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STUDY OF WHOLE PLANT OF *VERNONIA CINEREA* LESS. FOR *IN VITRO* ANTIOXIDANT ACTIVITY

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ABSTRACT

In vitro antioxidant activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of whole plant of *Vernonia cinerea* Less. have been tested using various antioxidant model system viz, DPPH, hydroxyl, superoxide, ABTS and reducing power. Methanol extract of *V. cinerea* is found to possess higher DPPH radical scavenging activity. Petroleum ether extract of *V. cinerea* exhibited highest hydroxyl, superoxide and ABTS radical cation scavenging activity. Petroleum ether extract of *V. cinerea* showed the highest reducing ability. This study indicates significant free radical scavenging potential of *V. cinerea* which can be exploited for the treatment of various free radical mediated ailments.

Key words: *Vernonia cinerea*, Antioxidant activity, Methanol, DPPH, ABTS.

INTRODUCTION

Medicinal plants are commonly rich in phenolic compounds, such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins. These compounds have multiple biological effects including antioxidant activity.[1] *In vitro* experiments on antioxidant compounds in higher plants show how they protect against oxidation damage by inhibiting or quenching free radicals and reactive oxygen species.[2] The role of these compounds as potential antioxidants can be inferred by their similarity to synthetic antioxidants of related structures. To be able to identify the medicinal potency of any plants, the *in vitro* antioxidant activity assay must be carried out on the selected plants as the first step. The present study deals with evaluation of the antioxidant activity of whole plant of *V. cinerea. V. cinerea* (Family: Asteraceae) is a terrestrial annual erect herb. It grows up to 80cm high. It can be found in roadside, open waste places, dry grassy sites and in perennial crops during plantation. It is located especially in different Asian countries such as India, Bangladesh and Nepal. Stems are rounded solid hairy. Leaves are alternate spiral, elliptic and the length is more than 2 cm long/wide. Flowers are bisexual grouped together in a terminal head.[3] It has many therapeutic uses in different traditional medicine of the world. Different parts of the plants are of different therapeutic values to mention a few it could be used as antimalarial, astringent, anthelmintic, anti-diarrhoeal and anti-viral activity. It is commonly known as sahdevi or little iron wood.[4] Root Decoction is used in the treatment of diabetes mellitus.[5] Stem is used in the treatment of human breast cancer.[6] A large number of different plants have been studied as new sources of natural antioxidant.[7-9] For the first time, we report here the antioxidant properties of different extracts of *V. cinerea.*

MATERIALS AND METHODS

Collection and processing: The whole plant of *Vernonia cinerea* Less. were collected from V. O. Chidambaram College campus, Thoothukudi, Tamil Nadu respectively. The collected samples were cut into small fragments and shade dried until the fracture is uniform and smooth. The dried plant material was granulated or powdered by using a blender, and sieved to get uniform particles by using

sieve No. 60. The final uniform powder was used for the extraction of active constituents of the plant material.

Preparation of plant extract: The coarse powder (100g) was extracted successively with petroleum ether, chloroform, ethyl acetate, acetone and methanol, each 250 ml in a Soxhlet apparatus for 24 hrs. All the extracts were filtered though Whatman No.41 filter paper. . All the extracts were concentrated in a rotary evaporator. The concentrated extracts were used for *in vitro* antioxidant activity. The methanol extract was used for the estimation of total phenolics and flavonoids.

Estimation of Total Phenolics: Total phenolic content was estimated using Folin-Ciocalteau reagent based assay as previously described [10] with little modification. To 1mL of each extract (100µg/mL) in methanol, 5mL of Folin-Ciocalteau reagent (diluted ten-fold) and 4mL (75g/L) of Na_2CO_3 were added. The mixture was allowed to stand at 20ºC for 30min and the absorbance of the developed colour was recorded at 765nm using UV-VIS spectrophotometer. 1mL aliquots of 20, 40, 60, 80, 100 µg/mL methanolic gallic acid solutions were used as standard for calibration curve. The absorbance of solution was compared with gallic acid calibration curve. The total phenolic content was expressed as gallic acid equivalents (GAE g/100g dry weight of extract).

