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A STUDY ON THE PHYTOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF METHANOL AND METHANOL/WATER EXTRACT OF AERIAL PART OF *PHYLLANTHUS NIRURI*

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ABSTRACT

This work focuses on the phytochemical and antimicrobial screening of the methanol and mixture of methanol/water extracts. The phytochemical screening of the methanol extract revealed the presence of Carbohydrate, Glycoside, Anthraquinones, Steroidal Triterpenes, Flavonoids, Tannins and Alkaloids while Cardiac glycoside and saponins were found to be absent. The Methanol/water mixture extract reveal the presence of Carbohydrate, Anthraquinones, Cardiac Glycoside, Saponins, Steroidal Triterpenes, Flavonoids, Tannins and Alkaloids with the absence of Anthraquinones. The antimicrobial activities of the extracts were carried out to ascertain which fraction of the extracts was more sensitive to the test organisms. The methanol extract was found to be sensitive with the following zone of inhibition to Staphylococcus aureus (19 mm), Streptococcus Pyrogenes (19 mm), Salmonella typhi (22 mm), Shigellia dysenteric (21 mm), Pseudomonas aeruginosa (20 mm) and Microsperum canis (15 mm) and resistant to Escherichia coli, Proteus Mirabilis and Trichophyton without zone of inhibition. While the methanol/water extract was found to be sensitive to Streptococcus Pyrogenes (21 mm), Escherichia coli (20 mm), Shigellia dysenteric (20 mm), Pseudomonas aeruginosa (19 mm), and Trichophyton (17 mm) while Staphylococcus aureus, Salmonella Typhi, Proteus mirabilis and Microsperum canis were resistant to the methanol/water extract without zone of inhibition. Based on the activities of these extracts on the selected microbes their Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration/Minimum Fungicidal Concentration (MBC/MFC) were carried out to ascertain their level of activities.

Keywords: Inhibition, Organisms, Resistance, Maceration, Flavonoid**,** *Salmonella Typhi*

INTRODUCTION

Medicinal plants are considerably useful and economically essential. They contain active constituents which are used in the treatment of many diseases. Plants have been used to treat common infectious diseases, their healing potential has been utilized in many countries of the world like Ayurveda and Unani (Jayshree & Manneemegalai, 2008).

Phyllantus Niruri is an annual herb of Euphorbiaceae family, have potential value in wide range of ailments like jaundice, gonorrhea, frequent menstruation and diabetes, as a poultice for skin ulcers, swellings and itching and in the treatment of chronic dysentery. It is a widespread tropical plant commonly found in coastal areas, known by the common names gale of the wind, stonebreaker or seed-under-leaf. It is a relative of the spurges, belonging to the genus *Phyllantus* of the family. It grows 50–70 cm (20–28 in) tall and bears ascending herbaceous branches. The bark is smooth and light green. It bears numerous pale green flowers which are often flushed with red. The fruits are tiny, smooth capsules containing seeds.

The plant is also known as Bhumyamalaki, Keezhanelli (Malayalam, Tamil) has been used in Ayurveda, Siddha for problems of the stomach, genitourinary system, liver, kidney, and spleen, and to treat chronic fever (Patel *et al*, 2011). Amazonian tribes of Brazil historically used this plant to treat kidney stones and gallstones, naming it Chanca piedra, the Spanish term for stonebreaker. *Phyllanthus niruri* is nowadays marketed as an herbal remedy under the name Chanca piedra (Donal, 2010). The plant has been investigated for its potential medicinal benefits. A 2010 review found some evidence that it might be effective at inhibiting kidney stone formation (Boim *et al*, 2010).

The aim of this study is to screen the Methanol and Methanol/Water Extract of the aerial Part of *Phyllantus Niruri* against some selected microorganisms to know their efficacy against such common disease causing organism.

MATERIAL AND METHOD

Plant Materials

The plant material was collected from Okene in Kogi State, Nigeria and the plant identified at the herbarium of Ahmadu Bello University, Zaria, Nigeria as *Phyllantus Niruri* with voucher No. 035. The plant was air dried under the shade in the laboratory. The air dried aerial plant part was pounded using a local mortar and pestle until a finely grounded plant part was obtained which was kept in an air tight polythene bag and kept away for subsequent analysis.

Extraction procedure

The pulverized dried aerial plant part was extracted using cold maceration method. Two hundred grams (250 g) of the powdered plant material were placed in a covered bottle and extracted with 500 ml of the methanol followed with Methanol/Water in the cold successively after which it was filtered using Whatman No.1 filter paper into round bottom flask. The filtrate was poured into beakers and allowed to evaporate to obtain the crude extracts.

