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Effect of seasonal variation on phenolics, flavonoids and terpenoids in *Mesosphaerum suaveolens* (L.) Kuntze., and *Ocimum basilicum* L. using GC-MS and LC-MS/MS

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Abstract

Mesosphaerum suaveolens (L.) Kuntze. and *Ocimum basilicum* L., species belonging to Lamiaceae, are used in cosmetics and folk medicine. Most of the available studies focus on the phytochemical profile of these plants. The present study was aimed at understanding the metabolic content of both plants collected in summer, winter and rainy season. The plants were collected in all three seasons and analysed using gas chromatography -mass spectrometry (GC MS) and liquid chromatography using Agilent Jet Stream and Electrospray Ionization Quadrupole time of flight mass spectrometric detection. The total phenolics were found to be highest in the monsoon samples for *M. suaveolens* at 5.3 mg/mL ± 0.124 and in the summer samples for *O. basilicum* at 7.46 mg/mL ± 0.23. Total flavonoids were to be highest in summer samples in both *M. suaveolens* and *O. basilicum* with 3302 mg/mL ± 112.6 and 5210 mg/mL ± 74.57 respectively. Total terpenoids were found to be highest in the summer samples in both plants with 72 mg/mL ± 1.55 and 58 mg/mL± 1.413 in *M. suaveolens* and *O. basilicum* respectively. In total, more than 950 compounds were identified, with majority of the compounds identified being phenolics, flavonoids and terpenoids. Metabolic profiling revealed the presence of compounds such as ethers, esters, steroids, catechins, imidazoles, retinols, ketones, amino acids, alcohols and aldehydes. The comparative analysis also revealed that *M. suaveolens* showed more accumulation of compounds compared to *Ocimum*. This study led to an in-depth understanding of the metabolite content in *Mesosphaerum*, which is a less studied wild plant. It also helped in understanding how seasons influence the metabolite content of both plants which has also not been studied at length.

Keywords

gallic acid; Lamiaceae; linalool; phytochemicals; quercetin; secondary metabolites

Introduction

Lamiaceae, the family of mints and aromatic compounds, consists of 236 genera and 7000 species (1) includes *Salvia*, *Mesosphaerum*, *Ocimum*, *Lavandula*, *Vitex*, *Scutelleria, Thymus*, *Stachys*, *Plectranthus*, *Ocimum, Teucrium* etc. (2). These plants include essential oils, which are rich in volatile monoterpenes, sesquiterpenes and diterpenes (3). Plants in this family are cultivated worldwide for their essential oils, which are used as flavorings, herbs and cosmetics due to their aroma (4). Large populations of *Mesosphaerum suaveolens* (L.) Kuntze grow alongside ponds, marshes and along roadsides. It grows as a weed, hence mostly remains unexploited (5). Ursolic acid present in these plants is found to inhibit the HIV-integrase enzyme (6).

Ocimum basilicum L. is an ornamental plant which is also used for religious purposes in some countries. The plants act as antioxidants, antimicrobials, carminatives, galactogogue and provide relief from stomach pain (7). Some of the major phytochemicals found in *Ocimum basilicum* L. are linalool, eugenol, limonene, camphor, geraniol, germacrene, beta-elemene and beta-caryophyllene (8). Due to the vast array of secondary metabolites, the plant has important applications in the pharmaceutical industry. Additionally, the production of pharmaceuticals, cosmetics, functional foods with higher nutritional value and other goods can use plant metabolites that share structural similarities with the original metabolites (9). Based on their chemical structures, the metabolites are grouped into major classes. The first are nitrogen-containing molecules (alkaloids) and the second are nitrogen-deficient molecules (terpenoids and phenolics) (10).

There are few scientific studies on the metabolites of *M. suaveolens* and *O. basilicum.* Nonetheless, studies have been done on the chemical makeup of a closely related species from the Lamiaceae. For example, thymol, cymene and cineole have been found in the *mint,* while caryoplyllene, manool, viridoflorol have been found in the Sage. Cymene, terpinene and thymol have been found in Thyme. *Thymol* has also been found in the *Oregano* (11,12). Due to high antioxidant properties, these species have been used for cosmetic purposes (13, 14). Analyzing data from chemical examinations of other Lamiaceae species has revealed a greater interest in phenolic chemicals, which can be explained by their high biological activity The strong chemical and biological activity of these plants emphasize on the need for analysis of other plants of the family (15,16). The essential oils studied included monoterpenes, sesquiterpenes and diterpenes (17). Terpenoids, which are generated by the combination of two (monoterpene), three (sesquiterpene), or four (diterpene) isoprene units and phenylpropanoids are the two primary structural families of common essential oils in terms of hydrocarbon skeleton. Both the terpenoid and phenylpropanoid families contain phenolic chemicals, which are sometimes recognized as the primary components of many essential oils (18,19). There is also an increasing interest in plant essential oils as natural alternatives to synthetic additives. Flavonoids are yet another class which has been known to have anti-inflammatory, antibacterial, antioxidant and analgesic properties, which makes them-prospective components for scientific investigations (20-22).

