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Polyphenolic Compounds and Free Radical Scavenging Activity in Eight *Lamiaceae* Herbs of Manipur

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Abstract

Eight plants of *Lamiaceae* under subfamily *Nepetoideae* found in Manipur, India were selected for estimation of their polyphenolic compounds and free radical scavenging activity which is expressed on dry weight basis. In this present study, the total phenol and flavonoid contents as well as the free radical scavenging activity were studied using spectrophotometric method. The total phenol content was determined based on Folin-Ciocalteau reagent, flavonoid was determined by aluminium chloride spectrophotometric method and tannin by Folin Dennis Method. The free radical scavenging activity was determined by using DPPH radical which is expressed as IC_{50} (µg/ml). The total phenolic content varied from 21.39±0.927 to 46.28±0.543 mg/g, flavonoids content in the selected samples varied from 13.30±0.684 to 26.03±0.217 mg/g and tannin content varied from 8.72±0.160 to 17.04±0.206 mg/g. The free radical scavenging activity among the selected samples varied from 11.67±0.221 to 38.29±0.532 µg/ml. The correlation between the free radical scavenging activity with total phenol content (R^2=0.511), with flavonoids (R^2=0.241) and with tannin (R^2=0.690) was calculated and maximum correlation value was found between tannin content and the free radical scavenging activity of the plant samples. The result supports that tannins were more responsible for free radical scavenging activity in the presently selected plants.

Keywords: antioxidant, flavonoids, IC_{50}, Indo-Myanmar hotspot, *Nepetoideae*, tannin

Introduction

Polyphenols are a group of secondary metabolites involved in the H_2O_2 scavenging in plant cells. Interest in plant materials rich in polyphenolic compounds are on the increase due to their high antioxidant potency, which may offer protection against cancer, through the inhibition of oxidative damage, known to be a potential cause of mutation. Free radicals cause oxidative damage to lipids, proteins, and nucleic acids (Shui and Leong, 2004). The antioxidative property of polyphenols is a predominant feature of their radical-scavenging capacity (Yang *et al*., 2001; Cotelle, 2001; Facino *et al*., 1990). They possess ideal structural chemistry for radical scavenging activity and are more effective than tocopherol and ascorbate (Pandhair and Sekhon, 2006). In the aerobes, due to large generation of reactive oxygen species such as superoxide radical, hydrogen peroxide, and hydroxyl radical leads to severe effects on the cardiovascular system either through lipid peroxidation or vasoconstriction and other ailments such as inflammation, cancer, diabetes mellitus etc (Lachance *et al*., 2001; Nickavar *et al*., 2007).

Phenolic acids, flavonoids and tannins are the most commonly found polyphenolic compounds in plant extracts (Wolfe *et al*., 2003; Naik *et al*., 2006). Flavonoids are 15-carbon compounds generally distributed throughout the plant kingdom (Harborne, 1988). Flavonoids and many other phenolic compounds of plant origin have been reported as scavengers of reactive oxygen species (ROS), and are viewed as promising therapeutic drugs for free radical pathologies (Parshad *et al*., 1998; Chang *et al*., 2007). Tannins are naturally occurring, high molecular weight polyphenols which can be divided into hydrolysable tannins and condensed tannins. Tannins are the most abundant antioxidants in the human diet and they exhibit many biologically important functions which include protection against oxidative stress and degenerative diseases (Atanassova and Christova-Bagdassarian, 2009). The oxidation inhibiting activities of tannins have been known for a long time (Edeoga *et al*., 2006).

The family *Lamiaceae* is represented by about 236 genera and 7172 species in the world (Harley *et al*., 2004). It is a well known fact that many members under this family are useful economically for medicinal, culinary, ornamental and various commercial utilizations. Many plants, especially those belonging to the *Lamiaceae* family show strong antioxidant activity (Marinova and Yanishlieva, 1997; Hirasa and Takemasa, 1998; Triantaphyllou *et al*., 2001). Thus, members of the family are very important due to their medicinal and aromatic properties leading to production of the herbal products and food supplements.

