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Evaluation of antimicrobial, antioxidant and phytochemical screening of some Sudanese medicinal plants

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Abstract
The present study was carried out to investigate possible antibacterial; antioxidant activities and phytochemical screening of ethanol extract some Sudanese medicinal plants. Phytochemical analysis of the crude extract was performed to detect presence of different kinds of phytoconstituents. The antibacterial activity was investigated against two Gram positive and two Gram negative bacteria and two fungi by using disc diffusion method. All extracts showed moderate or week antimicrobial activity against all strains used except *M. pigra* extract, which showed promising activity. Antioxidant activity showed that M. pigra was the most active one (85±0.05) compare to the other sample and standard propyl gate (83±0.05). Pytochemical screening showed the presences and absence of the secondary metabolites among all sample tested.

Keywords: antioxidant, phytochemical, antimicrobial

Introduction
'Health is dearer than wealth'; so, the value of medicinal plants is more than what it is in the marketplace, i.e. it can be said to be essentially nfinite. Human beings have been utilizing plants for basic preventive and curative health caresince time immemorial (Holley & Cherla, 1998) [8]. As many as 35,000 – 70,000 species of planthave been used at one time or another for medicinal purposes (Farnsworth & Soejarto, 1991) [5]. From historical records, the human use of plants or floral parts to enhance physicaland spiritual well-being goes back thousands of years and is difficult to date precisely. However, from those records, it is apparent that most of the early people, such as the Assyrians, Babylonians, Egyptians and ancient Hebrews, were familiar with the properties8and use of many medicinal plants (Ghani, 2003) [6]. The practice of medicine using medicinal plants flourished most during the Greek civilization, when historical personalities like Hippocrates (born 460 BC) and Theophrastus (born 370 BC) practiced herbal medicine.
Medicinal plants grow naturally around us. Over centuries, cultures around the world have learned how to use plants to fight illness and maintain health. These readily available and culturally important traditional medicines form the basis of an accessible and affordable health-care regime and are an important source of livelihood for indigenous and rural populations. Increasingly, medicinal species that reside in natural areas have received scientific and commercial attention (Marinelli, 2005) [11].
Sudan has a wide diversity climate which is responsible for its varied vegetation and very rich flora. In Sudan as well as elsewhere, people prefer to use traditional medicine rather than synthetic drugs, because herbs can easily be obtained from nature and are cheap and have less side effects. It is estimated that Sudan encompasses more than 3156 plant species belonging to 1137 genera and 170 families (El Amin 1990). Many authors studied different biological activities of many Sudanese medicinal plants such as hepato-protective, antimicrobial activity, anti-parasitic, anti-diabetic, antioxidant and antiinflamatory (Ali *et al.*, 2011, Sadaabi and Moglad 2011, Ahmed *et al.*, 2010. Taha *et al.*, 2010) [3, 16, 2, 20] and (Musa *et al.*, 2011) [14].

Materials and Methods
Plant Material
All plant samples were collected from Shendi's area (Northern Sudan) expect *Vangueria magagscariensis* which collected from Southern Kordufan, identified by Dr. Waiel Elsadig (taxonomist of the Medicinal and Aromatic Plant Research institute) and herbarium vouchers were deposited at the herbarium of Medicinal and Aromatic Plants Research Institute.

Table 1: Selected Samples

Sample	Family	Vernacular name	Part used	Traditional uses
Heliotropium hypercam	Boraginaceae	Danab alagrab	whole plant	Animal wound healing
Heliotropium sudanicum	Boraginaceae	Danab alagrab	whole plant	Animal wound healing
Mimosa pigra	Mimosaceae	Alfas	fruits	Asthma
Solaen stemma argrl (Del.)	Asclepiadaceae	Alhargel	seeds	Infection, and abdominal pain
Vangueria madagascariensis	Rubiacea	Alkerker	seeds	Infection dieses

Preparation of crude ethanolic extracts

Extraction was carried out according to method described by Sukhdev *et al.* (2008): 50 g of each botanical coarsely powdered sample was extracted by soaking in one liter of 80 % ethanol (national distillation company, Sudan) for about seventy two hours with daily filtration and evaporation. Solvent was evaporated under reduced pressure to dryness using rotary evaporator apparatus (buchii, Switzerland) and the extract allowed to air till complete dryness and the yield percentages were shown in table (2). Extracts were stored in ambulant bottles in a refrigerator till used.

