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Comparative Antioxidant Activity of Ethanolic extracts of Whole Plant and Leaf Callus of *Mollugo oppositifolia* L. A Potent Traditional Medicinal Herb

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Abstract : Natural products from dietary components such as Indian species and medicinal plants are known to possess antioxidant activity. Antioxidants are inhibitors of oxidation are compounds which prevent the oxidation and in general prolong the life of the oxidizable matter. Majority of the diseases/disorders are mainly linked to oxidative stress due to free radicals. The free radicals (oxidants) are species with very short half-life, high reactivity and damaging activity towards macromolecules like proteins, DNA and lipids. In general, the reactive oxygen species circulating and react with the electron of other molecules in the body and these also affect various enzyme systems and cause damage which may further contribute to conditions such as cancer, ischemia, ageing, adult respiratory distress syndromes, rheumatoid arthritis etc. Dietary plants contain variable amounts of antioxidants. It has been proved that plant antioxidants may contribute to the beneficial health effects of dietary plants. The present study was to evaluate antioxidant activity of ethanolic extract of whole plant and leaf callus of *Mollugo oppositifolia* L. is an important traditional medicinal herb belonging to the family Molluginaceae using 2,2-diphenyl-1-Picryl-hydrazyl (DPPH) radical scavenging assays. The results obtained showed that the ethanolic extracts of whole plant and leaf callus showed significant DPPH activity with IC_{50} value of 52.82± 0.0017 µg/mL and 58.66±0.004 µg/mL respectively, while IC_{50} of vitamin C as standard was 84.84±11.54µg/mL. Present study revealed that an antioxidant activity was higher leaf callus extract compare to whole plant extract of *Mollugo oppositifolia* L..
Keywords : Antioxidant activity, Free radicals, leaf callus, 2,2-diphenyl-1-Picrylhydrazyl (DPPH) , *Mollugo oppositifolia* L., and whole plant.

1. Introduction

Traditional medicine from plant extracts has proved to be clinically effective and relatively less toxic than the existing synthetic drugs. Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Many solvents have been used to extract active materials from plants such as alcohols (ethanol or methanol), diethyl ether, chloroform, ethyl

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acetate, *n*-butanol, and water. Phytochemicals are bioactive compounds of plant origin. They are naturally present in all parts of the plant body: bark, leaves, stems, roots, flowers,fruits, seeds, and so on. They have been recognized as the basis for traditional herbal medicine practiced in the past and now[1]. Oxidative stress is an important risk factor in the pathogenesis of numerous chronic diseases. Free radicals and other reactive oxygen molecules are recognized as agents involved in the pathogenesis of sicknesses such as asthma, inflammatory arthropathies, diabetes, parkinson's, Alzheimers diseases and cancers as well[2]. Reactive oxygen molecules are also said to be responsible for the human ageing [2, 3]. An antioxidant can be broadly defined as any substance that delay or inhibits oxidative damage to a target molecule. The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavanoids scavenge free radicals such as per-oxide, hydroper-oxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to are degenerative diseases. Herbal plants are considered as good anti oxidant since ancient times.
The plant used in treating stomache, earache, aperients, skin diseases. Leaves are bitter in taste and antiseptic. It has been reported that species of *Mollugo* used to treat gastric ulcer[5] and posses antimicrobial, antibacterial[6], anticancerous and antitumor[7,12], anti-inflammatory[8], hepatoprotective[9], Hypoglycemic[10] and antidiabetic activities[11]. Hence, the study was undertaken to evaluate the comparative antioxidant activities of ethanolic extracts of whole plant and leaf callus of *Mollugo oppositifolia* L. by using DPPH scavenging assay[15].

A

B

Figure1. A. Habit- *Mollugo oppositifolia* L. B. Leaf callus induced from leaf explant on MS+BAP+NAA (0.8mg/L).

2. Materials and Method

2.1 Collection of Plant material and extraction

The plant of *Mollugo oppositifolia* L., was collected from Karnatak University campus, Dharwad, Karnataka state, India during the month of June and authenticated by one of the authors in the Department of Botany, Karnatak University Dharwad (Figure: 1 A).

Mollugo oppositifolia L., is an diffuse, prostrate or ascending, stems numerous, dichotomously branched, with long internodes, slender, glabrous or pubescent near ends. Leaves in whorls of 4 to 5, unequal, oblanceolate or linear-lanceolate or sometimes spathulate, rounded or acute and apiculate at the apex, much tapered into petiole which is therefore obscure, flowers white, in axillary fascicles of two or more. Capsules ellipsoid, a little shorter than a sepals, 3-celled. Seeds very numerous, subreniform, with raised tubercular points, dark brown, appendages with a very small white scale at the hilum extended into a bristle which curves round the seed.

