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Antimicrobial and Phytochemical Screening of Phyllantus Niruri

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ABSTRACT

Theorigin of *Phyllanthus niruri* is tropical America; from there it spread as a weed to other tropic and sub-tropics. It is a tropical annual herb shrub which grows as weed in moist humid waste land. *Phyllanthus niruri* is among more than 500 *Phyllanthus* species that are widely spread in temperate and tropical climates region Lizuka *et al.,* 2007. It grows 30 - 40 cm in height, has small leaves and yellow flowers; the stem has green capsule, and blooms with flowers with 5 white sepals and apical acute anther.38g of Mueller Hinton Agar was dissolved in 1000ml distilled water in a conical flask, the mouth of the conical flask was plugged with cotton woo wrapped in aluminium foil. This was sterilized in an autoclave at 121^{o}C for 15mns. The media was removed and allowed to cool to 45^{o}C, later poured into a sterilized plastic petri plates which were appropriately labeled. The present study revealed the antimicrobial activity and phytochemical screening of phyllanthus niruri. The antimicrobial activity of phyllanthus niruri shows great significant against pathogens which are responsible for common infections of skin, respiratory, urinary and gastrointestinal tracts. The phytochemical screening of oxalate, terpenoids, tannins, phenols, quinones, flavonoids, alkaloids, saponins and steroids were all found to be active within the plant. This bioactive phytochemicals present in P. niruri can be useful for further researches on the plant (P. nururi) since the phytochemicals have shown preclinical efficacies for treating human diseases' which include hepatitis and HIV/AIDS. This work has compiled the chemical constituents present and can be useful for further researches

KEYWORDS: *phyllanthus niruri, E. coli, salmonella typhi, antimicrobial*

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INTRODUCTION

Phyllanthus niruri is a valuable medicinal plant which has been used for centuries in ancient Hindu system of medicine i.e. "Ayurveda" to cure gallstones, jaundice and diseases of urogenital system. It is a subtropical plant of great value, which plays an important role in health improvement around the world. Every part of this plant has been investigated as a source of valuable compounds medicinal herb. Origin of *Phyllanthus niruri* is tropical America; from there it spread as a weed to other tropic and sub-tropics. It is a tropical annual herb shrub which grows as weed in moist humid waste land. *Phyllanthus niruri* is among more than 500 *Phyllanthus* species that are widely spread in temperate and tropical climates region Lizuka *et al.,* 2007. It grows 30 - 40 cm in height, has small leaves and yellow flowers; the stem has green capsule, and blooms with flowers with 5 white sepals and apical acute anther. The fruit has green capsules, and smooth and fruiting pedicels while seeds are longitudinally rogues(Obianime &Uche, 2009).

It is found throughout the tropics and sub- tropics such as West Africa (including Nigeria and Ghana), Europe, Asia (including China, Pakistan, India and Malaysia Indian ocean), central and south America as medicinal plant for the treatment of various diseases. The plant has been used for a long period of time (thousands of years) in *Ayurvedic* traditional medicine for various illnesses (Adedapo et al., 2005). In India, *Phyllanthus niruri* is one of the most important traditional medicines used for the treatment of jaundice, asthma, hepatitis and urolithic disease (Ishimaru et al., 1992).Among the *Phyllanthus* species, *P. niruri* is a small erect annual herb growing

up to 30–40 cm in height and is indigenous to the Amazon rainforest and other tropical areas, including South East Asia, Southern India and China (Gupta et al., 2003). Its leaves are 7–12 cm long and they are alternate, sessile oblong. It has small off-white-greenish flowers, which are solitary, auxiliary, pedicellate, apetalous and monoecious. *P. amarus* and *P. sellowianus* are closely related to *Phyllanthus niruri* in appearance, phytochemical contents and history, but they are found in drier regions of India and Brazil, and even in Florida and Texas. In a recent report, cladistic analysis indicated that the *Phyllanthus* genus is paraphyletic and therefore the two problematic and confusing species, *Phyllanthus niruri* and *Phyllanthus amarus*, are two individual species (Lee et al., 2011).

