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ANTIMICROBIAL AND PHYTOCHEMICAL SCREENING OF *CORDIA AFRICANA* IN SUDAN

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ABSTRACT

The present study was conducted to investigate the antimicrobial activity and phytochemical screening for different parts (leaves, stem, park and fruit) of *Cordia africana*. The extracts of *C. africana* was screened for its antimicrobial activity against four standard bacteria, two Gram-positive bacterial strains (*Bacillus subtilis* and *Staphylococcus aureus*), two Gram-negative bacterial strains (*Escherichia coli* and *Pseudomonas aeruginosa*) and two fungal strains (*Apergillus niger and Candida albicans*) using the cup plate agar diffusion method. The methanol extract of *C. Africana* extracts exhibited inhibitory effects against most of the tested organisms with zone of inhibition ranging from (11-30 mm). Phytochemical investigation for the methanolic extracts showed the presences of cumarins, saponins, sterols and triterpens, while anthraquienones glycosides, alkaloids and cyanogenic glycosides were absenced. In conclusion: These studies conducted for both *C. africana* in the treatment of bacterial and fungal *in vitro*.

Keywords: *In vitro*, antimicrobial activity, phytochemical screening, *C. Africana*, in Sudan.

I. INTRODUCTION

Natural products are, generally, either of periodic origin or originate from microbes, plants, or animal sources (Nakanishi, 1999). As chemicals, natural products include such classes of compounds as terpenoids, alkaloids ,flavonoids polyketides, amino acids, peptides, proteins

,carbohydrates, lipids, nucleic acid bases, ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and so forth (Jarvis, 2000).

Medicinal plants are plants or plant parts or its exudates having medicinal properties. In fact, it is the chemical constituents in plants that yield the medicinal prosperities (Maryum, 2004). Like other developing countries, Sudanese traditional medicine represents a unique blend of indigenous cultures with Islamic, Arabic and African traditions. Consequently, a variety of diseases –epidemic and endemic – are known. To face them, people have tapped the environmental resources, e.g. plants, minerals and animal products for the management of health (El-Hamidi, 1970; Banthorpe *et al*., 1976; Antoun *et al*.,1977; Antoun *et al* ,1981; El Sheikh *et al*.,1982).

Herbal drugs are of major importance in Sudanese traditional medicine. Floristic studies (Broun *et al*, 1929, Andrews 1950, 1952, 1956, El Amin 1990) in sudan revealed that more than 3156 species belonging to 1137 genera and 170 families exist. The documentation of medicinal plants of sudan was performed by Medicinal and Aromatic plants Research Institute (MAPRI), where the medicinal plants of certain districts were published ; Erkawit , Nuba Mountains ,White Nile ,North kordofan , and of Angasana (EL Ghazali, 1986; EL Ghazali et al.,1987;1994;1997;2003, respectively).

Sudanese medicinal plants have been reported as a source of antibacterial and antiviral agents (Ietidal *et al*., 2010), while (koko *et al*., 2000) reported the fasciolicidal properties of some of these plants. Comprehensive studies of antimicrobial properties of Sudanese medicinal plants were conducted by (Almagboul *et al*., 1995).

Used of *C. Africana* firewood, timber (furniture, beehives, boxes, mortars, church, drums),food (fruit),medicine (bark, roots), fodder (leaves), bee forage ,mulch , soil conservation , ornamental, shade. (Bein *et .al* 1996). The present study was conducted to investigate the antimicrobial activity and phytochemical screening of *C. Africana* in Sudan.

II. MATERIALS AND METHODS

Plant materials: Different parts of *C. Africana* sample were collected from Algalabat (East of Sudan), authenticated by Dr. Hayder Abd Algader and Herbarium specimens were deposit at the Herbarium of Medicinal and Aromatic Plants Research Institute.

Preparation of crude extracts: Extraction was carried out according to method described by Sukhdev et. al. (2008): 500 g of each part was coarsely powdered using mortar and pestle. Coarsely powdered samples were successively extracted with petroleum ether and 80 % methanol using shaker extractor apparatus. Extraction carried out for about three days with daily filtration and evaporation the solvent for petroleum ether and five days for methanol. Solvents were evaporated under reduced pressure using rotary evaporator apparatus. Finally extracts allowed to air in Petri dishes till complete dryness and the yield percentages were calculated as followed:

Weight of extract obtained / weight of plant sample X100

Fractionation of methanolic extract: 50 g of leaves and bark methanolic extract were separately dissolved in 500 ml of distilled water and shacked, three times with 100 ml of chloroform in each time using separator funnel. Chloroform layers were combined together and evaporated under reduced pressure using rotary evaporator apparatus. Aqueous layers were then re-shacked three times with 100 ml of ethyl acetate in each time using separator funnel Ethyl acetate layers were combined together and evaporated under reduced pressure using rotary evaporator. Aqueous layers were lyophilized using freeze-drier machine till dryness and the yield percentage of each fraction were calculated.

