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Research Article

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Comparative Study on the Phytochemical Constituents and Antimicrobial Assessment of Water and Methanol Leave Extracts of *Phyllanthus amarus* and *Phyllanthus urinaria* on Some Microbial Pathogens

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ABSTRACT

Phytochemical analysis of Phyllanthus urinaria and Phyallanthus amarus were studied. The results revealed the presence of flavonoid, tannin, alkaloid, terpenoid, steroid, resin, glycoside, saponin and phenolic nucleus at different intensities. All the phytochemical parameters tested were present in methanol extract of P. amarus. Glycoside and tannin were absent in water extract of P. urinaria, water extract of P. amarus and methanol extract of P. urinaria while resin was absent only in the water extract of P. urinaria. The methanol and hot water extracts of these plant leaves were used to assess the antimicrobial effect on Staphylococcus aureus, Bacillus cereus, Candida albicans and Aspergillus niger. The antimicrobial activity of ethanol extract of Phyllanthus urinaria against Staphylococcus aureus had a highest value of 1.71 ± 0.283, while that of ethanol extract of Phyllanthus amarus against Aspergillus niger had the highest value of 1.47 ± 0.012. The Minimum Inhibitory Concentration (MIC) of Ethanol and hot water extracts of P. urinaria on Staphylococcus aureus and Bacillus aureus were (19.00, 15.00) and (17.20, 14.00) while P. amarus were (21.50, 18.00) mg/ml and (20.50, 17.80) mg/ml. The MIC of ethanol and hot water extract of P. urinaria on Candida albicans and Aspergillus niger were (11.00, 10.00) mg/ml and (9.60, 9.50) mg/ml while on P. amarus (9.40, 9.10) mg/ml and (7.10, 7.00) mg/ml.

Keywords: *Phyllanthus urinaria* extract; minimum inhibitory concentration; phytochemical

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INTRODUCTION

Phyllanthus is one of the largest genera in the family *Euphorbiaceae* of the flowering plants [1]. Phyllanthus species are widely distributed in all tropical and sub-tropical regions of the earth [2]. Phyllanthus species are used in folk remedies in the treatment of liver, kidney and bladder problems, diabetes and intestinal parasites [3]. Other common

names phyllanthus includes gripe weed, stonebreaker, and leaf flower among others [3]. The medicinal values of these plants lie in some chemical substances that produce a definite physiological action on the human body. The most important of these substances are alkaloids, glycosides, tannins, flavonoids, saponins, phenols, oils and many others [4]. Some drugs have been obtained from natural sources while some may be prepared by the modification of the natural ones hence; there is need for the local herbs to be evaluated for phytochemicals so as to determine the potential of indigenous sources of medicines [5]. Intra-specific variation in phytoconstituents has been documented extensively among the plants [6]. These medicinal plants have been underutilized in orthodox medicine but have confirmed to be used worldwide in the food and pharmaceutical industries. Alkaloids are very important in medicine and constitute most of the valuable drugs. They have marked physiological effect on animals and show considerable pharmaceutical activity. They act by prolonging actions of several hormones which require phosphodiesterase [7]. Tannins are useful in medicine because of their astringent properties. Tannins and Alkaloids are known to have anti-herbivore defense functions in plants and medicinal plants could be serving as a deterrent to grazers [8]. Herbs that contain tannins are recommended for a wide range of treatments including inflammation, liver injury, kidney problems, arteriosclerosis, hypertension, stomach problems and inhibition of active oxygen and are commonly recommended as diuretics and anti-diarrheas. Saponins are glycosides widely occurring in a variety of plants and are characterized by their bitter taste and foaming in aqueous solution. They prevent disease invasion of plants by parasitic fungi [9]. Flavonoids are the commonest phenolic constituents having 15-compounds generally distributed throughout the plants kingdom and the presence of flavonoids in plants have shown some effects like antibacterial, antiviral, antitoxin, antioxidant and anti-inflammatory anti-carcinogenic activities [5]. Phenolic compounds are known to have anti-fungal and anti-microbial effects. This work aims at analyzing the phytochemical constituents of phyllanthus amarus and phyllanthus urinaria extracted with cold water and ethanol extract and testing their antimicrobial activities against selected microbial pathogens.

MATERIALS AND METHODS

Preparation of Plant Extracts

Fifty grams (50 g) of the dried, blended samples of *Phyllanthus amarus* and *Phyllanthus urinaria* were dispensed into 150 ml of ethanol and sterile distilled water (hot) and added separately to each of the 500 ml conical flask. The mixture was covered with a cork, properly homogenized and allowed to stand for 24 hrs before it was filtered with Whatmann no. 1 filter paper. The filtrates were concentrated using a rotary evaporator and stored at 4°C for further use.

Sterility of Plant Extracts

Each of the concentrated plant extract was tested for microbial growth by plating them on Nutrient agar and incubated at 37°C for 24 h [10].

