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Antioxidant Activity and Cytotoxicity of *Heliotropium bacciferum* Forssk Leaves and Stem

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Abstract: This work investaged and determined the cytotoxicity and antioxidant of extracts produced from *Heliotropium bacciferum* forssk leaves and stem. The leaves and stem was extracted successively with diethyl ether, ethyl acetate and methanol by using orbital shaker apparatus. The radical scavenging activity of *Heliotropium bacciferum forces* was screened by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH), the antioxidant results was expressed as concentration of inhibition (IC_{50}) of the free radical DPPH, the ethyl acetate stem and methanol stem extracts and showed the highest scavenging activity with IC_{50} = 36.10 µg/ml and 31.31 µg/ml respectively compared to the control standard gallic acid which yield IC_{50} = 4.5 µg/ml. The scavenging activity of *Heliotropium bacciferum* forces stems extract was increased correspondingly with the increasing polyphenolic content. The cytotoxicity of *Heliotropium bacciferum* forces leaves and stem extracts was carried out by brine shrimps lethality and the results showed that the all extracts were nontoxic.

Key words: Heliotropium Bacciferum · Antioxidant · Radical Scavenging Activity · 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) · Brine Shrimp · Cytotoxicity

INTRODUCTION

Heliotropium bacciferum forssk leaves and stem extracts contain proteins, amino acids, polyphenols, carbohydrates, terponoids and alkaloids[1, 2]. As Sudan is rich of mulitpe medicinal plants in need to rediscovered [3 - 6], Heliotropium bacciferum is one of important plants used in local treatments.

Oxidants or Free radicals sometimes are chemical species containing unpaired electrons, are abundant in nature and have significant role in chemical reactions, combustion and food spoilage [7]. Superoxide radical is also another radical involved in the immune response against pathogens [8].

Antioxidants are substances that help protect the body from oxidative damage caused by free radicals [9]. Herbal medicines can be potentially toxic to human health. In this way, scientific research has shown that many plants used in traditional and folk medicine are potentially toxic, mutagenic and carcinogenic [10, 11].

The dried leaves and stem of *Heliotropium bacciferum* forces, which belong to family Boraginaceae and locally known as (danab alagrab) was chosen for this study due to its reputed use in traditional medicine for treatment of many diseases the Aerial parts used to treat Snake bites, Wound bleeding and wound healing. Also the whole plant use to treat stomachic The present research work aimed to evaluate the antioxidant and cytotoxicity of leaves and stem of *Heliotropium bacciferum* forces.

MATERIALS AND METHODS

Sample Collection and Preparation: The leaves and stem of the *Heliotropium bacciferum* forssk were collected from Soba region, southwest Khartoum. The plant samples were identified in medicinal and aromatics plants-national research institute (National Research Center). The plant materials were dry under shade and after optimum drying, coarsely powdered and stored in air tight, well-closed container until further use. The *Heliotropium bacciferum* forssk coarsely powdered were extracting with different solvents (diethyl ether, ethyl acetate and methanol). The plant Materials were extracting by cold extraction use magnetic stirring.

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Antioxidant activity

Diphenyl Picryl Hydrazine (DPPH) Radical-scavenging Activity: The antioxidant activities of extracts were determined by using the method of [12] with slight modification. To 1mg/ml extracts a sample was added 2ml of DPPH radical and 0.9ml of Tris-HCL. Control tube was added 0.1ml absolute methanol, 2ml of DPPH radical and 0.9ml of Tris-HCL To last tube was added 2ml of absolute methanol and 0.9ml Tris-HCL this tube use as blank tube. Then all extracts samples, blank and control tubes were incubated at room temperature in dark place for 30 min. measured the absorbance (activity) use UV-Vis spectrophotometer at 517nm. Decrease in the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. The extract concentration showing 50% inhibitions (IC50) were calculated from the graph of percentage RSA against extract concentration with gallic acid used as standards.

(% RSA) was calculated as follows:

%RSA = (control absorbance - sample absorbance)/ control absorbance x 100

Inhibition Concentration Assay (IC_{50}): Serial dilutions of the high Percentage antioxidant were prepared from tested extracts samples (500µg/ml) to give the final concentrations of 500, 250, 125 and 62.5 µg/ml, in methanol respectively and analysis for both radical scavenging activities DPPH. Triplicate assays were carried out.

