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RESEARCH ARTICLE

Morpho-anatomical, fluorescence, phytochemical and antibacterial studies of *Phyllanthus myrtifolius* Moon. and *Phyllanthus reticulatus* Poir. of Kerala

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ABSTRACT

This study aims to find out the differences between two species of *Phyllanthus* i.e. *P. myrtifolius* Moon. and *P. reticulatus* Poir. in their morpho-anatomical, fluorescent, phytochemical and antibacterial properties. A comparative analysis of morphological and anatomical characters were worked out using dissection and compound microscopes; fluorescence analysis done under a UV cabinet; qualitative phytochemical analysis and antibacterial studies were conducted as per standard procedures. Although the fundamental characters remained almost the same, there were many small differences in their morpho-anatomical characters especially the colour of the fruit, the number of tepals, presence of ridges and furrows in the stem, type of stomata etc. The study of the plant powder brings to light several distinguishing characters which enable us to differentiate between the two species when treated them with several chemical reagents. A complete absence of fluorescence was revealed in both the plant powder extracts. Phytochemical analysis revealed that both the plants possessed secondary metabolites which have strong pharmacological properties and can be utilized for medicinal purposes. *P. myrtifolius* less commonly used as a medicinal plant exhibited at several instances, more appreciable antibacterial results when compared to *P. reticulatus* against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Escherichia coli* bacteria and produced inhibition zones of 20 mm, 18 mm, 17 mm and 15 mm respectively. The leaf extracts of both the plants exhibited greater antibacterial potency than either stem or root extracts. However, acetone root extracts of *P. myrtifolius* and acetone and distilled water root extracts of *P. reticulatus* showed antibacterial effects against both *P. aeruginosa* and *P. vulgaris* bacteria.

Introduction

Medicinal plants have long been used for the treatment of diseases all over the world. They were used by our forefathers before the emergence of modern medicine-Allopathy. In recent years, there has been an increasing awareness about the importance of medicinal plants and efforts are taken for their conservation. In certain cases, the plant parts (stem/ rhizome, root, bark, leaves, fruits or even seeds) or even the whole plant may be useful. *Phyllanthus* is a genus of Euphorbiaceae family which has over 6500 species in 300 genera (1). The members of *Phyllanthus* include herbs to small trees. *Phyllanthus* species are generally well known for the biologically active compounds they possess. They are useful in economic botany, medicinal chemistry and pharmacognosy. Some drugs have been obtained from natural sources while some may be prepared by the modification of the natural ones (2). The aliments treated with these medicinal plants include asthma, cough, diarrhoea, diabetes, malaria, eye and ear problems, indigestion, constipation, nausea, vomiting, bleeding, child care, healing of wounds and sores, in tooth extraction, gonorrhea and syphilis. In many countries around the world, *Phyllanthus* species are used in folk remedies (3). Various species of the genus have been reported to be used in the treatment of liver, kidney and bladder problems, diabetes, appendix inflammation, prostrate problems and intestinal parasites (4). They are also a good source of biopesticides (5–7). In our study, we

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considered two species of *Phyllanthus* viz. *P. myrtifolius* Moon. and *P. reticulatus* Poir. and a comparative study was made to rule out the fundamental differences in their morphological, anatomical, phytochemical, antibacterial and fluorescent characteristics. These species were selected because they were abundantly available in and around our nearby areas.

Phyllanthus myrtifolius Moon. is a small shrub about 50 cm tall while *Phyllanthus reticulatus* Poir. is a shrub and rarely becomes a tree. In *P. myrtifolius*, leaflets are alternately arranged with 1.4 cm long and 0.4 cm width and leaves are linear and lanceolate. Flowers are small pendulous, red and the number of tepals is 6. Fruit is a capsule, pale green with axile placentation. It is mostly grown as an ornamental plant particularly as a bonsai in north east India.

Phyllanthus reticulatus Poir is a scandent shrub and leaves are alternately arranged. Leaflets have an optimum length of 3.1 cm and 1.1 cm width. Flowers are small and yellowish and are borne in clusters. The number of tepals is five in number. The flowering shoots and pedicels are covered in short velvety hairs. Fruit is berry like and becomes blackish when ripened. The leaves and roots are used as medicine for the fractures and traumatic injury, used in diabetic control, as diuretic and has anti-diarrhoeal potency.

Metcalfe and Chalk dealt extensively on the relative importance of anatomical features in the systematic positions of groups of angiosperms (8). Anatomical studies are a systematic line of evidence used in combination with other systematic lines to arrive at a good taxonomic condition (9). In many instances, morphological and anatomical peculiarities proved to be efficient tools in bringing about the correct identification of medicinally valuable plants from their wild relatives of less medical efficacy.

