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Research article

IN VITRO ANTIOXIDANT STUDIES OF *CARDIOSPERMUM HALICACABUM* L. VAR. *LURIDUM* (BLUME) ADELB SEEDS

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ABSTRACT: *Cardiospermum halicacabum* Linn. (Sapindaceae) is a climber, commonly found throughout India, used as vegetable as well as traditional medicine for rheumatism, lumbago and nervous diseases. In the current study, we investigated the antioxidant potency of successive extracts of *C. halicacabum* seeds employing various established *in vitro* systems, such as the lipid peroxidation, nitric oxide radical scavenging and reduced glutathione assay. Furthermore, all the extracts exhibited concentration dependent inhibition in all the models at tested doses 25-1000 µg/ ml. The data obtained in the *in-vitro* models clearly establish the antioxidant potency of *C. halicacabum* seed extract.

Key words: Antioxidant, *Cardiospermum halicacabum* Linn, Seeds

INTRODUCTION

Cardiospermum halicacabum Linn. is an important medicinal twining herb distributed throughout India. The whole plant as such has a high potential medicinal value (Kirtikar and Basu, 1935). Roots and leaves are used to treat fever, arthritis and chronic rheumatism. Seeds are diaphoretic and used in tonics. The plant has sedative action on the central nervous system (Varier, 1993). Two glasses of a 1-2 h. maceration of aerial parts of the plant are drunk or used for bathing in the treatment of hyperthermia, and in some areas water extracts of the seed are taken (Neuwinger, 2000). Flavone aglycones have been isolated from an extract of this plant which also demonstrated insecticidal activity (Shabana *et al.*, 1990). Extracts of this plant have been reported to contain different triterpenoids, glycosides, and a range of fatty acids (Ahmed *et al.*, 1993; Ferrara *et al.*, 1996; Srinivas *et al.*, 1998). Phytochemical constituents such as oleic acid, erucic acid, hexadecanoic acid, octadecanoic acid and a range of other compounds have been reported from this seeds (Jayanthi and Jegadeesan, 2008) According to World Health Organisation (WHO) still about 80% of the world populations mostly on plant based drugs. Low coast and easy availability, these factors has generated a renewed interest in plant medicine in the last decade. Many plants often contain substantial amounts of antioxidants including Vitamin C and E, carotenoids, flavonoids and tannins etc., and thus can be utilized to scavenge the excess free radicals from human body (Pratt, 1992).

But the importance of *C. halicacabum* seeds as an effective antioxidant agent is still to be established. The present study was undertaken to evaluate the antioxidant activity of various solvent extracts of *C. halicacabum* seeds *in vitro* on a three models.

MATERIALS AND METHODS

Chemicals

All chemicals and solvents were analytical grade and were obtained from Ranbaxy Fine Chemicals, Mumbai, India.

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Plant material

Seeds of *Cardiospermum halicacabum* var. *microcarpum* were collected from Thirumalairayanpattinam, Karaikal, Pondicherry, Union Territory of India and authenticated by Dr.M.Jegadeesan and the plant specimen was lodged in the Tamil University herbarium, Voucher No.TUH 51B.

Seed extracts

The seeds of *C. halicacabum* var. *microcarpum* **(**100 kg) were shade dried and powdered coarsely. The powder was extracted successively using soxhlet apparatus with petroleum ether (60-80°C), benzene (60°C), chloroform (60°C) and alcohol (78°C). Each time before extracting with next solvent, powdered material were dried in an air-oven below 50°C. The extracts were dried over anhydrous sodium sulfate, stored in sealed vials in refrigerator (5-8°C) until analysis. Finally, marc was macerated with chloroform water for 24 h. to obtain the aqueous extract. The extract was concentrated by distilling the water and then evaporating to dryness on a water bath (Anonymous, 1996).

