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Research Article

Free radical scavenging potential of *Cardiospermum halicacabum* L. var. *microcarpum* (Kunth) Blume seeds

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ABSTRACT
The free radical scavenging activity of the seeds of *C. halicacabum* was studied by using different antioxidant models. All the extracts showed good dose-dependent free radical scavenging property in all the models at tested doses (25-1000 µg/1ml). Thus augmenting the wide use of plant in the indigenous system of medicine, which may partly be due to antioxidant and free radical scavenging activity of the extracts.

Key words: Free radicals, *Cardiospermum halicacabum*, antioxidant, Sapindaceae.

INTRODUCTION

Free radicals have been implicated in causation of ailments such as liver cirrhosis, atherosclerosis, cancer, diabetes etc. Antioxidants that scavenge free radicals have great potential in ameliorating these disease processes[1]. Together with other derivatives of oxygen they are inevitable by products of biological redox reactions[2]. Reactive oxygen species has been known to cause tissue injury through covalent binding and lipid peroxidation[3], which has been shown to augment collagen synthesis and fibrosis. The increased production of toxic oxygen derivatives is considered to be a universal feature of stress conditions. Although medicinal plants are rarely used as antioxidants in traditional medicine, their claimed therapeutic properties could be due, in part to their capacity for scavenging oxygen free radicals, which may be involved in many diseases.

Cardiospermum halicacabum L. var. *microcarpum* (Kunth) Blume is a plant of Sapindaceae family widespread in tropical and sub-tropical countries. In India, this plant leaves are commonly consumed leafy vegetable. Indian system of medicine recommends *C. halicacabum* leaves for rheumatism, chronic bronchitis, stiffness of limbs and snakebite[4]. Roots and leaves are used to fever, arthritis and chronic rheumatism. Seeds are diaphoretic and used in tonics. The plant has sedative on the central nervous system[5]. In the line of the pharmacological validation of this plant, the toxicological evaluation of *C. halicacabum* revealed that the drug is safe and is not toxic upto 40 g/kg in rats[6]. It is known to contain saponin, quebrachitol, apigenin, proanthocyanidin and stigmosterol[7]. Phytochemical constituents such as olieic acid, erucic acid, hexadecanoic acid, octadecanoic acid and a range of other compounds have been reported from this seed [8, 9].

As a detailed review of literature afforded no information on the antioxidant potential of the plant, it was therefore thought worthwhile to investigate the *in vitro* antioxidant potential of ***C. halicacabum*** var. ***microcarpum*** seeds.

MATERIALS AND METHODS

Chemicals

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

Plant material – Seeds of ***Cardiospermum halicacabum*** var. ***microcarpum*** were collected from Thirumalairayanpattinam, Karaikal, Pondicherry, Union Territory of South India and authenticated by Dr.M.Jegadeesan Department of Environmental and Herbal Science

and the plant specimen was lodged in the Tamil University herbarium, Voucher No.TUH 51A.

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.

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[10]

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[11,12].

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 minutes. 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)[13] – 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.

RESULTS

Several concentrations ranging from 25 to 1000 µg/ml of all the extracts of ***C. halicacatum*** var. ***microcarpum*** 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, the activity was exhibited as follows, petroleum ether, ethanol, benzene, water and chloroform at a tested doses (Table 1 and Fig.1). The higher inhibition was noted in 1000 µg/ml in Nitric oxide scavenging method. However, the water extract had only moderate inhibition compared to other extracts (Table 2 and Fig.2). In GSH method among the five extracts, petroleum ether, benzene and chloroform extracts exhibited higher level of inhibition than the ethanol and water extracts (Table 3 and Fig.3).

DISCUSSION

Interest in the search for natural antioxidants has increased over the past few years as the reactive oxygen species production and oxidative stress have been show to play vital role in a number of disorders[14]. Antioxidants may after resistance against the oxidative stress by scavenging free radicals, inhibiting lipid peroxidation and by many other mechanisms and

thus prevent disease[15]. Also the restriction laid on the use of synthetic antioxidants have been the important incentive for such research work. These studies are more pertinent with regard to therapeutic agents of plant origin employed in treating a wide range of diseases[16].

From the results, it was observed that the all the extracts of the ***C. halicacabum*** var. ***microcarpum*** were found to act as radical scavengers against different free radicals under the conditions of oxidative stress. Initiation of lipid peroxidation by ferrous sulphate takes place either through ferryl perferryl complex[17] or through OH radical by Fenton reaction[18]. Ferryl-perferryl complex can also initiate lipid peroxidation in a similar manner as OH^-, although it is less reactive than OH[19].