2.2 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 using doubled distilled water. The stock solutions of the test samples were prepared using ethanol. Ascorbic acid is used as a standard.

2.3 Processing of whole plant material

The whole plant was washed thoroughly in tap water to remove all debris in the laboratory and the whole plant was spread out and dried in the shade (room temperature) for 4 to 5 weeks. The dried plant samples were ground into a fine powder using the grinder (electronic machine) and the powder thus obtained was stored in an airtight container with label. Dried plant material was extracted with the solvent of ethanol using soxhlet apparatus for 24 hrs. The extracts were then dried at room temperature and used to determine antioxidant activity by DPPH.

2.4 Induction and processing of Leaf callus

Healthy leaves were selected and washed thoroughly under running tap water for 15 mins to wash off the debris and microbes present on the surface and they were washed with two drops of Tween 20 detergent solution for 10 mins. After, that they were thoroughly washed under running tap water until the traces of Tween 20 were removed. Then it was rinsed with distilled water. The remaining steps of surface sterilization were carried out under aseptic conditions in the Laminar airflow chamber. The plant was then subjected to 70% ethanol treatment for 30 sec and again washed with distilled water at least three to four times. 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. Murashige and Skoog (MS) medium used 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 (0.2-0.8 mg/L). The pH of the media was adjusted to 5.8 and autoclaved at 121ºC for 15 mins. The cultures were incubated in a growth room at a temperature of 25±2 ºC, relative humidity 55±5%, and 16-h photoperiod. Callus induction from leaf explant on MS medium with plant growth regulator observed after one week of incubation. Three replicates of cultures were established (Figure: 1 B andTable-1).

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

2.5 Evaluation of Antioxidant activity (DPPH)

The antioxidant activity of ethanolic extracts against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined by UV spectrophotometry at 517 nm. Different concentrations of both samples were prepared using ethanol (100, 200, 300, 400 and 500 µg/L). Vitamin C was used as standard (Control) antioxidant. 1.0 mL DPPH working solution(0.2 mM) was mixed with 0.5 mL of different concentrations (100, 200, 300, 400 and 500µg/L) directly from the test samples and the standard (5, 10, 20, 40, 80 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 = $[(Ac - As)/Ac] \times 100$; where, Ac and As are the absorbance of control and sample, respectively.

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

2.6 Statistical analysis

The experimental data were analyzed statistically using IBM SPSS Statistics v 20 software (Table-1).

3. Results

Ethanolic extract of whole plant and leaf callus showed significant DPPH activity with IC_{50} value of 52.82± 0.0017 µg/mL and 58.66±0.004µg/Ml respectively, while IC_{50} of vitamin C as standard was 84.84±11.54µg/mL. Antioxidant activity was higher leaf callus (IC_{50} 58.66 µg/mL) compare to whole plant (IC_{50} 52.82 µg/mL) of *Mollugo oppositifolia* L. The results were expressed as IC_{50}, 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 (Table-2 and Figure: 2).

Table-1: Induction of leaf callus of *Mollugo oppositifolia* L.

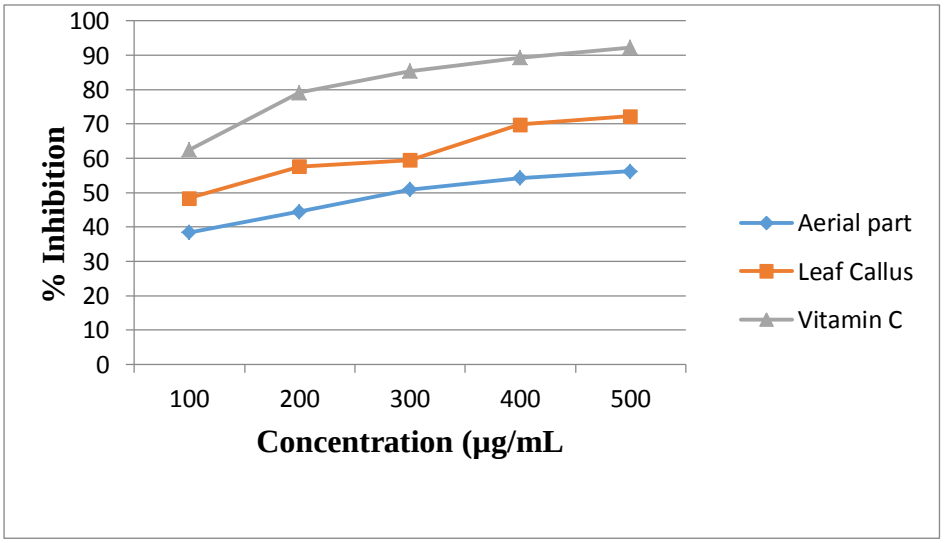
Sl. No.	MS medium supplemented with combination and concentrations of hormones (mg/L)		Weight of leaf callus(mg)
	BAP	NAA	
1.	0.2	0.2	44.26 ±0.007[a]
2.	0.3	0.3	60.01 ±1.62[a]
3.	0.4	0.4	69.08 ±1.31[c]
4.	0.5	0.5	74.12 ±0.12[b]
5.	**0.8**	**0.8**	**79.18 ±4.01[b]**

