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INVITRO STUDY ON ANTIPLATELET AND ANTIOXIDANT ACTIVITIES OF FLESHY STEM JUICE OF *CARALLUMA ATTENUATA*

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Abstract

The fleshy stem Juice of *Caralluma attenuata* were evaluated for their antioxidant activity and antiplatelet effects. ABTS radical scavenging, lipid peroxidation and superoxide scavanging methods were used for the antioxidant activity tests. Total flavonoids and total phenolics were also evaluated spectrophotometrically. The fleshy stem Juice of *Caralluma attenuata* contained the highest total flavonoid and total phenolic content and exhibited the highest antioxidant activities. The total phenolic and total flavonoid content ranged from 59.32 and 38.24 mg/g dry extract, respectively. The results of ABTS assay showed that fleshy stem Juice of *Caralluma attenuata* had the scavenging 71.23% values followed by standard. For fleshy stem Juice of *Caralluma attenuata*, the antioxidant activity and contents of total phenolics and total flavonoids correlated well with each other. In vitro investigation of the antiplatelet effect showed that fleshy stem Juice of *Caralluma attenuata* has the highest inhibition (63.64 %).

Keywords: Antioxidant, Platelet aggregation, Total phenolics, *Caralluma attenuata*

1.Introduction:

Platelets play a key role in maintaining physiologics play a key role in maintaining homeostasis in blood. Platelets also play a major role in initiating thrombus formation which occurrs with various thrombotic disorders, including hypertension, atherosclerosis and ischemic heart diseases. Thrombosis may occur if the hemostatic stimulus is improperly regulated either due to impaired capacity of inhibitory pathway or more commonly when the capacity of natural anticoagulant mechanism is overwhelmed by the intensity of stimulus. Platelets are primed by several common risk factors and thrombosis is also an important factor in the pathophysiology of unstable angina and myocardial infarction (Tepper et al., 2000). Therefore, agents with anti-platelet and anti-thrombotic effects may have wide therapeutic potential for circulatory diseases.

Antioxidants play important roles in preventing the diseases induced by reactive oxygen species, which result in oxidative damage to DNA, proteins and other macromolecules and are associated with degenerative or pathological events, such as aging, asthma, and cancer (Balaban et al. 2005). However, there are serious concerns about the carcinogenic potential of synthetic antioxidants widely used in the food industry, for example, butylated hydroxyanisole and butylated hydroxytoluene. Therefore, intensive research has been carried out to develop natural alternatives, which may serve as potent candidates in combating carcinogenesis and aging processes.

Plants, which are sources of phytochemicals with strong antioxidant activity, have attracted a great deal of attention in recent years. Antioxidants, which inhibit the oxidation of organic molecules, are very important, not only for food preservation, but also for the defence of living systems against oxidative stress (Masuda et al., 2003). Phenolic antioxidants interrupt the propagation of the free radical autoxidation chain by contributing a hydrogen atom from a phenolic hydroxyl group, with the formation of a relatively stable free radical that does not initiate or propagate further oxidation processes (Kaur & Kapoor, 2001).

Phenolic compounds account for most of the antioxidant activity of plant extracts. Plants vary in content and structure of phenolic components (number of phenolic rings, aromatic substitution, glycosylation, conjugation with other phenolic compounds or organic acids) and thus vary in their antioxidant properties. The identification, quantification and characterization of phytochemicals is important for applications of plant extracts as new food additives. This allows the development of efficient quality control measures to ensure the authenticity and standardization of product composition and quality. Identification of the phytochemicals which make a major contribution to the antioxidant activity of plant extracts is often difficut because of the large number of phenolic components that are present. Masuda et al., 2003).

Diverse methods are currently used to assess the antioxidant activity of plant phenolic compounds. Chemical assays are based on the ability to scavenge synthetic free radicals, using a variety of radical-generating systems and methods for detection of the oxidation end-point. 2,20-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)- or DPPH radical-scavenging methods are common spectrophotometric procedures for determining the antioxidant capacities of plant components. These chromogens (the violet DPPH radical and the blue green ABTS radical cation) are easy to use, have a high sensitivity, and allow for rapid analysis of the antioxidant activity of a large number of samples. These assays have been applied to determine the antioxidant activity of food, wine and plant extracts and pure components (Awika, et al., 2003; Van den Berg, Yu et al., 2002). Unfortunately, results from these methods often do not correlate with the ability of a compound to inhibit oxidative deterioration of foods. This is because the antioxidant activity in food and biological systems is dependent on a multitude of factors, including the colloidal properties of the substrates, the conditions and degree of oxidation and the localization of antioxidants in different phases. Hence, it is pertinent to use different assay systems to assess and compare the antioxidant effectiveness in plant extracts (Kaur & Kapoor, 2001).

