



ORIGINAL ARTICLE

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GC-MS Analysis of essential oils extracted from the leaves of *Pelargonium graveolens* L. Herit and *Murraya koenigii* (L.) sprengel

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ABSTRACT

Leaves of *Pelargonium graveolens* and *Murraya koenigii* were extracted from Clevenger method by hydro distillation, using Diethyl ether as a solvent and using NaSO_4 as a moisture content as remover. The extract was subjected to screen the phytochemical constituents through gas chromatography mass spectrometry (GC-MS) analysis. The GC-MS analysis of the leaf essential oil showed a total of 18 chemical compounds accounting for 99.976% of the oil in the leaves of *Pelargonium graveolens* the major compounds identified in the leaf oil of *Pelargonium graveolens* were, semicarbazone, caryophyllene oxide, citronellol and bicyclo [5.2.0] Nonane, 2-Methylene-4,8,8-Trimethyl-4-Vinyl. 22 chemical compounds accounting for 100% of the oil in *Murraya koenigii*, the major compounds identified in the leaf oil of *Murraya Koenigii* was caryophyllene, nicotinimidamide, linalool, pinene, ethyl ester, 1h-Indazole, 4,5,6,7-Tetrahydro-7-Methyl, cadinol and many other notable compounds were identified as in the range of less than 5%. From the present study it can be concluded that *P. graveolens* and *M. koenigii* leaves contain effective bioactive of pharmacological importance.

Key words: Essential oils, *Pelargonium graveolens*, *Murraya koenigii*, GC-MS, bioactive compounds, NIST.

Received 09.07.2024

Revised 17.08.2024

Accepted 20.09.2024

INTRODUCTION

Essential oils (Eos) are sweet-scented volatile liquids synthesised by oil secreting cells, cavities, canals, epidermal cells or glandular trichomes. They are synthesized in plants through secondary metabolic activity (1). Essential oils are most habitually produced from hydro distillation (2). Components present in EOs mostly comprise volatile terpenes and hydrocarbons (3). Essential oils have received increasing recognition to have natural additives for the shelf- life prolonging food products due to risk in making use of synthetic preservatives. Essential oils are good fount of bioactive compounds, which possesses antioxidative and antimicrobial properties, so the use of essential oils can be convenient to extend shelf-life in food products (4). *Pelargonium graveolens* commonly known as geranium which belongs to Geraniaceae family, is a bushy, aromatic plant, the stem and leaves are pubescent. The leaves are highly aromatic in nature. In the industries like perfumery, cosmetic and aromatherapy have a great extent use of *P. graveolens* essential oil in all over the world. It has since become a vital role in aromatherapy oil. Geranium oil reacts well to the effects of constipation, insomnia, restlessness, nervousness, anxiety, worry, high blood pressure, anger, frustration, emotional upsets, hypercholesterolemia, slow to lose weight and low metabolic forces (5). *Murraya koenigii* belongs to the family Rutaceae, is a shrub to small tree upto 8m in height. The main stem is dark green to brownish, with numerous dots on it. The leaves are usually with pellucid glands. Different examines were performed on the Bark, root, leaves, fruit and fruit pulp of *Murraya koenigii* for medicinal uses and in ayurveda (6). Hence the present work carried was to analyse the chemical composition of essential oils from leaves of *Pelargonium graveolens* and *Murraya koenigii* by GC-MS respectively.

Objectives of the study

1. To screen the phytochemicals through GC-MS analysis
2. To know the possible phytochemical compounds, present in the given plant samples.

MATERIAL AND METHODS

Collection of plant materials

The fresh leaves of *Pelargonium graveolens* and *Murraya koenigii* Sprengel were collected from Kolavanahalli village, Chikkaballapur. The plants were authenticated by Sri B. S. Chandrashekar, Scientist E, SFM Division, IWSST, Bangalore.

Those leaves were washed through the distilled water and kept for shade-drying till the water droplets evaporate under room temperature. The leaves were chopped into small pieces.

