IJP (2014), Vol. 1, Issue 10 **(Research Article)**

Received on 19 July 2014; received in revised form, 13 September 2014; accepted, 29 September 2014; published 01 October 2014

AN EVALUATION OF THE ANTIOXIDANT PROPERTIES OF SOME OIL YIELDING LAMIACEOUS PLANTS FROM MORNI HILLS (HARYANA, INDIA)

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Keywords:

Lamiaceous plants, Essential oils, Antioxidant activity, Free radical Scavenging activity

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ABSTRACT: The present study was conducted to explore the antioxidant and radical scavenging activities of essential oil extracted from the leaves of plants of family Lamiaceae from Morni hills in the north-eastern part of the state of Haryana, India. Ten plants- *Hyptis suaveolens, Mentha longifolia, M. pulegium, Ocimum basilicum, Plectranthus incanus, P. striatus, Pogostemon benghalensis, Rabdosia rugosa, Salvia plebeia* and *Scutellaria angulosa* of the family Lamiaceae were explored from the area and the essential oil from their leaves was extracted. The maximum oil yield was found in leaves of *O. basilicum*, while the oil yield was the minimum in leaves of *P. incanus*. The density of oil was found to be the maximum in *H. suaveolens*, while it was the minimum in *P. incanus* and *S. angulosa*. The essential oils of all the plants exhibited DPPH radical scavenging activity; however, it was best exhibited by *S. angulosa* (with least IC_{50} value) in comparison to standard butylated hydroxytoluene (BHT). Further, the oil also exhibited total antioxidant activity, which was found to be the greatest in *H. suaveolens*, *O. basilicum* and *P. benghalensis* (with lesser IC_{50} values compared to standard ascorbic acid). The study concludes that the Morni hills ecosystem has a rich diversity of Lamiaceous plants that possess a good antioxidant /radical scavenging activity that is worth exploiting in future.

INTRODUCTION: Human body is exposed to various environmental stresses like pollution, smoke or radiations that may trigger the generation of various free radicals like reactive oxygen species or nitrogen-based free radicals causing significant cellular damage. Antioxidants are thus required to scavenge these harmful free radicals and protect living organisms from their undesirable toxic effects like diseases and accelerated aging.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.IJP.1(10).640-45

Article can be accessed online on: www.ijpjournal.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.1(10).640-45

Normally, the antioxidants are produced within the body as a part of normal metabolism; yet, these are sometimes insufficient to scavenge enhanced free radicals generated under external stresses. Thus, these have to be supplied externally through food or medicines. Most external sources of antioxidants are synthetic like butylated hydroxytoluene (BHT) and may have their side effects.

Plant-based natural chemicals as antioxidants are catching the attention worldwide for being environmentally safer and without any toxicity of their own. In this regard, essential oils, the volatile oily liquids, have been the focus of current research since these also serve as functional ingredients of food [1, 2]. The members of the plant families,

Asteraceae, Lamiaceae, Rutaceae, and Myrtaceae, are the rich source of essential oils; however, the family Lamiaceae is the most important source.

Family Lamiaceae- one of the widespread families of flowering plants- is represented by ~ 240 genera over 6000 species [3, 4]. It comprises mostly of aromatic plants that serve as the source of volatile essential oil. The plants of the family occur in most tropical, sub-tropical and temperate regions of the world, especially the Mediterranean regions. The family serves as a source of culinary herbs, medicine and commercially important oil [5]. Many plants of the family are grown in gardens or parks for their aesthetic value, while a few occur as weeds. The family also includes some commercially important trees like teak in its extended version [4]. The family is an important source of essential oils that find extensive use in food, fragrance and pharmaceutical industries. Besides, the oils also possess various useful properties like anti-inflammatory, antimicrobial or antiseptic, *etc*. [6, 7, 8] Of late; their use as the natural antioxidants is also being explored [1].

The present work, therefore, aimed to explore the essential oil from several wild plants of family Lamiaceae from Morni hills (Haryana) and their antioxidant potential. Morni hills were selected as the study site based on the wealth of wild flora it harbors. Though there are some scanty reports on the vegetation of Morni hills [9, 10], yet detailed information regarding the utility of its flora, particularly with regards to the essential oil from aromatic plants, is lacking.

