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A comparative study of morpho-anatomical, fluorescent characteristics, phytochemical and antibacterial studies of two different *Phyllanthus* species of Kerala

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Abstract
Two species of *Phyllanthus* ie *P. amarus* and *P. urinaria* were selected for their Morpho-anatomical, Fluorescent characteristics, Phytochemical and Antibacterial studies. There were minute differences in their Morpho-anatomical characters and study of their powder extracts brings into light several distinguishing characters which enable us to differentiate between the two species. Fluorescent characteristics of the plant powders were studied, but it revealed the complete absence of fluorescence in the powder extracts. Phytochemical analysis revealed that both the plants possessed Alkaloids, Tannins, Flavonoids, Phenols and Terpenoids which have strong pharmacological properties such as antioxidant, anti-inflammatory, anticancer, antimicrobial attributes which can be utilized for medicinal purposes. *P. amarus*, the most popular plant against several dreadful diseases as well as the less popular *P. urinaria* possess several phytoconstituents that has the capability to resist gram positive as well as gram negative bacteria. At several instances, *P. urinaria* showed appreciable antibacterial results comparable to *P. amarus*.

Keywords: morpho-anatomical, phytoconstituents, phytochemical, antibacterial, zone of inhibition, fluorescence assay

Introduction
Since time immemorial, medicinal plants were used for the treatment of several dreadful diseases all over the world. As we all know, they are the store houses of different phytocompounds that are able to cure several abnormal conditions. *Phyllanthus* is a genus of Euphorbiaceae family which has over 6500 species in 300 genera [1]. The healing powers of *Phyllanthus* as claimed by local medicinal practitioners range from head ache, skin diseases to gonorrhea and syphilis [2, 3]. Besides *P. amarus* possess strong antioxidant, antitumor, anticancer and antibacterial properties [4]. In our study we considered two species of *Phyllanthus* that is *Phyllanthus amarus and Phyllanthus urinaria* and a comparative study of significant differences among them in their morphological, anatomical, fluorescent, phytochemical and medicinal attributes were carried out.
Phyllanthus amarus is a medicinally important species and is often adulterated with others due to their close similarities with other species. *Phyllanthus amarus* and *Phyllanthus urinaria* are erect herbs, in *P. urinaria* young stem and leaves are reddish in appearance. *Phyllanthus amarus* is a branching annual glabrous herb which is 30-60 cm high and have slender, leaf-bearing branch lets, distichous leaves which are sub sessile elliptic-oblong, obtuse with rounded base. Flowers are yellowish, whitish or greenish, males flowers in groups of 1-3 whereas females are solitary. Fruits are depressed-globose like smooth capsules present underneath the branches and seeds are trigonous, pale brown with longitudinal parallel ribs on the back [5]. *P. amarus* can be considered weed like since it is very common along the road sides and in high traffic areas. *P. urinaria* is less commonly available species whose leaves are large at the tip and smaller towards the petiole. Flowers are greenish white minute and appears at the axiles of the leaves. Fruits are greenish in colour with vegetable carving like outer rough surface appears to be several layered along the underside of the stem which are erect and red.

Materials and Methods
The materials for study were collected from Maharaja's college campus and nearby places. The plants were identified by using the flora, "Flowering plants of Kerala" by N. Sasidharan, Database KFRI Version 2 [6]. The plants taken for the study were *Phyllanthus amarus* and *Phyllanthus urinaria.*

Phyllanthus amarus Schum. & Thonn. *Phyllanthus urinaria* L.

Fig 1: Habit of *P. amarus* and *P. urinaria*

Methodology

1. 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. Minute characters were observed with hand lens or dissection microscope. Anatomy of stem, root, node and stomata were observed using compound microscope and photographs were taken.

2. Fluorescent properties

Fluorescence analysis of the plant powder was observed in day light and UV light (254nm) with the help of a UV cabinet.

3. Phytochemical analysis

The various steps involved in this study were:

1. Collection of materials: Healthy plant materials were collected, species were differentiated with respect to their morphological characters in consultation with flora and other literature available.
2. Drying procedure: The collected plant materials were washed in tap water and again in distilled water for removing dust particles. Then they were kept in shade for drying and grinded it. Then the powder is stored in airtight containers for future studies.
3. Preparation of extract from the plant and analysis of extracts : 5 gms of plant powder were weighed accurately and separately dissolved in 50 ml ethanol and acetone and distilled water and kept for 72 hours with constant stirring (10% concentration). After that it was filtered with filter paper and then kept in refrigerator and used for further studies. Ethanol, Acetone and distilled water extracts of *P. amarus* and *P. urinaria*, were used for phytochemical studies [7].

4. Antibacterial studies

The various steps involved in this study were

a) Preparation of culture medium: Weighed 28 gms of Nutrient agar and transferred into a beaker containing 1 litre distilled water. Gently heated the contents to dissolved the medium and covered the mouth of the beaker with aluminium foil. Petriplates and nutrient agar containing beaker placed into autoclaved and sterilized. Further operations were done in laminar air flow chamber. The sterilized agar medium were poured into petridishes and allowed to solidify at room temperature and kept in an incubator in inverted position for 24 hours.

b) Antimicrobial assay: Four strains of bacteria were used for the study. The bacterial srains selected for study were *E. coli*, *Pseudomonas*, *Proteus*, and *Staphylococcus aureus*. The inoculum were collected from Microbiology lab of Maharaja's College, Ernakulam for study.