For the extraction of essential oils from *Pelargonium graveolens* and *Murraya koenigii* Sprengel by hydro-distillation under optimal operating conditions and a quantity of 1Kg of plant material is added to round bottom flask and distilled water is added till the material immerse then the water inlet and outlet pipes are attached to the condenser of Clevenger and kept for 3 to 4 hours maintaining temperature of 45°C- 50°C.

The distillation two phases were observed: an aqueous phase- aromatic water and an organic phase-essential oil, which is less dense than water then the oil is separated and collected in test tubes or conical flask and sealed vials, as it is aromatic. Experiment is conducted till the required oil is obtained.

Separation of oil

The oil and water which forms two separate layers when the mixture is poured into separating funnel. Diethyl ether is added as a solvent so non polar compounds are generally soluble more than in alcohols such as ethanol, the solvents are shaken vigorously for 20-30sec, which greatly increases the surface area in contact between two liquids. After separating, the air is released through a stopper, the dense water collected at the bottom of the funnel and it is collected in a separate beaker. Then oil is collected, anhydrous sodium sulphate is added to absorb moisture content from oil and filtered using Whatman filter paper and then the oil is kept open under room temperature for solvent evaporation. The oil obtained was stored in vials at 40°C in a refrigerator for further analyses.

Identification of Phytochemical compounds

GC-MS Analysis

GC-MS (Gas chromatography-Mass spectrometry) analysis of leaves of *Pelargonium graveolens* and *Murraya koenigii* essential oils was performed. The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite -5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1µL of extract sample injected into the instrument the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min⁻¹; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

RESULTS

GC-MS analysis

GC-MS analysis showed the presence of 18 and 22 phytocompounds accounting for 99.976% and 100% in *Pelargonium graveolens*, *Murraya koenigii* (Table:1-2). The identification of phytochemical compounds was confirmed on the basis of retention time, peak area and molecular weight and formula. Thus, phytoconstituents obtained through GC-MS analysis was interpreted using the database of (NIST) library. The GC-MS chromatogram of oil extract of two different plants are shown in Figure 1-2.

Chemical composition of essential oil from leaves of *Pelargonium graveolens*

The GC-MS analysis of the leaf essential oil showed a total of 18 chemical compounds accounting for 99.976% of the oil (Figure 1). The major compounds identified in the leaf oil of *Pelargonium graveolens* were, semicarbozone (40.45%), caryophyllene oxide (22.58%), citronellol (13.06%) and bicyclo [5.2.0] Nonane, 2-Methylene-4,8,8-Trimethyl-4-Vinyl (6.335%). The other notable compounds found in the leaf oil in concentration less than 5% were, linalool, geraniol, rose oxide, eudermol, limonene.

Chemical composition of essential oil from leaves of *Murraya Koenigii*

The GC-MS analysis of the leaf essential oil showed a total of 22 chemical compounds accounting for 100% of the oil (Figure 2). The major compounds identified in the leaf oil of *Murraya Koenigii* was caryophyllene (16.136%), nicotinimidamide (9.313%), linalool (8.429%), pinene (8.379%), ethyl ester (7.485%), 1h-Indazole, 4,5,6,7-Tetrahydro-7-Methyl (6.424%) and cadinol (5.0253%). The other notable compounds found in the leaf oil in concentration less than 5% were, histamine, benzoic acid, phenethyl alcohol, Z-jasmone, viridifloral, neryl acetate, beta elemene, spathulenol, selinene, 2'-Ethylacetoacetanilide.

DISCUSSION

Essential oils are made up different components of volatile compounds biosynthesized by plants, containing a large number of components (7). GC-MS was used to separate chemical components, quantify components, identify unknown components. Gas chromatography (GC) coupled with mass spectrometry (MS) has been one of the most useful techniques of choice to identify variations in the composition of essential oil due to the volatility and polarity of its components (8). A new component Semi-carbazone was the major component found in *P. graveolens* in present studies and it is not found in any review of literature. Semi-carbazone was found in 40.45% in the present studies. Semi-carbazone is effective means for removing harmful metals from the environment has been studies through the use of thiosemicarbazone and semi-carbazone chelating resins (9).

