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DETERMINATION OF PHYTOCHEMICAL CONTENTS AND ANTIOXIDANT ACTIVITIES OF ETHANOL EXTRACT OF THE LEAVES OF *SCURRULA PARASITICA* L.

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Keywords:

Scurrula parasitica, Anti-oxidant activity, DPPH, ABTS, Phytochemical screening, Phenols and Flavonoids

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ABSTRACT: The phytochemical analysis of ethanol extract of *Scurrula parasitica* showed positive results for alkaloid, saponins, tannin, terpenoids, flavonoids, phenol, reducing sugar and phytosterols whereas glycoside is found to be absent. Total phenol and flavonoid contents were evaluated and found to be total phenolic compounds (101.9-379.1 µg/g tissue) and total flavonoids (72-174.1 µg/g tissue), respectively. The free radical scavenging activity of ethanol extracts of *Scurrula parasitica* (Loranthaceae) was determined to validate the medicinal potential of the plants. The antioxidant activity was performed by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay and ABTS + cation. The optimum concentration for both DPPH and ABTS was obtained at 100 µg/ml with an IC value of 53.28100 µg/ml and 160.8100 µg/ml, respectively. Therefore, the high content of both phenols and flavonoids might contribute to the anti-oxidative activity.

INTRODUCTION: Free radicals are unpaired electrons or unstable molecules spinning around the nucleus. When their production increases in large amounts, they are called oxidative stress which causes oxidative damage and leads to diseases like cancer, aging, diabetes, atherosclerosis and neurodegenerative disorders [1, 2]. The discrepancy between the formation and detoxification of pro-oxidants leads to the development of oxidative stress which causes many diseases. Natural antioxidants present in plants are important tools in obtaining and maintaining good health origin protect against these radicals [3]. Antioxidants such as polyphenols, phenolic acids, tannins, flavonols, isoflavones, and curcuminoids are found to be present on herbs and spices [4].

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The natural antioxidants like flavonoids and phenolics acids are found present in plants [5, 6, 7, 8]. Medicinal plants having antioxidant property have been the main focus for researchers because plants carry natural antioxidants which reduce lipid peroxidation from harmful effects caused by free radicals. Anti-oxidant scavenging properties are exerted by a great number of ethnomedicinal herbs. *Scurrula parasitica* is an herbaceous growing shrub of the family Loranthaceae. They are found growing on *Dendrophthoe falcate* and *Mangifera indica* [9, 10].

Anti-cancerous and analgesic activities are found to be present on *S. parasitica* [11, 12]. Parasitic genera vary considerably in their habits and host ranges as plant parasites have originated multiple times during angiosperm evolution [13]. Loranthaceae consists of about 900 species and 75 genera, the majority of which are found in the southern regions of India. The plant also displays anticancer, anti-diabetic, anti-hepatotoxic, anti-oxidant, immune-modulatory and cytotoxic activity [14, 15]. Phytochemicals screening of *S. parasitica* reveals

the presence of different major components like catechin, rutin, icariside, aviculin, flavonoids, oleanolic acid, lupeol, *etc*. [16] In this study, we investigate the results of the phytochemical screening along with the antioxidant actions of ethanolic extract of *Scurrula parasitica*. The result commencing from this research might insert into the general significance of the therapeutic prospective of the plant.

MATERIALS AND METHODS:

Preparation of Plant Extract: Fresh leaves of *Scurrula parasitica* were collected from Kolasib district, Mizoram during the month of February 2018. The plant was identified at Botanical Survey of India (BSI), Shillong and authenticated at the Department of Environmental Science with voucher number MZU 742, Mizoram University. The leaves of the plant sample were washed with water, dried and made into powder form and kept in a beaker. Around 400 to 500 ml of ethanol was taken in a beaker, to it 100 gm of *Scurrula parasitica* powder was added, sealed properly and then kept for 4 days. Then, the solvents were filtered using Whatman's filter paper to remove any impurities and evaporated into a crude form using a rotary evaporator. The extract was stored at a low temperature until further use.

Phytochemical Screening for Active Metabolites: The following tests were conducted to identify the different phytochemicals present as the research follows the methods done by Trease and Evans [17].

Alkaloids:

Mayer's Test: To 3ml of plant extract, few drops of Mayer's reagent are added, the formation of yellow precipitate signifies the presence of alkaloids.