Preparation of rat liver homogenates

Wister strain albino rats (160-180 g) were taken from the inbred group maintained at Tamil University animal house, Thanjavur. The animals were fed with standard pellet diet supplied by Lipton and Co. Ltd., Bangalore. Water was made available to animals ad-libitum. The animal experiments were carried out in accordance with animal ethical committee norms. Randomly selected male rat, was fasted overnight and was sacrificed by cervical dislocation, dissected and abdominal cavity was perfused with 0.9% saline. Whole liver was taken out and visible clots were removed and weighed amount of liver was processes to get 10% homogenate in cold phosphate buffered saline, pH 7.4 using glass Teflon homogenizer and filtered to get a clear homogenate.

Inhibition of lipid peroxidation (Prasanth Kumar et al., 2008)

The different concentration of all extracts (25-100µg/ml) were added to the liver homogenate. Lipid peroxidation was initiated by adding 100 µl of 15 mM $FeSO_4$ solution to 3 ml of liver homogenate (final concentration was 0.5 mM). After 30 min. 100 µl of this reaction mixture was taken in a tube containing 1.5 ml of 10% TCA. After 10 min. tube were centrifuged and supernatant was separated and mixed with 1.5 ml of 0.67% TBA in 50% acetic acid. The mixture was heated in a hot water bath at 85°C for 30 min. and in a boiling water bath to complete the reaction. The intensity of pink coloured complex formed was measured at 535 nm in a spectrophotometer. The percentage inhibition of lipid peroxidation was calculated by comparing the results of the test with those of controls not treated with the extracts, as per the following formula.

$$\text{Inhibition (\%)} = \frac{\text{(Control – test)}}{\text{Control}} \times 100$$

Scavenging of nitric oxide radical (Sreejayan and Rao 1997; Shirwaikar Annie and Someshekar 2003).

Nitric acid scavenging activity was measured by using a spectrophotometer. Sodium nitroprusside (5 mM) in phosphate buffered saline was mixed with different concentrations of all the extracts (25-100µg/ml) and incubated at 25°C for 30 min. A control without test compound was taken. After 30 min 1.5 ml of the incubation solution were removed and diluted with 1.5 ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid, and 0.1% naphthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphthylene diamine was measured at 546 nm.

Reduced glutathione assay (GSH) (Tripathi and Sharma 1998)

Liver homogenate with different concentration of all extracts (25-100µg/ml) were mixed with 0.5 ml of 5% Trichloro acetic acid in 0.1 mM EDTA. The sample was centrifuged at 2000 g for 10 min. and the supernatant was mixed with 2.5 ml of 0.1 M phosphate buffer (pH 8) the colour was developed by adding 100 µl of 0.01% DTNB. Absorbance was measured at 412 nm with the help of Spectrophotometer. The percentage reduction was calculated by comparison with the control with the above mentioned formula.

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RESULTS

Several concentrations ranging from 25-1000 µg/ml of all the extracts of *C. halicacabum* var. *luridum* were tested for their antioxidant activity in different *in-vitro* models. It was observed that free radicals were scavenged by the test compounds in a concentration dependent manner in all the models. In LPO method, among all extracts, the petroleum ether extract showed only less activity than other extracts. In the other extracts, activity was found to increase when the concentration of the extract was increased from 25 to 1000 µg/ml. The higher reduction was noted at the concentration of 500 and 1000 µg/ml. (Table 1 and Fig.1) the higher inhibition was noted in 1000 µg/ml in nitric oxide scavenging method. (Table 2 and Fig.2) In GSH method all the extracts showed significant ($P \leq 0.001$) inhibition. Among the five extracts, petroleum ether, benzene and chloroform exhibited higher level of inhibition than the ethanol and water extracts. (Table 3 and Fig.3).