MATERIALS and METHODS:

About the Study Site: The present study was conducted at Morni hills (30° 35′ to 34° 45′N and 70° 00′ to 75° 15′ E), the only hill station in the state of Haryana, and is about 35 km from Chandigarh. The study area has an altitudinal range of 1550 to 3600 feet above sea level and is bordered in the north by Shivalik (outer Himalaya) and in the south by plains of Haryana state. The climate of Morni Hills is generally sub-tropical to and characterized by a diversity of flora and fauna [10].

Collection of Plants: Ten plants, *viz.*, *Hyptis suaveolens* (L.) Poit., *Mentha longifolia* (Linn.) Huds., *M. pulegium* Linn., *Ocimum basilicum* Linn., *Plectranthus incanus* Link., *P. striatus* Benth., *Pogostemon benghalensis* (Burm. F.) O. Ktze, *Rabdosia rugosa* (Wall. ex Benth.) H. Hara, *Salvia plebeian* R. Brown and *Scutellaria angulosa* Benth., belonging to Lamiaceae were located in different parts of the Morni hills. These were collected and identified at College of Forestry (Y.S Parmar University of Horticulture and Forestry, Solan, H.P., India) and their voucher numbers were obtained **Table 1**. The essential oil was extracted from the leaves of the plants in their vegetative stage. For this, collections were made carefully using non-destructive methods taking care that the whole plant is not harmed.

Extraction of the Essential Oils: The essential oil was extracted from the chopped leaves of the plants by hydro-distillation using Clevenger's apparatus. Nearly two kg of freshly collected leaves were mixed with 2 L of water. The mixture was boiled for 3 h and oil was collected from the nozzle of the condenser. The essential oil so obtained was dried over anhydrous sodium sulphate and stored at 4 °C until tested. Different concentrations (50, 100, 200 and 400 µg/ml) of the oil were prepared for the determining its antioxidant properties.

DPPH (2, 2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity: DPPH scavenging activity was determined as per the method of Bozin *et al.* [7] This assay is based on the reduction of a methanolic solution of colored free radical DPPH by any free radical scavenger. For the test, 200 µl sample solution of different concentrations (50, 100, 200 and 400 µg/ml) of oil was taken in test tubes, and 3 ml of 0.01mM alcoholic DPPH solution was added. A parallel blank and positive control (BHT) was also maintained. The samples were incubated in the dark. The absorbance of the yellow colored solution so developed was measured at 517 nm. The decrease in absorbance of the samples with increasing concentration indicated DPPH scavenging activity of the oil. The activity oil was calculated as per Singh *et al.*, [11] and expressed as IC_{50} (Concentration of oil at which the activity was measured to be 50%).

Total Antioxidant Activity (TAA): The total antioxidant capacity of the essential oil was measured as per the method of Prieto *et al.* [12] using phospho-molybdenum. Briefly, 1 ml of reagent

solution (6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added to the 0.1 ml of essential oil. The reaction mixture was incubated at 95°C for 90 min. The absorbance of the solution was measured at 695 nm. The antioxidant capacity of the extract was expressed as IC_{50}.

RESULTS AND DISCUSSION: Ten plants of the family Lamiaceae were collected and identified from different locations of the Morni hills. *Hyptis suaveolens*, commonly known as bush mint or vilayati tulsi, was located from the roadsides and forest margins. The oil extracted from the leaves was clear with a strong aroma, and its density was calculated to be 0.98 g/ml. The yield of the oil was measured to be 2.00 ± 0.05 ml/kg on a fresh weight basis **Table 1**.

Mentha longifolia, commonly known as horsemint, was collected from the sides of the river passing through the region. The leaves of the plant possessed peppermint like aroma and yielded ~ 3.00 ml oil/kg with a density of 0.92 g/ml **Table 1**. Another species of *Mentha, M. pulegium*, commonly known as pennyroyal or squaw mint, was also collected from the area growing near the river.