Procedure

The experiment was done in an inoculation hood. The bacterial culture in nutrient broth was swabbed using buds over the solidified agar medium. Then by using cork borer prepared well in the medium. The medium was kept in the incubator for three hours. Stem, root, leaf extracts of *Phyllanthus* species were filled in the wells prepared. Positive control was ampicillin antibiotic (0.2 gm in 100ml distilled water) and negative control (the solvents in which the extract was prepared ie acetone, alcohol and distilled water) and kept in the incubator for 24 hours after inoculation. After the stipulated period, take out the petriplates and the zone of inhibition was observed and measurements were taken using scale.

Calculation of inhibition zone

Well diffusion assay method was used to investigate the antibacterial activity of plant extracts.

Results and Discussion

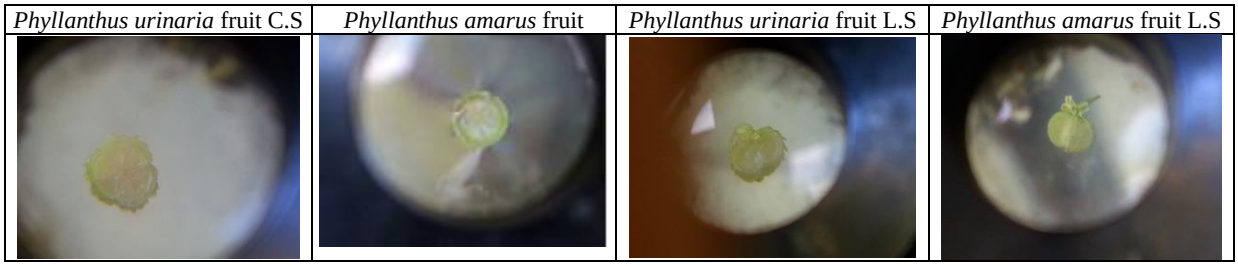
Morphological study: The morphology of two *Phyllanthus* species were examined, many minute differences were found. The morphological similarities and differences observed were presented in Table 1

Table 1: Details of vegetative and reproductive characters of *P. amarus* and *P. urinaria.*

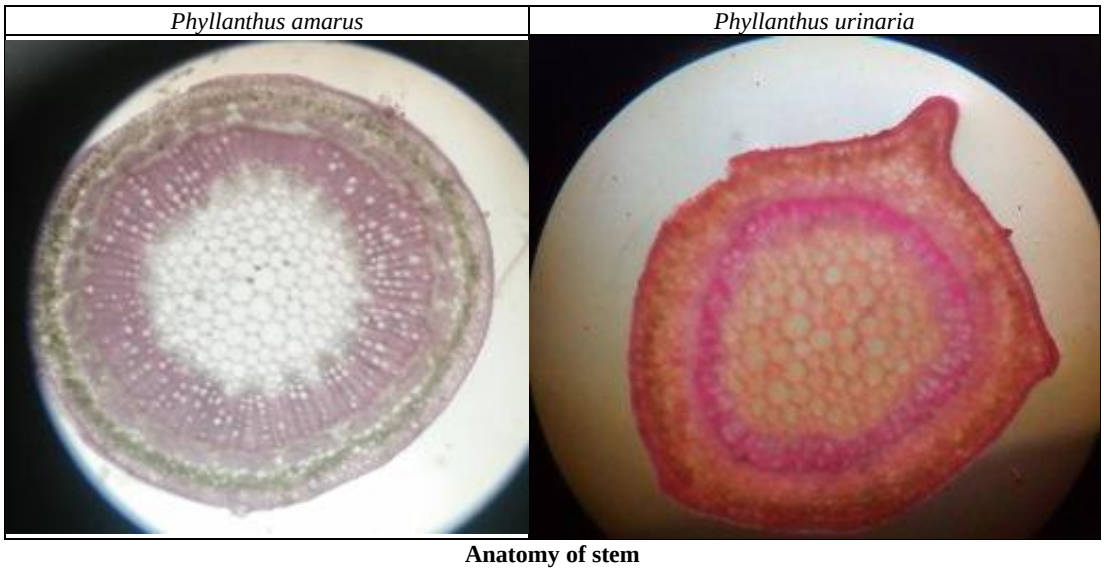
Character	*Phyllanthus amarus*	*Phyllanthus urinaria*
Habit	Herb	Herb
Plant height	Up to 65 cm	Up to 50 cm
Stem colour	Pale green to white	Light rose to pinkish
Leaf Type	Simple, Under touch it does not exhibit seismonasty.	Simple, exhibits seismonastic changes when touched.
Leaf let size	8mm long and 4mm broad. Midrib divides the leaflet into two equal l portions.	8mm long and 3mm broad. Midrib divides leaflet unequally.

Leaflet shape	Oblong	Oblong
Leaflet Tip	Rounded	Pointed
Leaf stipules	Lanceolate	Linear-- subulate
Leaflet Length	0. 8 cm	1.1 cm
Leaflet Width	0. 4 cm	0.5 cm
Petiole length	2 mm	1 mm
Phyllotaxy	Alternate	Alternate
Male flowers	Solitary	Axillary clusters
Stamen	Three, Filaments connate	Five, filaments united.
Female Flowers	Solitary, Tepals 5	Solitary, Tepals 6
Ovary	Globose	Globose
Fruit Type	Capsule	Capsule
Fruit surface	Smooth	Rough
Fruit colour	Green	Green
Fruit Surface	Smooth	Rough
No of Locules	6	6
Margin wall	Smooth	Wart like projections.
Placentation	Axile	Axile
Number of tepals	5	6

All leaflets are found in alternate phyllotaxy. All leaves are simple. The tip of leaflet of *P. amarus* is rounded while that of *P. urinaria* is with pointed end. Fruit is a capsule in both while the fruit surface of *Phyllanthus urinaria* is rough, wart like projections appeared on its surface, while that of *P. amarus* has smooth. surface. Axile placentation is observed in both species while there are 5 tepals in *P. amarus* and 6 tepals in *P urinaria*. Thus we can differentiate both the species easily with respect to these morphological characters.



