour (fig. 1)[25].

MATERIALS AND METHODS

Collection of plant material:

Selected *Phyllanthus* species fruits were collected from Tirumala hills, Chittoor district, Andhra Pradesh, India and were identified and authenticated by a taxonomist.

Preparation of extract:

Phyllanthus fruits were harvested, washed, cut into little pieces, and dried in the shade. The dried fruits were then pulverised into a fine powder and kept in airtight bottles using a mixer grinder. Weighing around 10 g of three species were soaked in 100 ml of methanol and incubated for 3 d in an Orbital shaker at 3000 rpm for 72 h at 37°. After the incubation period, the solution was filtered through Whatman no.1 filter paper to remove any solid particles. The resulting filtrate was then concentrated by evaporation using a Rota evaporator (Buchi) under reduced pressure at

40°. For subsequent examination, the dried crude extract was kept at 4°[26].

Chemicals and reagents

Nutrient agar and Potassium bromide (KBr) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Dimethyl sulphoxide were purchased from Himedia, India. All reagents were used for analytical quality (SD fine with 99.8 % purity).

Apparatus:

Orbital shaker, rotary evaporator, Fourier Transform Infrared spectroscopy (FTIR), Ultraviolet Visible (UV-Vis) spectrophotometer were used for the study.

Test organisms:

Escherichia coli, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Salmonella typhi* species were isolated from Sri Venkateswara Institute of Medical Sciences (SVIMS) Tirupati and IMTECH Chandigarh, respectively. The Kirby-Bauer Test, commonly known as the zone of inhibition test, was a quantitative antibacterial activity test. By using the spread plate method[27], bacteria cultures were spread on to Nutrient agar plates after 24 h. The disc diffusion method was used to investigate antibacterial activity. As a positive control for antibacterial activity, chloramphenicol was used.

Selection of reference antibiotics:

DUEL, an approved medical business, provided reference standard antibiotics such as Gentamycin, Tetracycline, and Ciproflloxacin was purchased from Bross scientifics. The antibiotic's purity was 99.8 %.

Phytochemical screening:

Alkaloids: Alkaloids were detected by using 50 mg of fruit extract was dissolved in 5 ml of methanol and 2 N HCl, filtered, and then treated with Meyer's and Wagner's reagents. If a precipitate formed, it indicated the presence of alkaloids.

Flavonoids: Flavonoids were detected by 50 mg of extracts were heated with 5 ml of ethyl acetate at 40°-50° on a water bath for 5 min. The filtrate was then treated with 1 % aluminium chloride in methanol, and the appearance of a yellow color indicated the presence of flavonoids.

Tannins: Tannins were detected by 50 mg of all extracts were boiled in 10 ml of water and a few drops of 5 % FeCl3 were added to the filtrates. The formation of a brownish-green color indicated the presence of tannins.

Fig. 1: Plants of (a) *Phyllanthus acidus*; (b) *Phyllanthus emblica* and (c) *Phyllanthus niruri*

Steroids: Steroids were detected by adding 2 ml of chloroform and concentrated H2SO4 were added with the 5 ml aqueous leaf extract solution of *Phyllanthus* sp. In the lower chloroform layer red color appeared that indicated the presence of steroids.

Glycosides: Glycosides were identified by the formation of a reddish-brown color ring at the junction of glacial acetic acid to $FeCl_3$ and concentrated H_2SO_4 reaction mixture.

Tannins: To 2 ml of the filtrate, 10 ml of distilled water was added followed by few drops of $FeCl_3$, formation of blue colour indicates the presence of tannins.

Saponins: Saponins were detected by adding 2 ml of filtrate, 1 ml of ammonia solution and 1 ml of lead acetate. Formation of lack green precipitate or deep green foam indicates the presence of saponins.

Resins: 1 ml of solvent extracts were treated with few drops of acetic anhydride solution followed by 1 ml of concentrated H_2SO_4. Colouration ranging from orange to yellow indicates the presence of resins.

Terpenoids: To 1 ml of extract added 0.5 ml chloroform followed by few drops of concentrated H_2SO_4. Formation of reddish brown precipitate indicates the presence of terpenoids.

Carbohydrates: Carbohydrates were detected by adding 2 drops of Molisch reagent and few drops of concentrated H_2SO_4 to 2 ml of filtrate. Formation of violet or reddish colour indicates the presence of carbohydrates (Table 1).

