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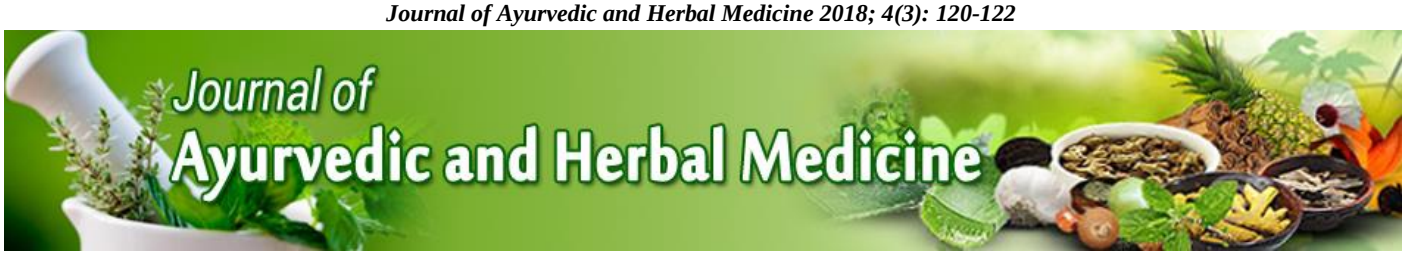

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***Corresponding author:**
Dr. L.D.A.M Arawwawala
Industrial Technology Institute, Research & Development Complex, No 503A, Halbarawa Gardens, Thalahena, Malabe, Sri Lanka
Email: menuka@iti.lk

Investigation of in vitro antioxidant activities of Sri Lankan grown *Glinus oppositifolius* (L.) Aug. DC

M.N.F. Rizniya[1], A.H.M. Mawjood[2], L.D.A.M. Arawwawala[2]

1 *Institute of Indigenous Medicine, University of Colombo, Rajagiriya, Sri Lanka*

2 *Industrial Technology Institute, Research & Development Complex, No 503A,Halbarawa Gardens, Thalahena, Malabe, Sri Lanka*

ABSTRACT

Agrimonia pilosa Ledeb (AL) has received considerable attention as a herbal medicine for its applications in ethnopharmacology with heath benefits. This study aimed to investigate antioxidant activities and A549 growth inhibitory capacity from its root extract (RE) and aerial parts extract (AE). The 50% ethanol extracts were used for the tests. The total polyphenol content and the antioxidant effects comprising ABTS+ and DPPH free radical scavenging activities were evaluated. Phenolic compounds in the extracts were isolated using high performance liquid chromatography (HPLC). Lactate dehydrogenase released in medium was also evaluated. Total phenolic and flavonoid content, and *in vitro* antioxidant potential of the RE were higher ($p<0.01$) than that of the AE. Two individual phenolic compounds consisting of 4-hydroxybenzoic acid and p-coumaric acid were firstly identified in both by HPLC. The RE exhibited higher A549 inhibitory capacity compared to the AE and activated the apoptotic proteins of bcl-2, bax, bad, caspase-3 and caspase-9 in A549. In conclusion, the AL extracts were more effective in antioxidant and A549 cells inhibitory capacity.

Keywords: *Glinus oppositifolius*, Radicals, Antioxidants.

INTRODUCTION

Glinus oppositifolius (L.) Aug. DC (Family: Mollungincaeae) is an unconventional leafy vegetable found in Asian countries including Sri Lanka. It is commonly found as a wild creeper in the fields of the East and Southeast coastal areas of Sri Lanka. *G. oppositifolius* is a branched herb containing linear to obovate, opposite leaves and greenish white flowers. This plant is being used as a leafy vegetable by the people in Sri Lanka from the time immemorial for nutritious and medicinal purposes. Further, people believe that *G. oppositifolius* has anti-diabetic, glactogogue, and blood purifying properties.[1] According to Unani system of medicine the concept of blood purifier means purification of blood from impurities which includes microorganisms, glucose, cholesterol, free radicalsetc. In Sri Lanka metabolic disorders and non-communicable diseases are becoming threatening health problem for the last few decades. This is mainly due to life style changes and dietary habits which lead to excessive production of free radical in the body. It is a well-established fact that many of the diseases like diabetes mellitus, hypertension, inflammation, myocardial damage, cardiac arrhythmias and cancer are due to free radical mediated oxidation of cellular components.[2] Therefore, the importance for finding of plant origin antioxidants have greatly increased in recent years. Antioxidant activity was evaluated for *G. oppositifolius* grown in India[3] and Bangladesh.[4] However, biological activities of the plants within in the same species can vary with origin of the country.[5]Therefore, an attempt was taken to investigate the antioxidant activity of *G. oppositifolius* grown in Sri Lanka.

