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Research Article

Phytochemical Screening and Antimicrobial Activity of *Hydnoraabyssinicia* Root Extract

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Abstract

This study was carried out in Khartoum state-Sudan, during March; this plant was collected from Al-Dubibat area, locality of algoze, South Kordofan State, western Sudan. The dried root of Hydnora abyssinicia was extracted successively with (petroleum ether, chloroform, and methanol), The phytochemical screening carried out for different plant roots extracts and showed that it contain high amount of alkaloids in all extract and moderate amount of flavonoids (in chloroform, methanol extracts) and moderate amount of tannins, sterol and triterpenes also moderate amount of cardiac glycoside and high amount of saponins. The antimicrobial activity of extracts were evaluated against four standard bacteria (Gram positive; Bacillus subtilis, Staphylococcus aureus) and (Gram negative; Escherichia coli, Pseudomonas aeruginosa).in addition of one standard fungi (Candida albicans).The result of antimicrobial tests indicated that the methanolic extract inhibited the growth of all microorganisms and most extracts showed same degree of antimicrobial activity. The result provides promising baseline information for the potential use of these crude extracts in the treatment of bacterial and fungal infections.

Keywords: Al-Dubibat area; Folk medicine; Medicinal plants; Phytochemical screening

Introduction

Sudan is the largest country in Africa, it has a wide diversity climate which is responsible for its varied vegetation and very rich flora. Many species of plants grow abundantly in the Sudan and other African countries and are used by the village populations for treatment of various disorders [1,2]. The Sudanese folkloric medicine represents a unique blend of indigenous cultures with Egyptian, Indian, Arabian, East and West African cultures [3,4]. In Sudan; plants are the main medicinal source to treat infectious diseases [5], in many developing countries like Sudan, the medicinal plants have played an important role in the treatment of diseases especially in rural areas. The medicinal and aromatic plants contain a number of chemical constituents such as alkaloids, flavonoids, tannins, saponins, glycosides and others isolated and used as an important source of indispensable drugs [6-8]. State that, medicinal plants are known by their required clinical effects on the abnormal living tissues or organs while toxic ones are known by their ability to cause a non-required physiological deviation in animals' bodies, the traditional medicinal plants are increase in both developing and industrialized countries [4,9-11] reported that both literate and illiterate people still use local plants as drugs in many conditions.

Many secondary metabolites of plant are commercially important and find use in a number of pharmaceutical compounds [12]. However, a sustained supply of the source material often becomes difficult due to the factors like environmental changes, cultural practices, diverse geographical distribution, labor cost and selection of the superior plant stock and over exploitation by pharmaceutical industry [13]. The species *Hydnoraabyssinicia* belong to family *Hydnoraceae* locally known as (tartous) was chosen because it's using traditionally in treatment of many abdominal diseases. Phytochemical activities were investigated to detect the effects of antimicrobial.

Objective

The objective of this study is to evaluate the phytochemical profile and the antimicrobial activities of *Hydnora abyssinica* root.

Material and Method

All the chemicals and reagents used in this study were of analytical grade such as chloroform, distilled water, ethanol, methanol, petroleum ether, acetic anhydride, sulphuric acid, gelatine salt, ferric chloride, reagents (Wagner, Hager, and Dragendorffs), aluminium chloride and potassium hydroxide.

Plant material, collection and identification

Hydnora abyssinicia were collected in Septembers 2015 from Al-Dubibat area, locality of algoze, South kordofan State- Sudan and identified in herbarium of natural research Centre and compared with herbarium of Faculty of Science University of Khartoum.

