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Chemical constituents and antioxidant activity of some Sudanese medicinal plants

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Abstract
Sudanese plants show potential as a source of extracts rich in phenolic constituents and natural antioxidants. This study came with the objective to investigate the chemical constituents and antioxidant activity of some Sudanese medicinal plants, used in traditional medicine for the treatment of various diseases. Using 96% ethanol as a solvent by means of soaking technique, extracts of fifteen Sudanese medicinal plants: whole plant, leaf, root, bark, and fruit were analyzed to determine their chemical constituents using conventional chemical tests where applicable. All the tested plants showed the presence of various secondary metabolites such as: tannins, flavonoids, coumarins, saponins, sterols, terpenes, cardiac glycosides, anthraquinones, alkaloids, carbohydrates, and reducing sugars. Cyanogenic glycoside were not detected. The antioxidant activity was investigated by measuring the scavenging activity of the DPPH (1, 1-diphenyl-2-picryl hydrazyl radical) radical scavenging method. The tested plants revealed promising sources of natural antioxidants for medicinal uses. The most active plants were: Euphorbia aegyptiaca, Euphorbia acalyphoides, *Francoeuria crispa*, Grewia tenax and Cissus quadrangular is.

Keywords: Sudanese medicinal plants, chemical constituents, antioxidant activity

Introduction
Plants produce various antioxidant compounds as protection against reactive oxygen species (ROS) and free radicals. ROS are various species of activated oxygen leading to oxidative damage of tissues. Free radicals in the cell may occur due to various external factors such as ultraviolet radiation, chemical reactions and some metabolic processes [1, 2, 3]. It has been established that oxidative stress is among the major causative factors in induction of many chronic and degenerative diseases, including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others [4]. Recently, plants have received much attention as sources of biological active substances including antioxidants.

Sudan being a large country extending from desert in the north to the rainfall forests in the south provides a large diversity of vegetation. Fifteen Sudanese medicinal plants from 14 families were selected for the present investigation in the study area White Nile state in Sudan: *Euphorbia aegyptiaca*, *Euphorbia acalyphoides*, *Francoeuria crispa*, *Grewia tenax*. *Cissus quadrangularis, Physalis angulate, Cadaba glandulosa*, *Nymphaea lotus, Leptadenia arborea*, *Cordia sinensis, Trianthema portulacastrum*, *Boscia senegalensis, Amaranthy graecizans, Cucumis dipsaceus* and *Tephrosia apollinea*. Selection was based primarily on their Ethnobotanic and Ethnopharmacologic uses as antioxidant and antimicrobial plants. The selected plants were subjected to preliminary phytochemical screening and assessment of their antioxidant activity. The Ethnopharmacologic data were collected by consulting traditional healers and herbalists and the natives in the study area [5]. The present paper reports the results of phytochemical screening and antioxidant activity of the selected Sudanese medicinal plants, used in traditional medicine for the treatment of various diseases.

Material and Methods
Sample collection
The selected plants were collected from different locations of White Nile state in Sudan, during February 2016, and were identified in the Medicinal and Aromatic Plants Research Institute (MAPRI), Khartoum, Sudan. Voucher specimens were deposited in the herbarium. The collected plants were shade dried for 15 days and size reduced by mechanical grinder into coarse powder and stored in well closed containers free from environmental, climatic changes till usage.

Extraction of plant material
Fifty grams of powdered sample of each plant were extracted exhaustively with 500 ml 96% ethanol and the contents of the conical flask were left at room temperature for 72 h. with frequent shaking. The extracts were filtered using Whatman filter paper no. 1 and the clear solution was evaporated and the residual extract was dried. The samples were kept in the refrigerator at 5°C before use.

Phytochemical Screening of extracts
The prepared 96% ethanol extracts were used for the following tests according to methods described by Harbone [6], Sofowora [7], Martinez & Valencia [8] with few modifications.

Test for Tannins
To 2 ml of ethanol extract of all plant parts, 2 ml of 10% $FeCl_3$ solution were added in a test tube, the formation of blue-black or green colour was taken as presumptive evidence for the presence of tannins.

