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PHYTOCHEMICAL AND ANTIMICROBIAL INVESTIGATIONS OF EXTRACTIVE FROM *PHYLLANTUS AMARUS*

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ABSTRACT

Phytochemical and antimicrobial investigation of *Phyllantus amarus* was carried out. *Phyllantus amarus* (eyin-olobe) family *Euphorbiaceae* were extracted with hexane and methanol using sohxlet extractor also water extract was carried out. The extracts were screened for the classes of organic compounds present and antimicrobial activity was carried out using five bacteria including *Klebsiella, Helicobacter pylori, Pseudomonas auruginosa, Escherichia coli* and *Shigella*. Phytochemical tests showed that aqueous and methanolic extracts of *Phyllantus amarus* contained flavonoids, tannins, saponins, glycosides and cardiac glycosides, while hexane extract does not contain any of these compounds. The methanolic and aqueous extracts were found to be effective against the tested organisms while the hexane extract was observed to show negative.

INTRODUCTION

Phyllantus amarus belongs to the family *Eupharbiaceae*. More than fifteen species of the genus *phyllantus* have been identified, among the species, the most common ones are *P. amarus, P. niruri, P. sellowianus, P. corcoradenisis and P. urinaria. Phyllantus amarus* has disk of female flower stellate with normally fine deep radiating lobes, styles short but distinct, deeply bifid at apex, not forming a closely packed circle, a top ovary, antherthecae with markedly oblique dehiscence, one female flower together with one male in each axil, leaves elliptic ablong, 5-10mm long, 2-4.5mm broad[1].

In vivo studies of crude extract of *P. amarus* in modifying the genotoxicity induced in *Vicia faba* by tannery showed a significant reduction in the frequency of chromosomal alteration. However, there was no significant variation in mitotic frequency, this suggests that *P. amarus* is antigenotoxic[2].

Also antitumor activity of *P. niruri* has been reported[3]. An Anticimicrobial activity of decoctions of different parts of *P. uninaria* when tested against *Bacillus subtills* was found to be active[4].

A novel cyclic metabolite named amarulones has being isolated from *P. amarus*.

Chemical examination of the polar extractives of the aerial part of P.amarus led to the isolation of ameriinic acid, a novel ellagitanium, together with 1-0-galloyl-2-4-de-hydrohexa-hydroxyddiphenyl-gluco-pyranose, elaeocarpusin, repadusinic acid. This structure of amariinic acid was established as a ring-opened oxidizing cyclohexentrionemoiety of dehydrohexa-hydroxydiphenyl attached to 0-4 of thyglucose.

Concoctions from this plant have been used traditionally in treatment of dysentery and diarrhea in some parts of Nigeria.

The therapeutic or medicinal activity of plants usually depends on the presence of what are known as “active principles or compounds”, and some understand of these is necessary in any study of the actions and uses of plants and plant parts as drug. The compound of chief interest in medicine may classified into several groups according to their chemical nature and their action on the animal body, some of these compounds are; Alkaloid, tannins, saponins, glycosides, fixed oil and fats, mucillages and gum.

In this study, the phytochemical screening of hexane, methanol and aqueous extracts of the various parts (roots, stem and leaves) of the plant was carried out with a view to identifying the presence of various chemical components. The

Table - 1 : Phytochemical screening of the extracts of *Phyllantus amarus*

Compounds	Aqueous Extract	Methanolic Extract	Hexane Extract
Alkaloids	-	-	-
Tannins	+	+	-
Flavonoids	+	+	-
Reducing Sugar	+	+	-
Glucosides	+	+	-
Cardiac	+	+	-
Glycosides	+	+	-

Key + Present;
- Absent

antimicrobial activities of extracts is also investigated using five bacterial pathogen to find out the potency of these towards inhibiting bacteria growth of other diseases caused by bacterial pathogens apart from dysentery and diarrhea that is being known and used for in some parts of Nigeria.

MATERIALS AND METHODS

The various parts (leaves, stem,root,etc) of *P. amarus* were collected on the University of Ibadan, Ibadan campus and identify in the forestry Herbarium of the University. Prior to extraction, the samples were crushed and air-dried under controlled condition to avoid occurrence of too many chemical changes. Hexene,methanol and aqueous extract were used for the analysis. Aqueous extract were obtained from 160g of the dried powder sample using 500ml of distilled water. The procedure is as outlined by NIPRD[7] the hexane and the methanolic extract were obtained using soxhlet apparatus. The powder sample, 184g was extracted for 24hours as outlined in USP[8]

Phytochemical Analysis:

Hexane, methanolic and aqueous extracts were tested separately for the following classes of organic compound; Alkaloid, Glycosides, Saponins, Tanins, Flavonoids, and steroid.