Phytochemical screening of the crude extracts and fractions: Methanolic extracts of each part, which showed the highest antimicrobial activity were subjected to qualitative chemical screening for the identification of the various classes of phyto-constituents using methods described (Martinez & Valencia (1999), Sofowora (1993), Harborne (1984) and Wall et al (1952)The screening was covered for alkaloids, saponins, flavonoids, tannins, sterols, triterpenes, cumarins and anthraquinone.

Test of Alkaloids: 0.5 g of each extract was dissolved in 2 ml of 2N HCL, stirred while heating in water bath and for 10 minutes, cooled, filtered and divided into two test tubes. To one test tube a few drops of Mayer’s reagent were added, while to the other tube a few drops of Valser’s reagent were added. A slight turbidity or heavy precipitate in either one or the two test tubes was taken as presumptive evidence for the presence of alkaloids.

Test of Saponins: 0.5 g of each extract was placed in a clean test tube and 10 ml of distilled water was added. The tube was stoppered and vigorously shaken for 30 seconds, then allowed to stand for 15-20 minutes, and classified for saponins content as follows: no froth =

negative ; froth less than 1 cm = weakly positive; froth 1cm = medium; froth 1-2 cm = high positive; and froth greater than 2 cm = strong.

Test of Flavonoids: 0.5 g of each extract was dissolved in 15 ml of 96% ethanol and filtered. The filtrate was used for the following tests:

a) To 3 ml of the filtrate in a test tube, 1 ml of 1% aluminium chloride solution in methanol was added .Formation of a yellow colour indicated the presence of flavonoids.
b) To 3 ml of the filtrate in a test tube, 1 ml of potassium hydroxide solution was added. A dark yellow colour indicated the presence of flavonoids compounds.
c) To 3 ml of filtrate, 0.5 ml of concentrated HCL and a few magnesium turnings (0.5 g) were added .Production of definite color change to pink or red was taken as presumptive evidence that flavonoids compounds were present in the plants sample.

Test of Tannins

0.5 g of each extract was stirred with 10 ml of hot saline solution. The mixture was cooled and filtered. About 5 ml of this solution was treated with few drops of the Gelatin-salt reagents. Formation of an immediate precipitate was taken as evidence for the presence of tannins. Positive test confirmed by the addition of few drops of 1% FeCL3, test reagent to another portion of the solution and should result in a characteristic blue, blue-black, green or blue-green colour and precipitate.

Test of Sterols and triterpenes

0.5 g of each extract was dissolved in 10 ml chloroform, 0.5 ml acetic anhydride (0.5 ml) was added and the solution was transferred into a dry test tube. Few drops of concentrated sulphuric acid were poured carefully down the walls of the test tube so as to form a lower layer. Brownish-red or violet ring at the zone of the contact with supernatant and green or violet coloration denoted the presence of sterols and /or triterpenes pink to purple.

Test of Coumarins

0.5 g of each extract was dissolved in 10 ml distilled water in test tube and filter paper was attached to the test tube to be saturated with the vapor after a spot of 0.5N KOH was put on it. Then the filter paper was inspected under ultra violet (UV) light, the presence of coumrins was indicated if the spot was found to adsorbe the ultra violet (UV) light.

Test for Anthraquinone glycosides: 0.5 g of each 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. A 5ml of the benzene solution was shacked with 3ml of 10% ammonium hydroxide solution and the tow layers were allowed to separate. The presence of anthraquinones was indicated if the alkaline layer was found to have assumed pink or red color.

Antimicrobial activity of *C. Africana* extracts

Test microorganisms: The antibacterial activity of plants extract was assessed against four bacterial species: Gram positive (+ve) bacteria *Staphylococcus aureus* (ATCC 25923 American Type Culture Collection, Rockville, Maryland, USA) and *Bacillus subtilis* (NCTC 8236 National Collection of Type Culture, Colindale, England) and two Gram negative (-ve) bacteria: *Pseudomonas aeruginosa* (ATCC 27853 American Type Culture Collection, Rockville, Maryland, USA) and *Escherichia coli* (ATCC 25922), in addition to two fungal species: *Candida albicans* (ATCC 7596 American Type Culture Collection, Rockville, Maryland, USA) and *Aspergillus niger* (ATCC 9763 American Type Culture Collection, Rockville, Maryland, USA).