Nitric oxide is a free radical produced in mammalian cells, involved in the regulation of various physiological processes. However, excess production of NO is associated with several diseases[20, 21]. In the present study, all the seed extracts of ***C. halicacabum*** var. ***microcarpum*** showed significant ($P > 0.001$) in *in vitro* antioxidant activities. This may be due to the antioxidant principles in the extracts which compete with oxygen to react with nitric oxide[22] thereby inhibiting the generation of nitrite.

Glutathione (GSH), a tripeptite present in all the cells is an important antioxidant[23]. GSH also functions as free radical scavenger and in the repair of radical caused biological damage[24, 25]. ***C. halicacabum*** var. ***microcarpum*** inhibited the oxidation of reduced glutathione in a dose-dependent manner, which may be due to the presence of other phytochemicals in the extracts. The importance of thiols especially of cysteine and glutathione to lymphocyte function has been known for many years. GSH is non-enzymatic mode of defense against the free radicals.

CONCLUSION

The antioxidant and free radical scavenging activities of all extracts of ***C. halicacabum*** var. ***microcarpum*** used as a anti-inflammatory and antipyretic drug in the Indian System of Medicine were determined. The results obtained suggest that the use of this seed extract widely in the treatment of many diseases which include poisoning, rheumatism, bronchitis, stiffness of the limbs and nervous disease, may be due to its antioxidant and free radical scavenging ability. This report undoubtedly elucidated antioxidant potential of ***C. halicacabum*** substantiating the already known utility of this wonder herb.

Fig. 1Antioxidant activity of seed extracts of C. halicacabum var. microcarpum (LPO method)

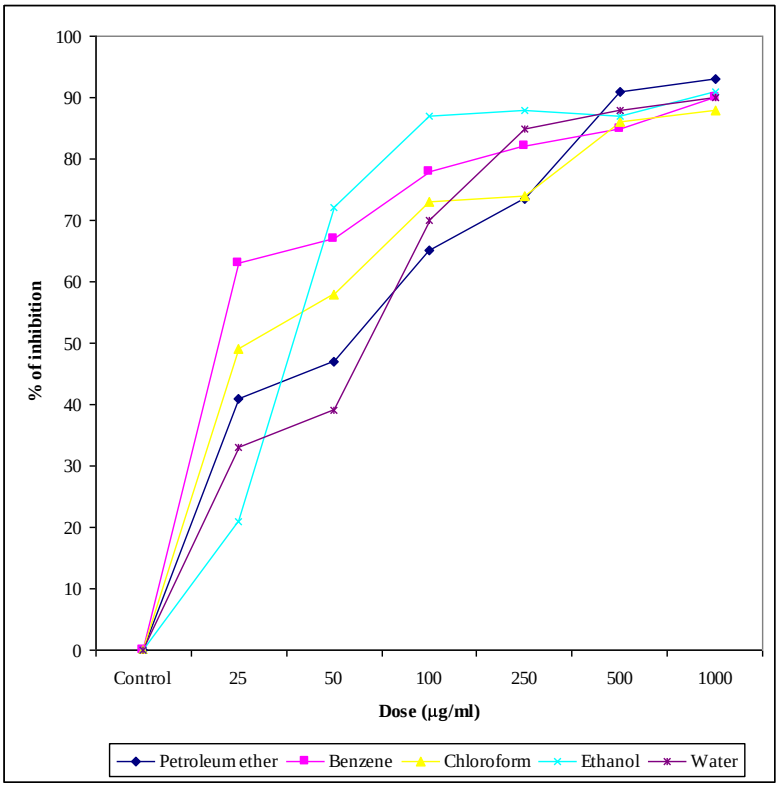

Fig. 2Antioxidant activity of seed extracts of C. halicacabum var. microcarpum (Nitric oxide method)