Inoculum preparation:

To prepare adequate dilution, 10 mg/ml crude extracts of *Phyllanthus acidus*, *Phyllanthus emblica*, and *Phyllanthus niruri* were weighed and diluted in sterile distilled water. *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Salmonella typhi* inoculums were produced in nutrient broth medium and incubated at 37° for 8 h. After growth was observed, the cultures were stored in the refrigerator at 2-8° for examination[28].

Anti-bacterial susceptibility test:

The antibacterial activity was tested using the Kirby-Bauer disc diffusion and the well plate techniques[27]. Nutrient Agar Media (NAM) was used to test antibacterial activity *in vitro*. NAM plates were made by pouring 15 ml of molten medium into sterilised Petri plates. The plates were allowed to harden for 15 min before being swabbed uniformly with 100 µl of inoculum suspension and allowed to dry for 5 min. On a 6 mm sterile disc, 100 µl/disk of extract was loaded. As conventional antibiotics, 20 g of Gentamycin, 20 g of Ciprofloxacin, and 20 g of Tetracycline discs were utilised. The compounds were allowed to diffuse for 5 min after the loaded discs were placed on the medium's surface. After 24 h of growth, the plates were incubated at 37° and the zone of inhibition was measured in ml. Drops of sterile distilled water were used in place of different plant extracts in a control experiment. Inhibition zones produced around the disc were measured at the end of the incubation (Table 2).

TABLE 1: PHYTOCHEMICAL SCREENING OF Phyllanthus SPECIES OF METHANOLIC EXTRACT OF FRUITS

Phytochemical constituents	*Phyllanthus niruri*	*Phyllanthus acidus*	*Phyllanthus emblica*
Carbohydrates	++	++	++
Glycosides	+	++	++
Steroids	-	-	-
Alkaloids	+	++	++
Tannins	-	-	+
Flavonoids	+	++	++
Resins	-	-	-
Saponins	++	++	++
Terpenoids	+	++	++

Note: (++): Higher amounts; (+): Lower amounts and (-): Absent

TABLE 2: THE ANTI-BACTERIAL ACTIVITY OF Phyllanthus SPECIES OF METHANOLIC EXTRACT OF FRUITS

Plants	Micro organisms				
	Escherichia coli	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Salmonella typhi*
Phyllanthus emblica [a]	+	+	+++	+++	+
Phyllanthus niruri[b]	+++	+	+++	+	+
Phyllanthus acidus[c]	+	+	+	+	+++
Synergistic activity[a+b+c]	++++	+++	++	+++	++

Note: (+): Lower inhibition; (++): Moderate inhibition; (+++): Higher inhibition and (++++): Very high inhibition

FTIR:

All extracted powders and different fractions obtained after column chromatography were scanned in the range of 4000-6500 cm-1 with a resolution of 4 per cm-1 using 2.8 FTIR. By attaching an ATR accessory to an FTIR spectrometer, attenuated total reflection (ATR)/ FTIR spectra were acquired at ambient temperature (Perkin Elmer, Spectrum 100)[29].

UV-Vis spectrophotometric analysis:

E1-E3 (1 mg) separated chemicals were diluted in 10 ml methanol. A UV-Vis spectrophotometer was used to scan the sample solution from 200-750 nm (Evolution, Thermo fisher, USA).

RESULTS AND DISCUSSION

The various phytochemicals are present in the methanolic extract of selected *Phyllanthus* fruits were identified from methanolic extract. The fruit contains alkaloids, flavonoids, polyphenols, and proteins in higher amount according to a preliminary qualitative analysis. However, saponins, tannins, terpenoids, glycosides and steroids are present in methanolic extract.

The antimicrobial investigation found that among the three *Phyllanthus* species, *Phyllanthus emblica* showed the highest activity against *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. *Phyllanthus niruri* showed the maximum activity against *Pseudomonas aeruginosa* and *Escherichia coli*. *Phyllanthus acidus* showed the maximum activity against only *Salmonella typhi*. Synergistic activity of three *Phyllanthus* species was found to be the most effective against all the bacterial species.