Phytochemical Screening

Phytochemical screening for the active constituents was carried out using the methods described by (Martinez & Valencia (1999) [13], Sofowora (1993) [18], Harborne (1984) [7] and Wall *et al* (1952) [21].

Test of tannins

0.5 g of the extract was washed three times with petroleum ether (S. D. Fine, India), dissolved in 10 ml hot saline solution and divided in two tests tubes. To one tube 2-3 drops of ferric chloride reagent was added and to the other one 2 – 3 drops of gelatin salts reagent was added. The occurrence of a blackish blue colour in the first test tube and turbidity in the second one denotes the presence of tannins.

Test of sterols and triterpenes

0.5 g the extract was washed three times with petroleum ether and dissolved in 10 of chloroform (S. D. Fine, India). To 5 ml of the solution, 0.5 ml acetic anhydride (Sharlu, Spain) was added followed by 3 drops of conc. Sulphuric acid (Sharlu, Spain) at the bottom of the test tube. At the contact zone of the two liquids, a gradual appearance of (green to blue) color was taken as an evidence of the presence of sterols and (pink to purple) colour for presence of triterpenses in the sample.

Test for Alkaloids

0.5 g of the extract was heated with 10 ml of 2 N Hcl (Sharlu, Spain) in water bath, stirred for about 10 minutes, cooled, filtered and divided into two test tubes. To one test tube few drops of Mayer's reagent was added while to the other tube few drops of Valser's reagent was added. A slight turbidity or heavy precipitate in either of the tow test tubes was tanked as presumptive evidence for the presence of alkaloids.

Tests for Flavonoids

0.5 g of the extract was washed three times with petroleum ether, dissolved in 30 ml of 80% ethanol. The filtrate was divided in three test tubes and used for following tests: -

A/ to 5 ml of the filtrate in a test tube 1ml of 1% aluminum chloride solution was added. Appearance of a yellow color indicated the presence of Flavonoids.

B/ to 5 ml of the filtrate in a test tube 1ml of 1% potassium hydroxide solution was added. Appearance of a yellow color indicated the presence of Flavonoids.

C/ to 5 ml of the filtrate in a test tube 1ml of 10 % lead acetate solution was added. Appearance of a yellow color indicated the presence of Flavonoids.

Test for Saponins

0.5 of the extract was placed in a clean test tube. 10 ml of distilled water was added, vigorously shaken for about 30 seconds. Tube was then allowed to stand and observed for the formation of foam, which persisted for least an hour, was taken as evidence for presence of saponins.

Test for Cumarins

0.5 g of the extract dissolved in 10 ml distilled water in test tube and filter paper attached to the test tube to be saturated with the vapor after a spot of 0.5N KoH spotted on it. Then the filter paper was inspected under UV light, the presence of coumrins was indicated if the spot have found to be adsorbed the UV light.

Test for Anthraquinone glycoside

0.5 g of thee extract was boiled with 10 ml of 0.5N KoH containing 1ml of 3% hydrogen peroxide solution. The mixture was extracted by shaking with 10 ml of benzene (S. D. Fine, India). To 5 ml of the benzene solution 3 ml of 10 % ammonium hydroxide solution was added and the two layers were allowed to separate. Appearance of pink or red color in the lower layer indicated the presence of anthraquinones.

Test for cyanogenic glycoside

0.5 g of the extract was placed in Erlenmeyer flask and sufficient distilled water was added to moisten the sample, followed by 1ml of chloroform. A piece of freshly prepared sodium picrate paper was carefully inserted between a split crock which was used to stopper the flask, a change in color of the sodium picrate paper from yellow to various shades of red was taken as an indication of the presence of cyanogenic glycoside.

Results of phytochemical screening are shown in table (3).

Antimicrobial activity

Tested microorganisms

Gram positive bacteria *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC25923), gram-negative bacterial strains *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), and two fungal strains *Apergillus niger* (ATCC 9763) and *Candida albicans* (ATCC 7596) were used. Bacterial and fungal strains used in the study were obtained from the Department of Microbiology, of the Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI) and National Health Laboratory of Khartoum in Sudan. The bacterial cultures were maintained on nutrient agar and incubated at 37°C for 18 h and then used for the antimicrobial test.

