ISSN 0975-6299

Vol 2/Issue 2/Apr-Jun 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

PHARMACOGNOSY

ANTIOXIDANT ACTIVITY, PHENOL AND FLAVONOID CONTENT OF SOME LESS KNOWN MEDICINAL PLANTS OF ASSAM

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ABSTRACT

Ethanolic extract of four medicinal plants *Polygonum microcephalum, Moringa oleifera, Croton tiglium* and *Gomphrena globosa* were examined for antioxidant activity, and phenol and flavonoid content. Total phenol and flavonoid content and DPPH radical scavenging activity of the extracts were spectrophotometrically determined. Catechol, Quercetin, and ascorbic acid were taken as standard in case of phenol, flavonoid content and antioxidant activity respectively. The total phenol and total flavonoid content was observed highest in *P. microcephalum*. The DPPH radical scavenging activity was highest in *M. oleifera*. There observed a relationship between phenol and flavonoid content but failed to show relationship between phenolic content and antioxidant activity of the ethanol extracts of the plants.

KEY WORDS

antioxidant, phenol, flavonoid, medicinal plants.

INTRODUCTION

Medicinal plants have been playing a vital role on the health and healing of man since down of human civilization. In spite of tremendous development in the field of allopathic medicines during the 20th century, plants still remain one of the major sources of drugs in modern as well as in traditional system of medicine. Medicinal plants are source of certain bioactive molecules which act as antioxidants and antimicrobial agents[1-4]. There is an upsurge in demand of plant materials containing phenolics as they retard oxidative degradation of lipids and thereby improving quality and nutritional value of food[4-6].

Free radicals are responsible for several disorders in human body[7-8]. Oxidative process is one of the most important routes for producing free radicals in food, drug, and even in living systems. The free radicals in the human body have adverse effects on its immune system[9]. Consumption of natural oxidants as free radical scavengers may become necessary to improve the depleted immune system[7, 10-12]. It is reported that the antioxidant constituents of plant materials provide protection from coronary heart disease and cancer[13] and protect the body from damage caused by free radical induced oxidative stress[14-15].

Recently, more attention has been given in medicinal plants of therapeutic potentials as antioxidants in reducing free radical induced tissue injury. Many plants have been investigated in the search for novel antioxidants [16-24]. The synthetic antioxidants have restriction for use, as they are suspected to be carcinogenic. Therefore, the importance of searching for and exploiting natural antioxidants has increased greatly in present years [25].

P. microcephalum, M. oleifera, C. tiglium and *G. globosa* are commonly used in various ailments by different ethnic group of Assam. Leaves of *P. microcephalum* and *M. oleifera* are used as vegetable. *P. microcephalum* is believed to be appetizer and it releafs from acidity in stomach. Leaves of *M. oleifera* leaf are believed to cure jaundice and prevent viral infection that cause measles. *C.tiglium* leaves are used to cure fungal infection in rotten nails. Leaves of *G. globosa* are used to stop bleeding due to cut injury.

The purpose of the present study was to investigate the antioxidant activity, phenol and flavonoid content of some potential medicinal plants *P. macrocephalum, M. oleifera, C. tiglidium* and *G. globosa* .

MATERIALS AND METHODS

Leaves of *P. microcephalum, M.oleifera, C. tiglium* and *G. globosa* were collected from Dibrugarh district, Assam. Voucher specimen (DUL.Sc.2530, 2531, 2532, 2533) were deposited in the Department of Life Sciences, Dibrugarh University. Leaves were shade dried, powdered and ground with a pestle and mortar in the measured volume of solvents (80: 20 ethanol –water). The extract was filtered through Whatman No. 1 filter paper. Each extract was prepared just before the analysis for prevention of any degradation. Folin-Ciocalteau reagent and all other chemicals used were Merck products.

DPPH radical scavenging activity[26]

Antioxidants react with 1, 1- diphenyl -2-picryl-hydrazyl (DPPH) radical and convert it to 1, 1- diphenyl -2-picryl hydrazine. The degree of change in colour from purple to yellow can be used as a measure of the scavenging potential of antioxidant extracts. Aliquots of extract solutions were taken and made up the volume to 3ml with methanol. 0.15ml of freshly prepared DPPH solution was added, stirred and left to stand at room

temperature for 30 minutes in dark. The control contains only DPPH solution in methanol instead of sample while methanol served as the blank (negative control). Absorbance was noted at 517 nm by using UV-Vis spectrophotometer. The capacity of scavenging free radicals was calculated as follows:

Scavenging activity (%) = {(Control abs.-sample abs.)/Control abs.} X 100.