Studies have mostly focused on the changes in metabolic content when the plants were subject to *in vitro* studies under the influence of plant growth regulators (PGRs) wherein the Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and Total Terpenoid Content (TTC) content was found to be differentially expressed compared to control (23). This work sought to assess the potential of *M. suaveolens* and *O. basilicum* leaves as a source of important metabolites. Thus, a thorough metabolomic profiling of *M. suaveolens* and *O. basilicum* leaves extract in three seasons (winter, summer and monsoon) was done in the present study to understand the diversity of metabolites across various seasons. Given that most of the metabolites present in Lamiaceae were essential oils, *M. suaveolens* and *O. basilicum* leaves were also investigated using GC-MS to identify the active volatile compounds. Most of the studies on Lamiaceae focused on estimating the phytochemical content in various plants. However, the seasonal influence on the content of phytochemicals and other metabolites needs to be investigated. Hence this study would help in understanding the time of accumulation of these compounds as well as the effect of climate on the content of these compounds. Both the plants have been less studied for effect of season on phytochemical variations; hence this study helps in bridging the gap.

Materials and Methods

Collection and identification of plant material

M. suaveolens and *O. basilicum* leaves were collected from the hedges of Navrachana University. To study the seasonal profile of the phytochemicals, samples of both plants were collected during various seasons: Winter, Summer and Monsoon. All the samples were collected between 9 and 10 a.m. in the morning. The collected samples were kept in a plastic container with ice. The leaf samples were air dried for 20 days at 24˚C in a ventilated fume hood with moisture content of 10-14%. The obtained leaf samples were then stored at 4˚C till further analysis (24).

Plant authentication

For herbarium preparation, the collected plants were compressed in dry newspaper sheets till all moisture was absorbed. Thereafter, the plants were cleaned with 0.1% mercuric chloride (HiMedia, #GRM1067, India) in 70% ethyl alcohol (HiMedia, #MB106, India). Once the plant was firmly placed in the herbarium, the data of its scientific name, common name, family name, collecting location and season were put on the sheet in the lower right corner. After poisoning and attaching the poison stamp, the authorization of plants was done at the BARO Herbarium, Maharaja Sayajirao University of Baroda.

Extraction of metabolites from Mesosphaerum suaveolens (L.) Kuntze. and Ocimum basilicum L.

20 g each of *M. suaveolens* and *O. basilicum* leaves were separately extracted with methanol as a solvent system in a Soxhlet apparatus at 20-30˚C for 36 hr. Once the leaves were completely dried at room temperature, the methanolic extracts were filtered through Whatmann filter paper No. 1 and then concentrated on a heating mantle to remove all the traces of methanol. The samples were then stored at -20˚C for further phytochemical analysis (25). The extraction was done separately for all three seasons for both *M. suaveolens* and *O. basilicum*.

Total phenolic content

The total phenolic content was performed using Folin-Ciocalteu reagent by the method of (26) with few modifications. 10 mg of the plant extract was dissolved in 10 mL of methanol (1 mg/mL) and 0.5 mL of FC reagent (Folin-Ciocalteu reagent) was added. The samples were mixed properly and incubated at room temperature for 3 min. Thereafter, 2 mL of 20% sodium carbonate was added to the reaction tubes and the tubes were vortexed and incubated for 30 min in dark. The absorbance of the samples was measured

at 650 nm using a microcontroller-based UV-visible spectrophotometer (CL-1320 Chemiline) against a blank containing distilled water. The standard curve was plotted using different concentrations of gallic acid in methanol. Total phenolic content was expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW).

Total flavonoid content

The total flavonoid content was determined by the method of (27). 0.1 mL of the plant extract was added to 0.3 mL of distilled water. 30 µL of 5% $NaNO_2$ was added to the tube and incubated for 5 min at room temperature. 0.2 mL of 1 mM NaOH was added to the tube, followed by 0.34 mL of distilled water. The absorbance was measured at 510 nm using a microcontroller-based UV-visible spectrophotometer (CL-1320 Chemiline). The standard curve was plotted with different concentrations of quercetin in ethanol. Total flavonoid content was expressed as milligrams of quercetin equivalent per gram of dry weight (mg quercetin/g DW).

Total terpenoid content:

The total terpenoid content was determined by the method of (28). 1 mL of plant extract was taken and 2 mL of chloroform was added to it. The sample mixture was properly mixed and kept at room temperature for 3 min. 200 µl of concentrated sulfuric acid was added to the mixture and incubated at room temperature in the dark for 2 hours. A reddish-brown precipitate was, formed which was left undisturbed. The supernatant from the tube was carefully removed and 3 mL of methanol was added. The tubes were shaken properly until the precipitate dissolved completely in methanol. The absorbance was measured at 538 nm using a microcontroller-based UV-visible spectrophotometer (CL-1320 Chemiline). The standard curve was plotted with different concentrations of linalool in methanol. Total terpene content was determined as milligrams of linalool equivalent per gram of dry weight (mg Linalool/g DW) (29).

GC-MS analysis

The gas chromatogram was recorded in the Perkin Elmer Clarus 680 with the Mass Detector SQ8C with ELITE 5MS, which has 95% polydimethylsiloxane with a 5% phenyl group. An ionization energy of 70 eV was used for electron ionization (EI). 99.99% helium gas was used as a carrier gas with a constant flow rate of 1 mL/min. The injection volume was 1 microliter. The sample preparation was done by completely dissolving 10 mg of the plant extract into 10 mL of methanol. The injector temperature was 250°C. The oven parameters were set as the initial temperature at 70°C for 2 min, then a ramp of 25°C/min to 150°C with a hold of 10 min and then, ending with a ramp of 25°C/min to 260°C, holding it for 40 min. The total run time was 60 min (30). The software used to determine the mass spectra and the chromatogram was Turbo Mass and the compounds detected were identified by MAINLIB of the Gujarat Biotechnology Research Centre, Gandhinagar.