Phytochemical analysis

The pulverized aerial plant part of *Phyllantus Nuriru* was screened for plant metabolites. Standard techniques of Brain and Turner (1975) were employed in the phytochemical screening. The presence or absence of typical metabolites such as flavonoid, terpenoid, cardiac glycosides, tannins, steroids, saponins, phlobatannins, Phytosterols, alkaloids, carbohydrates and glycoside were determined.

ANTIMICROBIAL SCREENING

Media preparation

The blood agar base was the medium used as the growth medium for the bacteria and Sabourand dextrose agar (SDA) for the growth of the fungi; all media were prepared according to the manufacturer's instruction. This was boiled to dissolves and was sterilized at 121^{O}C for 15minutes, the medium was cooled to 45^{O}C and was then poured into sterilized Petri dishes, the plates were allowed to cool and solidify.

Preparation of Microbial Culture

The following clinical microbes *Staphylococcus aureus, Streptococcus pyrogenes, Escherichia coli, Salmonella Typhi, Shigellia Dysenteric pseudomonas aeruginosa, Proteus mirabilis, Microsperum canis and Trichophyton* were obtained from the department of medical microbiology, Ahmadu Bello University, Teaching Hospital Shika, Zaria, Nigeria. All the microbes were checked for purity and maintained in slants of nutrient agar for the bacteria and the fungi were maintained in slants of S.D.A.

Preparation of solution of plant extracts for antimicrobial screening tests

The extracts were made into solutions of various concentrations using distilled water as solvent. Stock solutions of the plant extracts were prepared by initially dissolving 0.8 g of the extracts in 10 mls of distilled water to obtain a concentration of 80mg/ml. From the stock solution, concentration of 80-, 40-, 20-, 10- and 5 mg/ml were prepared by serial dilution. The well diffusion method was used to determine the anti-microbial screening activities of the extracts from the plant.

Determination of the anti-microbial activity of the extract from the plant using the well diffusion method.

The molten cooled agar media were seeded with the standardized suspension (0.1 ml) of the inoculums of the test micro-organism, the inoculums was spread evenly by the use of sterile swab over the surface of the agar medium; the seeded plates were then allowed to dry at 37 ^{0}C for 30 minutes. A standard cork borer of 6 mm diameter was used to cut a well at the center of each inoculated medium, the solution of the test extracts of known concentration was then inoculated into each well on the medium, the extract was then allowed to diffuse into the agar media for 2 hours. Thereafter, the plates were then incubated at 37 ^{0}C for 24 hours for the bacteria and 30 ^{0}C for the fungi for 1 – 7 days, after which the plates were observed for zones of inhibition of growth, the zones were measured with a transparent ruler and a pair of divider and the result recorded in millimeter.

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentrations of the crude extracts were carried out on the test-organisms and were done by the broth dilution nutrient method for the bacteria and the malt extract broth for the fungi. The broth was prepared according to the manufacturers instruction, 10 mls of each broth was dispensed into test-tubes and was sterilized by means of autoclave at 121 ^{0}C for 15minutes, after which the broth was allowed to cool.

Mc-farland's turbidity standard scale number 0.5 was prepared to give a turbid solution. Normal saline was used to make turbid suspension of the micro-organisms and was incubated at 37 ^{0}C and 30 ^{0}C for 6hours for the bacteria and fungi respectively. Dilutions of the micro-organisms in the normal saline was done continuously until the turbidity marched that of the normal saline by visual comparison, at this point the concentration of the bacteria is about $1.5x10^{8}$ cfu/ml and that of the fungi is about $1.5x10^{6}$ cfu/ml. two-fold serial dilution of the extract in the broth were made to obtained the concentrations of 80-, 40-, 20-, 10- and 5 mg/ml. the initial concentration of the extract was obtained by dissolving 0.8 g of the extract in 10 mls of the sterile broth.