Dragendorff's Test: To 3 ml of plant extract, few drops of Dragendorff's reagent are added. The development of brownish fluorescent precipitate indicates.

Glycosides:

Liebermann's Test: To a small quantity of aqueous pant extract, 2 ml of both chloroform and acetic acid was added, after it was cooled, concentrated H_2SO_4 was added again. The formation of green color shows the unit of aglycone, which is the steroidal part of glycosides.

Keller-Kiliani Test: To 10 ml of aqueous plant extract, 4 ml of glacial acetic acid and 1 drop of ferric chloride 2.0% was added, then 1 ml of concentrated H_2SO_4 was added. The formation of a brown ring between the layers shows the presence of cardiac steroidal glycosides.

Reducing Sugar:

Benedict's Test: To 1 ml of aqueous plant extract, 5 ml of Benedict's solution was added in a test tube, boiled for 10 min and allowed to cool, the formation of a brick-red precipitate indicates the presence of reducing sugar.

Fehling's Test (Standard Test): To 2 ml of aqueous plant extract, 1 ml of both Fehling's solutions A & B was added and boiled for 10 min and allowed to cool. The development of brick red precipitate shows the presence of reducing sugar.

Tannins: 1 ml of 5% ferric chloride solution was added to 0.5 ml of aqueous plant extract. Observation of blue-green or blue-black coloration indicates the presence of tannins

Flavonoids: To 5 ml of ethanolic plant extract, 1 ml of concentrated hydrochloric acid and 0.5 g of Mg was added. Red or pink color development indicates the presence of flavonoids.

Saponins: To 20 ml of distilled water, 5 ml of plant extract was added and shaken in a graduated cylinder for 10 to 15 min. The formation of foam shows the presence of saponins.

Terpenoids: This test followed the methods done by Watson L and Dallwitz MJ [18] in which 2 ml of acetic anhydride and concentrated H_2SO_4 was added to a small quantity of the extract. Blue, green rings formation indicates the presence of terpenoids.

Phenols: To a small quantity of aqueous plant extract, few drops of ferric chloride solution were added. The development of blue-black color confirms the presence of phenols.

Phytosterols:

Salkowski Test: Chloroform was added to a small quantity of plant extract in a test tube and filtered, and then few drops of concentrated H_2SO_4 were added, shaken and allowed to cool. The formation of a golden color shows the presence of triterpenes.

Liebermann-Burchard Test: Chloroform was added to a small quantity of plant extract in a test tube and filtered. A few drops of acetic anhydride was added to the filtrate and allowed to cool. Then concentrated H_2SO_4 was added along the sides of the test tube. Brown color development at the junction signifies the presence of phytosterols.

Total Phenolic Content: The phenolic content of *Scurrula parasitica* was determined by Folin–Ciocalteu's phenol reagent, which was reported by Kim *et al.* [19] Singleton and Rossi 196520. Firstly, 200 µl of appropriately diluted sample/ gallic acid standard was added to 2.6 ml of distilled water. Then, 200 µl of Folin–Ciocalteu's phenol reagent was added and mixed properly. After 6 min, 2 ml of 7% (w/v) Na_2CO_3 solution was further added and mixed.

Incubation was done at room temperature for 90 min and the absorbance was considered at 750 nm versus a prepared blank. The blank consisted of 200 µl 50% (v/v) ethanol as an alternative to the sample. Gallic acid in 50% (v/v) ethanol solution in standard concentrations of 0.1, 0.3, 0.5 and 0.8 mg ml^{-1} was used as a calibration curve; this was drawn each day of study. The total phenolic content was expressed as mg gallic acid equivalent (GAE)/ g of the dry weight (DW). The results obtained were performed in triplicate.

Total Flavonoids Content: The determination of the flavonoid content of *Scurrula parasitica* was measured according to the method specified by Zhishen *et al.* [21] The plant extract was added with 0.3 ml of 5% sodium nitrite and mixed properly. Then it is incubated for 5 min, after which 0.3 ml of 10% aluminum chloride solution was added. It is kept for 6 min and then 2 ml of 1 M sodium hydroxide was added and which make up the volume to 10 ml with water.