Estimation of Flavonoids: The flavonoids content was determined according to Eom *et al.*[11] An aliquot of 0.5ml of sample (1mg/mL) was mixed with 0.1ml of 10% aluminium chloride and 0.1ml of potassium acetate (1M). In this mixture, 4.3ml of 80% methanol was added to make 5mL volume. This mixture was vortexed and the absorbance was measured spectrophotometrically at 415nm. The value of optical density was used to calculate the total flavonoid content present in the sample.

DPPH radical scavenging activity: The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non radical form DPPH-H.[12] The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method.[12] Briefly, an 0.1mM solution of DPPH in methanol was prepared, and 1mL of this solution was added to 3 ml of the solution of all extracts in methanol at different concentration (50,100,200,400 & 800µg/mL).The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10S UV: Thermo electron corporation). Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability of scavenging the DPPH radical was calculated by using the following formula.

DPPH scavenging effect (% inhibition) = $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged

Hydroxyl radical scavenging activity: The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell *et al*.[13] Stock solutions of EDTA (1mM), FeCl3 (10mM), Ascorbic Acid (1mM), H_2O_2 (10mM) and Deoxyribose (10 mM), were prepared in distilled deionized water. The assay was performed by adding 0.1mL EDTA , 0.01mL of $FeCl_3$,0.1mL H_2O_2, 0.36mL of deoxyribose, 1.0mL of the extract of different concentration (50, 100, 200, 400 &800µg/mL) dissolved in distilled water,0.33mL of phosphate buffer (50mM , pH 7.9), 0.1mL of ascorbic acid in sequence . The mixture was then incubated at 37^0C for 1 hour. 1.0mL portion of the incubated mixture was mixed with 1.0mL of 10%TCA and 1.0mL of 0.5% TBA (in 0.025M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532nm. The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

Superoxide radical scavenging activity: The superoxide anion scavenging activity was measured as described by Srinivasan *et al*.[14] The superoxide anion radicals were generated in 3.0 ml of Tris – HCL buffer (16 mM, P^H 8.0), containing 0.5 mL of NBT (0.3mM), 0.5 ml NADH (0.936mM) solution, 1.0 mL extract of different concentration (50,100,200,400 & 800µg/mL), and 0.5 mL Tris – HCl buffer (16mM, P^H 8.0). The reaction was started by adding 0.5 mL PMS solution (0.12mM) to the mixture, incubated at 25^oC for 5 min and the absorbance was measured at 560 nm against a blank sample, ascorbic acid. The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula

Antioxidant activity by radical cation (ABTS. +): ABTS assay was based on the slightly modified method of Huang *et al*.[15] ABTS radical cation (ABTS+) was produced by reacting 7mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS + Solution were diluted with ethanol to an absorbance of 0.70+0.02 at 734 nm. After addition of 100µL of sample or trolox standard to 3.9 mL of diluted ABTS+ solution, absorbance was measured at 734 nm by Genesys 10S UV-VIS (Thermo scientific) exactly after 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC). The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

Reducing power: The reducing power of the extract was determined by the method of Kumar and Hemalatha.[16] 1.0 mL of solution containing 50,100,200,400 &800µg/mL of extract was mixed with sodium phosphate buffer (5.0 mL, 0.2 M, pH6.6) and potassium ferricyanide (5.0 mL, 1.0%): The mixture was incubated at 50^{o}C for 20 minutes. Then 5mL of 10% trichloroacetic acid was added and centrifuged at 980 g (10 minutes at 5^{o}C) in a refrigerator centrifuge. The upper layer of the solution (5.0 mL) was diluted with 5.0 mL of distilled water and ferric chloride and absorbance read at 700 nm. The experiment was performed thrice and results were averaged.

Statistical analysis: Antioxidant activities like DPPH radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical activity, ABTS radical cation scavenging activity and reducing powers were estimated in triplicate determinations. Data were analyzed using the statistical analysis system SPSS (SPSS software for windows release 17.5; SPSS Inc., Chicago IL, USA) Estimates of mean, standard error for aforesaid parameters were calculated.

