IJPSR (2011), Vol. 2, Issue 7 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 30 March, 2011; received in revised form 05 May, 2011; accepted 25 June, 2011

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF *LEUCAS ASPERA*

B. K. Das*, B. Das, F. K. Arpita, M. A. Morshed, A. Uddin, R. Bhattacherjee and J.M.A. Hannan

Department of Pharmacy, North South University, Bashundhara, Dhaka-1229, Bangladesh

Keywords:

Leucas aspera,
Phytochemical,
Antioxidant activity,
Ethanol extract

Correspondence to Author:

B. K. Das

Department of Pharmacy, North South University, Bashundhara, Dhaka-1229, Bangladesh

ABSTRACT

The purpose of the current work was to conduct phytochemical screening and antioxidant activity of different extracts of *Leucas aspera*. The dried powder of the whole plant was extracted with n-hexane, ethyl acetate and ethanol which were subjected to various chemical tests to ascertain the main constituents of the plant. The results revealed the presence of significant amounts of alkaloids, glycosides, tannins and flavonoids in ethanol extract while the other two extracts contain moderate amount of the chemical constituents. Our results indicate the presence of phytochemicals in the order of ethanol > ethyl acetate > n-hexane extract. Antioxidant activity of these extracts were performed by using DPPH free radical scavenging assay, total anti-oxidant capacity and the total phenol capacity where ascorbic acid (for DPPH scavenging and total anti-oxidant) and gallic acid (for total phenol) were used as standards. The results showed that the ethanol extract possesses more anti-oxidant activity than ethyl acetate and n-hexane extracts.

INTRODUCTION: Free radical reactions have been implicated in the pathology of numerous diseases including brain disorders, platelet aggregation, atherosclerosis, ageing, cancer, inflammatory diseases and a variety of other disorders. Ultraviolet light, ionizing radiation and chemical reactions can induce the production of reactive oxygen species (ROS), like superoxide radical anion, hydroxyl radicals, singlet oxygen and hydrogen peroxide, in the cells. These reactive oxygen species cause lipid peroxidation, protein peroxidation, DNA damage and cellular degeneration in the cells [1-6].

Antioxidants are scavenging or trapping agents capable of interfering with processes involved in oxidative stress and appear to be of primary importance in the prevention of these chronic diseases. Although synthetic antioxidants such as butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT) are useful, their potential health risks and toxicity have raised concerns to their wide spread use in the food industry [7, 8]. Thus, the need for alternative sources of antioxidants is paramount and the search for natural antioxidants, especially of plant origin, has received much attention, and efforts have been made to identify new natural resources for active antioxidant compounds [9].

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many of which based on their use in traditional medicine. It has been noted that the original source of many important pharmaceuticals currently in use have been plants used by indigenous people [10]. It has been reported that about 64% of the total global population remains dependent on traditional medicine and medicinal plants for provision of their health-care needs [11].

The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from ageing-related diseases has intrigued scientist for a long time. Therefore, the effectiveness of phytochemicals in the treatment of various diseases may lie in their antioxidant effects [12].

Phenolic compounds such as flavonoids, alkaloids, phenolic acids, stilbenes, lignans, lignin and tannins, found in both edible and nonedible plants, are well known as scavengers of free radicals and have multiple biological effects, including antioxidant activity [13-18]. The antioxidant activity of phenolics is principally due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers.

Leucas aspera (Family - Labiatae) is an annual herb found throughout India as a weed in cultivated fields, wastelands and roadsides. The plant is widely found in Bangladesh and its Bengali name is Dondokolos. The juice of the leaves is used as remedies for psoriasis, chronic skin eruptions and chronic rheumatism [19]. The flowers are given with honey to treat cough and cold in children. The leaves are applied to the bites of serpents, poisonous insects and scorpion sting. *L. aspera* leaves are also used as insecticides and mosquito repellent in rural area [20]. The plant extract with honey is a good remedy for stomach pain and indigestion.

