Research Article

In vitro Antioxidadive Profiling of *Smilax zeylanica* Aerial Parts

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ABSTRACT

In recent years, the search of natural antioxidants from plants has been of great interest to scientists. The present study was carried out to evaluate the antioxidant activities of different solvent extracts of aerial parts of *Smilax zeylanica* using different models viz: DPPH, hydroxyl, superoxide and ABTs radical cation scavenging activities. Methanol extract at 800µg/ml showed maximum superoxide radical scavenging (138.24%) of followed by hydroxyl radical scavenging activity (134.22%), ABTs scavenging activity (134.16%) and DPPH radical scavenging activity (106.36%) at the same concentration. Among solvent tested, methanol extract of *Smilax zeylanica* exhibited strong reducing ability (0.526). The results suggest that the methanol extract of *Smilax zeylanica* aerial part plays an important role in the modulation of oxidative stress.

Keywords: *Smilax zeylanica,* DPPH, free radicals, Total Phenolics.

INTRODUCTION

Antioxidants are substances that may protect your cells against the effects of free radicals. Free radicals are molecules produced when your body breaks down food, or by environmental exposures like tobacco smoke and radiation. Free radicals are atoms or molecules with singlet, i.e. unpaired electron which makes them highly reactive. Oxidative free radicals are generated by metabolic reactions create a chain of reaction leading to membrane and other lipid peroxidation, DNA damage, etc. This has been implicated in atherosclerosis, cancers, neurodegenerative and inflammatory bowel diseases. Many studies suggest that a diet high in antioxidants from fruits and vegetables is associated with a lower risk of cancer, cardiovascular disease, aging process, Parkinson's disease and Alzheimer's disease[1-3]. A plant based diet protects against chronic oxidative stress-related diseases. The antioxidants can interfere with the oxidation process by reaction with free radicals, chelating free catalytic metals and also by acting as oxygen scavengers. Dietary plant contains variable chemical families and considerable amount of antioxidants. It has been hypothesized that plant based antioxidants may contribute to the beneficial health effects.

Smilax zeylanica L. (Family- Smilacaceae), is a perennial climbing shrub which is locally known as Kumarilata and Indian smilax. It is used as medicinal plant in the treatment of various major and minor ailments. Traditionally, roots and leaves of *S. zeylancia* are used as a substitute for the official drug, Sarasparilla, in the treatment of venereal diseases; decoction is applied for rheumatism, pain in the lower extremities, sores swellings, and abscesses, and also used in the treatment of dysentery[4]. In the previous study, alcohol and aqueous extracts of roots and rhizomes of *S. zeylancia* have shown potential antiepileptic activity[5]. Phytochemical research has reported that it contains 1-3% steroidal saponins, phytosterols, starch, resin, sarsapic acid, and minerals[6]. Leaves and roots contain diosgenin[7]. Roots also contain large amounts of tannin, saponin, 31-norcycloartenol, beta-sitosterol, parillin, phenolic acid, and potassium nitrate. The saponin, on hydrolysis, yields the sapogenins, sarsasapogenin, asparagenin, and sapogenin[8].

Based on the above, the present study is conducted to quantify total phenolic and flavonoid content and to study *in vitro* antioxidant activity of various extracts of *S. zeylanica* aerial part so as to make researchers to route for other pharmacological activities.

MATERIALS AND METHODS

Collection and processing

The aerial parts of *Smilax zeylanica* L. were collected from Pechiparai, Kanyakumari District, Tamil Nadu, India. The plant specimen was identified and authenticated in Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. A voucher specimen was deposited in Ethno pharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin, Tamil Nadu. 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 through 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[9] 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*[10]. 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[11].