RESULT

Total phenolic content and total flavonoid content: The total phenolic and flavonoid content of methanol extract of *V. cinerea* was found to be 0.147g 100g^{-1} and 1.2g 100g^{-1} respectively.

DPPH radical scavenging activity: DPPH radical scavenging activity of petroleum ether, chloroform, ethyl acetate, acetone and methanol extracts of whole plant of *V. cinerea* are shown in figure 1. Among the solvent tested, methanol extract exhibited highest DPPH radical scavenging activity. At 800µg/mL concentration, methanol extract of whole plant of *V. cinerea* possessed 112.16% scavenging activity on DPPH. The IC_{50} values were found to be 28.16 and 19.14µg/mL respectively for methanol extract and ascorbic acid (Table 1).

Hydroxyl radical scavenging activity: Hydroxyl radical scavenging activity of petroleum ether, chloroform, ethyl acetate, acetone and methanol extracts of whole plant of *V. cinerea* are presented in figure 2. Petroleum ether extract of whole plant of *V. cinerea* (800µg/mL) exhibited the maximum hydroxyl scavenging activity (89.05%). IC_{50} values were found to be 23.16 and 21.94µg/mL respectively for petroleum ether extract and ascorbic acid (Table 1).

Superoxide radical scavenging activity: The *V. cinerea* whole plant extracts were subjected to the superoxide radical scavenging activity and the results are given in figure 3. Among the solvent tested, petroleum ether extract of whole plant of *V. cinerea* exhibited the maximum superoxide radical scavenging activity. At 800µg/mL concentration, petroleum ether extract of *V. cinerea* whole plant showed 113.85% scavenging activity on superoxide radical. IC_{50} values were found to be 28.31 and 23.16µg/mL respectively for petroleum ether extract and ascorbic acid (Table 1).

ABTS radical scavenging activity: The *V. cinerea* whole plant extracts were subjected to the ABTS radical cation scavenging activity and the results are presented in figure 4. The petroleum ether extract of whole plant of *V. cinerea* exhibited potent ABTS radical cation scavenging activity in concentration dependent manner. At 800µg/mL concentration, *V. cinerea* whole plant extract possessed 105.25% scavenging activity on ABTS which is higher than the standard trolox whose scavenging activity is 92.81%. IC_{50} values were found to be 26.81 and 21.67µg/mL respectively for petroleum ether extract and trolox (Table 1).

Reducing power: Figure 5 showed the reducing ability of different solvent extracts of *V. cinerea* whole plant compared to standard ascorbic acid. Absorbance of the solution was increased when concentration increased. Among the solvent tested, petroleum ether exhibited highest reducing power activity.

DISCUSSION

Phenolic compound, the principal antioxidant constitutes of natural plant products, are composed of phenolic acid and flavonoids.[17] These compounds are potent radical terminations by donating a hydrogen atom to the radical and preventing lipid oxidation at the initial step. The high potential of polyphenols to scavenge free radical may be because of their many phenolic hydroxyl groups. In this respect, polyphenolic compounds commonly found in plants have been reported to have multiple biological effects like anticancer, antiproliferative, antimicrobial, wound healing and antibacterial activities including antioxidant activity.[18-21] Hence the present study exposed that the methanol extract of *V. cinerea* hold high amount of phenols and flavonoids, petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *V. cinerea* is examined for their antioxidant property. The 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical is widely used as a model system to investigate the free radical scavenging activities of several plant extracts. DPPH is stable, nitrogen centered free radical which produces violet color in ethanol solution. It was reduced to a yellow colored product, diphenylpicryl hydrazine, with the addition of the extracts. The reduction in the number of DPPH molecules can be calculated with the number of available hydroxyl groups.[22] The result indicates that the different extract of *V. cinerea* showed concentration dependent scavenging effect against DPPH radical. This showed that the extract with their proton donating ability, could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.[23]