Cytotoxicity Test

Bioassay of A. Salina: For toxicity tests, ten nauplii were selected and transferred into each sample vial by means of a 23 cm disposable Pasteur pipette from the lighter side, having been separated by the divider from the shells and the final volume in each vial was adjusted to 5mL using artificial seawater. The vials were maintained under illumination. The surviving nauplii were counted with the aid of a 3x magnifying glass, after 24 hours and the percentage of deaths at the three dose levels and control were determined. In cases of control no deaths were observed to occur in the control after 48 h Positive controls were prepared using Potassium dichromate (K2Cr2O7) and negative control Dimethyl Sulphoxide (DMSO). Three replicates were prepared for each dose level. To begin the bioassay brine shrimp eggs (obtained from Directorate of Fisheries) were hatched in a shallow rectangular dish (22 - 32 cm) under the same conditions described in the literature except that natural instead of artificial. The data was corrected using Abbott's formula [6] as follows:

% deaths = [(Test-control)/control] x 100.

Preparation and Hatching of Brine Shrimp (A. Salina): Brine shrimp eggs (*A. Salina*) were hatched in shallow glass vessel covered with a foil then divided it into two unequal holes compartment (dark large part and light small part) and full with artificial sea water prepared from Sodium chloride salt (NaCl 35% = 35g were dissolved in 500 ml of distill water) The smaller compartment was illuminated by tungsten filament light and gently kept from air. After 24hours, hatched were transferred to fresh artificial seawater and incubated for further 24hours in a warm room 25°C- 29°C.

Sample Preparations for Cytotoxicity Test: Samples for the experiment were prepared by dissolving 20 mg of the different extracts in each 5mL of dimethyl sulphoxide. Appropriate amounts of this DMSO solution (5µL, 50µL and 500µL to give concentrations of 10, 100 and 1000 ppm or mg/mL respectively) were transferred into 10 ml vials (3 vials for each dose and 1 for control). Three replicates were prepared for each dose level then dried further in vacuum for 1 h to remove DMSO completely. Control was prepared using only DMSO.

LD_{50} Determinations: The lethal concentration fifty (LC_{50}), 95% confidence interval and slope were determined from the 24 hour counts using the probit analysis method [13, 14]. LC_{50} is indicative of toxicity level of a given plant extract to the brine shrimp larva.

RESULTS AND DISCUSSION

Antioxidant Activity by DPPH Radical Scavenging of Heliotropium Bacciferum Forces

Evaluation the Antioxidant Activity for Plant Extracts: The result of the DPPH radical scavenging activity and the IC_{50} of the diethyl ether, ethyl acetate and methanol extract leaf and stem of *Heliotropium bacciferum*, gallic acid are showed in Table 1.

The antioxidant activity of *Heliotropium bacciferum*, leaves showed the diether leaf extract (9.79%), followed by methanol leaf extract (33.37%) and ethyl acetate leaf extract (39.29%) inactivity for DPPH scavenging. While methanol stem extract showed high DPPH activity (82.35%), followed by moderate activity in ethyl acetate

Table 1: Radical-scavenging activity of *Heliotropium bacciferum* forces

Plants parts	Extracts	RSA. At Conc. 500µg/ml.	IC_{50} µg/ml	Inference
	Di. ether	9.79%	Not need detect	Inactive.
Leaves	E.A	39.29%	Not need detect	Inactive.
	MeOH	33.37%	Not need detect	Inactive.
Stems	Di. ether	38.38%	Not need detect	Inactive
	E.A	56.54%	36.10	Moderate% & Strong IC50.
	MeOH	$^{++}$82.35%	31.31	High% & Strong IC50.
Standard gallic acid		77%	4.5	Moderate% & strong IC50.

Where: RSA= radical scavenging activities; Means (n=3); +++= very high; ++= high; Di. ether= Dithyl ether, E.A= Ethyl acetate and MeOH= Methanol.

stem extract (56.54%) and inactivity in diether stem extract (38.38%). The high antioxidant activities of methanol stem extracts (82.35%) were comparable to control standard gallic acid (77%).