Higher plants have long been shown to be excellent and reliable sources for the development of novel antiplatelet aggregation and antioxidants drugs. In india, many plants have been used for treatment of various malignancies over centuries. *Caralluma attenuate* Wight. [Syn.: *C. fimbriata* Hook.] (Asclepiadaceae), is a thick, succulent perennial herb growing wild in and around south Tamilnadu. The juice of the plant along with black pepper is recommended in the treatment of migraine (Srinivasacharyulu, 1931). This extract helps relieve dermatitis, eczema and dandruff. It is an antioxidant, rejuvenating skin cells, and also has anti-rheumatic and anti-arthritic effects. (Sankaranarayanan, 2009). This plant was found to be a rich source of glycosides (Ramesh, 1995). Chemical investigations have resulted in the isolation of luteolin-4%-O-neohesperidoside, i.e. luteolin-4%-O-[a-(L-rhamnopyranosyl- (1.2)-b-D-glucopyranoside)] as the major chemical constituent of this plant. However, the properties of this plant, especially its antiplatelet aggregation and antioxidants activity, have not yet been investigated. Therefore, this prompted us to investigate the inhibitory effect of this plant on platelet aggregation and antioxidants activity.

2. Material and Methods:

2.1 Preparation of extracts

Fleshy stem Juice of *Caralluma attenuata* were prepared according to the method described by Boaky- Yiadon (1979) with little modifications. Twenty grams of fleshy stem Juice of *Caralluma attenuata* were air-dried, crushed and blended into powder using an electric blender for each solvent. The blended material was transferred to a beaker and soaked separately in 100 ml of the organic solvent at room temperature. The mixture was extracted by agitation on a rotary shaker. The extract obtained was vaccum-dried and used for further test.

2.2 Phytochemical Screening Test of fleshy stem Juice of *Caralluma attenuata*

Thin Layer Chromatography

The fleshy stem Juice of *Caralluma attenuata* were loaded on to pre coated TLC (60 F_2 54) and it was developed using solvent system in the ratio of 1:0.5:0.1 (Hexane, Chloroform and Methanol) visible and the non visible spot given and it is fluorescent with UV light at 360nm.

2.3 Total phenolic content of fleshy stem Juice of *Caralluma attenuata*

The total phenolic content of fleshy stem Juice of *Caralluma attenuata* was determined using the method by Gutfinger (1981). The DSM extract (1 mL, 1 mg/mL) was mixed with 1 mL of 50% Folin-Ciocalteu reagent and 1 mL of 2% Na_2CO_3, and centrifuged at 13400 X g for 5 min. The absorbance of upper phase was measured using a spectrophotometer (Model UV-1601; Shimadzu, Tokyo, Japan) at 750 nm after 30 min incubation at room temperature. TPC was expressed as a tannic acid equivalent.

2.4 Preparation of washed platelets

Blood samples were taken from the heart and transferred into plastic tubes containing acid–citrate–dextrose (ACD) (75 mM trisodium citrate, 38 mM citric acid and 138 mM glucose) as anticoagulant in a volume ratio 6:1. Platelet rich plasma was obtained by centrifugation of blood at 400 ×g for 10 min at room temperature. Platelet rich plasma was then centrifuged at 800 ×g for 10 min and the obtained platelets were washedwith Tyrode buffer (136mMNaCl, 2.7mMKCl, 10mM HEPES, 12 mM NaHCO3, 0.34 mM Na2HPO4, 1 mM MgCl2, 5.5 mM glucose) containing 0.35% albumin and 10% ACD, pH 6.5. The washed platelets were then centrifuged again at 800 ×g for 10 min and finally re-suspended in Tyrode buffer containing 0.1% albumin, pH 7.4. Platelet number was counted by a Coulter Counter (JT; Coulter Electronics, Inc., USA) and adjusted to 3×108 cells/ml.

