STUDIES ON *IN VITRO* ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF *ECBOLIUM LINNEANUM* KURZ. (ACANTHACEAE).

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Summary

Oxidative free radicals are formed constantly in the human system and have been implicated in several human diseases. These free radicals are scavenged, i.e. removed by enzymatic and non enzymatic antioxidants defense systems. When these defense mechanisms are inadequate oxidative stress can damage proteins, carbohydrates, lipids and nucleic acids. Various plant components like carotenoids, xanthophylls, phenolic acids, polyphenols, diketones etc. are recognized as plant antioxidants[1]. Current research is now directed towards finding naturally occurring antioxidants of plant origin from *Ecbolium linneanum* (Acanthaceae) in various *in vitro* models. The study aims in assessment of the *in vitro* antioxidants potentials of the *Ecbolium linneanum* ethanolic extract (ELEE) using DPPH radical scavenging activity determination, determination of nitric oxide radical scavenging activity and reducing power determination.

Key words: *Ecbolium linneanum*, antioxidant activity, DPPH, nitric oxide

Introduction

Free radicals are formed in tissue cells by many endogenous and exogenous causes such as metabolism, chemicals and ionizing radiations. Increased generation of free radicals and other reactive oxygen species or impaired antioxidants defense mechanism has been implicated in variety of pathological conditions. The term “oxidative stress” refers to a shift in the ratio of prooxidant / antioxidant balance. The imbalance can be due to excessive ROS production and / or to limited antioxidant defenses. Increased generation of free radicals and other reactive oxygen species are impaired antioxidant defense mechanisms have been implicated in a variety of pathological conditions including cancer, atherosclerosis, cataracts, inflammation & autoimmune disease, lung disease, neurological disorders, aging and cell death. Free radicals are formed constantly in the human system and have been implicated in several human diseases.

These free radicals are scavenged, i.e., removed, by enzymatic and no-enzymatic antioxidant defense systems. When these defense mechanisms are inadequate, oxidative stress can damage proteins, carbohydrates, lipids and nucleic acids [1, 2] Various parts of *Ecbolium linneanum* have been used in folk medicine .The roots of the plant are reported to be used for jaundice, menorrhagia and rheumatism. Decoction of the leaves is given for stricture and all parts of the plant for gout and disuria.[3,4] With reference to the folklore use of the plant in medicine, current research is now directed towards finding naturally occurring antioxidants of plant origin from *Ecbolium linneanum* (Acanthaceae) in various *in vitro* models.

Materials and methods

Chemicals and Instruments

L-ascorbic acid, 2, 2-diphenyl 1-picrylhydrazyl(DPPH), sodium nitroprusside, trichloroacetic acid (TCA), potassium ferricyanide, thiourea. All other chemicals and reagents used were of analytical grade. UV spectra were recorded in Shimatzu 2401 UV visible spectrophotometer.

Plant Material and Extract

The aerial parts (fresh leaves, flowers) of the plant *Ecbolium linneanum* were collected from Nagpur district; Maharashtra (India) in the month of August-September (flowering season) and the plant was identified and authenticated from the Botany Department R.T.M Nagpur University, Nagpur. The voucher specimen of the plant (no.9087) has been kept in the Department of Botany for further references.

The freshly collected plant parts were shade dried and subjected to size reduction. The powder was initially defatted with petroleum ether (60-80°C). The marc was further macerated with ethanol for 72h. The extract was preserved at room temperature for further studies.

Determination of free radical scavenging activity

The free radical scavenging activity of the extracts was evaluated based on the ability to scavenge the synthetic DPPH [5, 6, 7, 8]. This assay provided useful information on the reactivity of the compounds with stable free radicals, because of the odd number of electrons. DPPH shows a strong absorption band at 517 nm in visible spectrum (deep violet color). The bleaching of DPPH absorption is representative of the capacity of the test drugs to scavenge free radicals independently. 3ml ethanolic solution of each extract (20-100 g/ml) was mixed with 1.0ml 0.1M solution of DPPH (Sigma Aldrich) in ethanol. Similar additions were made for the ascorbic acid standard solution (20-100 g/ml).The mixture was kept for 30mins. Absorbance was read at 517nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in µg/ml) of extracts that inhibits the formation of DPPH radicals by 50%.