The DPPH assay was used to assess the antioxidant activity of the extracts of the tested indigenous traditional medicinal plants. DPPH is a stable free radical, which accepts an electron or hydrogen radical to form a stable diamagnetic molecule which is widely used to investigate radical scavenging activity. Antioxidants react with the DPPH radicals [15]. Positive results were obtained from this assay, with *Heliotropium bacciferum* forssk exhibiting the most activity. The crude methanol extract of *Heliotropium bacciferum* forssk stem (31.31µg/ml) possessed relatively the most effective DPPH radical antioxidant capacity with their IC_{50} value less than 50 µg/ml. This value was higher than gallic acid standard antioxidants analyzed. Therefore, the present study indicated that, the DPPH quenching ability of *Heliotropium bacciferum* forssk could be related the flavonids and tannins present, which might possibly donate hydrogen from phenolic hydroxyl groups in order to discontinue the free radical chain reaction and prevent damage from free radicals. Table 1. The Methanol extract a richest by polyphenols and phenolic compounds. Polyphenols and phenolic compounds are good antioxidants [16].

Phenolic compounds, for example, flavonoids are of fastidious interest because of their antioxidant activity through oxygen radicals scavenging and peroxidation inhibition. Antioxidants that scavenge free radicals have a key role in inflammatory disorders, cancer, aging and cardiovascular diseases [17]. Many antioxidant activities are due to the presence of coumarin lignans, flavonoids, flavones, anthocyanin, isocatechins, isoflavones and catechins [9]. Heliotrine alkaloid demonstrated temporary hypotension perse in dogs and extensively condensed the nicotine induced vasopressor spasmogenic responses [18].

The plant *Heliotropium bacciferum* revealed significant DPPH radical scavenging activity. Other plants of genus *Heliotropium* also showed antioxidant activity. Modak isolated three (3) flavonoids, 3-O-methylgalangin, 7-O-methyleriodictiol and naringenin from the plant *Heliotropium taltalense*. The isolated flavonoids exhibited DPPH radical scavenging activity which recommends that *Heliotropium bacciferum* may possess flavonoids accountable for radical scavenging activity [19-21].

The Results of cytotoxicity effects of the *Heliotropium bacciferum* forssk extracts, were determined against *Artemia salina* larvae the percentage of death (% mortality) at different concentrations (10, 100 and 1000 µg/ml) and LD_{50} values, were shown in Tables 3. Positive control was prepared using Potassium dichromate ($K_2Cr_2O_7$) and negative control Dimethyl Sulphoxide (DMSO) (Table 4).

Brine shrimp bioassay results of the *Heliotropium bacciferum* forces extracts showed that all diethyl ether, ethyl acetate and methanol extracts

Table 2: Total flavonoid content (TFC), Total Tannin content and total phenolic content (TPC) of *Heliotropium bacciferum* forssk leaf and stem in various solvents and their IC_{50} values in DPPH assay

Extract	TFC, QE (mg/mL)	TPC, GAE (mg/mL)	TTC, TA (mg/mL)	IC_{50} (mg/mL)
Diethyl ether leaf	211.66	322.48		0
Ethyl acetate leaf	178.64	415.40		0
Methanol leaf	77.53	406.49	236.21	0
Diethyl ether stem	226.11	145.49		0
Ethyl acetate stem	164.20	291.50		36.10
Methanol stem	172.45	415.40	225.77	31.31

TFC=Total flavonoid content, TPC= Total phenol content, TTC= Total Tannin contentQE=Qucrectin, GAE= Gallic acid, TA= Tannic aci

Table 3: Brine shrimp by Finney probity analysis (model) of *Heliotropium bacciferum forssk* leaves and stem extracts

Extraction	Conc. ppm	Total	Dead	Survive	Mortality, %	LD_{50}	Result
Diethyl ether leaf	10	30	5	25	17	2222.43	Safe
	100	30	3	27	10		
	1000	30	15	15	50		
Ethyl acetate Leaf	10	30	4	26	13	45435.52	Safe
	100	30	8	22	27		
	1000	30	9	21	30		
Methanol leaf	10	30	2	28	7	10804.73	Safe
	100	30	6	24	20		
	1000	30	9	21	30		
Diethyl ether stem	10	30	10	20	33	72908786317.23	Safe
	100	30	10	20	33		
	1000	30	11	19	37		
Ethyl acetate stem	10	30	9	21	30	44316.42	Safe
	100	30	9	21	30		
	1000	30	12	18	40		
Methanol stem	10	30	8	22	27	17612.14	Safe
	100	30	6	24	20		
	1000	30	13	17	43		

