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IN VITRO ANTIOXIDANT STUDIES OF ETHANOLIC EXTRACT OF LEAVES OF BLUMEA MOLLIS

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ABSTRACT

The antioxidant activity of defatted ethanolic extract of the leaves of *Blumea mollis* were studied for its free radical scavenging property on different *in vitro* antioxidant models like DPPH radical, hydroxyl radical, nitric oxide radical scavenging activity, reducing power, superoxide anion radical scavenging activity were analyzed. The extract showed free radical scavenging property in dose-dependent manner.

KEYWORDS: ***Blumea mollis*, Free radical, Anti oxidant and hydroxyl radical**

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INTRODUCTION

Blumea mollis (D.Don) Merr (F: Asteraceae) is an aromatic annual herb commonly found in the plains of India, outer Himalaya, Sri Lanka and Myanmar. The leaf of the plant is traditionally used for skin diseases and the boiled herb is used to treat diarrhea [1]. Tribal people of Chittoor district, A.P. use *Blumea mollis* (*B. mollis)* to treat such ailments where free radicals involved. Eg: Hepatotoxicity, asthma, dropsy. There were no reports on systematic study on the antioxidant activity of this plant. Therefore, the present study was carried out to screen the antioxidant activity of alcohol extract of the leaf of *B. mollis*.

MATERIALS AND METHODS

(i) chemicals

Thiobarbituric acid (TBA), trichloroacetic acid (TCA), sodium nitroprusside, N-napthylethylenediamine dihydrochloride all chemicals used are of high purity and were obtained from S.D. fine chemicals Ltd., India.1-1-diphenyl-2-picrylhydrazyl (DPPH), Griess reagent, NBT (Nitroblue tetrazolium) was obtained from Sigma Chemicals. Hexane, Chloroform, Petroleum ether, Diethyl ether was obtained from Merck, India. All other chemicals were of laboratory grade.

(ii) Plant material

Blumea mollis leaves were collected from Tirumala hills, Chittor Dt., A.P. and authenticated by botanist Dr. K. Madhava chetti, Asst. Professor, Dept. of Botany, S.V. University, Tirupathi and voucher specimen was deposited in S.V. University Botany Department, Tirupathi.

(iii)Preparation of Ethanolic Extract

The leaves of the plant were allowed to dry under shade. The dried leaves were powdered in a Wiley mill. 500gm of dry powder was extracted with petroleum ether (3 L, 60-80^{o}c) and refluxed for 3 hrs, then filtered and subjected to distillation under reduced pressure. The procedure was repeated for three times. Marc was extracted with ethanolic (3 L) and it is refluxed for 3 hrs. The extract was filtered procedure was repeated for three times and concentrated in *vacuum* to get the semi solid (Yield: 5%) which was used for anti oxidant studies.

(iv) IN VITRO ANTIOXIDANT ACTIVITY

The antioxidant activity of the ethanolic extract of leaves of *B. mollis* was studied by using different *in vitro* antioxidant models like DPPH radical and nitric oxide radical scavenging activity, superoxide anion radical, reducing power and hydroxyl radical scavenging activity.

The antioxidant profile of Ethanolic extract of leaves of *B. mollis* has been evaluated at concentrations of 50, 125, 250, 500, 1000 µg/ ml. The percentage inhibition activity was recorded in a graded response.

Percentage inhibition = $A_C - A_T / A_C \times 100$

Where A_C and A_T are the absorbance values of the control and of the Test sample, respectively. A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and expressed as IC_{50} value.

(a) DPPH radical scavenging activity

DPPH scavenging activity was measured by the spectrophotometric method [2]. To an ethanolic solution of DPPH (200µM), 0.05ml of test compound dissolved in ethanolic was added at different concentrations (50-1000µg/ml). An equal amount of ethanolic was added to the control. After 20min the decrease in absorbance of test mixtures was read at 517nm and the percentage inhibition was calculated [3].

