



Research Article

Variation in Total Phenolic and In-vitro Antioxidant Activities of *Mentha longifolia* L. From Azad Jammu and Kashmir Territory

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Abstract

The examination was done to decide the all-out phenolics, all-out flavonoids, and in-vitro antioxidants of *M. longifolia* gathered from various areas (Rawalakot, Khaigala, Akhorban, Alisojal, Phirkot, Bhaikh, and Singola) of Poonch, Azad Jammu, and Kashmir. The watery concentrates of *M. longifolia* were surveyed for absolute phenolics, all-out flavonoids, restraint against thiobarbituric acid reactive species (TBARS) prompt by various star oxidants (10 μ M FeSo₄ and 5 μ M sodium nitroprusside) in mice liver and brain homogenates, DPPH radical searching limit, iron-chelating power. The outcomes demonstrated that *M. longifolia* contained a generous level of phenolic (192.10 \pm 2.10 mg/g) and flavonoid compounds (4.23 \pm 0.03 mg/g) separately. Among various areas, the Alisojal indicated the most elevated antioxidant movement against lipid peroxidation prompted by iron in mice liver (IC₅₀, 23.67 \pm 2.67 μ g/ml) and brain (IC₅₀, 13.85 \pm 0.85 μ g/ml) and showed the most noteworthy cell reinforcement action against sodium nitroprusside in mice liver (IC₅₀, 14.76 \pm 1.76 μ g/ml) and brain (IC₅₀, 14.05 \pm 1.05 μ g/ml). The concentrates of various areas likewise demonstrated promising DPPH radical searching (IC₅₀, 30.47 \pm 1.47 - 71.77 \pm 2.77 μ g/ml) and metal-chelating activities (IC₅₀, 40.67 \pm 0.89 - 90.92 \pm 3.92 μ g/ml). In general, it was presumed that *M. longifolia* may be an expected wellspring of phenolic and flavonoid mixes bearing a considerate of the antioxidant character towards animal tissues.

Keywords: *Mentha longifolia*, antioxidant activities, phenolic substance, total flavonoid.

Introduction

Mild zone of Pakistan including Azad Jammu and Kashmir is blessed with the rich wellspring of sweet-smelling and restorative plants. For a considerable length of time, restorative plants include chemical segments of remedial and medicinal values since they have been utilized as medicine and drugs with the assistance of human diseases. Over 80% of the world's occupants depend on the regular solution for their essential medicinal services necessities (Derwich et al., 2010). Therapeutic plants are utilized against parasitic, bacterial, and viral contaminations in a few pieces of the world. Therapeutic plants are the shelter of nature to fix various ailments of people (Padmini et al., 2010). One of them *Mentha longifolia* L. has a place with family Lamiaceae is a quickly developing, lasting herb that regularly called wild mint and horsemint (Raymond et al., 2004). Wild mint plants can reach up to approach the tallness of 1.5 m and their leaves are masterminded in



