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R Shunmuga Vadivu
Research Scholar, Department of Biochemistry, Marudupandiyar College, Thanjavur, Tamil Nadu, India

S Velavan
Department of Biochemistry, Marudupandiyar College, Thanjavur, Tamil Nadu, India

Correspondence
S Velavan
Department of Biochemistry, Marudupandiyar College, Thanjavur, Tamil Nadu, India

Phytochemical screening, reactive oxygen and nitrogen species scavenging activities of *Caralluma indica* stem extract

R Shunmuga Vadivu and S Velavan

Abstract
The ethanolic extract of *Caralluma indica* was screened for phytochemical analysis and *in vitro* antioxidant activity. The antioxidant activity screened through DPPH, total antioxidant assay, super oxide metal chelating, iron reducing power activity and nitric oxide scavenging activity at different concentrations and ascorbic acid as a standard antioxidant. Phytochemical analysis of various extract (Aqueous, Methanol, Ethanol and Ether) showed that the presence of rich source of active chemicals. Bioflavonoid content of *Caralluma indica* attributed the concentration dependent antioxidant activity. Overall antioxidant activity of *Caralluma indica* was found to be the strongest. The present study reveals that the *Caralluma indica* would exert several beneficial effects of virtue of their antioxidant activity and could be rendering useful as drug formulation. This is the first report recording to *Caralluma indica* stem.

Keywords: Phytochemicals, antioxidant, radical scavenger, reactive oxygen species, *Caralluma indica*

Introduction
The oxidative damage of various biomolecules in the human body is associated with lipid peroxidation, cell structural injury, tissue impairment and gene mutation. In addition lipidperoxidation initiated by free radicals, is one of the major factors for food deterioration during processing and storage Donnelly and Robinson (1995) [5] Free radicals play a crucial role in aging as well as many disease conditions cardio vascular disorder, cancer, neuro degenerative disorder, inflammation. Pharm-huy *et al.* (2008). [17] The most effective path to eliminate and diminish the action of free radicals which cause the oxidative stress is antioxidative defence mechanism. Antioxidants are those substances which possess free radical chain reaction breaking properties Pourmorad *et al.* (2006) [18]. Reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), super oxide anion (O_2^-) and hydroxyl ($^{\cdot}OH$) radical and hydrogen peroxide (H_2O_2) are often generated as byproducts of biological reactions or from exogenous factors Kiritokar *et al.* (1998) [11]. Under stress, our bodies produce more reactive oxygen species (ROS) than enzymatic antioxidants (superoxide dismutase glutathione per oxidase) and non-enzymatic antioxidants (ascorbic acid (vit c) α tocopherol (vit E).This imbalance leads to cell damage Bhatia *et al.* (2003) [3], Peuchant *et al.* (2004) [16] and health problems Steer *et al.* (2002) [22] if excess ROS are not eliminated by antioxidant system these reactive species will exert oxidative damage effects by reacting with nearby every molecules found in living cells, include DNA. Plant extract and plant products such as flavonoids and other polyphenolic constituents have been reported to be effective radical scavenger and inhibitors of lipid per oxidation Velavan (2015) [25]. Many synthetic antioxidant compounds have shown toxic and/or mutagenic effects which have stimulated the interest in the use of natural antioxidants. The therapeautic potential of natural medicinal plants as an antioxidant in reducing such free radical induced tissue injury, suggests that many plants have antioxidant activities that can be therapeautically useful Kannatt *et al.* (2007) [10]. The quantitative determination of ascorbic acid in plant extracts shows that they are good source of ascorbic acid. Ascorbic acid acting as a chain breaking antioxidant impairs with the formation of free radicals in the process of formation of intracellular substances throughout the body, including collagen bone matrix and tooth dentine Beyar (1994) [2] and Aqil *et al.* (2006) [1].
The plant and its products are rich sources of a phytochemicals and have been found to possess a variety of biological activities including antioxidant and anticancer activity Velavan *et al.* (2007) [25] and Velavan (2015) [25]. There is an increasing interest in the antioxidant effects of compounds derived from plants, which could be relevant in the relation to their nutritional value. Different aromatic herbs have been investigated for their antioxidant activity. Particularly those belong to the Apocynaceae family have been found to be very effective with regard to natural antioxidant Zara iqbal (2017) [26].