The genus *Phyllanthus* is one of the most important groups of plants traded as a raw herbal drug in India. The genus *Phyllanthus* of family *Euphorbiaceace*, consists of approx. 1000 species, spread over tropical and sub-tropical continents like America, Africa, Australia and Asia. In India, *Phyllanthus amarus* is widely distributed as a weed in cultivated and waste lands. All three major habits i.e., trees, shrubs and herbs are seen amongst the *Phyllanthus* species. (Ravikant et al., 2011) have also described southern India to be the genetic hotspot of *Phyllanthus* species. *Phyllanthus niruri Schum*and *Thonn*. Has a long history of usage by people, because of its rich medicinal values. Commonly known by the name of *Bhumi amla*, belongs to a large family of upright or prostrate herbs or shrubs, often with milky acrid juice. In Unaniet al., 1995 literature, it is described by the name of *'Bhuti'* which means *Bhum Amlak - Amla* of Land. It plays important role in the development of green medicines which are safer to use and more dependable than costly synthetic drugs with no adverse effects. *P. niruri* has been described in Ayurveda by the Sanskrit name - *Bhoomy-aamlakee, Taamalakee* and *Bhoodha* tree. *P. niruri* uses are gaining momentum because of their novel antiviral activity against hepatitis B virus and for several other biological activities such as kidney and gallbladder stones, for cold, flu, tuberculosis, liver diseases, etc. Vernacular Names: The plant is known by different vernacular names in the different areas by the local people (Table 1). It is commonly called carry me seed, stone breaker, wind breaker, leaf flower or gale of wind. *Phyllanthus* means leaf and flower because of its appearance, where flower, fruit and leaf appear to be used.

Table 1: Botanical classification of phyllanthus niruri

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Euphorbiales
Family	Euphorbiaceae
Genus	Phyllanthus
Species	Nururi

Source: Author's analysis, 2021

Fig 1: Phyllanthus Niruri

METHODODOLOGY

Sampling

The samples of whole *Phyllanthus niruri* were collected from their natural habitat within the compound of Yobe state university. They were collected in a proper season and condition as recommended by W H O, 2003. The

plants were identified and authenticated by a technologist at Biological science department, of Yobe state University Damaturu, Yobe State. *Phyllanthus niruri*leaves stem, short and stem were thoroughly washed and rinsed with distilled water and were air dried at room temperature (25°C) for two weeks, they were further dried using oven until constant weight was ensured. The dried plant material was grounded to fine powder with a domestic electric grinder, packaged in glass jars and store until required for use.

Materials

The equipment's and instruments used in this study were all calibrated to check their status before and in the middle of the experiments. Apparatus such as volumetric flasks, measuring cylinder and digestion flasks were thoroughly washed with detergents and tap water and then rinsed with deionized water. All Glass wares were cleaned with 10% concentrated Nitric acid (HNO_3) in order to clear out any heavy metal on their surfaces and then rinsed with distilled-deionized water. The digestion tubes were soaked with 1% (w/v) potassium dichromate in 98% (v/v) H_2SO_4 and the volumetric flasks in 10% (v/v) HNO_3 for 24 hours followed by rinsing with deionized water and then dried in oven and kept in dust free place until analysis began. Prior to each use, the apparatus were soaked and rinsed in deionized water.

Preparation of extracts

The fresh weights of the plant samples were determined and then air dried for 7 days until constant weights were obtained. The dried plant samples were milled. For each sample, three extractions were made using cold ethanol, hot ethanol and hot distilled water. About 20 g each of the powdered samples were weighed into 500 ml round bottom flask. Cold extraction in 250 ml of 85% ethanol was done on each sample for three days using the mechanical shaker (Heidolf, Vibramax 100 and Germany). The mixtures were filtered and concentrated using rotatory evaporator (Heidolph type VVI rotary evaporator (Normschiff Geratelbau Werthein, Federal Republic of Germany) to form the crude cold ethanol extracts. Another 20 g of each sample was again weighed and extracted with hot 85% ethanol for 3 days. The third batch of extraction was done with hot distilled water on 20 g of each sample. Cold and hot ethanol extracts were filtered and concentrated as before.

Reagents and Chemicals

Reagents and chemicals used for the laboratory works were all analytical grade: Deionized water (chemically pure with conductivity 1.5µs/cm and below was prepared in the laboratory) was used for dilution of sample and intermediate metal standard solutions prior to analysis and rinsing glassware and sample bottles.