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

Table 4: Brine shrimp bioassay of Controls and lethality death (LD_{50}) levels analysis by Finny- Probit Model of *Heliotropium bacciferum* forssk leaves and stem extracts

	Extracts	Conc. ppm	No. of Nauplii	No of Survivors	Average	% Death	LD_{50} µg/mL	Inference
		10	30	0	0	100		
		100	30	0	0	100		
		1000	30	0	0	100		
K2Cr2O7	Positive	10	30	0	0	100		
	Control	100	30	0	0	100	1.7	Toxic
		1000	30	0	0	100		
		10	30	0	0	100		
		100	30	0	0	100		
		1000	30	0	0	100		
		10	30	30	10	0		
		100	30	30	10	0		
		1000	30	30	10	0		
	Negative	10	30	30	10	0		
DMSO	Control	100	30	30	10	0	726129 $X10^{11}$	Safe (Non toxic)
		1000	30	30	10	0		
		10	30	30	10	0		
		100	30	30	10	0		
		1000	30	30	10	0		

Notice Data is based on mean values of three replicate, Where:, Conc. = Concentrations, No. = Number, µg/ml= Microgram per milliliter.

Heliotropium bacciferum forssk leaf and stem were safe to brine shrimps at concentrations of 10, 100 and 1000 ppm. All these extracts were comparable to standard drug, ($K_2Cr_2O_7$) that gave 100 % mortality.

LD_{50}Determination of Heliotropium Bacciferum Forssk: The general toxic activity of the LD_{50}, 95% confidence interval was determined by using the Probit analysis method described by [22] and the lethal death fifty (LD_{50}) were determined from 48 hours counts of the samples and control. The references ranging of toxic activity were considered weak or (non-toxic) when the LD_{50} values of the crude extracts was determined greater than 500µg/ml, Moderate toxic when the LD_{50} was determined between 100-500 µg/mL and designated as strongly toxic when the LD_{50} was less than 100 µg/mL.

The results of the LD_{50} values Table 3 of *Heliotropium bacciferum* forssk leaves and stem were shown to be non-toxic in diethyl ether leaf extract (2222.43µg/mL) and stem (7290878631.23 µg/mL) followed by ethyl acetate leaf extract (45435.52 µg/mL) and stem (44316.42 µg/mL) and in methanol leaf extract

(10804.73µg/mL) and stem (17612.14 µg/mL). The results of LD_{50} values revealed that the total extracts of the leaves and stems displayed were Safe enough to be used against brine shrimp, as compared to standard drug (potassium dichromate= 1.70 µg/mL).

Generally, as compared to standard drug, ($K_2Cr_2O_7$) showed 100% mortality was obtained the maximum mortalities occurred at 1000 ppm concentration while the least mortalities happened to be at 10ppm concentration; the %mortality increased with an increase in concentration at 1000 ppm; however the results have shown that the brine shrimp survival is inversely proportional to the concentration of extract. The results of these tests confirm that leaves and stem of *Heliotropium bacciferum* forssk are nontoxic and hence safe for commercial utilization.

CONCLUSIONS

This study revealed the antioxidant potential of *Heliotropium bacciferum* forssk; the ethyl acetate extract stem gave positive results of scavenging of the free radical DPPH, methanol extract was presented the most reactive due to its high polyphenolic content. The cytotoxicity effects of the *Heliotropium bacciferum* forssk extracts, against *Artemia salina* larvae showed that the all plant extracts were safe.

