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EVALUATION OF *IN VITRO* ANTIOXIDANT ACTIVITY OF FLOWERS OF *BLEPHARIS MOLLUGINIFOLIA*

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ABSTRACT: The objective of this study was to characterize the non-enzymatic and enzymatic antioxidants and Scavenging activities of different extracts (Petroleum ether, Benzene, Chloroform, Acetone, Ethanol, Methanol and Water) of dried flowers powder of *Blepharis molluginifolia* (Acanthaceae). Enzymatic antioxidant activity such as Superoxide Dismutase, Glutathione –S-transferase, peroxidase and Catalase of *B. molluginifolia* flowers were high in methanol extract. Non enzymatic antioxidants like Tocopherol, Ascorbic acid, Phenols, Carotenoids and Lycopene were analysed. Acetone extract showed highest Total Phenol content and Vitamin-C were 100.66±1.5 mg GAE/gm & 52.08±2.42 mg/gm respectively. Scavenging activities like DPPH, ABTS and H_2O_2 were high in Methanol extract. In this study, the non-enzymatic antioxidant activity was found to be significant, which prove to be a better scavenger of free radical in comparison to enzymatic extracts in the shade dried extracts.

INTRODUCTION: Reactive oxygen species(ROS)such as Superoxide (O_2^-), Hydrogenradicals (OH^-), and Hydrogen peroxide ($H_2O_2^-$) are considered as important factors causing many diseases like cardiovascular, diabetes, inflammation, cancer and neurogenerative diseases [1, 2]. ROS are degraded to non-reactive forms by Enzymatic and Non-Enzymatic defence mechanisms. Free radicals react with known biological molecule and damage protein, breakdown of DNA strands and initiates peroxidation of various molecules.

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Antioxidants act as a major defence against radical-mediated toxicity, by protecting the damages caused by free radicals [3].

Antioxidative components of natural origin have attracted special interest because they can protect human body from free radicals.Enzymatic antioxidants include primary enzymes like Superoxide Dismutase, Catalase, Glutathione peroxidase and secondary enzymes include Glutathione reductase etc [4].

Non-Enzymatic antioxidants include either water-soluble (vitamin C and phenolic compounds) or lipid-soluble (vitamin E and carotenoids) compounds which act as defence against oxidative stress [5, 6]. However antioxidant compounds like Phenols, Flavonoids scavenge these free radicals and protect the system from the Oxidative mechanisms.

Blepharis molluginifolia belongs to the family Acanthaceae, is a threatened medicinal herb. This plant is used for Urinary discharges and also equated with Uttangana. Traditionally the plant is used for bone fractures, skin diseases and allergies.[7]Abundant occurrence of Phenols in seed samples has been reported. Steroids and Cardiac glycosides were found in seed samples of *Blepharis genus*.[8]Theaim of the present studywas to determine non-enzymatic and enzymatic antioxidants and scavenging activitiesof different flower extracts of *Blepharis molluginifolia*.

MATERIALS AND METHODS:

Chemicals and reagents: Folin-Ciocalteau Reagent, Nitroblue Tetrazolium (NBT), DL-Alpha-Tocopherols, Hydrogen Peroxide, Xylene,2,2`-Dipyridyl, Ferric Chloride, Pyrogallol, 2,2-Diphenyl-1-Picrylhydrazyl, Gallic acid, Riboflavin,Chloro-2,4-dinitrobenzene (CDNB), Standard ascorbate, Reduced glutathione were purchased from Merck.

Plant materials: The flowers of *Blepharis Molluginifolia* were collected from its natural habitat at Koyathanda in Nallamala forest region, Andhra Pradesh, in the month of December and January. The plants were authenticated by the Dr. N. Balahussaini, Agricultural College, Kadapa.

Preparation of extracts: The flowers of *Blepharis molluginifolia* (100g) were dried under shade, and undergone crushing in electric blender to form powder. After that this powder was used for extraction by using various polar and nonpolar solvents like Petroleum ether, Benzene, Chloroform, Acetone, Water, Ethanol and methanol by using Soxhlet extractor. These are dried and preserved for the further tests.

Enzymatic antioxidants

1) **Assay of Superoxide Dismutase (SOD)** [9, 10]: The activity of superoxide dismutase was assayed spectrophotometrically by the method of Misra and Fridovich (1972) in the flower extracts of the plant.

2) **Assay of Catalase [10, 11] (CAT):** Catalase activity was determined by adopting the method of Luck (1974).

3) **Assay of Peroxidase [10, 12] (POX):** The activity of peroxidase was determined by the method of Reddy *et al* (1995).

4) **Assay of Glutathione s-transferase [10, 13] (GST):** The activity of glutathione S-transferase activity was performed by the method of Habig *et al* (1974).

NON-ENZYMIC ANTIOXIDANTS:

1) **Estimation of Ascorbic Acid [14]**: The amount of ascorbic acid present in the flower extracts, of this plant was estimated by the method of Roe and Keuther (1943).