After the investigation of the optical properties using UV-Vis spectra data, it has been confirmed that the presence of polar functional groups in the isolated extracted samples. To further, we have been extending our observations for the detailed investigation of existing functional groups in the samples using FTIR. Fig. 2 represents the functional group analysis of (i) *Phyllanthus acidus*, (ii) *Phyllanthus emblica*, (iii) *Phyllanthus niruri*, and (iv) synergetic samples. As from the spectra, several characteristic stretching absorption peaks have been identified at 3450, 3293, 2936, 245, 2366, 1729, 1629, 1239, and 1066 cm^{-1}, that are ascertained for the presence of OH, NH_2 , ArC-H, C-H, SH, ester/amide, C=C, amide band-III, and C-O functionalities respectively in the *Phyllanthus acidus* (curve i). On the other hand, similar types of peaks with wave numbers were also resonated in the *Phyllanthus emblica* (curve ii), *Phyllanthus niruri* (curve iii) samples with slight modifications. However, considerable changes were notified in the synergetic samples (curve iv). Initially, the hydroxyl peak has been broadened after mixing the three samples, which might be responsible for the intermolecular hydrogen bonding between the molecules. Later, the aromatic extended conjugations also happened with the shifting of ArC-H peaks from 2936 to 3012 cm^{-1}. The thiol and amide/ester bonds were also shifter towards higher wave numbers and corresponding wave numbers with the list of possible functionalities were summarized in Table 3. Accounts of the above-mentioned changes, all samples have been enriched with the essential functionalizes, and

considerable changes were also happened before and after synergetic effects.

The preliminary optical observations were examined using UV-Vis spectroscopy as presented in fig. 3. According to the image, the optical properties of (i) *Phyllanthus acidus*, (ii) *Phyllanthus emblica*, (iii) *Phyllanthus niruri*, and (iv) synergistic were recorded. Herein, the synergistic samples were prepared by the mixing of the above three samples in a hydrothermal process. As from the spectra, all samples were showing two characteristic peaks in the range between 220-245, and 260-300 nm, which were accountable for the π–π*, and n–π* electronic transitions respectively. The aromatic double bonds are responsible for the π–π*, and on the other hand the n–π* electronic transitions are accountable for the presence of polar functional groups like hydroxyl (-OH), Amine (-NH_2), and amide (-CO-NH_2). Noteworthy, a broad curves have been notified for the *Phyllanthus acidus* (curve i), *Phyllanthus emblica* (ii) extract of n–π* electronic transitions may be due to the presence of hydroxyl (-OH), Amine (-NH_2) functionalities through hydrogen bonding and these extracts were completely enriched with the polar functional groups. On the other hand, despite having the similar functional groups in *Phyllanthus niruri* (curve iii), the less intense peaks were observed, which might be responsible for the presence of less number of before mentioned functionalities. Finally, however, significant differences have been notified in the synergistic sample (curve iv). Initially, a blue shift has been identified in the π–π*, n–π* electronic transitions as compared to the other spectra. In addition, there was an additional broad peak observed at in the range between 350-400 nm, which might be responsible for the presence of extended conjugation of the aromatic rings in the sample. In summary, presence of the extended peak in the synergistic sample confirms the combination of the remaining three *Phyllanthus acidus*, *Phyllanthus emblica*, and *Phyllanthus niruri* extracted samples.

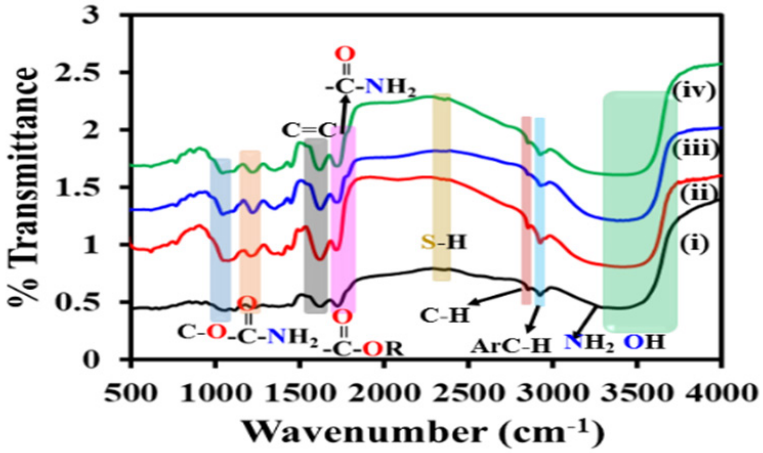

Fig. 2: FTIR spectrum of (i) *P. acidus* (ii) *P. emblica* (iii) P. niruri and (iv) Synergetic

TABLE 3: THE LIST OF OBSERVED FUNCTIONAL GROUPS WITH RESPECTIVE WAVE NUMBERS OF *Phyllanthus acidus*, *Phyllanthus emblica*, *Phyllanthus niruri* AND SYNERGETIC SAMPLES

