# Invitro Antioxidant Assay For Solvent Crude Extract Of Caralluma Fimbriata

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**ABSTRACT**

The phytochemistry of the genus Caralluma is characterized by many pregnane glycosides. Other chemical constituent s include flavones, glycosides, megastigmane glycosides, saponins and several flavonoids. Natural antioxidants play a key role in health maintenance and prevention of the chronic and degenerative diseases, such as atherosclerosis, cardia c and cerebral ischemia, carcinogenesis.Antioxidants exert their activity by scavenging the 'free,oxygen radicals' there by giving rise to a fairly 'stable radical'. The free radicals are metastable chemical species, which tend to trap electron s from the molecules in the immediate surroundings. These radicals if not scavenged effectively in time, they may dam age crucial bio molecules like lipids, proteins including those present in all membranes, mitochondria and, the DNA re sulting in abnormalities leading to disease conditions The RSA activity of secondary metabolite was determined using DPPH assay.ABTS radical,scavenging activity of the extract was determined.The ability to reduce ferric ions was mea sured using the FRAPassay.TRAP(Total Radical- Trapping Antioxidant Parameter) Assay was also performed.

**KEYWORDS:** Caralluma fimbriata, Caralluma Antioxidant Assay, DPPH Assay, Edible Cactus, ABTS Assay, FRAP, TRAP

## INTRODUCTION

Natural antioxidants play a key role in health maintenance and prevention of the chronic and degenerative diseases, su ch as atherosclerosis, cardiac and cerebral ischemia, carcinogenesis, neurodegenerative disorders, diabetic pregnancy, r heumatic disorder, DNA damage and ageing (Uddin et al., 2008; Jayasri et al., 2009).

Besides natural antioxidants, some synthetic antioxidants are also reported such as, butylated hydroxytoulene (BHT) a nd butylated hydroxyanisole (BHA). But these synthetic antioxidants possess greater risks of side effects; therefore, in vestigations on identifying the natural antioxidants have become very important issue (Alma et al., 2003). In the past f ew years, natural antioxidants have generated considerable interest in preventive medicine (Govindarajan et al., 2003). Plants produce a huge amount of antioxidants and they can represent a potential source of new compounds having anti oxidant properties (Mosquera et al., 2007).

Antioxidant capacity assays are classified into two groups in terms of principal of chemical reactions. These are hydro gen atom transfer (HAT) reaction based assays and single electron transfer (ET) reaction based assays (Huang et al., 2 005).The ET.based assays measure the reduction capacity of an antioxidant and when oxidant is
reduced, color changes occur (Apak et al., 2007). The HAT..and ET based assays determine
the radical (or oxidant) scavenging capacity, rather than the suppressive antioxidant effect of a sample (Huang et al., 2 005).Some of the common in vitro antioxidant assays are the 1,1-diphenyl-2-
picrylhydrazyl (DPPH) radical scavenging assay, superoxide anion radical scavenging activity, reducing power, ferric t hiocyanate assay and total antioxidant activity (Goze et al., 2009; Saha et al., 2008; Prasad et al., 2009; Li et al., 2008) Generation of the ABTS [2, 29-azinobis-(3-ethylbenzothiazoline- 6-
sulfonic acid)] radical cation constitutes the basis of one of the spectrophotometric methods that have been carried out to the measurement of the total antioxidant

## IN VITRO ANTIOXIDANT ASSAYS

### Estimation of Radical Scavenging Activity (RSA) using DPPH Assay

The RSA activity of secondary metabolite was determined using DPPH assay according to
(Alagumani et al., 2010), with slight modification. The decrease of the absorption at 517 nm of the DPPH solution afte r addition of the antioxidant (secondary metabolite) was measured in
a cuvette containing 2960 µl of 0.1 mM ethanolic DPPH solution and was mixed with 40 µl of 20 to 200 µg/mL of cr ude extract. Blank containing 0.1 mM ethanolic DPPH solution without secondary metabolite was vortexed thoroughly ; the setup was left at dark at room temperature.The absorption was monitored after 20 min. Ascorbic acid (AA) and B utylated hydroxytoluene (BHT) were used as references. The ability to scavenge DPPH radical was calculated by the following equation.

% of DPPH radical scavenging activity = Abs control □ Abs sample Abs control

Abs control is the absorbance of DPPH radical + ethanol;

Abs sample is the absorbance of DPPH radical + crude extract.