Anatomical study



Anatomy of stem

Fig 5: Anatomical section of stem of *P. amarus* and *P. urinaria***.**

Character	***Phyllanthus amarus***	***Phyllanthus urinaria***
Outline of stem	Round	Angular projections at one or several positions.
Ridges and furrows	Absent	Present
Epidermis	Single layered	Single layered
Hypodermis	2-3 Layered collenchyma followed by 2-3 layered Chlorenchyma	2-3 layered chlorenchyma followed by parenchyma.
Cambium	Appears as a wavy band one to two layered.	Appears wavy margin, two-three layered
Secondary Vascular tissues	Phloem in patches and xylem continuous. Occupies one third of the section.	Phloem in patches; secondary xylem possess less wider vessels opposite the ridges and wider vessels in the other regions.

		Occupies one fifth of the section.
Pith	Large parenchymatous pith. Centre of the pith contains some crystal depositions at the corners of the cells.	Very large pith. Pith occupies very large portion. Only centre of the pith contains crystal depositions at the corners..

Anatomy of root

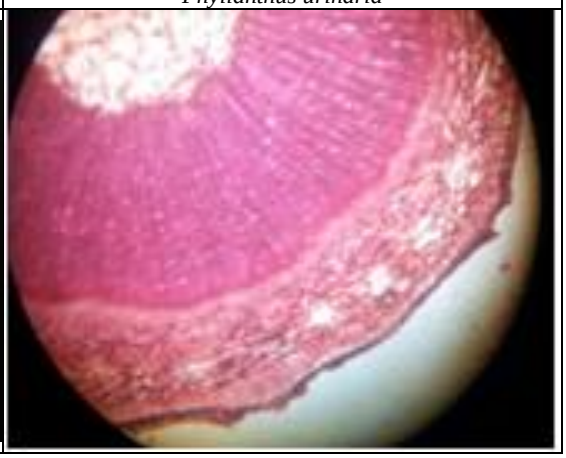
Phyllanthus amarus	*Phyllanthus urinaria*
	

Fig 6: Anatomical section of root of *P. amarus* and *P. urinaria*.

Root	***Phyllanthus amarus***	***Phyllanthus urinaria***
Cork	Wavy margin, well differentiated Periderm.	Wavy, well differentiated Periderm. Comparatively large corky region.
Vascular cambium	Appears as a wavy ring	Wavy ring, two-three layered
Secondary xylem and Phloem	Occupies major portion	Occupies one third portion
Rays	Numerous, Eccentrically placed.	Numerous, Not eccentrically placed.
Pith	Completely absent	Well demarcated pith, made up of parenchyma and occupies one third portion.

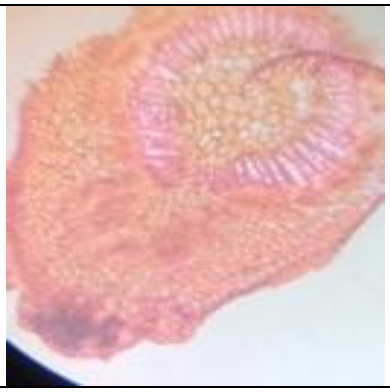
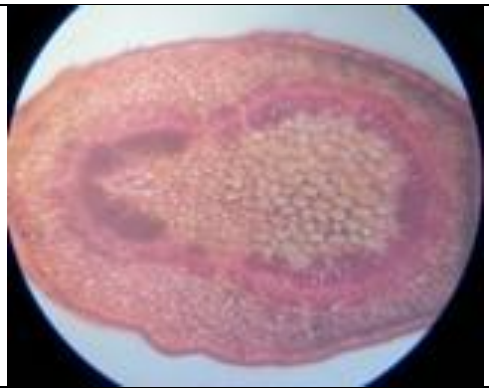
Nodal anatomy	
	
Phyllanthus amarus node	*Phyllanthus urinaria* node
Both showing unilacunar node	

Fig 7: Anatomical section of node of *P. amarus* and *P. urinaria*. Stomata – types

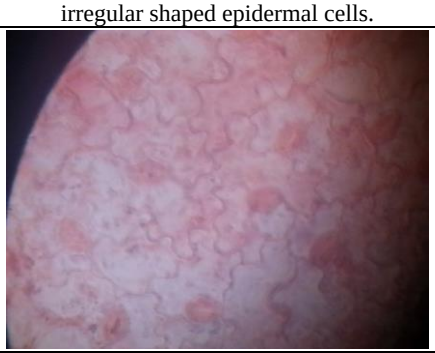
Phyllanthus amarus – anisocytic stomata with only **two** irregular shaped epidermal cells.	*Phyllanthus urinaria* – paracytic stomata with only **two** irregular shaped parallely arranged epidermal cells.
	

Fig 8: Anatomical section of stomata of *P. amarus* and *P. urinaria*

Both the species exhibit anatomical differences also. The stem of *P. amarus* is rounded in cross section while *P. urinaria* is having angular projections at several points around the stem. Ridges and furrows are present in *P. urinaria* while it is absent in *P amarus*. Pith of stem is large in *P. amarus* but comparatively largest pith is found in *P. urinaria*. Crystal like black depositions are found in the centre of pith at the corners of the cells in both *P. amarus* and *P. urinaria*.

