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BIOCHEMICAL ASPECTS OF SOME SELECTED SPECIES OF LAMIACEAE FAMILY OF IMPHAL VALLEY DISTRICTS OF MANIPUR: A REVIEW

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Abstract

Ten plants of Lamiaceae found in Manipur, India were selected for the estimation of their polyphenolic compounds and free radical scavenging activity which is expressed on dry weight basis. 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 (R₂ = 0.511), with flavonoids (R₂ = 0.241) and with tannin (R₂ = 0.690) was calculated and maximum correlation value was found between tannin content and the free radical scavenging activity in the presently selected plants. Up-to-date review of reports for family Lamiaceae plants were also incorporated.

Keywords: Lamiaceae, Antioxidant, Flavonoids, IC50, Indo-Myanmar Hotspot, Nepetoideae, Tannin.

Introduction

Lamiaceae is represented by 236 genera and 7,172 species (Harley et al., 2004) in the world with cosmopolitan distribution, however, whose centre is chiefly in the Mediterranean region, where they form a dominant part of vegetation (Lawrence, 1951). This family is regarded as being one of the highly evolved plant families, at least from the viewpoint of floral structure. Recently, there have been many workers dealing with taxonomic updation including nomenclature and classification of the family. The modern circumscription of Lamiaceae includes many genera which were formerly placed in the Verbenaceae (Cantino et al., 1992; Harley et al., 2004). Several phylogenists believed that angiosperms are monophyletic i.e. group originated from single ancestor (Dahlgren, 1983). Cantino (1992) observed that the family Lamiaceae as circumscribed by Bentham (1876) and Briquet (1895-1897) was polyphyletic. The phylogenetic imperative dictates that all supraspecific taxa should be monophyletic and it should be the central principle to the professional world view of many phylogenetic systematists (Cantino et al., 1999). The best classification would be a cladistic one, in which all recognized groups are strictly monophyletic. In an effort to circumscribe monophyletic groups of Lamiaceae, Cantino et al. (1992) introduced a revised classification following the earliest work of Junell (1934) which was modified by Harley et al. (2004).

Lamiaceae 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.

Manipur state also harbours a good diversity of Lamiaceae including one endemic species, Colquhounia elegans Wall. ex Benth. (Chauhan, 2000). George Watt, C.B. Clarke, A. Meebold and S.K. Mukerjee were the earliest plant explorers of Manipur where they collected many plant species including several Lamiaceae members from the state. Among the dominant families of dicots in Manipur, Lamiaceae S. stricto. is found to be the fourth abundant family by Deb (1961) who reported 63 species under 32 genera with 2 varieties from the state. Singh (1987) had studied 18 genera and 25 species from Tengnoupal district (Chandel) of Manipur, and work of Singh (1980) described 25 species distributed over 17 genera in the Tamenglong district in the said family. Sinha (1996) described 41 species under 24 genera and Singh et al. (2003) discuss 12 genera and 16 species under this family. Also, Khan (2005) noted 16 species from Thoubal district under this family excluding the recently shifted members from Verbenaceae regarding their ethnomedicinal uses. Singh (1997) described Leucas manipurensis Singh as new species from Manipur which is found to be endemic to India and confined to Assam, Nagaland, Manipur, Tripura and Meghalaya (Chauhan, 2000).

Over and above these, three species Clerodendrum lasiocephalum C.B.Clarke, Orthosiphon wattii Prain, Premna milleflora C.B.Clarke are also found to be endemic to North-East India (Anon., 2010). Kumar (2008) described a new species of Pogostemon



Desf. while revising the subfamily Pogostemonoideae for Flora of India as Pogostemon manipurensis which was earlier reported as P.purpurescens Dalz. from Kassome range, Manipur by Watt. Some species reported earlier by Deb (1961) could not be traced from their collection site or locality.

Materials and Methods Plant material collection

Ten 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 IC₅₀ (µg/ml). The seeds of selected ten 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., Clerodendrum colebrookianum Walp., Clerodendrum indicum(L.) Kuntze and Perilla frutescens L.) (Table 1), under the subfamily Nepetoideae of family Lamiaceae will be collected locally and will be planted in experimental fields for this study. All the selected taxa are used as culinary herbs in Manipur and are cultivated, the aerial parts of these plants will be collected just before the flowering time. The collected samples will be dried in shade and make it into powder form by a grinder. Specimens will be identified and the vouchers will be deposited at the Manipur International University. The information regarding the selected ten plants (Table 1) are collected from the Manipur University Museum of Plants (MUMPS), Department of Life Sciences, Manipur University, Canchipur, Imphal.