Rabdosia rugosa ***Plectranthus striatus***

Pogostemon benghalensis ***Plectranthus incanus*** ***Ocimum basilicum***

Nepeta floccosa ***Mentha pulegium*** ***Scutellaria angulosa***

FIG. 1: PHOTOS OF THE SELECTED LAMIACEOUS PLANTS OF THE MORNI HILLS, HARYANA, INDIA

The plant has been reported to be the smallest of all the mints and had spearmint like fragrance. The oil yield was measured to be ~2.50 ml/kg whereas its density was 0.93g/ml. Another plant of the family *Ocimum basilicum* or sweet basil was collected from sides of the hilly tract with moist soil. The leaves of *O. basilicum* yielded 3.50 ml/kg oil with a density of 0.96 g/ml. Two species of Genus

Plectranthus, *P. incanus*, commonly known as soft-stem mint, and *P. striatus*, commonly known as crested flower isodon, were collected from the damp shady places near the forest areas. The leaves of *P. incanus* yielded ~0.5 ml/kg oil, whereas those from *P. striatus* yielded ~ 1.0 ml/kg oil **Table 1**. The density of oil from *P. incanus* was lesser than that of *P. striatus* **Table 1**. *Pogostemon benghalensis*, the common name of patchouli, was another plant of family Lamiaceae that commonly occurred along roadsides or in the open areas. The yield of oil extracted from the leaves was 1.20 ml/kg while the density was calculated to be 0.89 g/ml **Table 1**. *Rabsosia rugosa*, commonly known as wrinkled-leaf isodon, was collected from the rocky slopes and exhibited shrubby nature. The yield of the oil was 0.80 ml/kg, whereas the density was 0.87 g/ml **Table 1**.

TABLE 1: LIST OF PLANTS OF FAMILY LAMIACEAE, THEIR COMMON ENGLISH NAMES, HABIT AND HABITAT, PLACE AND DATE OF COLLECTION, AND THE AMOUNT AND DENSITY OF OIL

S. no.	Botanical and Common English Name	Habit and Habitat	Place and date of collection /Field book number	Amount (ml/kg) and Density of oil (g/ml)
1	*Hyptis suaveolens* (L.) Poit.	Shrub	Bir-Ghaggar	2.00±0.05
	Pig nut	Roadsides, forest areas	24-12-2012 12445	0.98
2	*Mentha longifolia* (L.) Huds.	Herb	Bir-Ghaggar	3.00±0.07
	Horsemint	Banks of river	12-2-2012 12440	0.92
3	*Mentha pulegium* L.	Herb	Mandhana	2.50±0.08
	English Pennyroyal	Sides of flowing streams and ponds	25-4-2011 12447	0.93
4	*Ocimum basilicum* L.	Herb	Bir-Ghaggar	3.50±0.50
	Sweet basil	Slopping sides of hills, moist soil	29-9-2011 12448	0.96
5	*Plectranthus incanus* Link.	Herb	Barha Sher	0.50±0.02
	Soft-stem mint leaf	Damp shady areas of forest	11-11-2012 12450	0.84
6	*Plectranthus striatus* Benth.	Herb	Mandhana	1.00±0.08
	Crested flower isodon	Damp shady sides of hills	26-1-2011 12446	0.95
7	*Pogostemon benghalensis*	Herb	Mandhana	1.20±0.04
	(Burm. f.) Kuntze	Near roadsides,	11-10-2011 12442	0.89
	Patchouli	open forests		
8	*Rabdosia rugosa* (Wall. ex	Shrub	Near forest check	0.80±0.03
	Benth.) Hara	Rocky slopes of hills,	post	0.87
	Wrinkled Leaf Isodon	sunny sides	20-6-2012 12439	
9	*Salvia plebeia* R.Br.	Herb	Berwala	0.90±0.05
	Sage weed	Open forest	14-7-2012 12444	0.91
10	*Scutellaria angulosa* Benth.	Herb	Berwala	0.90±0.03
	Himalayan skull cap	Near to roads, hanging from slopes of mountains	4-3-2012 12443	0.84

* Common English names and field book numbers are given in bold letters

Salvia plebeia, commonly known as sage weed, was collected from the open areas near the forests. Its leaves yielded around 0.90 ml oil/kg with the density of 0.91 g/ml. *Scutellaria angulosa*, commonly known as Himalayan skull-cap, was found growing near the roads or slopes of mountains. The oil yield and density from *S. angulosa* were measured to be 0.90 ml/kg and 0.84 g/ml, respectively.