2.5 Assay of platelet aggregation in vitro

Aggregation experiments were performed using a lumi-aggregometer (Chronolog Corp., Havertown, PA). Washed platelets were incubated with various tested compounds or vehicles at 37 °C for 15, 30 and 60 min. Then aggregation was induced by addition of an agonist, thrombin, while stirring at 1000 rpm in a silicone-treated glass cuvette, in a final volume of 250 µl. The reaction was then allowed to proceed for 4 min, and the extent of aggregation was expressed as the percentage of aggregation of the control values. For antiplatelet experiments, the aggregation was performed using the threshold concentration of thrombin (0.1 U/ml). Threshold is defined as the lowest concentration of an agonist that evokes irreversible aggregation, with amplitude between 65% and 85% of the potential maximum deflection in light transmission.

2.6 ABTS assay

ABTS 2,2-azinobis (3-ethyl benzothiazoline-6-sulfonic acid) radical scavenging activity of AS extracts was determined according to Re et al. (1999). ABTS radical was freshly prepared by adding 5 ml of a 4.9 mM potassium persulfate solution to 5 ml of a 14 mM ABTS solution and kept for 16 h in dark. This solution was diluted with distilled water to yield an absorbance of 0.70 at 734 nm and the same was used for the antioxidant assay. The final reaction mixture of standard group was made up to 1 ml with 950 µl of ABTS solution and 50 µl of Vit-C. Similarly, in the test group 1 ml reaction mixture comprised 950 µl of ABTS solution and 50 µl of the extract solutions. The reaction mixture was vortexed for 10 s and after 6 min absorbance was recorded at 734 nm against distilled water by using an ELICO (SL150) UV–Vis Spectrophotometer and

compared with the control ABTS solution. Ascorbic acid was used as reference antioxidant compound.

2.7 Lipid peroxidation assay

A modified thiobarbituric acid-reactive species (TBARS) assay (Ohkowa, Ohisi, & Yagi, 1979) was used to measure the lipid peroxide formed, using egg yolk homogenates as lipid rich media (Ruberto, Baratta, Deans, & Dorman, 2000). Malondialdehyde (MDA), a secondary end product of the oxidation of polyunsaturated fatty acids reacts with two molecules of thiobarbituric acid (TBA) yielding a pinkish red chromogen with an absorbance maximum at 532 nm (Janero, 1990). 0.5 ml egg homogenate (10% in distilled water, v/v) and 0.1 ml of extract were mixed in a test tube and volume was made up to 1 ml by adding distilled water. Finally 0.05 ml $FeSO_4$ (0.07 M) was added to the above mixture and incubated for 30 min to induce lipid peroxidation. Thereafter, 1.5 ml of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5 ml of 0.8% TBA (w/v) (prepared in 1.1% sodium dodecyl sulphate) and 0.05 ml 20% TCA were added, vortexed and then heated in a boiling water bath for 60 min. After cooling, 5.0 ml of butan-1-ol was added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm by using an ELICO (SL150) UV–Vis Spectrophotometer. As leaves may also contain other phytochemicals, which may also absorb at 532 nm, therefore to eliminate this non- MDA interference, another set of samples were run as above without TBA. The absorbance value was subtracted.

2.8 Superoxide radical scavenging property

This assay was based on the capacity of the extract to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) (Beauchamp & Fridovich, 1971) in the presence of the riboflavin-light-NBT system with slight modification, as described earlier (Tripathi & Pandey, 1999; Tripathi & Sharma, 1999). In brief, each 3 ml reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 2 µM riboflavin, 100 µM EDTA, NBT (75 µM) and 1 ml test sample solution. It was kept in front of fluorescent light and absorbance was taken after 6 min at 560 nm by using an ELICO (SL150) UV–Vis Spectrophotometer. Identical tubes with reaction mixture were kept in the dark and served as blanks. The percentage inhibition of superoxide generation was measured by comparing the absorbance of the control and those of the reaction mixture containing test sample solution.

