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Free radical scavenging activity of methanolic extract of *Ecbolium viride* (Forssk). Alston roots

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ABSTRACT

Anti oxidants are emerging as prophylactic and therapeutic agents for various diseases The present study deals with an antioxidant potential of methanolic extract of Ecbolium viride (Forssk).Alston roots. The antioxidant potential of methanolic extract of Ecbolium viride (Forssk).Alston roots was investigated by employing three different established in vitro methods, such as DPPH radical scavenging activity, Nitric oxide radical scavenging activity and reducing power assay. Total flavonoid content was also determined by colorimetric method. The extract was found to be rich in flavonoid content (78±4.8mg quercetin equivalent/g dry weight of extract) and the data obtained in these three methods (78.25%, 69.79% and 0.2756(absorbance) at 100µg concentration respectively) comparable to standard. Results obtained in the present study reveal methanolic extract of Ecbolium viride (Forssk).Alston posses significant antioxidant activity.

Key words: *Ecbolium viride* (Forssk).Alston, DPPH, Nitric oxide radical scavenging, Reducing power assay, Quercetin.

INTRODUCTION

Free radicals are generally unpaired electron containing species produced by chemical toxicants, the metabolism of xenobiotics and environmental pollutants. They cause deleterious effects to cells by oxidizing crucial and functional biomolecules, which leads to the cell damage and tissue injury. They involve in the many pathological conditions like cardiac, neuro, hepatodisorders,

cancer and diabetes [1]. Current research into free radicals has confirmed that food rich in antioxidants play an essential role in prevention of cardiovascular diseases and cancer [2]

Ecbolium viride (forssk).Alston commonly known as kappubobbili in Kannada belongs to the family Acanthaceae and the roots of the plant are reported to be used for jaundice, menorrhagia and rheumatism [3, 4]. The roots are reported to contain glycoflavones such as Orientin, Vitexin, Isoorienten, and Isovitexin [5].Based on the uses and phytoconstituents, the plant was selected for this study to prove its antioxidant potential.

MATERIALS AND METHODS

1. Plant material

The plant material was collected from vicinity of Tirumala hills, Chittor district of Andhra Pradesh, identified and authenticated by Dr. Madhava chetty, Asst.Professor, Botany Dept, Sri Venkateswara University, Tirupati (voucher specimen Ev -1768)

2. Preparation of plant extracts

The roots were collected, washed and dried at room temperature. After complete drying, it was powdered in a multi mill grinder and passed through a 60 mesh sieve. Dried powdered drug was subjected to successive solvent extraction (petroleum ether, benzene, chloroform, methanol and water) and subjected to phytochemical screening for the detection of various constituents

3. Determination of total flavonoid content [6]

The total flavonoid content was determined spectrophotometrically at 430nm by creating a complex with $AlCl_3$.0.5 ml solution of methanolic extract mixed with 1.5 ml of methanol, 0.1 ml 10% aluminum chloride, 0.1ml of 1M potassium acetate, 2.8 ml of distilled water and left at room temperature for 30 min.The absorbance of the reaction mixture was measured at 430 nm. The content was calculated as quercetin equivalents/g dry extract

4. *In vitro* Antioxidant evaluation

a. DPPH radical scavenging activity [7]

The stable 1, 1- diphenyl-2-picryl hydrazyl radical (DPPH) was used for the determination of free radical scavenging activity of the extract. 1 mM solution of DPPH in methanol was prepared and 1ml of this solution was added to 3 ml of various concentrations of methanolic extract of *Ecbolium viride* (5, 10, 25, 50 and 100 µg) and the reference compound. After 30 min, absorbance was measured at 517 nm. Ascorbic acid (100µg) was used as the reference material. All the tests were performed in triplicate and the percentage of inhibition was calculated

b.Nitric oxide radical scavenging activity [8]

This method is based on the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric ions that can be estimated using Griess reagent. The reaction mixture (3 ml) containing sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) and methanolic extract of *Ecbolium viride* in different concentrations (5, 10, 25, 50 and 100 µg) and the reference compound were incubated at 25°C for 150 min. Each 30 min, 0.5 ml of the incubated sample was removed and 0.5 ml of the Griess reagent (1% sulphanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2%H_3PO_4) was added. The absorbance of the

chromophore formed was measured at 546nm. All the tests were performed in triplicate and the results averaged. Ascorbic acid used as the reference compound. The percentage decrease in absorbance was calculated.

Reducing power method [9]

Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action. Different concentration of methanolic extract of *Ecbolium viride* (5, 10, 25, 50 and 100 µg) extract in 1ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%).The mixture was incubated at 50 ^{o}C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Ascorbic acid was used as the reference material. All the tests were performed in triplicate and the results averaged. Increased absorbance of the reaction mixture indicates increase in reducing power.

Table1: Antioxidant Potential of methanolic extract of *Ecbolium viride* roots

Concentration (µg)	DPPH (%)	Nitric oxide (%)	Reducing power (absorbance)
20	22.01±0.003	20.21±0.004	0.1723 ± 0.031
40	34.36±0.006*	27.40±0.006*	0.1992 ± 0.021
60	45.12±0.01*	43.49±0.007*	0.2181 ± 0.019*
80	59.34±0.009*	58.90±0.003*	0.2341 ± 0.001*
100	78.25±0.004*	69.79±0.009*	0.2756 ± 0.042*
Std(100 µg)	94.11±0.003*	84.66±0.009*	0.3056±0.031*

*Values are the mean ± S.E.M., n=3, Significance *P<0.001 compared to control*

RESULTS AND DISCUSSION

Phytochemical screening of different extracts reveal methanolic extract was good source of flavonoids and other phenolic compounds. The flavonoid content of methanolic extract was found to be 78±4.8mg quercetin equivalent/g dry weight of extract. The flavonoids have been shown to posses significant anti oxidant activity [10]. Hence the methanolic extract was selected for the present study and it posses significant antioxidant activity in all the three methods (Table1) and comparable with standard at concentration of 100 µg.In DPPH and Nitric oxide radical scavenging method, it was found that the radical scavenging activity of methanolic extract increases with increase in concentration in dose dependant manner and exhibited significant activity at 100 µg. In reducing power assay method, the presence of anti oxidant in the sample would result in reducing Fe^{3+} to Fe^{2+} by donating an electron and amount of Fe^{2+} complex can be monitored by measuring the formation of Perl's Prussian blue at 700nm. Increase in the absorbance at 700nm indicates an increase in reductive ability and table 1 shows the dose dependant response of reducing power where the activity of methanolic extract of *Ecbolium viride* roots increased steadily with increase in concentrations. The flavonoid compounds in methanolic extract may contribute to the antioxidant activity although other antioxidants may probably be present in the extract.

CONCLUSION

The results obtained in the present study indicated that the methanolic extract of *Ecbolium viride* (Forssk).Alston roots posses significant antioxidant activity.

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