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ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACTS OF AERIAL PART AND LEAF CALLUS OF MOLLUGO PENTAPHYLLA L. - A POTENT NUTRACEUTICAL HERB

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ABSTRACT

Antioxidants are the phytochemicals that have the ability to trap free radicals. They could be either synthetic or natural. These compounds scavenge the free radicals such as per-oxide, hydroper-oxide or lipid per-oxyl and they inhibit the oxidative mechanisms. Free radicals generated in aerobic metabolism are involved in a series of regulatory processes such as cell proliferation, apoptosis, and gene expression. Antioxidant activity of ethanolic extracts of aerial part and leaf callus has been evaluated by using DPPH assay. Leaf callus (IC_{50} 77.01%) showed significant antioxidant activity compare to areiral part (IC_{50} 53.02%) of the plant *Mollugo pentaphylla* L. This may be due to higher concentration of secondary metabolites present the in leaf callus, this is confirmed with DPPH IC_{50} of vitamin C as standard (85.03%).

Keywords: Aerial part, Antioxidant activity, DPPH, leaf callus and *Mollugo pentaphylla* L.

INTRODUCTION

Medicinal plants used in traditional medicine are well known for significant sources of natural antioxidants. Which are very efficient to block the process of oxidation by neutralizing free radicals. Plants and plant products are part of the vegetarian diet and a number of them exhibit medicinal properties and it is also commonly accepted that medicines taken from plant products are safer than the synthetic medicines. However,

the toxicity profile of most medicinal plants have not been comprehensively assessed **[1, 2]**. Free radicals are produced by exogenous and endogenous factors in the human body **[5].** The most common reactive oxygen species (ROS) includes superoxide anion (O^-_2), hydrogen per-oxide (H_2O_2), per-oxyl radicals (ROO) and nitric oxide (NO) **[1, 10 and 13].** ROS plays an important role in cell metabolism including energy production, phagocytosis and intercellular signaling. These ROS produced by sunlight, ultraviolet light, ionizing radiation, chemical reactions and metabolic processes have a role in a wide variety of disorders such as DNA damage, carcinogenesis, cardiovascular diseases, aging and neurodegenerative diseases, atherosclerosis and rheumatoid arthritis **[11, 16]**. Antioxidant scavenge the free radicals, such as per-oxide, hydroper-oxide and they inhibit the oxidative mechanism that leads to degenerative disorders **[6, 8]**. Natural antioxidants can protect the human body from free radicals and prevent the progress of many chronic diseases and also considered as molecules that can effectively fight the damage caused by oxidative stress in human cells with little or without side effects compared to synthetic antioxidants. Since *Mollugo pentaphylla* L. is used as both nutritional and medicinal plant, for the treatment of eye diseases, to treat sprue and mouth infections, the leaves are used to make a soup that is said to promote the appetite, used as a stomachic and in earaches, skin disorders and abdominal pains. The plant is an antipyretic and hypoglycemic potential **[17]**, antiseptic, appetizer, antidiabetic **[9]**, anticancer, antitoxic, antioxidant potential **[3]** and diuretic agent **[19]**. The present study is undertaken to evaluate antioxidant activity of aerial part and leaf callus.

The DPPH **(G. P, Vandnere; *et al* 2011)** assay was performed for *Mollugo pentaphylla* L., the results were expressed as IC50, which represents the sample concentration in µg/mL required to reduce 50% of the DPPH free radicals added to the reaction medium. All the measurements were performed in triplicate. DPPH is a free radical compound which has the scavenging ability for antioxidants sample and shows good absorbance at 517 nm. Vitamin C **[18]** is used as a standard antioxidant and it has strong DPPH scavenging property.