The aim of the present work was to evaluate the antioxidant potential of *L. aspera* extracts using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging capacity, total antioxidant capacity and total phenol determination assays to support the pharmacological effects and phytochemical investigation of this plant. Although numerous studies have shown the medicinal values of *L. aspera*, there still remains ample scope for further in depth research. Accordingly, we disclose herein the antioxidant effects of the whole parts of *L. aspera* to further establish the scientific basis of the traditional uses of the plant.

MATERIALS AND METHODS:

Collection of the Plant Materials: *Leucas aspera* was collected from the side of the cultivated lands of Allardarga village of Daylatpur upazilla in Kushtia district of Bangladesh in April 2010 and the plant was identified by Bangladesh National Herbarium, Mirpur, Dhaka on 1st May 2010. The Accession no is DACB 35032. The plant is very much well known and widely distributed throughout Bangladesh especially in Kushtia areas.

Extraction of the Plant: The whole plant was first sun dried for several weeks, crushed by hands and dried again. Then the crushed parts of the plant were ground into coarse powder with the help of a mechanical grinder. By using the concept of the nature of solubility and distribution of the active ingredients, powdered material was extracted successively with n-hexane, ethyl acetate and ethanol (95%) as non-polar, medium-polar and polar solvents by cold extraction method for a period of 3 days with occasional shaking and stirring.

The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material followed by a second filtration through whatman filter paper. The filtrates (n-hexane, ethyl acetate and ethanol extract) obtained were evaporated by rotary evaporator (Bibby RE-200, Sterilin Ltd., UK) at 5 to 6 rpm and at 68ºc temperature. It rendered a gummy concentrates of chocolate black color. The gummy concentrate was designated as crude extract which was then freeze dried and preserved at $4^{o}C$ [21].

Phytochemical Analysis: Qualitative phytochemical tests for the identification of alkaloids, flavonoids, steroids, glycosides, saponins, tannins and terpenoids were carried out for all the extracts by the method described by Harborne and Sazada *et al* [22, 23].The freshly prepared extracts of *L. aspera* were qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extracts was performed using the following reagents and chemicals: alkaloids with Wagner reagent, flavonoids with the use of concentrated hydrochloric acid, tannins with 5% ferric chloride, saponins with ability to produce suds, gum with Molish reagents and concentrated sulfuric acid, steroids with sulfuric acid, reducing sugar with the use α-napthol and sulfuric acid and terpenoids with chloroform and concentrated hydrochloric acid.

Assessment of Antioxidant Activity:

DPPH Radical Scavenging Activity: The free radical scavenging capacity of the extracts was determined using DPPH by the established method [24]. DPPH solution (0.004% w/v) was prepared in 95% methanol. The crude extracts of *Leucas aspera* were mixed with 95% methanol to prepare the stock solution (5 mg/ml). The test samples were prepared from stock solution by dilution with methanol to attain a concentration of 10µg/ml, 50µg/ml, 100µg/ml and 500µg/ml respectively. Each sample solution (2 ml) was mixed with 1 ml of DPPH solution. The mixture was shaken vigorously and maintained for 30 min in dark. The absorbance was measured at 517 nm. The absorbance of the control was obtained by replacing the sample with methanol. Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration (5 mg/ml). % scavenging of the DPPH free radical was measured using the following equation:

% DPPH radical-scavenging = [(Absorbance of Control - Absorbance of test Sample) / (Absorbance of Control)] x 100

Determination of total antioxidant capacity: The antioxidant activity of the extracts of *Leucas aspera* was evaluated by the phosphor- molybdenum method according to the procedure of Prieto *et al.* [25]. 0.3 ml (the conc. of the solution is 5 µM/ml) of extract was mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95^{o}C for 90 min and cooled to room temperature. Finally, absorbance was measured at 695 nm using a spectrophotometer against blank. A typical blank solution contains 3ml reagent solution and appropriate volume (0.3ml) of the same solvent in place of the extract. The total antioxidant capacity was expressed as the number of equivalents of ascorbic acid.