Survey, collection and identification of species of Lamiaceae

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 km² lies in the North Eastern part of India falls under Indo-Myanmar hotspot regions of the world (Meyers et al., 2000) with extraordinarily 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.

Frequent surveys had been conducted covering the nine districts of Manipur between the periods of 2003 to 2009 to collect the plant specimens during their flowering season (Devi, et al., 2012). Plants belonging to the family were collected and noted down their related information. By comparing the character of these plants with various available floras (Hooker, 1885; Cooke, 1906; Duthie, 1911; Gamble, 1924; Kanjilal et al., 1939; Mukerjee,1940; Deb, 1961, 1983 and Li and Hedge, 1994) and other literatures (Harley and Paton, 2001; Rajendran and Daniel, 2002; Harley et al., 2003; Suddee et al., 2004a, b; Suddee et al., 2005) the collected plants were preliminarily identified. Classification provided by Harley et al. (2004) will be followed for the study.

SL. NO.	Species	Local Name	Voucher No.
1	Clerodendrum colebrookianum Walp.	Kuthap	004269
2.	Clerodendrum indicum(L.) Kuntze	Moirang khanum	004262
3.	Elsholtzia blanda Benth.	Kanghuman	004302
4.	Elsholtzia communis (Coll. and Hemsl.) Diels var.	Lomba purple flower	004328
5.	Elsholtzia communis (Coll. and Hemsl.) Diels var.	Lomba white flower	004301
6.	Elsholtzia stachyodes (Link) Wu	Tekta	004303
7.	Hyptis suaveolens Poit.	Tukma	004311
8.	Ocimum americanum L.	Mayangba	004313
9.	Ocimum basilicum L.	Naoseklei	004312
10.	Perilla frutescens L.	Thoiding Angouba	004309

Table 1. Ten selected species of Lamiaceae of Manipur with their local names and voucher numbers.



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Extraction

Extraction method for Total Phenolics and Tannins

Total phenol content and tannin will be 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 will be weighed and kept in magnetic stirrer for 3 h after adding 10 ml of 80% ethanol. The extracts will be centrifuged for 15 min at 10, 000 rpm. The supernatants has to be collected and stored for analyzing total phenol and tannin contents.

Extraction method for flavonoids

Hundred milligrams of the powdered samples will be weighed and mixed with 10 ml of 80% methanol by intermittent maceration for 48 h. The solvents evaporated and reduced up to 5 ml at room temperature. This extract will be 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 will be done by slight modification of method adopted by Yang et al. (2007). From the dried powdered samples, 3 g are weighed and put into 100 ml flasks. Each flask is added with 50 ml of 80% methanol. After one week of storage at room temperature the supernatants will be filtered and these filtered extracts are dried at room temperature (30°C). The dried samples are then weighed. The extracted samples are then dissolved in 10 ml methanol and stored in refrigerator for further experiments.

Estimation

Estimation of total phenolics

From the supernatants of phenolic extracts, 1 ml of each sample is collected then evaporated in a petriplate to dryness. Then the dried residue is dissolved in 1 ml of distil water; 100 μ l 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 is added. Then after 2 min, 20% of Na₂ CO_{3 is} added and mixed thoroughly. The contents are kept in a boiling water bath for about 1 min. Then the test tubes are cooled in running tap water and the absorbances of the blue coloured complex are taken against blank at 650 nm with the help of UV-VIS Double Beam Spectrophotometer Version 6.51. The total phenol content is calculated and expressed in mg/g using a standard curve prepared from catechol.

Estimation of flavonoids

From the supernatants of flavonoids extract, 100 μ l of the supernatant is taken and it is 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 is kept at room temperature for 30 min. The absorbance is measured at 415 nm using UV-VIS Double Beam Spectrophotometer Version 6.51. The calibration curve is prepared using different concentrations of quercetin which is expressed in mg/gm dry weight.