IC_{50} value was calculated from the plotted graph of scavenging activity against the concentrations of the samples. IC_{50} is defined as the total antioxidant necessary to decrease the initial DPPH radical by 50%. Triplicate measurements were carried out and IC_{50} was calculated for all the extracts based on the percentage of DPPH radicals scavenged. Ascorbic acid was used as the reference compound (positive control) with concentrations 20 to 500 µg/ml.

Determination of total phenolics

The total phenolics content of extracts of the plants were determined according to the method described by Malik and Singh[27]. Aliquots of the extracts were taken in a 10 ml glass tube and made up to a volume of 3 ml with distilled water. Then 0.5 ml folin ciocalteau reagent (1:1 with water) and 2 ml Na_2CO_3 (20%) were added sequentially in each tube. The tubes with solution were warmed for 1 minute, and then cooled. A blue color was developed in each tube because the phenols undergo a complex redox reaction with phosphomolibdic acid in folin ciocalteau reagent in alkaline medium which resulted in a blue colored complex. Absorbance was measured at 760 nm. A standard calibration plot was generated at 760 nm using known concentrations of catechol. The concentrations of phenols in the test samples were calculated from the calibration plot and expressed as mg catechol equivalent of phenol/g of sample.

Determination of total flavonoids

The aluminum chloride method was used for the determination of the total flavonoid content of the extracts[25]. Aliquots of extract solutions were taken and made up the volume 3ml with methanol. Then 0.1ml $AlCl_3$ (10%), 0.1ml Na-K tartarate and 2.8 ml distilled water were added sequentially. The solution mixture was vigorously shaken. Absorbance at 415 nm was recorded after 30 minutes of incubation. A standard calibration plot was generated at 415 nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent /g of sample.

RESULTS AND DISCUSSION

Total phenol and total flavonoid content and the antioxidant activity of ethanol extracts of the plants are shown in table 1 and table 2. Total phenol content in terms of catechol equivalent (the standard curve equation: y =0.0966x, r^2 = 0.9878) were between 3.6 /g and 19.0 mg /g dry material while total flavonoid content (the standard curve equation: y = 0.0148x, r^2 = 0.975) in terms of quercetin equivalent were between 17.2mg/g and 39.6mg/g dry wt. Similar result on phenol content of *Asparagus* extract in different cultivars was obtained by Rodriguez et al. [29]. It is observed that phenol and flavonoid content of the plants differ among them. Highest phenol and flavonoid content were noted in the extracts of plants *P. microcephalum* (Table 1). The phenolic compounds act as free radical terminators[30] and mechanism of action of flavonoid are through scavenging or chelating process [8, 31]. The antioxidant activity of the plants varied considerably in terms of IC_{50} value (Table 2). The highest antioxidant activity was noted in the extracts of plants *M. oleifera* followed by the plants *P. microcephalum, G. globosa* and *C.tiglium* respectively.There was no correlation between total phenolic content and antioxidant activity in this study but there are some reports[23, 29], which showed correlation between antioxidant activity and phenolics content of certain medicinal plants. The results of the present study supported the findings of some other investigators in

certain other medicinal plants [1, 32]. The reason for lacking correlation between phenolics

Table-1

Total phenol & flavonoid content of P.microcephalum, M.oleifera, C. tiglium and G. globosa

Plants	Phenol (mg catechol equivalent/g dry material)	Flavonoid content (mg quercetin equivalent /g dry material)
P.microcephalum	19.0	39.6
M. oleifera	13.4	37.0
C. tiglium	7.48	26.6
G. globosa	3.6	17.2