The progressive shift of chemotherapy from first line to second and third world as the costly replacement drugs for treating the highly resistant infectious diseases are unaffordable (10). This brought about the need for developing new drugs against many resistant microbes. Even though there are numerous literature available regarding the antimicrobial properties of many plant extracts, none has been so far exploited for clinical studies. The present study also aims to bring out the antimicrobial potency of *P. myrtifolius* a plant less known for its antimicrobial properties and compare its antibacterial efficacy with a well known medicinal plant *P. reticulatus*.

Materials and Methods

The materials for study *Phyllanthus myrtifolius* Moon. was collected from Kottakkal, Kerala GPS coordinates: 11°00'08.9"N 75°59'51.3"E and *Phyllanthus reticulatus* Poir. from Perumbavoor, Kerala GPS coordinates: 10°06'51.7"N 76°29'15.2"E (Fig. 1). These plants were identified using relevant literature organized as a Database KFRI version 2 (11).

Fig. 1. Habit of *Phyllanthus myrtifolius* Moon. (above) and *Phyllanthus reticulatus* Poir. (below)

Morpho-anatomical studies

Fresh plants were collected and the simple macroscopic as well as microscopic characters of the foliar and floral parts were observed for morphological studies. Morphological characters of leaves like length, width, type, size, shape, apex, margin, length of the petiole, phyllotaxy, fruit characters like colour, type, surface, placentation type, number of tepals etc. were observed with hand lens or dissection microscope. Anatomy of stem, root, node and stomata were observed using compound microscope and photographs were taken.

Fluorescent analysis

The leaf, stem and root powders were treated with several chemical reagents like concentrated nitric acid, concentrated sulphuric acid, concentrated hydrochloric acid, glacial acetic acid etc. and observed the colour combinations produced in them and a comparison table has been made out of it. Fluorescence analysis of the plant powder was also observed with the help of a UV cabinet.

Preparation of extract and phytochemical analysis

Healthy plant specimens were collected, species were identified with respect to their morphological characters in consultation with flora and other literature and keys available. The collected plant materials were washed under running tap water,

shade dried, powdered and then stored in air tight containers. For phytochemical analysis, 5 g of plant powder were weighed accurately and separately dissolved in 50 ml ethanol, acetone and distilled water and kept for 72 h in a rotary shaker for constant stirring (10% concentration) and centrifuged. The supernatant was collected and kept in refrigerator for future use. Alcohol, acetone and distilled water extracts of *P. myrtifolius* and *P. reticulatus* were used for phytochemical studies as per standard procedures (12).

Antibacterial studies

Preparation of culture medium: Nutrient agar powder manufactured by Central Drug House, Private Limited, New Delhi was used for the preparation of the culture medium. The major ingredients were, Peptic digest of animal tissue (5 g/l), Sodium Chloride (5 g/l), Beef extract (1.50 g /l), Yeast extract (1.5 g/ l). Agar (15 g/l) with a final pH at 25 °C of 7.4 ± 0.2.

28 g of nutrient agar was weighed and transferred into a beaker containing 1 litre of distilled water. Gently heated the contents to dissolve the medium and covered the mouth of the beaker with aluminium foil. Petriplates and nutrient agar containing beaker was placed in autoclave and sterilized. Further operations were done in laminar air flow chamber. The sterilized agar medium was poured into petridishes and allowed to solidify at room temperature of 33 °C and kept in an incubator in inverted position for 24 h.

Four strains of bacteria available at Botany laboratory of Maharaja's College, Eranakulam were used for the study. These strains of bacteria were originally collected from the Microbiology lab of Cochin University of Science and Technology, Kalamassery, Eranakulam. The bacterial strains selected for study were *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Staphylococcus aureus*. Here, *S. aureus* was gram positive and the rest were gram negative. Since only these strains were present in our lab, we have decided to conduct our experiments on them.

The experiment was done in a laminar airflow chamber (Model No. VLF 3 from Frendz Inc, Kochi, India). The bacterial culture in nutrient broth was swabbed using buds over the solidified agar medium. A cork borer was used to prepare wells in the medium. The medium was kept in the incubator for 3 h. Stem, root, leaf extracts of *Phyllanthus* species (5 g in 50 ml of acetone, alcohol and distilled water) of 10% concentration were filled in the wells prepared. Ampicillin antibiotic (0.2 g in 100 ml distilled water) was kept as positive control and the solvents in which the extract was prepared i.e. acetone, alcohol and distilled water was taken as the negative control respectively. The petriplates were then inverted and kept in the incubator at 37 °C for 24 h for the optimum growth of the organisms. After the stipulated period of time, petriplates were taken out, zone of inhibition was recorded using scale.

