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Antioxidant Activity and Brine Shrimp Lethality of *Bacopa monnieri* L.†

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Various extracts of *B. monnieri* have shown superoxide free radical, hydroxyl radical and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activities and inhibition of lipid peroxidation. Alcohol extract, however, showed better antioxidant activity compared to other extracts of *B. monnieri*. Methanol and commercial Bacopa extracts are equipotent to amla extract in inhibition of lipid peroxidation in rat liver, but Bacopa extracts have shown superior activity in rat brain tissue. Bacopa extracts have also shown brine shrimp lethality.

Key Words: *Bacopa monnieri* L., Antioxidant activity, Brine shrimp lethality.

INTRODUCTION

Bacopa monnieri L. (Brahmi: Scrophulariaceae) is an important herb in the Indian systems of medicine: the Ayurveda and the Unani, for its tranquilizing and memory enhancing effects[1,2]. It is used clinically for memory boosting[3] and has proved to be useful in treating epilepsy[4], cardiotonic[5], insomnia, etc. Alcohol soluble saponin fraction was established as the active fraction responsible for memory enhancing activity[2]. Antioxidant activity of *B. monnieri* was also reported earlier for its effect on lipid peroxidation and liver glutathione levels[6].

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 increasingly recognized for their contribution to tissue injury and degenerative diseases, including arthritis, hemorrhagic shock, atherosclerosis, diabetes, hepatic injury, ageing, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, tumor promotion and carcinogenesis[7,8]. The present study was undertaken to evaluate antioxidant activity and brine shrimp lethality of hexane, ethyl acetate, methanol, aqueous methanol extracts of *B. monnieri* in addition to the commercially available Bacopa extracts, standardized to bacosides A and B. Antioxidant activity of these extracts was studied in different mechanisms

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like superoxide, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), hydroxyl radicals, scavenging activity, lipid peroxidation in rat liver and brain tissues.

Brine shrimp lethality (BSL) assay is a simple bench-top bioassay developed by McLaughlin *et al.*[1, 2] and the results obtained by this assay have been reported to be corroborative with the cytotoxicities determined in 9KB and 9PS cells[14, 15]. We have investigated the brine shrimp lethality of various Bacopa extracts also and the results are presented in this note.

EXPERIMENTAL

Nitro blue tetrazolium (NBT), 1,1-diphenyl-2-picryl-hydrazyl (DPPH), thiobarbituric acid and tris(hydroxymethyl)amino methane were obtained from Sigma Chemicals Co., St. Louis, MO 63178 USA. EDTA, NaCN, $FeCl_3$, ascorbic acid, (SDS), H_2O_2, NaOH, acetic acid, *n*-butanol, pyridine and other reagents of AR grade were procured from Qualigens Fine Chemicals, Mumbai, India.

Extraction: Powdered material (400 g) of *B. monnieri*, kindly supplied by Laila Impex, was extracted with petroleum ether (2.5 L), ethyl acetate (2 L), methanol (2 L) and aqueous methanol (70%, 2 L) using a Soxhlet apparatus and extracts were concentrated, independently, under reduced pressure to obtain the crude residues 7, 4, 17 and 74.7 g, respectively.

Commercial Bacopa extracts having 30 and 54% bacosides A and B, amla extract, podophyllotoxin, bacoside-A and bacoside-B were generously provided by Laila Impex.

Determination of superoxide radical scavenging activity: Superoxide radical scavenging activity of various extracts of *Bacopa monnieri* was determined by the method of McCord and Fridovich[9]. The assay mixture contained EDTA (6.6 μM) containing 3 μg NaCN, 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 firom the plot drawn concentration (μg) *vs.* percentage inhibition.

Determination of DPPH free radical scavenging activity: DPPH radical scavenging activity was measured by the method of Lamaison *et al.*[10] based on the reduction of coloured methanolic solution of DPPH. 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. Drug activity is expressed as 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 of concentration (μg) *vs.* percentage inhibition.

Determination of hydroxyl free radical scavenging activity: Hydroxyl radical scavenging activity was measured by studying the competition between

deoxyribose and the test compounds for hydroxyl radicals generated by Fenton reaction. The hydroxyl radicals attack deoxyribose that eventually results in TBARS (thiobarbituric acid reactive substances) formation. The reaction mixture contained deoxyribose (2.8 mM), $FeCl_3$ (0.1 mM), EDTA (0.1 mM), H_2O_2 (1 mM), ascorbic acid (0.1 mM), sodium phosphate buffer (20 mM, pH 7.4) and various concentrations of test substance in a final volume of 1 mL. The reaction mixture was incubated for 1 h at 37°C. Deoxyribose degradation was measured as TBARS by the method of Ohkawa *et al.*[11] The percentage inhibition was determined by comparing the absorbance values of test and control tubes. IC_{50} values were obtained from the plot of concentration (μg) *vs.* percentage inhibition.