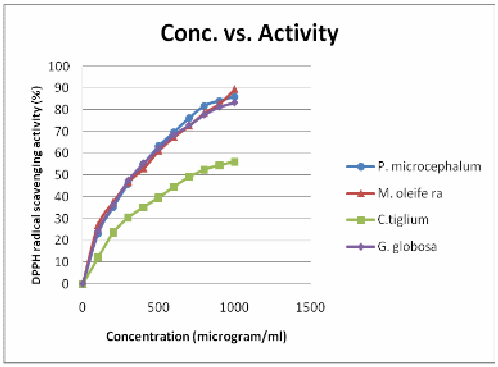
Table-2

Antioxidant activity of P. microcephalum, M.oleifera, C. tiglium and G. globosa

Plants	Antioxidant activity IC_{50} in ug/ml (lower IC_{50} value indicate higher antioxidant)
P. microcephalum	431.23
M. oleifera	429.31
C. tiglium	764.15
G. globosa	433.33

Fig1

Conc. of ethanol extract Vs. DPPH free radical scavenging activity of P. microcephalum, M.oleifera, C. tiglium and G. globosa



content and antioxidant activity in the present study may be due to the presence of some other phytochemicals such as ascorbic acid, tocoferol and pigments as well as the synergistic effects among them[1]. These phytochemicals as a whole contribute to the total antioxidant activity of the extracts. The result also showed that the percentage of antioxidant activity of the ethanol extracts increases with increasing concentration of the extracts in 200 µl to 1000 µl in all the samples

(Fig1) and the results are in agreement with findings of others[4].

In the present study, all the plants showed phenol and flavonoid content and exhibit antioxidant activity. Consumption of *M. oleifera*, *P. microcephalum* leaves as vegetable is likely to be benefit by scavenging and reducing free radicals in the body. Use of *C. tiglium* leaves as natural antiinfectant and the leaves of *G. globosa* as natural blood coagulatory is of immense importance among the ethnic people. Natural antioxidants of plants origin have greater application and they also find use as nutraceuticals and phytoceuticals as they have significant impact on the status of human health and disease prevention[32]. The present study provides scientific basis of the use of these plant extracts in traditional health care system. Detail work by using different methods will be the aim of further investigation. Further, studies on other medicinal plants would be of great importance.

ACKNOWLWDGEMENT

Authors are thankful to the U.G.C. for financial support and Dibrugarh University, Assam for providing necessary facilities.

REFERENCES

1 Sengul M, Yildiz H, Gungor N, Cetin B, Eser Z, & Ercisli S, Total phenolics content, antioxidant and antimicrobial activities of some medicinal plants. Pak J Pharm Sci, 22(1):102-106, (2009).

2 Chopra RN, Nayer SL & Chopra IC, Ed. Glossary of Indian Medicinal Plants, 3rd Edn, Council of Scientific and Industrial Research, New Delhi : 7-246, (1992)

3 Bruneton J, Pharmacognosy Phytochemistry Medicinal plants, France: Lavoisiler Publishing Co.: 265-380, (1995).

4 Khalil MY, Moustafa AA & Naguib NY, Growth, phenolic compounds and antioxidant activity of some medicinal plants grown under organic farming condition. World Journal of Agricultural Sciences, 3(4) : 451-457, (2007).

5 Landry LG, Arabidopsis mutants lacking phenolic sunscreens exhibit enhanced UVB injuri and oxidative damage. Plant Physiology:1159, (1995).

6 Rice-Evans CA, Millar NJ, Bolwell PG, Bramley PM & Pridham JB, The relative antioxidant activities of plant derived polyphenolic flavonoids. Free Radical Res., 22: 375-383, (1996).

7 Kumpulainen JT, & Salonen JT, Natural Antioxidants and Anticarcinogens in Nutrition, Health and Disease, The Royal Society of Chemistry, UK,: 178- 187, (1999).

8 Cook NC & Samman S, Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. Nutritional Biochemistry, 7: 66- 76,(1996).

9 Pourmorad F, Hosseinimehr SJ & Shahabimajd N, Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. African Journal of Biotechnology, 5(11) : 1142-1145, (2008).

10 Halliwell B, Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? Lancet, 344 : 721- 724, (1994).

11 Kuhnan J, The flavonoids. A class of semi-essential food components; their role in human nutrition. World Review of Nutrition and Dietetics, 24 :117- 191, (1976).

12 Younes M, Inhibitory action of some flavonoids on enhanced spontaneous lipid peroxidation following glutathione depletion. Planta Medica, 43 : 240-245,(1981).

13 Loringer J, The use of Antioxidants in food, In: free Radicals and Food Additives, edited by O.J.Halliwel, O.I.B. Taylor and Francis, London,:129-150, (1999).

