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PHYTOCHEMICAL AND ANTI MICROBIAL EVALUATION OF METHANOLIC AND ETHANOLIC EXTRACTS OF PHYLLANTHUS AMARUS.

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ABSTRACT

This study aimed to assess the antibacterial efficacy of methanolic and ethanolic extracts of Phyllanthus amarus. The leaves were meticulously cleaned in clean water, rinsed with sterile distilled water, and permitted to dry at room temperature for several days. It was oven-dried at 45°C for approximately one hour until deemed sufficiently brittle to fracture. The final dilutions employed were 40 mg/ml, 60 mg/ml, and 80 mg/ml. Four clinical microorganisms were isolated and identified as Klebsiella pneumoniae, Bacillus subtilis, Staphylococcus aureus, and Escherichia coli. The agar well diffusion technique was employed for the in vitro antibacterial bioassay. The methanolic and ethanolic plant extracts shown significant antibacterial activity at a concentration of 80 mg/ml. Phytochemical screening assays indicated the presence of saponins, alkaloids, and Salkowski in the plant extract. The results demonstrated that all extracts exhibited enhanced antibacterial activity with a progressive rise in their concentrations. The methanolic extracts of Phyllanthus amarus exhibited the greatest efficacy against all tested species. The ethanol extracts exhibited the lowest antibacterial activity in comparison to the methanolic extracts.

Key words : Methanol extract, Ethanol extract, phytochemical, antimicrobial, Phyllanthus amarus.

INTRODUCTION

Plants with medicinal properties remain a significant therapeutic resource for treating human ailments. Nature has bestowed upon us an abundant botanical diversity, with several varieties of plants flourishing in various regions of the country. Since ancient times, various components of numerous medicinal plants have been utilised in India to treat certain illnesses. According to the World Health Organisation (WHO), over 80% of the global population relies on natural products for their health due to their minimal side effects and cost-effectiveness. Phyllanthus amarus Plays a significant function in human health. This medicinal herb treats dengue fever, infertility, haemorrhoids, jaundice, and urinary tract inflammation. In Southeast Asia, young shoots and leaves are used as vegetables. Despite the longstanding role of green plants in traditional medicine for preventive and therapeutic purposes, targeted research is ongoing to explore the antibacterial properties of underutilised plants, aiming to employ them in human applications to mitigate bacterial infections. The genus Phyllanthus is a significant category of plants utilised as raw herbal medicine in India (Veddk et al., 2008). The genus Phyllanthus, belonging to the family Euphorbiaceae, comprises over 1000 species distributed across tropical and subtropical continents such as America, Africa, Australia, and Asia. Phyllanthus amarus is extensively prevalent as a weed in agricultural and barren lands in India (Patel et al., 2008). All three primary growth forms, namely trees, shrubs, and herbs, are observed within the Phyllanthus species. Phyllanthus amarus Schum. and Thonn. has a longstanding history of utilisation due to its significant therapeutic properties. Commonly referred to as Bhumi amla, it is part of a vast family of erect or prostrate herbs or shrubs, frequently characterised by a milky, bitter sap. In Unani literature, it is referred to as 'Bhuti,' signifying

BhumAmlak - Amla of Land. It plays a crucial part in the advancement of green medications, which are safer and more reliable than expensive synthetic treatments that have no harmful effects. P. amarus is referred to in Ayurveda by the Sanskrit names Bhoomy-aamlakee, Taamalakee, and Bhoodha tree. The applications of P. amarus are increasing because to its unique antiviral properties against the hepatitis B virus and various other biological activities, including the treatment of kidney and gallbladder stones, colds, influenza, tuberculosis, and liver illnesses (Unander et al., 1993).

Vernacular Names

The plant is recognised by many vernacular names among local populations in different regions (Table 1). It is commonly referred to as carry me seed, stone breaker, wind breaker, leaf flower, or storm of wind. Phyllanthus denotes leaf and flower due to its morphology, wherein the flower, fruit, and leaf appear to be combined.

S.no.	Language	Vernacular Names
1	Hindi	Bhumiamla,Jangliamli
2	English	Blackcatnip, carrymeseed, Childpick-a-back, Galeofwind, Gulfleafflower, Hurricaneweed, Shatter stone, Stonebreaker
3	Tamil	Keelanelli (Keezhanelli)
4	Bengali	Bhuiamla
5	Rajasthani	Gugario
6	Oriya	Bhuiaola

Botanical Characteristics

Branching annual glabrous herb Phyllanthus amarus is 30–60 cm high and bears thin leaf-bearing branchlets, distichous subsessile leaves with elliptic-oblong, obtuse, rounded bases (Fig. 1). Axillary flowers are yellow, pale, or green. Female blooms are solitary, while males are 1–3. Fruits are depressed-globose smooth capsules under the branches, and seeds are trigonous, pale brown, and longitudinally parallelribbed. Stalk capsules are 1–2 mm length, spherical, smooth, 2 mm broad, six seeds. Explosive seed capsules launch seeds away from the plant. Triangular, light brown, 1 mm-long seeds have 5–6 ribs on the back (Wessels et al., 1976).