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inverse sets, from oval to lanceolate, regularly wool, and with a serrate edge. Leaf hues run from dim green and dim green to purple, blue, and once in a while light yellow (Brickell et al., 1997). For the most part, individuals use it as a drug since it is estimated as stomachic carminatives, diuretic, and energizer and organic exercises like stomach related, choleric, pain-relieving, antioxidant, calming antiviral, antibacterial just as insecticidal, antiallergenic, antiulcer, detoxicant, tonic, and sedative, it is excessively utilized for mouth wash, gas torment, muscle torment, and toothache (Duke et al., 2002; Phatak et al., 2002). Dry mint leaves are significant in the drug of stomach tremors of a nutritious channel (Fialova et al., 2008). Antioxidant mixes in plants assume an imperative job as wellbeing ensuring factor. Numerous antioxidant mixes, normally happening from plant sources, have been recognized as a free radical or dynamic oxygen foragers (Zheng and Wang, 2001). The antioxidant exercises are identified with the structure of phenolic mixes, and rely upon the positions and number of hydroxyl gatherings and glycosylation and different substitutes of the phenolic atom (Rice-Evans et al., 1996; Heim et al., 2002). As of late, the interest for normal antioxidants is expanded in view of their potential for ailment counteraction, wellbeing advancement, and shopper worthiness. The significance of the antioxidant constituents of plant materials in the support of wellbeing and assurance disease is likewise raising enthusiasm among researchers, food producers, and shoppers as the pattern of things to come are pushing toward utilitarian food with explicit wellbeing impacts (Aruoma, 1991). Plants are acceptable wellsprings of common antioxidants, for example, phenolic substances and expanded dietary admission of normal phenolic antioxidants connects with diminished coronary illness (Rimm et al., 1993; Middleton et al., 2000). Numerous examinations demonstrated that antioxidant exercises of plant phenolics were more grounded than that of manufactured antioxidants, for example, BHA and BHT (Oktay et al., 2003). The intake of antioxidants, for example, polyphenols, has been successful in the anticipation of these diseases (Cao et al., 1997; Vinson et al., 1995). The phytochemical investigation has demonstrated the nearness of flavonoids in the leaves of the plant (Higa et al., 2007). Flavonoids likewise assume essential jobs in barriers against predators and pathogens and add to physiological capacities, for example, dominance (torpidity) and seed development, and furthermore significant in the plant for typical development advancement and protection against disease and injury (Singh, 2010). Poonch division of Azad Kashmir is a hilly territory and situated towards the north of Islamabad/Rawalpindi and in the North – East of Murree, Pakistan. The geographic zone plotted is arranged in the lower regions of the Himalayas between 730 to 750 East longitudes and 330 to 350 North scopes. The range in geology and climatic states of this area support the vegetation of sweet-smelling and therapeutic plants going from herbs to enormous trees. Perfect and good climatic states of this district are well appropriate for the development of restorative verdure and are richly started in good countries, run earthbound, and slopes. These assets are being persecuted in the Unani arrangement of medication and are utilized by the neighborhood occupants of Kashmir itinerant families (Ahmad et al., 2011). The region of Azad Jammu and Kashmir is honored with a rich wellspring of sweet-smelling verdure, huge numbers of which have not been recently investigated for their characteristic possibilities. The current examination was arranged with the intent to explore the antioxidant exercises of indigenous specie of mint including *M. longifolia* gathered from Rawalakot.

Methodology

The Readiness of the Leave Extracts

Leaves of *Mentha longifolia* were gathered from various locales of Poonch i.e., Rawalakot, Khaigala, Akhorban, Alisojal, Phirkot, Baikh, and Singola during April-May, 2012. The new plant tests were brought to the Laboratory of Food Technology (Innovation), Faculty

of Agriculture, Azad Jammu, and Kashmir in polyethylene bags. The plant was distinguished and validated by a botanist. The leaves were washed and dried were washed in a stove at 45 °C and ground to the mesh size of 30 mm. The samples (5 g) were absorbed in high-temp water (250 ml) for 30 minutes and sifted utilizing the Whatman filter paper. All animal study was utilized in exacting agreement with the NIH Guide for the Consideration and Utilization of Laboratory Animals. Male BALB/c mice (2.0–2.5 months and 24–30 g), were bought from the National Institute of Health Islamabad and were utilized for in vitro examinations. The animals were kept in independent cages with consistent access to food and water in a stay with controlled temperature (22 ± 3 °C) and on a 12-h light/dark cycle with lights turned on at 7:00 a.m.

Creation of TBARS from Liver and Brain Tissues

Creation of TBARS was controlled by an adjusted technique (Ohkawa et al., 1979). The mice were anesthetized with chloroform, yielded by execution, tissues were immediately evacuated and put on ice. One gram of brain and liver tissue was homogenized in chill 100 mM Tris buffer pH 7.4 (1:10 w/v) and centrifuged. The homogenates (100 µl) were incubated with or without 50 µl of the newly arranged oxidant (iron and sodium nitroprusside) and various concentrations of the plant extracts along with an appropriate volume of deionized water to give a complete volume of 300 µl at 37 °C for 1 h. The shading reaction was done by including 200, 500 µl, 500 µl every one of the 8.1% Sodium dodecyl sulphate (SDS), acetic acid (pH 3.4), and 0.6% TBA individually. The reaction blends, including those of sequential dilutions of 0.03 mM standard MDA was incubated at 97 °C for 1 h. The absorbance was perused in the wake of cooling the tubes at 532 nm in a spectrophotometer.