The hydroxyl radical (generated secondarily by the reaction of superoxide and hydrogen peroxide) actually brings about depolymerisation of hyaluronic acid as an OH^-scavenger can return the viscosity of hyaluronic acd solution. Hydroxyl radical (OH-) is closely associated with inflammatory disorders like arthritis where a progressive loss of hyaluronic acid in joint is an important feature disease.[24] The results of the present study showed that hydroxyl radical scavenging effect of *V. cinerea* is dependent on the concentration of the extract. Superoxides are produced from molecules oxygen due to oxidative enzymes.[25] of body as well as via non enzymatic reaction such as antioxidation by catecholamines.[26] The decrease in absorbance at 560nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. Based on the result, it appears that *V. cinerea* scavenges superoxide radicals by combining with superoxide radical ions to form stable radicals, thus terminating the radical chain reaction.[27] ABTS radical is a blue chromophore produced by the reaction of ABTS and potassium persulphate after incubation in the dark environment.[28] The decolorization of ABTS cation reflects the capacity of an antioxidant species donate electrons or hydrogen atoms to deactivate these radical species.[29] The ABTS assay is based on the inhibition of the absorbance of the radical cation ABTS, which has a characteristic long wavelength absorption spectrum. The present study indicates that the different extracts of *V. cinerea* showed higher ABTS radical scavenging activity in concentration dependent manner.

The reducing capacity of a compound may serve as an important indicator of its potential antioxidant activity.[30] The reducing properties are generally associated with the presence of reductones.[31] According to Gordan,[32] antioxidant action of reductones is based on the breaking of the free radical chain by donating a hydrogen atom. In the present study, the higher absorptions at higher concentration indicate that the strong reducing power potential of the different extracts of *V. cinerea*. It is suggested that the extract have high redox potentials and can act as reducing agents.

Several reports have conclusively shown close relationship between total phenolic content and antioxidative activity of the plant extracts.[33-35] Since the chemical composition and structures of active extract components are important factors governing the efficacy of natural antioxidants, the antioxidant activity of an extract could not be explained on the basis of their phenolic content, which also needs their characterization.[36] For instance, it has been reported that phenolic compounds with ortho and para dihydroxylation or a hydroxyl and a methoxy group are more effective than simple phenols.[37-38] Polyphenolic compounds such as flavonoids, phenolic acids and tannins are considered to be the major contributors to the antioxidant activity of fruits, vegetables and medicinal plants. Phenol and phenolic compounds such as flavonoids have been shown to possess significant antioxidant activities and their effects on human nutrition and health are considerable.[39] However, synergistic or additive actions of the phenolics present in the extracts cannot be ruled out. This is the first report that envisages the antioxidant activities of different solvent extracts of whole plant of *V. cinerea*. Hence, the whole plant of *V. cinerea* could be a good source of antioxidant which can be used as a potential tool in the treatment of disorders associated to oxidative stress, cardiovascular arrests, inflammation, cancer and diarrhea.

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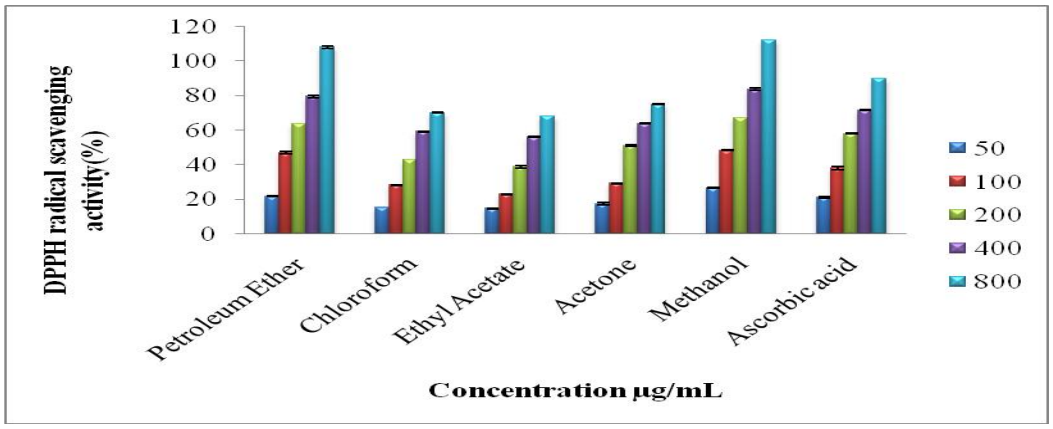