LC-MS/MS (Liquid Chromatography with tandem Mass Spectrometry) analysis

An Agilent 6200 series TOF/6500 series Q-TOF system with a binary gradient pump, degasser, column thermostat, autosampler and UV detector was used for the analysis. The injection amount of the samples was 5 µL. The draw speed was 100 µL per min, the eject speed was 400 µL per minute and the run time was set to 20 min. The solvents used as a mobile phase to run the system were 100% acetonitrile and 0.1% formic acid (v/v). The flow rate of the system was set to 0.300 mL/min. The pressure bar maximum was set to 1200 bars. The work temperature was set to 40°C and was unchanged throughout the run. The MS operation was done using an electrospray ion source in positive mode and the ion source used was a dual AJS ESI. The detection was performed in MS mode. The chromatographic data was processed using Mass Hunter software. The MS spectra obtained from this analysis were integrated and compared with the LibSearch and DBSearch of the GBRC (Gujarat Biotechnology Research Centre, Gandhinagar). The samples that matched the compounds, when compared to the spectra of the library, were noted down and a positive identification list of all the compounds was made. The method by Moldovan was used for LC-MS/MS and a few modifications were made and the procedure was performed (31).

Statistical analysis

All the experimental data were recorded and analyzed. The statistical analysis for all the sets was done in triplicate using one-way ANOVA. The significance of the mean difference was established by Dunnet's test comparison where *$p<0.05$ and **$p<0.01$. The results were presented as the mean value ± standard deviation (SD). The software used for the analysis was GraphPad Prism 8.4.2.

Result and Discussion

Plant authentication

The authentication of *M. suaveolens* and *O. basilicum* was done at The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat's (India) BARO Herbarium. The voucher specimens were named as RM1 and RM2 for *M. suaveolens* and RM3 and RM4 for *O. basilicum* and were submitted for authentication. The specimens *M. suaveolens* and *O. basilicum* were examined with BARO123450027128, K000509912! and BARO123450026912, K000479656! Respectivel (Supplementary File 1).

Total phenolic content

In *Mesosphaerum suaveolens* (L.) Kuntze., it was seen that the monsoon samples had the maximum amount of phenolic content and the winter samples had the least amount of phenolic content. The summer and winter samples had 4.489 mg/mL ± 0.23 of total phenolics, while the monsoon samples had 5.314 mg/mL ± 0.23 of total phenolic content. In *Ocimum basilicum* L., it was seen that the summer samples had the maximum amount of phenolic content, while the winter samples had the least amount. The winter sample had 6.146 mg/mL ± 0.124 of total phenolic content, the summer sample had 7.464 mg/mL ± 0.124 of total phenolic content and the monsoon samples had 6.864 mg/mL ± 0.124 of total phenolic content (Fig. 1).

Total flavonoid content

In *Mesosphaerum suaveolens* (L.) Kuntze., it was seen that the summer samples had the maximum amount of flavonoid content and the winter samples had the least amount of flavanols. The summer samples had 3302 mg/mL ± 112.6 of flavonoids. The winter samples had 2045 mg/mL ± 112.6 of

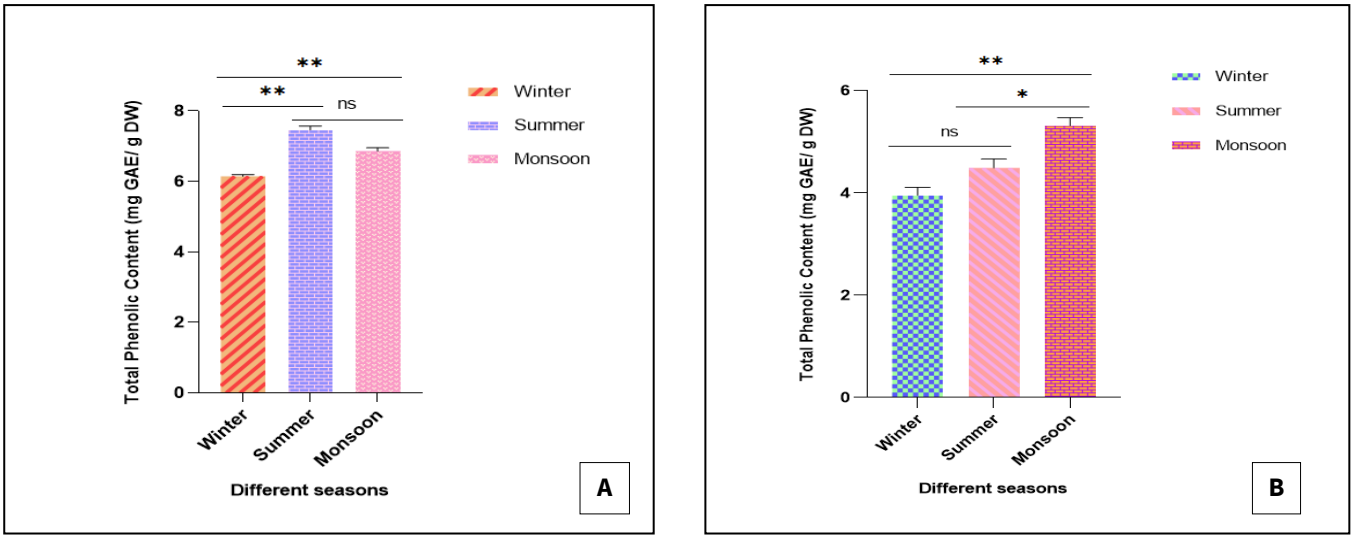

Fig. 1. Total phenolic Content in A. *Mesosphaerum suaveolens* (L.) Kuntze. B. *Ocimum basilicum* L. where *p<0.05 **p<0.01 and ns represents non-significant value.

flavonoid content and the monsoon samples had 2581 mg/mL ± 112.6 of flavonoids. While in *Ocimum basilicum* L., it was also seen that the summer samples had the maximum amount of flavonoids. The winter sample had 4202 mg/mL ± 74.57 of flavonoid secretion, the summer sample had 5210 mg/mL ± 74.57 and the monsoon samples had 4902 mg/mL ± 74.57 of flavonoid secretion. It was found that summer samples showed the highest levels of flavonoids. Monsoon samples showed moderate levels of flavonoids and winter samples showed the least levels of flavonoids (Fig. 2).