Tests for Flavonoids
To 20 ml of each extract was evaporated to dryness on a water bath, cooled and the residue was defatted by several extractions with petroleum ether. The defatted residue was dissolved in 30 ml of 80% ethanol and filtered and the filtrate was used for following tests: -

1. To 3 ml of the filtrate in a test tube 1ml of 1% $AlCl_3$ solution was in methanol was added. Formation of a yellow color indicated the presence of flavones or and chalcones.
2. To 3 ml of the filtrate in a test tube 1ml of 1% KOH solution was added. A dark yellow color indicated the presence of flavones or flavonenes. chalcone and or flavonols.
3. Shinoda's test: to 2 ml of the filtrate 0.5 g of magnesium turnings were added flowed by 1 ml of conc. hydrochloric acid. Pink or red colouers was taken as presumptive evidence that flavonenes were present in the plant sample.

Test for Coumarins
Three ml of extract were evaporated to dryness and the residue was dissolved in hot water. After cooling; the solution was divided into two test tubes: one tube contained the reference, and the aqueous solution of the second tube was made alkaline with 0.5 ml of ammonia solution (10%). The formation of an intense florescence under UV light indicates the presence of coumarins and /or derivatives.

Test for Saponins (Forth Test)
Two ml of extract were placed into a clean, dry test tube and 2 ml of distilled water were added, and the tube was stoppered and shaken vigorously for about 30 seconds. The tube was allowed to stand in a vertical position and observed. Formation of persistent foam, remained stable for 15 minutes, indicates the presence of saponins.

Test for Steroids (Liberman-Buchard test)
To two ml of the extract in chloroform and 2ml acetic anhydride few drops conc. H_2SO_4 were added from the side of the test tube. Blue-green ring between layers indicates the presence of steroids.

Test for terpenoids (Salkowski test)
Five ml of the extract were mixed with 2 ml of chloroform followed by careful addition of 3 ml conc. H_2SO_4. Formation of pink- purple ring indicates the presence of terpenes.

Test for cardiac glycosides (Keller-Kiliani test)
To two ml of the extract of all plant parts, 1 ml glacial acetic acid, 6 drops of 10% ferric chloride solution and 6 drops of conc. H_2SO_4 were added in a test tube. Green-blue color indicates the presence of cardiac glycosides.

Test for Anthraquinones (modified Borntrager's test)
Five ml of the extract of all plant parts were heated with 10 ml of 0.5N KOH and 1ml of 3% of H_2O_2 solution for 10 minutes. After filtering the cooled mixture, 10 drops of glacial acetic acid were added to acidify the mixture. The mixture was extracted with 10ml of benzene using a separatory funnel. To a 5ml of the benzene layer, 4 ml of ammonia solution were added. Red or pink colour in the alkaline layer is an indication of the possible presence of Anthraquinones.

Test for Alkaloids
To ten ml of the extract of all plant parts, 1ml of 2N HCl was added in a test tube, and heated in a water bath for 10 minutes. 1ml from each solution was taken and a few drops of Mayer's reagent/Wagner's reagent/ Dragendorff's reagent were added and mixed separately. Appearance of yellow precipitate/ brownish-red precipitate/ red precipitate or turbidity indicates the presence of alkaloids.

Test for Cyanogenic Glycosides (Guignard test)
The fresh sample (5gram) was placed in a 125 ml Erlenmeyer flask and sufficient distilled water was added to moisten the sample, followed by 1 ml of chloroform to enhance enzyme activity. A piece of freshly prepared sodium picrate paper was carefully inserted between a split corks which was used to stopper the flask, and left at room temperature for up to 3hrs. A change in colour of sodium picrate paper from yellow to various shades of red was taken as a presumptive evidence for the presence of the cyanogenic glycosides.

Test for carbohydrates: (Molisch's test)
To three ml of the extract of all plant parts, 1 ml of Molish reagent and 3 ml conc. H_2SO_4 were added and the mixture was heated at 45°C. The formation of a violet ring in the interface layer is an indication for the presence of the carbohydrates.

Test for reducing sugars: (Fehling's test)
One ml of the extract of all plant parts were diluted with 2ml distilled water and 1ml of Fehling's solution 1ml was added and heated. A brick-red precipitate denotes the presence of reducing sugars.