Figure 1. DPPH radical scavenging activity of different extracts of *Vernonia cinerea*

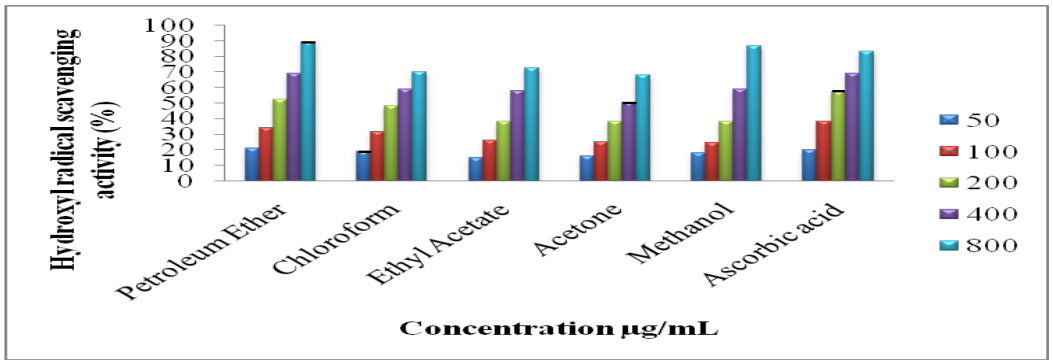

Figure 2. Hydroxyl radical scavenging activity of different extracts of *Vernonia cinerea*

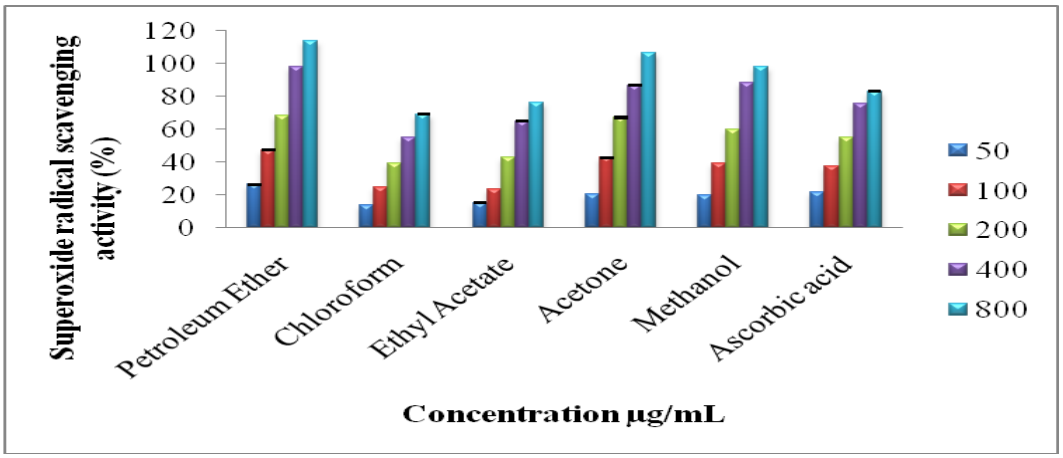

Figure 3. Superoxide radical scavenging activity of different extracts of *Vernonia cinerea*