Functional groups	*Phyllanthus acidus* (cm^{-1})	*Phyllanthus emblica* (cm^{-1})	*Phyllanthus niruri* (cm^{-1})	Synergistic (cm^{-1})
OH	3450-3550	3450-3550	3450-3550	3400-3570
R-NH_2	3293	-	-	-
ArC-H	2926	2926	2926	3902
C-H	2845	2845	2845	2945
S-H	2366	2366	2366	2422
Ester/amide-II	1710	1713	1713	1735
C=C	1629	1629	1629	1640
Amide band-III	1239	1239	1239	1275
C-O	1066	1066	1066	1082

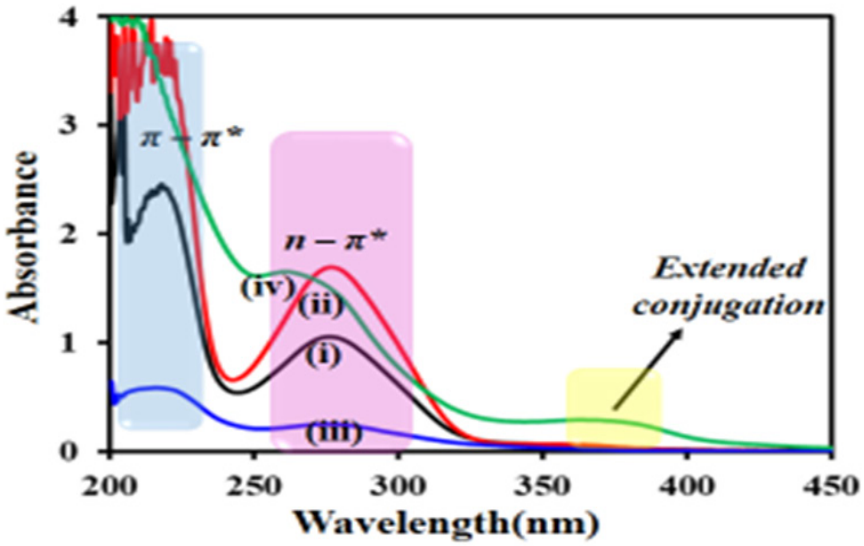

Fig. 3: UV-Visible spectrum of (i) P.acidus; (ii) P.emblic; (iii) P.niruri and (iv) Synergistic

In conclusion, the antibacterial activity of *Phyllanthus emblica* and *Phyllanthus niruri* against *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumonia* was found to be the maximum among the three *Phyllanthus* species studied in this study. Antibacterial activity against *Salmonella typhi* was highest in *Phyllanthus acidus*. Synergistic activity of three *Phyllanthus* species was found to be the most effective against *Escherichia coli*. The synergistic bioactive compound might be play a prominent role for the anti-bacterial activity because, which possess antimicrobial properties are phenolics and polyphenols (flavonoids, tannins,) terpenoids, alkaloids, These compounds capable of exerting antibacterial activities *via* various mechanisms of action i.e., suppress nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism.

UV-Vis spectroscopic analysis of methanolic extracts shows the presence of hydroxyl (-OH), amine (-NH_2) groups in the major peaks. FTIR results indicated the hydroxyl peak has been broadened in the synergistic sample and the aromatic extended conjugations also appeared with the shifting of ArC-H peaks from 2936 to 3012 cm^{-1}.

As a result of these findings, researchers have a great scope to investigate the novel bioactive compounds from these methanolic fruit extracts and further development of prospective formulations to combat the diseases caused by microorganisms.

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Conflict of interest:

The authors declared no conflict of interests.

REFERENCES

1. Adil MD, Kaiser P, Satti NK, Zargar AM, Vishwakarma RA, Tasduq SA. Effectof *Emblica officinalis* (fruit) against UVB-induced photo-aging in human skin fibroblasts. J Pharmacol 2010;132(1):109-14.
2. Anderson D, Phillips BJ, Yu TW, Edwards AJ, Ayesh R, Butterworth KR. Butterworth: Effects of vitamin C supplementation in human volunteers with a range of cholesterol levels on biomarkers of oxygen radical-generated damage. Pure Appl Chem 2000;72(6):973-83.
3. Bauer AW, Kirby WMM, Sheries JC, Truck M. Antibiotic susceptibility testing by standardized single disc method. Am J Clin. Pathol 1966;45(4):493-96.
4. Chang CC, Lien YC, Liu KCSC, Lee SS. Lignans from *Phyllanthus* urinaria. Phytochemistry 2003;63(7):825-33.
5. Center for New Crops and Plants Products. Otaheite gooseberry. 2011.
6. Cooper KE. Theory of antibiotic inhibition zones in Agar media. Nature 1955;176:5101.
7. Ganguly R, Mishra P, Sharmaa, Microbes and infection. Indian J Microbiol 2001;41:211-3.
8. Harborne JB. Phytochemical methods: A Guide to modern techniques of plant analysis. Chapman and Hall 1998;1-302.
9. Hukuri VI, kalyani GA, Kakrani HK. Hypoglycemic activity of flavonoids of *Phyllanthus* frametus in rats, Fitoterapia 1988;59:68-70.
10. Janick J, Paull RE. The encyclopedia of fruit and nuts. CABI 2008.