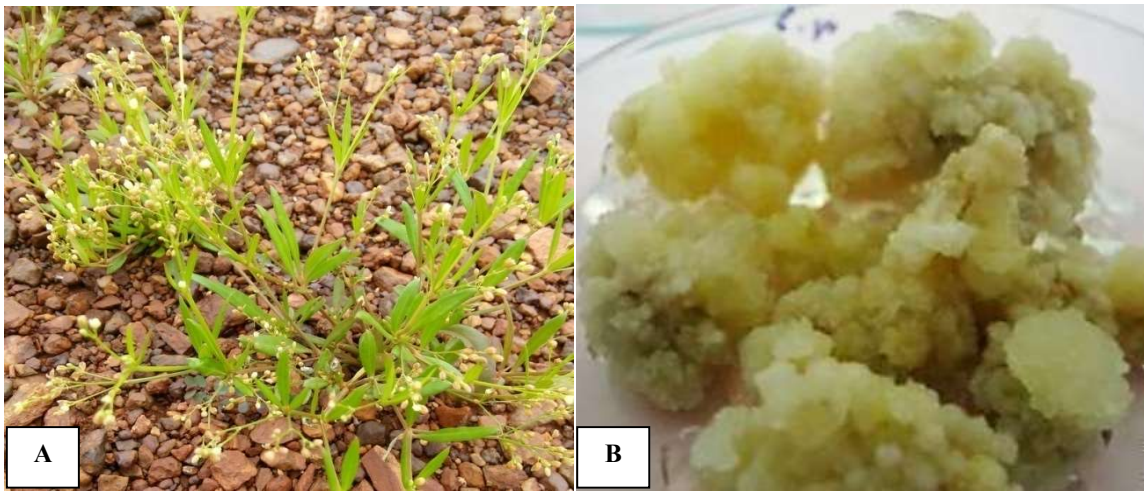


Figure 1: A. Habit- *Mollugo pentaphylla* L. and 1 B. Leaf callus induction from leaf explant on MS+BAP+NAA (0.5 mg/L)

MATERIALS AND METHOD

Mollugo pentaphylla L., belonging to family Molluginaceae **[4]** is an annual, glabrous pubescent, diffuse weed found throughout India. It is found in wet rocks, sandy and roadside footpaths **(Figure 1)**. It is an erect slender annual herb up to 10-20 cm. high and arid areas. Leaves are whorled or opposite, linear-lanceolate to obovat.e. This plant characterized with pentangular stem and five leaves with different size at the node. Flowers are white in terminal compound cymes. Stamens three, style three, capsules sub-globose with numerous seeds and are roundish rainy form, compreesed covered with raised tubercular point, dark brown **(Figure: 1 A)**.

Collection of Plant material

The plant of *Mollugo pentaphylla* L., was collected from Karnatak University campus, Dharwad, state of Karnataka, India during the month of June and authenticated by one of the authors in the Department of Botany, Karnatak University Dharwad.

Processing of aerial part

The whole plant was washed thoroughly in tap water to remove all debris in the laboratory and the aerial part was spread out and dried in the shade (room temperature) for 4 to 5 weeks. The dried plant aerial parts were ground into a fine powder using the mechanical machine. Powder thus obtained was stored in an airtight container with label and subjected for extraction using ethanol with the help of soxhlet apparatus for 24 hrs. The extracts were then dried at room temperature and used to determine antioxidant activity by DPPH assay **(Figure 1A)**.

Induction and processing of Leaf callus

Healthy leaves were selected and washed thoroughly under running tap water for 15 mins to wash off the dirt and microbes present on the surface and they were washed with two drops of Tween 20 detergent solution for 10 mins. Subsequently, they were thoroughly washed under running tap water until the traces of Tween 20 is removed and then rinsed with distilled water. The remaining steps of surface sterilization were carried out under aseptic conditions in the Laminar airflow chamber. The leaf explant was then subjected to 70% ethanol treatment for 30 sec and again washed with distilled water at least three minutes. After washing with distilled water, surface sterilization was done with mercuric chloride (0.1%w/v $HgCl_2$) solution for 2 min and rinsed four to five times with sterilized distilled water to remove traces of mercuric chloride. Thus sterilized leaf explants were inoculated on to Murashige and Skoog (MS) medium for induction of callus. The medium was also supplemented with various plant growth regulators, which include auxins NAA (naphthalene acetic acid) and cytokinin BAP (6 benzylaninopurine) in different concentrations and combination (0.1-0.5 mg/L). The pH of the media was adjusted to 5.8 before autoclaving. All media were autoclaved at 121ºC for 15 mins. The cultures were incubated in a growth chamber lab temperature of 25±2 ºC with relative humidity 55±5 and 16-h photoperiod. Callus induction from leaf explant on MS medium with plant growth regulators observed regularly till required quantity of callus is available or grown, **(Figure 1 B and Table 1)**.

The leaf callus thus obtained was carefully taken out from the culture tubes and washed with slightly warm sterile distilled water to remove the agar traces and air-dried in the oven in the laboratory at lab temperature for 4 to 5 weeks. The dried callus were ground into a fine powder using the mechanical machine and the powder thus obtained was stored in an airtight container and labelled. The dried leaf callus was subjected extraction using ethanol in a soxhlet apparatus for 24 hrs. The extracts were then dried at room temperature and used to determine antioxidant activity by DPPH.