Amount of total phenolic compounds: The content of total phenolic compounds in plant extracts was determined by Folin–Ciocalteu method [26]. For the preparation of calibration curve 1 ml aliquots of 50, 100, 150 and 200 µg/ml ethanolic gallic acid solutions were mixed with 5 ml Folin–Ciocaleu reagent (diluted ten-fold) and 4 ml (75 g/l) sodium carbonate. The absorption was read after 30 min at 20^{o}C at 765 nm and the calibration curve was drawn. One ml aqueous ethanol, ethyl acetate and n-hexane extract at 200 µg/ml was mixed with the same reagents as described above, and after 1 h the absorption was measured for the determination of plant phenolics. All determinations were performed in triplicate. Total content of phenolic compounds in ethanol, ethyl acetate and n-hexane extracts in gallic acid equivalents (GAE) was calculated by the following formula:

$$C = c. V/m$$

Where: C = total content of phenolic compounds, mg/g plant extract, in GAE; c = the concentration of gallic acid established from the calibration curve, mg/ml; V = the volume of extract, ml; m = the weight of pure plant ethanolic, ethyl acetate and n-hexane extract, g.

RESULTS: Preliminary phytochemical screening of the extract of *L. aspera* revealed the presence of various bioactive components of which alkaloid, cardiac glycosides, terpenoids and tannins were the most prominent and the result of phytochemical test has been summarized in the **table 1**. Our results indicate the presence of phytochemicals in the order of ethanol > ethyl acetate > n-hexane extract. The above data shows the higher yield of phytochemicals in ethanol extract.

TABLE 1: QUALITATIVE ANALYSIS OF THE PHYTOCHEMICALS OF *L. ASPERA* EXTRACTS

Chemical Constituent	n-hexane extract	Ethyl acetate extract	Ethanol extract
Alkaloids	++	++	+++
Glycosides	+	+++	+++
Flavonoids	-	+	++
Saponins	-	+	++
Tannins	+	++	+++
Terpenoids	+++	+++	+++

Symbol (+) indicates presence and (-) indicates absence of phytochemicals

DPPH Scavenging Activity: The ethanol, n-hexane and ethyl acetate extracts of *L. aspera* exhibited a significant dose dependent inhibition of DPPH activity. A concentration-dependent assay was carried out with these extracts and the results are presented in **fig. 1**. Among five different concentrations used in the study (5 to 500 µg/ml), ethanol extract showed scavenging activity of 31.68%, 46.40%, 49.44%, 53.92%, and 83.67% at 5, 10, 50,100, 500 µg/ml concentration respectively.

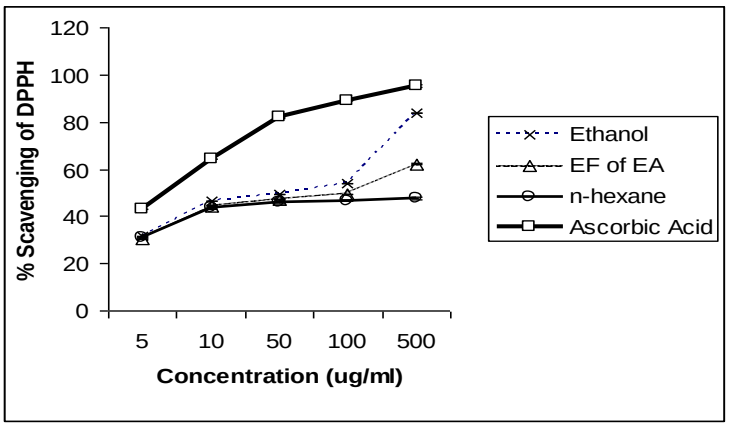

FIG. 1: %DPPH SCAVENGING ACTIVITY OF *L. ASPERA* EXTRACTS AND ASCORBIC ACID

On the other hand, ascorbic acid showed 53.19%, 64.10%, 81.91%, 89.28%, and 95.36% activity with the same concentration. Percent (%) scavenging activity or % inhibition was plotted against log concentration and from the graph IC_{50} (inhibition concentration 50) value was calculated by linear regression analysis. The IC_{50} value of ascorbic acid, ethanol, n-hexane and ethyl acetate extract was found to be 125.57 µg/ml, 176.46 µg/ml, 461.62 µg/ml and 277.97 µg/ml, respectively **(table 2)**.