***In vitro* testing of extracts for antimicrobial activity**

Testing for antibacterial Activity

The cup-plate agar diffusion method (Kavanagh, 1972) was adopted with some minor modifications to assess the antibacterial of the prepared extracts. One ml of the standardized bacterial stock suspension 10^8–10^9 C.F.U/ ml were thoroughly mixed with 100 ml of molten sterile nutrient agar which was maintained at 45 ºC. 20 ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-

dishes. The agars was left to set and in each of these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were removed. Alternate cups were filled with 0.1 ml sample of the oil using automatic microlitre pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 ºC for 18 hours. Two replicates were carried out for the oil against each of the tested organisms. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were shown in table (4).

Testing for antifungal activity

The same method as for bacteria were adopted, instead of Nutirent agar, Sabouraud dextrose agar was used the inoculated medium was incubated at 25ºC for two days for *Candida albicans* and three days for *Aspergillus niger*. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were shown in table (4).

Antimicrobial activity of standard reference drugs

Four standard reference drugs (ciprofloxacin and gentamicin for antibacterial and nystatin and clotimazole for antifungal were used. Different concentrations were prepared by dissolving specific weight of each drug in specific volume of distilled water and tested against standard organism using the same method above. Results are shown in table (5).

Antioxidant Activity

DPPH radical scavenging assay

The DPPH free radical scavenging activity Principle: The antioxidant activity was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. Experiments were carried out according using the method of Shimada *et al*. (1992), with slight modification. Active samples can reduce the stable radical DPPH to the yellow-colored diphenyl- picrylhydrazine.

Assay

Test samples were allowed to react with 2.2 di (4-tretoctylphenyl)-1-picrylhydrazyl stable free radical (DPPH) for half an hour at 37oc in 96-wells plate. The concentration of DPPH was kept at (300µM). The test sample was dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using multiplate reader spectrophotometer. Percentage of radical scavenging activity of the sample was determined in comparison with a DMSO treated control. All tests were conducted in triplicate. Results are shown in table (6).

DPPH radical scavenging (%) = 100-{(Ac-At)/Ac} × 100

Where, At= Absorbance value of test compound; Ac=Absorbance value of control.

Results and discussion

Table 2: Yield percentages of crude ethanolic extracts

Sample	Weight of sample	Weight of extract	Yield percentage	Color	Texture
H. egyptyacium	50 g	9.8 g	19.6 %	green	gummy
H. sudanicum	50 g	9.2 g	18.4 %	green	gummy
M. pigra	50 g	8.9 g	17.8 %	brown	powder
S. argel	50 g	3.611 g	7.222 %	brown	gummy
V. madgascariensis	50 g	8.6 g	17.2 %	brown	gummy

Table 3: Results of phytochemical screening

Sample Test	*S. argel*	*H. egyptyacium*	*H. sudanicum*	*M. pigra*	*V. madgascariensis*
Alkaloids	+	+++	++	-	+
Anthraqunone glycosides	-	-	-	-	-
Cummarins	++	++	++	+	+
Cyanogenic glycosides	-	-	-	-	-
Flavonoids	+	+++	++	+++	+
Saponins	++	++	+	++	++
Sterols	+	+++	++	+	-
Taninns	+	++	++	++	+
Triterpens	+++	++	+	+	++

Key: + Trace, ++ Moderate, +++ High, Negative.

Table 4: Antimicrobial activity of different sample extracts against Standard Organisms using (100 mg/ml)

Sample	Standard tested organisms* /M.D.I.Z (mm)**					
	E. c	***Ps.* a**	***S. a***	***B. s***	***C. a***	***A. n***
H. egyptyacium	12 - 14	11 - 11	11 - 11	13 - 14	-	13 - 14
H. sudanicum	-	-	-	12 - 14	-	11 - 11
M. pigra	16 -17	18 - 20	18 - 19	17 -17	16 - 16	17 18
S. argel	11 - 11	14 - 15	14 -15	13 - 15	12 -12	12 - 13
V. mad gascariensis	-	-	14 - 15	15 - 15	11 -11	11 - 12

*Standard organisms tested: *B.S. = Bacillus subtilis, S.a. =Staphylococcus aureus, E.c. = Escherichia coli, Ps.a. = Pseudomonas aeruginosa, A.n= Aspergillus niger, C.a= Candida albicans* MeOH=Methanol. P.E=Petrolium ether ** M.D.I.Z=: Mean diameter of growth inhibition zone in (mm). Result: >18 mm: Sensitive, 14 to 18 mm: Intermediate: <14 mm: Resistant. (-): No inhibition zone.