***Preparation of bacterial suspensions*:** One ml aliquots of a 24 h broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 h. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8 to 10^9 CFU/ml. The suspension was stored in the refrigerator at 4°C till used.

The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (Miles *et al*; 1938). Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37°C for 24 h. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension. Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

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 activity of the prepared extracts. One ml of the standardized bacterial stock suspension 10^8 to 10^9 CFU/ml were thoroughly mixed with 100 ml of molten sterile nutrient agar which was maintained at 45°C. 20 ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes. The agar was left to set and all of these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were removed. The cups were filled with 0.1 ml sample of each extracts using automatic microlitre pipette, and allowed to diffuse a room temperature for two hours. The plates were then incubated in the upright position at 37°C for 18 h. Two replicates were carried out for each extract against each of the test organisms. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were tabulated.

Testing for antifungal activity: The same method as for bacteria was adopted. Instead of nutrient agar, Sabouraud dextrose agar was used. The inoculated medium was incubated at 25°C for two days for the *Candida albicans* and three days for *Aspergillus niger.*

III. RESULTS

Table (1): Yields percent of successive extracts

Sample	Weight of sample	Petroleum ether		Methanol	
		Weight of extract	Yield %	Weight of extract	Yield %
Bark	500 g	1.16 g	0.232	33.6 g	6.72
Leaves	500 g	28.342 g	5.668	78.776 g	15.755
Fruits	500 g	7.985 g	1.597	16.74 g	3.348
Stem	500 g	3.94 g	0.788	62.76 g	12.552

Table (2): Yields percents of Fractionation

Sample	Chloroform		Ethyl acetate		Aqueous	
	Weight of extract	Yield %	Weight of extract	Yield %	Weight of extract	Yield %
Leaves	4.868g	9.736	3.219g	6.438	25.6g	12.8
Stem	1.854g	3.708	4.558g	9.116	38.4 g	19.2

Table (3): Phytochemical screening results for methanolic extract for different parts of plant

No.	Test	Part used			
		Bark	Fruits	Stem	Leaves
1	Saponins	+	+	+++	+
2	Coumarins	+	+	++	+
3	Tannins	++	+	+++	+
4	Alkaloids	-	-	-	-
5	Sterol	-	-	+	-
6	Triterpenes	++	+	++	+
7	Flavonoids	+++	+	+++	+
8	Cyanogenic glycosides	-	-	-	-
9	Anthraquinone glycosides	-	-	-	-

(-): Absent (+): Low concentration (++): Moderate concentration (+++): High concentration

Table (4): Antimicrobial activity of different parts extracts of *C. africana* against Standard Organisms (100 mg/ml)

Part Used	Solvent	Standard tested organisms* /M.D.I.Z (mm)**					
		B. s	*E. c*	*Ps*	*S. a*	*C. a*	*A. n*
Leaves	MeOH	16-17	18-19	16-17	16-17	17-18	15-16
	P.E	-	-	-	-	-	-
	chloroform	-	-	-	-	-	-
	Ethyl acetate	16-17	14-15	17-17	20-21	12-13	14-15
	water	-	-	-	-	-	-
Fruits	MeOH	14-15	16-17	16-17	15-16	13-14	17-18
	P.E	-	-	-	-	-	-
Stem	MeOH	14-15	16-17	15-16	17-18	13-14	16-17
	P.E	-	-	-	-	-	-
	chloroform	-	-	-	-	-	-
	Ethyl acetate	17-20	14-15	14-15	17-18	25-26	13-14
	water	-	-	-	-	-	-
Park	MeOH	17-18	15-17	16-17	17-18	15-16	14-15
	P.E	-	-	-	-	-	-

*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): Minimum Inhabitation concentration of *C. africana* different parts' methanolic extracts against the standard bacterial and fungal

Extracts	Concentration (mg/ml)	*E.c*	*p.s*	*B.s*	*s.a*	*c.a*	*A.n*
Leaves	**50**	18	20	20	21	20	30
	25	16	18	17	17	18	25
	12.5	15	17	16	16	17	20
	6.25	14	16	15	15	16	18
stem	**50**	17	19	20	22	20	28
	25	15	15	19	20	18	25
	12.5	14	14	14	18	16	22
	6.25	14	14	14	16	15	20
Bark	**50**	14	16	18	20	17	14
	25	13	15	19	18	16	13
	12.5	12	14	17	17	15	12
	6.25	11	13	16	16	14	11
Fruit	**50**	17	15	20	22	20	17
	25	16	14	18	20	19	16
	12.5	15	13	18	17	18	15
	16.25	14	12	17	16	17	13