Chemical used

All the chemicals and plant growth regulators used are of high analytical grade. 2,2-Diphenyle-1-picryl hydrazyl (DPPH) from Sigma Aldrich Ltd. Mumbai. All solutions were prepared freshly from doubled distilled water. The stock solution of the test sample

was prepared in ethanol. Ascorbic acid is used as a standard.

Evaluation of Antioxidant activity (DPPH)

The antioxidant activity of ethanolic extracts of both aerial and leaf callus against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was used to determine by UV spectrophotometry at 517 nm. Different concentrations of the both extracts were prepared by using ethanol (100, 200, 300, 400 and 500 mL). Vitamin C was used as an antioxidant standard (Control). 1.0 mL DPPH working solution(0.2 mM) was mixed with 0.5 mL of different concentrations (100, 200, 300, 400 and 500 µg/mL) directly from the test samples and the standard (100, 200, 300, 400 and 500 µg/mL) solution and incubated for 30 minutes in dark at room temperature. The absorbance was measured at 517 nm (Labman UV Visible Spectrophotometer). The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The radical scavenging activity was calculated using the following formula:

% Antioxidant activity = [(Ac − As)/Ac] × 100
where, Ac and As is the absorbance of control and sample, respectively.

Extract concentrations providing 50% inhibition (IC_{50}) was calculated from the plot of inhibition for percentage of inhibition against extract concentration.

Statistical analysis

The experimental data were analyzed statistically using IBM SPSS Statistics v 20 software. The experimental results were expressed as a mean±standard deviation of the mean of three replicates **(Table 2)**.

RESULTS

The antioxidant activity of ethanolic extract of aerial part and leaf callus was evaluated by using DPPH assay with a standard vitamin C. Antioxidant activity of leaf callus (IC_{50} 77.01%) was higher than the aerial part (IC_{50} 53.02 %) of *Mollugo pentaphylla* L. **(Table 2)**. The standard vitamin C antioxidant activity was 85.03 %. This may be due to accumulation of higher concentrations of secondary metabolites in the leaf callus. This is confirmed with DPPH IC_{50} value of vitamin C as standard 85.03 %, **(Figure 2)**. Higher the IC_{50} values reflects higher DPPH radical scavenging activity.

Table 1: Induction of leaf callus of *Mollugo pentaphylla* L

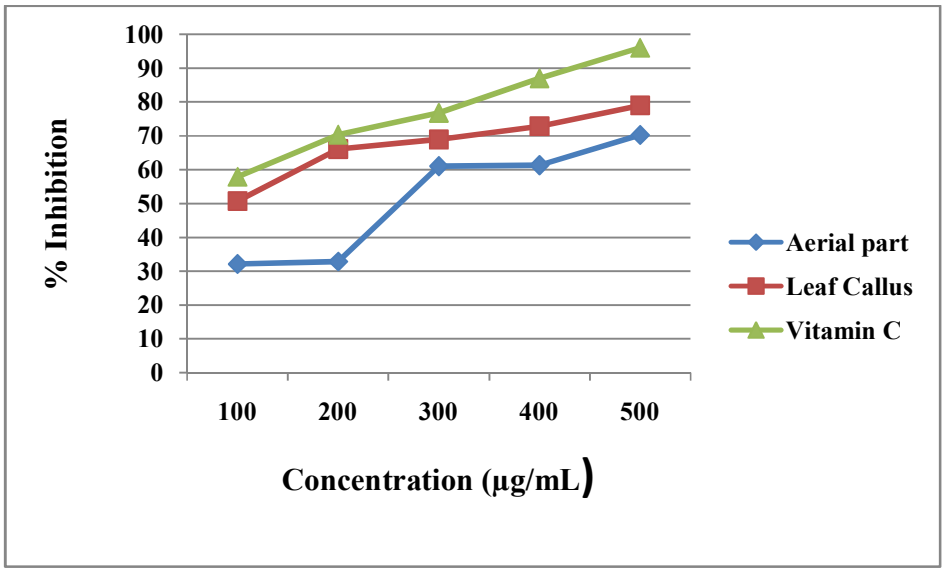
Sl. No.	MS medium supplemented with combination and concentrations of hormones (mg/L)		Weight of leaf callus(mg)
	BAP	NAA	
1.	0.2	0.2	49.02 ±1.67[a]
2.	0.3	0.3	62.16 ±2.03[a]
3.	0.4	0.4	78.19 ±4.20[b]
4.	0.5	0.5	83.05 ±5.49[c]
5.	1.0	1.0	51.38 ±4.22[b]