Estimation of tannin

From the supernatants of tannin extracts, $100 \ \mu l$ of aliquot of each sample is taken and 7.5 ml of distil water are added. After that, 0.5 ml of Folin Denis Reagent (FDR) followed by 1 ml of 35% Na₂ CO₃ is 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 will be 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 (6x10-5M) is freshly prepared, where 3 ml of this solution is mixed with 100 µl of methanolic plant extracts ($3-50 \mu g/ml$). The samples are incubated for 20 min at $37^{\circ}C$ 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 is measured (A_B). The experiment is carried out in triplicate. Radical scavenging activity is calculated using the formula given below:

% inhibition of DPPH = $[AB-AE /An] \times 100$

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



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Essential oil extraction

The dried materials which is kept separately, for each and every species are taken for the estimation of crude oil by using Hydro-distillation method apparatus.

Crude fat extraction

From the dried materials, Crude fat is extracted by using Soxhlet apparatus, using the medium solvent by Petroleum Ether.

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 are considered to be significant. Correlation analyses of free radical scavenging activity (Y) versus the total phenolic content, flavonoids and tannin (X) are also carried out by using Microsoft Excel-2007.

Results and Discussion

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., 2002, 2007). Among the Lamiaceae plants, with the exception of Hyptis suaveolens, which has high medicinal values and is widespread as a noxious weed (Devi et al., 2008). 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. communisvar. purple flower (21.39 ± 0.927). The flavonoid content in the selected species ranged from 13.30 ± 0.684 (Perilla frutescens) to 26.03 ± 0.217 (O. basilicum) (Devi et al., 2011) (Tab. 2).

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

Tab. 2. Polyphenolic Contents (mg/g DW (Dry Weight)±SEM)in mature aerial parts of Lamiaceae plants (n=3) **Note:** Different letters between species denote significant differences (LSD test, P<0.05)

Source: Devi, K.S., Singh, P. K. (2011). Polyphenolic Compounds and Free Radical Scavenging Activity in Eight Lamiaceae Herbs of Manipur, Notulae Scientia Biologicae, 3(2):108-113.

Flavonoids exhibit inhibition of mutagenicity induced by chemical mutagens and have anticarcinogenic, antioxidant and antiinflammatory activities (Miyazawa et al., 2000, Cotelle, 2001). In five Salvia species, Nickavar et al. (2007) reported the total flavonoid content which ranged from 8.58 ± 0.99 to 53.16 ± 1.95 mg/g DW due to the difference in species. 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).

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). 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 B₁₂ (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).



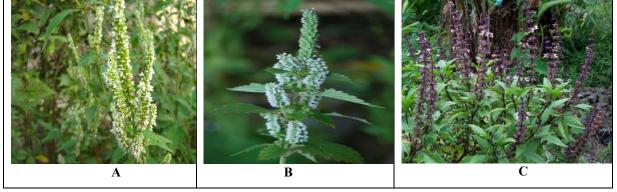
Fig. 2, presents the free radical scavenging activity of the selected plants under Lamiaceae found in Manipur which is expressed as IC50 (μ g/ml). The maximum free radical scavenging activity was found in Hyptis suaveolens (11.67±0.221) and minimum in E. communis var. purple flower (38.29±0.532) with more free radical scavenging activity in H. suaveolens as lesser is the IC₅₀ of the sample, more is the free radical scavenging activity and vice versa. The IC50 of five Salvia species ranged from 23.53 to 129 μ g/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 sub1strate (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. 3). 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 their hydroxyl groups (Chen and Ho, 1997, Yang et al. 2007). 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. 4) and with tannins (X) and free radical scavenging activity (Y) is found to have a correlation coefficient of $R^2 = 0.690$ (Fig. 5). 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 use as anti-carcinogenic, anti-mutagenic and in treatment of cancer patients (Ramakrishnan et al., 2006, Sui et al. 2004).

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Conclusions

These ten 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 research programme 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 and other biochemical and physiological parameters.



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Figure 1. Eight selected plant species of Lamiaceae of Manipur.

*A. Elsholtzia blanda Benth., B. Elsholtzia stachyodes (Link) Wu, C. Ocimum basilicum L., D. Hyptis suaveolens Poit., E. Ocimum americanum L., F. Perilla frutescens L., G. & H. Elsholtzia communis (Coll. and Hemsl.) Diels var. (purple & white flower), I. Clerodendrum indicum (L.) Kuntze, J. Clerodendrum colebrookianum Walp.