Antioxidant activity
DPPH radical scavenging assay
The DPPH radical scavenging assay was performed according to the method of Shimada *et al.*, (1992) [9], with some modification. In 96-wells plate, the test samples were allowed to react with 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37ºC. The concentration of DPPH was kept as (300µM). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at λ: 517nm using multiplate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

IC_{50} Calculations

The IC_{50} the concentration of test material, which possess 50% inhibition of free radicals of all the extracts and their fractions, was determined by monitoring the effect of different concentrations ranging from 0.5-0.0035mg/ml. The IC_{50} of the extracts and their fractions were calculated using EZ-Fit Enzyme Kinetic Program (Perrella Scientific Inc, U. S.A).

Results and Discussion

This study came with the objective investigation of chemical constituents and antioxidant activity of 15 Sudanese medicinal plants. The Scientific name, family, local name, plant part used and medicinal uses of the selected plants have been presented in Table1.

Table 1: Summary of Selected Sudanese plants used in traditional medicinal

Scientific name	Family	Local name	Part used	Medicinal uses
Euphorbia aegyptiaca Boiss	Euphorbiaceae	Um lebaina. (malbein)	WP	Against scorpion bites, anti-inflammatory, rheumatoid arthritis, dermatitis and conjunctivitis
Euphorbia acalyphoides Hochst. ex. Boiss	Euphorbiaceae	Um lebaina. (labien)	WP	Against scorpion bites, anti-inflammatory.
Francoeuria crispa (Forssk.) cass.	Asteraceae.	Al-tagar.	WP	Against alopecia, treatment of inflammation, repellent to insects.
Grewia tenax. (Forssk.) fiori,	Tiliaceae.	Godhaim, guddaim	R L F	Treat jaundice, pulmonary infections, asthma, curing various skin diseases Against trachoma. Treat anemia and chest diseases.
Cissus quadrangularis L.	Vitaceae.	Salala	WP	Dyspepsia, hemorrhoid, menstrual disorder, antioxidant, ant flatulence, antibacterial, antifungal, bone fracture, anti-osteoporotic.
Physalis angulate L.	Solanaceae.	Karm karm,	WP	Stomach disorders, Bright's Disease.
Cadaba glandulosa Forssk.	Capparaceae.	kurmut.	L	Haemorrhoids, urinary tract infections, stems and roots are used as antirheumatic.
Nymphaea lotus L.	Nymphaeaceae	Suteib.	R L	diarrhoea, dysentery, dyspepsia and general weakness. Aphrodisiac, astringent, cardiotonic, sedative, antiinflammatory agent, Indigestion, heart diseases, stomach aches, cancer and as anti-hemorrhagic.
Leptadenia arborea (Forssk.) schweinf.	Asclepiadaceae	Lewais.	L	Leaves and stems are used against snake bites, the stem is used for nose and tooth swelling.
Cordia sinensis Lam	Boraginaceae.	Anderab.	B R L	Inhance wound healing, stomach disorders, treat conjunctivitis in cattle. Treat malaria, but can cause an abortion. Kidney and bladder disease, ulcers, diuretic, dysentery, diarrhea, asthma, coughs and dental disorders.
Trianthema portulacastrum L.	Aizoaceae.	Al-Raba'a kabeer	WP L R	Against scorpion bites. Diuretic property, applied in the treatment of jaundice, strangury, dropsy. Eye cures, corneal ulcers, itching, dimness of sight and night blindness.
Boscia senegalensis (Pers.) Lam. ex. Poir.	Capparaceae	Mukheit	L	the whole plants are used as anthelmintic, Cancer, ulcer and swellings
Amaranthy graecizans (vill). Bolos & vigo	Amaranthaceae	lisan El Tair	WP	Tenderizer in cooking tough vegetables such as cowpea leaves and pigeon peas.
Cucumis dipsaceus Enernb.	Cucurbitaceae	Agour el-kelab.	F	Its fruit juice is topically applied to prevent hair loss. whole plant is used as anti-emetic.
Tephrosia apollina Delile. Link.	Fabacea	Amayoga.	WP	Used as a tonic, insecticidal, antiviral, antiplasmodial.