**ABTS Assay**

ABTS radical,scavenging activity of the extract was determined according to (Re et al., 1999). The $ABTS^{\cdot+}$ cation radical was produced by the reaction between 5 ml of 14 mM ABTS solution and 5 ml of 4.9mM potassium persulfate ($K_2S_2O_8$) solution, stored in the dark at room temperature for 16 h. Before use, this solution was diluted with ethanol to get an absorbance of 0.700 ± 0.020 at 734 nm. The plant extract at various concentrations with 1ml of ABTS solution was homogenized and its absorbance was recorded at 734 nm. Ethanol blanks were run in each assay, and all measurements were done after at least 6 min. Similarly, the reaction mixture of standard group was obtained by mixing 950µl of $ABTS^{\cdot+}$ solution and 50µl of BHT. As for the antiradical activity, ABTS scavenging ability was expressed as $IC_{50}$(µg/ml). The inhibition percentage of ABTS radical was calculated using the following formula:

ABTS scavenging activity (%) = $(A_0 - A_1) / A_0 \times 100$

Where $A_0$ is the absorbance of the control and $A_1$is the absorbance of the sample.

**Ferric Reducing Antioxidant Power (FRAP) Assay**

The ability to reduce ferric ions was measured using the method described by (Benzie and Strain, 1996). The FRAP reagent was generated by mixing,300 mM sodium acetate buffer (pH 3.6), 10.0 mM (tripyridyl triazine) TPTZ solution and 20.0 mM $FeCl_3.6H_2O$ solution in a ratio of 10:1:1 in volume. Samples at different concentrations (100,200,300,400 and 500 µg/ml) was

then added to 3 ml of FRAP reagent and the reaction mixture was incubated at 37 °C for 30 min. The,increase in absorbance at 593 nm was measured. Fresh working solutions of $FeSO_4$ were used for calibration. The antioxidant capacity based on the ability to reduce ferric ions of sample was calculated from the linear calibration curve and expressed as mmol $FeSO_4$ equivalents per gram of sample (DW).

**TRAP (Total Radical-Trapping Antioxidant Parameter) Assay**

The TRAP was determined according to the method of (Ghiselli et al., 1995) based on the protection provided by antioxidants on the fluorescence decay of Rphycoerythrin (lagphase) during a controlled peroxidation reaction. Briefly, 120 µL of diluted sample were added to 2.4mL of phosphate buffer (pH 7.4), 375µL of bi distilled water, 30µL of diluted R,PE and 75µL of ABAP (2,2′azobis(2..amidinopropane) dihydrochloride); the reaction kinetics at 38°C were recorded for,45 min (or more, if necessary) by a LS..55 luminescence spectrometer

(Perkin Elmer, Wellesley, MA). TRAP values were calculated from the length of the lag,phase due to the sample compared with that of Trolox and expressed as □mol of Trolox per 100 g of dry weight of crude extract sample.

**EXPERIMENTAL RESULTS**

**1. ESTIMATION OF RADICAL SCAVENGING ACTIVITY (RSA) USING DPPH ASSAY**

The DPPH free radical scavenging activity (in %) of different concentrations of ethyl acetate extracts of both Carallum a fimbriata and ascorbic acid is shown in Figure 1. The degree of stable DPPH* decolorization to DPPHH (reduced form of DPPH) yellow indicated the scavenging efficiency of the extract. The radical scavenging ability of different concentrations, namely 0.02, 0.04, 0.06, 0.08, 0.1, 0.12, 0.14, 0.16, 0.18, 0.2 mg/ml, methanol extracts of Caralluma,fimbriata was found to be 64, 65, 68, 69, 73,75,76,77,81 and 85% respectively, followed by ethyl acetate and hexane(82% and 70%) Ascorbic acid exhibited marked antioxidant activity of 82% in 0.2 mg/ml as compared to ethyl acetate extracts. Both ethyl acetate extracts and the standard exhibited dose dependent activity.

**2. ABTS CATION FREE RADICALS SCAVENGING ACTIVITY**

The free radical scavenging capacities of both solvent crude extract were tested using ABTS radical cation decolorization assay (Re et al., 1999). Both the solvent extract showed significant free radical scavenging activity with greatest extent (Figure 2). Among the two solvent extract, methanol showed $ABTS^{*+}$ scavenging activity as 67.96%, whilst ethyl acetate showed 49.35%. The standard ascorbic acid showed 98.25% scavenging activity against $ABTS^{*+}$.

