Research Article

Available online at www.JGTPS.com

ISSN: 2230-7346
Journal of Global Trends in Pharmaceutical Sciences
Volume- 5, Issue -2, pp – 1603-1611 - April –June (2014)

PRELIMINARY PHYTOCHEMICAL SCREENING, TOTAL PHENOL CONTENT AND IN-VTRO ANTIOXIDANT ACTIVITY OF *CARALLUMA UMBELLATA HAW*

G.V. Sampath Kumar[1]
Ch. Sandhya[2]

[1]Reaserch Scholar, Department of Pharmacology, A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, A.P, India-530003
[2]Research scholar, Department of Pharmacology, A.U.College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, A.P, India-530003

Journal of Global Trends in Pharmaceutical Sciences

ABSTRACT

Objective: To evaluate the *in-vitro* antioxidant capability of the hydro-alcoholic (70% v/v methanol) extract of the stems of *Caralluma umbellata Haw* and the determination of their total phenolic content as well as phytochemical screening.
Method: *In-vitro* antioxidant activity was evaluated by studying total phenol content, alkaloid content, DPPH radical scavenging activity, super oxide scavenging activity, hydroxyl radical scavenging activity. The amount of total phenols was analyzed with the Folin-Ciocalteau reagent. Gallic acid and ascorbic acid were used as standard for all the experiments.
Results: Phytochemical analysis revealed the presence of flavanoids, terpenoids, steroids, glycosides, alkaloids, and phenols in stem extract. It was identified that the phenolic component is responsible for antioxidant activity. IC_{50} values of the extract for DPPH radical scavenging activity, super oxide scavenging activity, hydroxyl radical scavenging activity were determined to be175.00, 190.50 and 266.30 µg respectively. Each experiment was carried out in triplicate and results were expressed as mean ± SEM.
Conclusions: The study suggests that hydro-alcoholic (70% v/v methanol) extract of the stems of *Caralluma umbellata Haw* exhibit great potential for antioxidant activity and may be useful for their medicinal functions.

Keywords: *In-vitro* antioxidant activity, *Caralluma umbellata Haw,* phytochemical screening, total phenolic content.

1. INTRODUCTION

Oxygen is essential to life, but usage of oxygen generates by-products known as reactive oxygen species (ROS) are more commonly formed free radicals. These compounds are a normal part of the body's stress response, but they can damage healthy cells and are especially likely to attack the fats that provide structure to the membranes surrounding body cells. Antioxidants neutralize the effects of free radicals, but activity may be limited to specific antioxidants. The body produces a range of its own protective antioxidants. Some foods are also rich in antioxidants and these may boost the body's own supply. Plants produce hundreds of different antioxidants for their own protection, some may also be useful to human are present in fruits, herbs and spices, nuts etc. Oxidative stress due to free radicals and other reactive oxygen species are recognized as risk factors in the pathogenesis of numerous chronic diseases such as asthma, Parkinson's and Alzheimer's diseases, cancers as well as atherosclerosis. In the past decade, several natural antioxidant compounds have been isolated from medicinal plants have aroused much attention and resulted on increasing efforts to search for plant derived antioxidants against free radicals like reactive oxygen (ROS) and nitrogen species (RNS) [1-3]. Free radicals such as reactive oxygen species are also reason for the ageing in human [4, 5]. Although the antioxidant defense system include both endogenously and exogenously derived compounds, plant based antioxidant have recently received a great attention [6].Hence, many studies have been carried out to identify antioxidant compounds with pharmacological activity and a limited toxicity

Address for correspondence

***G.V. Sampath Kumar**
Mobile: +91 9908725742
Email: sampath.venu9@gmail.com

from medicinal plants. These include phenolic compounds [7]. Plants consist of a numerous bioactive compounds that are used for the treatment of large number of diseases in human by traditional healers. Phytochemicals are natural bioactive compounds which are present in plants. These are certain non-nutritive plant chemicals which have some disease preventive properties. Tribal people treat many disorders with the one or combination of plant extracts from locally available plants. Some of these claims by the traditional healers have been substantiated with scientific evidence. These natural compounds work with nutrients and dietary fibers to protect animals and man against diseases. Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances [8-10]. Phenolic compounds which are secondary metabolites in plants are one of the most widely occurring groups of phytochemical that exhibit antiallergenic, antimicrobial, anti-artherogenic, antithrombotic, anti-inflammatory, vasodilatory and cardio protective effects [11, 12].