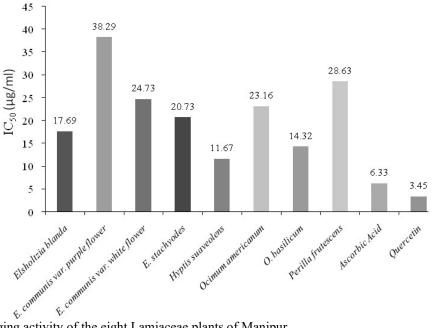
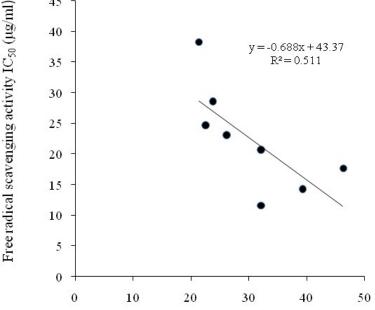


Fig. 2. Free radical scavenging activity of the eight Lamiaceae plants of Manipur. Source: Devi, K.S., Singh, P. K. (2011). Polyphenolic Compounds and Free Radical Scavenging Activity in Eight Lamiaceae Herbs of Manipur, Notulae Scientia Biologicae, 3(2):108-113.





Total phenolic content (mg/g DW)

Fig. 3. Correlation of free radical scavenging activity (Y) versus total phenolic content (X) of eight Lamiaceae plants of Manipur.
Source: Devi, K.S., Singh, P. K. (2011). Polyphenolic Compounds and Free Radical Scavenging Activity in Eight Lamiaceae Herbs of Manipur, Notulae Scientia Biologicae, 3(2):108-113.

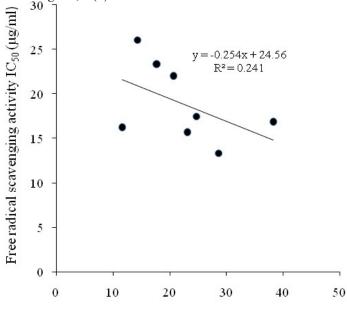
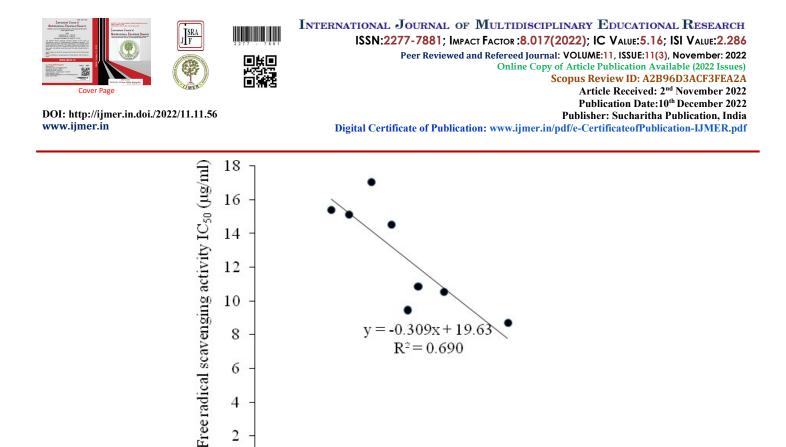
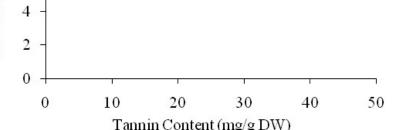




Fig. 4. Correlation of free radical scavenging activity (Y) versus flavonoid content (X) of eight Lamiaceae plants of Manipur. **Source:** Devi, K.S., Singh, P. K. (2011). Polyphenolic Compounds and Free Radical Scavenging Activity in Eight Lamiaceae Herbs

of Manipur, Notulae Scientia Biologicae, 3(2):108-113.





y = -0.309x + 19.63

 $R^2 = 0.690$

Fig. 5. Correlation of free radical scavenging activity (Y) versus tannin content (X) of eight Lamiaceae plants of Manipur.

Source: Devi, K.S., Singh, P. K. (2011). Polyphenolic Compounds and Free Radical Scavenging Activity in Eight Lamiaceae Herbs of Manipur, Notulae Scientia Biologicae, 3(2):108-113.

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