14. Yoshida T, Ahmed AF, & Okuda T, Tannins of tamaricaceeous plants. II-

New dimeric hydrolysable tannins from Reaumuria hirtella Chemi and Pharmaco, In : Khalil MY, Moustafa AA, Naguib NY, Growth, phenolic compounds and antioxidant activity of some medicinal plants grown under organic farming condition. World Journal of Agricultural Sciences, 3(4): 451-457, (2007).

15. Souri E, Amin G, Sherifabadi AD, Nazifi A, & Farsam H, Antioxidative activity of sixty plants from Iran. Journal of Pharmac Res., 3: 55-59, (2004).
16. Bol'shakova IV, Lozovskaia EL & Sapezhinskii II, Antioxidant properties of plant extracts, Biefizika, 43 :186-188,(1998).
17. Kahkonen MP, Hopia AI, Vuorela JH, Rauha JP, Pihlaja K, Kujala TS, & Heinonen M, Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem, 47: 3954-3962, **(**1999)
18. Chu Y, Flavonoid content of several vegetables and their antioxidant activity, J. Sci. Food and Agricul, 80 : 561 – 566, (2000).
19. Mantle D, Eddeb F, & Pickering AT, Comparison of relative antioxidant activities of British medicinal plant species in vitro. J. Ethnopharmacol., 72 : 47- 51,(2000).
20. Trojakova L, Reblova Z, Nguyen HT, & Okorny JP, Antioxidant activity of rosemary and sage extracts in rape oil, Jour. Food Lipids, 41: 5181-5190, (2001).
21. Koleva II, Van Beek TA, Linssen JPH, Groot A de & Evstatieva LN, Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochemical Analysis, 13: 8-17,(2002).
22. Oke JM & Hamburger MO, Screening of some Nigerian medicinal plants for antioxidant activity using 2, 2- diphenyl-picryl- hydrazyl radical. African J. Biomed. Res., 5 :77- 79,(2002).
23. Galvez M, Martim-Cordero C, Houghton PJ & Ayuso MJ, Antioxidant activity of methanol extracts obtained from Plantago species. J. Agric.Food Chemistry, 53: 1927-1933, (2005).
24. Erdemoglu N, Turan NN, Cahoco I, Senor B, & Aydon A, Antioxidant activities of some Lamiaceae plant extracts. Phytother Res., 20 : 9-13, (2006).
25. Mervat MMElFar & Hanan AATaie, Antioxidant activities, total anthrocynins, phenolics and flavonoids contents of some sweet potato genotypes under stress of different concentrations of sucrose and sorbitol, Australian J Basic Applied Sc, 3: 3609-3616,(2009).
26. Anti-Stanojevic L, Stanojevic M, Nikolic V, Nikolic L, Ristic J & Canadanovic-Brunet V, Antioxidant activity and total phenolic and Flavonoid contents of Hieracium pilosella L. extracts, Sensors, 9 : 5702-5714, (2009).
27. Malik EP & Singh MP, Plant Enzymology and Hittoenzymology, Kalyani Publishers, New Delhi, 286, (1980).
28. Rodriguez R, Jaramillo S, Rodriguez G, Espejo JA, Guillen R, Fernandez-Bolanos J, Heredia A & Jimenez A, Antioxidant activity of ethanolic extracts from several asparagus cultivars. J. Agric. Food Chem., 53 : 5212-5217,(2005).
29. Shahidi F & Wanasundara PKJPD, Phenolic antioxidants, Critical Reviews in Food Science and Nutrition, 32 : 67-103, (1992).
30. Kessler M, Ubeaud G & Jung L, Anti- and pro-oxidant activity of rutin and quercetin derivatives, J. Pharm and Pharmacol., 55 : 131-142,(2003).
31. Bajpai M, Pande A, Tewari SK, & Prakash D, Phenolic contents and antioxidant activity of some food and medicinal plants. International Journal of Food Sciences and Nutrition, 56(4) : 287-291, (2005).
32. Baruah IC & Baruah M, Non-conventional food plants of medicinal use in Northeast India, In: Medicinal plants of Northeast India edited by Borthakur SK, Ahmed A, Gogoi P, Dutta DN & Ahmed GU, North eastern development finance corporation LTD., Guwahati, Assam: 318, (2010).