Caralluma umbellata Haw grows wild in dry and arid regions and several Districts of Andhra Pradesh, in India. It is a thick, erect, leafless, branching, and succulent a perennial herb [13]. It is medicinally important and rich in pregnane glycosides, which may possess different biological activities [14] including anti-inflammatory activity [13, 15]. A significant analgesic was exhibited by Carumbelloside-I, isolated from *C. umbellata* [16]. Previously, the tribal people of Chittoor District, Andhra Pradesh, India used *Caralluma umbellata Haw* stem juice warmed and mixed with turmeric powder for alleviation of stomach disorder and abdominal pains [17-19].

However to the knowledge of authors there are no sufficient scientific reports available in literature regarding the phytochemical constituents of the plants that are responsible for disease healing process. Hence, in the present investigation, an effort is made to identify the phytochemical constituents and antioxidant property of the extract by using DPPH scavenging assay, super oxide scavenging activity, hydroxyl radical scavenging activity and determination of total phenolic content. The results are reported in this paper along with assigning the reasons there off.

2. MATERIALSAND METHODS

2.1. Chemicals and Standards

DPPH (1, 1-diphenyl-2-picryl hydrazyl), Gallic acid, ascorbic acid, Follin-Ciocalteu's phenol reagent were obtained from Sigma Chemical Company Ltd. (USA). Riboflavin was procured from Loba chemie Pvt Ltd., Mumbai. Deoxyribose and Nitro blue tetrazolium was procured from Sisco Research Laboratories Pvt Ltd., Mumbai. All other chemicals and reagents used were of analytical grade.

2.2. Plant samples:

Fresh stems of the plant *Caralluma umbellata* were collected from Araku Valley, near Visakhapatnam (District), Andhra Pradesh (State), India in the month of November 2013. The plant was taxonomically identified and authenticated by Dr. P. Venkaiah, Professor of the Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh, India. Voucher specimens (GVSK/KLB/11/2013) have been kept in the laboratory for future reference.

2.3. Preparation of Hydro-alcoholic extract of Caralluma umbellata Haw:

Freshly collected plant material was dried under shade and the dried material was milled to obtain a coarse powder. To the coarse powder (500gms) in maceration chamber, 2.5 liters of alcohol (70% v/v Methanol) was added and macerated for 5 days at room temperature. The macerated extract was obtained and concentrated under vacuum at a temperature of 45^0C by using rotary evaporator, dried completely, weighed and stored in a desiccator.

2.4. Preliminary phytochemical analysis:

Screening tests were carried out for the hydro-alcoholic crude extract of *Caralluma umbellata Haw* stem using standard procedures to identify the constituents by methods described by Trease [20] and Evans and Harbone [21]. Preliminary phytochemical analysis on plant extracts was performed using the following chemicals and reagents: flavonoids (Mg metal and HCl), phenolics (FeCl3), protein and amino acid (Millon's and Ninhydrin reagent), alkaloids (Mayer and Dragendorff's reagent), saponins (Foam test), phytosterols, triterpenoids (Liebermann-Burchard Test) and carbohydrates (Fehling's solution A and B) [22 - 24].

2.5. Determination of antioxidant activity

The antioxidant activity was evaluated by the following methods.

2.5.1. Quantification of Total phenol content:

Total phenolic content of each extract was determined by Folin-Ciocalteau reagent method[25] with some modifications.10 µl of extract along with standard solution of gallic acid (10, 20, 40, 80, 160, 320 µg/ml) was incubated with 500 µl of diluted Folin-Ciocalteau reagent and 350 µl of 1N Na2CO3 for 30 min. at room temperature. The tubes were vortexed for few seconds and allowed to stand for 30 min at 20°C for color development. The Folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols, thereby producing a blue color upon reaction.The products of the metal oxide reaction have a blue absorption with a maximum at 765nm against blank using double beam bio-spectrophotometer (Elico BL198). A typical blank solution contained the solvent used to dissolve the plant extract. The total phenolic contents were determined from the linear equation of a standard curve prepared with gallic acid. The intensity of the light absorption at that wavelength is proportional to the concentration of phenols. All measurements were done in triplicate. The total phenol content was expressed in terms of Gallic acid equivalent (GAE in the units: mg/g) by using standard Gallic acid calibration curve [26].

2.5.2. Quantification of Total Alkaloid content:

The plant extract (1mg/ml) was dissolved in 2 N HCl and then filtered. The pH of phosphate buffer solution was adjusted to neutral with 0.1 N NaOH. 1ml of this solution was transferred to a separating funnel and then 5ml of BCG solution along with 5ml of phosphate buffer were added. The mixture was extracted with chloroform by vigorous shaking. The extracts were collected in a 10ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470nm. All measurements were done in triplicate and the results were expressed as Mean ± SEM.