Antioxidant Activity by DPPH Radical Rummaging

The antioxidant movement of fruit extract was estimated and utilizing the scavenging of stable DPPH radical as indicated by the strategy for Hatano et al. (1998). Briefly, 0.25 mM arrangement of DPPH radical (0.5 ml) was added to the sample solution in ethanol (1 ml) at various fixations (25-200 µg/ml) of concentrates. The blend was shaken vigorously and left to represent 30 minutes in dark, and the absorbance was estimated at 517 nm. The ability to scavenging the DPPH radical was determined to utilize the following equation: (%) scavenging = $[(A_0 - A_1)/A_0] \times 100$, Where, A_0 is the absorbance of the control response reaction and A_1 is a sample and the absorbance itself. The IC₅₀ values (separate fixations that cause 50% scavenging) were resolved from the graph of scavenging impact rate plotted against the concentrated focus. All judgments were completed in triplicate.

Metal-Chelating Action

The Fe (II) chelating capacity of the concentrates was resolved to utilize an adjusted strategy for Puntel et al. (2005). Briefly, 150 µL of newly arranged 2 mM FeSO₄·7H₂O was added to a reaction blend containing 168 µL of 0.1 M Tris-HCl (pH 7.4), 218 µL of saline, and plant extracts (25-200 µL). The reaction blend was incubated for 5 min, before the expansion of 13 µL of 0.25% 1, 10-phenanthroline (w/v). The absorbance was in this manner estimated at 510 nm utilizing a spectrophotometer.

Assurance of Phenolics Content

The all-out phenolics content as Gallic acid equal was dictated by the technique for Singleton et al. (1999). The concentrates (0.5 ml) were added to 2.5 ml, 10% Folin-Ciocalteu's reagent (v/v), and 2 ml of 7.5% sodium carbonate. The reaction blend was incubated at 45 °C for 40 minutes and the absorbance was estimated at 765 nm in the spectrophotometer. Gallic acid was utilized as a standard phenol. The absolute phenol content was briefed as milligrams of Gallic acid counterparts/g extract.

Assurance of Flavonoid Content

The all-out flavonoids as quercetin counterparts were controlled by the technique for Kosalec et al. (2004). Quercetin was utilized to make the adjustment bend [0.04, 0.02, 0.0025

and 0.00125 mg/ml in 80% ethanol (v/v)]. The standard solutions or concentrates (0.5 ml) was blended in with 1.5 ml of 95% ethanol (v/v), 0.1 ml of 10% aluminum chloride (w/v), 0.1 ml of 1 mol/l sodium acetate, and 2.8 ml of water. The volume of 10% aluminum chloride was snubbed by a similar volume of refined water in the clear. After incubation at room temperature for 30 min, the absorbance of the reaction blend was estimated at 415 nm. The complete flavonoid content was communicated as milligrams of quercetin counterparts/g of concentrate.

Statistical Analysis

The software package Statistical was utilized for the investigation of data. The outcomes were communicated as means \pm standard deviation. The data were broken down by one way ANOVA and diverse gathering implies were looked at by Duncan multiple range (DMR) test where important. $P < 0.05$ was viewed as noteworthy in all cases.

Result and Discussion

Lipid Peroxidation Actuated by Iron and Sodium Nitroprusside in Mice Liver and Brain