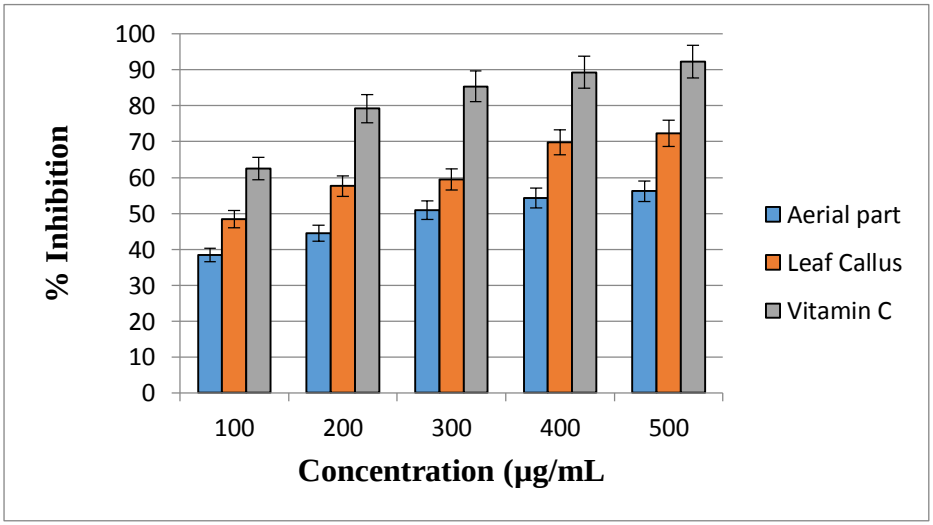
Table 2: Percentage of inhibition of DPPH and IC_{50} for ethanolic extracts of whole plant and leaf callus of *Mollugo oppositifolia* L. at different concentrations (µg/mL) compared with vitamin C (standard/control).

Concentration µg/mL	% inhibition by plant		Concentration µg/mL	% inhibition by vitamin C
	Whole plant	Leaf callus		
100	47.84±0.0001[a]	48.42±0.001[a]	5	62.5±0.0002[b]
200	52.73±0.0005[b]	57.62±0.0005[b]	10	89.52±00
300	53.19±0.0005[b]	59.45±0.0005[b]	20	96.52±5.77[b]
400	54.33±0.0005[b]	62.57±0.0005[b]	40	96.10±5.77[b]
500	55.45±0.0001[a]	65.26±0.0015[c]	80	97.48±0.0003[a]
IC_{50} (µg/mL)	52.82± 0.0017 µg/Ml	58.66±0.004µg/mL	IC_{50} (µg/mL)	84.84±11.54µg/mL

Values are mean±SD of triplicates.

Figure 2. Antioxidant activity of ethanolic extract of whole plant and leaf callus of *Mollugo oppositifolia* L. at different concentrations (µg/mL) compared with vitamin C.





4. 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 free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution, which shows good absorbance at 517 nm[13]. Vitamin C is usually used as a standard because of its availability in different food sources and it has a strong DPPH scavenging property[1] and is used as standard in the present study on whole plant and leaf callus extracts of *Mollugo oppositifolia* L.The present study showed promising antioxidant activity in whole plant ethanolic extract as 52.82± 0.0017 µg/mL and ethanolic extract of leaf callus as 58.66±0.004µg/mL, similar result obtained with whole plant and leaf callus methanolic extract of *Mollugo nudicaulis*[14] with IC_{50} values of DPPH radical scavenging as 48 and 190 µg/mL respectively and also with whole plant and leaf callus ethanolic extract of *Meconopsis quintuplinervia*[17], Tibetan herb with IC_{50} values of DPPH radical scavenging as 375.57 and 713.27 µg/mL.

5. Conclusion

It could be concluded that ethanolic extract of whole plant and leaf callus of *Mollugo oppositifolia* L. has higher potential and are of significant sources of antioxidants, thus both whole plant and leaf callus can be used as antioxidants. Hence, *Mollugo oppositifolia* L. is a valuable source of natural antioxidants as well as in the pharmaceutical industries.

