# Screening of Seven Medicinal Plants of Family Lamiaceae for Total Phenolics, Flavonoids and Antioxidant Activity

Touseef Rehan[1*], Riffat Tahira[1], Tabassum Rehan[2,] Ayesha Bibi[3] and Muhammad Naeemullah[1]
[1]Plant Genetic Resources Program, National Agricultural Research Centre, Park Road, Islamabad, Pakistan
[2]Department of Biotechnology, Quaid-i-Azam University, Islamabad, Pakistan
[3]Department of Genetics, Hazara University, Mansehra, Pakistan
*Corresponding Author: touseefxyz@gmail.com

## ABSTRACT

In order to prolong the storage stability of foods and to reduce the damage to human body, synthetic antioxidants are used in industrial processing. However, the side effects of some synthetic antioxidants are documented as they are reported to be carcinogenic. Present study was carried out to explore the antioxidant potential of phenolics, flavonoids and terpenes present in crude extracts and essential oils of seven selected medicinal herbs of family Lamiacae. Highest oil yield was observed for *Oreganum vulgare* followed by *Melissa officinalis*. Lowest oil yield was recorded for *Lavendula officinalis*. Methanolic extract of *M. officinalis* showed highest phenolic content (243.9mg GE/100g DW), while highest flavonoid content was recorded in methanolic extract of *Mentha pulegonium* (256.2mg CE/100g DW). Highly significant differences were found among different plants and concentrations for plant extracts and essential oils. Highest antioxidant activity was recorded for essential oil (68.523%) and methanolic extract (60.017%) of *M. officinalis*. *O. vulgare* showed lowest antioxidant activity in both essential oil (46.583%) and methanolic extract (38.585%).

**Key words:** DPPH, Total Phenolics, Total flavonoids, Essential oils

## 1. Introduction

Polyphenols are major secondary metabolites having important role in scavenging free radicals in plant cells. Oxidative radicals are reported to be a potential cause of mutations, damage to lipids, DNA and proteins which in turn lead to certain disorders including cancer. Higher antioxidant potency of polyphenolic compounds leads to growing interest in isolation and use of natural products as antioxidants [1]. Plant extracts are rich sources of polyphenolic compounds including phenolic acids, tannins and

flavonoids [2, 3]. Flavonoids are mainly 15-C compounds found generally throughout the plant kingdom [4]. Many natural compounds isolated from plants having free radical scavenging potential are reported to be promising therapeutic agents for several free radical pathologies [5, 6]. Tannins, high molecular weight polyphenols are also found naturally in medicinal herbs and have a major role in free radical scavenging. Human diet contain tannins most abundantly and various biologically important functions including protection against oxidative damage and certain degenerative diseases, are reported to be exhibited by tannins [7].

The family Lamiaceae is represented by about 236 genera and 7172 species in the world [8]. Many members of this family are beneficial economically and are frequently useful for several medicinal, ornamental, commercial and culinary purposes. Several previous studies reported strong antioxidant potential of members of lamicea family [9-11]. 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. Medicinal herbs belonging to family of lamiacae are rich in essential oil content. Eseential oils are considered to be one of the potential agents having strong antioxidant, antibacterial and anticancer activities [12-14] The present study was carried out to screen out and compare the essential oils and methanolic extracts of the members of lamiace family including *Melisa officinalis, Mentha pulegonium, Napeta cateria, Oreganum vulgare, Rosemarinus officinalis, Thymus vulgaris and Lavendula officinalis* for their phytochemical constituents and antioxidant potential.

## 2.1. Materials and Methods

### 2.1.1. Preparation of Plant Material

Leaves of the selected seven medicinal plants were collected and were shadow dried under glass house conditions at National Agriculture Research Centre (NARC), Islamabad, Pakistan. Dried leaves were subjected to grinding to make coarse powder for extraction process. 70g of each of the dried powdered plant material was macerated in 200ml of 70% methanol for 48 hours in air tight bottles. Filtration of this was carried out using Whatman filter paper 1. Methanol was evaporated from the filtrate of each plant using Rotary evaporator (Bibby, RE200B). The dried plant material was collected, weighed, labeled and stored at 4˚C.