Total terpenoid content

In *Mesosphaerum suaveolens* (L.) Kuntze., it was seen that the summer samples had the maximum amount of terpenoid content and the winter samples had the least amount of terpenes. The summer samples had 72.11 mg/mL ± 1.55 of Linalool, the winter samples had 43.15 mg/mL ± 1.55 of Linalool and the monsoon samples had 62.08 mg/mL ± 1.55 of Linalool. Similarly, in *Ocimum basilicum* L., the summer samples had the maximum amount of terpene content and the winter samples had the least amount of terpenes. The winter sample had 29.47 mg/mL ± 1.413 of Linalool, the summer sample had 58.06 mg/mL ± 1.413 of Linalool and the monsoon samples had 49.31 mg/mL ± 1.413. It was found that summer samples showed the maximum levels of terpenoid content. Monsoon samples showed moderate levels of terpenoids and winter samples showed the least levels of terpenoids (Fig. 3).

GC-MS analysis

The representation of the comparative volatile essential oils among all the three seasons were done using GC-MS in both the plants. It was seen that seasonal variation was less in *Ocimum* than in *Mesosphaerum.* There were five compounds that were found in all three seasons of *Mesosphaerum,* such as phytol, 1 -Phenanthrenemethanol, 1,2,3,4,4a,5,6,9,10,10a-decahydro-1,4a-dimethyl-7-(1-methylethyl)-, [1R- (1à,4aá,10aà)] -, 1-Phenanthrenemethanol, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-, [1R- (1à,4aá,10aà)] Squalene and ç-Sitosterol, whereas only two compounds from *Ocimum* were found to be present in all three seasons, such as Estragole and ç-Sitosterol (Table 1). It was found that the samples from the summer and monsoon seasons showed the most unique essential oils when compared to the winter season.

It was found in both the plants, *Mesosphaerum suaveolens* (L.) Kuntze. and *Ocimum basilicum* L., that the summer season had the maximum number of secondary metabolites secreted when compared to the other two seasons. The peak flavonoid concentrations in *Melittis melissophyllum* L. occurred in may, when the plant was in the flowering stage and the lowest levels occurred in september, when the plant was nearing the end of its vegetative phase

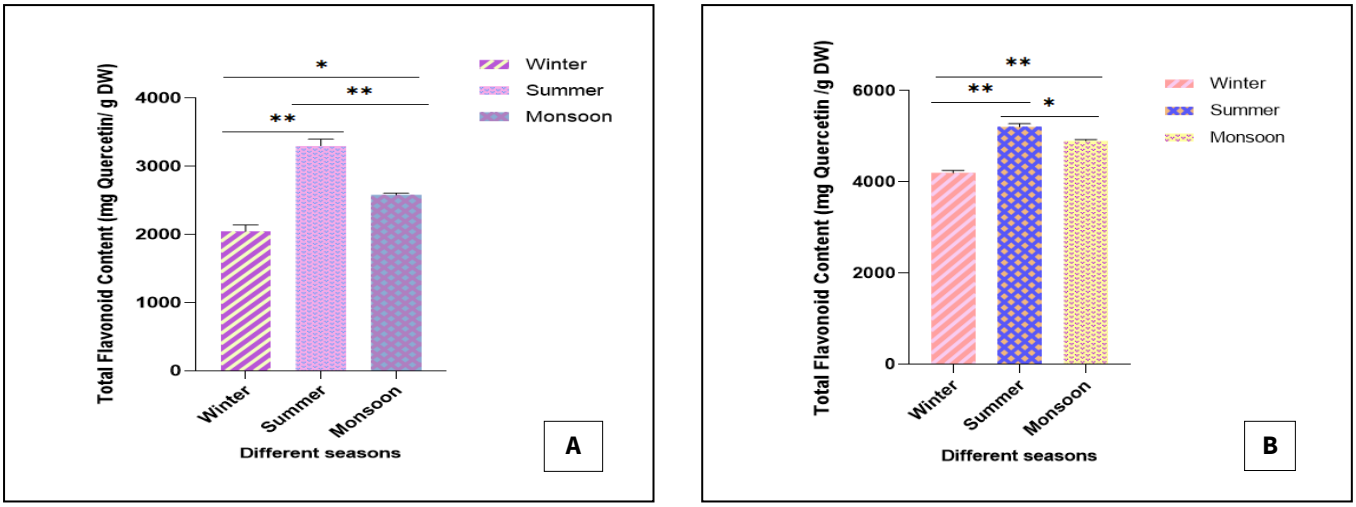

Fig. 2. Total Flavonoid Content in A. *Mesosphaerum suaveolens* (L.) Kuntze. B. *Ocimum basilicum* L. where *p<0.05 and **p<0.01.