Results and Discussion

Morphological study

The morphology of two *Phyllanthus* species were examined and many minute differences were found. The morphological similarities and differences observed were presented (Table 1).

P. myrtifolius and *P. reticulatus* were shrubs. All leaflets were found in alternate phyllotaxy. Leaves were simple. The tip of leaflet of *P. myrtifolius* was acute while that of *P. reticulatus* was with obtuse ends. Fruit was a capsule in *P. myrtifolius* while in *P. reticulatus*, a berry. Axile placentation was observed in both the species. There were 5 tepals in *P. reticulatus* and 6 tepals in *P. myrtifolius*. The fruit surface of both the plants were smooth. Fruits of *P. reticulatus* were black when ripened. Long stalk was found in the fruit of *P. myrtifolius* (Fig. 2). Thus, we can differentiate both the species easily based on these morphological characters.

Both the species exhibited anatomical differences also. The stem of *P. myrtifolius* was rounded in cross section while *P. reticulatus* was having angular projections at several points around the stem (Table 2; Fig. 3). Ridges and furrows were present in *P. reticulatus* while it was absent in *P. reticulaus*. Pith of stem was very small in *P. myrtifolius* but comparatively very large pith was found in *P. reticulatus*. Crystal like black depositions of calcium oxalate were found in the centre of pith at the corners of the cells in *P. reticulatus* (Fig. 3).

Both the species exhibited well differentiated periderm with a wavy margin in their root section. In both plants, vascular cambium appears as a complete ring, but pith was completely absent in *P. reticulatus* but a small pith was present in *P. myrtifolius* (Table 3; Fig. 4).

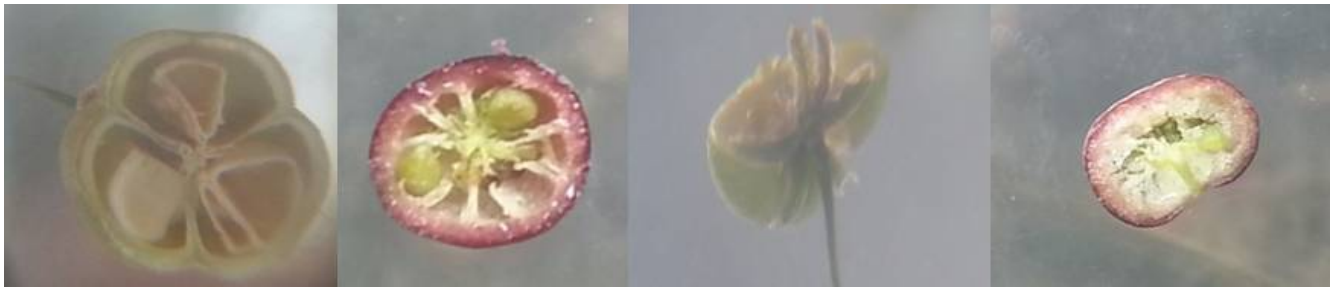
Unilacunar node was present in both the species as can be inferred from (Fig. 5). Formerly morphology and anatomy of *Phyllanthus* species were studied (13). They studied the morphology and anatomy of *Phyllanthus amarus* along with six other *Phyllanthus* species. Previous studies showed that the stomata present in *P. amarus* was anisocytic while that of *P. urinaria* was paracytic, and in *P. mullerianus* showing absence of stomata in the upper epidermis (14). In our studies, we found that *P. myrtifolius* has paracytic stomata and *P. reticulatus* has very less number of stomata (Anisocytic type) in the lower epidermis with irregular shaped subsidiary cells and complete absence of stomata in the upper epidermis as per (Fig. 6).

Fluorescence analysis

When the powder of different species of *Phyllanthus* mixed with different chemical reagents, the following colour changes occurred in day light and no fluorescence was recorded when placed under UV light. In UV light, all appeared black i.e. analysis revealed the complete absence of fluorescence in any of the plant powder extracts (Supplementary Figs. 1, 2 & 3; Supplementary Tables 1 & 2) The powder study can be used as an aid in differentiating the two

Table 1. Details of vegetative and reproductive characters of *P. myrtifolius* and *P. reticulatus*