### 2.1.2. Extraction of Essential Oils

Extraction of essential oils was carried out through hydrodistillation using clavenger apparatus. 250g of each of the dried plant material was used with 3 liter water. Oil was collected from the upper surface of condensed water in separating funnel. Oil was purified with n-hexane and sodium sulfite.

EO (%) = Volume of essential oil (ml) ×100/ Weight of dried plant material (g)

### 2.1.3. Determination of the total phenolic content

Total phenolic content in dry material of selected seven medicinal plants was determined through Folin cicalteu assay. 1 ml of the extract of each plant and gallic acid standard solution (20, 40, 60, 80, and 100 mg/1} was added to 9 ml ddH2O in a 25ml volumetric flask. dd$H_2O$ was used as a blank. To each of the flask 1ml of Folin cicalteu reagent was added and shaken vigorously. 10 ml of 7% $Na_2Co_3$ solution was added to each of the mixture after 5 minutes. Volume of the solution was raised up to 25ml with distilled water. Samples were incubated for 90 minutes at room temperature. UV-VIS Spectrophotometer Lambda 5 was used to measure the absorbance against the prepared reagent blank at a wavelength of 750nm. The data for the total phenolic contents of methanolic extracts of all the seven medicinal herbs was expressed as milligrams of gallic acid equivalents (GAE) per 100 grams dry mass (mg GAE/100 g DW).

### 2.1.4. Determination of the total flavonoid

Aluminium chloride colorimetric assay was used to determine flavonoid content of selected medicinal herbs. An aliquot (1ml) of extracts or standard solution of catechin was added to 10ml volumetric flask containing 9ml dd$H_2O$. 300µl of 5% $NaNo_2$ was added. After 5 min incubation 300µl of 10% $AlCl_3$ was added. 2ml of 1M NaOH were added to the flask at the sixth minute and volume of the mixture was raised up to 10ml with dd$H_2O$. The solution was shaken vigorously and absorbance was measured at 510nm against the prepared reagent blank using UV-VIS Spectrophotometer Lambda 5. The data of the total flavonoid contents of the dry herbs were expressed as milligrams catechin equivalents (CE) per 100 grams dry mass (CE/100 g DW). All samples were analyzed in duplicates.

### 2.1.5. DPPH Assay

Standard spectrophotometric 1, 1-diphenly - 2- picrylhydrazyl (DPPH) assay was carried out to measure the free radical scavenging activity of essential oils and methanolic extracts of selected medicinal herbs. Four concentrations of essential oils and plant extracts (50, 100, 150 and 200ppm) were used. 25 µl of each concentrations of essential oil and plant extract was added to 2975 µl of 0.1mM methanolic solution of DPPH. 60 minutes incubation was given to all the samples at room temperature. DMSO was used as blank. Absorbance of the samples was measured against blank at 517nm using UV-VIS Spectrophotometer Lambda 5. All the samples were analyzed in triplicates.

### 2.2. Calculation

Inhibition of free redical by DPPH in percent (I %) were calculated in following way.

$$I\% = [(A_{blank} - A_{sample}) / A_{blank}] * 100$$

$A_{blank}$ is the absorbance of the control reaction (containing all reagents excepts the test compound ) $A_{sample}$ is the absorbance of the test compound.

### 2.3. Statistical analysis

Data was expressed as means of three replicates and was subjected to analysis of variance followed by LSD using software statistix version 8.

## 3. Results and Discussion

A plethora of previous studies indicate that certain plant derived compounds such as vitamins, phenolic acids, flavonoids, dietary fibers and free radical scavengers play an important role in the prevention of common diseases like cancer, inflammation, dibetes, certain cardiovascular disorders and aging process [15,16]. Such natural compounds are considered to be promising health promoting agents due to their antioxidant attibutes [17-19].

The family Lamiacae lists about 250 genera and more than 6700 species [20]. Certain members of Lamiaceae family are used as dietary supplements and are considered to be important preventive agents of many disorders [21-23]. Essential oils and extracts of these plants are known to possess antiseptic, anti-inflammatory and antimicrobial activities [24-26]. Phenol sand flavonoids have been implicated as natural antioxidants in fruits and vegetables, possessing health maintaining and protecting abilities [27].