The state of Manipur which extends between 23°59′N-25°47′N and between 92°59′E-94°46′E with total geographical area of 22,327 km^2 lies in the North-Eastern part of India falls under Indo-Myanmar hotspot regions of the world (Meyers *et al*., 2000) with extraordi-

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narily rich flora. A total of 39 genera with 110 species of *Lamiaceae* are distributed all over the state which includes some endemic plants. Among these species, some are found in cultivated form only and some are found in wild forms. Measurement of the polyphenols and free radical scavenging activity of herbs has become important tools for the understanding of the relative importance of plant species especially from the health point of view (Chang *et al*., 2007). This paper provides an evaluation and comparison of the polyphenolic contents and their relative free radical scavenging activity of the eight selected taxa of *Lamiaceae*.

Materials and methods

Plant material collection

The seeds of selected taxa (*i. e*. *Elsholtzia blanda* Benth.*, E. communis* (Coll. and Hemsl.) Diels var. purple flower, *E. communis* (Coll. and Hemsl.) Diels var. white flower, E. *stachyodes* (Link) Wu*, Hyptis suaveolens* Poit*, Ocimum americanum* L.*, O. basilicum* L. and *Perilla frutescens* L.) Tab. 1 under the subfamily Nepetoideae of family *Lamiaceae* were collected locally and planted in experimental fields for this study. All the selected taxa are used as culinary herbs in Manipur and are cultivated, with the exception of *H. suaveolens*, which has high medicinal values and is widespread as a noxious weed (Devi *et al*., 2008). The aerial parts of these plants were collected just before the flowering time. The collected samples were shade dried and ground into powder form by a grinder. Specimens were identified and the vouchers were deposited at the Manipur University Museum of Plants (MUMPS), Department of Life Sciences, Manipur University.

Tab. 1. Eight selected species of *Lamiaceae* of Manipur with their local names and voucher numbers

Species	Local Name	Voucher No.
Elsholtzia blanda Benth.	Kanghuman	004302
E. communis (Coll. and Hemsl.) Diels var. purple flower	Lomba	004328
E. communis (Coll. and Hemsl.) Diels var. white flower	Lomba	004301
E. stachyodes (Link) Wu	Tekta	004303
Hyptis suaveolens Poit.	Tukma	004311
Ocimum americanum L.	Mayangba	004313
O. basilicum L.	Naoseklei	004312
Perilla frutescens L.	Thoiding Angouba	004309

Extraction

Extraction method for Total Phenolics and Tannins

Total phenol content and tannin were estimated by using Folin-Ciocalteau reagent (FCR) and Folin Dennis method respectively (Thimmaiah, 1999). One hundred milligram of the ground powder of the plant samples were weighed and kept in magnetic stirrer for 3 h after adding 10 ml of 80% ethanol. The extracts were centrifuged for 15 min at 10, 000 rpm. The supernatants were collected and stored for analyzing total phenol and tannin contents.

Extraction method for flavonoids

Hundred milligrams of the powdered samples were weighed and mixed with 10 ml of 80% methanol by intermittent maceration for 48 h. The solvents were evaporated and reduced up to 5 ml at room temperature. This extract was stored for the estimation of flavonoids contents. Aluminium Chloride spectrophotometric method was used for flavonoids determination (Chang *et al*., 2002) with slight modification.

Extraction method for free radical scavenging activity

Plant extraction was done by slight modification of method adopted by Yang *et al*. (2007). From the dried powdered samples, 3 g were weighed and put into 100 ml flasks. Each flask was added with 50 ml of 80% methanol. After one week of storage at room temperature the supernatants were filtered and these filtered extracts were dried at room temperature (30°C). The dried samples were then weighed. The extracted samples were then dissolved in 10 ml methanol and stored in refrigerator for further experiments.

