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Antioxidant and cytotoxicity activity of *Cordia africana* in Sudan

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ABSTRACT

Cordia africana Lam. (family- Boraginaceae) is a small to medium-sized evergreen tree, 4 to 15 (30) m high, heavily branched with a spreading, umbrella-shaped or rounded crown. Bole typically curved or crooked. Bark grayish-brown to dark brown, smooth in young trees, but soon becoming rough and longitudinally fissured with age; young branchlets with sparse long. Uses 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. The present study was conducted to investigate the *in-vitro* antioxidant (DPPH assay) and cytotoxic (brine shrimp) of different parts (leaves, stem, park and fruit) of *C. africana*. The different parts of *C. africana* was screened for antioxidant screening for their free radical scavenging properties using 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl (DPPH), while propyl galate was used as standard antioxidant and screened for their cytotoxicity using brine shrimp. The inhibition percentage of antioxidant against (DPPH assay) varied from (37 ± 0.10 to 95 ± 0.00% RSA). The test of cytotoxicity was done using brine shrimp lethality, verified the toxic extracts except stem by water and leaves by methanol and water extracts. This study was conducted to evaluate the antioxidant activity and cytotoxicity (brine shrimp) of C*. africana*.

Keywords: *Cordia africana,* antioxidant activity*,* brine shrimp.

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INTRODUCTION

Natural products are generally either of prebiotic origin or originated from microbes, plants, or animal sources (Nakanishi, 1999). As chemicals, natural products include classes of compounds such 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 and Taha, 1981; El Sheikh et al., 1982).

Herbal drugs are of major importance in Sudanese traditional medicine. Floristic studies (Bruno and Massey, 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).

Sudanese medicinal plants have been reported as a source of antibacterial and antiviral (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).

Uses of *C. africana* include 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 purpose of the study was to evaluate the antioxidant activity and cytotoxicity (brine shrimp) activity of *C. africana* (leaves, stem, park and fruit).

MATERIALS AND METHODS

Plant materials

Different parts of the plant sample were collected from Algalabat and authenticated by Dr. Haidar Abd-Elgader and Herbarium specimens were deposited at the Herbarium of Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), Khartoum Sudan.

Preparation of crude extracts

Extraction was carried out according to method described by Sukhdev et al. (2008): 500 g of each different plant parts (leaves, stem, park and fruit) 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 of the solvent for petroleum ether and five days for methanol. Solvents were evaporated under reduced pressure using rotatory evaporator apparatus. Finally, extracts allowed to air dry in Petri dishes till complete dryness and the yield percentages were calculated as followed equation:

$$\text{Yield \%} = \frac{\text{Weight of extract obtained}}{\text{Weight of plant sample}} \times 100$$

Fractionation of methanolic extract

50 g of leaves and bark methanolic extract were separately dissolved in 500 ml of distilled water and shaken 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.

Toxicity testing against the brine shrimp

Storage of Artemia salina eggs

Eggs of *Artemia salina* were stored at low temperatures (4°C), they will remain viable for many years.

Hatching shrimp

Brine shrimp eggs, *A. salina* were hatched in artificial seawater prepared by dissolving 38 g of sea salt in one liter of distilled water. After 24 to 72 h incubation at room temperature (22 to 29°C), the larvae were attracted to one side of the vessel with a light source and then collected with pipette. Larvae were separated from eggs by aliquoting them three times in small beakers containing artificial seawater.

Brine shrimp assay

Bioactivity of the extract was monitored by the brine shrimp lethality test (Meyer et al., 1982). Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of plant extracts. 50 mg of *A. salina* (leach) eggs were added to a hatching chamber containing artificial sea water (75 ml). The hatching chamber was kept under an inflorescent bulb for 48 h for the eggs to hatch into shrimp larvae. 20 mg of test extracts of the various plant species were separately dissolved in 2 ml of methanol, then 500, 50, and 5 µl of each solution was transferred into vials corresponding to 1000, 100, and 10 µg/ml, respectively. Each dose was tested in triplicate. Ten larvae of *A. salina* Leach (taken 48 to 72 h after the initiation of hatching) were added to each vial. The final volume of solution in each vial was adjusted to 5 ml with sea water immediately after adding the shrimps. One drop of dimethylsulphoxide (DMSO) was added to the test and control vials before adding the shrimps to enhance the solubility of test materials. LD_{50} values were determined at 95% confidence intervals by analyzing the data on a computer loaded with a “Finney Programme.” The concentration at which it could kill 50% larvae (LD_{50}) was determined.

Statistical analysis

All data were presented as means ± S.D. Statistical analysis for all the assays results were done using Microsoft Excel program and Finney Programme.

RESULTS AND DISCUSSION

The present study was conducted to investigate the antioxidant and cytotoxicity for different parts (leaves, stem, park and fruit) of *Cordia africana* (Tables 1 to 4).

Antioxidant activity of *C. africana* extracts

Different parts of *C. africana* (leaves, stem, park and fruit) were screened to show their antioxidant activity via DPPH assay. The extract of methanol leaves, stem, bark and fruit gave antioxidant activity of 80, 88, 74 and 37%, respectively. Also extract of chloroform leaves and stem gave antioxidant activity of 79 and 78%, respectively. The extract of ethyl acetate leaves and stem gave antioxidant activity of 95 and 91% respectively and extract of water leaves and stem gave antioxidant activity of 82 and 89% respectively.

Table 1. Yield % of successive extracts.

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

Table 2. Yield % of different fractions form 80% methanolic extract of leaves and stem.

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

Table 3. Results of antioxidant activity of different part extracts of *C. africana*.

No.	Sample	%*RSA ± SD (DPPH)
1	Stem, methanol	88 ± 0·01
2	Stem chloroform	78 ± 1014
3	Stem ethyl acetate	91 ± 9746
4	Stem water	89 ± 0.08
5	Leaves, methanol	80 ± 0·03
6	Leaves chloroform	79 ± 0.01
7	Leaves ethyl acetate	95 ± 0.00
8	Leaves water	82 ± 0.06
9	Fruit, methanol	37 ± 0·10
10	Bark, methanol	74 ± 0·02
11	PG	93 ± 0·01

Key: *RSA = Radicals scavenging activity. PG = Propyl Galate.

Table 4. Result (brine shrimp) by Finney probity analysis (model).

No.	Sample	Solvent	LD_{50}	Result
1	Fruit	Methanol	139.817	Highly toxic
2	Bark	Methanol	0.0000414	Highly toxic
3	Stem	Methanol	74.965	Highly toxic
4	Stem	Chloroform	131.084	Highly toxic
5	Stem	Ethyl acetate	101.203	Highly toxic
6	Stem	Water	1175.795	Non-toxic
7	Leaves	Methanol	1646.0573	Non-toxic
8	Leaves	Chloroform	15.653	Highly toxic
9	Leaves	Ethyl acetate	464.545	Moderate
10	Leaves	Water	2056.735	Non-toxic

Key: ≤ 249: highly toxic; 250 – 499: Moderate; 500 - 1000: non-toxic.

Cytotoxicity assay of *Cordia africana* extracts

Cytotoxicity for different parts of *C. africana* (leaves, stem, park and fruit) fractions and extracts were conducted via brine shrimp lethality test except chloroform fraction of branches and leaves. The results stated that LD_{50} values below 249 µg/ml ware considered as highly toxic, 250 to 499 µg/ml as medium toxicity and 500 to 1000 µg/ml as light toxicity. Values above 1000 µg/ml were regarded as non-toxic (McLaughlin et al., 1998).

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