Crude methanolic extracts of selected seven medicinal plants (*Melisa officinalis, Mentha pulegonium, Napeta cateria, Oreganum*

*vulgare, Rosemarinus officinalis, Thymus vulgaris and Lavendula officinalis)* were screened for phytochemical analysis. The quantitative estimation of phytochemical constituents of these five herbs showed that these medicinal plants are rich source of total phenolic and flavonoids according to the data shown in Fig 1. Methanolic extract of *M. officinalis* showed highest phenolic content (243.9mg GE/100g DW), while higest flavonoid content was recorded in methanolic extract of M. pulegonium (256.2mg CE/100g DW).

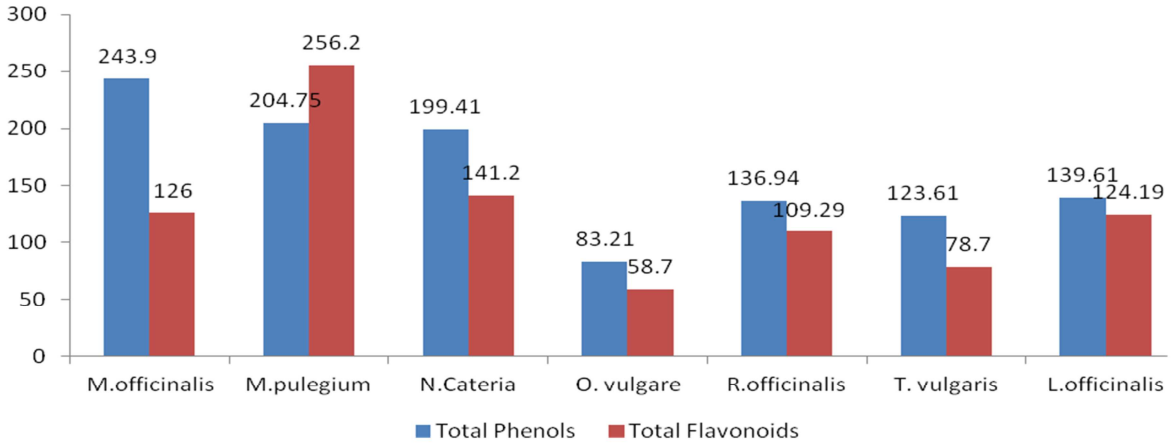


**Fig. 1.** Total Phenols and Flavonoids in medicinal plants in methanolic extracts

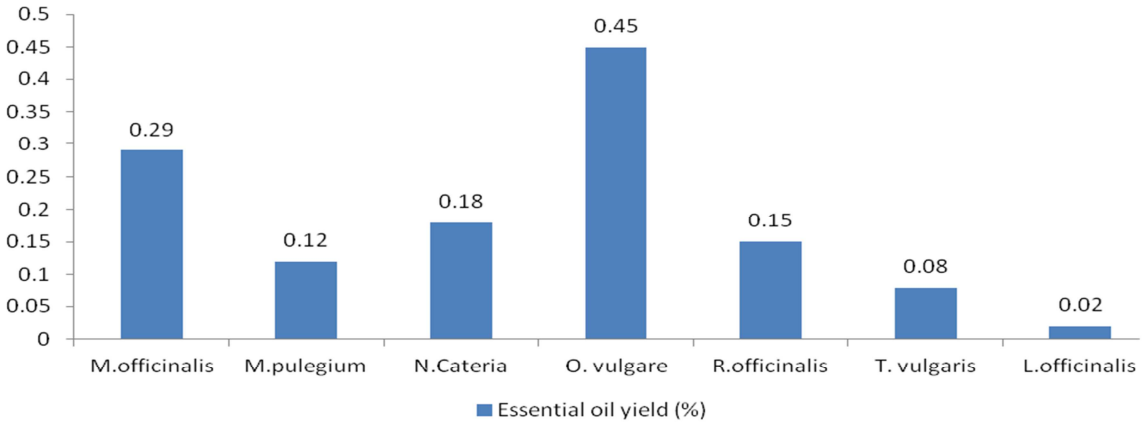


**Fig. 2.** Essential oil yield from different medicinal plants of family Lamiaceae

Lamiaceae plants are a source of aromatic oil responsible for specific flavour and aroma. In spite of the other secondary metabolites such as alkaloids, ursolic acid and iridoids [28], terpenoides and flavonoides are the major compounds studied so far in this family [29]. Many of the biological activities are associated with the terpene constituents of the essential oils of these plants [30]. Terpenoids are also linked to the chemical defenses of these plants against the attack of herbivores and pathogenicmicroorganisms [31]. In the present study percent essential oil yield was determined of the selected seven medicinal herbs of family Lamiacae.

