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Research Article

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Phytochemical screening and antimicrobial activity of the solvents' fractionated leaves extract of *Olax subscorpioidea*

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ABSTRACT

A powdered sample of the leaves of Olax subscorpioidea was parculated with ethanol to produce a crude extract. Four fractions were marcerated from the crude extract with n-hexane, chloroform, ethyl acetate and methanol. Phytochemical result of the fractions confirmed the distribution of tannins, saponnins, flavonoids, alkaloids, reducing sugar and cardiac glucosides among the fractions. The antibacterial activities of the fractions were determined against Escherichia coli, Staphylococcus aureus, Salmonella typhi and Klebsiellapnuemoniae at 10mg/disc, 5mg/disc, 2mg/ml and 1mg/disc. The fractions indeed, demonstrated broad activity against all the test bacteria and the activities of the fractions were concentration dependent. The presence of the secondary metabolites in the plant sample and the activities of the plant fractions justified the use of the plant as an antibacterial therapy by traditional disease healers.

Keywords: *Olax subscorpioidea*, fractionation, Phytochemical screening, Agar disc diffusion Antimicrobial test

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INTRODUCTION

Medicinal plants are the richest and commonest natural resource used in traditional medicine. Of the 250, 000 higher plant species on earth, more than 80,000 are medicinal [1]. Although plants had been priced for their medicine, flavouring effect and aromatic qualities for centuries, but the synthetic products of the modern age had for some time surpassed their importance. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security [1].

Drug resistant infectious microbes have become an important public health concern warranting organizations in public and private sectors worldwide to work together [2]. Apart from the public health threat, the search for newer microbial sensitive treatments to overcome resistant microbes is usually very expensive and contributes to the higher costs of health care. The fact that newer treatment regime use more expensive pharmaceuticals and it also has side effect and demand longer hospital stays for infected individual [2]. Traditionally used medicinal plants were known to produce variety of compounds of known therapeutic properties [3]. The antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world [4]. These substances that can either inhibit the growth of pathogen or kill them and have least or no toxicity to host cells are considered candidates for developing new antimicrobial drugs [4]. Newer treatment regime use more expensive pharmaceuticals and demand longer hospital stays for infected individual [2]. However, People no longer depend on synthetic drugs and they are turning to the natural products with the hope of safety and security [1].

Olax subscorpioidea belongs to a family *Olacaceae*. It is called 'tsamiyarbiri' in Hausa. It can either be a shrub or a tree. It is up to 10 m or more in height. This plant is awidely distributed in Nigeria, Zaire and Senegal [5]. The ethanolic extract of the stem of *O. subscorpioidea* were evaluated and shown to have considerable activity on both fungi and bacteria isolates with zone of inhibition ranging from 7.2 mm to 21.5 mm [5]. Jaloos is a herbal preparation which comprises of the root *O.subscorpioidea* and parts of other plants. It is used in the management of

breast tumor in sourthern part of Nigeria [6]. Phytochemistry of the root of *O. subscorpioidea* revealed the presence of alkaloids, steroids, glycosides and terpenoids and the ethanolic root extract of the plant had shown potent antiulcer activity on mice that were induced with ulcer [7].

The traditional use of the different parts of the plant for medication and the empirical proof for some of the plant medicinal prompted this research to explore further the medicinal values of the plant.

EXPERIMENTAL SECTION

Plant Materials

The leaves of *Olax subscorpioidea*, were freshly collected at an uncultivated land in Damanko village about 9km west of Zaria main town, Zaria Local Government, Kaduna State. The plants were identified and authenticated by Mallam Umar ShehuGalla of the Herbarium unit, biological science, Ahmadu Bello Univesity, Zaria

Preparation of Plant Samples

The leaves of *O. subscorpioidea* were plucked from their stems and collected separately. The leaves were dried under-shade for seven days and ground into powder using clean pestle and mortar. The powdered sample was stored in a closed container and kept in the dark at room temperature until it was required for use [8].

Extraction and Fractionation of Plant Materials

A as part of the appropriate measure taken to guarantee that potential active constituents are not lost, altered or destroyed during the preparation of the extract [9], cold extraction (Percolation) was adopted in this research. The plant sample (200g) was percolated with 1000ml of ethanol for 14 days. Each extract was then decanted, filtered and concentrated using Rota vapor machine (RVO) at 40^0C. The concentrated crude ethanol extract was collected in a beaker and allowed to dry. The total extract was macerated with n-hexane for number of times. Each time, 30cm3 of n-hexane was added to the crude extract stirred gently for 5min. The process was repeated until a clear solution is obtained from the crude extract and the solutions obtained each time were collected in the same beaker and evaporated to dryness. The residue was further macerated consecutively using the same procedure with chloroform, ethyl acetate and methanol so that four fractions of varied polarity were obtained.

Phytochemical Screening of Plant Materials

The phytochemical analyses of the fractions were conducted by subjecting the fractions to different standard confirmatory tests. This is to determine the presence of certain phytochemical classes.