2) **Estimation of Tocopherol [15]:** The level of tocopherol was estimated Spectrophoto-metrically by the method reported by Rosenberg (1992).

3) **Estimation of total Carotenoids and Lycopene [16]:** The estimation of total carotenoids and lycopene was done by the method described by Zakaria *et al* (1979).

4) **Determination of total Phenols [17]:** Total phenols was determined by the method (Folin-Ciocalteu) proposed by Mallick and Singh (1980).

EVALUATION OF RADICAL SCAVENGING ASSAYS:

1) **DPPH Radical Scavenging Assay [18]:** The ability of the plant extracts to scavenge the stable free radical DPPH was assayed by the method of Mensor *et al* (2001).

2) **ABTS Radical Scavenging Assay [19]:** The ability of all the plants flower extracts to scavenge the free radical ABTS (2, 2-azino-bis 3-ethyl benz thiazoline-6-sulfonic acid) was studied using the method adopted by Shirwaikar *et al* (2006).

3) **Hydrogen Peroxide Radical Scavenging Effect** [20]: The scavenging activity of hydrogen peroxide by the plant extracts was determined by the method of Ruch *et al* (1989).

RESULTS & DISCUSSION:

Assay of Enzymatic Antioxidants: The levels of enzymatic antioxidants assessed in *B. molluginifolia* flowers in different extracts (petroleum ether, benzene, chloroform, acetone, ethanol, methanol and water) were collectively represented in **Fig. 1**. The activity of GST, CAT, SOD and POX was high in methanol extract. Reports showed Methanolic extracts of *Nerium indicum* flowers have more Cellular antioxidant enzymes [21].

Least activity was shown by chloroform extract for GST and CAT. Acetone extract showed least activity for SOD and Benzene extract for POX. In our experiment, there is a correlation between catalase activities and scavenging of hydrogen radical and superoxide dismutase and glutathione-s-transferase, peroxidase activity, which are indicators OH radical scavengers. This indicates this medicinal plant is highly potential antioxidant.

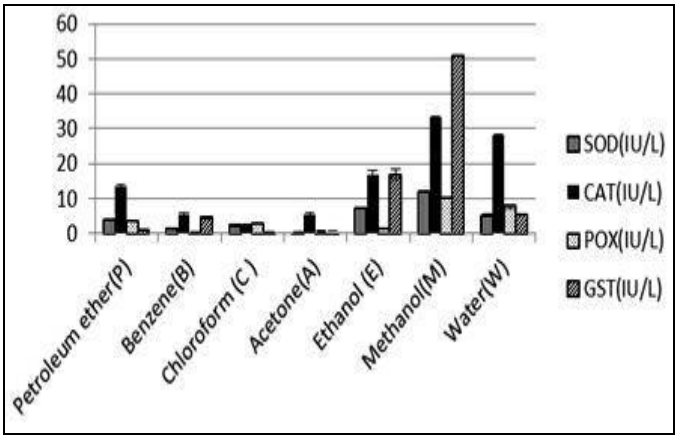

FIG. 1: ENZYMATIC ANTIOXIDANT ACTIVITY OF *BLEPHARIS MOLLUGINIFOLIA* FLOWERS. Values are mean ± SD of three parallel measurements

Assay of non-enzymatic antioxidants: In the non-enzymatic activity, the total phenol content, vitamin-C, vitamin-E and carotenoids and lycopene were analysed in different extracts, collectively represented in **Table 1**.

Total phenol compounds, as determined by Folin Ciocalteu method, are reported as Gallic acid equivalents by reference to standard curve ($y = 19.473x-17.582$, $r^2 = 0.9979$). Acetone extract showed highest activity for total phenol content with; chloroform extract showed least activity for total phenol content with. Similarly Acetone extract showed highest activity in *Bombax malabaricum* flowers [22].

Vitamin-C content was expressed as ascorbic acid equivalents per gm. Highest Ascorbic acid were present in Acetone extract and least is in chloroform extract with. Similarly Acetone extract showed highest activity in *Bauhinia tomentosa* flowers [23]. In plant cells, the most important reducing substrate for H_2O_2 removal is ascorbic acid [24].

Carotenoids have capacity for quenching singlet oxygen and free radicals [25]. Highest activity was in Benzene and least value in water extract. Same activity was seen in *Tagetes erecta* dried flowers [26]. Carotenoids are therefore efficient free-radical scavengers.

Lycopene is a bright red carotene and carotenoid pigment and phytochemical found in tomatoes and other red fruits and vegetable. Lycopene's eleven conjugated double bonds give it its deep red colour and are responsible for its antioxidant activity. Lycopene may also interact with reactive oxygen species, such as hydrogen peroxide and nitrogen dioxide [27]. Lycopenes and vitamin- E) showed highest activity with methanol extract.

α–tocopherol interact with the polyunsaturated acyl groups of lipids, stabilize membranes, scavenge and various reactive oxygen species (ROS) and lipid soluble by products of oxidative stress [28].

