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Antioxidant Activity and Brine Shrimp Lethality of *Tragia involucrata* L.¶

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Various extracts of *T. involucrata* have shown superoxide free radical, DPPH radical scavenging activities and brine shrimp toxicity. Ethyl acetate and methanol extracts showed poor to moderate antioxidant activity. However the aqueous methanol extract showed potent antioxidant activity and the fractions obtained from bioactivity guided fractionation of aqueous methanol extract showed potent antioxidant activity when compared to other extracts of *T. involucrata* and known commercial antioxidants like BHT, BHA. Methanol and aqueous methanol extracts also showed moderate brine shrimp lethality and fractions obtained from aqueous methanol extracts showed potent brine shrimp toxicity.

Key Words: Antioxidant activity, *Tragia involucrata,* BHA, BHT, *Artemia salina*, Brine shrimp lethality.

INTRODUCTION

Tragia involucrata L. (Vrischikali: Euphorbiaceae) is an important herb in the traditional Indian Systems of Medicine for its use in the treatment of a variety of diseases as a diuretic, antiperiodic and for venereal diseases[1]. The methanolic extract of *Tragia involucrata* has been studied in different experiments found that the extract possess significant, antimicrobial activity, analgesic, antiinflammatory and wound healing properties[2,3].

Free radicals and their metabolites, which are formed in the body as a consequence of normal metabolic reactions, exposure to pollutants and UV radiation, are recognized for their contribution to tissue injury and degenerative diseases, including arthritis, hemorrhagic shock, atherosclerosis, diabetes, hepatic injury, aging, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, tumour promotion and carcinogenesis[4,5]. The present study was undertaken to evaluate antioxidant activity using superoxide and DPPH radical scavenging assays and brine shrimp lethality of hexane, ethyl acetate, methanol, aqueous methanol extracts of *T. involucrata*. The other objective of the present study is to identify potent antioxidant fraction using bio-activity guided fractionation.

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EXPERIMENTAL

Nitrobluetetrazolium (NBT), 1,1-diphenyl-2-picryl-hydrazyl (DPPH) were obtained from Sigma Chemicals Co., St. Louis, MO 63178 USA. Brine shrimp eggs (*Artemia salina* cysts) were obtained from Argenta Chemical Laboratories, Redmond, (USA). EDTA, NaCN, ascorbic acid and other reagents of AR grade were procured from Qualigens Fine Chemicals, Mumbai, India. Silica gel (100-200 mesh) chromatography grade was obtained from Acme Laboratory Chemicals, Mumbai, India. Podophyllotoxin, was generously provided by Laila Impex. HPLC was carried out using Shimadzu system, equipped PDA detector LC-10ATVP pumps and SPD M-10 AVP-PDA detector and auto injector and loaded with Class VP software using C_{18} phenomenex luna (250 × 4.6, 5 μM) column and 0.1 % H_3PO_4-CH_3CN (60:40) mobile phase, UV absorbance detector (205 nm). The plant material was collected at Sriramapuram near Bhimavaram, India and the plant material was authenticated by Dr. Kanumuri Gopala Raju, Botany Department, DNR College, Bhimavaram and a herbarium specimen # 153/8/420C was deposited in the Taxonomy Department.

Extraction: The shade dried plant material was pulverized and 120 g of the powder, was extracted successively with hexane (1 L), ethyl acetate (0.75 L), methanol (0.75 L) using a Soxhlet apparatus. The exhausted material was then taken out of the Soxhlet apparatus and extracted with aqueous methanol (80 %, 2 L) under reflux for 2 h. All extracts were filtered and concentrated independently, under reduced pressure to obtain the crude residues as summarized in Table-1.

TABLE-1
DETAILS OF SOXHLET EXTRACTION AND ANTIOXIDANT ACTIVITY

Solvent used	Volume (L)	Dry weight (g)	Antioxidant activity IC_{50} (μg/mL)*	
			DPPH	NBT
Hexane	1.00	5.56	> 100	> 100
Ethylacetate	0.75	3.02	52.00	> 100
Methanol	0.70	20.5 0	48.50	> 100
Aqueous methanol	1.00	12.42	11.50	27.0
Standard (BHA)			3.85	110.7
Standard (BHT)			24.00	140.1

*Concentration required to scavenge 50 % of the free radical and values obtained are mean of 3 tests.

The aqueous methanol extract (5 g) was subjected to column chromatography on silica gel (150 g) using eluents of increasing polarity starting from EtOAc to MeOH. The fractions were monitored by TLC and grouped in to 8 fractions and concentrated independently under vacuum. Details of the fractions and their free radical scavenging activities were reported in Table-2.

TABLE-2
ANTIOXIDANT ACTIVITY OF THE FRACTIONS OF *T. involucrata* MeOH EXTRACT

Fraction	Antioxidant activity IC_{50} (μg/mL)*	
	DPPH	NBT
1	> 50	> 100
2	3.1	7.0
3	4.2	40.0
4	13.0	18.0
5	> 50	>100
6	> 50	51.0
7	19.0	82.0
8	17.0	59.0
BHT	4.0	115.0
BHA	25.0	150.0

*Concentration required to scavenge 50 % of the free radical and values obtained are mean of 3 tests.