Semi-carbazone show broad range of application in medicine, pharmacy, coordination chemistry, biological activities, industries, food packages, dyes, and polymer and also used as an O₂ detector. Numerous applications in pharmacology such as antiviral, antifungal, antimicrobial, antimalarial, antituberculosis, anticancer, anti-HIV, catalytic application in oxidation of organic compounds, and nanotechnology (10). Caryophyllene oxide is the bicyclic sesquiterpene is one of the minor components found in the essential oil extracted from leaves of *P. graveolens* with 3.7% (11) where as in the present study 22.58% was found in the leaf oil of *P. graveolens* as second major component. Caryophyllene possesses anticancer activities, affecting growth and proliferation of numerous cancer cell, and analgesic properties (12). Citronellol (48.44%) was the major component present in the essential oil extracted from leaves of *Pelargonium graveolens* (13), 14% of citronellol as oxygenated monoterpene as a main representative (14), 27.53% (15), 37.55% (11) where as in the present study 13.06% was found in the leaf oil of *P. graveolens*. Citronellol contribute to various activities- antimicrobial, anthelmintic, antioxidant, anticonvulsant antitrypanosomal and wound healing and mosquito repellent action (16). Geraniol 50.2% (14), 25.85% (15) where as in the present study it is less than 5%. The other notable compounds found in the leaf oil in concentration less than 5% were, linalool, geraniol, rose oxide, eudermol, limonene was found. The variation in the chemical composition of geranium is may due to variations in geographical location, temperature, rainfall, soil, etc. (17). The essential oil of *P. graveolens* is one of the most expensive essential oil used in the perfumery, flavouring and cosmetic industries and therefore the plant is widely cultivated (11). Caryophyllene (9.5%) were the major components of *M. koenigii* (18), 6.5% from the plains of Northern India, 3.35% oil from the lower Himalayan range, 53.9%) from Eastern India, 53.9% Southern India (Kozhikode, Kerala) (19), where as in the present study 16.136%, the present investigation slightly varies with respect to the literature review. Nicotinimidamide primarily used as a nutritional supplement for vitamin B₃. Supplementation of nicotinamide restores cellular NAD⁺ pool and mitochondrial energetics, attenuates oxidative stress and inflammatory response, enhances extracellular matrix and skin barrier, and inhibits the pigmentation process in the skin (20). Linalool was the major component present in the essential oil leaves of *M. koenigii* with 32.83% (21), 8.0% (22), 0.04% (23) where as in the present study 8.429% found in the leaves of *M. koenigii* essential oil. Linalool and linalool-rich essential oils are known to exhibit various biological activities such as antimicrobial, anti-inflammatory, anticancer, anti-oxidant properties and several in vivo studies have confirmed various effects of linalool on the central nervous system. The applications of linalool are specific scent to domestic products such as soaps, detergents and shampoos. Linalool also plays an import role in nature as a key compound in the complex pollination biology of various plant species to ensure reproduction and survival. Linalool is a key compound for the industrial production of a variety of fragrance chemicals such as geraniol, nerol, citral and its derivatives, as well as a lead compound in the synthesis of vitamins A and E. The repellent properties of linalool on various crop-destroying insects has been well documented accentuating the application of this molecule in eco-friendly pest management (24). Pinene 61.4% is obtained in the leaves of *M. koenigii* (25), 75.4% (26), 55.7% (27), where as in the present study 8.379% found in the leaves of *M. koenigii* essential oil. Pinene pharmacological activities are antibiotic resistance modulation, anticoagulant, antitumor, antimicrobial, antimalarial, antioxidant, anti-inflammatory, anti-Leishmania, and analgesic effects (28), (29).