TABLE 2: FREE RADICAL SCAVENGING ACTIVITY OF DIFFERENT EXTRACTS OF *LEUCAS ASPERA*

Sample	Conc. mg/ml	Mean % DPPH scavenging activity ± SD [a]	IC_{50} Value (mg/ml)
n-hexane extract	5	30.79 ± 0.11	461.627
	10	43.45 ± 0.11*	
	50	46.18 ± 0.07*	
	100	46.27 ± 0.03*	
	500	47.70 ± 0.11*	
Ethyl acetate extract	5	30.71±0.17*	277.972
	10	44.39±0.12*	
	50	47.29±0.07*	
	100	49.44±0.07*	
	500	62.21±0.08*	
Ethanol extract	5	31.68±0.11*	176.46
	10	46.40±0.08*	
	50	49.44±0.38*	
	100	53.92±0.07*	
	500	83.67±0.43*	
Standard ascorbic acid	5	43.19±0.41*	125.575
	10	64.10±0.21*	
	50	81.91±0.70*	
	100	89.28±0.014*	
	500	95.36±0.66*	

*Data are expressed as the mean of % inhibition at different concentrations (n=3)

Total Antioxidant Activity: The antioxidant activity of different plant extracts at 5, 50, 100, 200 and 500 µg/ml concentrations was measured by the phosphor-molybdenum method. Total antioxidant capacity of the three extracts of *L. aspera* was calculated using the standard curve of ascorbic acid (y = 0.0079x - 0.1751; R^2 = 0.9811) **(fig. 2)** and is expressed as number of equivalent of ascorbic acid per gram of plant extract (AAE). The total antioxidant capacity of ethanol, n-hexane and ethyl acetate extract was found to be 195 ±8.05, 159.56 ± 8.95 and 186.77 ± 11.63 mg/g of plant extract respectively (expressed as ascorbic acid equivalents) as shown in **table 4**. The phosphor-molybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm.

TABLE 3: ABSORBANCE OF ASCORBIC ACID AT 695nm

Conc. (µg/ml)	Absorbance at 695nm	Mean Absorbance ± SD
500	4.000 3.913 3.925	3.946±0.047
200	1.237 0.936 1.135	1.103±0.153
100	0.429 0.414 0.425	0.423±0.007
50	0.205 0.215 0.210	0.210±0.005
5	0.045 0.043 0.033	0.040±0.006

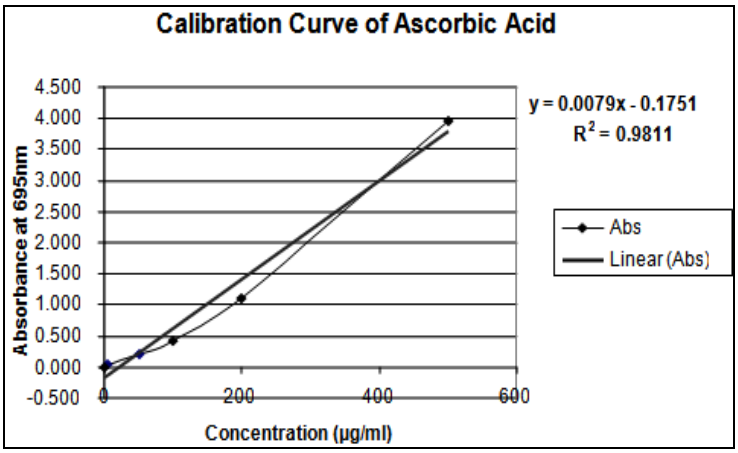

FIG. 2: CALIBRATION CURVE OF ASCORBIC ACID

TABLE 4: TOTAL ANTIOXIDANT CAPACITY OF THE THREE EXTRACTS

Extract	Total antioxidant capacity, mg/g plant extract in AAE±SD
Ethanol	195.00± 8.056*
Ethyl acetate	186.77± 11.636*
n-hexane	159.56 ± 8.951*

Results are expressed as mean ± SD (*p <0.05).