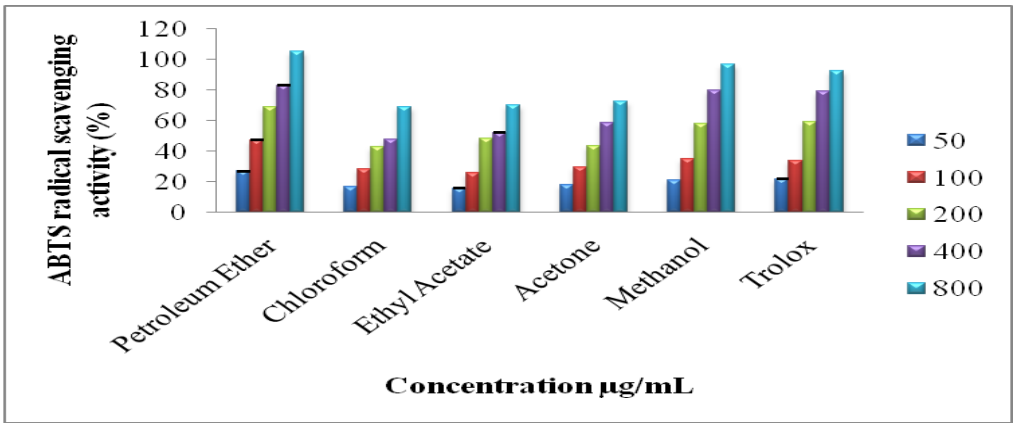

Figure 4. ABTS radical cation scavenging activity of different extracts of *Vernonia cinerea*

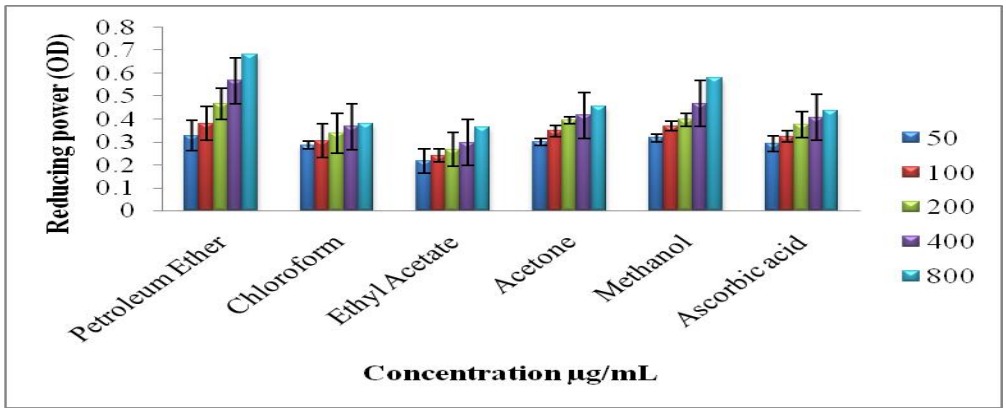

Figure 5. Reducing power ability of different extracts of *Vernonia cinerea*

Table 1. IC_{50} values of different extracts of *Vernonia cinerea*

Solvent	IC_{50} (µg/mL)			
	DPPH	**Hydroxyl**	**Superoxide**	**ABTS**
Petroleum ether	27.26	23.16	28.31	26.81
Chloroform	17.28	19.02	17.13	19.54
Ethyl acetate	16.13	19.36	18.93	19.89
Acetone	18.65	18.33	28.81	20.85
Methanol	28.16	22.34	26.16	24.13
Ascorbic acid	19.14	21.94	23.16	-
Trolox	-	-	-	21.67