Mice liver and brain homogenates were convincing with iron and sodium nitroprusside (SNP) to cause lipid peroxidation and the antioxidant impact of *Mentha longifolia* leaves from various areas was examined. There was a measurably significant ($p < 0.05$) increase in the arrangement of TBARS in iron (II) sulfate (10 μ M) and sodium nitroprusside (5 μ M) incited lipid peroxidation contrasted with the basal (Figs. 1 and 2). However, treatment with various genotypes leaves extracts essentially diminished the lipid peroxidation, in a dose-dependent way at a fixation scope of 25-200 μ g/ml (Fig. 2). In mice liver the IC₅₀ estimations of various genotypes leave extracts followed the order Alisojal > Rawalakot > Akhorban > Phirkot \geq Khaigala > Singola > Baikh against iron-instigated lipid peroxidation (Table 2). For sodium nitroprusside instigated lipid peroxidation, the IC₅₀ estimations of the concentrates followed the order Alisojal > Rawalakot > Singola \geq Baikh > Phirkot > Khaigala > Akhorban (Table 2). A comparable pattern in the antioxidant movement was appeared in mice brain by treatment with various concentrates (Fig. 2). In mice brain the IC₅₀ estimations of various genotypes leave separates followed the request Alisojal > Khaigala > Singola \geq Baikh > Rawalakot > Phirkot > Akhorban against iron-induced lipid peroxidation (Table 3). For sodium nitroprusside initiated lipid peroxidation, the IC₅₀ estimations of the concentrates followed the order Alisojal > Baikh \geq Singola > Rawalakot > Khaigala > Akhorban > Phirkot (Table 3). Oxygen is basic for the survival of high-impact cells; however, it has for quite some time been known to be poisonous to them when provided at fixations more prominent than those in ordinary air (Halliwell and Gutteridge, 1981). The biochemical mechanism is responsible for O₂ poisonousness includes lipid peroxidation. Free radical mischief to polyunsaturated lipids, DNA, certain amino acids, and sugars can achieve the arrangement of TBA-receptive intermediates. These intermediates, when warmed under an optimal state of acidity of causticity, separate to free malondialdehyde, which within the sight of TBA structures a trademark adduct demonstrating absorbance at 532 nm (Gutteridge and Wilkins, 1982). The brain is especially powerless against oxidative damage in light of its high oxygen usage, its high substance of oxidizable polyunsaturated unsaturated fats, and the nearness of redox-dynamic metals (Cu and Fe). Hints of iron salts are available in every single natural framework, and an expansion in the typical focus will potentiate the poisonous impacts of oxygen (Halliwell and Gutteridge, 1981). Increase in the development of TBARS in iron (II) sulphate (10 μ M) - initiated oxidative pressure to recommend conceivable harm of tissues with iron over-burden. As superoxide creation expanded the free iron in the mitochondria and cytosol can cause impressive oxidative demolition which can respond with Fe (III) to recover Fe (II) that adds to the Fenton response (Fraga and Oteiza, 2002). The development of lipid peroxidation items happened because of an over-burden of iron which has been seen in various issues like the brain, liver, and kidneys (Houglum et al., 1990; Sabir

et al., 2012). The concentrates of *Mentha longifolia* leaves from various zones can give assurance against oxidative concern in the mice brain and liver. The decrease in the Fe (II) induced lipid peroxidation in the mice brain and liver homogenates within the sight of the concentrates could be because of the capacity of the concentrates to chelate Fe (II) or potentially scavenge free radicals created by the Fe (II) catalyzed the creation of receptive oxygen species (ROS). Here the concentrates of *Mentha longifolia* from various areas showed a more prominent capacity to diminish TBARS in mice brain contrasted with the liver. Plant concentrates may substitute manufactured food antioxidants, which may impact human health when devoured constantly (Martinez-Book et al., 2001). Plant-inferred food added substances, particularly polyphenolic mixes, have additionally been attributed health advancing properties, as regarding avoidance of constant cardiovascular infections (Harborne and Williams, 2000).

Antioxidant Activity by DPPH Radical Scavenging of Mentha Longifolia Leaf Removes

The impact of concentrates from various areas on DPPH decrease appear in Fig. 3. The diverse genotype leaves separate displayed solid antioxidant action against DPPH radical searching in a portion subordinate way (Fig. 3). As indicated by the IC50 esteems, the capacity to rummage DPPH radicals of the concentrates could be positioned as Alisojal > Akhorban > Phirkot ≥ Khaigala > Singola > Rawalakot > Baikh (Table 4). Free radicals are engaged with the procedure of lipid peroxidation, are considered to assume a cardinal job in various incessant pathologies, for example, malignancy and cardiovascular infections among others, and are associated with the maturing procedure. Along these lines, the leaf extracts were assessed against DPPH• radicals to decide their free radical searching properties. In this test, DPPH• radical fills in as the oxidizing substrate, which can be diminished by an antioxidant compound to its hydrazine subordinate by means of hydrogen gift and as the response pointer particle (Dorman et al., 2003). All the *Mentha* separates were equipped for searching DPPH• radicals in a portion subordinate way. These outcomes are in accordance with (Dorman et al. 2003) they saw that the *Mentha* spp indicated high antioxidant movement against DPPH• radicals. (Saha et al. 2008) additionally saw that the antioxidant exercises expanded by expanding the convergence of leaves of *Mimusops elengi* linn. The phenoxide gathering of a deprotonated phenolic compound has a high charge thickness which can tie a reasonably exceptionally charged cation (Hider et al., 2001).