Key: WP= Whole Plant, L= Leaves, R= Roots, B= Bark, F= Fruit.

Chemical Constituents

The phytochemical screening of the 96% ethanolic extracts of the tested plants revealed the presence of various secondary metabolites: tannins, flavonoids, coumarins, saponins, sterols, terpenes, cardiac glycosides, anthraquinones, alkaloids, carbohydrates, and reducing sugars. Cyanogenic glycoside were not detected. Phytochemical constituents of the plants under study have been depicted in Table 2.

Table 2: Preliminary of Phytochemical screening of the Ethanolic extracts of 15 Sudanese Medicinal plants.

NO.	Scientific name	Part Used	Detected Metabolites															
				Flavonoids									Alkaloids					
			Tannins	$ALCL_3$ 1%	KOH 1%	Mg turning	Coumarins	Saponins	Sterols	Terpenes	Cardiac glycosides	Anthraquinones	Mayer's reagent	Wagner's reagent	Dragendorff's reagent	Cyanogenic glycosides	Carbohydrates	Reducing Sugars
1	*Euphorbia aegyptiaca*	WP	+++	++	++	+++	++/	-	++	+	++	+	+	+	+	-	++	+++
2	*Euphorbia acalyphoides*	WP	+	++	+	-	++	-	+	++	+++	-	+	+	+	-	++	+
3	*Francoeuria crispa*	WP	+++	++	++	++	++	-	-	+++	+++	++	+	+	+	-	++	++
4	*Grewia tenax*	R	+	-	+	-	+	-	-	+	++	-	-	-	-	-	++	-

5	*Cissus quadrangularis*	WP	++	++	+	++	++	++	-	+	++	++	++	++	++	-	+	+
6	*Physalis angulate*	WP	++	++	+	+++	+++	+++	++	++	++	+	+	++	+	-	++	+++
7	*Cadaba glandulosa*	L	++	+	-	+	+++	++	+	+	++	+	+	++	++	-	-	+++
8	*Nymphaea lotus*	R	+	+	+	-	+	-	-	+	-	-	+	+	++	-	+	-
9	*Leptadenia arborea*	L	++	++	+	-	+++	++	++	-	+	+++	++	++	++	-	++	+++
10	*Cordia sinensis*	B	+++	+	++	-	+	-	-	++	-	++	-	-	-	-	++	++
11	*Trianthema portulacastrum*	WP	++	-	-	++	++	+	+++	-	++	-	-	-	-	-	+	+++
12	*Boscia senegalensis*	L	++	++	+	+	++	+++	++	-	+	+	+++	+++	+++	-	++	++
13	*Amaranthy graecizans*	WP	++	+	+	++	++	-	++	-	++	-	-	-	-	-	+++	+++
14	*Cucumis dipsaceus*	F	+	++	++	+	-	+	-	+++	++	-	+	+	+	-	++	++
15	*Tephrosia apollina*	WP	+++	+++	+++	+++	+	-	-	-	-	-	-	-	-	-	+	++

Key: WP= Whole Plant, L= Leaves, R= Roots, B= Bark, F= Fruit.
+ =Trace, ++ =Moderate, +++ =High, - =Negative