2.5.3. Free radical scavenging activity (DPPH method):

The scavenging activity for DPPH free radicals was measured [27]**.** Ethanolic solution of the sample extract at various concentrations (20, 40, 80, 160, 320 and 640 µg/mL) was added separately to each 5 mL of 0.004% solution of DPPH and allowed to stand for 30 min at room temperature. After incubation, the absorbance of each solution was determined at 517 nm using spectrophotometer. Ascorbic acid was used as the reference standard. The corresponding blank reading was also taken and DPPH radical scavenging activity was calculated by using the formula 1. IC50 value is the concentration of the sample required to scavenge the 50% DPPH free radical.

2.5.4. Superoxide Anion Radical Scavenging Activity (Riboflavin photo reduction method):

Superoxide scavenging activity of the plant extract was determined by McCord and Fridovich method [28] 1969, which depends on light induced superoxide generation by riboflavin and the corresponding reduction of nitro blue tetrazolium. 0.1 ml of different concentrations (50, 100, 250, 500, 750 and 1000µg) of plant extract and 0.1 ml of 6 µM ethylene-di-amine tetra-acetic acid containing NaCN, 0.1 ml of 50 µM nitro blue tetrazolium, 0.05 ml of 2 µM riboflavin were transferred to a test tube, and final volume was made up to 3 ml using phosphate buffer. Then the assay tubes were uniformly illuminated with an incandescent light (40 Watts) for 15 minutes and thereafter the optical densities were measured at 560 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ascorbic acid. The percentage inhibition of superoxide production was evaluated by comparing the absorbance values of control and experimental tubes. The percentage inhibition was calculated from the below Formula 1.

2.5.5. Hydroxyl Radical Scavenging Activity (Deoxyribose degradation method):

Hydroxyl radical scavenging activity was measured according to the method of Elizabeth and Rao [29] 1990, by studying the competition between deoxyribose and test extract for hydroxyl radicals generated by Fenton's reaction. Hydroxyl radical was generated by

the Fe^{3+}-ascorbate-EDTA-H_2O_2 system (the Fenton reaction). The hydroxyl radical attacks deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances (TBARS). The reaction mixture in a final volume of 1.0 ml contained 100 µl of 2-deoxy2ribose (28 mM in 20 mM KH_2PO_4 buffer, pH 7.4), 200 µl of the plant extract at various concentrations (20, 40, 80, 160, 320 & 640 µg/ml) in buffer, 200 µl of 1.04 mM EDTA and 200 µM $FeCl_3$ (1:1v/v), 100 µl of 1.0 mM hydrogen peroxide (H_2O_2) and 100 µl of 1.0 mM ascorbic acid. Test samples were incubated at 37°C for 1h. The free radical damage imposed on the substrate, deoxyribose was measured using the thiobarbituric acid test. One ml of 1% thiobarbituric acid (TBA) and 1.0 ml 2.8% trichloro-acetic acid (TCA) were added to the test tubes and were incubated at 100°C for 20 min. After cooling, the absorbance was measured at 532 nm against a blank containing deoxyribose and buffer. Ascorbic acid (20, 40, 80, 160, 320 & 640 µg/ml) was used as a positive control. The percentage inhibition was calculated from the below Formula 1.

Calculation of percentage inhibition

The percentage inhibition was calculated using the formula:

Formula-1: Inhibitory ratio = $\frac{(A0 - A1)}{A0} \times 100$

Where, A0 is the absorbance of control; A1 is the absorbance with addition of plant extract/ ascorbic acid.

Calculation of 50% inhibition concentration

The optical density value obtained with each concentration of the extract/ ascorbic acid was plotted taking concentration on X-axis and percentage inhibition on Y-axis. The graph was extrapolated to find the 50% inhibition concentration of extract/ ascorbic acid.

2.6. Statistical analysis

Data were analyzed by SPSS 6.0 (SPSS Inc.). Equations for best fitted line to estimate IC50 values obtained by linear regression statistics based on least squares method. Mean values were compared using one-way analysis of variance (ANOVA) using post hoc comparison test.

RESULTS

Preliminary phytochemical analysis:

Results of preliminary phytochemical analysis of hydro-alcoholic crude extract of *Caralluma umbellata Haw* stem are presented in (Table1). Phenols, flavanoids, alkaloids, steroids, terpenoids and glycosides were observed in qualitative analysis.