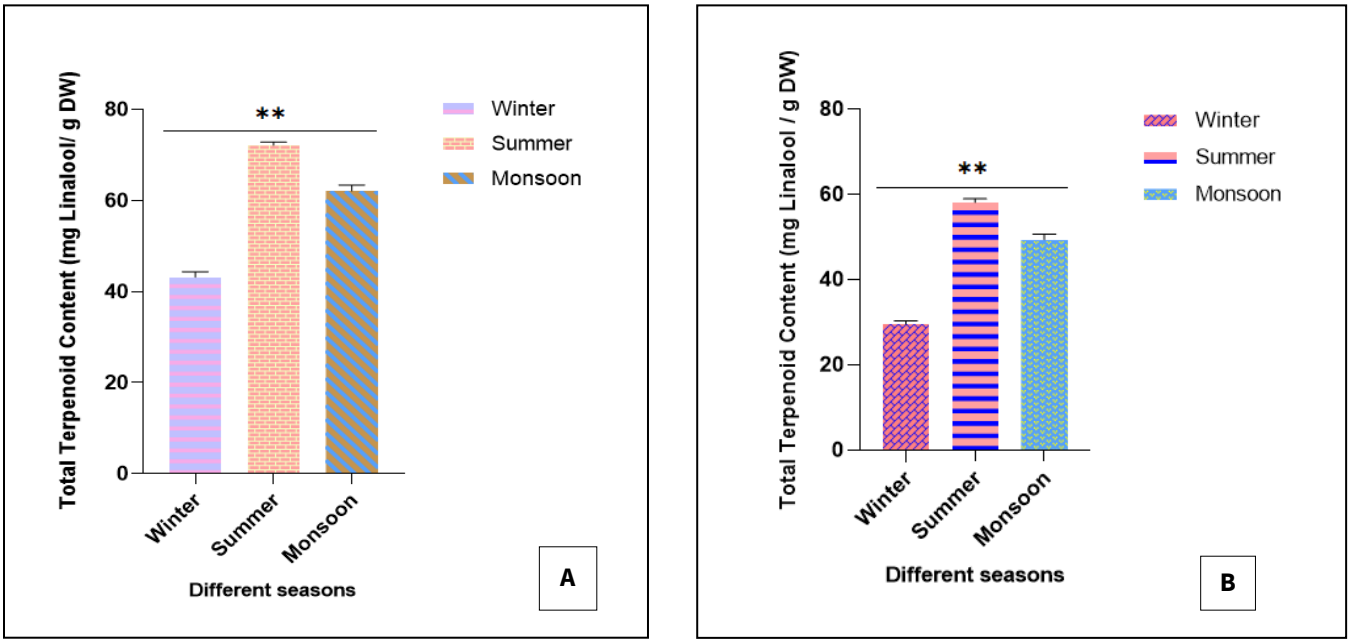

Fig. 3. Total terpenoid Content in A. *Mesosphaerum suaveolens* (L.) Kuntze. B. *Ocimum basilicum* L. where *p<0.05 **p<0.01 and ns represents non-significant value.

Table 1. Comparative Seasonal Variation in *Mesosphaerum suaveolens* (L.) Kuntze. and *Ocimum basilicum* L.

Name of the Compound	***Mesosphaerum suaveolens* (L.) Kuntze.**	***Ocimum basilicum* L.**
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Winter	Summer
7-Isopropyl-1,1,4a-trimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene (Dehydroabietan)	Winter, Summer	
Phytol	Winter, Summer, Monsoon	Winter
1-Phenanthrenemethanol, 1,2,3,4,4a,5,6,9,10,10a-decahydro-1,4a-dimethyl-7-(1-methylethyl)-, [1R- (1à,4aá,10aà)]-	Winter, Summer, Monsoon	
1-Phenanthrenemethanol, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-, [1R- (1à,4aá,10aà)]-	Winter, Summer, Monsoon	
1-Heptatriacotanol	Winter	
2-[4-methyl-6-(2,6,6-trimethylcyclohex-1- enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	Winter	
Squalene	Winter, Summer, Monsoon	Summer
ç-Sitosterol	Winter, Summer, Monsoon	Winter, Summer, Monsoon
Sulfurous acid, pentyl tetradecyl ester	Winter	
Caryophyllene	Summer	
ç-Elemene	Summer	
Neophytadiene	Summer, Monsoon	Summer
Androst-5,7-dien-3-ol-17-one, acetate	Summer	
7,8-Epoxylanostan-11-ol, 3-acetoxy-	Summer	
n-Hexadecanoic acid	Monsoon	Monsoon
Campesterol	Monsoon	
Stigmasterol	Monsoon	
Octadecane, 2-methyl- (Palmitic acid)	Monsoon	
Estragole		Winter, Summer, Monsoon
1,15-Pentadecanediol		Winter
Glycine, N-[(3à,5á)-24-oxo-3- [(trimethylsilyl)oxy]cholan-24-yl]-, methyl ester		Winter
17-Octadecynoic acid (Stearic acid)		Winter
Heptadecane, 9-hexyl-		Winter
9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4- yl)methyl ester, cis-		Winter
5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 4,9, 9a-tris(acetyloxy)-3-[(acetyloxy)methyl]-1,1a,1b,4, 4a,7a,7b,8,9,9a-decahydro-4a,7b-dihydroxy-1,1,6, 8-tetramethyl-		Winter
à-Amyrin		Winter
Z,Z,Z-1,4,6,9-Nonadecatetraene		Summer
Phenol, 4-(2-propenyl)-		Summer
E-7-Tetradecenol		Summer
dl-à-Tocopherol		Summer
24-Norursa-3,12-diene		Summer
Bicyclo[4.1.0]heptan-2-ol, (1à,2á,6à)-		Monsoon
1H-Imidazole		Monsoon
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6- methyl-		Monsoon
Bufa-14,16,20,22-tetraenolide, 3-(acetyloxy)-, (3á, 5á)-		Monsoon
Cyclohexasiloxane, dodecamethyl-		Monsoon
3-Methyl-2-(2-methyl-2-butenyl)-furan		Monsoon
d-Glycero-d-ido-heptose		Monsoon