11. Jayaprakasha GK, Rao LJ. Phenolic constituents from lichen *Parmontremastuppeum*. (Nyl.) hale and their antioxidant activity. Z Naturforsch C J Biosci 2000;55(11-12):1018-22.
12. Edible: An illustrated guide to the world's food plants. 2008.
13. Lim, T.K. (2012). *Phyllanthus emblica*. Edible Medicinal and Non-Medicinal Plants. 2012;258-96.
14. Moreira J, Klein-Júnior LC, Cechinel Filho V, de Campos Buzzi F. Anti-hyperalgesic activity of corilagin, a tannin isolated from *Phyllanthus niruri* L. (Euphorbiaceous). J Pharmacol 2013;146(1):318-23.
15. Nain P, Saini V, Sharma S, Nain J. Antidiabetic and antioxidant potential of *Emblica officinalis* gaertn. Leaves extract in streptozotocin-induced Type-2 Diabetes Mellitus (T2DM) rats. J Pharmacol 2012;142(1):65-71.
16. Omulokoli E, Khan B, Chhabra SC. Antiplasmodial activity of four Kenyan medicinal plants. J Pharmacol 1997;56(2):133-7.
17. Ott M, Thyagarajan SP, Gupta S. *Phyllanthus*amarus suppresses hepatitis B virus by interrupting interactions between HBV enhancer I and cellular transcription factors. Eur J Clin Invest 1997;27(11):908-15.
18. Mao X, Wu LF, Guo HL, Chen WJ, Cui YP, Qi Q, *et al.* The genus *Phyllanthus*: An ethnopharmacological, phytochemical, and pharmacological review. Evid Based Complement Alternat Med 2016;7584952.
19. Patel JR, Tripathi P, Sharma V, Chauhan NS, Dixit VK. *Phyllanthus* amarus: Ethno medicinal uses, phytochemistry and pharmacology: A review. J Ethno pharmacol 2011;138(2):286-313.
20. USDA-ARS. Germplasm Resources Information Network (GRIN).
21. Poompachee K. Chudapongse N. Comparison of the antioxidant and cytotoxic activities of *Phyllanthus*virgatus and *Phyllanthus*amarus extracts. Med Princ Pract 2011;21(1):24-9.
22. Rahman MAA, Moon SS. Antioxidant Polyphenols glycosides from the Plant Drabanemorosa. Bull Korean Chem Soc 2007;28(5):827-31.
23. Rai V, Khatoon S, Bisht SS, Mehrotra S. Effect of cadmium on growth, ultramorphology of leaf and secondary metabolites of *Phyllanthus*amarus and thonn. Chemosphere 2005;61(11):1644-50.
24. Sousa M, Ousingsawat J, Seitz R, Puntheeranurak S, Regalado A, Schmidt A, *et al.* An extract from the medicinal plant *Phyllanthus acidus* and its isolated compounds induce airway chloride secretion: A potential treatment for cystic fibrosis. Mol Pharmacol 2007;71(1):366-76.
25. Tseng TH, Kao ES, Chu CY, Chou FP, Wu HW, Wang CJ. Protective effects of dried flower extracts of *Hibiscus sabdariffa* against oxidative stress in rat primary hepatocytes. Food Chem Toxicol 1997;35(12):1159-64.
26. Unander DW, Webster GL, Blumberg BS. Usage and bioassaysin *phyllanthus* (euphorbiaceous). IV. Clustering of antiviral uses and other effects. J Pharmacol 1995;45(1):1-18.
27. Unander DW, Webster DW, Blumberg BS. Uses and bioassays in *Phyllanthus* (euphorbiaceous): A compilation II. The subgenus *Phyllanthus*. J Ethnopharmacol 1991;34(2-3):97-133.
28. Xia Q. A pharmacognostic and ethno pharmacological studies of Chinese *Phyllanthus* [Ph. D. thesis]. Peking Union Medical College, Beijing, China 1997.
29. Zhang YJ, Nagao T, Tanaka T, Yang CR, Okabe H, Kouno I. Antiproliferative activity of the main constituents from *Phyllanthus emblica*. Biol Pharm Bull 2004;27(2):251-5.