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[11].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 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.

$$\% \text{ inhibition} = \{(A_0 - A_1)/A_0)*100\}$$

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*[12]. Stock solutions of EDTA (1mM), $FeCl_3$ (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 (50 mM, pH 7.9), 0.1mL of ascorbic acid in sequence . The mixture was then incubated at 37^{0}C 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*[13]. The superoxide anion radicals were generated in 3.0 ml of Tris – HCL buffer (16 mM, pH 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, pH 8.0). The reaction was started by adding 0.5 mL PMS solution (0.12mM) to the mixture, incubated at 25^{o}C 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 ($ABTS^{\cdot+}$)

ABTS assay was based on the slightly modified method of Huang *et al*[14]. 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[15]. 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°C for 20 minutes. Then 5mL of 10% trichloro acetic acid was added and centrifuged at 980 g (10 minutes at 5°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 AND DISCUSSION

Total phenolic and flavonoid content

Polyphenolic compounds contribute significantly to the antioxidant activity of plant extract[16]. Phenols are one of the major groups of non-essential dietary components that have been associated with the inhibition of atherosclerosis and cancer, as well as age-related degenerative brain disorders[17]. Flavonoids are water soluble polyphenolic compounds, it can directly react with superoxide anion and lipid peroxyl radical and consequently inhibit or break the chain of lipid peroxidation[18].Flavonoids play some important pharmacological roles against diseases, such as cardiovascular disease, cancer, inflammation and allergy[19]. The total phenolic and flavonoid content of methanol extract of *Smilax zeylanica* aerial part were found to be 1.23 GAE g 100 g^{-1} and 1.01g 100 g^{-1} respectively. The high phenolic and flavonoid contents of methanol extract of *S. Zeylanica* aerial part may have contributed towards its antioxidant activity. The high potential of polyphenols to scavenge free radical may be because of their many phenolic hydroxyl groups. However, further studies are required to isolate the major phenolics and flavonoids present in the *S. Zeylanica* for its potent antioxidant activity.

DPPH radical scavenging activity

DPPH radical scavenging activity of petroleum ether, benzene, ethyl acetate, and methanol and ethanol extracts of aerial part of *Smilax zeylanica* is showed in fig. 1. This assay is one of the tests used to prove the ability of the components of plant extracts to act as donors of hydrogen atoms. DPPH, a protonated radical, has characteristic absorbance maximal at 517 nm, which decreases with the scavenging of the proton radical. This property has been widely used to evaluate the free radical scavenging effect of natural antioxidants[20]. All the extracts of *S. zeylanica* showed potent DPPH radical scavenging activity depending upon the increasing concentration. Among all the solvents tested, methanol extract at 800 µg/mL showed highest scavenging activity (106.36%) against DPPH radical with lowest IC_{50} value (25.77 µg/mL) when compared to standard ascorbic acid which shows 102.16% of scavenging activity against DPPH (IC_{50} value=28.65 µg/mL) (Table 1). The result indicates that this plant has a significant free radical scavenging activity on DPPH owing to its hydrogen donating ability.

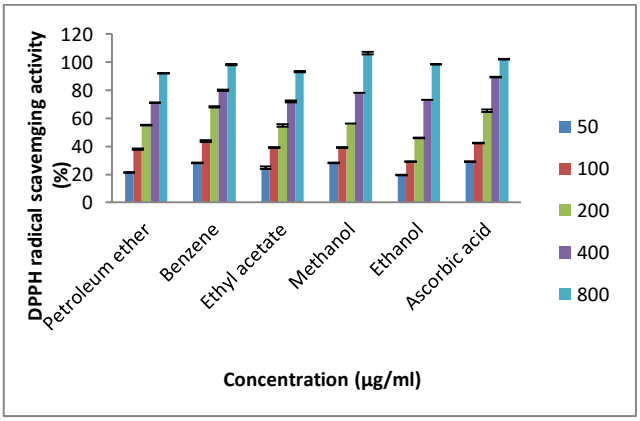

Figure 1: DPPH radical scavenging activity of different extracts of *Smilax zeylanica*