2.9 Hydroxyl radical scavenging assay

The deoxyribose method was used for determining the scavenging effect on hydroxyl radicals as described by Halliwell, Gutteridge, and Aruoma (1987). The reaction mixtures contained ascorbic acid (50 µM), $FeCl_3$ (20 µM), EDTA (2 mM), H_2O_2 (1.42 mM), deoxyribose (2.8 mM) with different concentrations of the test extracts in a final volume of 1 ml in potassium phosphate buffer (10 mM, pH 7.4). It was incubated at 37 °C for 1 h and then 1 ml of 2.8% TCA (w/v in water) and 1 ml of 1% thiobarbituric acid (TBA) (w/v) were added. The mixture was heated in a boiling water bath for 15 min. It was cooled and absorbance was taken at 532 nm. Thiourea was taken as positive control.

3. Statistics

All datas are expressed as means ± SD. Pearson's correlation analysis (SPSS 7.5 forWindows, SPSS Inc.) was used to test for the significance of relationship between the concentration and percentage inhibition at a $p < 0.05$ significance level.

4. RESULT

4.1. PHYTOCHEMICAL SCREENING OF JUICE OF *CARALLUMA ATTENUATA*

The phytochemical screening of the fleshy stem Juice of *Caralluma attenuata* studied showed the presence of flavonoids terpenoids, saponins and tannins (Table -1).

Table 1. Phytochemical screening of Juice of *Caralluma attenuata*

S.NO.	CHEMICAL CONSTITUENTS	JUICE OF *CARALLUMA ATTENUATE*
1	Alkaloid - Mayers reagent	+
2	Flavonoids	+
	Alkalai Reagent - Lead acetate	
3	Glycosides	+
	Bornbager's test	
4	Tannin	+
	$FeCl_3$ test	
5	Saponins	-
	Frothing test	
6	Terpenoids	+
	Nollers test	
7	Test for polyphenol - Ammonia Test	+
8	Test for anthocyanin - Ammonia test	+

- = Negative (absent)
+ = Positive (slightly present)

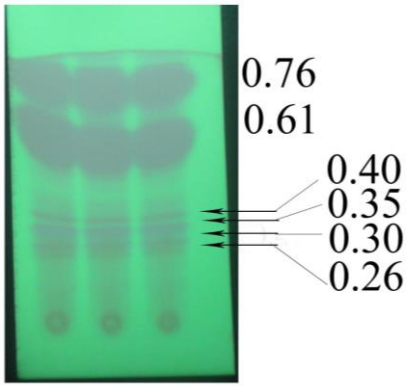
4.2 THE PARTIAL CHARECTERIZATION OF METHANOL LEAF EXTRACT OF *CARALLUMA ATTENUATA* BY TLC

The fleshy stem Juice of *Caralluma attenuata* loaded on Pre-coated TLC plates (60 F_2 54 Merck) and developed with a solvent system of hexane, chloroform and methanol in the ratio of 1:0.5:0.1 was efficient to extract the antibacterial compound it is used for further studies. The developed plate was viewed under UV 240nm and 360nm. (Table-2).

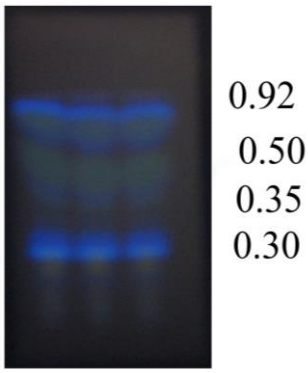
Table-2 Partial characterization of fleshy stem Juice of *Caralluma attenuata* by TLC

JUICE OF *CARALLUMA ATTENUATE*		
Component No.	UV Light 360nm Rf value	UV light 240nm Rf value
1	0.92	0.76
2	0.50	0.61
3	0.35	0.40
4	0.30	0.35
5	-	0.30
6	-	0.26

Fig-1. Partial characterization of fleshy stem Juice of *Caralluma attenuata* by TLC



TLC plate viewed 240 nm



TLC plate viewed 360 nm

4.3. TOTAL PHENOLIC COMPOUND OF *CARALLUMA ATTENUATA*

In this context, the preliminary experiments revealed that 80% acetone was the best solvent for the extraction of phenolics from beans at 60 °C for 60 min since it afforded a maximum yield of phenolics. The yields fleshy stem Juice of *Caralluma attenuata* ranged from 59.23% (w/w), Literature data and preliminary studies before this work indicated the presence of catechin or related compounds in beans. Therefore, the total phenolic contents were reported as catechin equivalents. In addition, catechin was used as a reference antioxidant in other experiments (Table-3).