(32). In *Mentha x villosa* Hudson., it was found that high levels of polyphenols and flavonoids were seen in September (Monsoon season), while in *Plectranthus amboinicus* (Lour.) Spreng, the maximum amount of rosmarinic acid and phenols was seen in july (Monsoon season). It was also seen that the summer season also influenced the polyphenols on many levels, which led to an increase in antioxidant activity (33). In *Teucrium cirsimaritin*, six flavones- luteolin, apigenin, diosmetin, cirsiliol, cirsimaritin and cirsilineol-were identified by the HPLC method in highest quantity from May to July. It was also found that out of all the secondary metabolites, the maximum number of flavonoids were secreted in the summer season (34). In may (summer season), maximum monoterpenes (α-pinene, camphene, β-pinene, camphor, myrcene, β-Phellandrene and cineole) were secreted in higher amounts in *Rosmarinus officinalis* (35).

Carvacrol, thymol and linalool were found in very high amounts in *Zataria multiflora* and showed high seasonal variation (36). In *Salvia officinalis,* the summer season had the maximum amount of monoterpenes and the growth was uniform in other seasons, while the sesquiterpenes were higher in both the summer and cold seasons (37). In the month of october (the end of the monsoon), the polyphenols, particularly rosmarinic acid and methylapigenin, were found in the highest quantities and also showed higher antioxidant and anti-inflammatory activities (38). The harvesting (summer) season (april, may and june) showed 65 new essential oils out of which 10 were found in maximum amounts (concentrations exceeding 2%) when compared to other seasons in *Teucrium ramosissimum* (39). The leaves of *Tetradenia riparia* (Hochst.) showed the highest levels of accumulation of essential oils during the months of winter and summer, while the autumn season showed a significantly lower amount of essential oils (40).

In *Tetradenia riparia* (Hochst.), it was found that different shading conditions showed different results. 80% shading application showed the least amount of essential oils when compared to 30% and 50% shading and the amount of essential oils increased by 3-5% (41). The levels of polyphenols also increased during the summer season (june month) by about 36 mg GAE gr^{-1} DW when compared to the winter and monsoon seasons (28 mg GAE gr^{-1} DW) and the levels of anthocyanins also increased abruptly at the onset of the summer season in the leaves of rosemary (42). There was a 3.0-fold increase during the summer season and a 3.1-fold increase during the spring season in monoterpenes and sesquiterpenes in *Ocimum gratissimum* L. (43). In *Trecium polium* L., during the flowering stage (june month), there was a higher amount of various essential oils such as α-pinene (17.04%), β-pinene (12.68%), limonene (6.65%), β-myrcene (6.07%) and germacrene D (5.89%) when compared to the vegetative stage (March month) (44). Studies in the leaves and inflorescences of the five species of Lamiaceae, i.e., *Origanum vulgare* subsp. *hirtum* (Link) Ietswaart, *Salvia officinalis* L., *Mentha spicata* L., *Salvia rosmarinus* Spenn and *Mentha × piperita* L., showed the highest essential oil content in the summer months, with the highest in the month of july. In *Salvia rosmarinus* Spenn, the highest essential oil content was in the month of june (45). Few essential oils such as germacrene D, α-copaene, β-cubebene, β-caryophyllene, α-bergamotene, γ-muurolene, γ-cadinene, cadinol, epi-α and α-cadinol were seen highest in the month of summer in *Ocimum basilicum* L. (46).

In plants such as *Agastache rugosa*, *Melissa officinalis*, *Ocimum basilicum Purpurascens* and *Salvia officinalis,* a high level of antioxidant activity was seen due to high amounts of rosmarinic acid accumulated during the flowering process of these plants (june and july months) (47). Various metabolites such as isoforskolin, forskolin, 1,9-dideoxyforskolin and 1-deoxyforskolin were secreted in the highest amount in the winter season in *Coleus froskholii* Briq. (48). In *Mentha longifera*, metabolites such as pulegone, 1,8-cineole and L-menthone were seen in the highest amounts in the winter season (49). The content of various essential oils (around 50 EOs) was found to be maximum in the flowering stage, i.e., the months of june and july, out of all the five stages in *Mentha longifera* (L.) L. (50). LC-MS studies in *Ocimum basilicum* L. showed rosmarinic acid as a major compound and in significant amounts when compared to other phenolic compounds (51). HPLC analysis of *Ocimum basilicum* L. with six different fractions of the extract showed 19 different phenolic compounds which have been found in this study as well with additional phenolic compounds (52). About 33 compounds with a combination of phenolics and terpenoids were found in the extract of *Mesosphaerum suaveolens* leaves by HPLC analysis (53).