The absorbance was considered at 510 nm with UV–visible spectrophotometer. The content of flavonoids was measured from quercetin (0–0.3 mg) standard curve furthermore expressed as mg catechin equivalents/g of the dry weight. The samples were made in triplicate.

Anti-oxidant Activity:

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Assay: The antioxidant action of the ethanolic extract of *Scurrula parasitica* polyphenolic extract/ascorbic acid to scavenge DPPH radical as described by Leong and Shui [22] with some modifications. Briefly, a 1 ml ethanol solution of 0.1 Mm DPPH was added to the different concentration of the ethanol extracts (5-100 µg/ml). The prepared mixture was vortexes and incubated for 30 min at room temperature and then the reading was recorded using a spectrophotometer at 523 nm. 80% (v/v) ethanol was used as the blank. Ascorbic acid (Vitamin C) was used for comparison with the plant extracts. The measurements were performed in triplicate. DPPH scavenging activity was performed using the equation:

$$\text{Scavenging (\%)} = (\text{A control} - \text{A sample})/\text{Acontrol} \times 100$$

Where A = absorbance of the test sample and Control = absorbance of the control.

Free Radical-Scavenging Capability by the use of ABTS•+ Radical Cation (ABTS Assay): ABTS scavenging activity of *Scurrula parasitica* was measured by reported method given by Re R *et al.* [23] with minor modification. Approximately, 37.5 mg of potassium persulphate was dissolved in 1 ml of distilled water. On preparation of the ABTS solution, a 44 µl of the solution was added to 9.7 mg of ABTS dissolved in 2.5 ml of distilled water. The ABTS solution was then kept at room temperature for around 15 h. Then, 88 ml of 50% ethanol is mixed with 1 ml of the ABTS solution to prepare the working solution. An entire amount of 50 µl of ethanol extract of special concentration ranging from 5-100 µg/ml was mixed with 100 µl of the ABTS working solution. It was allowed to stand for 4 min and the absorbance was examined at 734 nm. The readings were expressed as ascorbic acid equivalent, which was used as a standard.

RESULTS AND DISCUSSION:

Phytochemical Screening: The ethanolic extract of *Scurrula parasitica* confirm the presence of alkaloids, tannins, saponins, flavonoids, reducing sugar, phytosterols, terpenoid, and phenol but glycosides were absent **Table 1**. Medicinal plants contain secondary metabolites, which play a pivotal role against diseases and pathogens, phytochemical screening reveals the active constituents present in plants that are known to be responsible for various activities such as anti-microbial, anti-cancer, anti-oxidant, anti-diabetic and anti-fungal [24].

The result of the preliminary screening of ethanol extract of *Scurrula parasitica* proved to be efficient in exposing the important metabolites, so it will be further carried out for its antioxidant property.

TABLE 1: PHYTOCHEMICAL SCREENING RESULT OF ETHANOL EXTRACT OF *SCURRULA PARASITICA*

Test	*S. parasitica* extract
Alkaloid	+
Terpenoid	+
Flavanoid	+
Glycoside	-
Tannin	+
Saponin	+
Reducing Sugar	+
Phenols	+
Phytosterols	+

Total Phenol and Flavonoids Contents: The activity of *Scurrula parasitica* might be due to the occurrence of polyphenolic compounds, which are recognized to possess antioxidant activity according to the research done by (Okudu *et al.*, and Tepe *et al.* [25, 26] Phenols are plant metabolites which are denoted by the occurrence of several phenol groups, several of which are extremely reactive in chelating metal ions in aqueous solutions and neutralizing free radicals by donating a hydrogen atom or an electron [27].

Furthermore, the phenolic compounds acquire numerous pharmacological properties like anti-mutagenic, antibacterial and antitumor properties, all of which might be related to their antioxidant property [28]. Flavonoids are exceedingly effective antioxidants and considered as the most significant and broadly distributed single group of phenols present in plants [29]. Flavonoids are forming complexes with metal ions by inhibiting metal-initiated lipid oxidation [30].