Inhibition of lipid peroxidation induced by Fe^{2+}/ascorbate system: The reaction mixture contained rat liver/brain homogenate (0.1 mL, 25% w/v) in tris-HCl buffer (20 mM, pH 7.0), KCl (150 mM), ferrous ammonium sulphate (0.8 mM) and ascorbic acid (0.3 mM) in a final volume of 0.5 mL and was incubated for 1 h at 37°C. The lipid peroxide formation was measured by the method of Ohkawa *et al.*[11] The procedure follows: 0.4 mL of the incubated reaction mixture was treated with 0.2 mL of SDS (8%), acetic acid 1.5 mL (20%, pH 3.5) and thiobarbituric acid 1.5 mL (8%). The total volume was then made up to 4 mL by adding distilled water and kept in a water bath at 97°C for 1 h; after cooling, 1 mL of distilled water and 5 mL of mixture of *n*-butanol and pyridine (15 : 1, v/v) were added and shaken vigorously and centrifuged at 3000 rpm for 10 min. After centrifugation, absorbance of the organic layer was measured at 532 nm. The percentage inhibition was determined by comparing the absorbance values of test and control tubes. IC_{50} values were obtained from the plot of concentration (μg) *vs.* percentage inhibition.

Determination of brine shrimp lethality: 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 volume was made up to 5 mL using brine solution and maintained at 37°C 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 6 tubes and the average results are reported (Table-2). The percentage lethality was determined by comparing the mean surviving larvae of test and control tubes. LC_{50} values were obtained from the plot of concentration (μg) *vs.* percentage inhibition. Podophyllotoxin was used as a positive control.

RESULTS AND DISCUSSION

Antioxidant activity of various extracts of *B. monnieri* and commercial extracts having 30 and 54% bacosides was studied in comparison with vitamin-C and vitamin-E in different mechanisms and the results are presented in Tables 1 and 2. The study revealed that the commercial bacopa extracts, ethyl acetate and

TABLE-I
SUPEROXIDE, DPPH AND HYDROXYL RADICAL SCAVENGING ACTIVITY DATA*

S.No.	Test substance	Superoxide radical assay			DPPH radical assay			Hydroxyl radical assay		
		D[a]	% inh.[b]	IC_{50}	D[a]	% inh.[b]	IC_{50}	D[a]	% inh.[b]	IC_{50}
1	Hexane ext.	100	13.28		125	6.748				
		200	20.56	350	250	14.45	> 400			
		400	65.34		400	18.20				
2	Ethyl Acetate ext.	50	34.13		10	20.31				
		100	56.35	90	25	49.42	26			
		200	75.19		50	82.91				
	MeOH ext.	25	25.09		10	23.09		25	43.08	
		50	48.81	51	25	51.21	24.5	50	52.25	48
		100	68.00		50	90.28		100	59.87	
4	Aq. MeOH ext.	100	19.05			24.72		50	39.46	
		200	42.74	410		48.57	52	100	50.91	96
		400	48.74			86.38		200	56.75	
5	30% Com. ext.[c]	25	27.37		10	21.09		25	37.57	
		50	48.46	52	25	46.89	28	50	43.64	77
		100	78.87		50	90.57		100	55.69	
6	54% Com. ext.[c]	25	30.30		10	21.52		25	38.49	
		50	50.30	50	25	51.70	24	50	47.44	55
		100	39.50		50	91.72		100	56.05	
7	Vitamin C	50	17.67		2.5	43.46				
		100	67.51	150	5	78.85	2.5			
		200	97.52		10	91.63				
8	Vitamin E	100	22.12		1000	9.58				
		200	32.03	312.5	2500	23.87	5,500			
		400	62.96		5000	45.34				

*Values are mean of 3 tubes for each dose.
[a]Total concentratim of test substance in µg per mL.
[b]Percentage inhibition.
[c]Commercial extracts having 30 and 54% bacosides A and B.

methanol extractives have shown significant antioxidant activity in superoxide, DPPH, hydroxyl radicals scavenging activities and lipid peroxidation. Bacopa extracts exhibited several-fold potent antioxidant activity compared to those of vitamin-C and vitamin-E in all the mechanisms of antioxidant activity except in DPPH radical scavenging activity.