Table 1: IC_0 values of different extracts of *Smilax zeylanica*

Extracts	IC_{50} (µg/mL)			
	DPPH	Hydroxyl	Superoxide	ABTS
Petroleum ether	32.42	35.22	32.42	38.66
Benzene	29.91	36.23	38.67	45.82
Ethyl acetate	31.45	34.29	44.80	31.73
Methanol	25.77	29.49	28.93	22.74
Ethanol	28.94	30.18	29.56	24.29
Ascorbic acid	28.65	41.03	36.64	--
Trolox	-	-	-	33.56

Hydroxyl radical scavenging activity

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species capable of damaging bio molecules of the living cells[21-22]. Hydroxyl radical has the capacity to cause DNA strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity. In addition, this radical species is considered as one of the quick initiators of the lipid oxidation process[23-24]. Fig. 2

demonstrates hydroxyl radical scavenging activity of different extracts of *Smilax zeylanica.* Hydroxyl radical scavenging activity of different extracts of *S. zeylanica* increases with increasing concentration of extract. At 800 µg/mL methanol extract which has lowest IC_{50} value (29.49 µg/mL) showed highest scavenging activity (134.22%) against hydroxyl radical. At 800 µg/mL, ethanol, ethyl acetate and petroleum ether extract of *S. zeylanica* exhibited maximum scavenging activity of 126.16% ,118.26% and 103.13% respectively against hydroxyl radical with lowest IC_{50} value (30.18 µg/mL, 34.49 µg/mL and 35.22 µg/mL respectively) than standard ascorbic acid (98.56% and IC_{50} value = 36.23 µg/mL) (Table 1).The ability of the extracts to quench OH seems to be directly related to the prevention of lipid peroxidation and appears to be moderate scavenger of active oxygen species, thus reducing rate of chain reaction[25]. Hence, the *S. zeylanic a*aerial part extract can be considered as a good scavenger of hydroxyl radicals.

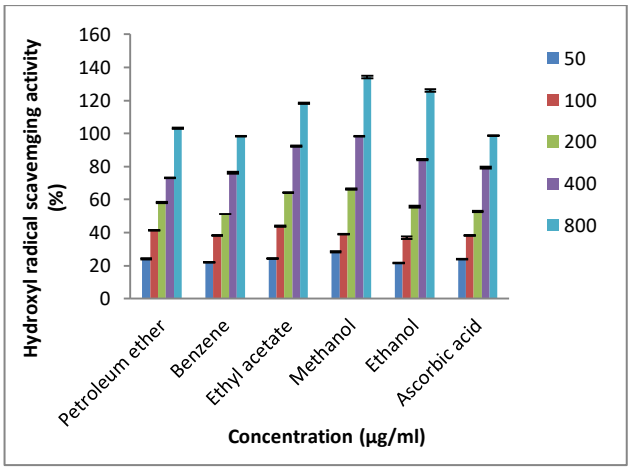

Figure 2: Hydroxyl radical scavenging activity of different extracts of *Smilax zeylanica*

Superoxide radical scavenging activity

Different extracts of *Smilax zeylanica* aerial part were subjected to superoxide radical scavenging activity and the results were expressed in fig. 3. Superoxide radicals are generated during the normal physiological process mainly in mitochondria. Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals that are very harmful to the cellular components[26] Hence superoxide radical scavenging by antioxidants has physiological implications. All the extracts showed effective scavenging activity against superoxide radical in concentration dependent manner. At 800 µg/mL, methanol, ethanol and petroleum ether extract of *S. zeylanica* exhibited maximum scavenging activity of 138.24%, 131.67% and 126.22% respectively against superoxide radical which have lowest IC_{50} value (28.93µg/mL, 29.56µg/mL and 32.42 µg/mL respectively) than standard ascorbic acid (36.64 µg/mL) (Table 1). High superoxide radical scavenging ability of *S. zeylanica* might be due to the high flavonoid content.