RERERENCES

1. Najeeb, T.M., A.E. Musa and T.O. Khider, 2020. Antibacterial and Antifungal Activities of Heliotropium bacciferum Forssk Leaves and Stem. Advances in Biological Research, 14(1): 1-7.
2. Telal M. Najeeb, Ali Elnaeim Musa and Tarig Osman Khider, 2020. Thin Layer Chromatography and Quantification of Total Contents of Phenol, Flavonoid, Tannin, Carbohydrates and Amino Acids of Heliotropium bacciferum Forssk Leaves and Stem Extracts. Academic Journal of Plant Sciences, 13(1): 08-14.
3. Khider, T. and M. Hubbe, 2018. Towards Rational Utilization of Indigenous Plant Resources. Bio. Res., 13(4): 7172-7174.
4. Issa, T., Y. Mohamed, S. Yagi, R. Ahmed, T. Najeeb, A. Makhawi and T. Khider, 2018. Ethnobotanical investigation on medicinal plants in Algoz area (South Kordofan), Sudan J. Ethnobiol. Ethnomed., 14: 31.
5. Telal M. Najeeb, Tahani O. Issa, Yahya S. Mohamed Reem H. Ahmed, Abdelrafie M. Makhawi and Tarig O. Khider 2018. Phytochemical Screening, Antioxidant and Antimicrobial Activities of Dalberegia melanoxylon Tree. World Applied Sciences Journal, 36(7): 826-833.
6. Fatma Sir ElKhatim, Tahani Osman Issa, Suilman I. Suliman, Yahya Sulieman Mohamed, Abdelrafie Mohamed Makhawi and Tarig Osman Khider, 2019. Phytochemical Screening and Antimicrobial Activity of Different Extractions of Sudanese Stylochiton borumensis Roots (Araceae). World Applied Sciences Journal, 37(9): 700-709 DOI: 10.5829/ idosi.wasj.2019.700.709.
7. Prasad, K.N., J. Hao, C. Yi, D. Zhang, S. Qui and Y. Jiang, 2009. Antioxidant and anticancer activities of Wampee (Clausena lansium (Lour.) Skeels) peel. Journal of Biomedicine and Biotechnology. 10.1155/612805
8. Solomons, T.W.G. and C.B. Fryhle, 2008. Organic Chemistry (9th ed.). United States of America. John Wiley and Sons, Inc., pp: 1288.
9. National Cancer Institute, 2004. Antioxidants and cancer prevention: Fact sheet.
10. Mengs, U., 1988. Toxic effects of sennosides in laboratory animals and *in vitro*, Pharmacology, 36(1): 180-187.
11. Ferreira-Machado, S.C., M.P. Rodrigues and A.P.M. Nunes, 2004. Genotoxic potentiality of aqueous extract prepared from Chrysobalanus icaco L. leaves, Toxicology Letters, 151(3): 481-4.
12. Pelka, M., C. Danzl, W. Distler and A. Petschelt, 2000. A new screening test for toxicity testing of dental materials. J. Dent., 28: 341-345.
13. Goze, I., A. Alim, A.S. Tepe, M. Sokmen, K. Sevgi and B. Tepe, 2009. Screening of the antioxidant activity of essential oil and various extracts of Origanum rotundifolium Boiss. From Turkey. Journal of Medicinal Plant Research, 3(4): 246-254.
14. Cioffi, G., M. D'Auria and A. Brac, 2002. Antioxidant and free radical scavenging activity of constituents of the leaves of Tachigalia paniculata, Journal of Natural Products, 65(11): 1526-1529.
15. Aqil, F., I. Ahmad and Z. Mehmood, 2006. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants, Turkish Journal of Biology, 30(3): 177-183.
16. Pandey, V.B., J.P. Singh, Y.V. Rao and S.B. Acharya, 1982. Isolation and pharmacological action of heliotrine, the major alkaloid of Heliotropium indicum seeds, Planta Medica, 45(4): 229-233.

17. Modak, B., M. Salina, J. Rodilla and R. Torres, 2009. Study of the chemical composition of the resinous exudate isolated from heliotropium sclerocarpum and evaluation of the antioxidant properties of the phenolic compounds and the resin, Molecules, 14(11): 4625-4633.
18. Valerio, Jr, G. and G.F. Gonzales, 2005. Toxicological aspects of the South American herbs cat's claw (Uncaria tomentosa) and maca (Lepidium meyenii): a critical synopsis, Toxicological Reviews, 24(1): 11-35.
19. Shyur, L.F., J.H. Tsung, J.H. Chen, C.Y. Chiu and P.C. LO, 2005. Antioxidant properties of extracts from medicinal plants popularly use in Taiwan, App. Sci. and Eengineering, 3: 195-2002.
20. Molina, A., M.J. Reigosa and A. carballeira, 1998. Release of Allelochemical Agents from litter, though fall and top soil in plantations of Euclyptus Globules, 12: 79-87.
21. McLaughlin, J.L., LL. Rogers and JE. Anderson, 1998. Use of biological assay to evaluate botanicals. Drug. Inf. J, 32: 513-524.
22. Finney, D., 1971. Probit analysis: a statistical treatment of the sigmoid response curve. Cambridge: Cambridge University Press, pp: 256. [University of Oxford, Oxford, England.