Both the species exhibit well differentiated periderm with a wavy margin in their root section. In both plants, vascular cambium appears as a wavy ring, but pith is completely absent in *P. amarus* but a well differentiated pith is present in *P. urinaria*.

Formerly morphology and anatomy of *Phyllanthus* species were studied.[8] The morphology and anatomy of *Phyllanthus amarus* along with six other *Phyllanthus* species were studied and found that the stomata present in *P. amarus* is anisocytic while that of *P. urinaria* is paracytic, both having only two subsidiary cells only.[9] Former studies revealed that *Phyllanthus amarus* showing anisocytic stomata and *Phyllanthus urinaria* showing paracytic stomata and Phyllanthus *mullerianus* showing absence of stomata in the upper epidermis [10]. Our studies was in confirmation with the above findings.

Unilacunar node is observed in both the *Phyllanthus* species selected for studies.

2. Fluorescence analysis

When the powder of different species of *Phyllanthus* mixed with different reagents, the following colour changes occur in day light and no fluorescence was recorded when placed under UV light. The powder study can be used as an aid in differentiating the two species. Formerly fluorescence analysis of *Sida* was studied.[11]

Under UV light, all appeared black in colour ie it revealed the complete absence of fluorescence in any of the plant powder extracts.

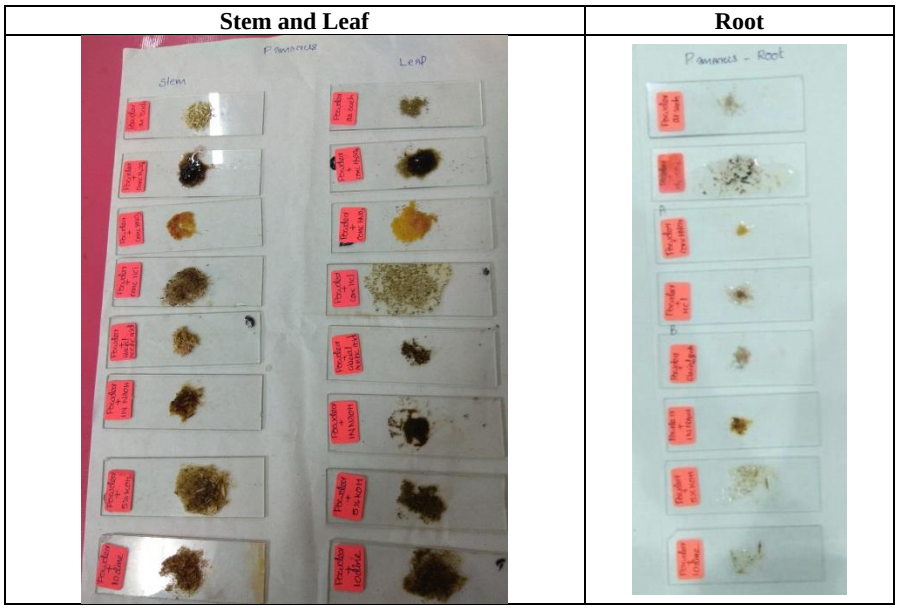


Fig 9: Powder of *P. amarus* leaf, stem and root treated with different chemicals, exhibiting variations in colour.

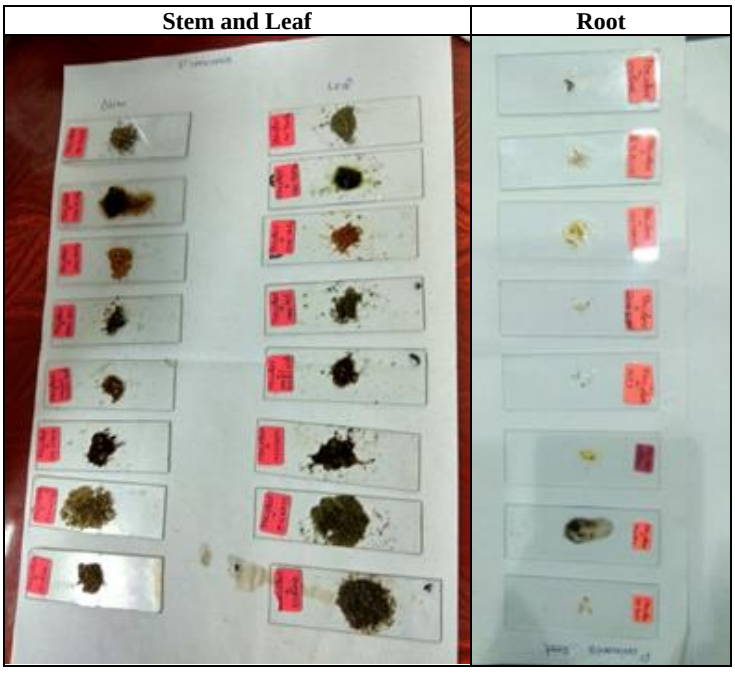


Fig 10: Powder of *P. urinaria* leaf, stem and root treated with different chemicals, exhibiting variations in colour.