TABLE 2: TOTAL PHENOL AND FLAVONOIDS CONTENTS IN ETHANOL EXTRACT OF *SCURRULA PARASITICA*

Conc. (µg/ml)	Phenol content (µg/g tissue)	Flavonoid content (µg/g tissue)
5	101.907456	72
10	143.112384	87.9
20	206.528328	99.83333333
40	284.047536	120.7666667
80	329.038248	154.0333333
100	379.130832	174.1333333

The result of the research revealed the level of phenol and flavonoids compounds in ethanol extract of the leaves of *Scurulla parasitica* as shown in **Table 2**. The overall phenolic contents of all the formulations of the extract is found to be around 101.9 to 379.1 (µg/g tissue) at concentrations from 5 to 100 µg/ml plant extracts. The entire flavonoid content of the extract is found to be around 72 at 5 µg/ml to 174.1 (µg/g tissue) at 100 ml concentration.

This activity in ethanol extract of *Scurulla parasitica* is assumed to be largely due to their redox properties in decomposing peroxides, adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen [31]. Medicinal plants contain important metabolites like polyphenols, which are acting as antioxidants. In this regard, flavonoids and phenolic acids in plants were reported to possess considerable antioxidant capacity and consist of several biological properties, together [32]. The results from the study strongly suggest that polyphenols are essential components of *Scurulla parasitica* and the presence of these important constituents should be credited to its pharmacological properties.

Anti-oxidant Activity: DPPH method, the ABTS radical scavenging process is an important and comprehensively used antioxidant assays for performing on plant extracts. The frequently useful assays, differing within their effective principles were employed as a part of our research. Specifically, antioxidant activities of the inspected plant were determined as free radical scavenging capacity. The DPPH and ABTS scavenging activity exhibited an increase in the concentration manner up to a certain concentration. The optimum concentration for both DPPH and ABTS were obtained at 100 µg/ml with an IC value 53.28100 µg/ml and 160.8100 µg/ml, respectively. The results of the scavenging action of *Scurulla parasitica* are given in **Table 3**.

TABLE 3: DPPH AND ABTS SCAVENGING ACTION OF *SCURRULA PARASITICA*

Conc. (µg/ml)	ABTS	Ascorbic acid	DPPH	Ascorbic acid
5	0.460337	25.30457	10.92739	29.04385
10	2.129638	29.06166	15.74407	40.00719
20	2.613224	65.02371	38.40762	67.14594
40	3.022412	90.16089	53.01941	67.8289
80	17.92523	95.03394	54.36736	68.2243
100	25.63471		57.09921	
IC_{50}	160.8	2.723	53.28	2.805

DPPH Radical Scavenging Activity: The DPPH radical scavenging activity was performed with the ethanol extract of the leaves of *Scurrula parasitica* and compared with ascorbic acid. Its activity increases with increased concentration manner and maximum activity was observed at 57.09% at 0.1 mg/ml concentration as shown in **Fig. 1**. The value of IC_{50} was found to be 53.28 µg/ml. The ascorbic was a potent antioxidant and its IC_{50} value was 2.8 µg/ml. Free radicals are evaluated using DPPH [33]. The process of lipid peroxidation of free radicals is considered to play a key role in the development of chronic diseases [34]. It further showed the potential of the plant extract to scavenge diverse free radicals in different systems and could serve as an important therapeutic agent for controlling radical pathological damage.