Source of E. coli and salmonella typhi

The two bacteria were clinical isolates associated with patient obtained in specialist hospital Damaturu.

Media preparation

38g of Mueller Hinton Agar was dissolved in 1000ml distilled water in a conical flask, the mouth of the conical flask was plugged with cotton woo wrapped in aluminium foil. This was sterilized in an autoclave at 121^{o}C for 15mns. The media was removed and allowed to cool to 45^{o}C, later poured into a sterilized plastic petri plates which were appropriately labeled. The MHA was allowed to solidify using Bunsen burner flame, inoculating was sterilized where a colony was picked from the bacterial isolate of E. coli and selmonella typhi, these were streaked on the surface of the MHA entirely.

Preparation of plant extract and sensitivity discs

Whatman No.1 was punched using a paper puncher and a disc of 6.0mm/dm was obtained. These were placed in a beaker and sterilized in an oven at 140^{o}C for 1hr. the discs were allowed to cool until use.

The stock solution of the plant – extract was prepared in a sterilized screw scrapped bijon bottles using dimethyl sulfoxide (DMSO). 1g of each fraction was weighed on analytical weighing balance (OHAUS) and dissolved in 10ml DMSO which arrived at 100,000ug/ml concentration of stock solution.ss

From the stock solution, 0.1ml, 0.2ml, 0.3ml, 0.4ml, and 0.5ml of each fraction was added into 0.9ml, 0.8ml, 0.7ml, 0.6ml, and 0.5ml respectively of DMSO in a test tube which made 1ml of each.

The sterilized discs were placed into each of the bottles which arrived at disc potency of 100ug/disc, 200ug/disc, 300ug/disc, 400ug/disc, 400ug/disc, and 500ug/disc respectively.

Test organism

The test organism used for antimicrobial activity was Escheria coli and Salmonella typhi. These were obtained from Yobe state specialist hospital Damaturu as a clinic isolate from various samples obtained from clinical patients. Gram staining and biochemical tests were carried out to confirm the isolate and grown on differential

medium Eosyn methylene blue Agar (EMB) and Deoxychocolate Agar (DCA) for E. coli and Salmonella typhi respectively.

Method of Data Analysis

Statistical analysis was performed using the t-test. The values are means ± SD for three replicate values in each group. P values < 0.05 were considered to be statistically significant. Results were expressed as mean +_. Statistical analyses was carried out by one-way ANOVA (Graph Pad Prism 3.0), data was further subjected to Dennett's post hoc test and differences between treated groups and control accepted as significant at P<0.01 and P<0.05.

RESULT AND DISCUSSION

Table 2: Antibacterial Activity of Phyllantus Amarus Root against Escherichia Coli and Salmonella Typhi

Test Organism	Concentration of Extract (ug/ml)	Zone of Inhibition(Mm/Dm)
E. COLI	100ug/ml	no zone of inhibition observed
	200ug/ml	no zone of inhibition observed
	300ug/ml	4mm/dm
	400ug/ml	10mm/dm
	500ug/ml	13mm/dm
S. TYPHI	100ug/ml	zone of inhibition observed
	200ug/ml	zone of inhibition observed
	300ug/ml	zone of inhibition observed
	400ug/ml	zone of inhibition observed
	500ug/ml	zone of inhibition observed

Source: Author's analysis, 2021

Table 3: Antibacterial activity of phyllantus nururi stem against escherichia coli and salmonella typhi

Test Organism	Concentration of Extract (ug/ml)	Zone of Inhibition (mm/dm)
E. COLI	100ug/ml	no zone of inhibition observed
	200ug/ml	2mm/dm
	300ug/ml	6mm/dm
	400ug/ml	9mm/dm
	500ug/ml	15mm/dm
S. TYPHI	100ug/ml	no zone of inhibition
	200ug/ml	no zone of inhibition observed
	300ug/ml	no zone of inhibition observed
	400ug/ml	no zone of inhibition observed
	500ug/ml	no zone of inhibition observed

Source: Author's analysis, 2021

Table 4: Antibacterial activity of phyllantus amarus leaves against escherichia coli and salmonella typhi