Determination of Antioxidant Activity: DPPH radical Scavenging Activity

DPPH radical scavenging assay is the most common method used in the study of antioxidant activity of plant extracts. It results in the formation of stable free radical which can be detected by common spectrophotometric technique [10]. Decrease in absorbance shows the more efficient antioxidant activity of the extract in terms of hydrogen atom donating capacity. This assay is more indirect type as it measures the inhibition of reactive species (free radicals) generated in the reaction mixture and its results depend on the type of reactive species used [11]. Studies conducted on the free radical scavenging activity of medicinally important plants have shown that the efficiency of each plant species differ depending on the particular assay methodology, reflecting the complexity of the mechanisms involved in total antioxidant capacity. The observed antioxidant activity of the extracts may be due to the neutralization of free radicals (DPPH), either by transfer of hydrogen atoms or by transfer of an electron [12]. Scavenging activity of different plant extracts on DPPH radical has been reported in Table 3. There was noticeable variability in the antioxidant activity of plant extracts. Out of fifteen plants screened, *Euphorbia aegyptiaca*, *Euphorbia acalyphoides*, *Francoeuria crispa*, *Grewia tenax*. *Cissus quadrangularis*, *Physalis angulate*, *Cadaba glandulosa*, *Nymphaea lotus*, *Leptadenia arborea*, *Cordia sinensis Trianthema portulacastrum*, *Boscia senegalensis*, *Amaranthy graecizans*, *Cucumis dipsaceus* and *Tephrosia apollina* exerted the following activities: 90% (IC_{50} 0.040 µg/ml), 87% (IC_{50} 0.040 µg/ml), 90% (IC_{50} 0.123 µg/ml), 83% (IC_{50} 0.134 µg/ml), 80% (IC_{50} 0.184± µg/ml), 62% (IC_{50} 0.359 µg/ml), 61% (IC_{50} 0.394 µg/ml), 58% (IC_{50} 0.473 µg/ml), 52%, (IC_{50} 0.589 µg/ml), 53%, 31%, 30%, 20%, 5% and 54% respectively. The percent inhibition of Propyl Gallate was 93%, which is used as a standard. While *Euphorbia aegyptiaca* showed the highest activity 90% with least (IC_{50} 0.040 µg/ml), *Cucumis dipsaceus* showed the lowest activity 5%.

Table 3: DPPH radical scavenging activity and IC_{50} Value of 96% ethanol extracts of 15 Sudanese Medicinal Plants.

No.	Scientific name	Part Used	%RSA ±SD (DPPH)	IC_{50} ±SD µg /ml (DPPH)
1	*Euphorbia aegyptiaca*	Whole Plant	90± 0.01	0.040 ± 0.01
2	*Euphorbia acalyphoides*	Whole Plant	87± 0.02	0.056 ± 0.02
3	*Francoeuria crispa.*	Whole Plant	90± 0.02	0.123 ± 0.03
4	*Grewia tenax.*	Root	83± 0.02	0.134 ± 0.01
5	*Cissus quadrangularis*	Whole Plant	80± 0.04	0.184± 0.03
6	*Physalis angulate*	Whole Plant	62± 0.02	0.359± 0.06
7	*Cadaba glandulosa*	Leaf	61± 0.06	0.394 ± 0.01
8	*Nymphaea lotus*	Root	58± 0.01	0.473 ± 0.09
9	*Leptadenia arborea*	Leaf	52± 0.02	0.589 ± 0.09
10	*Cordia sinensis*	Bark	53± 0.09	-
11	*Trianthema portulacastrum*	Whole Plant	31± 0.09	-
12	*Boscia senegalensis*	Leaf	30± 0.03	-
13	*Amaranthy graecizans*	Whole Plant	20± 0.03	-
14	*Cucumis dipsaceus*	Fruit	5± 0.03	-
15	*Tephrosia apollina*	Whole Plant	54± 0.01	-
Standard	Propyl Gallate		93± 0.01	0.077µg/ml±0.01

Conclusion

The results of phytochemical screening revealed the presence of flavonoids and tannins in all extracts. The antioxidant activity of Phenolic compounds is due to their ability to reduce free radical formation and scavenge free radicals. The results of antioxidant activity, have indicated that *Euphorbia aegyptiaca* 90% (IC_{50} 0.040 µg/ml) and *Francoeuria crispa* 90% (IC_{50} 0.123 µg/ml) are two potential plants having strong antioxidant activity. This activity may be attributed to the tannins and flavonoids, detected in their extracts. The results of our study revealed good therapeutic potentials of some plants such as *E. aegyptiaca, E. acalyphoides, F. crispa, G. tenax* and *C. quadrangularis*, which might be used as antioxidant plants. The results presented in this report will also provide a suitable guide in choosing natural plant by the medical practitioners as natural antioxidants treating and controlling diseases.

It can be concluded that these plants could serve as a natural source of antioxidants in the food industry in addition to other pharmacological properties.

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