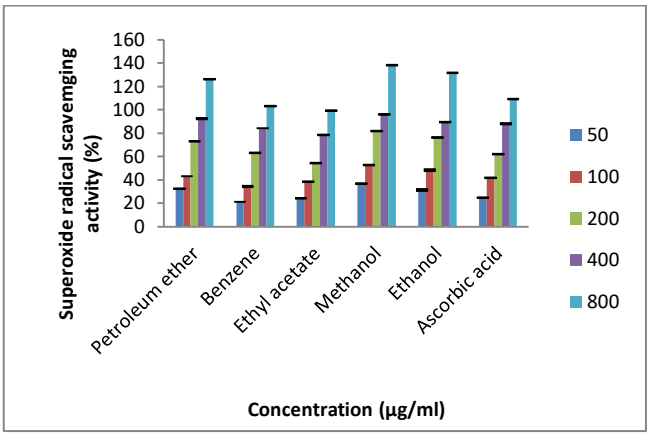

Figure 3: Superoxide radical scavenging activity of different extracts of *Smilax zeylanica*

ABTS radical cation scavenging activity

Decolourization of $ABTS^{\bullet+}$ reflects the capacity of antioxidant species to donate electrons or hydrogen atoms to inactivate this radical cation. $ABTS^{\bullet+}$ discoloration assay have been applied to the measurement of the total antioxidant activity of various sub-stances and wildly used in many recent studies for the detection of total antioxidant activity of essential oils[27-28]. At various concentrations ABTS radical cation scavenging activity of different extract of aerial part of *Smilax zeylanica* is shown in figure 3. It represents concentration dependent increased scavenging effect of different extracts of *S. Zeylanica* and standard trolox. Among the solvent tested, methanol and ethanol extract at 800 µg/mL showed highest scavenging activity (134.16% and 128.22% respectively) against ABTS radical with lowest IC_{50} value (22.74 µg/mL and 24.29µg/mL respectively) (Table 1). ABTS radical scavenging activity of ethyl acetate extract (102.16%) is higher than the standard trolox (101.22%). The $ABTS^{\bullet+}$ scavenging data of the present study suggests that the components present in *S. Zeylanica* are capable of scavenging free radicals through a mechanism of electron/hydrogen donation and should be able to defend free radical-mediated oxidative degradation.

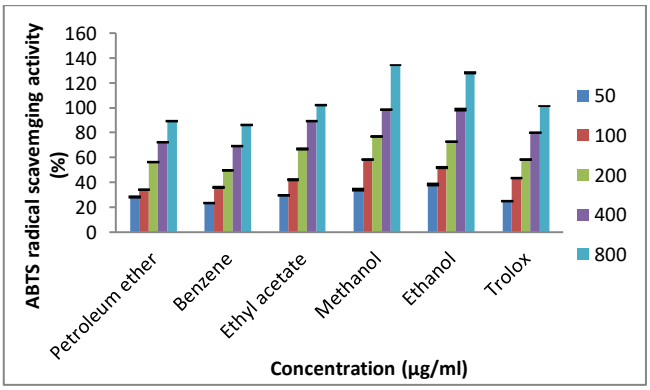

Figure 4: ABTS radical cation scavenging activity of different extracts of *Smilax zeylanica*

Reducing power

Reducing power indicates compounds that are electron donars, which can act as primary and secondary antioxidants. Reducing power of a compound may serve as a significant indicator of its potential antioxidant activity. Different studies have been shown that the reducing properties are generally associated with the presence of reduct ones, which have been shown to exert antioxidant action by breaking the free radical chain through the donation of a hydrogen atom[29-30]. Fig. 5 described the reducing power of different extracts of *Smilax zeylanica* aerial part. Reducing power of different extracts of *S. zeylanica* increased with increasing concentration of extracts. At 800 µg/mL, methanol, ethanol and petroleum ether extract of *S. zeylanica* showed potent reducing power of 0.526%, 0.498% and 0.456% respectively than standard ascorbic acid (0.451%). The antioxidant activity has been reported to be concomitant with the development of reducing power[31]. High reducing power of *S. zeylanica* aerial part extracts indicates its free radical scavenging ability.