Table – 1 : Antioxidant activity of seed extracts of *C. halicacabum* var. *luridum*(LPO method)

S. No.	Dose µg/ml	Antioxidant activity (OD)									
		Petroleum ether		Benzene		Chloroform		Ethanol		Water	
		Mean ± SD	% of inhibition	Mean ± SD	% of inhibition	Mean ± SD	% of inhibition	Mean ± SD	% of inhibition	Mean ± SD	% of inhibition
1.	Control	0.122 ± 0.0007	0	0.122 ± 0.0007	0	0.122 ± 0.0007	0	0.122 ± 0.0007	0	0.122 ± 0.0007	0
2.	25	0.0728 ± 0.0007	40	0.083 ± 0.0005	32	0.068 ± 0.0005	45	0.039 ± 0.026	68	0.045 ± 0.0005	63
3.	50	0.065 ± 0.0013	46	0.027 ± 0.0008	78	0.060 ± 0.001	51	0.042 ± 0.0006	66	0.033 ± 0.001	73
4.	100	0.0511 ± 0.0007	31	0.025 ± 0.0005	79	0.008 ± 0.0006	93	0.035 ± 0.0005	71	0.027 ± 0.0005	78
5.	250	0.036 ± 0.0005	70	0.013 ± 0.0005	89	0.003 ± 0.005	97	0.030 ± 0.0008	75	0.023 ± 0.0005	81
6.	500	0.031 ± 0.0004	74	0.007 ± 0.0005	93	0.002 ± 0.0005	98	0.013 ± 0.0007	89	0.013 ± 0.0004	87
7.	1000	0.025 ± 0.0005	79	0.003 ± 0.0005	97	0.002 ± 0.0005	98	0.012 ± 0.0005	90	0.005 ± 0.0006	96

Values are expressed as mean ± SD, n = 6. $P \leq 0.001$ compared to normal control.

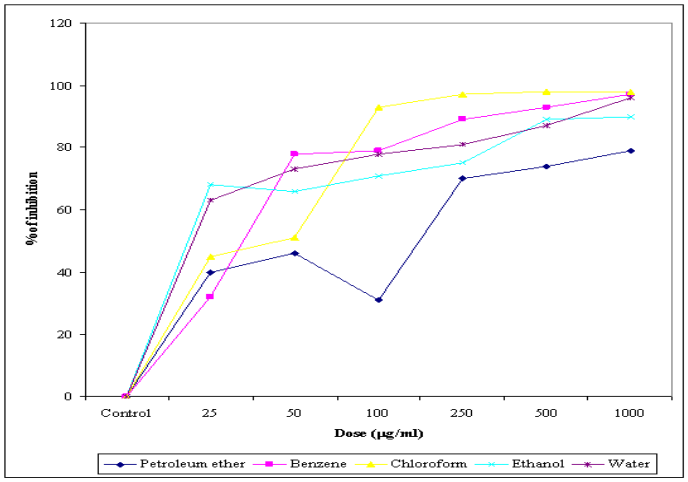

Figure – 1 : Antioxidant activity of seed extracts of C. halicacabum var. luridum (LPO method)

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Table – 2 : Antioxidant activity of seed extracts of *C. halicacabum* var. *luridum* (Nitric oxide method)

S. No.	Dose µg/ml	Antioxidant activity (OD)									
		Petroleum ether		Benzene		Chloroform		Ethanol		Water	
		Mean ± SD	% of inhi-bition	Mean ± SD	% of inhi-bition	Mean ± SD	% of inhi-bition	Mean ± SD	% of inhi-bition	Mean ± SD	% of inhi-bition
1.	Control	0.12 ± 0.0006	0	0.12 ± 0.0006	0	0.12 ± 0.0006	0	0.12 ± 0.0006	0	0.12 ± 0.0006	0
2.	25	0.093 ± 0.0008	22	0.051 ± 0.0004	57	0.092 ± 0.001	23	0.098 ± 0.0008	19	0.056 ± 0.0004	53
3.	50	0.042 ± 0.0006	65	0.047 ± 0.0005	60	0.084 ± 0.001	30	0.074 ± 0.003	38	0.051 ± 0.0009	57
4.	100	0.036 ± 0.0005	69	0.043 ± 0.0005	64	0.073 ± 0.0009	39	0.070 ± 0.0001	41	0.041 ± 0.0004	67
5.	250	0.021 ± 0.0008	82	0.021 ± 0.0005	82	0.041 ± 0.0007	54	0.041 ± 0.0005	66	0.031 ± 0.0002	74
6.	500	0.016 ± 0.0005	86	0.016 ± 0.0005	86	0.015 ± 0.0005	87	0.049 ± 0.0009	59	0.024 ± 0.0002	82
7.	1000	0.006 ± 0.0005	95	0.010 ± 0.0008	91	0.005 ± 0.0005	96	0.021 ± 0.0005	82	0.014 ± 0.0001	88

Values are expressed as mean ± SD, n = 6. P ≤ 0.001 compared to normal control.