Sl. No	*P. amarus*	Leaf (Day light)	Stem (Day light)	Root (Day light)
1	Powder as such	Dark green	Creamy green	Light green
2	Powder +conc HNO_3	Orange yellow	Orange yellow	Turmeric yellow
3	Powder + conc H_2SO_4	Brownish black	Black	Black
4	Powder + conc HCl	Pale olive green	Greenish black	Soil colour
5	Powder + Glacial acetic acid	Black	Creamy yellow	Light green
6	Powder + 1N NaOH	Brown	Yellowish red	Yellow brown
7	Powder + 5% KOH	Olive green	Olive green	Olive green
8	Powder + Iodine	Black Green	Olive green	Olive green

Sl. No	*P. urinaria*	Leaf (Day light)	Stem (Day Light)	Root (Day Light)
1	Powder as such	Greyish green	Greyish green	Creamy green
2	Powder +conc HNO_3	Yellow	Turmeric yellow	Light yellow
3	Powder + conc H_2SO_4	Dark green	Black	Black
4	Powder + conc HCl	Greyish green	Greenish black	Pale yellow
5	Powder + Glacial acetic acid	Black	Brown	Creamy green
6	Powder + 1N NaOH	Black	Deep black	Yellow
7	Powder + 5% KOH	Greyish green	Olive green	Creamy white
8	Powder + Iodine	Brownish green	Brown	Brownish black

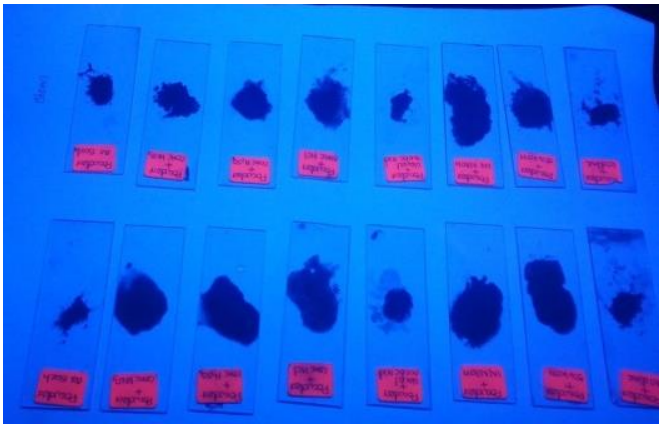


Fig 11: Powder of *P. amarus* & *P. urinaria* leaf, stem and root under UV Cabinet. No fluorescence was reported**.**

Phytochemical Analysis

Phytochemical analysis of the whole plant of both the species in different solvents (distilled water, ethanol and acetone,) revealed almost all the major Phytoconstituents.

Distilled water extracts of both species possess Flavonoids, Protein, Tannins, Phenol and Phytosterols while *P. urinaria* revealed the presence of Carbohydrates and Saponins in addition to it. The ethanol extract of *P. amarus* and *P. urinaria* revealed the presence of Flavanoids, Tannins, Phenols, and Phytosterols in common while *P. amarus* has Alkaloids and Carbohydrates and *P urinaria* has Saponins in addition to it. The acetone extract of *P. amarus* and *P. urinaria* revealed the presence of Alkaloids, Carbohydrates, Tannins and Phenol in common while *P. amarus* has Flavonoids, Proteins, Diterpenes and Phytosterols in addition to it.

From our study, Phytochemical analysis of *P. amarus* revealed the presence of almost all phytochemicals like Alkaloids, Flavonoids, Tannins, Phenol, Phytosterols, Carbohydrates, Proteins and Diterpenes, only Anthocayanin and Saponins were completely absent and more phytochemicals were obtained in acetone extraction. *Phyllanthus amarus* have numerous phytocompounds alkaloids, flavanoids, tannin, lignin, polyphenolic compounds and tetracyclic compounds.[12] Studies of aqueous extract of leaves and roots of *Phyllanthus amarus* showed the presence of alkaloids, phytosterols, phenolic compounds, tannins, proteins, carbohydrates, and aminoacids.[13] Phytochemical analysis of ethanolic leaf extracts of *Phyllanthus amarus* revealed the presence of alkaloids, cyanogenic glycosides, saponins, tannins and oxalates.[14] Our study recognised calcium oxalate like crystals in the transverse section of stem of *P. amarus* and *P. urinaria.*

From our study, Phytochemical analysis of whole plant of *Phyllanthus urinaria* revealed the presence of Alkaloids, Flavonoids, Tannins, Phenol, Phytosterols, Carbohydrates, Protein and Saponins. Previous phytochemical analysis showed that alkaloids, tannins, flavonoids, and saponins were present in the leaves, stem and roots of *P. urinaria*. [15] Anthocaynin and Diterpenes were completely absent in all solvents. From our studies comparatively aqueous extracts showed almost all phytochemicals than both ethanol and acetone extracts. Studies on *P. urinaria* revealed that aqueous extracts has more phytochemicals than the chloroform extract [16].

Our study revealed the presence of all major phytoconstituents like alkaloids, flavonoids, phenols, tannins, saponins and phytosterols which have profound pharmacological properties such as antioxidant, anti-inflammatory, anticancer, antimicrobial, antiviral properties present in both the species thus indicating potential me dicinal uses of these plants.

Antibacterial Assay

In this study, analysis of antibacterial activity of *P. amarus and P. urinaria,* against 4 strains *of* bacteria *(E. coli, Pseudomonas, Proteus vulgaris, Staphylococcus aureus)* was done. *S. aureus* is gram positive and all others are gram negative. Antibacterial activity of root, stem, and leaf of the above plants in ethanol, acetone and distilled water extracts was measured separately.

a) Antibacterial activity of ethanol extract of *P. amarus*

From the table given below, it can be inferred that against *E. coli*, both leaf and stem obtained an inhibition zone (7mm & 8mm) which is greater than the negative control (6mm). Against *Pseudomonas*, leaf, stem and root obtained inhibition zone of (9mm, 8mm, 7mm) which is greater than the negative control (6mm). Against *Proteus bacteria*, the plant extract obtained comparatively high results. Leaf and stem obtained an inhibition zone of 12 mm and root showed 10 mm where the inhibition zone is much higher than the negative control which was 6mm only. Against *S. aureus*, leaf, stem and root

obtained (7mm, 10mm and 9mm) higher than the negative control which evidently brings out the anti-bactericidal properties of the extracts.

