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Free Radical Scavenging Potential of leaf extracts of *Capparis grandiflora* wall.ex Hook

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ABSTRACT

In-vitro antioxidant activity of various extracts of the leaves of Capparis grandiflora Wall.Ex Hook was determined by DPPH free radical scavenging assay. The Reducing power of extracts was also determined. Ascorbic acid was used as standard and positive control for both the analysis. The chloroform and ethanolic extracts of Capparis grandiflora showed very significant DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging activity compared to that of standard antioxidant. The DPPH radical scavenging activity of the extract was increased with the increasing concentration. In DPPH free radical scavenging assay, IC_{50} value of chloroform and ethanol leaf extracts was found to be 39.2 µg/mL and 30.7 µg/mL respectively. The results concluded that the extracts have a potential source of antioxidants of natural origin.

Key words: Antioxidant, *Capparis grandiflora*, DPPH, free radical, reducing power.

INTRODUCTION

Free radicals of different forms are constantly generated for specific metabolic requirement and quenched by an efficient antioxidant network in the body. When the generation of these species exceeds the levels of antioxidant mechanism, it leads to oxidative damage of tissues and biomolecules, eventually leading to disease conditions, especially degenerative diseases. [1] Reactive oxygen species (ROS), sometimes called active oxygen species, are various forms of activated oxygen, which include free radicals such as superoxide ions and hydroxyl radicals, as well as non free-radical species such as hydrogen peroxide. [2,3] Antioxidants act as a major defense against radical mediated toxicity by protecting the damages caused by free radicals. Antioxidant based drugs or formulations for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer, have appeared in the last three decades.[4] Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals, and also by acting as oxygen scavengers. [5,6] Antioxidant supplements or foods containing antioxidants have been reported to protect the human body by

reducing oxidative damage. [7] There are some synthetic antioxidant compounds, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), commonly used in processed foods. However, it has been suggested that these compounds have some side effects. [8,9] In addition, it has been suggested that there is an inverse relationship between dietary intake of antioxidant rich foods and the incidence of human disease.[10] Many plant extracts and phytochemicals have been shown to have antioxidant/free radical scavenging properties, [11] and it has been established as one of the mechanisms of their action. Some of the non-nutritive antioxidants of plants are phenolic compounds, flavonoids, coumarins, benzyl isothiocyanate etc.[12] This has attracted a great deal of research interest in natural antioxidants. In continuation of our research on natural antioxidants, we found that leaf extracts of *Capparis grandiflora* a climbing shrub with spreading branches found mainly in the adjacent regions of Coimbatore, Nilgiris and Tiruchirappalli [13,14] has been used by the traditional practitioners as stomachic, anti-rheumatic, anti-tumor, anthelmintic, antimicrobial and anti inflammatory activities. [15-19] All the mentioned activities could probably due to anti-oxidant potential of *Capparis grandiflora* and hence an attempt was made to evaluate the antioxidant activity of leaf extracts.

MATERIALS AND METHODS

Plant material and extraction

The fresh leaves of *Capparis grandiflora* wall. ex Hook.(Capparidaceae), collected at the flowering stage in the month of March 2010 from the tribal areas of Attapady, Palakkad district, Kerala state, South India were authenticated by the Botanical survey of India, Coimbatore, Tamilnadu (BSI). A voucher specimen (no.BSI/SRC/5/23/10-11/Tech-565) was deposited in the departmental herbarium. Leaves were dried in shade for 20 days and then powdered to get a coarse powder. This powder was stored in air tight container and used for extraction. The dried and powdered plant material were successively extracted with chloroform and ethanol using a soxhlet apparatus and extracted with water by cold maceration. The extraction was carried out for 24 h at room temperature with mild shaking. [15] The extracts were filtered and concentrated by rotary vacuum evoparator at 40°C, and the weight of each residue was recorded and percent yield was calculated.

DPPH Radical Scavenging Activity [20]

The free radical scavenging capacity of the different extracts of *Capparis grandiflora* was determined using DPPH. [22,23] DPPH scavenging activity was measured by spectrophotometric method at 517 nm. Chloroform, ethanolic and aqueous extracts of the leaves of *Capparis grandiflora* (0.05 ml each) and standard compound, ascorbic acid were added in different concentrations (50-1000 µg/ml) to the methanolic solution of DPPH (100 µM, 2.95 ml). After 30 min, absorbance was measured in triplicate. [26] Percentage scavenging of the DPPH free radical was measured using the following equation:

% DPPH radical-scavenging = [(Absorbance of control - Absorbance of test Sample) / (Absorbance of control)] x 100

Assay of Reducing Power [21,24,25]

Extracts (25-300 µg) in 1 mL of distilled water were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1%), and incubated at 50°C for 30 min. Then, 2.5 mL of trichloroacetic acid (10% v/v) was added to the mixture and then centrifuged at 3000 rpm for 10 min. Finally, 2.5 mL of upper layer solution was mixed with 2.5 mL of distilled water and 0.5 mL $FeCl_3$ (0.1%) and the absorbance was measured at 700 nm. Ascorbic acid was used as reference.