Determination of superoxide radical scavenging activity: Superoxide radical scavenging activity of various extracts *T. involucrata* was determined by the method of McCord and Fridovich[6]. The assay mixture contained EDTA (6.6 mM) containing NaCN (3 μg), riboflavin (2 μM), NBT (50 μM), various concentrations of test substances and phosphate buffer (67 mM, pH 7.8) in a final volume of 3 mL. The tubes were mixed well and optical densities were measured at 560 nm. The tubes were uniformly illuminated with an incandescent lamp for 15 min and the optical densities were measured again at 560 nm. The percentage inhibition of superoxide radical generation was measured by comparing the absorbance values of control and those of the test substances. IC_{50} values were obtained from the plot drawn concentration (μg) *versus* percentage inhibition.

Determination of DPPH free radical scavenging activity: DPPH radical scavenging activity was measured by the method of Lamaison *et al.*[7], based on the reduction of coloured methanolic solution of the DPPH monitored at 517 nm. Free radical scavenging ability of the test substances added to the methanolic solution of DPPH is inversely proportional to the difference in initial and final absorption of DPPH solution at 517 nm. Antioxidant activity is expressed as the 50 % inhibitory concentration (IC_{50}). The reaction mixture contained 1×10^{-4} mM methanolic solution of DPPH and various concentrations of the test substances. Percentage inhibition was determined by comparing the absorbance values of test and control tubes. IC_{50} values were obtained from the plot, drawn for concentration (μg) *versus* percentage inhibition.

Determination of brine shrimp lethality: Brine shrimp lethality (BSL) assay is a simple bench top bioassay developed by McLaughlin *et al.*[8,9] and the results obtained by this assay have been reported to be corroborative with the cytotoxicities determined for 9KB and 9PS cells[10,11]. Brine shrimp (*Artemia salina*) nauplii were hatched using brine shrimp eggs in a conical shaped vessel (1 L), filled with sterile

artificial sea water (prepared using sea salt 38 g/L and adjusted to pH 8.5 using 1 N NaOH) under constant aeration for 48 h. After hatching, 10 nauplii were drawn through a pipette and placed in each vial containing 4.5 mL brine solution and added various concentrations of drug solutions and final volume was made up to 5 mL using brine solution and maintained at 37 ºC for 24 h under the light of incandescent lamps and surviving larvae were counted. Each experiment was conducted along with control (vehicle treated), at various concentrations of the test substance in each set that contains 3 tubes and the average results are reported (Table-3). The percentage lethality was determined by comparing the mean surviving larvae of test and control tubes. LC_{50} values were calculated by finney's software for probit analysis of quantal data. Podophyllotoxin was used as a positive control.

TABLE-3
BRINE SHRIMP LETHALITY DATA*

Test substances		Percentage lethality (dose in µg/mL)											
		0	1	2.5	5	10	25	50	75	100	150	200	IC_{50}
Hexane ext.	AA	9.33	9.33	9.33	9.33	8.67	8	7	6.33	5.67	5	3.67	137.5
	BB	C[a]	0	0	0	7.14	14.29	25	32.14	39.29	53.57	60.71	
EtoAc ext.	AA	9.33	9.33	9.33	9	8.33	8.00	7.67	6.33	5.67	4.33	3.67	175
	BB	C[a]	0	0	3.22	10.71	14.29	17.86	39.29	39.29	42.86	57.14	
MeOH ext.	AA	8.67				10	9.33	8.67	8.33	5.33	3.67	1.33	110
	BB	0	0	0	0	0	3.33	13.33	16.67	46.67	63.33	86.67	
Aq. MeOH ext.	AA	10	10	10	9.33	8.33	8.0	6.33	5	2	0.67	0	75
	BB	0	0	0	6.66	16.66	20	36.67	50	80	93.33	100	
Podophyllo-toxin	BB	C[a]	3.45	6.90	20.69	41.38	62.07	-	-	-	-	-	
	AA	8.33	5.6	4.8	1.0	0	0	-	-	-	-	-	3.1
	BB	C[a]	32.8	42	94	100	100	-	-	-	-	-	

AA = MSL[b]; BB = % Lethality.
*Values are mean of six tubes; [a]Considered as zero percent lethality; [b]Mean survival larvae.

RESULTS AND DISCUSSION

Antioxidant activity of various extracts of *T. involucrata* and fractions obtained in bioactivity guided fractionation were studied in comparison with known standards like vitamin C, BHA and BHT in different mechanisms and the results are given in Tables 1 and 2. On the basis of results obtained in the present study it is concluded that the aqueous methanol extract of *T. involucrata* and fractions 2, 3, 4 obtained by bioactivity guided fractionation showed moderate to potent antioxidant activity by two mechanisms. Brine shrimp lethality assay of various extracts and fractions of *T. involucrata* were studied in comparison to a known standard (podyphyllotoxin) as summarized in Table-3. The methanol and aqueous methanol extracts showed poor and moderate brine shrimp lethality respectively, whereas fraction 1s exhibited potent brine shrimp lethality. However the components responsible for antioxidant activity and brine shrimp toxicity is not clear. Further studies are required to isolate active compounds, which are responsible for bio activity.

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