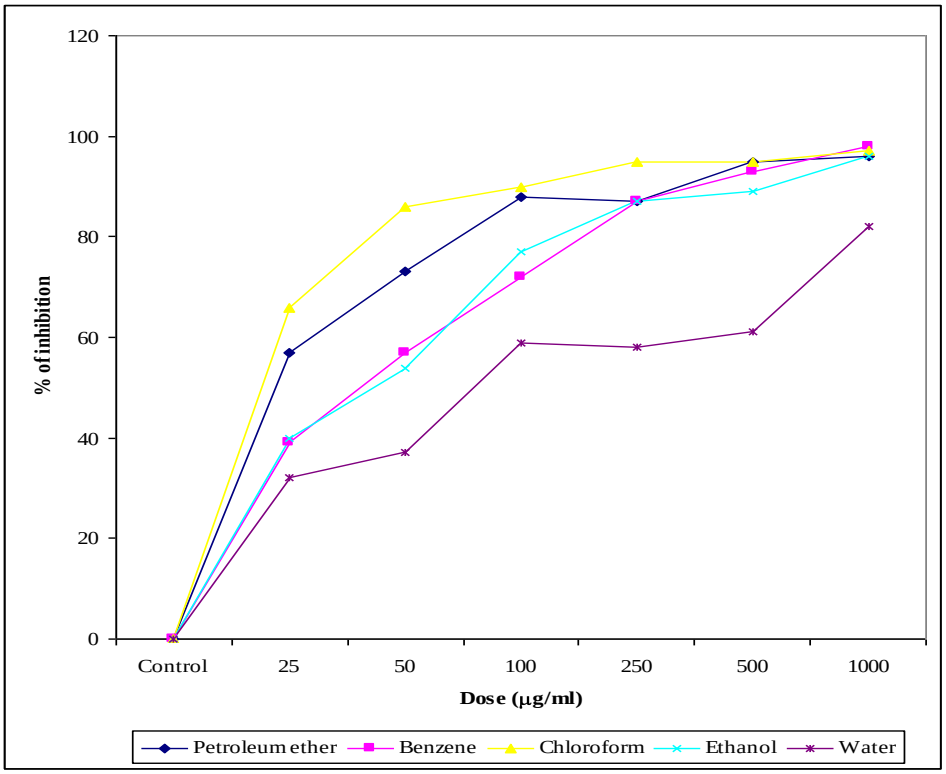

Fig. 3 Antioxidant activity of seed extracts of C. halicacabum var. microcarpum (GSH method)

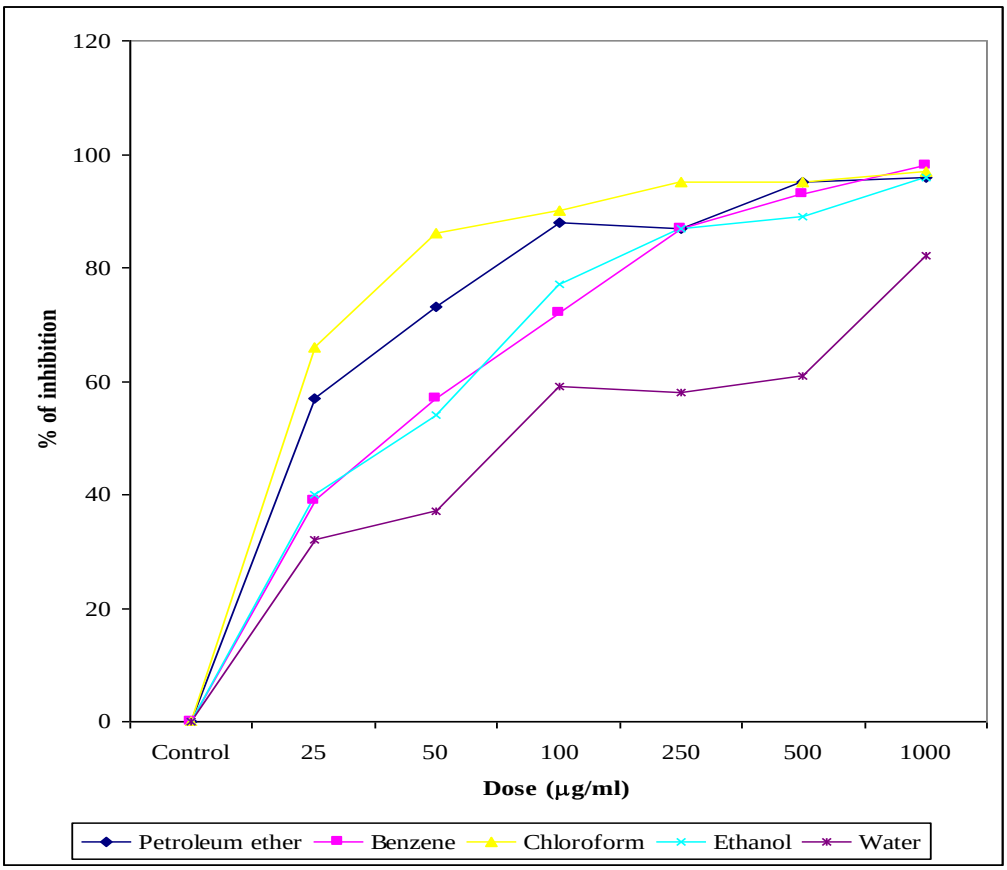

Table 1: Antioxidant activity of seed extracts of *Cardiospermum halicacabum* var microcarpum (LPO method)