Characters	*Phyllanthus myrtifolius*	*Phyllanthus reticulatus*
Habit	Shrub	Shrub to small tree
Plant height	3–4 m	6–7 m
Stem colour	Light rose to pinkish	Pale green
Leaf type	Simple	Simple
Leaflet size	14 mm long and 4 mm broad	31 mm long and 11 mm broad
Leaflet shape	Elliptic to lanceolate	Elliptic
Leaflet apex	Acute	Obtuse
Leaflet length	1.4 cm	3.1 cm
Leaflet width	0. 4 cm	1.1 cm
Petiole length	3 mm	2 mm
Phyllotaxy	Alternate	Alternate
Male flowers	Fascicles	Axillary fascicles
Female flowers	Solitary, Tepals 6	Solitary, Tepals 5
Ovary	Globose	Globose
Fruit type	Capsule	Berry
Fruit surface	Smooth	Smooth
Fruit colour	Green	Black when ripened
No of locules	3	6
Placentation	Axile	Axile
Number of tepals	6	5



Phyllanthus myrtifolius fruit C. S | *Phyllanthus reticulatus* fruit C. S | *Phyllanthus myrtifoilius* fruit L. S | *Phyllanthus reticulatus* fruit L. S

Fig. 2. Longitudinal and cross section of fruits of *P. myrtifolius* and *P. reticulatus.*

Table 2. Differences in characteristics of stem anatomy of *P. myrtifolius* and *P. reticulatus*

Characters	*Phyllanthus myrtifolius*	*Phyllanthus reticulatus*
Outline of stem	Round	Angular projections at one or several positions
Ridges and furrows	Absent	Present
Cork	Hyaline epidermal hairs present. Well differentiated periderm	Epidermal hairs absent Well differentiated periderm
Cambium	Not having a wavy margin. Circular in outline	Appears as a wavy band
Secondary vascular tissues	Occupies major portion. 80–90% of the section is occupied by secondary xylem and phloem	Phloem in patches; secondary xylem almost uniformly present throughout
Pith	Small pith. lens shaped schlerenchymatous/thick walled pith in the centre. Crystal like depositions absent	Very large pith. Every cell of the pith contains crystal depositions of calcium oxalate at the corners that strengthen the pith

Table 3. Differences in characteristics of root anatomy of *P. myrtifolius* and *P. reticulatus*

Root	*Phyllanthus myrtifolius*	*Phyllanthus reticulatus*
Cork	Wavy margin, well differentiated periderm Comparatively large corky region	Wavy, well differentiated periderm
Vascular cambium	Circular outline, not wavy	Circular outline, not wavy
Secondary xylem and Phloem	Occupies one third portion	Occupies major portion of the section
Rays	Numerous, not eccentrically placed	Numerous, Not eccentrically placed
Pith	Very small pith made up of parenchyma	Reduced or even absent