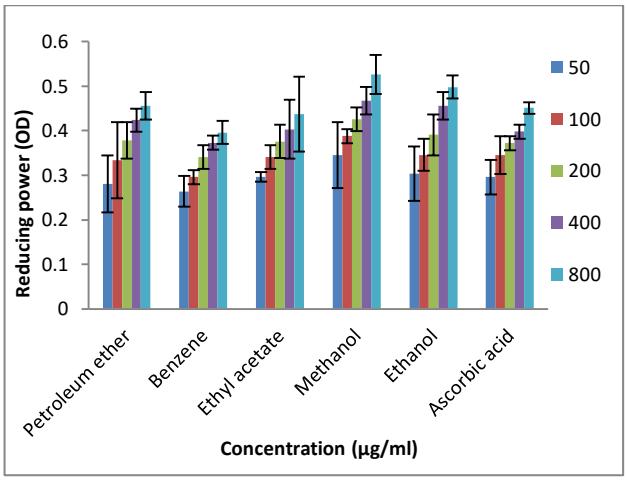

Figure 5: Reducing power of different extracts of *Smilax zeylanica*

CONCLUSION

This result of the present study suggests that the alcoholic extracts of *Smilax zeylanica* contain promising antioxidant substance which is responsible for its antioxidant activity. So, it may be considered in future to replace synthetic preservatives in food and pharmaceutical products. Further studies are needed to clarify the *in vivo* potential of this plant in the management of human diseases resulting from oxidative stress

REFERENCES

1. Vant Veer P, Jansen MC, Klerk M, Kok FJ. Fruits and vegetables in the prevention of cancer and cardiovascular disease. Public Health Nutr, 3, 2003, 103-107.
2. Bokov A, Chaudhuri A, Richardson A. The role of oxidative damage and stress in aging. Mech Ageing Dev, 125, 2004, 811-826.
3. Madamanchi NR, Vendrov A, Runge MS. Oxidative stress and vascular disease. Arterioscler. ThrombVascBiol, 25, 2005, 29-38.
4. Chopra RN. Indigenous drugs of India. Academic Publishers,Calcutta, 1992.
5. Madhavan V, Hemalatha HT, Murali A, Yoganarasimhan SN. Antiepileptic activity of alcohol and aqueous extracts ofroots and rhizomes of *Smilax zeylancia* Linn. Pharmacology online, 3, 2008, 263-272.
6. Chevallier A. The Encyclopedia of Medicinal Plants, 1st edn.DK Publishing Inc., New York, 1996, 55-281.
7. Kar DK, Sen S. A procedure for regeneration & propagation of *Smilax zeylanica* L. CurrSci, 53, 1984, 661.
8. Leung AY. Encyclopedia of common natural ingredients usedin food, drugs, and cosmetics. John Wiley, New York, 1980.
9. McDonald S, Prenzler PD, Antolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. Food Chem, 73, 2001,73-84.
10. Eom SH, Cheng WJ, Hyoung JP, Kim EH, Chung MI, Kim MJ, Yu C, Cho DH. Far infrared ray irradiation stimulates antioxidant activity in *Vitis flexuosa* Thunb. Berries. Kor. J. Med Crop Sci, 15, 2007, 319-323.
11. Shen Q, Zhang B, Xu R, Wang Y, Ding X, Li P. Antioxidant activity *in vitro* of selenium-contained protein from the se-enriched. *Bifodobacterium animalis* 01. Anaerobe, 16, 2010, 380-386.
12. Halliwell B, Gutteridge JMC, Aruoma OI. The deoxyribose method: a simple test to be assay for determination of rate constants for reaction of hydroxyl radicals. Ana Biochem, 65, 1987, 215-219.
13. Srinivasan R, Chandrasekar MJN., Nanjan MJ, Suresh B. Antioxidant activity of *Caesalpiniadigyna* root. J Ethnopharmacol, 113, 2007, 284-291.
14. Huang MH, Huang SS, Wang BS, Sheu MJ, Hou WC. Antioxidant and anti-inflammatory properties of *Cardiospermum halicacabum* and its reference compounds *ex vivo* and *in vivo.* J. Ethnopharmacol, 133, 2011,743-750.
15. Kumar RS, Hemalatha S. *In vitro* antioxidant activity of alcoholic leaf extract and subfractions of *Alangium lamarckii* Thwaites. JChem and Pharm Res, 3, 2011, 259-267.
16. Rao KS, Munjuluri PR, Kumar BVVR, Keshar NR. Evaluation of *in vitro* antioxidant activity and total phenolic content of methanol bark extract of *Mimus opselengi.* Free Rad Antiox, 1, 2011, 62-71.
17. Cheung LM, Cheung PC, Ooi VEC. Antioxidant activity and total phenolics of edible mushroom extracts. Food Chem, 81, 2003, 249-255.
18. Pareek A, Nagori BP. *In vitro* antioxidative profiling of different extract of *Fagonia schweifurthii* (Hadidi) Hadidi. Int J Res Ayurveda Pharm, 4, 2013, 533-540.
19. Mustafa AG, Singh IN, Wang J, Carrico KM, Hall ED. Mitochondrial protection after traumatic brain injury by scavenging lipid peroxyl radicals. J. Neuro chem, 114, 2010, 271-280.