Si. No	Microorganisms	Zone Of Inhibition (mm)				
		Leaf (L)	Stem (S)	Root (R)	Positive control (+ve)	Negative control (-ve)
1	*E. coli*	7	8	6	25	6
2	*Pseudomonas*	9	8	7	25	6
3	*Proteus vulgaris*	12	12	10	25	6
4	*S. aureus*	7	10	9	30	6

b) Antibacterial activity of acetone extract of *P. amarus*

The acetone extract of *P. amarus* leaf and root showed no inhibition against *E. coli*, while stem obtained 13mm inhibition zone much higher than that of negative control (7mm). Acetone extract of *P. amarus* against *Pseudomonas bacteria*, leaf, stem and root showed similar zone of inhibition of 15mm which is half the value of positive control (30mm). Against *Proteus*, leaf obtained higher value of inhibition (20mm) followed by stem (15mm) and root (12mm) while the positive control obtained 30mm zone of inhibition which can be considered as an effective result. Against *S. aureus*, too, leaf, root and stem obtained inhibition zones (13mm, 10mm, 8mm) higher than the negative control (7mm).

Microorganisms	Zone of Inhibition(mm)				
	Leaf (L)	Stem(S)	Root(R)	Positive control (+ve)	Negative control (-ve)
E. coli	0	13	0	30	7
Pseudomonas	15	15	15	30	10
Proteus vulgaris	20	15	12	30	11
S. aureus	13	10	8	30	7

c) **Antibacterial activity of distilled water extract of *Phyllanthus amarus***

Against *E. coli*, stem extract *of P. amarus* obtained (14mm) which in fact is much higher than both positive and negative control. Against *Pseudomonas*, leaf and root showed a clear zone of 10mm in diameter and stem obtained 12 mm inhibition zone while the Positive and negative control obtained a clear zone of 5mm and 8mm respectively which indicates effectiveness of the extract. Against *Proteus*, stem obtained 12mm inhibition zone while the Positive and negative control was 7mm and 8mm respectively. Against *S. aureus* stem extract was very effective ie 19mm which could be considered very high than even the positive control (9mm). Aqueous extracts of leaves and roots of *P. amarus* exhibited significant antibacterial activity against eight test bacteria like *Staphylococcus albus, Streptococcus faecals, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae and Proteus vulgaris.*[17] The extract of *P. amarus* showed significant antibacterial activity particularly against gram negative microbes. [18]

SI. NO	Microorganisms	Zone Of Inhibition(mm)				
		leaf	stem	root	Positive control	Negative control
1	*E. coli*	4	14	6	6	6
2	*Pseudomonas*	10	12	10	5	8
3	*Proteus vulgaris*	6	12	6	7	8
4	*Staphylococcus aureus*	10	19	11	9	15

d). **Antibacterial activity of ethanol extract of *Phyllanthus urinaria***

Against *E. coli*, *P. urinaria* stem obtained 19mm while the positive control obtained 27 mm zone of inhibition and negative control obtained 6mm inhibition zone and the result is commendable. Against *Pseudomonas*, the leaf obtained 15mm inhibition zone whereas the positive control obtained a clear zone of 25mm in diameter and there was no inhibition in the negative control. Against *Proteus*, leaf as well as the positive control (antibiotic solution) obtained 13mm zone of inhibition and that indicates the potential of leaves against microbes. Against *Staphylococcus aureus*, stem, root and leaf obtained inhibition zones (10mm, 7mm, 7mm) while the negative control failed to produce any results.

Si. No	Microorganisms	Zone Of Inhibition (mm)				
		leaf	stem	root	Positive control	Negative control
1	*E. coli*	8	19	7	27	6
2	*Pseudomonas*	15	8	7	25	0
3	*Proteus vulgaris*	13	11	8	13	6
4	*S. aureus*	7	10	7	27	0

e). Antibacterial activity of acetone extract of *Phyllanthus urinaria*

Against *E. coli*, leaf and root obtained 17mm inhibition zone diameter, stem (15mm) which indeed is lower than positive control (30mm) but higher than negative control (7mm) Against *Pseudomonas*, leaf (12mm), stem(8mm), both of which was higher than negative control (6mm), but leser than that of positive control. Against *Proteus* bacteria, leaf and stem obtained inhibition zone of 15mm, where the positive control obtained is 20mm and inhibition in the negative control. Against *Staphylococcus aureus*, leaf obtained a clear zone of (10mm), which in fact is higher than the negative control (6mm) but lower than that of positive control (20mm).

SI. NO	Microorganisms	Zone Of Inhibition (mm)				
		Leaf(L)	Stem(S)	Root(R)	Positive control(+ve)	Negative control(-ve)
1	*E. coli*	17	15	17	30	7
2	*Pseudomonas*	12	8	6	20	6
3	*Proteus vulgaris*	15	15	6	20	0
4	*S. aureus*	10	0	6	20	6

f). Antibacterial activity of distilled water extract of *Phyllanthus urinaria*

Against *E. coli*, leaf, stem and root obtained inhibition zone (10mm, 7mm, 5mm) which was greater than the positive control (4mm). Against *Pseudomonas*, root obtained an inhibition zone (7mm) which was equal to both positive and negative control. Against *Proteus*, both stem and root obtained inhibition zone equal to that of positive control (6mm). Against *Staphylococcus aureus*, root obtained 15 mm followed by stem (10mm) which is greater than both positive and negative control (7mm).