Drug % Scavenging (Mean ± SEM) of triplicates

Conc.	20 µg	40 µg	60 µg	80 µg	100µg
ELEE	51.1133 ± 0.1203**	52.6366 ±0.1697**	53.4566±0.1299**	54.6633±0.3548**	55.45±0.1997**
VIT C	20.403 ± 0.3180*	37.503 ± 0.3812*	50.1933± 0.2924*	64.35 ±0.077*	74.63± 0.2676*

Table 1: *In vitro* Free Radical Scavenging Effect of Extracts by DPPH Method

Determination of Nitric Oxide Radical Scavenging Activity

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide (Green et al, 1982; Marcoci *et al*) which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent [5, 6, 8]. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide (Marcocci *et al.*) Sodium nitroprusside (5mM) in phosphate-buffered saline (PBS) was mixed with 3.0ml of different concentrations (20-100 µg/ml) of the drugs dissolved in the suitable solvent systems and incubated at 25°C for 150 min. The samples from the above were reacted with Greiss reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% napthylethylene diamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine was read at 546 nm and referred to the absorbance of standard solutions.

Drug % Scavenging (Mean ± SEM) of triplicates

Conc.	20 µg	40 µg	60 µg	80 µg	100µg
ELEE	44.37333±0.6214	45.51333±0.8858	46.48 ±1.086	50.21 ± 1.226	51.2966 ± 0.608
VIT C	40.09 ± 0.3378	44.43 ±0.04041	49.7233 ± 0.1533	53.1 ± 0.0577	65.31 ±0.0346

Table 2: *In vitro* Nitric oxide scavenging effect

Determination of Reducing Power

The reducing power of the extracts was determined according to the method of Oyaizu (1986)[5, 8]. Various concentrations of the extracts (20-100 µg/ml) in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml, 0.2M, pH 6.6) and 1% potassium

ferricyanide (2.5ml). The mixture was incubated at 50 C for 20min. Aliquots of trichloroacetic acid (2.5 ml,10%) were added to the mixture, which was then centrifuged at 1036 x g for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared FeCl3solution (0.5 ml, 0.1%). The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Reducing power is given in ascorbic acid equivalent (ASE ml-1) that shows the amount of ascorbic acid expressed in mM those reducing power is the same than that of 1 ml sample.

Extract	**Concentration**	**Absorbance**
VIT C	20 µ g	0.398
	40µ g	0.511
	60µ g	0.678
	80µ g	0.747
	100µ g	0.857
ELEE (ELEE)	20 µ g	0.74
	40µ g	0.786
	60µ g	0.818
	80µ g	0.826
	100µ g	0.866

Table 3: Determination of Reducing Power

Statistical analysis

Tests were carried out in triplicate for separate experiments. The amount of extract needed to inhibit free radicals concentration by 50%, IC_{50}, was graphically determined by a linear regression method using MS-Windows based graph pad Instat (version 3) software. Results were expressed as graphically / mean± standard deviation.

Result and Discussion

Determination of IC_{50} values and Antioxidant activity:

The reduction capability of DPPH radical was determined by decrease in its absorbance at 517nm, which is induced by antioxidants.Table1: illustrates significant ($p<0.01$) decrease in the concentration of DPPH radical due to the scavenging ability of ELEE compared with the standard vitamin C which showed higher activity at all concentrations. IC_{50} value of ELEE, & vitamin C was found to be 13.94 µg/ml and 60.77 µg/ml respectively. Table: 2 illustrate the percentage inhibition of Nitric oxide generation by ELEE. The concentration which showed 50% inhibition for ELEE and vitamin C was found to be 86.17 µg /ml and 58.19 µg /ml respectively. The reducing capacity serves as significant indicator of its potential

antioxidant activity. The reducing power increased with increasing concentration of sample. All the amounts showed significant activities when compared to control. The standard for comparison was vitamin C.

Conclusion

Ethanotlic extract of *Ecbolium linneanum* showed significant antioxidant activity by inhibiting DPPH radicals, Nitric oxide scavenging and reducing power activities when compared with vitamin C as standard. Thus, the results of the study show that the aeriel parts of *Ecbolium linneanum* can serve as a good source of natural antioxidants along with its other traditionally accepted therapeutic values. Further work should be done on isolation and identification of the antioxidant components of ELEE.

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