REFERENCES

1. Packer L, Rimbach G and Virgili F. Free Rad. Biol. Med, 1999; 27: 704-724.
2. Ali BH, Blunden G, Tanira MO, and Nemmar A. Food Chem. Toxicol, 2008; 46: 409-420.
3. Gani A. Medicinal Plants of Bangladesh with chemical constituents and uses. 2nd ed., Asiatic Society of Bangladesh, 5 old Secretariate road, Nimtali, Dhaka, Bangladesh. pp .270-271, 2003.
4. Wealth of India, 1956, CSIR, Vol-IV, 448-449.
5. Mishra AS. J. crude res, 1984; 123: 1012-1028.
6. Yao-haur kuo, Yu-Jenkuo, Ang-Suyu, Ming-Derwu. Chem. Pharmaceu. Bull, 2003; 51: 425.
7. Cakir A, Mavi A, Yıldırım A, Duru ME, Harmandar M, Kazaz C. J. Ethnopharmacol, 2003; 87:73–83
8. Lee KY, Weintraub ST, Yu BP. Free Radical Biology & Medicine, 2000; 28: 261–265
9. Kumaran A, Karunakaran R. Food Chemistry, 2007; 100: 356–361
10. McDonald S, Prenzler PD, Antolovich M, Robards K. Food Chem. 2001; 73:73-84.
11. Eom SH, Cheng WJ, Hyoung JP, Kim EH, Chung MI, Kim MJ, Yu C, Cho DH. Kor J Med Crop Sci. 2007; 15: 319-323.
12. Shen Q, Zhang B, Xu R, Wang Y, Ding X, Li P. Anaerobe. 2010; 16: 380-386.
13. Halliwell B, Gutteridge JMC, Aruoma OI. Ana. Biochem. 1987; 65: 215-219.
14. Srinivasan R, Chandrasekar MJN, Nanjan MJ, Suresh B. J. Ethnopharmacol, 2007; 113: 284-291.
15. Huang MH, Huang SS, Wang BS, Sheu MJ, Hou WC. J. Ethnopharmacology. 2011; 133: 743-750.
16. Kumar RS, Hemalatha S. J. Chem and Pharm Res. 2011; 3: 259-267.
17. Gulcin I, Kulrevioglu OI, Oktay M, Buyukokuroglu ME. J. Ethnopharma. 2004; 90: 205-215.
18. Packia Lincy M, Daffodil ED, Pon Esakki D, Mohan VR. Int. J. of Adv. in Pharm. Sci. 2013; 4:578-586.
19. Daffodil ED, Packia Lincy M, Pon Esakki D, Mohan VR. J. Har. Res in Pharm. 2013; 2:112-120.
20. Jenecius AA, Uthayakumari F, Mohan VR. Int. Res. J. Pharmacy, 2012; 3: 256-259.
21. Nishanthini A, Agnel Ruba A, Mohan VR. Int J. Adv. Life Sci. 2012; 5:34-43.
22. Abirami B, Gayathri P, Uma D. Int. J. Chem Pharmaceau Sci. 2012; 3:17-24.
23. Marxen K, Vanselow KH, Lippemeier S, Hintze R, Ruser A, Hansen UP. Sensors. 2007; **7**:2080–2095.
24. Panda BR, Mohanta SR, Manna KA, Si S. J. Advan. Pharmaceu. Res. 2011; 2: 18-23.
25. Sainani GS, Manika JS, Sainani RK. Medicines update, 1997; 1:1.
26. Hemmani T, Parihar MS. Indian J Physio and Pharmacol, 1994; 42:440.
27. Wang T, Jonsdottir R, Olafsdottir G. Food Chem. 2009; 116: 240-248.
28. Mbaebie BO, Edeoga HO and Afolayan AJ. Asian Pac. J. Trop. Biomed, 2012; 2: 118-124.
29. Pellegrini N, Re R, Yang M, Rice-Evans C. Met Enzymol. 1999; 299: 379-389.
30. Ho YL, Huang SS, Deng JS, Lin YH, Chang YS, and Huang GH. Botan. Stud, 2012; 53: 55-66.
31. Krishnamoorthy M, Sasikumar JM, Shamna R, Pandiarajan C, Sofia P. and Nagarajan, B. Indian J Pharmacol, 2011; 43:557-562.
32. Gordan MH. Food antioxidants. London/New York; Elsevier:1990..
33. Kalpana Devi V, Mohan, VR. Asian Pac. J. Trop. Biomed. 2012; 2: S386-S391.
34. Tresina, PS, Mary Jelastin Kala S, Mohan VR. J. Appl. Pharmaceu. Sci. 2012; 2: 112-124.
35. Daffodil ED, Mohan VR. World J. Pharm. Pharmaceu. Sci, 2013; 2:3710-3722
36. Heinonen M, Lehtonen PJ and Hopla A. J Agri.Food Chem, 1998; 8: 25-31.
37. Frankel EN, Waterhouse AL, Teissedre PL. J. Agric. Food Chem, 1995; 43: 890-894.
38. Shahidi F, Wanasundara PKPD. Crit. Rev. Food Sci. Nutri, 1992; 32: 67 - 103.
39. Kessler M, Ubeaud G and Jung L. J. Pharm. Pharmacol, 2003; 55: 131-142.