Table 3-Yield and phenolic content of *Caralluma attenuata* extracts [a, b]

Sample	Yield of extract (g/100 g of fleshy stem Juice of *Caralluma attenuata*)	Total phenolic content (mg catechin equivalents per gram fleshy stem Juice of *Caralluma attenuata*)
Caralluma attenuata	59.23+1.23a	39.63±2.23[b]

[a] Data are expressed as mean ± standard deviation (n = 3) on a fresh weight basis.

[b] Means in each column sharing the same letter are not significantly (P = 0.05) different from 1 a other.

4.4 ANTI PLATELET AGGREGATION ASSAY

The fleshy stem Juice of *Caralluma attenuata* inhibited platelet aggregation in a concentration and time dependent manner. Fleshy stem Juice of *Caralluma attenuata* significantly decreased ($p<0.01$) platelet aggregation with the EC_{50} value between 25-100 µg/ml concentration. At 100 µg/ml concentration, *Caralluma attenuata* extract showed highly significant inhibition 64.23 percentage ($p<0.01$) on platelet aggregation at the incubation time of 60 min (Table-4). *Caralluma attenuate* has been widely used in many countries for the treatment of common cold and other diseases such as diarrhea, inflammation and diabetes. The present study demonstrated methanol extract of *Caralluma attenuate* remarkably decreased platelet aggregation induced by thrombin in a concentration-and time-dependent manner.

Table 4. Anti-platelet aggregation assay activity of fleshy stem Juice of *Caralluma attenuata*

Different Concentration of Juice	Inhibition (%) of fleshy stem Juice of *Caralluma attenuata*	Standard Vitamin-C
25	14.23±1.28	12.64±1.37
50	32.68±2.56	27.34±1.63
75	47.32±1.67	41.78±2.25
100	64.23±1.49	58.32±2.87
EC_{50}(µg/ml)	66.34	72.35

n = 3

All the observations in different groups showed significant ($P<0.01$) relationship between the concentration and percentage inhibition (Pearson's correlatioin analysis). [a]Mean ± SD.

4.5 ABTS ASSAY OF FLESHY STEM JUICE OF *CARALLUMA ATTENUATA*

Maximum inhibition was observed with the total higher concentration of fleshy stem Juice of *Caralluma attenuata* 100 µg/ml and minimum inhibition was observed with the 25 µg/ml fleshy stem Juice of *Caralluma attenuata*. Based on EC_{50} values, the order of scavenging activity of fleshy stem Juice of *Caralluma attenuata* was found to be: total 100, 300 and 500 µg/ml (Table-5). Free radicals are involved in the normal physiology of living organisms. They act as a messenger for signal transduction and also affect gene expression (Armario et al., 1990). Besides, Free radicals are also involved in the pathogenesis of several chronic diseases such as neurodegenerative diseases, ageing, rheumatoid arthritis, metabolic diseases like atherosclerosis, diabetes, hypertension, etc. (Misra et al., 1965).

Table 5. ABTS radical activity of fleshy stem Juice of *Caralluma attenuata*

Different Concentration Juice	Inhibition (%) of fleshy stem Juice of *Caralluma attenuata*	Standard Vitamin-C
25	17.25±7.12	15.34±1.78
50	38.65±5.71	34.78±1.89
75	51.46±5.91	47.34±2.34
100	71.23±2.36	63.35±1.48
EC_{50}(µg/ml)	62.34	68.25

n = 3

All the observations in different groups showed significant (P<0.01) relationship between the concentration and percentage inhibition (Pearson's correlatioin analysis). [a]Mean ± SD.

4.6 LIPID PEROXIDATION ASSAY OF FLESHY STEM JUICE OF *CARALLUMA ATTENUATA*

Different concentrated fleshy stem Juice of *Caralluma attenuata* also inhibited the lipid peroxidation induced by ferrous sulfate in egg yolk homogenates. Maximum inhibition was observed with total 100 µg/ml with EC_{50} value at 51.23 µg/ml and the standard were in the same order as described below that is: Total compound 25, 50, 75 and 100 µg/ml (Table-6).