Total Phenolic Content: Phenolic compounds are commonly found in both edible and inedible plants and plant parts. They have been reported to have multiple biological effects, including antioxidant activity. The content of phenolic compounds (mg/100g DW) in ethanolic, ethyl acetate and n-hexane extract was determined from regression equation of calibration curve (y = 0.0138x +0.1276, R^2 = 0.9881) and expressed in gallic acid equivalents (GAE). Among the three extracts, the total phenolic contents of *L. aspera* ethanol extract was found to be promising with a value of 15.36 ± 0.512 GAE/g dry weight of extract **(table 6)**.

Total phenol content of the three extracts of *Leucas aspera* were calculated using the standard curve of Gallic acid (y = 0.0138x + 0.1276; R^2 = 0.9881) and is expressed as Gallic acid equivalent (GAE) per gram of plant extract (**fig. 3**).

TABLE 5: ABSORBANCE OF GALLIC ACID AT 765nm.

Conc. (µg/ ml)	Absorbance at 765nm	Mean Absorbance ± SD
200	3.130 2.610 2.950	2.897±0.264
150	2.020 1.990 2.350	2.120±0.199
100	1.540 1.590 1.450	1.527±0.070
50	1.021 1.040 0.950	1.004±0.047

TABLE 6: TOTAL PHENOL CONTENT OF THE THREE EXTRACTS

Extract	Total phenol content, mg/g plant extract in GAE± SD
Ethanol	15.36 ± 0.512*
Ethyl acetate	-4.02 ± 0.512
n-hexane	-12.36 ± 0.256

Results are expressed as mean ± SD *

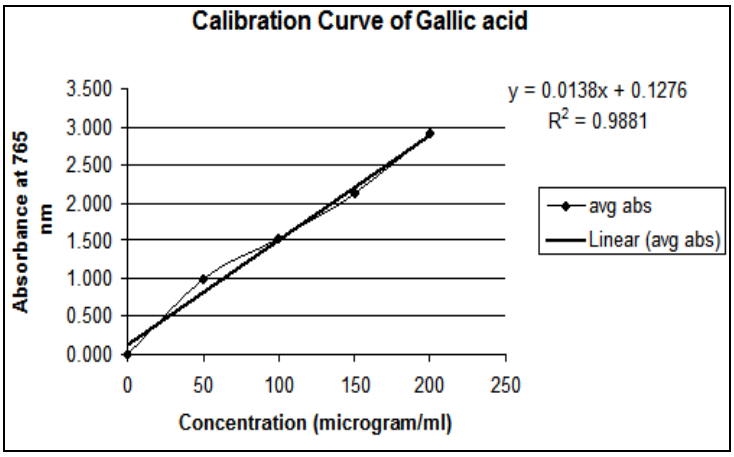

FIG. 3: CALIBRATION CURVE OF GALIC ACID

DISCUSSION: Isolation of pure, pharmacologically active constituents from plants remains a long and tedious process. For this reason, it is necessary to have methods available which eliminate unnecessary separation procedures. Chemical screening is thus performed to allow localization and targeted isolation of new or useful constituents with potential activities. This procedure enables recognition of known metabolites in extracts or at the earliest stages of separation and is thus economically very important.

Flavonoids, (a large group of naturally occurring plant polyphenolic compounds including flavones, flavonols, isoflavones, flavonones and chalcones), also known as nature's tender drugs, possess numerous biological and/or pharmacological activities. Recent reports of antiviral, anti-fungal, antioxidant, anti-inflammatory, antiallergenic, antithrombic, anticarcinogenic, hepatoprotective, and cytotoxic activities of flavonoids have generated interest in studies of flavonoid-containing plants. Flavonoids have demonstrated to exert beneficial effects on some diseases involving lipid peroxidation. The capability to interact with protein phosphorylation and the antioxidant, iron-chelating, and free radical scavenging activity may account for the wide pharmacological profile of flavonoids [27-32].