Each value represents the mean ± Standard error of three replicates, followed by superscript letters through columns that differ significantly at P<0.005 level when subjected DMRT followed by SPSS. BAP, benzylaminopurine; NAA, naphthaleneacetic acid

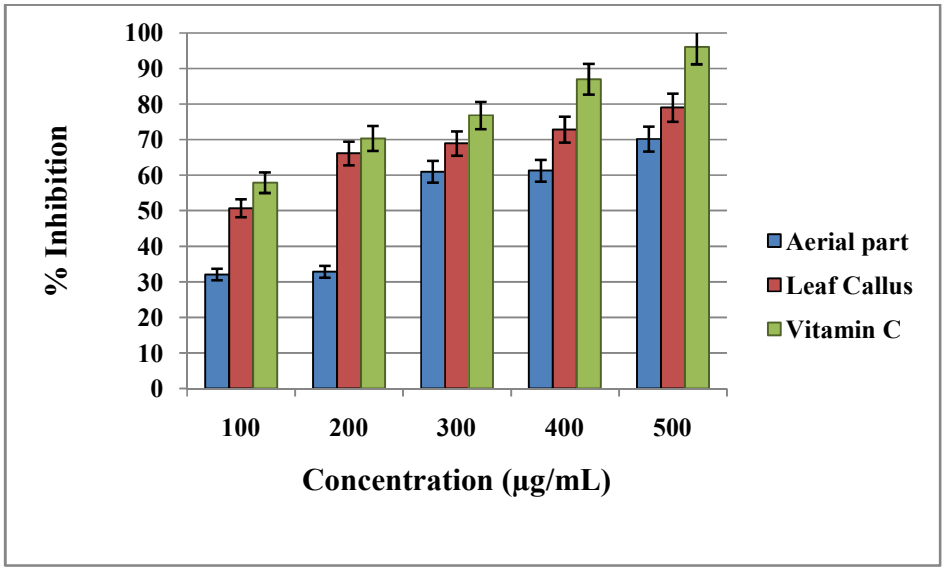
Table 2: Antioxidant activity of ethanolic extract of aerial part and leaf callus of *Mollugo pentaphylla* L. at different concentrations (µg/mL) compared with vitamin C

Concentration µg/mL	% Antioxidant activity of ethanolic extracts		Concentration µg/mL	% Antioxidant avtivity of std vitamin C
	Aerial part	Leaf callus		
100	40.82	50.72	100	68.89
200	45.11	66.12	200	78.00
300	54.00	78.00	300	84.56
400	61.28	91.00	400	95.72
500	65.24	99.23	500	99.76
IC_{50} (µg/mL/ Avg.)	53.02 %	77.01 %	IC_{50} (µg/mL/Avg.)	85.03 %

Values are mean±SD of triplicates



(A)



(B)

Figure 2: A, B; Antioxidant activity of Standard Vitamin-C (Control), aerial part and leaf callus of *Mollugo pentaphylla* L.

DISCUSSION

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol. This assay used in the present study on both aerial part and leaf callus of *Mollugo pentaphylla* L. to evaluate the differences of antioxidant activity in aerial part and leaf callus. Leaf callus has showed more significant activity compare to the aerial part. Similarly, methanolic extract of whole plant of *Mollugo pentaphylla* L., *Mollugo oppositifolia* **[11]** and *Mollugo nudicaulis* **[12, 14]** reported to have antioxidant avtivity. Further, methanolic leaf extracts of *Lantana camara* **[10]** and *Acalypha fruticosa* **[13]** are reported to have antioxidant activity. Furthermore, in case of *Phyllanthus amaraus* **[18]**, it is reported that antioxidant activity was greatest in *in vitro* plant extracts compare to *in vivo* plant extracts. It is also recorded that methanolic extract of whole plant *Torilis leptophylla* **[16]** and *Meconopsis quintuplinervia* **[7]** with potential antioxidant activity.

CONCLUSION

Based on finding it could be concluded that both aerial part and leaf callus of *Mollugo pentaphylla* L. can be used in nutraceuticals and pharmaceutical industry as they have significant antioxidant activity.

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