Metal chelation of Mentha longifolia Leaf Extracts

The impact of extracts from various areas on iron chelation appears in Fig. 4. The extracts showed solid chelating capacity in a portion subordinate way (Fig. 4). As per the IC50 value, the capacity to Fe (II) of the four examined extracts could be positioned Alisojal > Phirkot > Khaigala ≥ Rawalakot > Akhorban > Singola > Baikh (Table 4). Extracts rich in such parts ought to have the option to complex metals particles and balance out the type of the metal particle, hence ruining metal-catalyzed inception and hydroperoxide disintegration responses (Gordon, 1990). Due to the significance of metal chelation as an antioxidant property (Leong and Shui, 2001; Kehrer, 2000), the capacity of the *Mentha* extracts to contend with ferrozine for iron particles in the free arrangement was considered. Present outcomes are in concurrence with (Dorman et al. 2003) they portrayed that the *Mentha* fluid extracts showed a capacity to chelate iron (II) particles in a portion subordinate design; i.e., as the concentrate fixation expanded, the measure of iron (II) chelated comparably increased.

Total Phenolic and Flavonoid Substance

The phenolic content extended from 146.90±1.90 to 192.10±2.10 mg/g in leaves. While flavonoid content extended between 1.28±0.04 to 4.23±0.03 mg/g (Table 1). Phenolic compounds and flavonoids have been accounted for to be identified with anti-oxidative activity in organic frameworks, going about as scavenger of singlet oxygen and free

radicals (Rice-Evans et al., 1997; Jorgensen et al., 1999). Triterpenoids, steroids, steroidal glycosides, flavonoids, and alkaloids are contained in *Mentha* species (Jahan et al., 1995). Numerous naturally occurring triterpenoids showed a good anti-inflammatory action that has been disconnected from different plants (Fernandez et al., 2001; Ismaili et al., 2002). Pentacyclic triterpenoids have a wide range of organic exercises and some of them might be valuable in medication. There is developing enthusiasm for characteristic triterpenoids caused as much by the logical perspectives extraction and basic examination of these compound, as by the reality of their wide range of organic activities, they are bactericidal, fungicidal, antiviral, cytotoxic, pain-relieving, mitigating, and hostile to malignancy and anti-allergic (Patocka, 2003). The high substance of phenolic and flavonoids in the extracts of various areas of *Mentha* leaves extracts added to the antioxidant action. The phenolic content went from 146.90 ± 1.90 to 192.10 ± 2.10 mg/g in leaves. The most elevated phenolic content appeared in the Alisojal locale when contrasted with others. The flavonoid content went from 1.28 ± 0.04 to 4.23 ± 0.03 mg/g. All areas indicated high flavonoid substance however Alisojal displayed high flavonoids. This variety maybe because of the geography of soil. Prior investigations of (Dorman et al. 2008) announced an absolute phenolic substance in the *Mentha* spp. running from 285 mg/g to 366 mg/g. (Dragana et al. 2012) revealed that the concentrate of the normally dried *Mentha longifolia* had the most elevated by and large substance of phenols (113.8 ± 2.0 mg of Gallic acid/g of the dry concentrate) and flavonoids (106.7 ± 0.3 mg of rutin/g of the dry concentrate).

Conclusion

The outcome shows high viability of the crude extracts of *Mentha longifolia* leaves against free radical reaching, inhibition of reacted oxygen species, and lipid peroxidation which might be identified with their high beneficial use as a useful food and adequacy in the treatment of deteriorating conditions. It very well may be considered as a source of plant antioxidants with likely use in food and pharmaceutical fields.

References

- Ahmad, M., Akbar, F.M., Khan, M.R. and Nisar, H., 2011. *Exploitation and conservation of medicinal and aromatics plants from Azad Jammu and Kashmir. Proceeding of PARC and TASO-PGR workshop.*
- Aruoma, O.I. and Halliwell, B., 1991. Free radicals and food additives. *Taylor and Francis Limited.* p.201.
- Brickell, Christopher, Zuk and Judith, D., 1997. The American Horticultural Society: A-Z Encyclopedia of Garden Plants. *New York, NY, USA: DK Publishing, Inc.* pp.668.
- Cao, G., Sofic E. and Prior R. L., 1997. Antioxidant and pro-oxidant behavior of flavonoids: Structure–activity relationship. *Journal of Nutritional Biochemistry*, 22, pp.749-760.
- Derwich, E., Benziane, Z., Taouil, R., Senhaji, O. and Touzani, M. 2010. Aromatic plants of Morocco: GC/MS Analysis of essential oils of leaves of *Mentha piperita*. *Advances in Environmental Biology*, 4, pp.80-85.
- Dorman, D.J.H., Kosar, M., Kahlos, K., Holm, Y. and Hiltunen, R., 2003. Antioxidant properties and composition of aqueous extracts from *Mentha* species, hybrids, varieties and cultivars. *Journal of Agricultural Food Chemistry*, 51, pp.4563-4569.
- Dragana, M., Stanisavljević, S., Stojičević, S., Đorđević, S.M., Zlatković, B.P., Veličković, D.T., Karabegović, I.T. and Lazić, M.L., 2012. Antioxidant activity, the content of total phenols and flavonoids in the ethanol extracts of *Mentha longifolia* (L.) hudson dried by the use of different techniques. *Chemical Industry and Chemical Engineering Quarterly*, 18, pp.411-420.
- Duke, J.A., Godwin, M.J.B., Ducellier, J. and Duki, P.A.K., 2002. *Handbook of Medicinal Herbs.* 2nd ed., CRC Press, pp. 562-564