Estimation of total phenolics

From the supernatants of phenolic extracts, 1 ml of each sample were collected then evaporated in a petriplate to dryness. Then the dried residue is dissolved in 1 ml of distil water. 100 µl of the dissolved residue was taken and its volume was made up to 3 ml with distil water. In the test tubes containing test samples, 0.5 ml of folin-ciocalteau reagent was added. Then after 2 min, 20% of Na_2CO_3 were added and mixed thoroughly. The contents were kept in a boiling water bath for about 1 min. Then the test tubes were cooled in running tap water and the absorbances of the blue coloured complex were taken against blank at 650 nm with the help of UV-VIS Double Beam Spectrophotometer Version 6.51. The total phenol content was calculated and expressed in mg/g using a standard curve prepared from catechol.

Estimation of flavonoids

From the supernatants of flavonoids extract, 100 µl of the supernatant was taken and it was added with 0.1 ml of Aluminium chloride (10%), 0.1 ml of potassium acetate (1M) and 2.7 ml of distil water to make up volume to 3 ml. The reaction mixture was kept at room temperature for 30 min. The absorbance was measured at 415 nm using UV-VIS Double Beam Spectrophotometer Version 6.51. The calibration curve was prepared using different concentrations of quercetin which is expressed in mg/gm dry weight.

Estimation of tannin

From the supernatants of tannin extracts, 100 µl of aliquot of each sample was taken and 7.5 ml of distil water were added. After that, 0.5 ml of Folin Denis Reagent (FDR) followed by 1 ml of 35% Na_2CO_3 was added. The final volume was made up to 10 ml with distil water. The blue colour appeared was measured at 700nm by using UV-VIS Double Beam Spectrophotometer Version 6.51. The calibration curve was prepared using tannic acid expressed in mg/gm dry weight.

Estimation of free radical scavenging activity by DPPH method

Free radical scavenging activity was determined by using DPPH radical (Dudonne *et al*., 2009). The DPPH radical has been widely used to investigate the scavenging activities where the DPPH radical is scavenged by antioxidants through the donation of a hydrogen atom forming the reduced DPPH-H. The DPPH· solution in methanol ($6x10^{-5}$M) was freshly prepared, where 3 ml of this solution was mixed with 100 µl of methanolic plant extracts (3-50 µg/ml). The samples were incubated for 20 min at 37°C in a water bath, and then the decrease in absorbance at 515 nm was measured (A_E) in an UV-VIS Double Beam Spectrophotometer Version 6.51. A blank sample containing 100 µl of methanol in DPPH· solution was freshly prepared and its absorbance was measured (A_B). The experiment was carried out in triplicate. Radical scavenging activity was calculated using the formula given below:

$$\% \text{ inhibition of DPPH} = [\frac{A_B - A_E}{A_B}] \times 100$$

A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and represented as IC_{50} for each of test solution which is expressed as µg/ml.

Statistical analysis

Each parameter which are under consideration was carried out three times from which the mean values and their respective standard error mean (SEM) were calculated by using Microsoft Excel-2007. Significant differences of the data among the parameters were calculated by performing ANOVA test with the help of SPSS (9) and means were compared by least significant difference (LSD). Differences at $P<0.05$ were considered to be significant. Correlation analyses of free radical scavenging activity (Y) versus the total phenolic content, flavonoids and tannin (X) were also carried out by using Microsoft Excel-2007.

Results and discussion

Over the past few years, investigations for phenolics compounds in medicinal herbs have gained importance due to their high antioxidative properties (Zhu *et al*., 2004). Tab. 2 presented the total phenol, flavonoid and tannin content of the selected plants under *Lamiaceae* which is expressed on dry weight basis as mg/g. The amount of total phenols varied in different plants and ranged from 21.39 to 46.28 with maximum value in *Elsholtzia blanda* (46.28±0.543) and minimum in *E. communis* var. purple flower (21.39±0.927). Hakkim *et al*. (2008) reported the variable range of total phenolic content on dry weight basis as 42.1±3.1 in *O. selloi*, 123.1±2.3 in *Ocimum americanum* and 168.2±3.2 mg GA/g in *O. gratissimum*. In different parts of three *Coleus* species, the total phenol content ranged from 16.32 to 62.12 mg/g FW (Fresh Weight) (Rasineni *et al*., 2008).