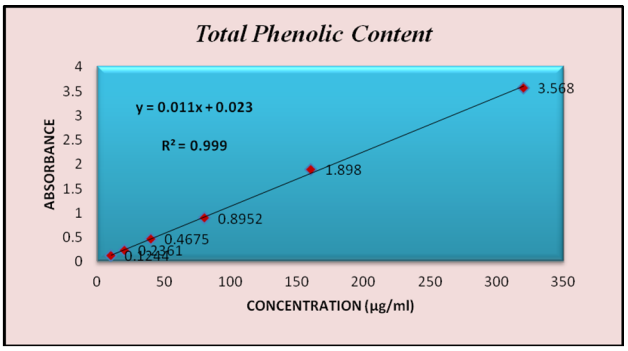
Table 1: Preliminary phytochemical analysis of hydro-alcoholic extract of *Caralluma umbellata haw*

S. No	Phytoconstituents	Present/Absent
1	Carbohydrates	-
2	Glycosides	+
3	Saponins	-
4	Tannins	-
5	Phytosterols & Terpenoids	+
6	Flavonoids	+
7	Alkaloids	+
8	Quinones	-

Quantification of Total phenol content:

The standard graph of Gallic acid yielded a curve with regression coefficient, r^2=0.999 (Graph 1)

The total phenolic content in the hydro-alcoholic extract of *Caralluma umbellata Haw* stem was estimated by Gallic acid equivalents (GAE). The total phenolic content was found to be 3.93 mg Gallic acid equivalent per gm weight of extract.



Graph.1: Total Phenols (Gallic acid) standard curve

Y= Absorbance of hydro-alcoholic extract of *Caralluma umbellata Haw*,
m = slope, X = concentration, c = constant.

Quantification of Total Alkaloid content:

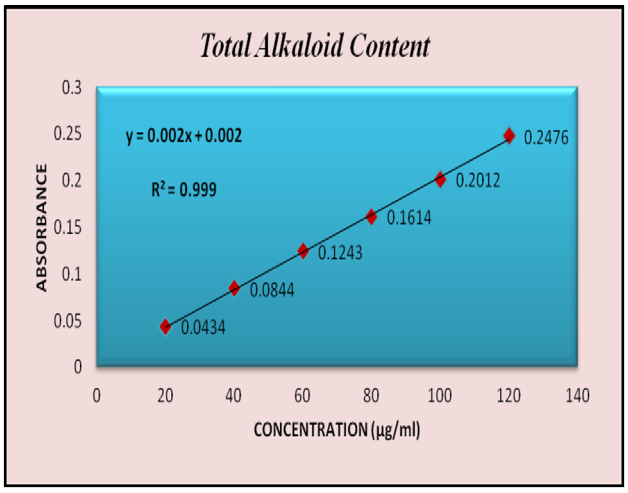
The standard graph of Gallic acid yielded a curve with regression coefficient, r^2=0.999

The quantified alkaloid content of hydro-alcoholic extract of *Caralluma umbellata Haw* stem was found to be 26.99 mg/gm from the standard graph (Graph 2).

$$Y = mx + c$$

Y= Absorbance of hydro-alcoholic extract of *Caralluma umbellata Haw*,

m = slope, X = concentration, c = constant.

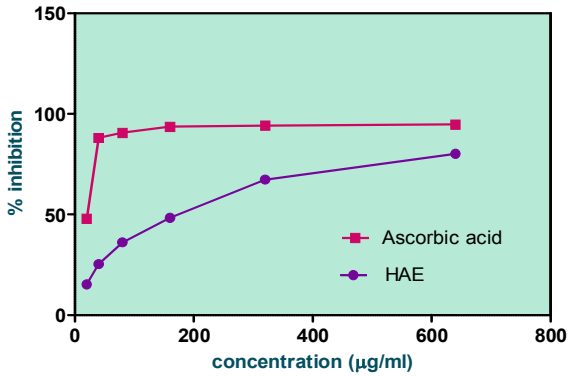


Graph 2: Total Alkaloids (Gallic acid) Standard curve

Free radical scavenging activity (DPPH method):

The radical scavenging capacity based on DPPH assay was determined and the results are shown in Table 2 for the species, *Caralluma umbellata*. The percentage of scavenging effect on the DPPH radical was (dose dependent) increased with the increase in the concentrations of the extract from 20 -640 µg/mL.