- Fernandez, M.A., De Las Heras, B., Garcia, M.D., Saenz, M.T. and Villar, A., 2001. New insights into the mechanism of action of the antiinflammatory Triterpene lupeole. *Journal of Pharmacy and Pharmacology*, 53, pp.1533-39.
- Fialova, S., Tekelova, D., Mrljanova, M. and Granci, D., 2008. The determination of phenolics compounds and antioxidant activity in mint and balms cultivated in Slovakia. Department of Pharmacognosy and Botany, Faculty of Pharmacy, Comenius University of Bratislava.
- Fraga, G.C. and Oteiza, P.I., 2002. Iron toxicity and antioxidant nutrients. *Toxicology*, 180:23-32.
- Gordon, M. H., 1990. The mechanism of antioxidant action *in Vitro*. In *Food Antioxidants*; Hudson, B. J. F., Ed. Elsev. Applied Science: London, U.K. pp 1-18.
- Gulluce, M., Sahin, F., Sokmen, M., Ozer, H., Daferera, D., Sokmen, A., Polissiou, M., Adiguzel, A. and Ozkan, H., 2007. Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp. longifolia. *Food Chemistry*, 103, pp.1449-1456.
- Gutteridge, J.M.C. and Wilkins, S., 1982. Copper-dependent hydroxyl radical damage to ascorbic acid: Formation of a thiobarbituric acid-reactive product. *FEBS Letters*, 137, pp.327-330.
- Halliwell, B. and Gutteridge, J.M.C., 1981. Formation of a thiobarbituric acid reactive substance from deoxyribose in the presence of iron salts: The role of superoxide and hydroxyl radicals. *FEBS Letters*, 128, pp.347-352.
- Harborne, J. and Williams, C., 2000. Advances in flavonoid research since 1992. *Phytochemistry* 55, pp.481-504.
- Hatano, T., Kagawa, H. Yasuhara, T. and Okuda, T., 1988. Two new flavonoids and other constituents in licorice root; their relative astringency and radical scavenging effects. *Chemical and Pharmaceutical Bulletin*, 36, pp.2090-2097.
- Heim, K.E., Tagliaferro, A.R. and Bobilya D.J., 2002. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *Journal of Nutritional Biochemistry*, 13, pp. 572-584.
- Hider, R.C. Liu, Z.D. and Khodr, H.H., 2001. Metal chelation of polyphenols. In *Methods in Enzymology*. Packer, L., Ed. Academ. Press, San Diego 335, pp.190-203.
- Higa, K.C., Lopes, L., Schild, M.X., Ana, L. and Haraguchi, M., 2007. Flavonóides glicosídicos nas folhas de *Solanum fastigiatum*. *Journal Brasil de Fitomed*, 5, pp.174-1174.
- Houglum, K., Filip, M., Witztum, J.L. and Chojkier, M., 1990. Malondialdehyde and 4-hydroxynonenal protein adducts in plasma and liver of rats with iron overload. *Journal of Clinical Investigation*, 86, pp.1991-1998.
- Ismaili, H., Sosa, S., Brkic, D., Fkih-Tetouani, S., Iidrrissi, A., Touati, D., Aquino, R.P. and Tubaro, A., 2002. Topical anti-inflammatory activity of extracts and compounds from *Thymus broussonettii*. *Journal of Pharmacy and Pharmacology*, 54, pp.1137-40.
- Jahan, N., Ahmed, W. and Malik, A. 1995. New steroidal glycosides from *Mimusops elengi*. *Journal of Natural Products*, 8, pp.1244-1247.
- Jorgensen L.V., Madsen, H.L. Thomsen, M.K., Dragsted L.O. and Skibsted, L.H., 1999. Regulation of phenolic antioxidants from phenoxyl radicals: An ESR and electrochemical study of antioxidant hierarchy. *Free Radical Research*, 30, pp.207-220.
- Kehrer, J. P., 2000. The Haber-Weiss reaction and mechanisms of toxicity. *Toxicol.*, 149: 43-50.
- Kosalec, I., Bakmaz, M., Pepeliniak, S. and Vladimir-Knezevic, S., 2004. Quantitative analysis of the flavonoids in raw propolis from northern Croatia. *Acta Pharmaceutica*, 54, pp.65-72.