Mean values for essential oil yield from different parts of lemon basil are presented in Fig.2.

Highest oil yield was observed for *O.vulgare* (0.45%) followed by *M.officinalis*. Lowest oil yield was recorded for *L.officinalis* (0.02%).

The antioxidative property of polyphenols is a predominant feature of their radical-scavenging capacity [32, 33]. Due to ideal structural chemistry of polyphenoles they possess more antioxidant potential than ascorbate and tochopherol [34]. Recently a highest rate of cardiovascular disorders due to lipid peroxidation has been recorded worldwide that is to the exposure of human population to reactive oxygen species in the environment and also due to their large generation in aerobes [35, 36].

**Table 1.** Analysis of variance for antioxidant activity of essential oils of different medicinal plants

| **Source** | **DF** | MS | F |
|---|---|---|---|
| Treatment | 6 | 1803.51 | 10.80** |
| Dose | 3 | 245.53 | 79.29** |
| Error | 18 | 22.74 | |
| Total | 27 | | |

** Highly significant at P≤0.05

**Table 2.** Analysis of variance for antioxidant activity of methanolic extract of different edicinal plants

| **Source** | **DF** | MS | F |
|---|---|---|---|
| Treatment | 6 | 1992.37 | 181.33** |
| Dose | 3 | 204.06 | 18.57** |
| Error | 18 | 10.00 | |
| Total | 27 | | |

** Highly significant at P≤0.05

Four different concentrations (50, 100, 150 and 200ppm) of the prepared methanolic extracts and essential oils of selected medicinal herbs were subjected to screening for their possible antioxidant activity through DPPH assay. Highly significant differences were found among different plants and concentrations forplant extracts and essential oils (Table 1 and 2). Highest antioxidant activity was recorded for for essential oil (68.523%) and methanolic extract (60.017%) of *M. officinalis*. *O.vulgare* showed lowest antioxidant activity in both essential oil (46.583%) and methanolic extract (38.585%). Antioxidant activity of extracts and essential oils increased in a dose dependendent manner. Highest antioxidant activity was depicted at 200ppm for both the extracts and essential oils of all the seven medicinal herbs, while lowest at 50ppm (Table 4).

Comparatively essential oils were having higher free radical scavenging potential than methanolic extracts. The antioxidant properties of the essential oil and different extracts of *lamiacae family plants* have been previously reported. Higher ratio of polar polyphenolic compounds and terpenes in essential oils is responsible for exhibition of higher antioxidant activity [37].

**Table 3.** Mean values for antioxidant activity (DPPH assay) of different medicinal plants

| S.NO | Plant | Antioxidant Activity of Essential Oils | Antioxidant Activity of Plant Extracts |
|---|---|---|---|
| 1 | Lemon Mint | 68.523[a] | 60.017[a] |
| 2 | Rosemary | 66.440[ab] | 47.742[ab] |
| 3 | Penneroyal | 61.265[bc] | 55.120[bc] |
| 4 | Thyme | 61.265[bc] | 43.638[bc] |
| 5 | Lavender | 55.800[cd] | 51.270[cd] |
| 6 | Napeta Cateria | 51.945[de] | 51.615[d] |
| 7 | Oregano | 46.583[e] | 38.585[e] |

*Means with same letters are statistically non-significant at P<0.05.

**Table 4.** Mean values for antioxidant activity (DPPH assay) of different medicinal plants at four different concentrations

| S.NO | Concentration | Antioxidant Activity of Essential Oils | Antioxidant Activity of Plant Extracts |
|---|---|---|---|
| 1 | 200ppm | 77.407[a] | 68.267[a] |
| 2 | 150ppm | 65.379[b] | 57.359[b] |
| 3 | 100ppm | 51.754[c] | 43.957[c] |
| 4 | 50ppm | 40.530[d] | 29.267[d] |

*Means with same letters are statistically non-significant at P<0.05.