Alkaloid itself plays very important role as a natural antioxidant. The isoquinoline alkaloids (stylopine, protopine, fumaritine, fumaricine, fumarophycine, fumariline, fumarofine) possess various kinds of pharmacological properties. The antimicrobial, antimalarial, cytotoxic, and anti HIV activities of the isoquinoline alkaloids have been reported and the

possible chemopreventive antitumor promoters are probably related to their radical scavenging activity against DPPH radical [33]. Phenolic alkaloids also serve as a new class of antioxidant agents of various medicinal plants. Antioxidant activities of different phenolic alkaloids, i.e., oleracein A, oleracein B and oleracein E, was also reported based on scavenging activity against DPPH radical and inhibitory effect on hydrogen peroxide-induced lipid peroxidation in rat brain homogenates [16]. Recent reports reveal that alkaloids can be used as good sources of natural antioxidants for medicinal and commercial needs [34].

Antioxidants that scavenge free radicals play an important role in cardiovascular disease, aging, cancer, and inflammatory disorders [35]. In addition, these naturally occurring antioxidants can be formulated to give nutraceuticals, which can help to prevent oxidative damage from occurring in the body. One way of estimating antioxidant activity is by the use of the stable free radical DPPH [36-38]. Results of the present study **(table 2)** show that the activities of the ethanol, ethyl acetate and n-hexane extract of *L. aspera* are moderately comparable to L-ascorbic acid. This is understandable since L-ascorbic acid is already in a pure form, while the plant extracts still need to be processed in order to isolate the compounds responsible for their antioxidant activity. However, this assay may be used to guide the fractionation and isolation of potential antioxidant compounds from these plant extracts.

Numerous plant constituents have proven to show free radical scavenging or antioxidants activity [39]. Phenols are very important plant constituents. There is a highly positive relationship between total phenols and antioxidant activity of many plant species, because of the scavenging ability of their hydroxyl groups [40]. The phenolic contents of the extract can also scavenge hydrogen peroxide by donating electrons and thereby neutralizing it to water [41]. It was also reported that phenolic compounds are effective hydrogen donors, making them very good antioxidants [42]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing super oxide anion ($O_2^{-\bullet}$), hydroxyl radical or peroxy radicals, quenching singlet and triplet oxygen or decomposing peroxides [43]. Polyphenolic contents appear to function as good electron and hydrogen atom donors, and therefore, be able to terminate radical chain reaction involved in lipid peroxidation by converting free radicals and reactive oxygen species to more stable products. Thus, the antioxidant activity of *L. aspera* extracts might be attributed to these modes of activity due to their flavonoid and alkaloid contents.

CONCLUSION: The present study indicates that the ethanol, ethyl acetate and n-hexane extracts of the whole plant *L. aspera* have got profound antioxidant effect and may have potential use in medicine. But ethanol extract was shown to possess more activity. From the previous studies and our current investigation it may be concluded that the flavonoids and alkaloids are responsible for aforementioned activity. This novel finding will aid us to conduct bioactivity guided isolation and characterization of leading compounds in due course.

ACKNOWLEDGEMENT: We gratefully acknowledge Professor SK Choudhury, Department of Pharmacy, Jahangirnagar University for his active support to conduct the antioxidant activity.