The aim of this work was to examine *Caralluma indica* from Apocynaceae family for their *in vitro* possible antioxidant activity. Keeping in view of its wide use and its chemical composition of the ethanolic extract of *Caralluma indica* is the commonly known as "Mullu oonan" in Tamil which are distributed in tropical and temperate region of India was determined for its invitro antioxidant activities. With this background and abundant source of unique, the present study were to investigate the free radical scavenging activity of *Caralluma indica* extract through the free radical scavenging, superoxide anion radical scavenging, total antioxidant, metal chelation iron reducing power activity and nitric oxide radical scavenging activity.

Materials and Methods

Chemicals

Nitro blue tetrazolium (NBT), Ethylene diamine tetra acetic acid (EDTA), Sodium nitroprusside (SNP), Trichloro acetic acid (TCA) Thio barbituric acid (TBA) Potassium hexacyno ferrate [K_3 Fe ($CN)_6$] and L-ascorbic acid were from Sisco research laboratories pvt ltd. India. All other chemicals and solvents used were of analytical grade available commercially.

Plant Materials

Plant materials were collected from Kathattipatti (Palaiyapatti North), Sengipatti Village at Thanjavur District in the month of Nov-2017. They were taken as whole plant. The whole plant was identified and authenticated by Dr. S. John Britto. The Director, the Rabiant Herbarium and centre for molecular systematic. St. Joshphe's college, Trichy - Tamil Nadu, India. A voucher specimen (RSV01) has been deposited at the Rapinat Herbarium St. Joshph's college, Trichy, Tamil Nadu, India.

Preparation of alcoholic extract

The stem of *Caraluma indica* was first washed several times with distilled water and traces of impurities were removed from the stem. Then old, infected and fungus damaged portion of the stems were removed. Healthy stems was spread out in a plain paper and dried in shade at room temperature for about 10 days. The collected stem was cut into small pieces and makes a fine powder using grinder mixture. The powder extracted with aqueous, methanol, ethanol and ether extract for 24 hours. After 24 hours, the supernatant was transferred into china dish. The supernatant was completely removed by keeping the china dish over a water bath at 45 ^{o}C. The extract was then concentrated until the solvent was completely removed. A semi solid extract was obtained after complete elimination of solvent. The obtained residue was kept in the refrigerator for phytochemicals screening. Different doses (20, 40, 60 and 80µg/ml) of ethanol extract used for *in vitro* antioxidant activity.

Preliminary Phytochemicals Screening

Chemical tests were carried out on the alcoholic extract using standard procedures to identify the preliminary phytochemical screening following the methodology of Sofowara (1993) [21], Trease and Evans (1989) [23] and Harborne (1973) [9].

In vitro Antioxidant activity

The free radical scavenging of antioxidants was evaluated by DPPH assay according to the procedure of Nuutila *et al.* (2003) [14]. The antioxidant activity of the extracts was evaluated by phosphomolybdenum method according to the procedure of Prieto *et al.* (1999) [19]. The scavenging activity of *Caralluma indica* towards superoxide anion radicals was assayed by the PMS-NADH system according to the method of Liu *et al.* (1997) [13]. The chelating activity of the extracts for ferrous ions Fe^{2+} was measured by the method of Dinis *et al* (1994) [4]. Nitric oxide radical scavenging activity was determined according to the method reported by Garrat (1964) [6].

Statistical analysis

Tests were carried out in triplicate for 3-5 separate experiments. The amount of extract needed to inhibit free radicals concentration by 50%. IC_{50} was graphically estimated using a linear regression algorithm.

Results and Discussion

The study reveals that tested plant materials have highest significant antioxidant activity and free radical scavenging activity, which contains of flavonoid and polyphenols. These phytochemicals are exhibited antioxidant and scavenging properties, which can be used as an accessible source of natural antioxidants with consequent health benefits.

Phytochemical Screening

The aqueous, methanol, ethanol and ether extract of *Caralluma indica* stem extracts were investigated. The aqueous, methanol and ethanol extracts shows the presence of tannins, saponin, flavonoids, anthroquinone, terpenoids, polyphenol, glycosides, coumarins while steroids present only in methanol and ethanol. Ether extract shows the presence of steroids, terpenoids, polyphenol and anthroquione. Among the various extract, ethanol extract has rich source of phytochemicals

In Vitro Antioxidant Activity

DPPH free radical scavenging activity determination

In the DPPH assay the antioxidant was able to reduce the stable radical DPPH to the yellow colored 1, 1 diphenyl 1, 2 picryl hydrazine. The molecule of 2, 2-diphenyl 1, 1- picryl hydrazine is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole The proton transfer reaction of the DPPH free radical scavenger causes a decrease in absorbance at 517 nm. which can be followed by a common spectrophotometer set in the visible region. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability Sindhu and Abhram (2006) [20] DPPH radical scavenging activity of *Caralluma indica* extract and standard as ascorbic acid presented in table 1 and fig 1. The half inhibition concentration (IC_{50}) *Caralluma indica* extract and ascorbic acid were 48.02 µg/ml and 34.89 µg/ml respectively. The plant extract exhibited a significant dose dependent inhibition of DPPH activity. The potential of ascorbic acid to scavenge DPPH radical is directly proportional to the concentration.