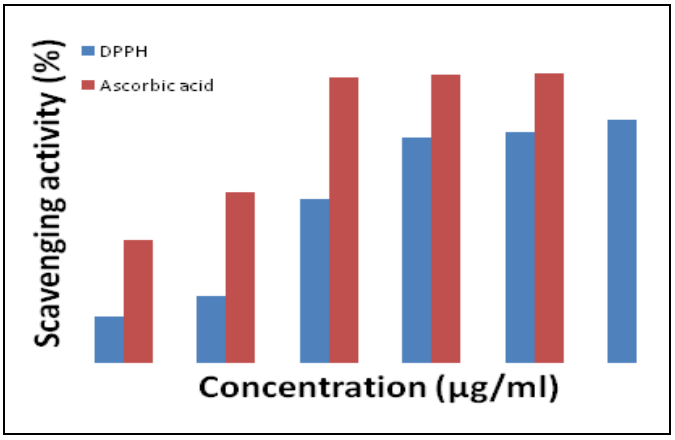

FIG. 1: DPPH ACTIVITY OF ETHANOL EXTRACT OF THE LEAVES OF *SCURRULA PARASITICA* AND ASCORBIC ACID

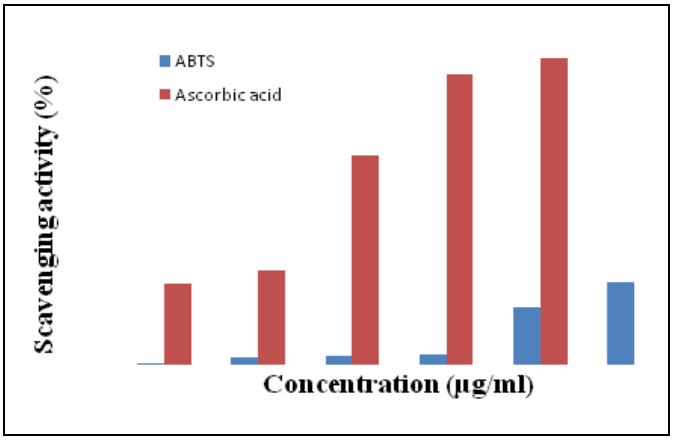

FIG. 2: ABTS ACTION OF THE ETHANOL EXTRACT OF THE LEAVES OF *SCURRULA PARASITICA* AND ASCORBIC ACID

ABTS Radical Scavenging Activity: The assessment of the scavenging activity of *Scurrula parasitica* was performed by ABTS assay method in which the ethanol extracts of the leaves was assessed compared to that of ascorbic acid. The activity exhibited a rise with an increase in concentration manner and the maximum was obtained at 25.63% (100 µg/ml) as shown in **Fig. 2**. The results of the ABTS scavenging capacity of the plant of the extracts were lower than those of ascorbic acid (100%). These indicate that the plant has the proton-donating capability and might serve as potential free radical inhibitors.

CONCLUSION: *Scurrula parasitica* possesses ethnomedicinal properties confirmed by our phytochemical studies such as alkaloid, saponins, tannin, terpenoids, flavonoids, phenol, and an antioxidant agent capable scavenging stable free radical like DPPH and ABTS cations. Those compounds are widely used and essential ingredients in pharmaceutical and development of new drugs. Therefore, *Scurrula parasitica* might provide such important resources for the development of new drugs or used as ethnomedicine locally.

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CONFLICTS OF INTEREST: There are no conflicts of interest.

REFERENCES:

1. Halliwell B and Gutteridge JM: Free radicals in biology and medicine, 3rd Edition. Oxford University Press, Oxford 1999; 1-25.
2. Kim M, Park J and Lim S: Anti-oxidant activity and cell toxicity of pressurized liquid extracts from 20 selected plant species in Jeju, Korea. Food Chemistry 2010; 122: 546-52.
3. Khan RA, Khan MR, Sahreen S and Ahmed M: Evaluation of phenolic contents and anti-oxidant activity of various solvent extracts of *Sonchus asper* (L.) Hill Chemistry Central Journal 2012; 6: 12.
4. Nobuji N: Phenolic anti-oxidants from herbs and spices. Biofactors 2000; 13: 141-46
5. Canadanovic-Brunet JM, Djilas SM, Cetkovic GS and Tumbas VT: Free-radical scavenging activity of wormwood (*Artemisia absinthium* L.) extracts. Journal of Science of Food and Agriculture 2005; 85: 265-72.
6. Konczak I, Okuno S, Yoshimoto M and Yamakawa O: Caffeoylquinic acids generated *in-vitro* in a high-anthocyanin-accumulating sweet potato cell line. Journal of Biomedicine and Biotechnology 2004; 5: 287-92.
7. Kosar M, Dorman D, Baser K and Hiltunen R: An improved HPLC post-column methodology for the