The percentage of inhibition of the DPPH radical was varying from 15.30% (in 20 µg/ml of the extract) to 80.23% (in 640 µg /mL of extract). The IC_{50} values of the hydro-alcoholic extract of this species and Ascorbic acid were determined to be 175.00µg/ml and 22.00µg/ml respectively. The extracts in all concentrations showed the percentage of inhibition of free radicals when compared to standard drug – Ascorbic acid. The results were graphically presented in Graph 3.



Graph 3: DPPH radical scavenging activity (HAE=hydro-alcoholic extract)

Table 2: DPPH radical scavenging activity of hydro-alcoholic (70% v/v methanol) extract of the stems of *Caralluma umbellata Haw*

S. No	Concentration (µg/ml)	% Inhibition of DPPH radical	
		HAE	Ascorbic acid
1	20	15.30±0.50	48.00±0.50
2	40	25.3±1.00	88.08±1.00
3	80	36.14±1.20	90.68±0.30
4	160	48.35±1.30	93.63±0.50
5	320	67.3±0.50	94.21±0.30
6	640	80.23±2.10	94.74±1.10
7	IC_{50}	175.00±0.50	22.00±0.50

Values are expressed as Mean ± SEM

Superoxide Anion Radical Scavenging Activity:

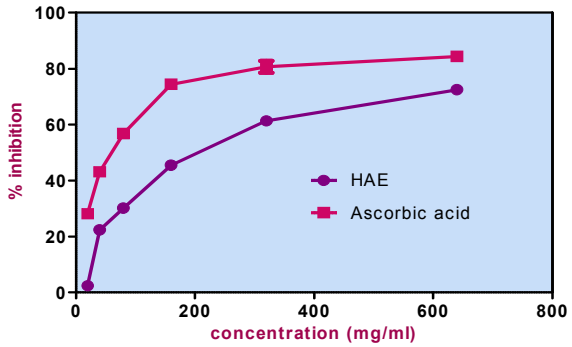
Percentage inhibition of superoxide radical generation was determined and compared with same doses of ascorbic acid. The percentage inhibition of superoxide generation by 20- 640 µg/ml concentration of hydro-alcoholic extract of *Caralluma umbellata Haw* was shown in Table 3.

The IC_{50} value of the extract of the study species and Ascorbic acid were found to be 190.50µg/ml and 54.40µg/ml respectively. The extract showed slightly significant activity when compared to that of the standard. This may be due to the presence of poly phenols. The results were graphically presented in Graph 4.

Table 3: Superoxide radical scavenging activity of hydro-alcoholic (70% v/v methanol) extract of the stems of *Caralluma umbellata Haw*

S. No	Concentration (µg/ml)	% Inhibition of Superoxide radical	
		HAE	Ascorbic acid
1	20	12.40±0.50	28.15±0.50
2	40	22.40±0.70	43.19±1.50
3	80	30.14±1.10	56.87±1.40
4	160	45.50±1.20	74.46±0.70
5	320	61.40±0.60	80.72±2.10
6	640	72.50±1.40	84.41±1.20
7	IC_{50}	190.50±1.20	54.40±1.10

Values are expressed as Mean ± SEM



Graph 4: Superoxide radical scavenging activity (HAE=hydro-alcoholic extract)

Hydroxyl Radical Scavenging Activity:

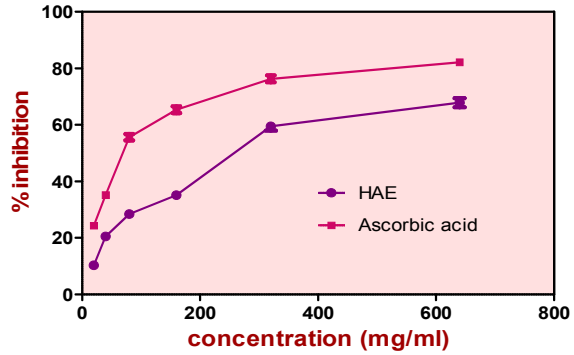
The hydro-alcoholic extract of *Caralluma umbellata Haw* produced dose-dependent inhibition of hydroxyl radicals ranging from 10.30% (in 20 µg/ml of the extract) to 67.80% (in 640 µg /mL of extract). The standard drug Ascorbic acid showed better percentage of inhibition of hydroxyl radicals than the hydro-alcoholic extract of *Caralluma umbellata Haw.*

The results of concentration dependent % inhibition of hydroxyl radical activity of the extract and the standard were compiled in Table 4 and shown in Graph 5. The IC_{50} value of the extract of the study species and Ascorbic acid were found to be 266.30µg/ml and 68.00µg/ml respectively.