Table 1: % of DPPH radical scavenging activity of *Caralluma indica* extract

Concentrations (µg/ml)	20	40	60	80	IC_{50} Value
Caralluma indica extract	20.23 ± 0.52	41.30 ± 0.29	63.00 ± 0.35	83.88 ± 0.64	48.02
Standard ascorbic acid	25.60 ± 2.04	61.26 ± 4.90	88.98 ± 7.11	99.34 ± 7.94	34.89

Values were expressed as mean ± Standard deviation for triplicates

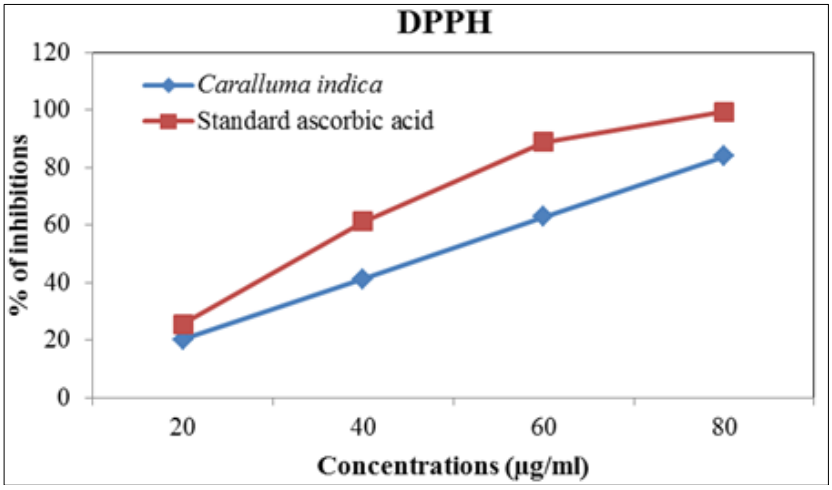

Fig 1: % of DPPH radical scavenging activity of *Caralluma indica* extract

Determination of Total antioxidant activity

The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al* (1999) [19]. The assay is based on the reduction of Mo (VI) –Mo (V) by the extract and subsequent formation of a green phosphate Mo (v) complex at 695 nm the antioxidant activity is expressed as the number the number of equivalents of ascorbic acid. The half inhibition concentration of plant extract and ascorbic acid were 52.76 µg/ml and 42.39 µg/ml respectively. The antioxidant activity is expressed as the number of equivalents of the ascorbic acid (Table 2 and fig 2).

Table 2: % of Total antioxidant activity of *Caralluma indica*

Concentrations (µg/ml)	20	40	60	80	IC_{50} Value
Caralluma indica extract	16.16 ± 1.30	38.88 ± 0.98	58.88 ± 0.74	75.18 ± 1.10	52.76
Standard ascorbic acid	22.35 ± 1.80	51.21 ± 4.09	72.54 ± 5.80	86.35 ± 6.95	42.39