*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 ** 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 (6): Determination of the Minimum Inhibitory Concentration (MIC) of methanolic extracts of different parts of *C. africana* against standard organisms

Part of plant	solvent	*B. s*	*E. c*	*Ps*	*S. a*	*A.n*	*C.a*
		Concentration (mg/ml)					
Leaves	MeOH	6.25	6.25	6.25	6.25	25	12.5
Stem	MeOH	12.5	12.5	6.25	6.25	25	25
Park	MeOH	12.5	6.25	6.25	6.25	6.25	25
Fruit	MeOH	6.25	12.5	6.25	6.25	25	25
Leaves	ethyl	12.5	12.5	12.5	12.5	25	25
Stem	ethyl	12.5	12.5	12.5	12.5	6.25	25

Key: MeOH=Methanol. Ethyl =ethylacetate.

Table (7): Antibacterial and antifungal activity of reference antibiotics against standard microorganisms

S. No	Drugs	Concentrations (µg/ml)	Standard microorganisms used MDIZ* (mm)			
			Tested bacteria used (M.D.I.Zmm)			
			B.s	S.a	E.c	Ps.a
1	Ampicillin	40	15	25	-	16
		20	14	20	-	13
		10	13	18	-	12
		5	12	15	-	-
2	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.Zmm)			
			A.n		*C.a*	
3	Clotrimazole	40	30		42	
		20	22		40	
		10	19		33	
		5	16		30	
4	Nystatin	50	28		17	
		25	26		14	
		12.5	23		-	

*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= Sensitive, 14-18 = Moderate, <= Resistant , - =NO inhibition zone

IV. DISCUSSION

The present study was conducted to investigate the antimicrobial, phytochemcal and screening of *C. Africana* in different parts (leaves, stem, bark and fruit).

Antimicrobial activity of *C. Africana* extracts

C. Africana in different parts (leaves, stem, park and fruit) was screened for its antimicrobial activity against four standard bacteria, two Gram-positive bacterial strains (*Bacillus cereus* and *Staphylococcus aureus*), two Gram-negative bacterial strains (*Escherichia coli* and *Pseudomonas aeruginosa*) and two fungal strains (*Apergillus niger and Candida albicans*) using the cup plate agar diffusion method. *C. Africana* at concentrations (50, 25, 12.5 and 6.25 mg/ml).

The mean diameter of inhibition zone produced by *C. Africana* extracts on the tested standard microorganism were presented. On the other hand, **Table (4 – 7)** showed antimicrobial activity of the reference chemotherapeutic drugs against the test microorganisms.

The leaves of *C. Africana* extract exhibited effects against most of the tested organisms with zones of inhibition ranging from (14-30 mm). The largest inhibition against *Apergillus niger* give (30 mm). The stem of *C. Africana* extract exhibited effects against most of the tested organisms with zones of inhibition ranging from (14-20 mm). The largest inhibition against *Candida albicans* gives (20 mm). The bark of *C. Africana* extract exhibited effects against most of the tested organisms with zones of inhibition ranging from (11-18 mm). The largest inhibition against *Bacillus subtilis* gives (18 mm). The bark of *C. Africana* extract exhibited effects against most of the tested organisms with zones of inhibition ranging from (12-22 mm). The largest inhibition against *Staphylococcus aureus* gives (22 mm). It is clear from Table (6) that the extracts of *C. Africana* showed high activity all bacteria and fungi.

Phytochemical screening of C. Africana extracts

Preliminary phytochemical screening of *C. Africana* under study **(Table 3)** showed that the presence of saponons, cumarins, tannins, triterpenes and flavonoids in the four different parts used, while anthraquienones glycosides and cyanogenic glycoside were absence in the four parts also. Sterol showed positive result only in the stem and negative result in the other parts. The concentration of the positive results ranged between high in stem, moderate in bark and low in leaves and fruits though all the results. There were no phytochemical studies found in the previous literature review.

The obtained results were found to be on line with findings of many authors on some plants of the family Boraginaceae. Nawal *et. al* (2011) isolated different compound from *Cordia sinensis*. She isolated some flavonoids, saponins, sterols and sugars. Vijayakumari (2013) reported that *Rotula aquatica* contains alkaloids , Flavonoids , Phenols , saponins , tannins and terpenoids, Anthraquinones and anthocyanin.

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