LC-MS/MS analysis

The analysis of all the unique phytochemicals identified in both *Mesosphaerum suaveolens* (L.) Kuntze and *Ocimum basilicum* L. across three seasons: winter, summer and monsoon were done via LC-MS/MS. Interestingly, the highest amount of unique phytochemicals was observed during summer season for both plants, followed by the monsoon and winter. This suggests a seasonal variation in phytochemical composition, with summer being the most prolific period (Supplementary Table 2).

In *Mesosphaerum suaveolens* (L.) Kuntze, terpenoids were the predominant class of phytochemicals, constituting 47% of the total identified compounds, followed by flavonoids (12%) and phenols (5%). Similarly, in *Ocimum basilicum* L., terpenoids were the most abundant phytochemicals, comprising 54% of the total, followed by flavonoids (9%) and phenols (3%). This trend underscores the significance of terpenoids in both plants, indicating their potential role in mediating biological activities and contributing to the plants' therapeutic properties (Fig. 4).

Flavonoids

Flavonols such as Quercetin, tricetin, morin, 6-hydroxykaempferol, 5,7,8,3',4'-Pentahydroxyisoflavone, 5,6,7,3',4'-Pentahydroxyisoflavone, herbacetin, 2'-Hydroxypseudobaptigenin, 6-Hydroxyluteolin, hypolaetin, isoetin, robinetin were determined only in summer samples of *M. suaveolens.* By comparing their mass spectra with (54) and with standard references, these flavonols were found to be derivatives of flavonoids and hydroxy flavones. The Quercetin group of flavonoids is one of the biggest with acylated and non-acylated fragments with linking carbohydrate groups which gives them antioxidant properties (55). Quercetin and Ayanin were found in *M. suaveolens* and *O.*

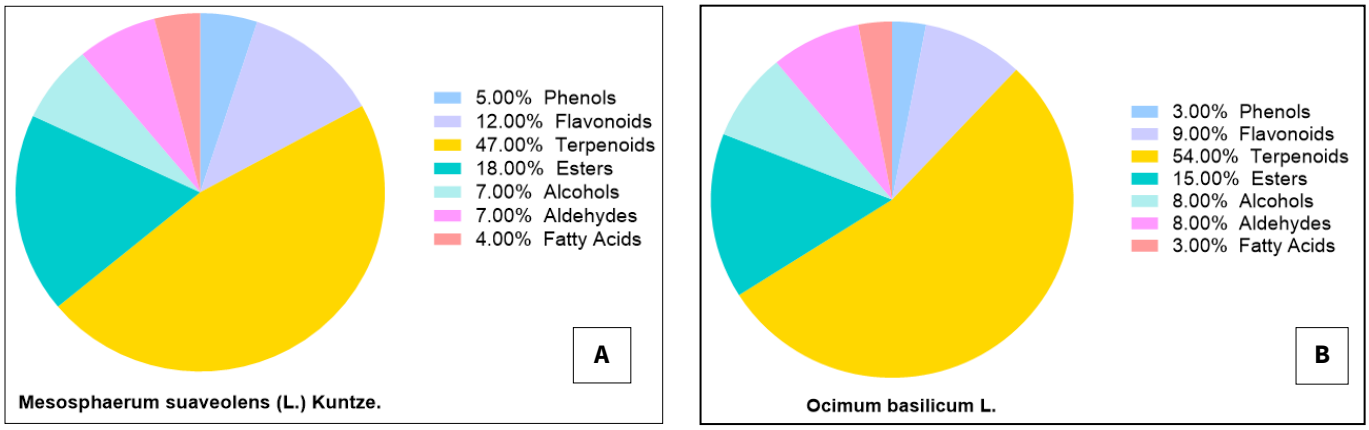

Fig. 4. Bioactive metabolites found in (A) *Mesosphaerum suaveolens* (L.) Kuntze. and (B) *Ocimum basilicum* L. during summer season.

basilicum respectively. Ayanin, quercetin and many derivatives of quercetin are known to have antiparasitic and antitrypanosomal activities (56). In *O. basilicum,* flavonoids such as nevadensin, pachypodol, questin, tambulin, santin, eupatilin and xanthomicrol were detected. Methoxyflavonoids such as 8-Hydroxy-4',5,7-trimethoxyflavone and 5-Hydroxy-4',4,6 -trimethoxyflavone were also found in *O. basilicum.* Different derivates of methoxyflavonoids have also been found in other plants of Lamiaceae family (57) and are known to have antioxidant properties. The two most common flavonoids in the Lamiaceae family are quercetin and kaempferol, together with their derivatives (58). In our study, both quercetin and 6-hydroxykaempferol have been isolated from *M. suaveolens.* Kaempferol derivates were also found in both the plants and were compared with mass spectra and standard references. (59).