It is interesting to note that the active fractions have shown higher percentage of inhibition of lipid peroxidation in rat brain compared to rat liver, perhaps, signifying their memory boosting effects. In fact, the commercial amla extract showed lower inhibition of lipid peroxidation in rat brain compared to rat liver (Table-2).

TABLE-2
INHIBITION OF LIPID PEROXIDATION IN RAT BRAIN AND LIVER*

S.No.	Test substance	Lipid peroxidation					
		Rat brain			Rat liver		
		D[a]	% inh.[b]	IC_{50}	D[a]	% inh.[b]	IC_{50}
1	MeOH ext.	25	41.86	46 µg	25 µg	27.80	83 µg
		50	51.28		50 µg	31.79	
		100	55.35		100 µg	59.66	
2	30% Com. ext.[c]	25	46.19	39 µg	25 µg	16.74	98 µg
		50	53.08		50 µg	28.93	
		100	62.19		100 µg	51.09	
3	54% Com. ext.[c]	25	44.97	36 µg	25 µg	22.69	85 µg
		50	55.72		50 µg	33.71	
		100	60.94		100 µg	56.95	
4	Amla DP	25	11.77	>100 µg	25 µg	11.05	83 µg
		50	19.78		50 µg	24.05	
		100	30.66		100 µg	52.41	

*Values are mean of 3 tubes for each dose.
[a]Total concentratim of test substance in µg per mL.
[b]Percentage inhibition.
[c]Commercial extracts having 30 and 54% bacosides A and B.

Brine shrimp lethality assay of various bacopa extracts and saponin fractions (bacoside-A and B) revealed that the saponin fractions are more potent compared to the commercial extracts or various bacopa fractions (Table-3).

TABLE-3
BRINE SHRIMP LETHALITY DATA*

Test substance		Percentage lethality (dose in µg/mL)											
		0	1	2.5	5	10	25	50	75	100	150	200	IC_{50}
Hexane ext.	MSL[b]	9.33	9.33	9.33	9.33	8.67	8	7	6.33	5.67	5	3.57	137.5
	% Lethality	C[a]	0	0	0	7.14	14.29	25	32.14	39.29	53.57	60.71	
EtOAc ext.	MSL[b]	9.33	9.33	9.33	9	8.33	8.00	7.67	6.33	5.67	4.33	3.67	175
	% Lethality	C[a]	0	0	3.22	10.71	14.29	17.86	39.29	39.29	42.86	57.14	
MeOH ext.	MSL[b]	10	10	10	10	10	9.33	8.67	8.33	5.33	3.67	1.33	110
	% Lethality	0	0	0	0	0	3.33	13.33	16.67	46.67	63.33	86.67	
Aq MeOH ext.	MSL[b]	10	10	10	9.33	8.33	8.0	6.33	5	2	0.67	0	75
	% Lethality	0	0	0	6.66	16.66	20	36.67	50	80	93.33	100	
30% Com ext.	MSL[b]	10	10	10	9.67	8.67	8.33	7	5.33	4.33	2.33	0	82.5
	% Lethality	0	0	0	3.33	13.33	16.67	30	46.67	56.67	76.67	100	
50% Com ext.	MSL[b]	10	10	10	10	9.33	8	7	5.67	4	2.33	0.67	85
	% Lethality	0	0	0	0	6.66	20	30	43.33	60	76.67	93.33	
Bacoside-A	MSL[b]	9.67	9.67	9.67	9	8	6.67	5.67	3.67	3.67	0	0	70
	% Lethality	C[a]	0	0	6.90	17.24	31.03	41.37	62.07	62.07	100	100	
Bacoside-B	MSL[b]	9.67	9.33	9.00	7.67	6.33	3.67	—	—	—	—	—	12.13
	% Lethality	C[a]	3.45	6.90	20.69	41.38	62.07	—	—	—	—	—	
Podophyllotoxin	MSL[b]	8.33	5.60	4.80	1.00	0	0	—	—	—	—	—	3.1
	% Lethality	C[a]	32.80	42	94	100	100	—	—	—	—	—	

*Values are mean of six tubes. [a]Considered as zero per cent lethaliy. [b]Mean survival larvae.

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