Values were expressed as Mean ± Standard deviation for triplicates

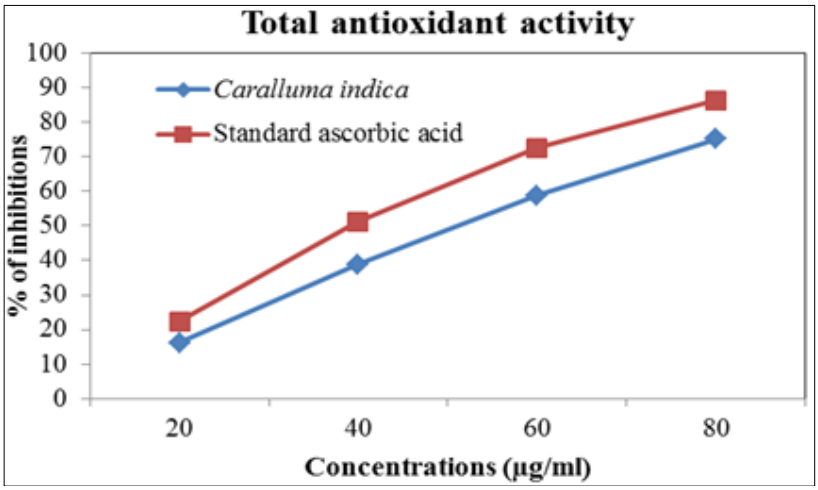

Fig 2: % of Total antioxidant activity of *Caralluma indica*

Superoxide anion Scavenging activity

Super oxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals are very harmful to the cellular components in a biological system Korycka Dhal and Richardon (1978) [12]. The scavenging activity of the *Caralluma indica* towards superoxide anion radicals was measured by the method of *Liu et al* (1997) [13]. Superoxide anion were generated in a non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system analysed by the reduction of nitro blue tetrazolium (NBT). In these experiments the reaction was initiated by adding 0.75ml of PMS (120µm) to the mixture. After 5 minutes of incubation at room temperature, the absorbance read at 560 nm was measured in spctrophotometer. The superoxide anion scavenging activity of *Caraluma indica* was increased markedly with the increase of concentration (Table 3 and fig 3).

Table 3: Superoxide radical scavenging activity of *Caralluma indica* extract

Concentrations (µg/ml)	20	40	60	80	IC_{50} Value
Caralluma indica extract	27.15 ± 0.24	33.01 ± 0.56	68.55 ± 0.42	75.43 ± 0.40	48.85
Standard ascorbic acid	31.25 ± 2.50	64.23 ± 5.13	89.54 ± 7.16	98.51 ± 7.88	31.60

Values were expressed as Mean ± Standard deviation for triplicates

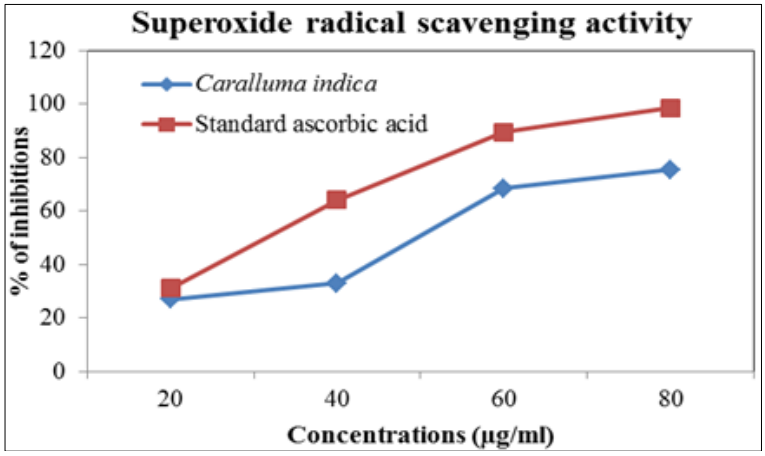

Fig 3: Superoxide radical scavenging activity of *Caralluma indica* extract

Fe^{2+} Chelating activity assay

Ferrozine can make complexes with ferrious ion. Ferrozine reacted with the divalent iron to form stable magenta complex species that was very soluble in water. In the presence of chelating agents, complex (red colored) formation is interrupted and as a result the red color of the complex is decreased. Thus the chelating effect of the coexisting chelators can be determined by the measuring rate of color reaction. The formation of ferrozine –Fe $^{2+}$ complex is interrupted in the presence of aqueous extract of *Caralluma indica* indicating that have chelating activity with an IC_{50} of 55.07 µg/ml and ascorbic acid was 30.93 µg/ml respectively. (Table 4 and Fig 4).Ferrous ion can initiate lipid peroxidation by the Fenton reaction as well as accelerating peroxidation by decomposing lipid hydro peroxides into peroxides and alkoxyl radicals Halliwell (1991) [8]. Metal chelating activity can contribute in reducing the concentration of the catalyzing transition metal in lipid per oxidation furthermore chelating agents that forms bonds with a metal are effective as secondary antioxidants because they reduce the redox potential and thereby stabilize the oxidized form of the metal ion Gordan (1990) [7]. Thus *Caralluma indica* demonstrate a marked capacity for iron binding suggesting their ability as a peroxidation protector that relates to the iron binding capacity At room temperature, the absorbance of the Fe^{2+}-Ferrozine complex was measured at 562nm. All the results are presented as means ± standard deviation of three determinations.