Having obtained different concentrations of the extracts in the broth, 0.1 ml of the standard inoculums of the micro-organism in the normal saline was then inoculated into the different concentrations in the test-tubes and the test-tubes were then incubated at 37 ^{0}C for 24 hours and 30^{O}C for 1-7days for the fungi. The lowest concentration of the extract in the test-tubes which shows no turbidity is recorded as the Minimum Inhibitory Concentration (MIC)

Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) of the crude extract

The Minimum Bactericidal and Fungicidal Concentration (MBC/MFC) of the extracts was carried out in order to determine whether the test micro-organisms were killed or only their growth were inhibited. Blood and Sabourand dextrose agars were prepared according to manufacturer's instructions and sterilized at 121 ^{O}C for 15 minutes and poured into sterile Petri dishes to cool and solidify. The content of the MIC in the serial dilution were sub-cultured onto the agars, the blood agar plates were incubated at 37 ^{O}C for 24hours and the Sabourand dextrose agar at 30 ^{O}C for 1-7 days after which the plates were

observed for growth. The MBC/MFC is the plate with the lowest concentration without colony growth.

RESULTS

Table 1: Preliminary Phytochemical Screening of the Methanol and Water Extract of Aerial Part of *Phyllantus niruri*

PROPERTY TESTED	METHANOL	METHANOL/WATER
Carbohydrate	+	+
Glycoside	+	_
Anthraquinones	+	+
Cardiac Glycoside	_	+
Saponins	_	+
Steroidal Triterpenes	+	+
Flavonoids	+	+
Tannins	+	+
Alkaloids	+	+

KEYS: + → Present – → Absent

Table 2: Antimicrobial activities of the Methanol and Methanol/water extract of *Phyllanthus Nuriru*

TEST ORGANISM	Methanol and methanol/water extract of *Phyllanthus nuriru* 80mg/ml	
	Methanol	Methanol/water
Staphylococcus aureus	S	R
Streptococcus pyrogenes	S	S
Escherichia coli	R	S
Salmonella typhi	S	R
Shigellia dysenteric	S	S
Pseudomonas aeruginosa	S	S
Proteus mirabilis	R	R
Microsperum canis	S	R
Trichophyton	R	S

Key: - S = sensitive R = resistance

Table 3: Zone of Inhibition of the Methanol and Methanol/water extracts of *Phyllanthus nuriru* against the test micro-organism (mm)

TEST ORGANISM	Zone of Inhibition (mm)	
	Methanol extract	Methanol/water extract
Staphylococcus aureus	19	0
Streptococcus pyrogenes	19	21
Escherichia coli	0	20
Salmonella typhi	22	0
Shigellia dysenteric	21	20
Pseudomonas aeruginosa	20	19
Proteus Mirabilis	0	0
Microsperum canis	15	0
Trichophyton	0	17

Table 4: MIC of the methanol extract for *Phyllanthus nuriru*

TEST ORGANISM	CONCENTRATIONS (mg/ml)				
	80	40	20	10	5
Staphylococcus aureus	-	-	20*	+	++
Streptococcus pyrogenes	-	-	20*	+	++
Escherichia coli					
Salmonella typhi	-	-	-	10	+
Shigellia dysenteric	-	-	-	10	+
Pseudomonas aeruginosa	-	-	-	10	+
Proteus mirabilis					
Microsperum canis	-	-	20*	+	++
Trichophyton					

Key: - = no turbidity (no growth). *= mic. + =turbid (light growth). ++ =moderate turbidity

Table 5: MIC of the methanol/water extract for *Phyllanthus nuriru*

TEST ORGANISM	CONCENTRATIONS (mg/ml)				
	80	40	20	10	5
Staphylococcus aureus					
Streptococcus pyrogenes	-	-	-	10	+
Escherichia coli	-	-	-	10	+
Salmonella typhi					
Shigellia dysenteric	-	-	-	10	+
Pseudomonas aeruginosa	-	-	20	+	++
Proteus mirabilis					
Microsperum canis					
Trichophyton	-	-	20	+	++

Key: - = no turbidity (no growth). O*= MIC. + =turbid (light growth). ++ =moderate turbidity

Table 6: MBC/MFC of the methanol extract for *Phyllanthus nuriru*

TEST ORGANISM	CONCENTRATIONS (mg/ml)				
	80	40	20	10	5
Staphylococcus aureus	-	40	+	++	+++
Streptococcus pyrogenes	-	40	+	++	+++
Escherichia coli					
Salmonella typhi	-	40	+	++	+++
Shigellia dysenteric	-	40	+	++	+++
Pseudomonas aeruginosa	80	+	++	+++	++++
Proteus mirabilis					
Microsperum canis	80	+	++	+++	++++
Trichophyton					

KEY: - = No colony growth, o*=MBC/MFC, + =scanty colonies, ++=moderate colonies growth, +++= heavy colonies growth