Test Organism Used

Four isolates namely *Staphylococcus aureus, Bacillus cereus, Candida albicans* and *Aspergillus niger* that were obtained from reference Laboratory at Michael Okpara University of Agriculture Umudike, Abia State.

Maintenance of the Microbial Isolates

The bacterial and fungal isolates were identified and sub cultured into fresh plates of nutrient agar and Saborauds dextrose agar and incubated for 24 hrs at 37°C and 30°C for 2-3 days respectively.

Standardization of Bacterial Inoculum

The inoculums size was standardized by transferring the pure cultures of each isolate to a sterile broth of the appropriate medium. This was incubated at 37°C for 24 h and 30°C for 48 hrs until a turbidity equivalent of 0.5 McFarland standards was achieved [10].

Preparation of control test

Dimethylsulfoxide (DMSO) was used as a control. This was to know if the extraction solvent has different effects on the organism and also to confirm any inhibition zone that may result in antimicrobial assay.

Screening for antimicrobial activity of ethanol and hot water extracts

This was achieved using agar well diffusion method [10]. A sterile cotton swab was used to collect standardized microbial isolates and plated on sterile Mueller Hinton agar and Sabourauds dextrose agar respectively. Wells made with 6 mm sterilized cork borer were filled with 400 mg/ml of each plant extract, solvent blanks and ciprofloxacin (standard drug). The petri dishes seeded with extracts were left to stand for 1 hour to enable the diffusion of the extracts into the media after which they were incubated at 37°C for 24 h and 30°C for 48 hours. The inhibition zone diameters were measured and recorded appropriately.

Determination of minimum inhibitory concentration

Varying concentrations of the extract (200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml) were prepared. Two milliliters (2 ml) of each concentration was added to each of 2 ml nutrient broth containing 0.1 ml of standardized test organisms of bacterial and fungal cells. The test tubes were incubated at the appropriate temperatures for the test organisms. The test tube with the lowest concentration of extracts which showed no growth after incubation was recorded as the MIC [10].

Phytochemical analysis

The aqueous (hot water) and ethanol extracts of these two plants were subjected to qualitative phytochemical screening.

RESULTS

Table 1 shows the qualitative phytochemical screening results of water and methanol extracts of *Phyllanthus urinaria* and *Phyllanthus amarus*. In the water extract of *Phyllanthus urinaria*, all the phytochemical parameters tested were present in varying degrees except tannin, resins and cardiac glycoside. However, in the methanol extract, flavonoid, alkaloid, steroid, resins, terpenoid, saponin and phenolic nucleus were all present, while cardiac glycoside and tannin were absent. In the methanol extract of *Phyllanthus amarus*, all the phytochemical parameters were present. However, only tannin and cardiac glycoside were absent in the water extract.

Table 1. Qualitative Phytochemical Screening of *Phyllanthus urinaria* and *Phyllanthus amarus*

Parameter	*Phyllanthus urinaria* Water Extract	*Phyllanthus amarus* Water Extract	*Phyllanthus amarus* Methanol Extract	*Phyllanthus urinaria* Methanol Extract
Flavonoid	++	++	+++	+++
Tannin	-	-	+	-
Alkaloid	+	+	++	+

Steroid	+	+	+	++
Resins	-	+	+	+
Terpenoid	+	++	+++	+
Saponin	++	++	++	+
Cardiac glycoside	-	-	+	-
Phenolic nucleus	+++	+++	+++	+++

Key: + = Present

++ = Moderately Present

+++ = Very Present

- = Absent

Table 2. Antibacterial inhibitory activity of Ethanol and Aqueous (Hot water) plant extract on bacterial isolates

Plant extracts	**Bacteria isolates/Diameters of zone of inhibition (mm)**			
	Ethanol extract		**Hot water extract**	
	Staphylococcus aureus	***B. cereus***	***Staphylococcus aureus***	***B. cereus***
Phyllantus urinaria	1.71 ± 0.282	1.56 ± 0.211	1.60 ± 0.000	1.66 ± 0.070
Phyllantus amarus	1.64 ± 0.210	1.49 ± 0.140	1.65 ± 0.071	1.29 ± 0.141

In Table 2, the ethanol extract of *Phyllantus urinaria* showed the greatest inhibitory activity on the bacterial isolates, S*taphylococcus aureus.*

Table 3. Antifungal activity of Ethanol and Aqueous (Hot water) plant extract on fungi

Plant extracts	**Fungal isolates/Diameters of zone of inhibition**			
	Ethanol extract		**Hot water extract**	
	Candida albicans	***Aspergillus niger***	***Candida albicans***	***Aspergillus niger***
Phyllantus urinaria	1.39 ± 0.050	1.33 ± 0.021	1.00+0.000	1.13 ± 0.020
Phyllantus amarus	1.41 ± 0.012	1.47 ± 0.010	0.97 ± 0.010	1.11 ± 0.010

In Table 3, Ethanol extract of *phyllantus amarus* showed the greatest inhibitory activity on the *Candida albicans*

Table 4. MIC of the active ethanol and aqueous (hot water) plant extract on selected bacterial isolates

Plant extracts	**Fungal isolates/Diameters of zone of inhibition**			
	Ethanol extract		**Hot water extract**	
	Staph aureus	***B. cereus***	***Staph aureus***	***B. cereus***
Phyllantus urinaria	19.00	15.00	17.20	14.00
Phyllantus amarus	21.50	18.00	20.50	17.80

The minimum inhibitory concentration of *P. amarus* on ethanol extract against *S. aureus* was the highest with the value of 21.50.