Table 4: % of Iron chelating activity of *Caralluma indica* extract

Concentrations (µg/ml)	20	40	60	80	IC_{50} Value
Caralluma indica extract	15.25 ± 1.17	30.76 ± 1.15	54.22 ± 1.55	78.20 ± 1.33	55.07
Standard ascorbic acid	35.23 ± 2.81	65.21 ± 5.28	78.51 ± 6.28	98.65 ± 7.89	30.93

Values were expressed as Mean ± Standard deviation for triplicates

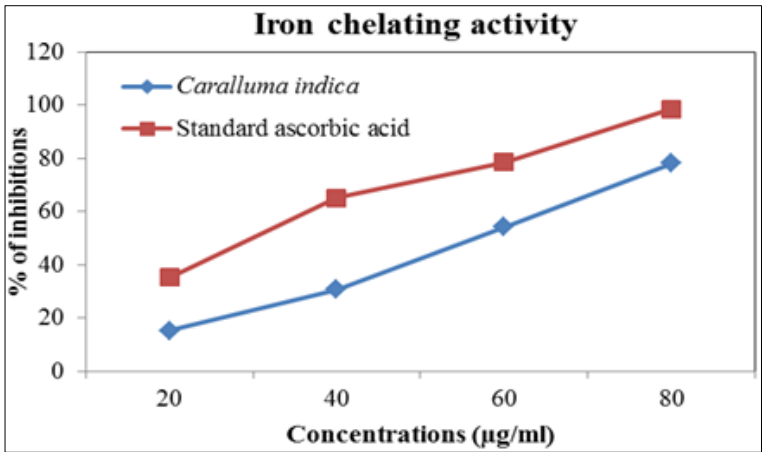

Fig 4: % of Iron chelating activity of *Caralluma indica* extract

Nitric Oxide Scavenging activity assay

Nitric oxide (NO^0) released from sodium nitro prusside (SNP) has a strong NO^+ character which can alter the structure and function of many cellular components. The extract of *Caralluma indica* exhibited good NO scavenging activity leading to the reduction of the nitrite concentration in the assay mediam. The NO scavenging capacity was concentration dependent. *Caralluma indica* in SNP solution significantly inhibited the accumulation of nitrite, a stable oxidation product of NO liberated from SNP in the reaction medium with time compared to the standard ascorbic acid. The toxicity of NO increase when it reacts with superoxide to form the peroxynitrite anion (ONOO-) which is a potential strong oxidant that can decompose to produce OH and NO_2 Pacher *et al* (2007) [15]. The present study shows that *Caralluma indica* increased with increasing concentration (Table 5 and fig 5).The half inhibition concentration (IC_{50}) of plant extract and ascorbic acid were 66.52 µg/ml and 40.92 µg/ml respectively.

Table 5: % of Nitric Oxide Scavenging activity of *Caralluma indica* extract

Concentrations (µg/ml)	20	40	60	80	IC_{50} Value
Caralluma indica extract	13.79 ± 0.08	22.79 ± 0.14	35.66 ± 0.34	68.88 ± 0.76	66.52
Standard ascorbic acid	26.19 ± 1.83	53.80 ± 3.76	71.42 ± 4.99	82.38 ± 5.76	40.92

Values were expressed as Mean ± Standard deviation for triplicates

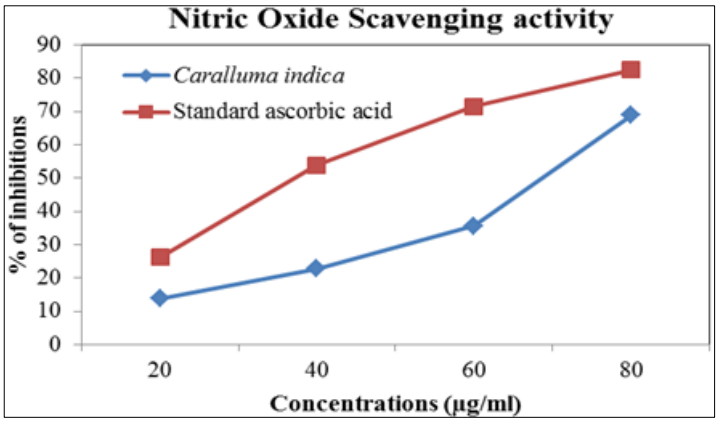

Fig 5: % of Nitric Oxide Scavenging activity of *Caralluma indica* extract

Conclusion

The result of this study shows that the *Caralluma indica* stem extract has rich source of phytochemicals and good antioxidant activities. This is the first report recording to *Caralluma indica* stem. The experimental evidence on the extract as natural antioxidant for its capacity to scavenge reactive oxygen and nitrogen species and protect organisms from oxidative damage and thus could be an effective against oxidative stress mediated diseases including cardiovascular diseases, cancer, diabetic, etc.,

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