Table-5. Inhibition of lipid peroxidation induced by $FeSO_4$ using egg yolk homogenates as lipid rich media by fleshy stem Juice of *Caralluma attenuata*

Different Concentration Juice	Inhibition (%) of fleshy stem Juice of *Caralluma attenuata*	Standard Vitamin-C
25	20.24±1.56	17.23±2.79
50	45.32±2.47	39.32±2.46
75	62.34±1.89	56.32±1.78
100	78.32±1.96	72.64±0.26
EC_{50}(µg/ml)	51.23	57.23

n = 3

All the observations in different groups showed significant (P<0.01) relationship between the concentration and percentage inhibition (Pearson's correlatioin analysis). [a]Mean ± SD.

4.7 SUPEROXIDE SCAVENGING ASSAY OF FLESHY STEM JUICE OF *CARALLUMA ATTENUATA*

All of the above fleshy stem Juice of *Caralluma attenuata*, exhibited potent scavenging activity for superoxide radicals in a concentration dependent manner. The fleshy stem Juice of *Caralluma attenuata* 100 µg/ml had EC_{50} values of 55.23 µg/ml and the standard Vitamin-C 100 µg/ml was least potent with an EC_{50} value 61.27 µg/ml. Based on their EC_{50} values, the order of scavenging activity was as follows: 25, 50, 75 and 100 µg/ml (Table-7). This project generated superoxide radicals by photochemical reduction of nitro blue tetrazolium (NBT) in the presence of a riboflavin-light-NBT system, which is one of the standard methods. These superoxide radicals (O_2) are highly toxic and may be generated either through xanthine activity or through mitochondrial reaction. Although it is a relatively weak oxidant, it may decompose to form stronger reactive oxidative species, such as singlet oxygen and hydroxyl radicals (Dahl & Richardson, 1978).

Table-7. Superoxide anion scavenging activity of fleshy stem Juice of *Caralluma attenuata* observed with a riboflavin - light – NBT system

Different Concentration Juice	Inhibition (%) of fleshy stem Juice of *Caralluma attenuata*	Standard Vitamin-C
25	16.32±1.02	13.25±2.20
50	37.45±2.13	33.64±2.17
75	51.32±1.47	42.36±1.38
100	74.32±2.59	66.34±1.49
EC_{50}(µg/ml)	55.23	61.27

n = 3
All the observations in different groups showed significant (P<0.01) relationship between the concentration and percentage inhibition (Pearson's correlatioin analysis). [a]Mean ± SD.

4.8 SITE SPECIFIC HYDROXYL RADICAL SCAVENGING ASSAY (with EDTA)

The fleshy stem Juice of *Caralluma attenuata* 100 µg/ml was found to be the most potent hydroxyl radical scavenger with EC_{50} values of 55.23 µg/ml and the 100 µg/ml standard was the least potent hydroxyl scavenger. Based on their EC_{50} values the order of scavenging activity for hydroxyl radicals was as follows: Total fleshy stem Juice of *Caralluma attenuata* > 25, 50, 75 and 100 µg/ml (Table-8). Hydroxyl radical is the most reactive radical known in chemistry. It can abstract hydrogen atoms from biological molecules, including thiols, leading to the formation of sulfur radicals capable to combine with oxygen to generate oxysulfur radicals, a number of which damage biological molecules (Halliwell, 1991).

Table-8. Hydroxyl radical scavenging activity of fleshy stem Juice of *Caralluma attenuata* in the deoxyribose assay in the presence and absence of EDTA

Different Concentration Juice	Inhibition (%) of fleshy stem Juice of *Caralluma attenuata*	Standard Vitamin-C
25	13.64±2.37	10.34±1.25
50	28.97±1.78	25.67±1.49
75	41.39±1.34	37.89±1.56
100	63.64±1.56	57.32±2.36
EC_{50}(µg/ml)	55.23	65.23

EC_{50}(µg/ml)of Thiourea: non-site specific-209 and site specific-290.
n = 3
All the observations in different groups showed significant (P<0.01) relationship between the concentration and percentage inhibition (Pearson's correlatioin analysis).
[a]Mean ± SD.