- Leong, L.P. and Shui, G., 2001. An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chemistry*, 76, pp.69-75.
- Martinez-Tome, M., Jimenez, A., Ruggieri, S., Frega, N., Strabbioli, R. and Murcia, M., 2001. Antioxidant properties of Mediterranean spices compared with common food additives. *Journal of Food Protection*, 64, pp.1412-1419.
- Middleton, E., Kandaswami, C. and Theoharides T.C., 2000. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacological Reviews*, 52, pp.673-751.
- Ohkawa, H., Ohishi, N. and Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, 95, pp. 351-358.
- Oktay, M., Gülçin İ. and Küfrevioğlu, Ö.İ., 2003. Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. *LWT-Food Science and Technology*, 36, pp.263-271.
- Padmini, E., Valarmathi A. and Rani, M.U., 2010. Comparative analysis of chemical composition and antibacterial activities of *Mentha spicata* and *Camellia sinensis*. *Asian Journal of Experimental Biological Sciences*, 1, pp.772-781.
- Patocka, J. 2003. Biologically active pentacyclic triterpenes and their current medicine signification. *Journal of Applied Biomedicine*, 1, pp.7-12.
- Phatak, S. V. and Heble. M.R., 2002. Organogenesis and terpenoid synthesis in *Mentha arvensis*. *Fitoterapi*, 73, pp. 32-39.
- Puntel, R.L., Nogueira, C.W. and Rocha, J.B.T. 2005. Krebs cycle intermediates modulate thiobarbituric acid reactive species (TBARS) production in rat brain in vitro. *Neurochemical Research*, 30, pp.225-235.
- Raymond, M. H., Atkins, S., Budantsev, A.L., Cantino, P.D., Conn B.J., Grayer, R.J., Harley, M.M., De Kok, R.P.J., Krestovskaja, T.V., Morales, R., Paton, A.J. and Ryding, P.O., 2004. "Labiatae" In: Klaus Kubitzki (editor) and Joachim W. Kadereit (volume editor). *The Families and Genera of Vascular Plants volume VII*. Springer-Verlag: Berlin; Heidelberg, Germany, pp.167-275.
- Rice-Evans, C.A., Miller, N.J. and Paganga, G., 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine*, 20, pp.933-956.
- Rice-Evans, C., Sampson, J., Bramley P.M., and Holloway, D.E., 1997. Why do we expect carotenoids to be antioxidants in vivo. *Free Radical Research*, 26, pp.381-398.
- Rimm, E.B., Stampfer, M.J. Ascherio, A., Giovannucci, E., Colditz G.A. and Willett, W.C., 1993. Vitamin E consumption and the risk of coronary heart disease in men. *The New England Journal of Medicine*, 328, pp.1450-1456.
- Singh, S. 2010. Phytochemical investigation of *Sonchus oleraceus* leaves and *Citrullus colocynthis* root. *Journal of Herbal Medicine and Toxicology*, 4, pp.159-162.
- Singleton, V. L., Orthofer, R. and Lamuela-Raventos, R.M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin– Ciocalteu Reagent. *Methods in Enzymology*, 299, pp.152-178.
- Vinson, J.A., Dabbagh, Y.A., Serry, M.M. and Jang, J., 1995. Plant flavonoids, especially tea flavonoids, are powerful antioxidants using an in vitro oxidation model for heart disease. *Journal of Agricultural Food Chemistry*, 43, pp.2800-2802.
- Zheng, W. and Wang, S.Y. 2001. Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural Food Chemistry*, 49, pp.5165-5170.