Table 4: Hydroxyl radical scavenging activity of hydro-alcoholic (70% v/v methanol) extract of the stems of *Caralluma umbellata Haw*

S. No	Concentration (µg/ml)	% Inhibition of Hydroxyl radical	
		HAE	Ascorbic acid
1	20	10.30±0.30	24.32±1.00
2	40	20.50±1.20	35.12±0.40
3	80	28.40±1.10	55.61±1.10
4	160	35.10±0.50	65.31±1.20
5	320	59.40±1.50	76.25±1.20
6	640	67.80±1.60	82.11±0.70
7	IC_{50}	266.30±1.30	68.00±1.30

Values are expressed as Mean ± SEM



Graph 5: Hydroxyl radical scavenging activity (HAE=hydro-alcoholic extract)

DISCUSSION:

Oxidants, commonly known as "free radicals," are a molecule with one or more unpaired electrons in the outer orbital. Many of these free radicals, which are the harmful byproducts generated during normal cell aerobic respiration in the form of reactive oxygen and nitrogen species, are an integral part of normal physiology [30]. An over production of these reactive species can occur, due to oxidative stress brought about by the imbalance of the bodily antioxidant defense system and free radical formation [31]. Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants. They are the first line of defense against free radical damage and are critical for maintaining optimum health [32]. Therefore, antioxidants that scavenge reactive oxygen species may be of great value in preventing the onset and/or the propagation of oxidative diseases [33].

The most common antioxidants present in vegetables and part of the plants are vitamins C, vitamin E, Carotenoids, phenols and 1609ttenuate1609. *In vitro* antioxidant activity of the 1609ttenuate extract leaves of *Boswellia ovalifoliolata* was investigated in the present study by total phenol content, flavonoid content ,DPPH, nitric oxide scavenging activity, hydroxyl radical scavenging activity, hydrogen peroxide scavenging activity , super oxide ion scavenging activity and metal chelating assays. The antioxidative effect is mainly due to phenolic components, such as phenolic acids,phenolic diterpenes and the presence of hydroxyl groups [34]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [35].

As per the literature various phytochemical compounds detected are known to exhibit medicinal activity as well as physiological activity [36, 37]. The Preliminary phytochemical screening of the extract reported the presence of Alkaloids, carbohydrates, 1609 ttenuate1609, phenols, terpenoids and phytosterols.

DPPH scavenging activity has been used by various researchers as a quick and reliable parameter to assess the in vitro antioxidant activity of crude plant extracts [20]. With the DPPH test the ability of a compound to act as a donor for hydrogen atoms or electrons is measured spectrophotometrically. Hydroxyl radicals are the major active species that cause lipid oxidation and significant biological damage [21]. The ability of the tested extracts to quench hydroxyl radicals seems to be directly related to inhibiting the process of lipid peroxidation seemed to be good scavengers of reactive oxygen species. The percentage of hydroxyl radical scavenging increased as the concentration of the extracts increased. The percentage of inhibition of the DPPH radical was varying from 15.30% (in 20 µg/ml of the extract) to 80.23% (in 640 µg /mL of extract). The IC_{50} values of the hydro-alcoholic extract of this species and Ascorbic acid were determined to be 175.00µg/ml and 22.00µg/ml respectively.

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Antioxidant on interaction with DPPH, transfer electron or hydrogen atom to DPPH and thus neutralizing its free radical character and convert it to 1 – 1 diphenyl – 2 – picryl hydrazine and the degree of discoloration indicates the scavenging activity of the drug [38]. In the present study, the hydro-alcoholic extract of *Caralluma umbellata Haw* had significant scavenging effect on the DPPH radical which was increasing with the increase in the concentration of the sample from 20 – 640µg/mL.

Super oxide is biologically important as it can form singlet oxygen and hydroxyl radical. Over production of super oxide anion radical contributes to redox imbalance and associated with harmful physiological consequences. Super oxide anion are generated in PMS-NADH system by the oxidation of NADH and assayed by the reduction of NBT (Nitro-blue tetrazolium) resulting in the formation of blue formazan. *In-vitro* super oxide radical scavenging activity is measured by riboflavin/light/NBT (Nitro blue tetrazolium) reduction. The super oxide radical reduces NBT to a blue colored formazan that can be measured at 360 nm. This compound donated their electrons to the superoxide and scavenges them to prevent their further interaction with NBT followed by inhibition of formation of blue colored formazan product [39]. The percentage inhibition of superoxide generation by 20- 640 µg/ml concentration of hydro-alcoholic extract of *Caralluma umbellata Haw* was shown in Table 3. The IC_{50} value of the extract of the study species and Ascorbic acid were found to be 190.50µg/ml and 54.40µg/ml respectively.