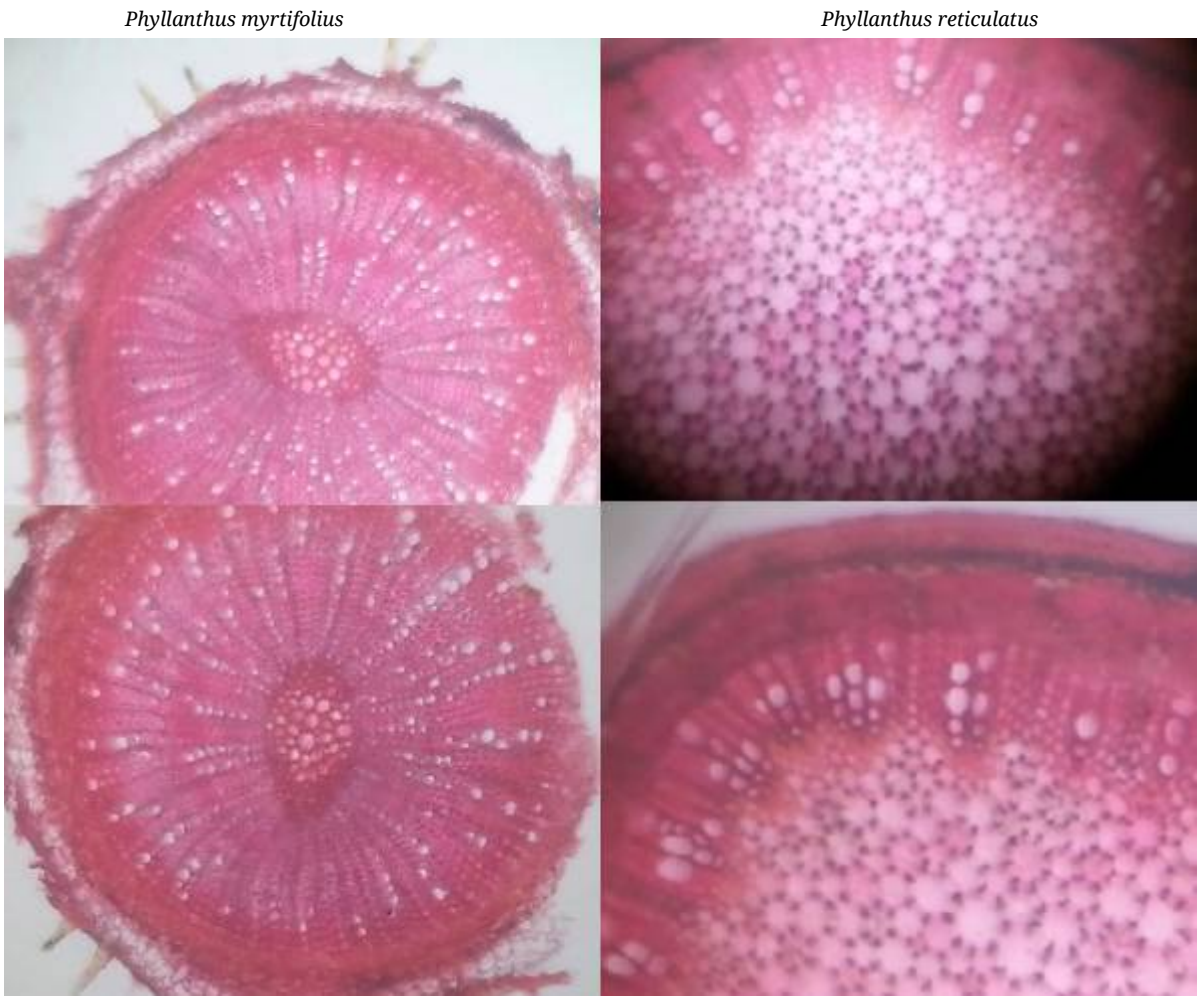


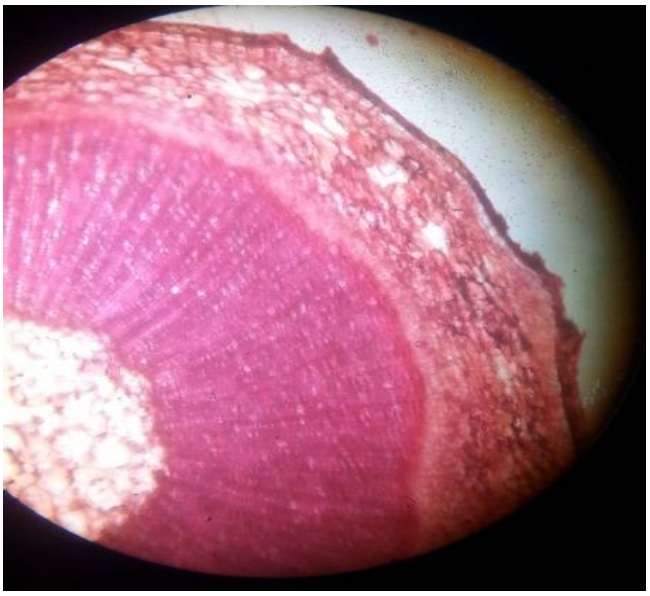
Fig. 3. Anatomical section of stem of *P. myrtifolius* and *P. reticulatus.*

species. Earlier, fluorescence analysis of *Sida* was studied and recorded (15).