MATERIALS AND METHODS

Plant material

Fresh *G. oppositifolius* plants were collected from South Eastern coastal area of Sri Lanka during the period of July – October 2013. *G. oppositifolius* was identified and authenticated by Curator of National Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka. A specimen voucher was deposited at Department of Ilmul Adviya, Institute of Indigenous Medicine, University of Colombo, Sri Lanka.

Preparation of methanolic extract of *Glinus oppositifolius*

Whole plants of *G. oppositifolius* were rinsed with running water and dried in the shade for 7-14 days. Dried samples were ground using a commercial grinder into coarse powder. *G. oppositifolius* (50 g) was

taken into a conical flask, added 150 mL of methanol and shaken well. After 1h, a reflux condenser was attached to the flask, boiled gently for 2 h, allowed to cool and filtered rapidly using a dry filter paper (Qualitative filter paper, 90 mm Diameter Whatman ®). Then the filtrate was transferred to a round bottom flask and evaporated to dryness under the reduce pressure (at 40 ^{o}C) using a rotor vapor and stored at 4 ^{o}C until used.

Antioxidant assays: By radical scavenging assays [1,1diphenyl 2-picrylhydrazyl (DPPH$^{\cdot}$) and 3-ethyl-benzothiazoline-6-sulfonic acid (ABTS$^{\cdot}$)] and (b) quantification of phytochemical classes responsible for antioxidant properties (flavonoids and phenols).

DPPH scavenging assay for *Glinus oppositifolius* methanolic extract

Preparation of standard solution: Trolox was used as an antioxidant standard. Required quantity of Trolox (1 mg) was dissolved in methanol to give up 1 mg/mL for use as a stock standard.

Preparation of test sample: Stock solution of sample was prepared by dissolving 5 mg of dried methanolic extract of *G. oppositifolius* in methanol to give concentration of 5 mg/mL. Stock solution was diluted with buffer (PBS) to get 5 concentrations (2.5 – 0.078 mg/ mL).

Preparation of DPPH solution: DPPH (10 mg) was dissolved in methanol (50 mL) and protected with an aluminum foil.

DPPH scavenging assay: The DPPH activity of methanolic extract of *G. oppositifolius* was carried out as described by Blois[6] with some modifications. In brief, different concentrations of the test solution (100 µL) were added into the micro plate and the pre-plate reading was recorded using a micro plate reader (Molecular Devicer – Spectra Max 384 plus) at 517 nm. Then 50 µL of DPPH (0.05mM) solution was added into the above test solutions and incubated at 25 ^{o}C for 20 min. After 20 min the absorbance was recorded at 517 nm using a micro plate reader. Trolox was used as the standard. A control reaction was carried out without the test sample.

ABTS scavenging assay for *Glinus oppositifolius* methanolic extract

Preparation of standard solution: Trolox was used as an antioxidant standard. Required quantity of Trolox (2 mg) was dissolved in 5 mM phosphate buffered saline; pH 7.4, (PBS) to give up 2 mg/mL for use as a stock standard.

Preparation of test sample: Stock solution of the sample was prepared by dissolving 5 mg of dried methanolic extract of *G. oppositifolius* in buffer (PBS) to give concentration of 5 mg/mL.

Preparation of ABTS solution: ABTS$^{\cdot+}$ solution (40 µL; 7 mM) was diluted with, PBS (5mM, pH 7.4).

ABTS scavenging assay: The ABTS activity of methanolic extract of *G. oppositifolius* was carried out with some modifications[7]. In brief, different concentration of the test solution (100 µL) was added into the micro plate as triplicate. Then 120 µL of phosphate buffer (5 mM), pH 7.4 was added into the test samples and the pre-plate reading at 734 nm was recorded using a Micro plate reader (Molecular Devicer – Spectra Max 384 plus). Then, 200 µL of diluted ABTS solution was added into the test sample and were incubated at 25 ^{o}C for 10 min. After 10 min. the absorbance was recorded at 734 nm. Trolox was used as the standard. A control reaction was carried out without the plant extract and IC_{50} values were calculated.

Quantitative determination of total polyphenolic content: Different concentrations of methanolic extract (0.1 mL) were mixed with distilled water (0.9 mL) and added 5 mL of Folin – Ciocalteu reagent (10 fold diluted solution). Saturated sodium carbonate solution (4 mL) was added to the above mixture and shaken. After 2 h, absorbance of the reaction mixture was measured at 765 nm. Total phenolic content was expressed as gallic acid equivalents (mg gallic acid/g extract)[8].

Quantitative determination of total flavonoid content: The total flavonoid content was determined according to Meda and co-workers.[9] In brief, 2% $AlCl_3$ (5 mL) in methanol was mixed with the same volume (5 mL) of methanolic extract in different concentrations. After 10 min. the absorbance of the reaction mixture was measured at 415 nm. Total flavonoid content was expressed as quercetin equivalents (mg quercetin/g extract).