5. CONCLUSION

In conclusion, many cardiovascular diseases can be attributed to excessive platelet aggregation, which has a critical role in thrombus formation (Lee et al., 1998). It appears that *Caralluma attenuate* methanol extract can inhibit platelet aggregation in vitro; therefore, they may be used to treat or prevent some cardiovascular diseases. The present study may lead to the development or synthesis of the new antiplatelet aggregation drugs from this medicinal plant that

is beneficial for cardiovascular disorder patients. However, this plant should be used with caution by patients with bleeding disorders as it may increase the risk of bleeding.

6. RECOMMENDATION

It may be suggested that aerial part of *Caralluma attenuate* juice extract was flavonoids group of antioxidant compound, which can effectively scavenge various reactive oxygen species/ free radicals under in vitro conditions. They also have mild metal chelation properties. This property may be attributed more to the polar fraction, because the juice fraction showed better response on all the tested parameters.

Reference

1. Armario, A., Company, L., Borras, M., & Hidalgo, J. (1990). Free Radical Research Communications, 9(2), 113.

2. Bast A, Haenen GRMM, Doleman CJA. 1991. Oxidants and antioxidants: state of the art. Amer J Med 91:2S-13S.

3. Chu Y (2000). Flavonoid content of several vegetables and their antioxidant activity, J. Sci. Food Agric. 80: 561-566.

4. Dahl, M. K., & Richardson, T. (1978). Photogeneration of superoxide anion in serum of bovine milk and in model systems containing riboflavin and amino acid. Journal of Dairy Science, 61, 400–407.

5. Dahl, M. K., & Richardson, T. (1978). Photogeneration of superoxide anion in serum of bovine milk and in model systems containing riboflavin and amino acid. Journal of Dairy Science, 61, 400–407.

6. Davies K (1995). "Oxidative stress: the paradox of aerobic life". *Biochem Soc Symp* **61**: 1–31.

7. Dharmasiri, M. G., Jayakody, J. R. A. C., Galhena, G., Liyanage, S. S. P., & Ratnasooriya, W. D. (2003). Anti-inflammatory and analgesic activities of mature fresh leaves of Vitex negundo. Journal of Ethno Pharmacology, 87, 199–206.

8. Emerti, J., Klein, J. M., Coutellier, A., & Congy, F. (1990). Pathology Biology Paris, 39(4), 316.

9. Exarchou V, Nenadis N, Tsimidou M, Gerothanassis IP, Troganis A, Boskou D (2002). Antioxidant activities and phenolic composition of extracts from Greek oregano, Greek sage and summer savory. J. Agric. Food Chem. 50: 5294-5299.

10. Farombi, EO., Ogundipe, O., & Moody, J. O. (2001). Antioxidant and anti-inflammatory activities of Mallotus oppositifolium in model systems. African Journal of Medicine and Medical Sciences, 30(3), 213–215.

11. Gey KF. 1994. The relationship of antioxidant status and the risk of cancer and cardiovascular disease: a critical evaluation of observational data. In: Nohl H, Esterbauer.

12. Gutfinger T. 1981. Polyphenols in olive oils. J Am Oil Chem Soc 58:966–8.
13. H, Rice-Evans C, editors. Free radicals in the environment, medicine, and toxicology. London: Richelieu Press. P 181-219.

14. Halliwell, B. & Gutteridge, J.M.C. (1989) Free Radicals in Biology and Medicine, 2nd edn. Clarendon Press, Oxford.

15. Halliwell, B. (1991). Reactive oxygen species in living systems: source, biochemistry and role in human disease. Annales Journal of Medicine, 91(Suppl. 3 C), 14S–22S.

16. Halliwell, B., Gutteridge, M. C., & Aruoma, O. I. (1987). The deoxyribose method: a simple test-tube assay for determination of rate constants for reactions of hydroxyl radicals. Analytical Biochemistry, 165, 215–219.

17. Haslam, E. (1996). Natural polyphenols (vegetable tannins) as drug: possible mode of action. Journal of Natural Products, 59, 205– 215.

18. Kaur, C., & Kapoor, H. C. (2001). Antioxidants in fruits and vegetablesthe millenium's health. International Journal of Food Science and Technology, 36, 703–725.

19. Koleva II, Van Beek TA, Linssen JPH, De Groot A, Evstatieva LN (2002). Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem. Anal. 13: 8- 17.