(b) Hydroxyl radical scavenging activity

The assay is based on quantification of the degradation product of 2-deoxyribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+}-ascorbate-EDTA-H_2O_2 system (the Fenton reaction) [4]. The reaction mixture contained, in a final volume

of 1 ml 2-deoxy-2-ribose (2.8 mM); KH_2PO_4-KOH buffer (20 mM, pH 7.4); $FeCl_3$ (100 µM); EDTA (100 µM); H_2O_2 (1.0 mM); Ascorbic acid (100 µM) and various concentrations (50-1000 µg/ml) of the test sample or reference compound. After incubation for 1 h at 37°C, 0.5 ml of the reaction mixture was added to 1 ml 2.8% TCA, then 1 ml 1% aqueous TBA was added and the mixture was incubated at 90°C for 15 min to develop the pink chromogen. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution. All tests were performed three times. Percentage inhibition was evaluated by comparing the test and blank solutions.

(c) Scavenging of nitric oxide radical

Nitric oxide was generated from sodium nitroprusside and measured by Griess' reaction [5-6]. Sodium nitroprusside (5mM) in standard phosphate buffer solution was incubated with different concentrations (63-1000µg/ml) of the ethanolic extract dissolved in phosphate buffer (0.025M, pH: 7.4) and the tubes were incubated at 25^0C for 5hrs. Control experiments without the test compounds but with equivalent amounts of buffer were conducted in an identical manner. After 5hrs, 0.5ml of incubation solution was removed and diluted with 0.5ml of Griess' reagent (1% napthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed was read at 546nm. The experiment was repeated in triplicate [7].

(d) Reducing power

The reducing power of Ethanolic extract of *B. mollis* was determined according to the standard method[8]. Different Concentrations of *B. mollis* extract was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and add potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50^0C 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. All the tests were performed in triplicate. Vitamin C was used as positive control.

(e) Superoxidase dismutase radical scavenging activity

The Scavenging activity towards the superoxide radical ($O_2^{\cdot-}$) was measured in terms of inhibition of generation of $O_2^{\cdot-}$ by following alkaline DMSO method [9-10]. Potasssium superoxide and dry DMSO were allowed to stand in contact for 24hr and the solution was filtered immediately before use. Filtrate (200 µl) was added to 2.8ml of an aqueous solution containing NBT (56 µM), EDTA (10 µM) and potassium phosphate buffer (10 mM). Test compound (1ml) at various concentration (50-1000 µg/ml) were added, the absorbance was recorded at 560 nm against a control.

STATISTICAL ANALYSIS

All measurements were repeated three times. The Values are expressed as mean±standard mean standard deviation. Statistical analysis was performed by the student t-test. Further, the data was analyzed by using linear regression analysis to calculate the IC_{50} values. $P < 0.01$ was regarded as significant.

RESULTS

Table-1
Anti-oxidant activity of ethanolicic extract of leaves of Blumea mollis

Concentration. Quantity(µg/ml)	DPPH method	Hydroxyl method	Nitric oxide method	Reducing Power method	Superoxide Dismutase method
50	16.95 ± 0.71	14.43 ± 0.22	12.53 ± 0.48	7.83 ± 1.26	19.50 ± 0.23
125	26.78 ± 0.24	18.65 ± 0.07	21.76 ± 0.59	12.74 ± 0.61	25.63 ± 0.34
250	40.86 ± 0.52	36.82 ± 0.73	39.84 ± 1.10	20.40 ± 0.42	44.21 ± 0.86
500	50.75 ± 0.83	60.67 ± 0.48	48.52 ± 0.07	44.61 ± 0.85	53.78 ± 0.69
1000	86.92 ± 0.67	77.48 ± 0.65	83.68 ± 0.28	68.83 ± 0.60	89.32 ± 0.77
Ascorbic acid 1000(µg/ml)	78.36 ± 0.96	68.31 ± 0.49	76.80 ± 0.83	62.70 ± 0.33	76.60 ± 0.42
IC_{50} (µg/ml)	500	525	520	670	480

Values are reported as Mean±S. D. (n=3), activity mentioned in terms of % inhibition. P<0.01

1. Inhibition of DPPH Radical

The extract was showed significant free radical scavenging activity and activity was dose dependent. The highest activity was observed at 1000µg/ml i.e., 86.92 % (Extract) and 78.36 % (Ascorbic acid). The IC_{50} value (the inhibitory concentration at which there is 50% reduction of free radical activity) of *B.mollis* was found to be 500µg/ ml.