Fig 1: Phyllanthusamarus Plant.

MATERIALS AND METHODS

Collection of plant sample: Fresh leaves and bark of Phyllanthus amaruswere collected from Acharya Nagarjuna University.

Preparation of plant extracts

Fresh plants were rinsed 4-5 times with tap water and then with sterile water and dried in shade at room temperature for 20-25 days. Crushed and sieved dried plant material was utilized for crude extraction. Extracting solvents included ethanol and methanol. All mixes were filtered with Whattman filter sheets and collected in sterile crucibles. Filtrates were placed in sterile reaction tubes and heated in water bath. The residues were stored at room temp.

Test organisms

Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, and bacillus subtilis were tested. They were isolated on sterile nutrient agar slants and transported to microbiology. Before extract bioassay, all test organism slants were held at 40C. All test bacterial strains were confirmed by extensive biochemical assays.

Antimicrobial activity of plants' extracts

Phyllanthus amarus methanol and ethanol extracts were tested for antibacterial activity using agar well diffusion. Muller Hinton agar media was put onto petri plates using a pour plate technique, incubated with a 24-hour bacterial culture swab, then smeared evenly with a sterile cotton swab. Agar medium wells were produced with a sterile corkborer (10mm). All plates were incubated at 37°C for 24 hours with extracts in the wells. Using mm-sized zones of inhibition, organism sensitivity was assessed.

Phytochemical screening method

Alkaloids, glycosides, tannins, saponins, anthraquinones, anthocyanins, flavonoids, reducing

sugars, and cyanogenic substances were qualitatively analysed. Additionally, mineral content was quantified.

RESULTS AND DISCUSSION

Methanolic and ethanol plant extracts were more efficient against pathogenic microorganisms Bacillussubtilis, Staphylococcus aureus, E.coli, and Klebsiella pneumoniae. (Fig 2). Phyllanthus amarus methanolic extracts were more antibacterial than ethanolic extracts. This suggests that they are prospective antibacterial agents, supporting Takazawaet al. (2007)'s recommendation to use a variety of solvents to extract phytochemicals from medicinal plants. His analysis suggests investigating these plants for active compounds that could be used in chemotherapy as commercial medicines with antibacterial properties are rare. Minerals are abundant in the vegetation. (Table 2).

Table 2: Qualitative Phytochemical screening of the medicinal plant samples

Plant	Lignans	Tannins	VolatileOils	Alkaloid	Saponin	steroid	flavonoids
Methanol	++	++	++	+	++	++	+ +
Ethanol	++	+	++	+	++	+	++

+ = present

The test plants we utilized in our investigation had no lead. It eliminates any risk of using these plants medicinally. Finally, our investigation confirms Phyllanthusamarus' antibacterial properties. This improves their infection-treatment potential. Further studies will examine how these plants' antibacterial properties vary with their environmental settings.

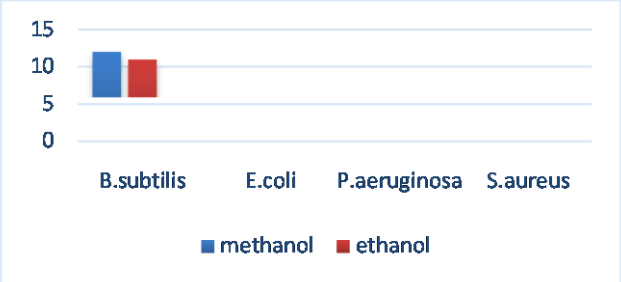

Fig 2:Antimicrobial activity of methanol, ethanol extracts ofP. amarus

CONCLUSION

The methanolic extracts of P. amarus showed stronger antibacterial activity against E. coli and S. aureus than the ethanol extracts. P. amarus' antibacterial action is mostly attributable to phyllantin (Jagtap, et al., 2009). Both P. amarus and P. niruri extracts inhibited four of the five E. coli isolates. A decoction or infusion of any herb may help treat UTI. The crude extract and fractions were tested for antibacterial and antifungal activity against Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, and Candida albicans (Itoro et al., 2008). Dichloromethane has activity against all test species with MIC of 100 µg/ml, while hexane, ethyl acetate, and aqueous methanol have no activity. The fraction may inhibit Candida albicans. This matches Foo and Wong's 1992 report. Due to its powerful pharmacological applications for jaundice, diabetes, syphilis, diarrhoea, fever, gonorrhoea, and more, P. amarus has attracted researchers for decades. Every culture has its unique traditional use of P. amarus, although disease treatment is almost universal. Antiviral, anti-diabetic, anti-hepatoxic, antibacterial, antifungal, and anti-inflammatory properties are found in plant extracts. This can help find new disease treatments. Further research is needed because this plant thrives in tropical and subtropical regions including India, Nigeria, and Malaysia. This review collected past studies to give a foundation for future work.

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