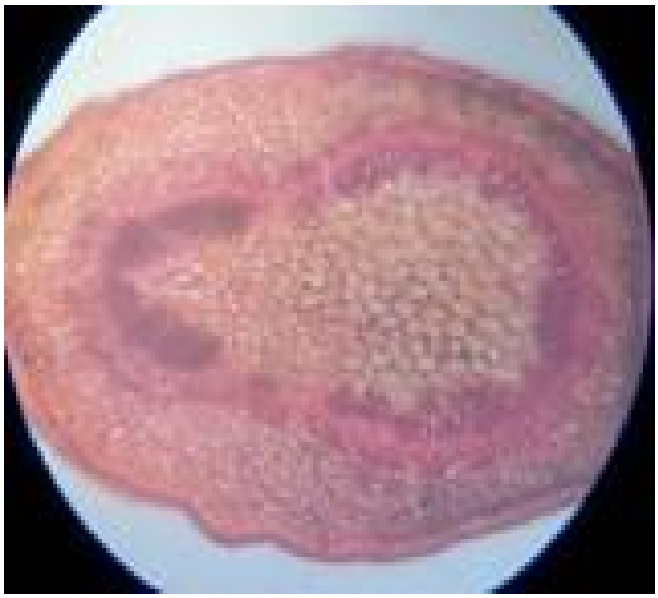
Phytochemical analysis

The phytochemical analysis of the whole plant of both the species in different solvents (distilled water, ethanol and acetone) revealed the presence of almost all the major phytoconstituents in it. The distilled water extracts of *P. myrtifolius* brings out the presence of flavonoids, tannins, phenols, phytosterols, carbohydrates, saponins and anthocyanin while alkaloids and diterpenes were absent in it. The ethanolic extract of the plant revealed the presence of almost all the major phytoconstituents except diterpenes and anthocyanin while in the acetonic extract of the plant saponins and anthocyanin were altogether absent. Protein was completely absent in all the solvent extracts of *P. myrtifolius* (Supplementary Table 3).

The distilled water, ethanol and acetone extracts of *P. reticulatus* brings out comparatively lesser number of phytoconstituents in it. However, Tannins and phenol were present in all the three extracts of *P. reticulatus* while, flavonoids, anthocyanin, diterpenes and phytosterols were present in distilled water extracts, carbohydrates and saponins were present in ethanolic extracts and saponins, diterpenes and phytosterols were present in acetonic extracts. Alkaloids and proteins were completely absent in all the three solvent extracts of *P. reticulatus* (Supplementary Table 4). Preliminary phytochemical screening of ethanolic extracts of *P. reticulatus* revealed the presence of terpenes, phytosterols, phenolic compounds, carbohydrates, flavonoids and minute amount of alkaloids while the distilled water extracts obtained all the above said compounds except alkaloids, gums and mucilages were found in addition (16). In yet another study, phytochemicals obtained were steroids, flavonoids, tannins, phenol and quinine and they reported that when compared to tooth brushes, commercial tooth powders, tooth pastes, chew sticks, the tooth powder made from *P. reticulatus* twigs and leaves showed better advantages (17). The acetone and methanol extracts of *P. niruri* and *P. reticulatus* were quite similar and contained all the necessary secondary metabolites such as flavonoids, tannins, glycosides, steroids and carbohydrates. Hexane and chloroform extracts showed the presence of alkaloids and saponins (18). Our studies obtained more number of Phytoconstituents like saponins while alkaloids were not obtained. According to the studies conducted, more than 510 compounds have been isolated from different *Phyllanthus* species, the majority of which are lignins, triterpenoids, flavonoids and tannins (19). GC-MS analysis of methanolic extract of *P. acidus* fresh leaves revealed 11 biomolecules (20). Similarly, the phytochemical analysis of leaf extract of *Phyllanthus fraternus*



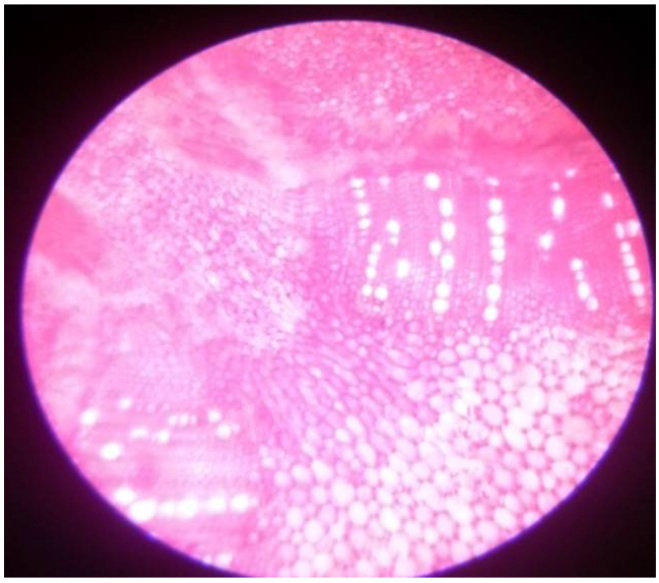
Phyllanthus myrtifolius



Phyllanthus myrtifolius

Phyllanthus reticulatus

Fig. 4. Anatomical section of root of *P. myrtifolius* and *P. reticulatus.*



Phyllanthus reticulatus

Fig. 5. Anatomical section of node of *P. myrtifolius* and *P. reticulatus* - Both showing unilacunar.

revealed that the chloroform extract contained tannins, saponins, terpenoids and steroids (21). Our studies revealed that the plants selected for the study contained almost all of these secondary metabolites in them. There were previous reports that the quantitative assay of three different *Phyllanthus* species revealed the fact that there was higher contents of tannins and saponins in the roots, flavonoids and phenols in the leaves and higher content of alkaloids in the stem compared with other parts of the plant (22).