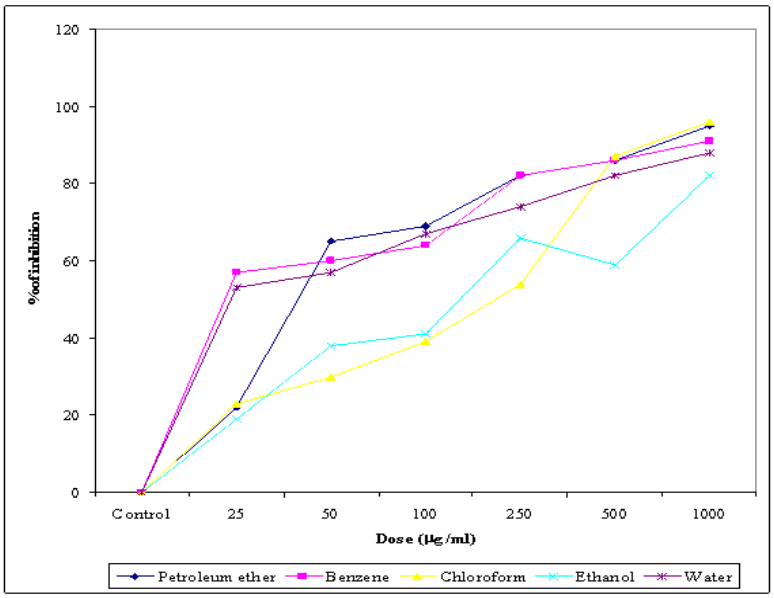

Figure – 2: Antioxidant activity of seed extracts of *C. halicacabum* var. *luridum* (Nitric oxide method)

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Table –3: Antioxidant activity of seed extracts of *C. halicacabum* var. *luridum* (GSH method)

S. No.	Dose µg/ml	Antioxidant oxidant (OD)									
		Petroleum ether		Benzene		Chloroform		Ethanol		Water	
		Mean ± SD	% of inhibition	Mean ± SD	% of inhibition	Mean ± SD	% of inhibition	Mean ± SD	% of inhibition	Mean ± SD	% of inhibition
1.	Control	0.134 ± 0.0005	0	0.134 ± 0.0005	0	0.134 ± 0.0005	0	0.134 ± 0.0005	0	0.134 ± 0.0005	0
2.	25	0.063 ± 0.0006	53	0.047 ± 0.0005	64	0.053 ± 0.001	60	0.066 ± 0.0008	50	0.092 ± 0.0005	31
3.	50	0.053 ± 0.0008	60	0.026 ± 0.0005	80	0.041 ± 0.0005	69	0.050 ± 0.0005	62	0.088 ± 0.0007	34
4.	100	0.050 ± 0.0008	62	0.018 ± 0.0007	86	0.032 ± 0.001	76	0.043 ± 0.001	68	0.081 ± 0.0005	39
5.	250	0.041 ± 0.0007	69	0.018 ± 0.0008	87	0.024 ± 0.0008	82	0.037 ± 0.0008	72	0.057 ± 0.001	58
6.	500	0.035 ± 0.0003	73	0.014 ± 0.0008	90	0.012 ± 0.0005	91	0.030 ± 0.001	77	0.031 ± 0.001	76
7.	1000	0.008 ± 0.0005	94	0.007 ± 0.003	95	0.008 ± 0.0008	94	0.002 ± 0.001	99	0.013 ± 0.0008	90

Values are expressed as mean ± SD, n = 6. P ≤ 0.001 compared to normal control.