6. References

1. Al-Rifai A, Aqel A, Al-Warhi T, Wabaidur S M, Al-Othman Z A, and Badjah-Hadj-Ahmed A Y. Antibacterial, Antioxidant Activity of Ethanolic Plant Extracts of Some *Convolvulus* Species and Their DART-ToF-MS Profiling. Evidence-Based Complementary and Alternative Medicine., 2017, 1-9 (5694305).
2. Mahdi-Pour B, Jothy S L, Latha L Y, Chen Y and Sasidharan S. Antioxidant activity of methanol extracts of different parts of *Lantana camara*. Asian Pacific J of Tropical Biomedicine., 2012, 2(12): 960-965.
3. Chiavaroli V , Giannini C, De Marco S, Chiarelli F, Mohn A. Unbalanced oxidant-antioxidant status and its effects in pediatric diseases. Redox Report Comm in Free Radical Research., 2011, 16(3): 101-107.
4. Cooke ,T. The Flora of the Presidency of Bombay, 1958: Vol-I.
5. Gopinathan, S. and Nija, S. Gastric ulcer curative potential of *Mollugo oppositifolia* Linn. extract–A preclinical study. World J of Pharm Research., 2014, 3(7): 929-948.
6. Martin-Puzon, J.J.R., Valle Jr, D.L. and Rivera, W.L. TLC profiles and antibacterial activity of Glinus oppositifolius L. Aug. DC.(Molluginaceae) leaf and stem extracts against bacterial pathogens. Asian Pacific J of Tropical Disease., 2015, 5(7): 569-574.
7. Vasincu, A., Ababei, D.C., Miron, A., Neamţu, M., Vasincu, I.M., Charalambous, C., Neophytou, C.M. and constantinou, A.I. Assessment of the potential antitumor effects of *Glinus oppositifolius* (l.) aug. dc. extracts. Farmacia., 2019, 67(4): 656-661.
8. Modi, K. and Shah, M. (2015). Pharmacognostic specifications and quantification of Oleanolic acid and Lupeol in *Mollugo oppositifolia* Linn. Pharmacognosy Journal., 2015, 7(2): 1-9.
9. Ramaseshan, S.T., Pitchaiah, P., Bharti, V., Ramakrishna, K.K., Gaddam, V., Tewari, D., Rath, C., Mangal, A., Mohan, P.M. and Singh, D.K. Pharmacognostical, Phytochemical and Nutritional Evaluation of *Glinus oppositifolius* (L.) Aug. DC. Pharmacognosy J., 2016, 8(1): 31-35.
10. Tanquilut, N.C., Alonzo, N.Q., James, A.E., Rowlands, D.K., Sanchez, G.C., San, R.D., Jose, E., Tanquilut, M.R.C. and Reyes, B.A.S. Hypoglycemic activity of Antidesma bunius (L.) Spreng and *Mollugo oppositifolia* L. fresh and alcoholic extracts in the db/db diabetic mouse model. J of Medicinal Plants Research., 2019, 13(17): 396-400.
11. Gopinathan, S. and Subha, S. Antidiabetic efficacy of *Mollugo oppositifolia* Linn. extract-an animal model study. World J of Pharma Research., 2014, 3(8),735-768.
12. Vasincu, A., Ababei, D.C., Miron, A., Neamţu, M., Vasincu, I.M., Charalambous, C., Neophytou, C.M. and constantinou, A.I. Assessment of the potential antitumor effects of *Glinus oppositifolius* (L.) aug. dc. extracts. Farmacia., 2019, 67(4), 656-661.
13. Juan, M Y and Chou, C C. Enhancement of antioxidant activity, total phenolic and flavonoid content of black soybeans by solid state fermentation with *Bacillus subtilis* BCRC 14715. Food Microbiol., 2010, 27 (5): 586–591.
14. Rameshkumar A. and Sivasudha T. *In vitro* antioxidant and antibacterial activity of aqueous and methanolic extract of *Mollugo nudicaulis* Lam. Leaves. Asian Pacific J of Tropical Biomed., 2012: S895-S900.
15. Sen Antara and Amla Batra. The study of *in vitro* and *in vivo* antioxidant activity and total phenolic content of *Phyllanthus amarus* Schum. And Thonn.: A medicinally important plant. International J of Pharmacy and Pharmaceutical Sci., 2013, 5(3): 942-947.
16. GP Vandnere, Patil AV, Jain S K and Wagh SS. Investigation on *in vitro* antioxidant activity of whole plant of *Cassia occidentalis* Linn. International J of Pharm Tech Research., 2011, 3(4): 1985-1991.
17. He Jingsheng, Bo Huang, Xiaoquan Ban, Jun Tian, Ling Zhu, Youwei Wang. In vitro and in vivo antioxidant activity of the ethanolic extract from *Meconopsis quintuplinervia*. J of Ethnopharmacology., 2012, 141: 104-110.

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