CONCLUSION

Pelargonium graveolens and *Murraya koenigii*, are some important medicinal plants used to extract essential oils. Hydro-distillation of leaves of *P. graveolens* and *M. koenigii* yielded coloured oils with strong odour but the yield was not significant. GC-MS analysis showed that the major class of chemical constituent present in essential oil of *P. graveolens* was semicarbozone, caryophyllene oxide, citronellol and bicyclo [5.2.0] Nonane, 2-Methylene-4,8,8-Trimethyl-4-Vinyl. The chemical constituent present in essential oil of *M. koenigii* was caryophyllene, nicotinimidamide, linalool, pinene, ethyl ester, 1h-Indazole, 4,5,6,7-

Tetrahydro-7-Methyl and cadinol. The results of the present investigation will pave the way to identify other bioactive compounds in *Pelargonium graveolens* and *Murraya koenigii* to resolve their efficacy by *in vivo* studies and to develop novel safer drugs.

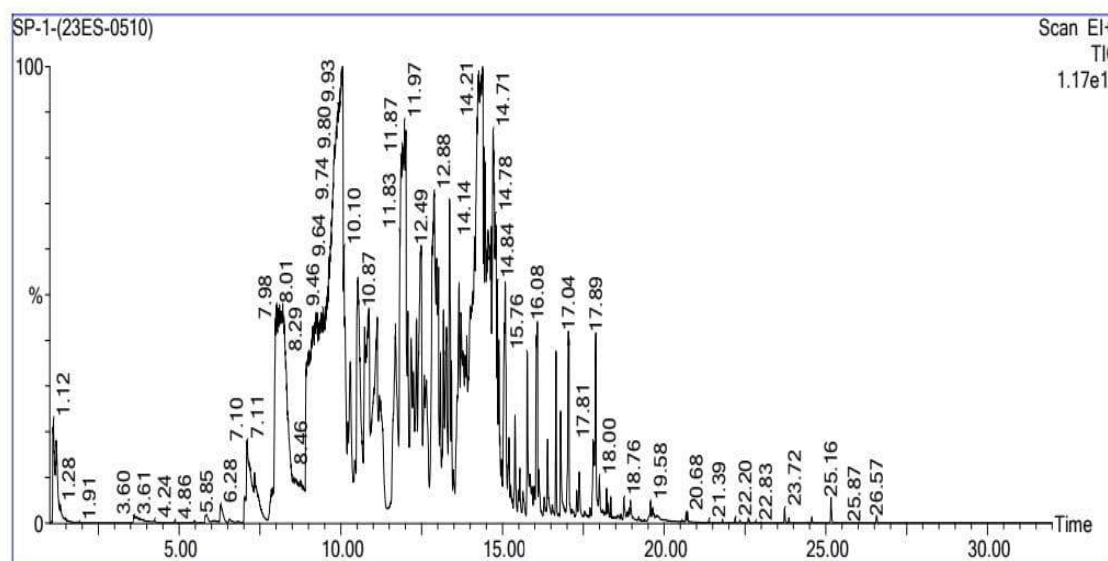


Figure 1: GC-MS chromatogram of essential oil of leaves of *P. graveolens*

Sl. No.	Compounds	Retention Time	Area %	Normality %
1	Z-(13,14-Epoxy) Tetradec-11-En-1-Ol Acetate	8.206	11.516	37.47
2	Ketone, 1-Cyclohexen-1-Yl Methyl, Semicarbazone	9.242	9.717	31.62
3	Ketone, 1-Cyclohexen-1-Yl Methyl, Semicarbazone	10.052	30.733	100.00
4	2-Pyridineethanol, 1-Oxide	10.102	1.785	5.81
5	2-Ethyl-5-N-Propylphenol	10.532	3.449	11.22
6	9,12-Octadecadien-1-Ol, (Z, Z)	11.132	2.480	8.07
7	Bicyclo [3.1.1] Heptane-2-Methanol, 6,6-Dimethyl	11.197	1.773	5.77
8	Bicyclo [5.2.0] Nonane, 2-Methylene-4,8,8-Trimethyl-4-Vinyl	11.693	2.582	8.40
9	Bicyclo [5.2.0] Nonane, 2-Methylene-4,8,8-Trimethyl-4-Vinyl	11.968	8.351	27.17
10	1,2,4-Metheno-1h-Indene, Octahydro-1,7a-Dimethyl-5-(1-Methylethyl) Trans-Anti-Trans-Tricyclo[7.3.0.0(2,6)]-7-Dodecene	12.488	2.879	9.37
11	Naphthalene, 1,2,3,4,4a,5,6,8a-Octahydro-7-Methyl-4-Methylene-1-(1-Methylethyl) Tricyclo[6.3.0.0(2,4)]Undec-8-Ene, 3,3,7,11-Tetramethyl	12.883	5.378	17.50
12	Not identified	12.963	2.255	7.34
13	Bicyclo [3.3.1] Nonan-9-Ol	13.363	1.858	6.04
14	Bicyclo [3.3.1] Nonan-9-Ol	14.389	6.335	20.61
15	1,4,7, -Cycloundecatriene, 1,5,9,9-Tetramethyl-, Z, Z, Z	14.709	3.390	11.03
16	1,4,7, -Cycloundecatriene, 1,5,9,9-Tetramethyl-, Z, Z, Z	15.084	2.421	7.88
17	5-Ethoxy-2-Methyl-Pyridine	16.079	1.533	5.05
18	6-[N-Aziridyl]-4-Methyl-2-Hexyn-4-Ol	17.040	1.544	5.02
Total			99.976%	