Si. No	Microorganisms	Zone of inhibition (mm)				
		Leaf	stem	root	Positive control	Negative control
1	*E. coli*	10	7	5	4	7
2	*Pseudomonas*	6	6	7	7	7
3	*Proteus vulgaris*	5	6	6	6	7
4	*S. aureus*	7	10	15	7	7

Maximum zone of inhibition observed in ethanol extracts followed by acetone, then distilled water. Previous studies revealed that the methanol, ethanol, petroleum ether, and aqueous extracts of *Phyllanthus amarus* were potent antimicrobials against all the microorganisms studied (*Bacillus cereus, Staphylococcus aureus, E. coli, Pseudomonas aeruginosa*.) among the solvents methanol and ethanol extracts showed high degree of inhibition, followed by petroleum ether and aqueous extracts [19]. Studies on methanol, ethanol and distilled water extracts of *P. amarus* indicated comparative high antimicrobial effects by methanol than the other two solvents [20].

Leaf and stem extracts have comparatively higher inhibition zone than root. When ethanolic extracts of both the plants were considered, against *E, coli* bacteria, highest inhibition was shown by ethanol extract of *Phyllanthus urinaria* stem -- 19mm. Here the positive control was 27mm and negative control was 6mm. Against *Pseudomonas* bacteria, leaves of *P. urinaria* showed high inhibition (15mm) where the positive control was 25mm and negative control was 0mm. Against *Proteus* bacteria, leaves of *P. urinaria* showed the highest inhibition (13mm) followed by leaves and stem of *P. amarus* (12mm) and the positive control was 25mm and negative control was 6mm. Against *S. aureus*, stems of both *P. amarus* and *P. urinaria* showed an highest inhibition zone of 10mm, where the positive control was 27mm and negative control was 0.

P. myrtifolius and *P urinaria* may indicate the presence of promising antibacterial agents that need to be further investigated [21, 22]. This study is in confirmation with our studies that *P. urinaria* possess strong antimicrobial agents.

In case of Acetone extracts, against *E. coli*, leaves and roots of *P. urinaria* showed the highest inhibition (17mm) followed by stem of *P. urinaria* (15mm), and the positive control was 30mm and negative control was 7mm. Against *Pseudomonas* bacteria, Leaves, stem and roots of *P. amarus* showed the highest zone of inhibition (15mm) where the positive control was 30mm and negative control was 10mm. Against *Proteus* bacteria, leaf extract of *P. amarus* showed the highest zone of inhibition (20mm) where the positive control was 30mm and negative control was 11mm. Against *S. aureus*, leaves of *P. amarus* showed the highest inhibition (13mm), where the positive control was 30mm and negative control was 7mm.

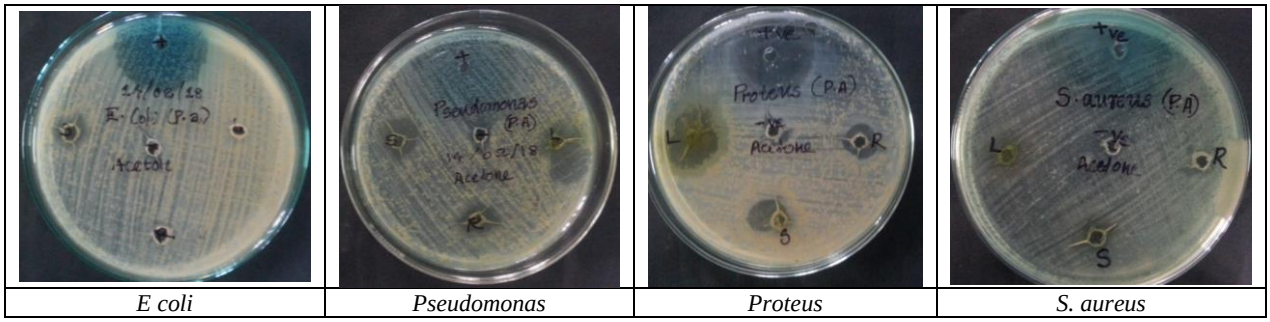
When Distilled water extracts of both the plants were considered, stem of *P. amarus* showed highest inhibition against *E. coli* bacteria (14mm) where the positive control and negative control was 6mm only. Against *Pseudomonas* bacteria also, the stem of *P. amarus* took the upper hand (12mm). Here also, the positive control was very less (5mm) which can be considerably appreciable. The same was the case against *Proteus* bacteria where the inhibition zone was 12mm and the positive control was 7mm and negative control was 8mm. Similarly, stem of *P. amarus* was found to be effective against *S. aureus* bacteria where inhibition zone was 19mm and the positive control was 9mm.

In conclusion it can be inferred that the Ethanolic extract of *P. urinaria* stem (19mm) showed highest action against *E. coli* bacteria followed by Acetonic extracts of *P. urinaria* leaf and root (17mm). Acetonic extract of *P. amarus* leaf, root and stem and Ethanolic extract of *P. urinaria* leaf showed 15mm inhibition zone against *Pseudomonas* bacteria. Acetonic extracts of *P. amarus* leaf showed high inhibition against *Proteus* bacteria (20mm) followed by stem of *P. amarus*(15mm) and *P. urinaria* leaf and stem (15mm). Against *S. aureus* bacteria, distilled water extracts of *P. amarus* stem showed high inhibition (19mm) followed by distilled water extract of root *of P. urinaria* (15mm).