20. Jao CH, Ko WC. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging by protein hydrolyzates from tuna cooking juice. Fish Sci, 68, 2002, 430–435.

21. Gordon MH. The mechanism of antioxidant action in vitro. London: Elsevier Applied Science, 1990.

22. Halliwell B. The biological toxicity of free radicals and other reactive oxygen species. In O. I. Aruoma, & B. Halliwell (Eds.), Free radicals and food additives. Oxford: Oxford University Press, 1991, 37-57.

23. Kappus H. Lipid peroxidation—Mechanism and biological relevance. In: O. I. Aruoma, & B. Halliwell (Eds.), Free radicals and Food Additives. London:Taylor and Francis, 1991.

24. Robak J, Gryglewski RJ. Flavonoids are scavengers of superoxide anions. Biochem Pharmcol, 37, 1998, 837-841.

25. Habbu PV, Mahadevan KM, Kulkarni PV, Daulatsingh C, Veerapur VP, Shastry RA. Adaptogenic and *in vitro* antioxidant activity of flavonoids and other fractions of *Argyreia speciosa* (Burm. F) Boj. in acute and chronic stress paradigms in rodents. Indian J ExpBiol, 48, 2010, 53-60.

26. Okhawa N, Ohishi K, Yagi H. Assay for lipid peroxides in animal tissues by thio-barbituric acid reaction. Anal Biochem, 95, 1979, 351-358.

27. Dastmalchi K, Dorman HJD, Laakso I, Hiltunen R. Chemical composition and antioxidative activity of Moldavian balm (*Dracocephalum moldavica* L.) extracts. LWT-Food SciTechnol, 40, 2007, 1655–1663.

28. Neffati A, Bouhlel I, Sghaier MB, Boubaker J, Limem I, Kilani S, Skandrani I, Bhouri W, Le Dauphin J, Barillier D, Mosrati R, Chekir-Ghedira L, Ghedira K. Antigenotoxic and antioxidant activities of *Pituranthos chloranthus* essential oils. Environ. Toxicol Pharmacol, 27, 2009, 187–194.

29. Li C, Lin E. Antiradical capacity and reducing power of different extraction method of *Areca catechu* seed. Afr J Biotechnol, 9, 2010, 7831-7836.

30. Lin E, Chou H, Kuo P, Huang Y. Antioxidant and antiproliferative activities of methanolic extracts of *Perilla frutescens.* J Med Plants Res, 4, 2010, 477-483.

31. Ebrahimzadeh MA, Nabavi SM, Nabavi SF, Bahramian F, Bekhradnia AR. Antioxidant and free radical scavenging activity of *H. officinalis* L. var. *Angustifolius, V. odorata, B. hyrcana* and *C. speciosum*. Pak J Pharm Sci, 23, 2010, 29-34.

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