**3. FERRIC REDUCING–ANTIOXIDANT POWER ASSAY**

The total antioxidant potential of a sample was determined using the ferric reducing ability of FRAP assay by Benzie and Strain (1996) as a measure of antioxidant power. The assay was based on the reducing power of a compound (antioxidant). Both solvent crude extract of C. fimbriata such as ethyl acetate and methanol showed 176.21□4.17 and 240.81 □6.48 of □m trolox/100 g dry weight, respectively (Table 1).

**4. TRAP(TOTALRADICAL-TRAPPING ANTIOXIDANT PARAMETER) ASSAY**

The TRAP was determined according to the method of Ghiselli et al. (1995) based on the protection provided by antioxidants on the fluorescence decay of R-phycoerythrin (lag-

phase) during a controlled peroxidation reaction. Among the solvent, the methanol extract showed strong TRAP activity of 65.02□2.46, whereas ethyl acetate showed only 43.24□1.85 of □m trolox/100 g dry weight (Table 1).

**Table 1 FRAP and TRAP assay of C. fimbriata solvent crude extracts**

| Crude extract | Antioxidant potential* | |
|---|---|---|
| | FRAP# | TRAP# |
| Ethyl acetate | 176.21□4.170 | 43.24□1.85 |
| Methanol | 240.81□6.48 | 65.02□2.76 |