Tab. 2. Polyphenolic Contents (mg/g DW (Dry Weight) ±SEM)in mature aerial parts of *Lamiaceae* plants (n=3)

Species	Total Phenol	Flavonoid	Tannin
Elsholtzia blanda	46.28±0.543	23.35^{a}±0.217	17.04±0.206
E. communis var. purple flower	21.39^{a}±0.927	$16.86^{b,c}$±0.976	8.72±0.160
E. communis var. white flower	$22.52^{a,b}$±0.591	17.45^{c}±0.369	10.87^{a}±0.167
E. stachyodes	32.07±0.566	22.00^{a}±0.856	14.52±0.201
Hyptis suaveolens	32.07±0.363	16.22^{b}±0.684	15.39^{b}±0.138
O. americanum	26.15±0.808	15.66^{b}±0.215	9.47±0.076
O. basilicum	39.31±0.439	26.03±0.217	15.13^{b}±0.187
Perilla frutescens	23.80^{b}±0.363	13.30±0.684	10.53^{a}±0. 138

Note: Different letters between species denote significant differences (LSD test, $P<0.05$)

The flavonoid content in the selected species ranged from 13.30±0.684 (*Perilla frutescens*) to 26.03±0.217 (*O. basilicum*) (Tab. 2). In five *Salvia* species, Nickavar *et al*. (2007) reported the total flavonoid content which ranged from 8.58±0.99 to 53.16±1.95mg/g DW due to the difference in species. Thus, the different values of flavonoids among the selected plants and also with other plants will be due to belonging to different taxa. Flavonoids exhibit inhibition of mutagenicity induced by chemical mutagens and have anticarcinogenic, antioxidant and anti-inflammatory activities (Miyazawa *et al*., 2000).

In the selected eight plants of *Lamiaceae*, the tannin content was found maximum in *E. blanda* (17.04±0.206) and minimum in *E. communis* var. purple flower (8.72±0.160) (Tab. 2). Tannin content among different tissues of three species of *Coleus* was found to be 0.085-0.210 mg/g FW (Rasineni *et al*., 2008). Recent studies have demonstrated that, low dosages of tannins (0.15-0.2%) in the diet can be beneficial to human health and will create a more astringent feel to the taste, although at higher concentration, they inhibit the digestive enzymes and reduce the bioavailability of iron and vitamin B_{12} (King-Thom *et al*., 1998). Tannins have shown potential antiviral, antibacterial and antiparasitic effects (Akiyama *et al*., 2001; Lu *et al*., 2004). In the past few years tannins have also been studied for their potential effects against cancer through different mechanisms (Yang *et al*., 2000; Tanimura *et al*., 2005).