All the phytochemical tests, with the exception of those for steroids, alkaloid and flavoniods were as outlined in NIPRD procedures. The test for steroid, alkaloids and Flavonoids were as described by Harborne[9] and Trease and Evans[10]. Also glycosides was tested as described by Goldfiem and De[11]

Antimicrobial Screening

The Kirby Bauer[12] disc diffusion method was used to determine the antimicrobial activity of the extracts.

Commercial prepared Nutrient Agar (NA) was used. 31g of powder agar were suspended in 1dm^3 of distilled water. This was heated to boiling to dissolve the powder completely. This was later sterilized in the autoclave for 15minutes at 1.5 bar and temperature of 121˚C. Disposable sterilized petri dishes were used. Prepared sterilised NA was poured into sterilised petri dishes in 20cm^3 amount. This was allowed to set or solidity and incubated at 37˚C for 24 hours for sterilizing test.

Assay of Extracts

2.0,1.0,0.5,and 0.2 grams of the herbal extracts were weighed. These dry weights were reconstituted in 20cm^3 of extractive solvent given a concentration of 0.1,0.05,0.025 and 0.01g/cm^3. Agar diffusion well method was used for the assay. The organism used were collected from the Department of Genetics, Nigerian institute of medical research, Yaba, Lagos. This NA was flooded with the standardardized innocullum and drained. The inoculated agar was bored with the use of cork borer aseptically, 0.4 cm^3 of each extract was transferred into the well aseptically and incubated for 24 hours at 37˚c. The zones of inhibition were measured.

RESULTS AND DISCUSSION

After extraction hexane extract was concentrated to give 2.7% yield and 8.2% for methanol extract and 7.3% yield for aqueous extract. The result of the photochemical analysis in Table 1 shows that reducing sugar, Glycosides, cardiac glycosides, saponin, tannins and flavoniods were present in methanolic and aqueous extracts but non of these compounds were found in hexane extract. Alkaloid was found

Table - 2 : Antimicrobial activity of *Phyllantus amarus*

Test Organisms	Concentration g/cm ^{3}	Zone of Inhibition (mm) Aqueous Extract	MethanolicExtract	Hexane Extract
Klebsiella spp.	0.10	25	24	R
	0.05	11	10	R
	0.025	3	6	R
	0.01	1	2	R
Helicobacter pylori	0.10	18	15	R
	0.05	6	6	R
	0.025	3	4	R
	0.01	0.5	1	R
P. aeruginosa	0.10	19	20	R
	0.05	9	14	R
	0.025	5	10	R
	0.01	2	4	R
E.coli	0.10	18	20	R
	0.05	8	13	R
	0.025	3	7	R
	0.01	1	3	R
Shigella spp.	0.10	22	21	R
	0.05	12	11	R
	0.025	8	8	R
	0.01	4	3	R

KEY : R - Resistant

to be absent in both methanolic, aqueous and hexane extracts. Tannins have the properties of precipitating proteins and mucus and constricting blood vessels, this astringent action gives them value in controlling hemorrhage, dysentery, checking diarrhea[13]. The presence of these compounds in this plant is responsible for the treatment of dysentery and diarrhea.

The result of the antimicrobial activity in Table 2 shows that all five bacterial isolates used were sensitive to the methanolic and aqueous extracts of the plant with different diameter of zones of inhibition of different concentration. The difference in zone of inhibition shows how active the extracts are to the tested organisms. The least zone of inhibition was found in *Helicobacter pylori* with 15,6,4 and 1mm at 0.1,0.05,0.025,and 0.01g/cm^3 concentration in methanol extract and 18,6,3,and 0.5mm at 0.1,0.05,0.0250 and 0.01g/cm^3 concentration in aqueous extract. The highest value was found in *Klebsiella spp* with 25,11,3, and 1mm at 0.1,0.05,0.025,and 0.01g/cm^3 concentration for aqueous extract and 24,10,6,and 2mm at 0.1,0.05,0.025,and 0.01g/cm^3 concentration for methanolic extract. The hexane extract has resistance to the organisms and none of the compound tested for were found in the hexane extract, this shows that the " active compound" in the extract is located in the aqueous and the methanolic extracts which are known to be polar compounds and can be inferred here that the active compounds are present in the polar phase. The inhibitory activity of these aqueous and methanolic

extracts show their potential application in the treatment of microbial induced ailments due to the present of certain groups of compound such as flavonoids and tannins[14].

CONLUSIONS

Hexane extracts contain none of the compound tested for, and show no inhibitory activity against the pathogen tested for. This means that the 'active compound' is in the polar solvent (water and methanol). The water and methanol extracts were found to inhibit the growth of other pathogens apart from the one that has been known and used to cure i.e. dysentery and diarrhea in some part of Nigeria. This means that the inhibitory activity of the extract promises potential application in the treatment of induced ailment like gastric ulcer and wound infection.

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