Phenols

Phenols such as gingerdione and 6- hydroxyshogaol were detected in both *M. suaveolens* and *O. basilicum.* Gingerdione and 6- hydroxyshogaol are the most abundant metabolites in Zinziberaceae family (60). These metabolites are known to have antioxidant potential (61). Bracteatin, 3-tert-Butyl-5-methylcatechol, butylated hydroxyanisole, 4-(Butoxymethyl) phenol and 2-Hydroxy-6-tridecylbenzoic acid were detected in *M. suaveolens.* Bracteatin is a type of polyphenol with glycoside groups and helps plant in flowering and their properties (62). Catechol and derivates of catechin aid in improving the circulation of blood flow, reducing cholesterol absorption and helps in improving the cardiac health (63). Anisol and its derivatives have been found in various other families such as Rutaceae, Asteraceae and Apiaceae (64). These metabolites have also been found in other species of Lamiaceae such as rosemary, mint, common sage, lavender and many others. Various derivatives of benzoic acid were found in *M. suaveolens,* which are used as antifungal and antibacterial preservatives and are also used as flavouring agents in food and colourings, cosmetics and pharmaceutical products (65). The polyphenol, salvianolic acid which is a dimer of rosmarinic acid was found in both *M. suaveolens* and *O. basilicum.* Rosmarinic acid is highly found in a variety of species of Lamiaceae such as *Rosmarinus officinalis* L., *Origanum vulgare* L., *Salvia officinalis* L., *Origanum majorana* L., *Lavandula angustifolia* Mill., *Ocimum* spp., *Mentha* × *piperita* L., *Melissa officinalis* L., *Thymus vulgaris* L. and *Hyssopus officinalis* L. Other phenol compounds such as 6-Shogaol and (1a,5b,6a)-7-Protoilludene-1,5,6,14-tetrol 14-(2,4-dihydroxy-6-methylbenzoic acid) were detected only in *O. basilicum*.

Terpenes

Terpenes were detected in both *M. suaveolens* and *O. basilicum.* Metabolites such as sericoside, lucyoside R, quercillicoside A, cyclopassifloside VI, palustric acid, L- pimaric acid, isopimaric acid, (ent-15beta,16beta)-15,16-Epoxy-3-kauranone, cascarillone, 8 (17),12-Labdadiene-15,16-dial, yucalexin A16, yucalexin B14, oryzalexin A and abietic acid were found in both *M. suaveolens* and *O. basilicum.* Sericoside is a natural metabolite which is known to rejuvenate skin by alterating DNA and reprogramming cells (66). Lucyoside B has antiinflammatory properties since it reduces the expression of mediators which are involved in pro-inflammatory activities (67). Cyclopassifloside VI has also been found in other species of Lamiaceae such as *Rosmarinus officinalis, Oreganum vulgare, Marrubium vulgare, Lavandula officinalis* L. and *Mesosphaerum sidifolium* and is known to have antioxidant properties (68). L- pimaric acid and isopimaric acid which are non- abietic acid diterpenoids are known to have antioxidant, antimicrobial, anti-inflammatory, antiulcer, antibacterial agents and cytotoxic and cardiovascular activities (69). They are also found in other species such as *Salvia caespitosa, Callicarpa americana, Salvia wiedemanni, Wedelia trilobata, Origanum ehrenbergii* and many others (70). Metabolites such as sugiol, isotretinoin, tokoronin, misoprostol, retinoic acid and (-)-spruceanol were detected in *M. suaveolens.* Sugiol is a type of diterpene which has antibacterial activities and is also found in *Hyptis lacustris* A. St.-Hil. ex Benth. and Hyptis *Jacq*. (71).

In conclusion, the extent secondary metabolite accumulation was different in all the seasons (Supplementary Table 2). In case of *M. suaveolens* and *O. basilicum,* highest metabolites accumulation was seen in the summer season. In other words, it was seen that at the onset of flowering phase, highest number of secondary metabolites were accumulated. Studies have shown that seasonal variation can be attributed to a variety of factors, including species, organ, climate pattern, region, habitat, altitude and climate variability. It can also be responsive to ecological stresses, meaning that it is not always directly related to the season (72). The reason being the precise ontogenic growth stage varies with the season and seasonal and maturity differences are related to each other. Due to variances in temperature and individual responses to local ecological conditions, different kinds of herbs-even those belonging to the same species-will not exhibit consistent phenological patterns and phytochemical composition (73).

Additionally, climate-related factors, such as drought and herbivores feeding stress, also have a direct impact on the biosynthesis of phytochemicals in plants. In cases where stress conditions persist, species-specific survival in natural ecosystems will rely on adaptation strategies, such as ecophysiological, structural and biochemical responses (74). This study can also be extended to examining seasonal variations of phytochemicals from plants in different temperate zones which could also provide insight into the effects of climatic changes in the future. Furthermore, it will be necessary to employ experimental methods to determine how herbs will react to various aspects of climate change.

Conclusion

Using the GS-MS and LC-MS/ MS techniques, more than 950 metabolites were identified. The study led to an understanding of numerous secondary metabolites expressed in both plants seasonally. Maximum number of phenolic and flavonoid compounds in these plant's attributes to the high antioxidant properties of the plants. Other categories of metabolites were also found but in scarce amount such as ethers, esters, steroids, catechins, imidazoles, retinols, ketones, amino acids, alcohols and aldehydes. As a result of the study on seasonal changes in these plants, it was revealed that the maximum accumulation of the secondary metabolites occurred in the summer season followed by monsoon and winter season. Metabolic profiling and comparative studies also found that among both the plants, *M. suaveolens* leaves showed the highest accumulation of secondary metabolite (summer leaves). The results of the study indicated that these plants could serve as an important source of various bioactive natural compounds. These wild plants could also be cultivated for their rich phytochemical constituents. The study undertaken could be extended to other plants of Lamiaceae for potential phytochemical constituents.

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Authors' contributions

RM contributed to the entire experimental work including standardization of the methods. RP contributed to Soxhlet extraction of both plants. RM and PP participated in the analysis and interpretation of the GC-MS and LC-MS/MS data analysis. MK designed the study and helped in editing and finalizing the draft. RM wrote the first draft of the manuscript and all the authors commented on this version. All authors have read the final manuscript and approved the submission.

Compliance with Ethical Standards

Conflict of interest: The authors report no financial or any other conflicts of interest in this work.

Ethical issues: None

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