Table 7: MBC/MFC of the methanol /water extract for *Phyllanthus nuriru*

TEST ORGANISM	CONCENTRATIONS (mg/ml)				
	80	40	20	10	5
Staphylococcus aureus					
Streptococcus pyrogenes	-	40	+	++	+++
Escherichia coli	-	40	+	++	+++
Salmonella typhi					
Shigellia dysenteric	-	-	20	+	++
Pseudomonas aeruginosa	-	40	+	++	+++
Proteus mirabilis					
Microsperum Canis					
Trichophyton	80	+	++ +++		++++

Key: - = no colony growth, O*=MBC/MFC, + =scanty colonies, ++=moderate colonies growth, +++= heavy colonies growth

DISCUSSION

The phytochemical screening (Table I) revealed the presence of Carbohydrate, Glycoside, Anthraquinones, Steroidal Triterpenes, Flavonoids, Tannins and Alkaloids while Cardiac glycoside and saponins were found to be absent in the Methanol extract. The Methanol/water extract contain Carbohydrate, Anthraquinones, Cardiac Glycoside, Saponins, Steroidal Triterpenes, Flavonoids, Tannins and Alkaloids. These phytochemicals had been found to have great pharmacological effect in the treatment of diseases. The presence of saponins, tannins and alkaloids in the plant calls for an in-depth study on the plant. The metabolites are of various pharmacological importance. Many triterpene saponins and their aglycones have been reported by Hostettmann and Martson, (1995) and in Ndukwe *et al.*, (2005) to have varied uses as antiulcer genic, anti-inflammatory, fibrinolytic, antipyretic, analgesic and anti-edematous in action. The presence of these compound may be responsible for the medicinal properties of this plant.

The antimicrobial activities of the extracts were carried out to ascertain which fraction of the extract was more sensitive to the test organism. The methanol extract was found to be sensitive to *Staphylococcus aureus, Streptococcus pyrogenes, Salmonella typhi, Shigellia dysenteric, Pseudomonas aeruginosa* and *Microsperum canis with* high record of zone of inhibition and resistant to *Escherichia coli, Proteus Mirabilis* and *Trychophyton* with low record of zone of inhibition. While the methanol/water extract was found to be sensitive to *Streptococcus pyrogenes, Escherichia coli, Shigellia dysenteric, Pseudomonas aeruginosa , and Trychophyton with high record of zone of inhibition while Staphylococcus aureus, Salmonella typhi, Proteus mirabilis* and *Microsperum canis* were resistant to the methanol/water extract with low record of zone of inhibition (Table 2 and 3).

The Minimum Inhibition Concentration (MIC) was determined to ascertain the lowest concentration level at which these extracts could inhibit the growth of these organisms. All the extracts were found to inhibit the growth of all the sensitive

organism at 20 mg/ml for *Staphylococcus aureus, Streptococcus pyrogenes and Microsperum canis and at 10 mg/ml for Salmonella typhi, Shigellia dysenteric and Pseudomonas aeruginosa* for the methanol extract. While the MIC for the methanol/water extract was found at 20 mg/ml for *Pseudomonas aeruginosa* and at 10 mg/ml for *Streptococcus pyrogenes, Escherichia coli* and *Shigellia dysenteric (*Table 4 and 5).

The Minimum Bactericidal Concentration (MBC) was carried out to determine and establish the minimum concentration at which the sensitive organism will be killed or completely exterminated. From table 6 and 7 the MBC/MFC was observed for *Staphylococcus aureus, Streptococcus pyrogens, Salmonella typhi and Shigellia dysenteric* at 40 mg/ml. While it was observed for *Pseudomonas aeruginosa* and *Microsperum canis* at 80 mg/ml in the methanol extracts. These values were noted to be higher than the MIC value obtained for the same extracts. The MBC/MFC values for the methanol/water extract were observed at 40 mg/ml for *Streptococcus pyrogens, Escherichia coli* and *Pseudomonas aeruginosa* while it was observed at 20 mg/ml and 80 gm/ml for *Shigellia dysenteric* and *Trichophyton canis* respectively.

CONCLUSION

The methanol and the methanol/water extracts shows significant antibacterial and antifungicidal properties as observed from the result. These properties could be collaborated with the presence of the phytochemicals observed to be present in the plant (Table 1) which has been shown to possess varying antibacterial and antifungicidal values. The inhibitory and bactericidal effect of the extracts justifies the traditional use of this plant. There is the need to embark on further research on the plant in order to find out and isolate the active medicinal component present in it.

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