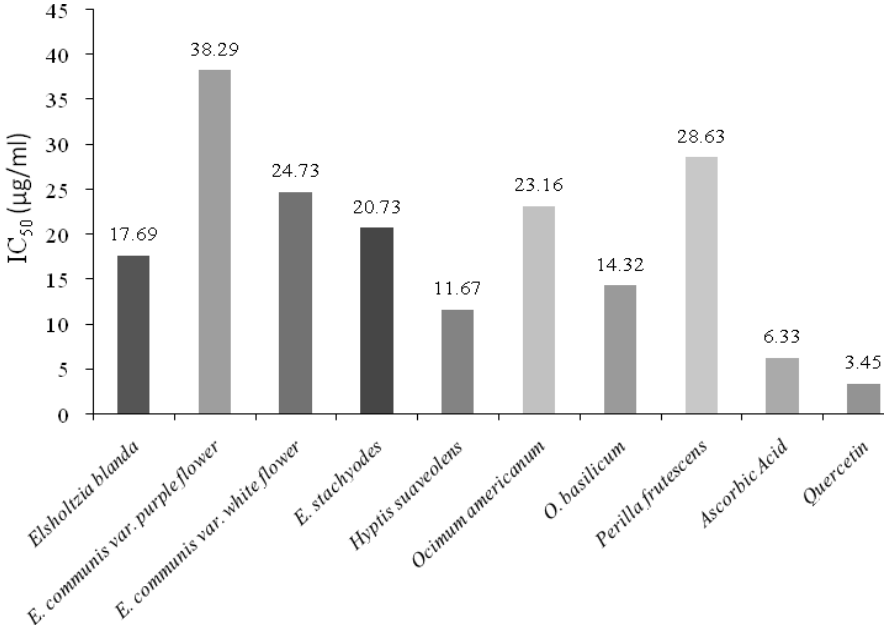

Fig.1. Free radical scavenging activity of the eight *Lamiaceae* plants of Manipur

Fig. 1 presents the free radical scavenging activity of the selected plants under *Lamiaceae* found in Manipur which is expressed as IC_{50} (µg/ml). The maximum free radical scavenging activity was found in *Hyptis suaveolens* (11.67±0.221) and minimum in *E. communis* var. purple flower (38.29±0.532) with more free radical scavenging activity in *H. suaveolens* as lesser is the IC_{50} of the sample, more is the free radical scavenging activity and *vice versa*. The IC_{50} of five *Salvia* species ranged from 23.53 to 129 µg/ml (Nickavar *et al*., 2007). The greater the free radical scavenging activity, the greater the antioxidant property as an antioxidant may be defined as any substance that when present at low concentrations compared with those of the oxidative substrate significantly delays or inhibit that substrate (Antolovich *et al*., 2002).

It has been reported that the antioxidant activity of many compounds of botanical origin is proportional to antioxidant content suggesting a correlation between total phenolics and antioxidant activity (Rice-Evans *et al*., 1997; Veglioglu *et al*., 1998). The correlation between free radical scavenging(Y) and total phenol content (X) of eight *Lamiaceae* plants found in Manipur had a correlation coefficient of R^2=0.511 (Fig. 2). It suggests that 51% of the free radical scavenging of these eight *Lamiaceae* plants is contributed by phenolic compounds. The antioxidative activity of polyphenols is generally ascribed to

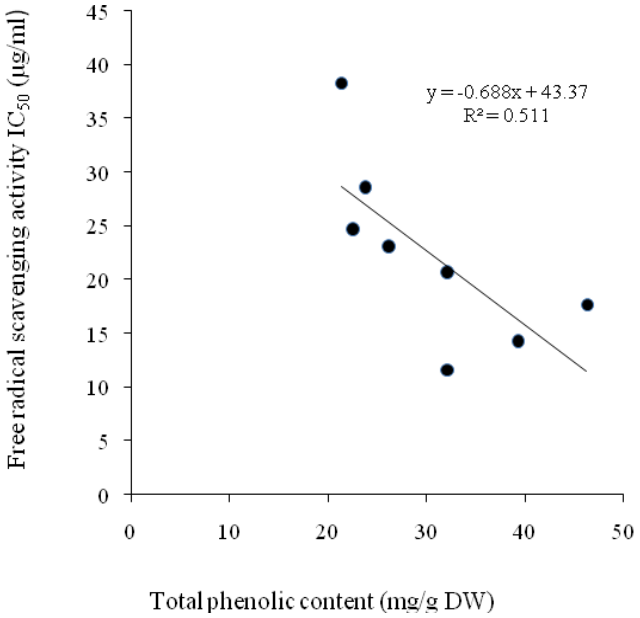

Fig. 2. Correlation of free radical scavenging activity (Y) versus total phenolic content (X) of eight *Lamiaceae* plants of Manipur