Table 5. MIC of the active ethanol and aqueous (hot water) plant extract on selected fungal isolates

Plant extracts	**Fungal isolates/Diameters of zone of inhibition**			
	Ethanol extract		**Hot water extract**	
	Candida albicans	***Aspergillus niger***	***Candida albicans***	***Aspergillus niger***
Phyllantus urinaria	11.00	10.00	9.60	9.5
Phyllantus amarus	9.40	9.10	7.10	7.1

The minimum inhibitory concentration of *P. Urinaria* on ethanol extract was highest on *Candida albicans.*

DISCUSSION

The result of the phytochemical screening of *P. urinaria* methanolic extract revealed the presence of steroid, flavonoid, alkaloid, saponin, resins, terpenoid, and phenolic nucleus at varying intensities (Table 1). *P. urinaria* water extract indicated the presence of flavonoid, alkaloid, steroid, terpenoid, saponin and phenolic nucleus in the screening result. The phytochemical screening result of *P. amarus* methanolic extract showed the presence of flavonoid, tannin, alkaloid, steroid, resins, terpenoid, saponin, cardiac glycoside and phenolic nucleus at varying intensities. Water extract of *P. amarus* showed the presence of all the phytochemicals screened except tannin and cardiac glycoside. This agrees with the studies conducted earlier [11] on the phytochemical constituents and anti-microbial studies of the two South African *Phyllanthus* species. The results of the phytochemical screening in this study are also agrees with the report of Lakshmi et al. [12] on the phytoconstituents of fifteen *Phyllanthus* species. The presence of these phytochemicals provides explanation for the use of *Phyllanthus* species as anti-bacterial, anti-protozoan, anti-malarial and anti-fungal agents. The biosynthesis of secondary metabolites varies among plants, even in different organs of plants and their biosynthesis depends on the environmental factors in which they grow [5]. Intraspecific variation of phytochemcials was observed in the three species studied [13]. It has been observed that, interspecific variation in phytochemical constituents has been documented extensively among plants. The differences in the phytochemical constituents in the different *Phyllanthus* species studied could be due to differences in genetic composition. The interspecific variation in phytoconstituents of plant could result to different levels of medicinal potentials for plants [14]. The ethonobotanical importance of the medicinal plants has been highlighted and it is used for various diseases. The antimicrobial activities of methanolic extract of *P. amarus* were tested against four organisms (Tables 3 and 4). The minimum inhibitory Concentration (MIC) of Ethanol and hot water extracts of *P. urinaria* on *Staphylococcus aureus* and *Bacillus aureus* were (19.00, 15.00) and (17.20, 14.00) while P. amarus were (21.50, 18.00) mg/ml and (20.50, 17.80) mg/ml (Table 5). The MIC of ethanol and hot water extract of *P. urinaria* on *Candida albicans* and *Aspergillus niger* were (11.00, 10.00 mg/ml and (9.60, 9.50) mg/ml while on *P. amarus* (9.40, 9.10) mg/ml) and (7.10, 7.00) mg/ml. Results obtained in this work do not agree with the findings of previous authors on antimicrobial status of *P. amarus* [15,16]. The result of this study highlighted the fact that methanolic extract of *P. amarus* exhibits antifungal properties. Presence of alkaloids, flavonoids and other phytochemicals in plant have amazing effect on man and has led to development of powerful pain killer medications [17]. Hence, *Phyllanthus amarus* can be used for treatment of infections caused by *Candida albicans*, *Staphylococcus aureus* which is a major pathogen of human infections, *Escherichia coli* which causes Uriniary Tract Infection (UTI), diarrhea, sepsis, and meningitis [18,19].

CONCLUSION

This results obtained from this study showed that the methanol extracts of *Phyllanthus urinaria and Phyllanthus amarus* species contain saponin, flavonoid, alkaloid, resins, steroid, terpenoid and phenolic nucleus at varying intensities, and this justifies their use in traditional medicine for the treatment of various diseases such as malaria, hepatitis B, jaundice, diarrhoea, dysentery and ulcer. There is variation in intensity of the phytochemical composition of the leaf part of the two *Phyallanthus* species and this establishes the fact that, these plants may not

have the same medicinal uses. The methanolic extract of *P. amarus* proved potent against *Candida albicans* and *Aspergillus niger*. This research shows that *Pyllanthus amarus* has potential antimicrobial agents which will be useful, in pharmaceutical Industries to develop a therapy against various diseases that are caused by both bacteria and fungi. The result of this study supports the development of new antimicrobial drugs from *Phyllathus amarus.*

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