2. Hydroxyl radical scavenging

The effect of *B. mollis* on hydroxyl radical and iron (II)-dependent deoxyribose damage was protected significantly at all concentrations; the percentage of inhibition of hydroxyl radical being 77.48 % (1000 µg/ml) and ascorbic acid 68.31 % (1000 µg/ml).

3. Nitric oxide scavenging activity

The scavenging of nitric oxide by *B.mollis* was concentration dependent. There was a potent inhibition of nitric oxide formation, with the maximum inhibition being at 1000µg/ml i.e., 83.68 % and ascorbic acid is 76.8 %. The IC_{50} value of the extract in this assay was found to be 520 µg/ml.

4. Reducing power activity

The percentage of inhibition is reducing Power 68.83 % and 62.7 % at 1000 µg/ml, *B.mollis* and ascorbic acid respectively. The IC_{50} value of the extract in this assay was found to be 670 µg/ml.

5. Superoxide radical scavenging activity

The IC_{50} values of the plant extract was found to be 480µg/ ml. The maximum percentage inhibition of the plant extract was

89.32% (1000 µg/ml) whereas ascorbic acid was exhibited 76.6% (1000 µg/ml). These data support the theory of free radicals' scavenging having responsibility for the antioxidant action of the extract.

DISCUSSIONS

In living systems, free radicals are constantly generated and they can cause extensive damage to tissues and biomolecules leading to various disease conditions, especially degenerative diseases and extensive lysis [11]. Many synthetic drugs protect against oxidative damage but they have adverse side effects. An alternative solution to the problem is to consume natural antioxidants from food supplements and traditional medicines [12-13]. Recent reports suggest that, many a plants are rich in antioxidants [14-16] which may be used to treat various diseases in where free radicals were involves. *B.mollis* is one such plant which has claims to treat various free radicals involving ailments such as hepatotoxicity, dropsy, throat infection, and asthma etc [1, 17]. On through literature survey it reveals that, there were no reports on *In vitro* antioxidant activity of *B.mollis*. Hence the present study is planned for *In vitro* antioxidant activity of leaves of *B. mollis*.

The ethanolicic extract of leaves of *B. mollis* was evaluated by using various *in-vitro* antioxidant models such as DPPH radical, hydroxyl radical, nitric oxide scavenging, reducing power, superoxide anion scavenging activities. These results were compared with the standard i.e., Ascorbic acid.

The DPPH radical scavenging ability of the extract was significantly ($P<0.01$) more than those of ascorbic acid. The study showed that the extract have the proton-donating ability and could serve as free radical inhibitors or scavengers.

The effect of *B. mollis* and Ascorbic acid on the inhibition of free radical-mediated deoxyribose damage was assessed by means of iron (II)-dependent DNA damage assay. When extract and ascorbic acid tested at same concentration level i.e., 1000 µg/ ml, extract showed more hydroxyl radical scavenging effect when compared with ascorbic acid.

The extract inhibited nitrite formation by directly competing with oxygen in the reaction with nitric oxide. The ethanolic extract of leaves of *B.mollis* significantly inhibited the production of nitric oxide.
Further it is evidenced that the extract showed more potent nitric oxide scavenging activity than Standard i.e., Ascorbic acid.

Reducing power is to measure the reductive ability of antioxidant, and it is evaluated by the transformation of Fe (III) to Fe (II), by donating an electron in the presence of the sample extracts [18]. The reducing power is increased with an increasing the concentration of extract. In present study the extract reduced the generation of free radicals, probably due to a combination of its abilities of free radicals scavenging, binding to Fe. The ethanolic extract of the *B.mollis* had shown good reducing power that was comparable with Vitamin C.

The superoxide radicals generated from dissolved oxygen by PMS-NADH coupling can be measured by their ability to reduce NBT. The decrease in absorbance at 560 nm with the plant extract and the reference compound, Ascorbic acid indicates their abilities to quench superoxide radicals in the reaction mixture.

The Capability of the *B.mollis* extract to interact with $O_2^{\cdot}$ radicals was measured as a function of its inhibitory effect on the NBT reduction caused by these radicals. It is evident that the *B.mollis* extract's capacity to react directly with $O_2^{\cdot}$ radicals depends on its concentration. In conclusion, the results of the present study suggest that tested plant material have potent antioxidant scavenging activity.

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