RESULTS AND DISCUSSION

Free radicals and other reactive oxygen species play a important role for causing diseases such as asthma, diabetes mellitus, hypertension, inflammation, myocardial damage, cardiac arrhythmias and aging.[2,10,11] In the present study, four *in vitro* antioxidant assays were performed and *G. oppositifolius* methanolic extract had shown potent antioxidant activity (Table 1). Antioxidants, by providing a hydrogen atom or by donation of electrons, can quench DPPH$\cdot$ free radicals and convert them to a colorless bleached product resulting in a reduction in absorbance.[12] ABTS$^{\cdot+}$ is a stable radical that not found in the human body. In this assay, a blue/green ABTS$^{\cdot+}$chromophore is generated by the oxidation of ABTS with potassium persulfate. Presence of hydrogen donating antioxidants, the blue/green color of ABTS$^{\cdot+}$ is reduced. This can be measured by spectrophotometrically at 745 nm.[13] DPPH$\cdot$ assay determines only the hydrophilic antioxidants whereas ABTS$^{\cdot+}$ assay measures both hydrophilic and lipophilic antioxidants.[7] In the present study, *G. oppositifolius* methanolic extract showed better radical scavenging activity against ABTS$^{\cdot+}$ than that of DPPH$^{\cdot}$. This indicates in addition to hydrophilic antioxidants, lipophilic antioxidants also play a major role in scavenging free radicals. In a previous study, Sri Lankan grown *G. oppositifolius* has shown to contain high amounts of proteins and vitamins.[14]

Table 1: Anti-oxidant activity of *Glinus oppositifolius* methanolic extract

Antioxidant assays	Results
DPPH assay	12.50 ± 0.68 mg Trolox equivalents/g of extract
ABTS assay	54.85 ± 0.48 mg Trolox equivalents/g of extract
Total flavonoid content	185.20 ± 0.80 mg quercetin equivalents/g of extract
Total phenol content	210.36 ± 0.45 mg gallic equivalents/g of extract

In conclusion, *G. oppositifolius* can be recommended as a useful green leafy vegetable and can get many health benefits by including in diet.

Conflict of interest- None declared

REFERENCES

1. Jayaweera DMA. Medicinal plants used in Ceylon. Publication of National Science Foundation. 1980, Part 1, 26-27.
2. Hans CP, Paul D, Chaudhary D, Bansal DD. Magnesium deficiency increased oxidative stress in rats. Indian Journal of Experimental Biology.2002, 40(11):1275-9
3. Asokkumar K, UmaMaheswari M, Sivashanmugam AT, Subhadra Devi V, Subhashini N, Ravi TK. Free radical scavenging and antioxidant activities of *Glinus oppositifolius* (carpet weed) using different in vitro assay systems. Pharmaceutical Biology.2009, 47: 474 – 482.
4. Hoque N, Imam MZ, Akter S, Md.Mazumder EH, RaquibulHasan SM, Ahmd J,. Md.Rana S. Antioxidant and antihyperglycemic activities of methanolic extract of *Glinus oppsitifolius* leaves. Journal of Applied Pharmaceutical Science.2011, 01 (07): 50-53.
5. Menkovic N, Savikin – Fodulovic K, Savin K. Chemical composition and seasonal variations in the amount of secondary compounds in

Gentiana lutea leaves and flowers. Planta Medica. 2000, 66: 178 – 180.
6. Blois MS. Antioxidant determination by use of stable free radical: Nature.1958, 181: 1199-1200.
7. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M. Antioxidant activity applying an improved ABTS radical cation decolonization Assay: Free Radical Biology and Medicine.1999, 26: 1231-1237.
8. Wolfe K, Wu X, Liu RH. Antioxidant activity of apple peels. Journal of Agricultural and Food Chemistry.2003, 51:609–614.
9. Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chemistry. 2005, 91:571–7.
10. Kanwar JR, Kanwar RK, Burrow H, Baratchi S. Recent advances on the roles of NO in cancer and chronic inflammatory disorders. Current Medicinal Chemistry. 2009,16:2373–2394.
11. Chiavaroli V, Giannini C, De Marco S, Chiarelli F, Mohn A. Unbalanced oxidant-antioxidant status and its effects in pediatric diseases. Redox Report. 2011, 16:101–107.
12. Alam Md N, Bristi NJ, Md. Rafiquzzaman. Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. Saudi Pharmaceutical Journal. 2013, 21: 143 – 152.
13. Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. BMC Complementary and Alternative Medicine.2012, 12: 221.
14. Rizniya MNF, Mawjood AHM, Arawwawala LDAM. A study on physico-chemical parameters and nutritional values of Glinus oppositifolius: A leafy vegetable. Sci Fed Journal of Herbal Medicine,2017, 1: 1-5.

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