Among the ROS, hydroxyl radicals are the most reactive and predominant radicals generated endogenously during aerobic metabolism. Due to the high reactivity, the radicals have a very short biological half-life. The presence of transition metal ions in a biological system could catalyze the Haber-Weiss and Fenton type reactions, resulting in the generation of hydroxyl radicals (OH). However, these transition metal ions could form chelates with the antioxidants, which results in

the suppression of OH generation and inhibition of peroxidation processes of biological molecules [40]. The hydro-alcoholic extract of *Caralluma umbellata Haw* produced dose-dependent inhibition of hydroxyl radicals ranging from 10.30% (in 20 µg/ml of the extract) to 67.80% (in 640 µg /mL of extract). The IC_{50} value of the extract of the study species and Ascorbic acid were found to be 266.30µg/ml and 68.00µg/ml respectively.

The results of the present study indicate that the hydro-alcoholic extract of *Caralluma umbellata Haw* has significant antioxidant activity which is comparable to that of the standard drug ascorbic acid. Thus, hydro-alcoholic extract of *Caralluma umbellata Haw* as promising natural source of antioxidants can be used in nutritional or pharmaceutical fields for the prevention of free radical- mediated diseases.

CONCLUSION

In conclusion, the results of the present study show that the hydro-alcoholic extract of *Caralluma umbellata Haw* contain significant amount of antioxidant agents. Therefore, the study suggests that the stems of the plant might be a potential source of natural antioxidants. The plant can be further harnessed for novel antioxidant/ bioactive compounds which may be very well evidenced by the present work.

REFERENCES:

1. Uang D, Boxin O, Prior LR, The chemistry behind antioxidant capacity assays, J Agric Food Chem, 53, 2005, 1841-185.
2. Pulido RJ, Orensanz A, Saura – Calixto L, Study of plasma antioxidant status in Alzheimer's disease, EurJ Neurol, 12 , 2005, 531-535.
3. Roginsky VA, Barsukova TK, Remorova AA, Moderate antioxidative efficiencies of 1610ttenuate1610 during peroxidation of methyl linoleate in homogenous and micellar solutions, J Am Oil Chem Soc, 73, 1996, 777-786.
4. Ivanova D, Gerova D, Chervenkov T, Yankova T, Polyphenols and antioxidant capacity of Bulgarian medicinal plants, Journal of Ethnopharmacology , 96(1-2) ,2005, 145-150.
5. Vasu K, Goud JV, Suryam A, Charya MS, Biomolecular and Phytochemical analysis of three aquatic angiosperm, African Journal of Microbiology Research , 3(8),2009, 418-421.
6. Enwuru, NV, Ogbonnia SO, Nkemehule F, Enwuru CA, Tolani O, Evaluation of antibacterial activity and acute toxicity of the hydroethanolic extract of *Stachytarpheta angustifolia* (Mill) Vahl, African Journal of Biotechnology , 7(11), 2008, 1740-1744.
7. Rice-EvansCA, Miller NJ, Paganga G, Antioxidant properties of phenolic compounds, Trends Plant Sci , 2, 1997, 152- 159.
8. Mojab F, Kamalinejad M, Ghaderi N, Vanidipour HR, Phytochemicals screening of some species of Iranian plants, Iran J Pharm Res, 2(2), 2003, 77-82.
9. Jigna P, Sumitra C, Antibacterial and phytochemical studies on twelve species of Indian medicinal plants, African Journal of Biomedical Research, 10(2), 2007, 175 – 181.
10. Jigna P, Sumitra C, Phytochemical screening of some plants from western region of India, Journal Plant Archives, 8(2), 2008, 657-662.
11. Elliottmiddleton JR, Chithan K, Theoharis CT, The effects of plant 1610ttenuate1610 on mammalian cells: Implications for Inflammation, Heart Disease, and Cancer, Pharmacol Rev , 52(4),2000, 673-751.
12. Dragan A, Dusankadavidovic A, Drago B, Nenad T, Structure- Radical Scavenging Activity Relationships of Flavonoids, Croatica Chemica Acta , 76(1) ,2003, 55-61.
13. Qiu SY, Cordell GA, Ravi Kumar B, Nageswara Rao Y, Ramesh M, Kokate C, et al. Bisdesmosidic Pregnane glycosides from *Caralluma Lasiantha*. Phytochem. 1999; 50:485-91.
14. Anitha K, Jayalakshmi G, Siva Rambabu S, Kiranmayee P. Antibacterial effect of *Caralluma 1610ttenuate Wt*. on Gram positive and Gram negative bacteria. Int Cong Chem Environ Sci. 2005;546-547.