Test for Alkaloids

Each fraction (0.5g) was stirred with 5ml of 1 percent aqeous hydrochloric acid on a steam bath; 1ml of the filtrate was treated with a few drop of Mayer's reagent and a second 1ml portion was treated similarly with Dragendoff's reagent. Turbidity or precipitation with either of these reagents was taken as evidence for the presence of alkaloids in the extract being evaluated [10].

Test for Saponins

Each fraction (0.5g) was shaken with water in a test tube. Frothing which persists on warning confirmed the presence of saponins [11].

Test for Tannins

Each fraction (0.5g) was stirred with 10ml of water. This was filtered and ferric chloride reagent was added to the filtrate, a blue-black precipitate indicated the presence of tannins [12].

Test for Flavonoids

A portion of each fraction was heated with 10ml of ethylacetate over a steam bath for 3min. The mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow colouration indicated the presence of flavonoid [13].

Test for Reducing sugar

1ml of each fraction was taken in five separate test tubes. These were diluted with 2ml of distilled water followed by addition of Fehling's solution (A+B) and the mixtures were warmed. Brick red precipitate at the bottom of the test tube indicated the presence of reducing sugar [14].

Test for Cardiac glycosides
2ml of each fraction was placed in a sterile test tube. This was followed by adding 3ml of 3.5% iron III chloride ($FeCI_3$), then 3ml ethanoic acid. This gave a green precipitate and a dark coloured solution respectively. Finally, concentrated H_2SO_4 was carefully poured down the side of the test tube which resulted in the formation of brownish red layer, at the interface. This confirms the presence of cardiac glycosides.

Antimicrobial Activity Test
The technique adopted for the sensitivity test was agar disc diffusion described by [15].

Preparation of test fractions' concentration
From whatman's No 1 filter paper, discs of about 6mm diameter were punched using a paper puncher and batches of 10 of the paper discs were transferred into vial bottles and sterilized in an oven at 140^0c for 60 minutes.

200mg/ml of each fraction was dissolved in 2ml of DMSO (Dimethyl sulphoxide) to produce stock solutions of 100mg/ml. From a stock solution of each fraction, 0.1ml, 0.2ml, 0.5ml and 1.0ml were transferred into labeled vial bottles which were preoccupied with 10 paper discs using 1ml sterile syringe and the solution were subsequently diluted with 0.9ml, 0.8ml, 0.5mland 0.0ml (i.e. without dilution) of DMSO that correspondingly resulted to 1mg/disc, 2mg/disc, 5mg/disc and 10mg/disc concentration. The prepared solutions of varied concentrations of the test fractions in the labeled bottles were kept in refrigerator until required for use.

Test microorganisms
The test organisms used for this research were *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Klebsiella pnuemoniae* obtained from Microbiology unit of Aminu Kano Teaching Hospital (AKTH) Kano. The identities of the microorganism were confirmed by standard biochemical test [16]. The cultures of the test organism were maintained in a nutrient agar slant at 4^0C. The organism were then inoculated into nutrient broth and incubated overnight at 37^0C for 24 hrs. The test bacteria were then diluted with normal saline until they give concentration of bacterial cells equivalent to 0.5 McFarland standard of Barium sulphate solution (1% v/v) [17].

Antibacterial susceptibility test (Bioasssay)
A suspension of nutrient agar (28g in 1000ml of distilled water) was made and autoclaved at 121^0C for 15mins according to the manufacturers' instruction. It was then carefully poured into sterile petridishes and were allowed to solidify. The suspension was used to streak for the confluent growth of the bacteria on the surface of nutrient agar plates using sterile swap. Four paper discs of 10mg/disc, 5mg/disc, 2mg/disc, 1mg/disc concentrations were taken from the prepared test fraction solutions and were carefully and aseptically placed on the inoculated surface of the nutrient agar and a positive control disc (Tetracycline 1mg/disc) was placed at the centre of the plate. The plates were incubated invertedly at 37^0C for 18 hours. The diameters of clear areas surrounding the discs where growths of the organisms were impeded (Zone of inhibition) were measured in millimeter and recorded. The assay was repeated two more times. The mean and the standard deviation (±SD) for the triplicate values were then calculated

RESULTS AND DISCUSSION

Table 4.1: Fractions obtained from the leaves of *O. Subscorpioidea*

Fraction	Weight(g)	Yield(%)	Nature	Colour
n- hexane	0.70	2.82	Oily	Yellow
Chloroform	0.50	2.06	Gummy	Black
Ethyl acetate	1.29	5.20	Gummy	Black
Methanol	22.32	89.9	Gummy	Brown

Table 2: Results of phytochemical analyses of the fractions of *O.subscorpioidea*

Fractions	Saponins	Tannins	Flavonoids	Reducing sugar	Alkaloids	Cardiac glucosides
n-Hexane	+	+	+	-	+	+
Chloroform	-	+	-	+	+	+
Ethylacetate	+	+	+	-	-	+
Methanol	+	+	+	+	-	-
(+) indicates present while (-) indicates absent						