## 4. Conclusion

A good array of secondary metabolites and antioxidant potential was exhibited by essential oils and methanolic extracts of members of family Lamiaceae. Therefore these essential oils and crude extracts of these medicinal plants would be more useful antioxidant agents as compared to synthetic antioxidants. Further studies are required to explore antioxidant potential of these purified natural products from these essential oils and crude extracts.

## References

[1] Shui GH, LP Leong.2004. Analysis of polyphenolic antioxidants in star fruit using liquid chromatography and mass spectrometry, J Chromatogr A., 1022:67-75.

[2] Wolfe K, X Wu, RH Liu. 2003. Antioxidant activity of apple peels, J Agric Food Chem., 51:609-614.

[3] Naik GH, KI Priyadarsini, H Mohan. 2006. Free radical scavenging reactions and phytochemical analysis of triphala, an ayurvedic formulation, Current Science., 90(8):1100-1105.

[4] Harborne JB. 1988. Introduction to ecological biochemistry, 3rd edn, Academic Press London.

[5] Parshad R, KK Sanford, FM Price, VE Steele, RE Tarone, GJ Kelloff, CW Boone.1998. Protective action of plant polyphenols on radiation-induced chromatid breaks in cultured human cells, anticancer Res., 18:3263-3266.

[6] Chang H, G Huang, DC Agrawal, C Kuo, C Wu, H Tsay. 2007. Antioxidant activities and polyphenol contents of six folk medicinal ferns used as “Gusuibu”, BOT STUD., 48:397-406.

[7] Atanassova M, V Christova-Bagdassarian. 2009. Determination of tannin content by titrimetric method for comparison of different plant species, J. Univ. Chem. Technol. Metallurgy., 44(4):413-415.

[8] Harley RM, S Atkins, A Budantsev, PH Cantino, B Conn, R Grayer, MM Harley, R Kok, T Krestovskaja, A Morales, AJ Paton, O Ryding and T Opson. 2004. Labiatae. *In*: Kadereit, J.W. (ed.). The families and genera of vascular plants (Kubitzki, K.: ed.). Vol. 7. Pp. 167-275.

[9] Marinova EM, NV Yanishlieva. 1997. Antioxidative activity of extracts from selected species of the family *Lamiaceae* in sunflower oil, Food Chem., 58:245-248.

[10] Hirasa K, M Takemasa. 1998. Spice science and technology. Marcel Dekker: New York.

[11] Triantaphyllou K, G Blekas, D Boskou. 2001. Antioxidative properties of water extracts obtained from herbs of the species *Lamiaceae,* Int J Food Sci Nutri., 52:313-317.

[12] Jie H, S Tao, H Jun, C Shuangyang, C Xiaoqiang and Z Guolin. 2007. Chemical composition, cytotoxic and antioxidant activity of the leaf essential oil of Photinia serrulata,Food Chem.,103: 355-358.

[13] Hussain, AI, F Anwar, STH Sherazi and R Przybylski. 2008. Chemical composition, Antioxidant and antimicrobial activities of basil (Ocimum basilicum) essential oils depends on seasonal variations, Food Chem., 108: 986-995

[14] Shabbir MK, R Nadeem, H Mukhtar, F Anwar and MW Mumtaz. 2009. Physico-chemical analysis and determination of various chemical constituents of essential oil in Rosa centifolia, PAKISTAN J BOT., 41(2): 615-620

[15] Marino M, C. Bersani and G Comi. 2001. Impedance measurements to study the antimicrobial activity of essential oils from Lamiacea and Compositae, INT J FOOD MICROBIOL., 67: 187-195

[16] Mata AT, C Proenca, AR Ferreira, MLM. Serralheiro, JMF Nogueira and M EM Araujo. 2007. Antioxidant and antiacetylcholinesterase activities of five plants used as Portuguese food species, Food Chem., 103: 778-786.

[17] Iqbal S, MI Bhanger and F Anwar. 2005. Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan, Food Chem., 93: 265-272.