***Phyllanthus amarus* in Ethanol extract**

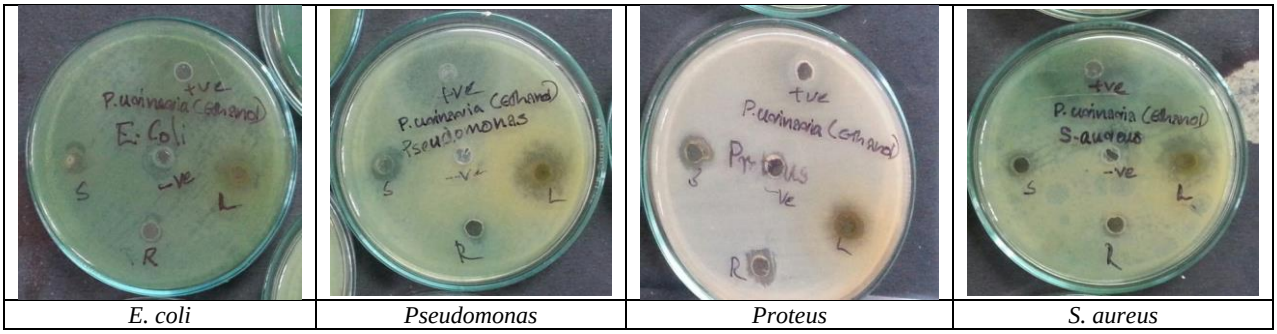
E. coli	*Pseudomonas*	*Proteus*	*S. aureus*

Fig 10: Antibacterial effects of ethanol extract of *Phyllanthus amarus* against 4 selected strains of bacteria. (S= Stem, R=Root, L=Leaf, += Positive control, -=Negative control). *Phyllanthus amarus* in Acetone



E coli	*Pseudomonas*	*Proteus*	*S. aureus*

Fig 11: Antibacterial effect of Acetone extract of *Phyllanthus amarus* against 4 selected strains of bacteria. (S= Stem, R=Root, L=Leaf, "+"= Positive control'-' =Negative control). *Phyllanthus urinaria* in Ethanol Extract.

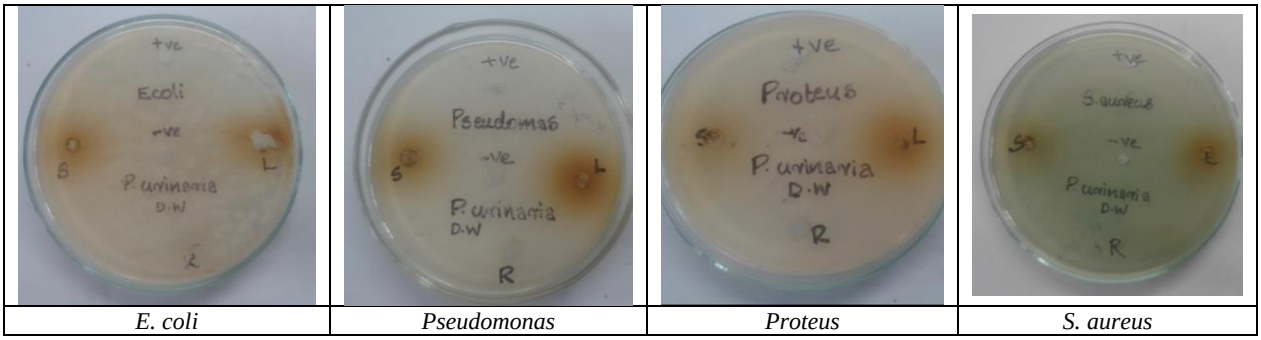


E. coli	*Pseudomonas*	*Proteus*	*S. aureus*

Fig 12: *Antibacterial effect of ethanol extract of Phyllanthus urinaria* against 4 selected strains of bacteria. (S= Stem, R=Root, L=Leaf, "+"= Positive control'-' =Negative control). *Phyllanthus urinaria* in Acetone extract.

E coli	*Pseudomonas*	*Proteus*	*S aureus*

Fig 13: Antibacterial effect of acetone extract of *Phyllanthus urinaria* against 4 selected strains of bacteria. (S= Stem, R=Root, L=Leaf, "+"= Positive control "-" =Negative control). *Phyllanthus urinaria* In Distilled water



E. coli	*Pseudomonas*	*Proteus*	*S. aureus*

Fig 14: Antibacterial effect of Distilled water extract of *Phyllanthus urinaria* against 4 selected strains of bacteria. (S= Stem, R=Root, L=Leaf, "+"= Positive control'-' =Negative control).

Si. No	Bacteria Used	Zone of Inhibition(mm)				
		Leaf	stem	root	Positive control	Negative control
1	*E. coli*	10	7	5	4	7
2	*Pseudomonas*	6	6	7	7	7
3	*Proteus vulgaris*	5	6	6	6	7
4	*S. aureus*	7	10	15	7	7

Conclusion

The two species of *Phyllanthus* – *P. amarus* and *P. urinaria, under* close observation of Morphological, Anatomical and Fluorescent studies could bring into light several minute differences which helps to differentiate the two species. Considerable differences were there in their Phytochemical and Antibacterial properties too. Many *Phyllanthus* species were often adulterated with others due to their close similarities in external appearance. Another significant factor was that the comparative study could bring into light the fact that the less valuable species of *Phyllanthus* like *P. urinaria* possess much more or higher antibacterial properties when compared to the traditional plant *P. amarus*. More scientific study of these plants hopefully could bring out many medicines/ drugs against many dreadful diseases

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