Preparation of crude extracts

50g of the dried roots was weighted and extracted successively with petroleum ether by shaker apparatus for four hours at room temperature. Then extracted with chloroform and was filtrated and dried after extraction, the residual of the powdered plants materials were dried and then extracted again with methanol for 18 hours. The extracts were air dried between each extraction 50g of the dried roots was weighted and extracted successively with petroleum ether by

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shaker apparatus for four hours at room temperature. Then extracted with chloroform and was filtrated and dried after extraction, the residual of the powdered plants materials were dried and then extracted again with methanol for 18 hours. The extracts were air dried between each extraction that has involved different solvents; each extract was filtrated through Whitman No 1 filter paper, followed by concentrated under vacuum room. The crude extracts were then kept at -20ºC in sterile universal bottles.

Preliminary Phytochemical screening of different extracts of the plant

General phytochemical screening for the active constituents was carried out for plant extracts using the methods carried by [14-16].

Antimicrobial activity

Preparation of nutrient agar media: 28g of powdered nutrient agar was weighted, dispersed in 1 liter of distilled water and allowed to soak for 10 minutes, swirl to mix then sterilized by autoclaving for 15 minutes at 121ºC cooled to 47ºC mixed well then poured into petri dishes.

Tested organisms

Bacterial organisms: *Bacillus subtitles* (NCTC 8236 Gram positive bacteria).

Staphylococcus aureus (ATCC 25923 Gram positive bacteria).
Escherichia coli (ATCC 25922 Gram negative bacteria).
Pseudomonas arginosa (ATCC 27853 Gram negative bacteria).

Fungal organisms: *Candida albicans* (ATCC 7596 Fungi).

In vitro testing of extract for antimicrobial activity

Testing for antibacterial activity: The cup-plate agar diffusion method [17] was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts. One ml of the standardized bacterial stock suspension 10^8-10^9 C.F.U/ml were thoroughly mixed with 100ml of molten sterile nutrient agar which was maintained at 45ºC. 20ml aliquots of the inoculated nutrient agar were distributed into sterile petri dishes. The agars was left to set and in each of these plates 5 cups (10mm in diameter) were cut using a sterile cork borer (No.5) and agar disk were removed. Alternate cups were filled with 0.1ml sample of each of the extract dilution in methanol using automatic micro liter pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37ºC for 18 hours. Three replicates were carried out for each extract against each of the test organism. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and mean values were tabulated.

Testing for antifungal activity: The same method for bacteria was adopted. Instead of nutrient agar. The incubated medium was incubated at 25°C for two days for the *candida albicans.*

Result and discussion

Phytochemical screening of *Hydnoraabyssinicia* and physical properties

Three solvents were used in successive polarities to extract secondary metabolites from *Hydnora abyssinicia* and their properties were cited in table 1.

Table 1 reported the result of extractives values of *Hydnora abyssinicia* as following for methanol 3.22% (Dark brown powder) followed by chloroform 1.24% (brown powder), petroleum ether 0.86% (brown powder) (Table 2).

Extracts	Characteristic	Colour of Extract	Weight	Yield
Methanol	Powder	Dark brown	6.44	3.22
Chloroform	Powder	Brown	2.48	1.24
Petroleum Ether	Powder	Brown	1.92	0.86

Table 1: Properties and extractives values of *Hydnoraabyssinicia* extract root Preliminary phytochemical screening of extract from *Hydnoraabyssinicia*.

Secondary Metabolite	Test	Successive Method of Extraction		
		Methanol	Chloroform	Petroleum
Alkaloids Acidic	H	-	-	-
	D	-	-	-
	M	-	-	-
	W	-	-	-
Alkaloids Basic	H	+++	+++	+++
	D	++++	++++	++++
	M	+++	+++	+++
	W	+++	+++	+++
Flavonoids	KOH	-	++	+++
	NH_4OH	-	++	+++
	ALCL3	-	++	+++
	Mg	-	-	-
Saponins	Foam test	+++	+++	+++
Coumarine	KOH/UV	-	-	-
Sterols & Triterpenes	Liebermann's	-	++	+++
	Salkowski	-	++	+++
Tannins	Ferric chloride test	-	-	+++
	Gelatin test	-	-	+++
Cardiac glycosides	Glacial acetic acid	+	+	+
Anthraquinone	Chloroform with NH_3OH	-	-	-

Table 2: Result of phytochemical screening.