REFERENCES:

1. Regine, K: In *Oxidative Stress: Oxidants and Antioxidants*; Helmut, S., Ed.; Academic Press: London, 1991; pp 245-273.
2. Finkel T and Holbrook NJ: Oxidants, oxidative stress and biology of ageing. Nature 2000; 408:239-247.
3. Halliwell B, Gutteridge JMC and Cross CE: Free-radicals, antioxidants and human diseases: Where are we now? J Lab Clin Med 1992; 119:598-620.
4. Knight JA: Diseases related to oxygen-derived free radicals. Ann Clin Lab Sci 1995; 25: 111-121.
5. Petrone WF, English DK, Wong K and Mccord JM: Free-radicals and inflammation: superoxide dependent activation of a neutrophil activating factor in plasma. Proc Natl Acad Sci USA 1980; 77:1159-1163.
6. Visioli F, Kearey JF and Halliwell B: Antioxidants and cardiovascular diseases: panaceas or tonics for tired sheep? Cardiovasc Res 2000; 47:409.
7. Branien AL: Toxicoly and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. JAOCS 1975; 52:59-63.
8. Ito N. Fukushima S. Hassegawa A. Shibata M. and Ogiso T. Carcinogenicity of butylated hydroxyanisole in F344 rats. J Natl. Cancer Inst. 1983; 70:343-347.
9. Dudonne S, Vitrac S, Coutiere P, Woillez M and Merillon JM: Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH,