Test Organism	Concentration of Extract (ug/ml)	Zone of Inhibition (mm/dm)
E. COLI	100ug/ml	4mm/dm
	200ug/ml	6mm/dm
	300ug/ml	10mm/dm
	400ug/ml	15mm/dm
	500ug/ml	26mm/dm
S. TYPHI	100ug/ml	no zone of inhibition observed
	200ug/ml	no zone of inhibition observed
	300ug/ml	no zone of inhibition observed
	400ug/ml	no zone of inhibition observed
	500ug/ml	no zone of inhibition observed

Source: Author's analysis, 2021

Table 5: Antibacterial activity of phyllantus amarus shoots against escherichia coli and salmonella typhi

Test Organism	Concentration of Extract (ug/ml)	Zone of Inhibition (mm/dm)
E. COLI	100ug/ml	no zone of inhibition observed
	200ug/ml	2mm/dm
	300ug/ml	3mm/dm
	400ug/ml	5mm/dm
	500ug/ml	8mm/dm
S. TYPHI	100ug/ml	no zone of inhibition observed
	200ug/ml	no zone of inhibition observed
	300ug/ml	no zone of inhibition observed
	400ug/ml	no zone of inhibition observed
	500ug/ml	no zone of inhibition observed

Source: Author's analysis, 2021

Antimicrobial activity of most commonly used standard antibiotic activity was studied against salmonella typhi and E.coli isolates. Pathogens are found to show comparable susceptibility towards the extract of *phyllanthus niruri*. Table 2 above shows Antimicrobial activity of root against E. coli with no zone of inhibition in salmonella typhi. In table 3 stem part of the plant shows greater antimicrobial activity than that of the root, in table 4 the leaves part of the plant shows greatest of all antimicrobial activity and table 5 shows the shoots of the plant shown least activity compared to the leaves (table 4) and similar to that of the stem. This was observed to be comparable to that of aqeous extracts of *phyllanthus niruri* with reference to the zones of inhibition shown by salmonella typhi and E. coli.

From the table below 6it shows that different parts of *phyllanthus niruri* exhibits active antioxidant activity to a varying degree with only oxalate that is only detected in leaves, and quinones is only detected in the stem part of the plant. Also flavonoids is also detected in the various part of the plant except in shoot of the plants and steroids found to be absent only in the leaves, while terpenoids, tannins, phenols, alkaloids and saponins are all detected in all the part of the plant.

Table 6: Presentation of phytochemical screening

	Oxalate	Terpenoids	Tannins	Phenols	Quinones	Flavonoids	Alkaloids	saponins	Steroids
PAS	-	+	+	+	+	+	+	+	+
PASH	-	+	+	+	-	-	+	+	+
PAL	+	+	+	+	-	+	+	+	-
PAR	-	+	+	+	-	+	+	+	+

Source: Author's analysis, 2021

The result of this study agrees with previous research that these plants (different parts of the plants) contain antimicrobial substances. The methanolic extract of the shows bioactive agents containing saponins, flavonoids, alkaloids, tannins, terpenoids, oxalates, phenols, quinones and steroids are present and are effective agents of medicinal values that are found in medicinal plant (Okwu & Eminike, 2006). Saponins is found to be natural antibiotics helping the body to fight infections and knocking out tumors especially lung and cancer cancers(Stray, 1998). Flavonoids are and free radical scavengers which prevent oxidative cell damage with strong anti-cancer activity and protect cells against all stages of carcinogenesis. Terpenoids have analgesic properties, terpenoids of methanolic extracts pf phyllanthus nururi are natural agent of antibacterial antifungal botanicals (Wagner & Bladts, 1996). Steroids have anti – inflammatory properties(Stanley, 2013). The plants also contain high percentage of carbohydrates and fibre and this was confirmed (Foo, 1993).

Conclusion

Phyllanthus niruri plant is widely used in Asia and Africa including Nigeria as medicinal herb. It has significant traditional uses, which include treating of hepatitis, cancer and kidney diseases among other related disease and it have been experimentally established and an attempt has been made to isolate its chemical constituents. This present work has shown the acitivity of the plant against pathogens and some of its chemical constituents (phytochemical screening) present in the plants, and can be useful information for further researches on this plant.

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