20. Larson R. A. 1988. The antioxidants of higher plants. Phytochemistry 27:969-78.

21. Larson RA. 1988. The antioxidants of higher plants. Phytochemistry 27:969–78.

22. Lee, J.Y., Lee, M.Y., Chung, S.M., Chung, J.H., 1998. Chemically induced platelet lysis causes vasoconstriction by release of serotonin. Toxicol. Appl. Pharmacol. 149, 235–242.

23. Li, C., & Xie, B. (2000). Evaluation of the antioxidant and pro-oxidant effects of tea catechin oxypolymers. Journal of Agricultural and Food Chemistry, 48, 6362–6366.

24. Lo liger J (1991). The use of antioxidants in food. In Aruoma OI, Halliwell B (Eds) Free radicals and food additives. Taylor and Francis, London. pp. 129–150.

25. Mantle D, Eddeb F, Pickering AT (2000). Comparison of relative antioxidant activities of British medicinal plant species, J. Ethnopharmacol. 72: 47- 51.

26. Masuda, T., Inaba, Y., Maekawa, T., Takeda, Y., Yamaguchi, H., Nakamoto, K., et al. (2003). Simple detection method of powerful antiradical compounds in the raw extract of plants and its application for the identification of antiradical plant constituents. Journal of Agricultural and Food Chemistry, 51, 1831–1838.

27. Misra, M. B., Terari, J. P., & Bapat, S. K. (1965). Journal of Science Technology, 3, 272.

28. Muggli R. 1993. Free radical tissue damage: the protective role of antioxidant nutrients. In: Corongiu F, Banni S, Dessi MA, Rice-Evans C, editors. Free radicals and antioxidants in nutrition. London: Richelieu Press. P 189-204.

29. Munasinghe, T. C. J., Seneviratne, C. K., Thabrew, M. I, & Abeysekera, A. M. (2001). Anti-radical and antipoperoxidative effect of some plant extracts used by Sri Lankan traditional medical practioner for cardioprotection. Phytother Res., 15, 519–523.

30. Oke JM, Hamburger MO (2002). Screening of some Nigerian medicinal plants for antioxidant activity using 2, 2 diphenyl picryl hydrazyl radical. Afr. J. Biomed. Res. 5: 77-79.

31. Pholphana, N., Rangkadilok, N., Thongnest, S., Ruchirawat, S., Ruchirawat, M., Satayavivad, J., 2004. Determination and variation of three active diterpenoids in Andrographis paniculata (Burm.f.) Nees. Phytochem. Anal. 15, 365–371.

32. Pratt D.E. and Hudson B.J.F. 1990. Natural antioxidants not exploited commercially. In: Hudson BJF, editor. Food antioxidants. London; Elsevier Applied Science. p 171-92.

33. Puppo, A. (1992). Effect of flavonoids on hydroxyl radical formation by Fenton-type reactions: influence of the iron chelator. Phytochemistry, 31, 85–88

34. Ramesh, M., 1995. Development of New Phytochemicals from Natural Sources—Ph.D., thesis submitted to Kakatiya University, Warangal, Andhra Pradesh, India.

35. Rhee SG (June 2006). "Cell signaling. H2O2, a necessary evil for cell signaling". *Science (journal)* **312** (5782): 1882–3.

36. Sankaranarayanan S., (2009). Medical Taxonomy of Angiosperm: Recent Trents Medicinal Uses and Chemical Constituent. Vol.1. Harishi Publication, Chennai. Pp. 555-556.

37. Sies H (1997). "Oxidative stress: oxidants and antioxidants" (PDF). *Exp Physiol* **82** (2): 291–5.

38. Srinivasacharyulu, Y., 1931. Yogarathnakaram, vol. 2, Swatantra Press, Nellore, Andhra Pradesh, India, p. 678.

39. Tepper, D., Ip, R. J., Ip, J. P., and Ip, J. H., (2000).The use of antiplatelet agents in cardiac disease. *Managed Care Interface*,13: 76-78.

40. Tondon, V., & Gupta, R. K. (2005). Effect of Vitex negundo on oxidative stress. Indian Journal of Pharmacology, 37, 37–45.

41. Velioglu YS, Mazza G, Gao L, Oomah BD (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products.J. Agric. Food Chem. 46: 4113-4117.

42. Vertuani S, Angusti A, Manfredini S (2004). "The antioxidants and pro-antioxidants network: an overview". *Curr Pharm Des* **10** (14): 1677–94.