15. Ray S, Nagaiah K, Khan NF. Anti-inflammatory activity of Carumbelloside-III, isolated from *Caralluma umbellata*. NSHM J Pham Health Mgt. 2011; 2:83-8.
16. Sawant BM, Sayad TD. N-Pentatriacontane from *Caralluma fimbriata*. J Shivaji Univ Sci. 1978; 18:87-91.
17. Vedavathy S, Mridula V, Sudhakar A. Tribal Medicine of Chittoor District of Andhra Pradesh, India, I edition, Herbal Folklore Research Centre; Tirupati; 1997.
18. Pullaiah T. Encyclopedia of world medicinal plants. Regency Publications New Delhi India. 2006; 2:437-9.
19. Basavaraju R, Vennel Raj J, Bhiravamurthy PV, Medicinal Plant Resources of Puttaparthi Mandal, Taxonomic Overview and Need for Conservation. Ethnobotanical Leaflets. 2009; 13:1382-1400.
20. Harborne,J.B, Phytochemical Methods. London: Chapman and Hall Ltd, 1973, 49-188.
21. Trease GE, Evans WC, Phamacognos 13thEd, Balliere- Tindal: London, 1989, 176-190.
22. Harborne JB. Phytochemical methods: A guide to modern technique of plant analysis. London: Chapman & Hill; 1998.
23. Harbone, J.B., Turner, B.L. Plant chemosystematics. Academic press, London. 1984: P: 61-62.
24. Gibbs, R.D. Chemotaxonomy of flowering plants. MC Gill Queens University press, Montreal and London. 1974.
25. McDonald S, Paul DP, Michael A, Kevin R, Phenolic content and antioxidant activity of olive extracts, Food Chemistry , 73(1), 2001,73-84.
26. Chandha S, Dave R, In vitro models for antioxidant activityevaluation and some medicinal plants possessing antioxidant properties: An overview, African Journal of Microbiology Research , 3(13), 2009, 981–996.
27. Blios MS. Antioxidant determinantions by the use of a stable free radical. Nature 1958; 26: 1199 – 1200.
28. McCord JM, Day Jr ED. Superoxide-Dependent Production of Hydroxyl Radical Catalyzed by Iron-EDTA Complex. FEBS Lett. 1978; 86:139-142.
29. Elizabeth K, Rao MNA: Oxygen radical scavenging activity of curcumin. ***Int J Pharmaceut* 1990,** 58:**2**37-240.
30. Gutteridge, J.M.C.&Halliwell, B. (2000). Free radicals and antioxidants in the year 2000 Ahistorical look to the future,, 899:136-147.
31. Lekameera, R., Vijayabaskar, P. & Somasundaram, S.T. (2008). Evaluating antioxidant property of brown ALGA (Derb. Et sol), 2: 126-130.
32. Cheeseman,K.H.& Slater,T.F. (1994). Free radical in medicine, 49: 479724.
33. Willet,W.C. (1994).Diet and healthwhat shouldwe eat ? , 264: 532 – 537
34. Shahidi F, Janitha PK and Wanasundara PD, Phenolic antioxidants. CRC Critical Rev. Food Science and Nutrition. 32 (1): 67103, (1992).
35. Shah R, *In vitro* antioxidant activity of roots of Tephosia purpurea Linn. International Journal of Pharmaceutical Sciences 2010; 2 (3): 30-33.
36. Rios JL, Recio MC, Medicinal plants and antimicrobial activit, J Ethnopharmacology, 100, 2005, 80-84.
37. Sofowora LA, Medicinal plants and traditional medicine in Africa.,Spectrum Books Ltd: Ibaban, 1993, p. 55-71.
38. Sochor J, Fully Automated Spectrometric Protocols for Determination of Antioxidant Activity: Advantages and Disadvantages. Molecules 2010; 15: 8618-8640.
39. Wang SY, Jiao H (2000). Correlation of antioxidant capacities to oxygen radical scavenging enzyme activities in blackberry. J. Agric. Food Chem. 48(11):5672-5676.
40. Chew YL, Assessment of in vitro antioxidant capacity and polyphenolic composition of selected medicinal herbs from Leguminosae family in Peninsular Malaysia. Food Chem 2009; 116: 13-18.