[18] Siddhuraju P and K. Becker. 2007. The antioxidant and free radical scavenging activities of processed cowpea (Vigna unguiculata (L.) Walp.) seed extracts, Food Chem., 101: 10-19.

[19] Liu H, N Qiu, H Ding and R Yao.2008. Polyphenols contents and antioxidant capacity of 68 Chinese herbals suitable for medicinal or food uses, FOOD RES INT., 41(4): 363-370.

[20] Hedge C. 1992. A global survey of the biogeography of the Labiatae. In Harley RM & Reynolds T (eds) Advances in Labiatae Science. Royal Botanic Gardens, Kew 7-17.

[21] Chalchat JC and MM Ozcan. 2008. Comparative essential oil composition of flowers, leaves and stems of basil (Ocimum basilicum L.) used as herb, Food Chem., 110: 501-503.

[22] Hussain AI, F Anwar, STH Sherazi and R Przybylski. 2008. Chemical composition. Antioxidant and antimicrobial activities of basil (Ocimum basilicum) essential oils depends on seasonal variations, Food Chemistry., 108: 986-995.

[23] Baser KHC, B Demirci, M Kurkcuoglu, F Satil and G Tumen. 2009. Comparative moropholigical and phytochemical characterization of Salvia cadmia and S. smyrnaea. PAKISTAN J BOT., 41: 1545-1555.

[24] Burt S. 2004. Essential oils: their antimicrobial properties and potential application in foods-a review. INT J FOOD MICROBIOL., 94: 223-253.

[25] Skocibusic M, N Bezic and V Dunkic. 2006. Phytochemical composition and antimicrobial activity of the essential oils from Satureja subspicata Vis. Growing in Croatia, Food Chemistry., 96: 20-28.

[26] Bozin B, N Mimica-Dukic, N Simin and G Anackov. 2006. Characterization of the volatile composition of essential oils of some Lamiaceae species and the antimicrobial and antioxidant activities of the entire oils, J. Agric. Food Chem., 54: 1822-1828.

[27] Kahkonen MP, AI Hopia, HJ Vuorela, J Rauha, K Pihlaja, ST Kujala and M Heinonen. 1999. Antioxidant activity of plant extracts containing phenolic compounds,J. Agric. Food Chem., 47: 3954-3962.

[28] Watson L, MJ Dallwitz. 1991. The families of angiosperms: automated descriptions, with interactive identification and information retrieval, Aust. Syst. Bot., 4:681-95.

[29] Cole MD. 1992. The significance of the terpenoids in the Labiate. In-Advances in Labiate Science, ed Royal Botânic Gardens KEW, Whitstable, pp 315-324.

[30] Richardson PM.1992. The chemistri of the Labiate: An introduction and overview. In-Advances in Labiate Science, ed. Royal Botanic Gardens KEW, Whitstable, pp. 291-297.

[31] Yang B, A Kotani, K Arai, F Kusu. 2001. Estimation of the antioxidant activities of flavonoids from their oxidation potentials, Anal Sci., 17:599-604.

[32] Cotelle N. 2001. Role of flavonoids in oxidative stress, Curr op Med Chem., 1:569-590.

[33] Facino RM, M Carini, L Franzoi, O Pirola, E Bosisio. 1990. Phytochemical characterization and radical scavenger activity of flavonoids from Helichrysum italicum G. Don(Compositae), Pharmacol Res., 22:709-721.

[34] Pandhair V, BS Shekhon. 2006. Reactive oxygen Species and antioxidants in plants: An Overview. J Plant Biochem Biot., 15:71-78

[35] Lachance PA, Z Nakat, WS Jeong. 2001. Antioxidants: an integrative approach, Nutrition., 17:835-838.

[36] Nickavar B, M Kamalinezad, H Izadpanah. 2007. In vitro free radical scavenging activity of five Salvia species, Pak J Pharm Sci., 20(4):291-294.

[37] Hamzeh A.2010. Antioxidant Activity of the Essential Oil and Methanolic Extract of *Teucrium orientale* (L.) subsp. *taylori* (Boiss.) Rech. F, Iran J Pharm Res Autumn., 9(4): 417–423.