ABTS, FRAP, SOD and ORAC assays. J. Agric. Food Chem. 2009; 57:1768-1774.
10. Balick JM and Cox PA: Plants, People and Culture: the Science of Ethnobotany. Scientific American Library, New York, 1996:228.
11. Cotton CM: Ethnobotany: Principle and Application. John Wiley and Sons, New York, 1996:399.
12. Akinmoladun AC, Ibukun EO, For EA, Obuotor EM and Farombi EO: Phytochemical constituent and antioxidant activity of extract from the leaves of Ocimum gratissimum. Sci. Res. Essay 2007; 2:163-166.
13. Kähkönen MP, Hopia AI, Vuorela HJ, Rauuha JP, Pihlaja K, Kujala TS and Heinonen M: Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem 1999; 47: 3954-3962.
14. Valenzuela A, Sanhueza J and Nieto S: Cholesterol oxidation: Health hazard and the role of antioxidants in prevention. Biol Res. 2003; 36:291-302.
15. Larson RA: The antioxidants of higher plants. Phytochemistry 1988; 27:969-978.
16. Zijuan Y, Cejia L, Lan X and Yinan Z: Phenolic Alkaloids as a New Class of Antioxidants in *Portulaca oleracea*. Phytother. Res. 2009; 23(7):1032–1035.
17. Racková L, Májeková M, Kost'álová D and Stefek M: Antiradical and antioxidant activities of alkaloids isolated from Mahonia aquifolium. Structural aspects. Bioorg Med Chem.2004; 12(17):4709-4715.
18. Ha SC and Won SW: A Quinolone Alkaloid with Antioxidant Activity from the Aleurone Layer of Anthocyanin-Pigmented Rice. J. Nat. Prod. 2001; 64:1579-1580.
19. Kirtikar KR and Basu BD: In: Indian Medicinal Plants. Vol III, Deharadun: Lalit Mohan Basu; 1991; 2019.
20. Reddy KM, Viswanathan S, Thirugnanasabmantham D, Santa R and Lalitha K: Analgesic activity of *L. Aspera*. Fitoterapia, 1993; 64:151-154.
21. Haque M, Haque ME and Rahman MM: Antibacterial and cytotoxic activities of *Capparis zeylanica* Linn roots. Ars Pharmaceutica 2008; 49:31-37.
22. Harborne JB (eds.): Phytochemical Methods: A guide to modern techniques of plant analysis. 3rd ed. Chapman and Hall, London ISBN: 0-412-57270-2, 1998: 302.
23. Sazada S, Verma A, Rather AA, Jabeen F and Meghvansi MK: Preliminary phytochemicals analysis of some important medicinal and aromatic plants. Adv. in Biol. Res. 2009; 3:188-195.
24. Hasan MS, Ahmed MI, Mondal S, Uddin SJ, Masud MM, Sadhu SK and Ishibashi M: Antioxidant, antinociceptive activity and general toxicity study of *Dendrophthoe falcata* and isolation of quercitrin as the major component. OPEM 2006; 6:355-360.
25. Prieto P, Pineda M, and Aguilar M: Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem. 1999; 269:337–41.
26. Folin O and Ciocalteu V: On tyrosine and tryptophane determination in proteins. J. Biol. Chem. 1927; 27:627-650.
27. Saija A, Scalese M, Lanza M, Marzullo D and Bonina F: Flavonoids as antioxidant agents: importance of their interaction with biomembranes. Free Rad Biol Med. 1995; 19(4):481-486.
28. Moon YJ, Wang X and Morris ME: Dietary flavonoids: Effects on xenobiotic and carcinogen metabolism. Toxicology 2006; 20:187-210.
29. Veitch NC: Isoflavonoids of the Leguminosae. Nat. Prod. Rep. 2007; 24:417-464.
30. Jiang H, Zhan WQ, Liu X and Jiang SX: Antioxidant activities of extracts and flavonoid compounds from *Oxytropis falcate* Bunge. Nat. Prod. Res. 2008; 22(18):1650-1656.
31. Kim HP, Son KH, Chang HW and Kang SS: Anti-inflammatory Plant Flavonoids and Cellular Action Mechanisms. J. Phamacol. Sci. 2004; 96:229-245.
32. van Acker SA, van den Berg DJ, Tromp MN, et al: Structural aspects of antioxidant activity of flavonoids. Free Rad Biol Med. 1996; 20:331-342.
33. Cui WH, Iwasa K, Tokuda H, Kashihara A, Mitani Y, Hasegawa T, Nishiyama Y, Moriyasu M, Nishino H, Hanaoka M, Mukai C and Takeda K: Potential cancer chemopreventive activity of simple isoquinolines,l-benzylisoquinolinjes, and protoberberines. Phytochemistry 2006; 67(1):70-79.
34. Fadila M-B, Sabiha K, Khalida B, Mohamed C, Sandrine A, Yves C, Henry M and Dominique L-M: Antioxidant activities of alkaloid extracts of two Algerian species of *Fumaria: Fumaria capreolata* and *Fumaria bastardii.* Rec. Nat. Prod. 2007; 1(2-3):28-35.
35. Cioffi G, D'Auria M, Braca A, Mendez J, Castillo A, Morelli I, De Simone F and De Tommasi N: Antioxidant and Free Radical Scavenging Activity of Constituents of the Leaves of *Tachigalia paniculata.* J. Nat. Prod. 2002; 65:1526-1529.
36. Molyneux P: The use of the stable free radical diphenylpicrylhydrazy (DPPH) for estimating antioxidant activity. Songklanakarin J. Sci. Technol. 2003; 26(2):211- 219.
37. Brand-Williams W, Cuevelier ME and Berset C: Use of a free radical method to evaluate antioxidant activity. Lebensm. Wiss. U-Technol. 1995; 28:25-30.
38. Moon JK and Shibamoto T: Antioxidant assays for plant and food components. J. Agric. Food Chem. 2009; 57(5):1655-1666.
39. Aruoma OI and Cuppett SL: Antioxidant methodology In vivo and In vitro concepts. AOCS press, Champaign, Illinois, 1997; 41-172.
40. Vinson JA, Yong H, Xuchui S and Zubik L: Phenol antioxidant quantity and quality in foods: vegetables. J. Agric. Food Chem. 1998; 46:3630-3634.
41. Ebrahimzadeh MA, Nabavi SM, Nabavi SF, Eslami B and Ehsanifar S: Antioxidant activity of *Hyoscyamus squarrosus* fruits. Pharmacologyonline 2009; 2:644-650.
42. Yen GC, Duh PD and Tsai CL: The relationship between antioxidant activity and maturity of peanut hulls. J. Agric. Food Chem. 1993; 41:67-70.
43. Osawa T: Novel natural antioxidants for utilization in food and biological systems; Post harvest biochemistry of plant food materials in the tropics, Japan Scientific Societies Press, Tokyo, Japan, 1994; 241- 251.

****