Table 3: Sensitivity test result of the leaves of *O. Subscorpioidea*

Fraction	Concentration mg/disc	Zone of inhibition (mm)			
		S. aureus	***E. coli***	***S. typhi***	***K. pneumoniae***
n- Hexane	1	10.50 ±0.43	10.90 ±0.70	08.80 ±0.42	08.85 ±0.35
	2	12.50 ±0.71	14.60 ±0.50	11.40 ±0.28	11.60 ±0.57
	5	16.10 ±0.14	18.45 ±0.64	14.85 ±0.36	14.55 ±0.78
	10	20.60 ±0.57	22.00 ±0.28	18.00 ±0.28	16.50 ±0.71
(+) Control	1	24.00 ±0.85	26.00 ±1.15	25.00 ±0.45	20.45 ±1.25
Chloroform	1	11.45 ±0.49	11.15 ±0.78	08.50 ±0.75	08.55 ±0.63
	2	14.15 ±0.49	13.65 ±0.49	12.25 ±0.35	12.25 ±0.35
	5	16.95 ±0.21	16.80 ±0.57	13.65 ±0.49	14.55 ±0.49
	10	20.60 ±0.57	19.05 ±0.21	16.55 ±0.49	17.50 ±0.42
(+) Control	1	25.80 ±0.25	27.05 ±0.35	24.85 ±0.24	21.00 ±0.34
Ethylacetate	1	11.80 ±0.57	09.80 ±0.43	08.80 ±0.28	11.40 ±0.57
	2	14.40 ±0.57	13.45 ±0.21	11.65 ±0.49	14.05 ±1.06
	5	17.30 ±0.42	16.80 ±0.57	13.85 ±0.64	16.60 ±0.71
	10	19.05 ±0.21	17.85 ±0.49	17.85 ±0.49	19.55 ±0.64
(+) Control	1	25.45 ±065	27.00 ±1.05	25.20 ±0.84	22 05 ±0.42
Methanol	1	13.20 ±1.13	12.70 ±0.85	10.85 ±0.92	11.95 ±0.21
	2	17.30 ±0.42	15.40 ±0.57	13.45 ±1.20	13.70 ±1.27
	5	19.50 ±0.71	19.35 ±0.49	17.80 ±0.28	18.35 ±0.92
	10	23.65 ±0.49	21.25 ±1.06	21.40 ±0.85	22.15 ±0.92
(+) Control	1	23.95 ±0.25	26.35 ±0.53	25.75 ±0.45	21.55 ±0.57

Mean of the triplicates ±S.D (standard deviation)

DISCUSSION

A total of 24.81g extract was yielded from 200g of the powdered sample. From marceration of the total extract with solvents of various polarities, methanol fraction has the highest percentage yield of 89.9% while chloroform fraction has the least percentage yield of 0.97% which is in line with report of several research works.

The phytochemical screening indicated the presence of some secondary metabolites in the plant fractions that account for the activities of the plant. This is in line with reports by scientists that plants have various biologically synthesized compounds that have pharmacological effects in the bodies of animals. Tannins that were reported to have anti-irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic effects were present in all the fractions. Plants that possess tannins are used to treat non-specific diarrhea, inflammations of mouth and throat and slightly injured skins [18] [19]. Flavonoids were contained in all the fractions except in chloroform fraction while cardiac glucosides which are used as lexative and carthatic drugs were confirmed in all the fractions except in methanol fraction. Alkaloids that act as antimalarial, anti-amoebic agents [20] were confirmed in n- hexane and chloroform fraction. The availability of the aforementioned secondary metabolites in the leaves' sample is supported by the report that the ethanol root's extract of the same plant contain among other metabolites alkaloids and glucosides [7].

The result of the bioassay showed that the fractions of the leaves' sample of the plant demonstrated abroad activity against the test organisms as the ethanol extract of the stem of *O. subscorpioidea* were evaluated and shown to have considerable activity on both fungi and bacteria isolates [5]. The activities of the sample fractions were found to be concentration dependent i.e. the activity of the plant fractions against the test organisms increases as the concentration of the fractions increases. The activities were also found to be comparatively more pronounced with methanol fraction of the sample which appeared to be most active among the test fractions against the microorganism and at almost all the concentrations. Methanol fraction recorded the highest activity of 23.65mm ±0.49 at 10mg/disc and 13.20mm ±1.13 at 1mg/disc against *S. aureaus* while n- hexane fraction recorded the lowest activity of 16.50mm ±0.71 at 10mg/disc against *K. pneumonia* and 08.50mm ±0.75 at 1mg/disc by chloroform fraction.

CONCLUSION

Highest yield of 89.9% fraction was obtained from methanol and since solvents dissolve solutes of their comparable polarity, it can be concluded that the plant sample contains more of polar compounds. The fact that the fractions of the leaves' sample of the plant contain some medicinally vital phytochemicals and also the remarkable activity the sample fractions exhibited against the four test organisms justified the popular use of the plant's leaves traditionally as a therapeutic agent for some bacterial ailments.

Recommendation

In view of the above, further study on the sample fractions of the plant can possibly lead to the isolation of compounds that if adequately modified, can serve as antibiotic drugs. It is also recommended that the plant sample be screened against other bacteria and also be tested for other medicinal parameters like antiparasite, antioxidant, antiulcer etc., in order to harness fully its medicinal potentials.

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