Antibacterial activity

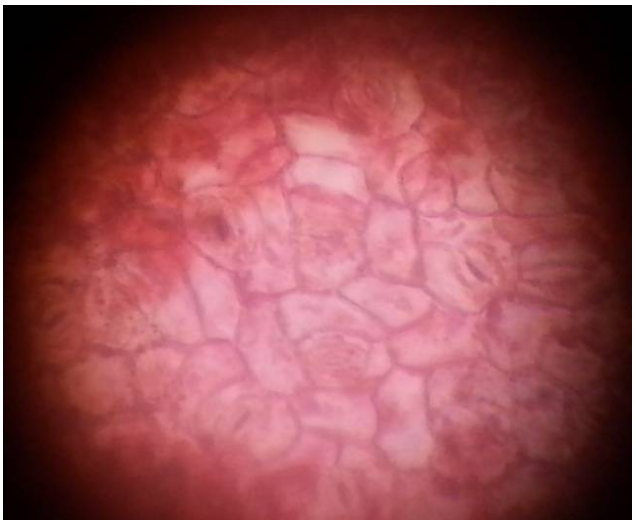
In our study, a comparative analysis of antibacterial activity of root, stem and leaf extracts in ethanol, acetone and distilled water of *P. myrtifolius* and *P. reticulatus* against 4 strains of bacteria (*Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus*) was done and the zone of inhibition was measured.

Antibacterial activity of *P. myrtifolius* was much higher when compared to *P. reticulatus* and another important fact was that the leaf extracts were having great inhibitory effect followed by root extracts against all the selected microbes. Stem extracts were exhibiting comparatively low rates of inhibition. Against *E. coli* bacteria, *P. myrtifolius* ethanolic leaf extract followed by acetonic leaf and stem extract showed high inhibition zone (15 mm, 12 mm and 7 mm respectively). Against *Pseudomonas*, *P. myrtifolius* exhibited high inhibition; both ethanolic and acetonic leaf extracts of *P. myrtifolius* showed an inhibition zone of 18 mm followed by acetonic root extract (16 mm) of the plant. Against *Proteus vulgaris* bacteria, both the ethanolic and acetonic leaf extract of *P. myrtifolius* showed an inhibition zone of 17 mm & 16 mm respectively followed by acetonic extract of root of the same plant (16 mm) whereas the stem extract showed an inhibition zone of 7 mm only. Against *S. aureus* bacteria, highest zone of inhibition

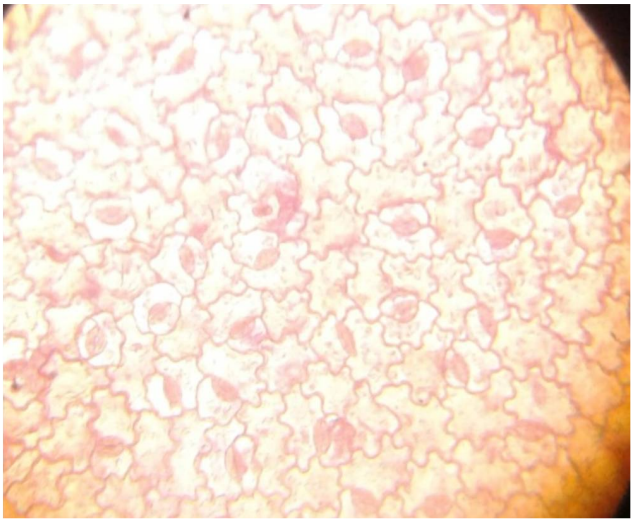
was shown by ethanolic leaf extract (20 mm) followed by acetonic leaf extract (12 mm) followed by distilled water extract of leaf of the same plant (11 mm) (Supplementary Tables 5, 6 & 7; Supplementary Figs. 4, 5 & 6). Previous studies proved that eighty percent methanol extracts obtained from seven *Phyllanthus* species were evaluated for antibacterial activity. Best antibacterial activity as indicated by MIC (Minimum Inhibitory Concentration) values was obtained by *Phyllanthus amarus* against *S. aureus* with a MIC value of 17.7 µg/ml; *P. myrtifolius* and *Phyllanthus urinaria* inhibited the growth of *Pseudomonas stutzeri* with MIC values of 7.8 µg/ml and 117 µg/ml (23). In our studies, *P. myrtifolius* exhibited most antibacterial effect against *S. aureus* (20 mm), *P. aeruginosa* (18 mm), *P. vulgaris* (17 mm) and *E. coli* bacteria (15 mm) and this could be considered as a confirmation of antibacterial potency of *P. myrtifolius*. The acetonic root extracts were also found to be effective against both *P. aeruginosa* and *P. vulgaris* bacteria (16 mm).