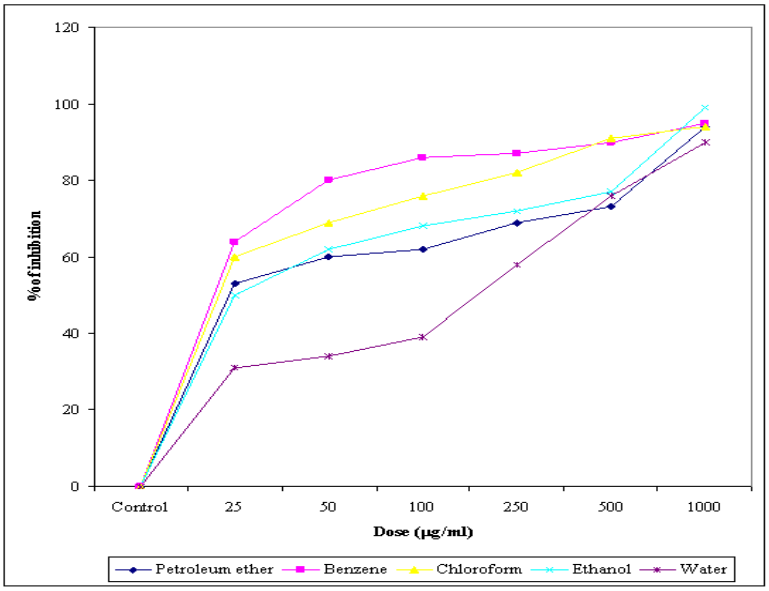

Figure –3: Antioxidant activity of seed extracts of *C. halicacabum* var. *luridum* (GSH method)

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DISCUSSION

Free radicals are chemical entities that can exist separately with one or more unpaired electron. The propagation of free radicals can bring about thousands of reactions and thus may cause extensive tissue damage (Cotran *et al.*, 1999; Yu *et al.*, 1992). Free radicals induced by peroxidation have gained much importance because of their involvement in several pathological conditions such as atherosclerosis, ischemia, liver disorder, neural disorder, metal toxicity and pesticide toxicity (Pandey *et al.*, 1994). Foods rich in antioxidants have been shown to play an essential role in the prevention of cardiovascular diseases, cancer, inflammation and problems caused by cell and cutaneous aging.

Lipid peroxidation is the oxidative degradation of polyunsaturated fatty acids and involves formation of lipid radicals leading to membrane damage. Free radicals induces lipid peroxidation in poly unsaturated lipid rich areas like brain and liver (Coyle and Puttafarcken, 1993). Inhibition of lipid peroxidation by ferrous sulphate takes place through hydroxyl radical by Fenton's reaction (Braugghler *et al.*, 1986). In this study, *in vitro* lipid peroxidation was induced in rat liver by using $FeSO_4$. The present results show that extracts of *C. halicacabum* var. *luridum* shows better dose-dependent prevention towards generation of lipid peroxides.

Nitric oxide is a free radical generated by endothelial cells, macrophages, neurons etc., and involved in the regulation of various physiological process (Lata and Ahuja, 2000). Excess concentration of NO is associated with several diseases (Ialenti *et al.*, 1993 and Ross, 1993). Oxygen react with the excess nitric oxide to generate nitric and peroxy nitrite anions, which act as free radicals (Cotran *et al.*, 1999 and Sainani *et al.*, 1997). In the present study, the extracts of *C. halicacabum* var. *luridum* showed better activity in computing with oxygen to react with nitric oxide and thus the inhibition of generation of anions. GSH constitute a major reducing substance of the cytoplasm and is known to protect the cellular system against toxic effects of lipid peroxidation. GSH and vitamins E and C exist in the interconvertible reduced and oxidized forms and thus participate in neutralizing free radicals as and when they are formed (McCay, 1985). In the present study, the extracts showed dose-dependent inhibition.

From the above results, it can be concluded that ethanolic extracts of leaves of *C. halicacabum* var. *luridum* showed that most potent *in vitro* antioxidant activity. This may be attributed to the presence of phytochemical like hexadecanoic acid, oleic acid, erucic acid, n-hexadecanoic acid etc., which probably play a role as an effective free radical scavenger and hence an effective anti-inflammatory agent.

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