S. No.	Dose µg/ml	Name of the extracts									
		Petroleum ether		Benzene		Chloroform		Ethanol		Water	
		Mean ± SE	% of inhi-bition	Mean ± SE	% of inhi-bition	Mean ± SE	% of inhi-bition	Mean ± SE	% of inhi-bition	Mean ± SE	% of inhi-bition
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	0.072 ± 0.0006	41	0.045 ± 0.0008	63	0.061 ± 0.0010	49	0.097 ± 0.0015	21	0.081 ± 0.0012	33
3.	50	0.064 ± 0.001	47	0.039 ± 0.0007	67	0.051 ± 0.001	58	0.034 ± 0.0005	72	0.074 ± 0.001	39
4.	100	0.042 ± 0.0008	65.2	0.026 ± 0.0008	78	0.033 ± 0.001	73	0.015 ± 0.0005	87	0.036 ± 0.0008	70
5.	250	0.032 ± 0.0009	73.6	0.021 ± 0.0008	82	0.032 ± 0.001	74	0.014 ± 0.0005	88	0.0175 ± 0.00082	85
6.	500	0.011 ± 0.0008	91	0.018 ± 0.0006	85	0.0165 ± 0.0008	86	0.016 ± 0.0005	87	0.014 ± 0.0009	88
7.	1000	0.008 ± 0.0005	93	0.012 ± 0.0008	90	0.014 ± 0.0008	88	0.011 ± 0.0008	91	0.012 ± 0.0005	90

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

Table 2: Antioxidant activity of seed extracts of *C. halicacabum* var microcarpum (Nitric Oxide method)

S. No.	Dose µg/ml	Name of the extracts									
		Petroleum ether		Benzene		Chloroform		Ethanol		Water	
		Mean ± SE	% of inhi-bition	Mean ± SE	% of inhi-bition	Mean ± SE	% of inhi-bition	Mean ± SE	% of inhi-bition	Mean ± SE	% of inhi-bition
1.	Control	0.122 ± 0.0006	0	0.122 ± 0.0006	0	0.122 ± 0.0006	0	0.122 ± 0.0006	0	0.122 ± 0.0006	0
2.	25	0.051 ± 0.0005	57	0.073 ± 0.0006	39	0.040 ± 0.0005	66	0.071 ± 0.0008	40	0.081 ± 0.0005	32
3.	50	0.033 ± 0.0005	73	0.051 ± 0.0007	57	0.016 ± 0.005	86	0.055 ± 0.0007	54	0.075 ± 0.0005	37
4.	100	0.014 ± 0.0005	88	0.034 ± 0.0004	72	0.012 ± 0.0004	90	0.027 ± 0.0005	77	0.051 ± 0.0004	59
5.	250	0.015 ± 0.0007	87	0.015 ± 0.0004	87	0.0065 ± 0.0005	95	0.016 ± 0.0004	87	0.050 ± 0.0004	58
6.	500	0.006 ± 0.0007	95	0.008 ± 0.0005	93	0.006 ± 0.0005	95	0.013 ± 0.0004	89	0.047 ± 0.0005	61
7.	1000	0.004 ± 0.0005	96	0.002 ± 0.0009	98	0.003 ± 0.0007	97	0.004 ± 0.0005	96	0.021 ± 0.0005	82

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

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

S. No.	Dose µg/ml	Name of the extracts									
		Petroleum ether		Benzene		Chloroform		Ethanol		Water	
		Mean ± SE	% of inhi-bition	Mean ± SE	% of inhi-bition	Mean ± SE	% of inhi-bition	Mean ± SE	% of inhi-bition	Mean ± SE	% of inhi-bition
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	0.052 ± 0.0006	61	0.039 ± 0.0008	70	0.085 ± 0.0008	36	0.094 ± 0.0005	29	0.080 ± 0.0008	40
3.	50	0.041 ± 0.0008	69	0.029 ± 0.0007	78	0.053 ± 0.0005	60	0.091 ± 0.0005	31	0.074 ± 0.0008	44
4.	100	0.029 ± 0.0008	78	0.027 ± 0.0005	80	0.041 ± 0.001	69	0.086 ± 0.0005	35	0.041 ± 0.001	69
5.	250	0.0155 ± 0.0008	88	0.014 ± 0.0008	89	0.040 ± 0.0008	70	0.074 ± 0.001	44	0.037 ± 0.001	72
6.	500	0.010 ± 0.0007	92	0.013 ± 0.001	90	0.031 ± 0.0007	77	0.0415 ± 0.0005	69	0.036 ± 0.0008	73
7.	1000	0.007 ± 0.0005	95	0.010 ± 0.0007	92	0.012 ± 0.0008	91	0.018 ± 0.0005	87	0.014 ± 0.0008	89

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

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