Key: Very high = (++++), High = (+++), Moderate = (++), Trace amount = (+) and absent = (-).

Hydnoraabyssinicia roots extract contain high amount of alkaloids, high amount of tannins in methanol extract and moderate amount of flavonoids, and triterpenes, and trace amount of cardiac glycosides and high amount of saponins.

The extract of *Hydnoraabyssinicia* root at concentrations (100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml), were subjected to antimicrobial tests by using cup plate agar diffusion method and inhibition zone were measured in (mm) against four bacterial strains and one fungi. The range of inhibition was found 11-25mm (Table 3).

Phytochemical screening of *Hydnoraabyssinicia*

The phytochemical screening were carried out on different extracts of *Hydnoraabyssinicia* roots extracts and they showed to contain high amount of alkaloids, high amount of tannins in methanol

extract , moderate amount of flavonoids, and triterpenes, trace amount of cardiac glycosides and high amount of saponins.

Extract	Concentration in mg/ml	Zone of inhibition in diameters (mm)				
		E.c	P.a	S.a	B.s	C.a
Methanol	100	19	-	20	25	19
	50	18	-	17	24	18
	25	17	-	14	23	16
	12.5	12	-	12	22	15
Chloroform	100	17	-	-	22	17
	50	16	-	-	20	14
	25	15	-	-	19	10
	12.5	11	-	-	18	10
Petroleum Ether	100	-	-	-	20	16
	50	-	-	-	19	15
	25	-	-	-	17	13
	12.5	-	-	-	15	10

Table 3: Result of antimicrobial activities.

Key: *B.s, Bacillus subtilis; S.a, staphyllo coccus aureus; E.c, Escherichia coli; P.a pseudomonas aeruginosa; C.a Candida albicans*; Concentration of extracts (100, 50, 25, 12.5mg/ml) .Zone of inhibition in (mm), - no inhibition, <9mm inactive, 9-12mm partially active, 13-18mm active, >18mm very active. The methanol extract showed high inhibition zone against four tested microorganisms (E.c, S.a, B.s, and C.a).

Antimicrobial activities of *Hydnoraabyssinicia*

The methanol extract showed high activity at all concentrations (100,50,25,12.5) against *bacillus subtilis* (25,24,23,22),low activity against E.c (19,18,17,12) respectively as well as *staphylococcus aureus* (20,17,14,12), and low activity against *candida albicans* (19,18,16,15), Chloroform extract cited low activity against E.c (17,16,15,11), as well as *candida albicans* (17,14,10,10) and high activity against *bacillus subtilis* (22,20,19,18),Petroleum ether extract showed high activity against *bacillus subtilis* (20,19,17,15) ,show low activity against *candida albicans* (16,15,13,10). This activity is due to presence of phytoconstituents present in roots extracts mainly saponins and phenolic compounds which was confirmed by phytochemical tests. African medicinal plants are well tested for their antimicrobial activity this activity is due to phytochemical class such as saponin, flavonoid, tannins and phenolic compounds (16).

Conclusion

Antimicrobial resistance is reported to be on the increase due to gene mutation of the disease pathogens. *Hydnora abyssinicia* was chosen for this study because of their reputation in folklore medicine as antimicrobial agents and usage in many diseases, this agreed with [15]. Phytochemical screening was carried out and lead to presence of some secondary metabolites the plant was showed to contain alkaloids, flavonoids, tannins, saponins, sterol, triterpenes, and cardiac glycosides. The crude extract was subjected to antimicrobial assays using cup plate diffusion method and the inhibition zone was measured in mm. The methanol extract gave good result against four tested microorganisms (E.c, S.a, B.s, and C.a).The petroleum ether extract showed absence of inhibition zone against four bacterial strains, and show low activity against *candida albicans*.

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