In our studies, the antibacterial properties of *P. reticulatus* revealed less inhibitory effects when compared to *P. myrtifolius*. The ethanolic and acetonic leaf extract of the plant could produce inhibition against *S. aureus* and *P. vulgaris* bacteria only (7 mm). The acetonic extracts of leaf, stem and root exhibited almost similar inhibition zones ranging between 4-5 mm. The root extracts in distilled water outcompete both stem and leaf extracts of the plant, against *P. aeruginosa* and *P. vulgaris* bacteria (10 mm and 13 mm) respectively while the stem and leaf extracts could produce inhibition zones of 8 mm and 7 mm against both *P. aeruginosa* and *P. vulgaris* bacteria (Supplementary Tables 8, 9 & 10; Supplementary Figs. 7, 8 & 9). Previous studies revealed that the ethanol, methanol and aqueous extracts of *P. reticulatus* were found to be bactericidal against *E. coli, P. aeruginosa, S. aureus* except *Klebsiella pneumoniae* species. The ethanolic and aqueous extracts showed minimum inhibitory properties when compared to methanolic extracts. Aqueous extracts had the least activity (24). But in our studies root extracts in distilled water has high antibacterial potency than both ethanolic and acetonic leaf and stem extracts of *P. reticulatus*. Here, the inhibition zone was 10 mm and 13 mm respectively against *P. aeruginosa* and *P. vulgaris* bacteria. Similarly in yet another study it was revealed that the methanol, chloroform and hexane extracts of *P. reticulatus* showed susceptibility against *S. aureus*, *E. coli*, *P. aeruginosa* and *Salmonella typhi* and found that MIC of 100-6.25 mg/ml and the zone of inhibition was 9.07 -30.18 mm, 8.17-24.57 mm and 5.60-14.67 mm for methanol, chloroform and hexane extracts respectively (25).

The antimicrobial efficiency of *P. amarus* was tested against eight human pathogens and found that the plant showed significant activity against all the pathogens tested but the alcoholic extract of *P. amarus* showed the maximum zone of inhibition and minimum inhibitory concentration against all the microbes. Among the solvents, methanol and ethanol extracts showed high degree of inhibition followed by petroleum ether and aqueous extracts (26). Our studies are in confirmation with the previous findings where, ethanolic and acetonic extracts produced good antibacterial results when compared with the distilled water extracts in case of *P. myrtifolius* while in case of *P. reticulatus* the distilled water extracts showed significant antibacterial properties especially the root extracts of *P. reticulatus.*



Phyllanthus myrtifolius – Paracytic stomata



Phyllanthus reticulatus – Anisocytic stomata

Fig. 6. Anatomical section of stomata of *P. myrtifolius* and *P. reticulatus*.

Conclusion

Our study revealed that flavonoids and phytosterols whenever present in the extracts produced good antibacterial results and that may be responsible for their better antibacterial effects as in case of both acetonic and ethanolic extracts of *P. myrtifolius* and also the distilled water extracts of *P. reticulatus*. However, the role of diterpenes, alkaloids and anthocyanins against bacteria could not be confirmed with this study, since some extracts in which diterpenes or alkaloids were not found exhibited good antibacterial properties. So in future, the quantitative estimation of the different phytoconstituents and a correlation between phytoconstituents and their

antibacterial potentialities could be studied in deep with respect to disease control. Further, the leaves of several *Phyllanthus* species which are medicinally important are often adulterated with some others due to close similarities with other groups of plants. So the unique characteristics of these plants could be identified which helped to demarcate and use in Ayurvedic preparations. A more elaborate study in this regard will definitely benefit mankind in combating several dreadful diseases.

Supplementary data

Table 1 | Table 2 | Table 3 | Table 4 | Table 5 | Table 6 | Table 7 | Table 8 | Table 9 | Table 10

Figure 1 | Figure 2 | Figure 3 | Figure 4 | Figure 5 | Figure 6 | Figure 7 | Figure 8 | Figure 9

Authors' contributions

NPC collected the specimens for study, conducted experiments and documented the data. JMJ designed and supported the experiments, supervised throughout the process and wrote the whole manuscript. TA critically analysed the work, provided technical help throughout the process.

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Conflict of Interest

The authors have no conflict of interest to declare.

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