*Values are means of triplicate determinations ± standard deviation

# □m trolox equivalent (TE)/100 g dry material (dm)

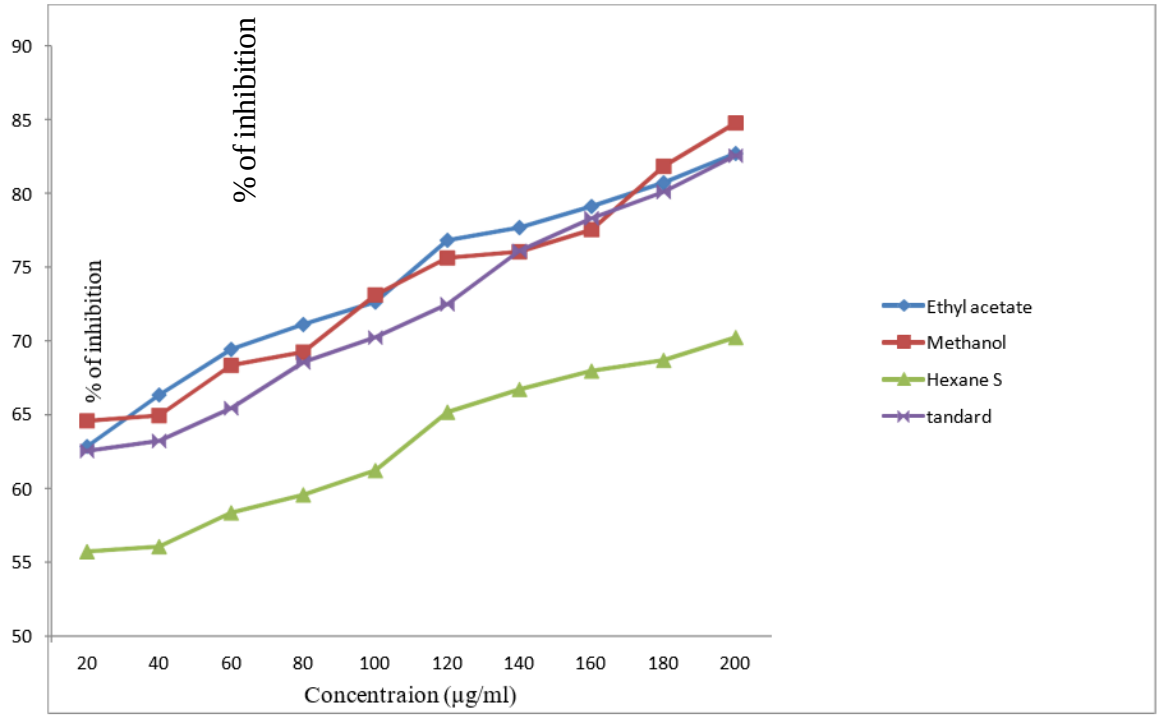


**Figure 1 Antioxidant activity of C. fimbriata solvent crude extract using DPPH method**

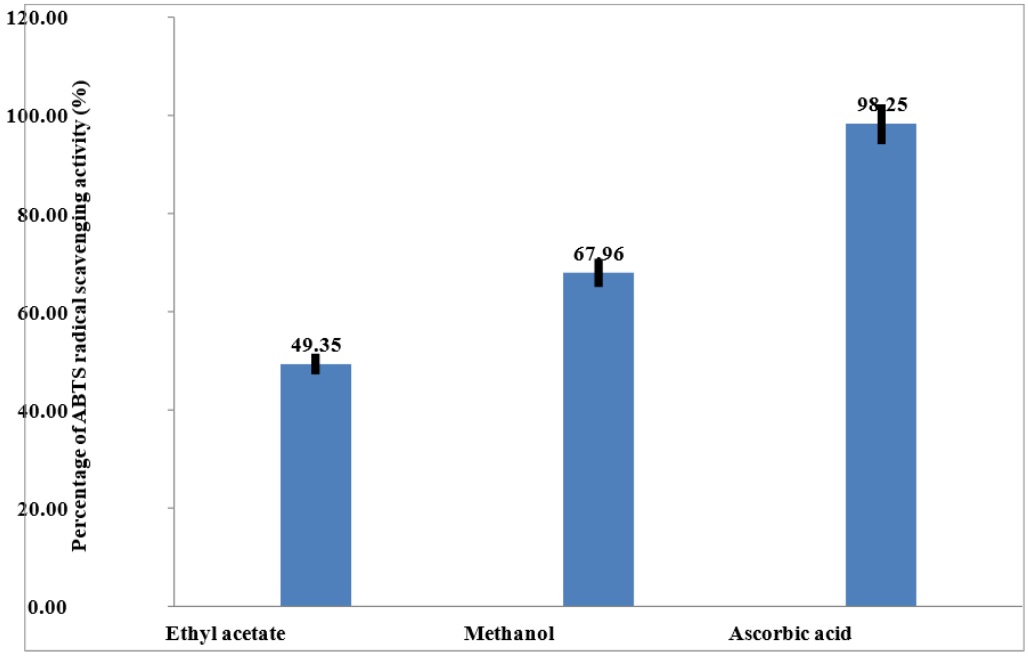


**Figure 2 ABTS free radical scavenging activity of C. fimbriata solvent crude extract**

## CONCLUSION

In this study, the scavenging activity of methanol extracts was found to be dose dependent that is higher the concentrat ion, more was the scavenging activity. Though the DPPH radical scavenging abilities of the extracts were less than tha t of ascorbic acid, the study showed that the extracts have the proton,donating ability and could serve as free radical in hibitors or scavengers, acting possibly as primary antioxidants. The radical scavenging ability of different concentratio ns, 0.02to0.2 mg/ml, methanol extracts of caralluma fimbriata was found to be 85%, followed by ethyl acetate and hex ane 82% and 70% Ascorbic acid exhibited marked antioxidant activity of 82% in 0.2 mg/ml as compared to ethyl aceta te extracts. Both ethyl acetate extracts and the standard exhibited dose dependent activity.

Aerial parts extract from Caralluma edulis were tested for its free radical scavenging activity (FRSA) in the DPPH (1, 1-diphenyl-2-
picrylhydrazil radical)screening assay. It showed a dose,dependent antioxidant activity comparable to the used control (Leopoldia comosa) which have been found to have remarkable antioxidant activity (Ansari et al., 2005).

Thus methanolic extract had better reduction capacity of DPPH radical than ethanolic, ethyl acetate and aqueous extrac t. In addition, antioxidant activity had a linear relationship with the total phenolic properties in some plants (Kalt et al., 1999 ). In the case of Gymnema sylvestre, decrease in the concentration of DPPH radicals due to the scavenging abilit y of gymnema methanolic extract. Maximum scavenging activity (57.10%) was observed at 100 µg/ml concentration a nd the $IC_{50,}$value of gymnema extract ( Rachh et al., 2009)were found to be 85.28 µg/ml.

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