The oils from the test plants possessed radical scavenging properties measured regarding DPPH radical scavenging and total antioxidant activity (TAA) and expressed as IC_{50}. The IC_{50} values of the essential oils were compared with standards like BHT and ascorbic acid. The standard BHT was used in the DPPH radical scavenging assay. The IC_{50} value of BHT was calculated to be 156.4ug/ml for DPPH scavenging activity. The IC_{50} value of *Scutellaria angulosa* oil was measured to be 83.6 (~half of that of the standard), whereas those of *Mentha longifolia*, *Ocimum basilicum*, and *Pogostemon beghalensis* were nearer to the standard **Table 2**.

These results indicated that oils from these plants have better or at least equal activity in comparison to BHT standard. The IC_{50} values of oils from other plants were calculated to be more than standards. The use of DPPH radical is an important assay to measure the scavenging activity of the chemicals including the essential oils.

It is one of the simple methods, and some workers consider it as the sole method for determining the antioxidant activity [1]. Some essential oils, including those from the plants of family Lamiaceae, have been evaluated for their radical scavenging activity following this method [1, 13].

TABLE 2: DPPH RADICAL SCAVENGING AND TOTAL ANTIOXIDANT ACTIVITY OF ESSENTIAL OILS OF SELECTED LAMIACEOUS PLANTS FROM MORNI HILLS (HARYANA, INDIA)

S. no.	Plant Name	DPPH Scavenging Activity (µg/ml)	Total Antioxidant Activity (µg/ml)
1	*Hyptis suaveolens*	237.3±6.37	125.1±2.24
2	*Mentha longifolia*	174.4±4.19	235.6±4.18
3	*Mentha pulegium*	262.2±7.32	195.7±6.01
4	*Ocimum basilicum*	160.4±2.62	111.4±3.07
5	*Plectranthus incanus*	264.1±4.74	276.2±8.12
6	*Plectranthus striatus*	209.2±5.23	234.8±8.34
7	*Pogostemon benghalensis*	171.3±5.74	89.5±2.37
8	*Rabdosia rugosa*	237.4±6.37	246.4±7.69
9	*Salvia plebeia*	163.7±6.37	193.8±7.12
10	*Scutellaria angulosa*	83.6±6.37	192.4±2.47
	Standard	156.4 (BHT)	165.7 (Ascorbic acid)

Another test used for determining the antioxidant activity of oil was total antioxidant activity, which is widely used to determine the antioxidant value, especially in plants. Previously, the antioxidant activity of the essential oils from fruit stalk of *Cinnamomum verum* J. Presl (=*C. zeylanicum*) [14], *Thymus vulgaris* L. [15] and *Allium sphaerocephalon* Linn. subspecies *sphaerocephalon* [16] have been determined by this method. The method is based on the reduction of molybdenum (Mo) (VI) to Mo (V), and subsequent formation of green phosphate / Mo (V) complex at acidic pH.

In this case, ascorbic acid was used as the standard, and the IC_{50} values of the plant oils were compared with it. The IC_{50} values of oil from *Hyptis suaveolens, Ocimum basilicum* and *Pogostemon benghalensis* were found to lower than the standard indicating that these oils possessed better activity than that of the standard. The other oils had IC_{50} values higher than the standard but not too high indicating that these oils could be useful as antioxidants.

CONCLUSION: From the above results, it is concluded that oils from the plants of Lamiaceae collected form Morni hills, Haryana, India, possess a good antioxidant /radical scavenging activity that is worth exploiting in future. However, the wild plants may not be necessarily used for this purpose as these could be cultivated for this purpose.

ACKNOWLEDGMENT: Sartaj Singh is thankful to Council of Scientific and Industrial Research (New Delhi, India) and University Grants Commission (New Delhi, India) for the financial assistance to carry out this work.

CONFLICT OF INTEREST: Nil

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How to cite this article:
Singh S, Batish DR, Kohli RK and Singh HP: An evaluation of the antioxidant properties of some oil yielding Lamiaceous plants from Morni hills (Haryana, India). Int J Pharmacognosy 2014; 1(10): 640-45. doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.1(10).640-45.