The strong relationship between the total phenol content and antioxidant activity in sweet basil was also reported [29]. The high amount of phenols in extracts may explain their high antioxidative activities.

TABLE 1: NON-ENZYMATIC ANTIOXIDANT ACTIVITY OF *BLEPHARIS MOLLUGINIFOLIA* FLOWERS

Extract	Phenol (mg/g)	Vitamin-C (mg/g)	Vitamin-E (mg/g)	Carotenoids (mg/g)	Lycopene (mg/g)
Petroleum ether	24.09±0.57	6.16±0.74	0.01±0.00004	26.7±3.06	2.62±0.09
Benzene	5.61±+0.57	16.14±1.26	0.00±0.0003	67.4±2.95	1.72±0.04
Chloroform	4.62±0.57	3.31±0.16	0.01±0.0001	24.7±4.62	1.23±0.03
Acetone	100.66±1.51	52.08+±2.42	0.02±0.0001	16.7±1.43	2.31±0.02
Ethanol	10.89±0.99	7.02±0.43	0.02±0.0001	36.7±1.15	1.49±0.01
Methanol	98.02±0.99	51.58±2.98	0.04±0.0007	60.00±2.00	5.69±0.01
Water	11.22±1.51	15.97±1.34	0.01±0.0002	3.3±1.15	1.34±0.03

Values are mean ± SD of three parallel measurements

Radical Scavenging activities:

1. **DPPH radical-scavenging activity:** DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts[30]. From **Fig. 2**, highest % of inhibition was shown by Methanol extract and least by ethanol extract. Usually, higher total phenol and flavonoids contents lead to better DPPH-scavenging activity.

2. **Hydrogen Peroxide Radical Scavenging activity:** H_2O_2 also forms OH.in the presence of metal ions and oxygen facilitates this reaction. Hence, metal chelating and H_2O_2 scavenging processes are important for living organisms [31]. The scavenging ability of different extracts on hydrogen peroxide was shown in **Fig. 2**. The radical scavenging capacity may be attributed to phenolic compounds in methanol extract with the ability to accept electrons, which can combine with free radical moiety to decrease hydroxyl radical compared with DPPH assay.

3. **ABTS Radical Scavenging activity:** ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants and of chain-breaking antioxidants [32]. Different artificial free radical species, such as $ABTS^{+}$ radical cation, has been used to assess radical scavenging ability and antioxidant activity. From Fig. 2, highest % of inhibition was shown by methanol extract and least by Benzene.

All tested extracts for DPPH, H_2O_2 and ABTS scavenging activities of this plant can inhibit the presence and production of free radicals but lesser when compared to reference standards Ascorbic acid and Butylated Hydroxy Toulene (BHT).

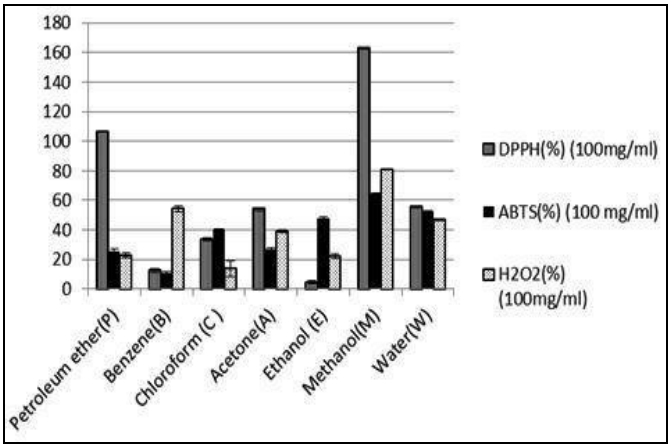

FIG. 2: RADICAL SCAVENGING ACTIVITIES OF *BLEPHARIS MOLLUGINIFOLIA* FLOWERS. Values are mean ± SD of three parallel measurements

Assay of standards:

Antioxidant assays	Ascorbic acid (100µg/ml)	BHT(100µg/ml)
DPPH	59.33	88.67
ABTS	68.5	68.8
H2O2	50.8	60.7

CONCLUSION: It could be concluded from the results of the present investigation that some oxidation parameters of assessing *in vitro* antioxidant effectiveness might be rapid and convenient depending on storage conditions, nature of oil and extent of oxidation. A number of different methods may be necessary to adequately assess the *in vitro* antioxidant activity of a specific plant material. By combining the knowledge of different antioxidant assays and assessment of oxidation parameters in the present study, it can be asserted that the investigated plant materials are a viable source of natural antioxidants and might have potential as "nutraceuticals" for the preparation of functional foods.

As India is rich in medicinally important flora, the meaningful exploitation of more indigenous plant materials and agricultural wastes is thus further recommended. An assessment of the toxicity and kinetic studies, as well as the function of these extracts in food and biological systems also needs to be investigated.

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