RESULTS AND DISCUSSION

DPPH radical scavenging activity of leaf extracts of *Capparis grandiflora* and ascorbic acid are presented in figure1. Reducing power of leaf extract of *Capparis grandiflora* and ascorbic acid are presented in figure 2. Both the methods have proven the effectiveness of various extracts compared to the standard antioxidant, ascorbic acid. The DPPH antioxidant assay is based on the ability of DPPH a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. The chloroform and ethanolic extracts of *Capparis grandiflora* exhibited a significant dose dependent inhibition of DPPH activity. The IC_{50} value of the chloroform and ethanolic extracts of the leaves of *Capparis grandiflora* were found to be 39.2 µg/mL and 30.7 µg/mL respectively. The reducing ability of a compound generally depends on the presence of reductants which have been exhibited antioxidative potential by breaking the free radical chain, donating a hydrogen atom. The presence of reductants (i.e. antioxidants) in *Capparis grandiflora* leaf extracts causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, the Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. The reducing power of *Capparis grandiflora* leaf extracts was very potent and the power of the extracts increased with quantity of sample.

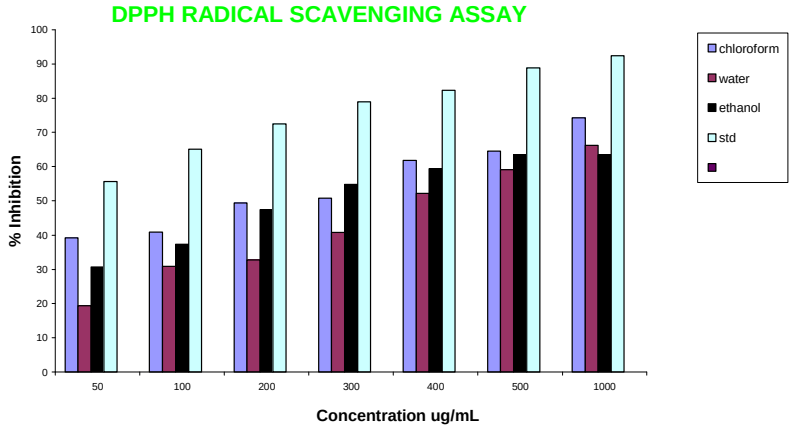

Figure 1. Bar diagram of DPPH radical scavenging assay of the leaf extracts of *C.grandiflora*

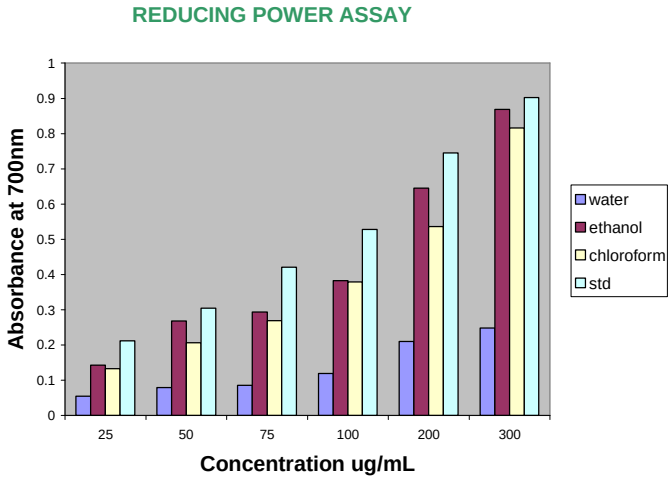

Figure 2. Bar diagram of reducing power assay of the leaf extracts of *C.grandiflora*

CONCLUSION

Chloroform and ethanolic leaf extracts of *Capparis grandiflora* showed significant antioxidant activity than the aqueous extract studied by both the methods. The activity may be due to the presence of tannins and flavonoids found in preliminary phytochemical analysis. Further studies are in progress for the isolation of active constituents responsible for antioxidant activity.

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