identification of free radical scavenging phytochemicals in complex mixtures. Chromatography 2004; 60: 635-38.
8. Oboh G, Raddatz H and Henle T: Anti-oxidant properties of polar and non-polar extracts of some tropical green leafy vegetables. Journal of Science of Food and Agriculture 2008; 88: 2486-92.
9. Bambaradeniya CNB, Ekanayake SP, Kekulandala LDCB, Fernando RHSS, Samarawickrama VAP and Priyadharshana TGM: An assessment of the status of biodiversity in the Maduganga mangrove estuary. Occasional Papers of IUCN Sri Lanka No.12002.
10. Weeraratna WG: The ecology and biology of parasitism of the Loranthaceae of Ceylon. In J.L. Harper (ed). the biology of weeds. British Ecological Society Symposium 1960; 1: 189-02.
11. Jing-jing L, Bing-hua C, Jun-rui L, Bing-jie Z and Yi-jun X: Free radical scavenging activities of three solvent extracts from *Scurrula parasitica* leaves. Natural Product Research and Development 2011; 23: 1127.
12. Mahajan N, Joshi P, Kondawar M, Kumar KS and Vaidhyalingam V: Anti-nociceptive potential of *Scurrula parasitica*: an unexploited parasitic plant. Research and Reviews: A Journal of Pharmacology 2013; 3: 4-8.
13. Kuijt J: University of California Press, Berkeley 1969; 246.
14. Schaefermeyer G: Complementary Therapies in Medicine1998; 6:72–177.
15. Taek JY: International Journal of Immunopharmacology 1988; 204: 163-72.
16. Sharma HK: Fitoterapia 2001; 72: 146-61.
17. Trease GE and Evans WC: Pharmacognosy Bailliere Tindall Press: London 1983; 309: 706.
18. Watson L and Dallwitz MJ: The families of flowering plants: descriptions, illustrations, identification and information retrieval, Version 25th 1992.
19. Kim JW, Lee JH, Hwang BY, Mun SH, Ko NY, Kim DK, Kim B, Kim HS, Kim YM and Choi WS: Morin inhibits Fyn kinase in mast cells and IgE-mediated type I hypersensitivity response *in-vivo*. Biochemical pharmacology 2009; 77: 1506-12.
20. Singleton V and Rossi JA: Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture 1965; 16: 144-58.
21. Zhishen J, Mengcheng T and Jianming W: The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food chemistry 1999; 64: 555-59.
22. Leong LP and Shui G: An investigation of antioxidant capacity of fruits in Singapore markets. Food Chemistry 2002; 76: 69-75.
23. Re R, Pellegrini N, Proteggenete A, Pannala A, Yang M, Rice-Evans C: Anti-oxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 1999; 26: 1231-37.
24. Hossain MA and Nagooru MR, Biochemical profiling and total flavonoids contents of leaves crude extract of endemic medicinal plant cordyline terminal is L. Kunth. Pharmacognosy Journal 2011; 3: 25-29.
25. Okudu T, Yoshida T and Hatano T: Food phytochemicals for cancer prevention II. In C.T 1994.
26. Tepe B, Sokmen M, Akpulat HA and Sokmen A: Screening of the antioxidant potentials of six Salvia species from Turkey. Food Chemistry 2006; 95: 200-04.
27. Petti S and Scully C: Polyphenols, oral health and disease: a review. Journal of Dentistry 2009; 37: 413- 23.
28. Shui G and Leong LP: Separation and determination of organic acids and phenolic compounds in fruit juices and drinks by high-performance liquid chromatography. Journal of Chromatography A 2002; 977: 89-96.
29. Maslarova YN: Inhibiting oxidation. In J. Pokorny, N. Yanishlieva, M. Gordon (Eds). Antioxidants in food practical applications. Cambridge Press Woodhead Publishing Ltd 2001; 35-59.
30. Lee VSY, Dou JP, Chen RJY, Lin RS, Lee MR and Tzen JTC: A massive accumulation of gallic acid and the unique occurrence of myricetin, quercetin and kaempferol in preparing old oolong black tea. Journal of Agricultural and Food Chemistry 2008; 56: 7950-56.
31. Zheng W and Wang SY: Antioxidant activity and phenolic compounds in selected herbs. Journal of Agricultural and Food Chemistry 2001; 49: 5165-70.
32. Gil MI, Ferreres F and Tomas-Barberan FA: Effect of postharvest storage and processing on the antioxidant constituents (Flavonoids and vitamin C) of fresh-cut spinach. Journal of Agricultural and Food Chemistry 1999; 47: 2213-17.
33. Porto CD, Calligaris S, Cellotti E and Nicoli MC: Antiradical properties of commercial cognacs assessed by the DPPH test. Journal of Agricultural and Food Chemistry 2000; 48: 4241-45.
34. Dorman HJD, Kosar M, Kahlos K, Holm Y and Hiltunen R: Antioxidant properties and composition of aqueous extracts from Mentha species, hybrids, varieties, and cultivars. Journal of Agricultural and Food Chemistry 2003; 51: 4563-69.

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