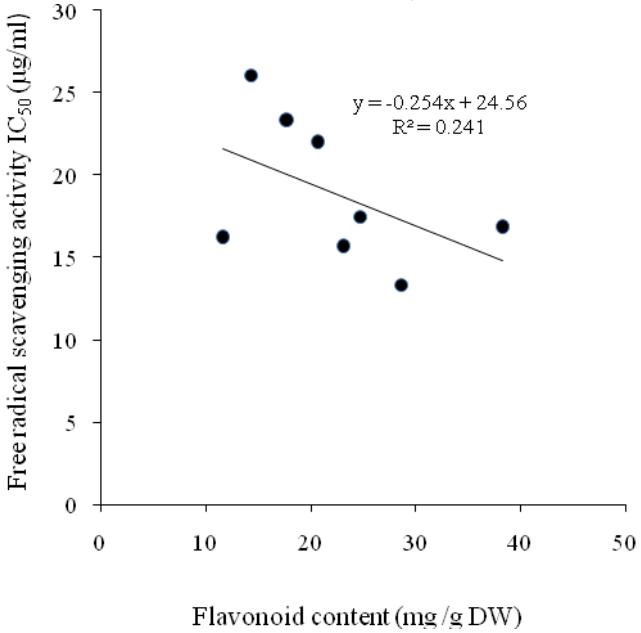

Fig. 3. Correlation of free radical scavenging activity (Y) versus flavonoid content (X) of eight *Lamiaceae* plants of Manipur

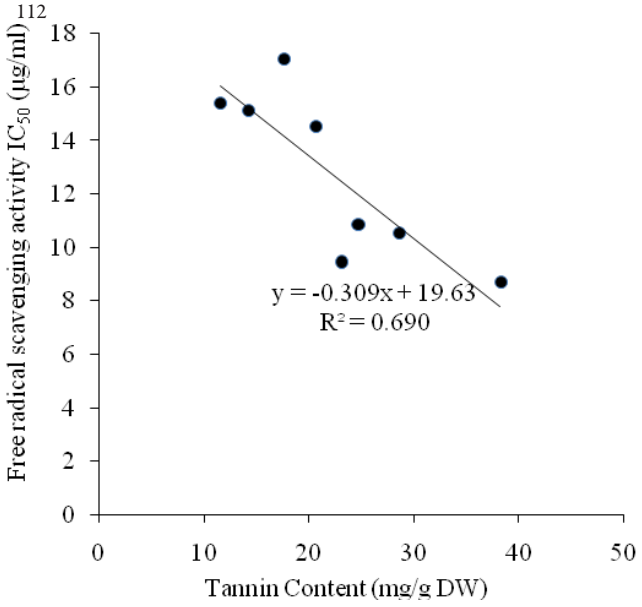

Fig. 4. Correlation of free radical scavenging activity (Y) versus tannin content (X) of eight *Lamiaceae* plants of Manipur

their hydroxyl groups (Chen and Ho, 1997). The remaining 49% of free radical scavenging activity may come from the presence of other active components like essential oils, carotenoids, vitamins and other glycosides. Among the phenolic compounds, the contribution of flavonoids as free radical scavenging activity as compared to with tannin in these eight selected *Lamiaceae* plants is found to be less. The correlation between flavonoids (X) with free radical scavenging activity(Y) is found to have a correlation coefficient of R^2=0.241 (Fig. 3) and with tannins(X) and free radical scavenging activity (Y) is found to have a correlation coefficient of R^2=0.690 (Fig. 4). In this case also, among the phenolic compounds also, the contribution of flavonoids is found to be 24.1% and tannin is found to be 69% in these eight selected plants of *Lamiaceae* found in Manipur. The present study shows that among the phenolic compounds also, tannins show high free radical scavenging activity and are good antioxidants which are also found to used as anti carcinogenic, anti mutagenic and in treatment of cancer patients (Ramakrishnan *et al*., 2006).

Conclusions

These eight *Lamiaceae* plants are often used in many local dishes of Manipur, are strong free radical scavengers and can be considered as good sources of natural antioxidants for many dishes, medicinal and commercial utility. Although, the plants taken belong to same family *Lamiaceae*, each of them whether under same genus or same species has different concentration of phenolic compounds with varied flavonoids and tannin content leading to differing amounts of antioxidants. The use of plants, foods and herbal products as antioxidants is increasing due to consumer awareness of their various health benefits. So, the paper will provide data on natural antioxidant sources in family *Lamiaceae* which is found in Manipur. However, further research is needed for isolation and identification of the phenolic compounds, flavonoids and tannins.

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