Table 5: Antimicrobial activity of standard drugs

Drugs	Concentrations (µg/ml)	Standard microorganisms used			
		Gram positive		Gram negative	
		Tested bacteria used			
		Mean Diameter of Growth Inhibition Zone (mm)			
		Bacillus subtilis	***Staphyococcus aureus***	***Escherichia coli***	***Pseudomonas aeruginosa***
Ampicillin	40	15	25	-	16
	20	14	20	-	13
	10	13	18	-	12
	5	12	15	-	-
Gentamicin	40	29	35	32	23
	20	22	33	30	22
	10	20	30	17	21
	5	17	28	-	19
Tested fungi used (M.D.I.Z. mm)					
		Apergillus niger		*Candida albicans*	
Clotrimazole	40	30		42	
	20	22		40	
	10	19		33	
	5	16		30	
Nystatin	50	28		17	
	25	26		14	
	12.5	23		-	

Table 6: Antioxidant activity of crude ethanolic extracts

No.	Sample	%RSA ±SD (DPPH)
1	*H. egyptyacium*	65±0.05
2	*H. sudanicum*	62±0.02
3	*M. pigra*	85±0.05
4	*S. argel*	36±0.02
5	*V. mad gascariensis*	74±0.05
6	Propyl galate	83±0.05

Results showed that difference between yield percentages of all sample under study. The highest yield was of *H. egyptyacium* (19.6 %) followed by *H. sudanicum* (18.4 %), *M. pigra* (17.8 %), *V. madgascariensis* (17.2 %) and at last of *S. argel* (7.2%). Yield percentages of extract depends mainly on the part of sample used and amount of extractable matter contained. *H. egyptyacium* and *H. sudanicum* showed week antimicrobial activity against all strains used and these results found to be on line with Elegami *et al*. (2001) [4], who reported that the methanolic extract of *H. egyptyacium* and *H. sudanicum* showed moderate activity against bacteria and fungi. *M. pigra* extract showed potent antimicrobial activity against all strains used and these resultsfound to be in agreement of Abdalla,. (2004) [1] and Mbatchou *et al* (2011) [12], who studied the antibacterial activity of *M. pigra* leaves different extracts. Methanolic extract showed the highest activity anti bacterial activity against organism used. *S. argel* and *V. mad gascariensis* showed week and moderate activity. *M. pigra* extract showed the highest antioxidant activity (85±0.05), followed by *V. madgascariensis* (74±0.05), *H. egyptyacium* (65±0.05), *H. sudanicum* (62±0.02) *and S. argel* (36±0.02). No literature about the antioxidant activity of *M. pigra*, *H. egyptyacium* and *H. sudanicum*. *V. madgascariensis* seeds extract showed good antioxidant activity (74±0.05) and the result obtained in this study found to be on line with sara *et al.* (2017) [17]. Phytochemical screening of *H. aegyptyacium* and *H. sudanicum* showed the presence of alkaloids, cumarins, flavnoids, saponins, triterpenes sterols, and tannins while anthraqunones glycosides and cyanogenic glycosides were absent. Content of alkaloid, tannins, sterols and flavnoids found to be high in *H. aegyptyacum.* This result is on line with many authors who reported that genus heliotropiym contains alkaloids, sterols, tannins, flavnoids and saponins Singh and Dubey (2011) and Jain and Singh (2001) [10]. *M. pigra* phytochemical screening showed the presence of tannins, flavonoids, sterols, triterpens, cumarins and saponins while, alkaloids, anthraquinones glycosides and cyanogenic glycosides gave negative results. These results are in a agreement with the findings of Mbatchou *et al* (2011) [12] and Oladosu *et al* (2015) [15] who reported that the whole plant contains alkaloids, amino acids, anthraquinones, flavonoids, general glycoside, saponins, sterols, tannins and triterpenoids. *S. argel* extract showed the presences of alkaloids, sterols, triterpenes, cumarins, flavnoids, tannins and saponins while anthraquinones glycosides and cyanogenic glycosides were absent. These results are on line with the previous studies of the other different parts of the plants phytochemical screening. *V. mad gascariensis* screening resulted the presences of alkaloids, flavonoids, tannins, saponins, cumarins and triterpens, while anthraquinones glycosides, sterols and cyanogenic glycosides were absent.

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