Table 1: Chemical composition of essential oil of leaf of *P. graveolens*

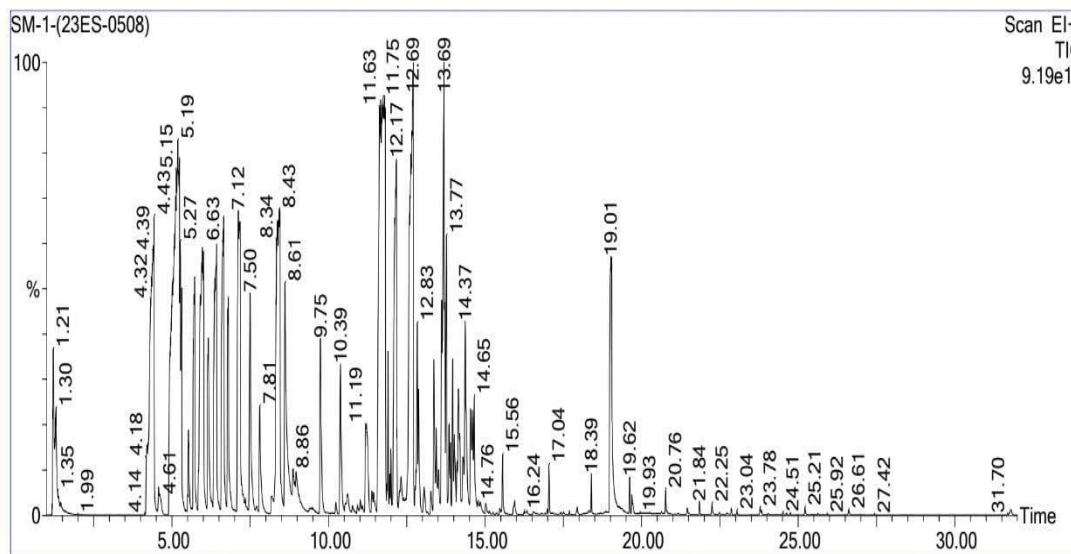


Figure 2: GC-MS chromatogram of essential oil of leaves of *M. koenigi*

Sl. No.	Compounds	Retention Time	Area %	Normality %
1	Silane, Dimethyldi-1-Propynyl	4.435	7.579	46.91
2	2-Amino-4-Methylpyrrole-3-Carbonitrile	5.190	9.313	57.65
3	Phenethyl Alcohol, P-Nitro- Alpha -Phenyl	5.730	2.588	16.02
4	1h-Indazole, 4,5,6,7-Tetrahydro-7-Methyl	5.975	6.424	39.76
5	2h-1b,4-Ethanopentaleno[1,2-B] Oxirene, Hexahydro Cyclopropane, Trimethyl(2-Methyl-1-Propenylidene)-	6.165	1.678	1039
6	Histamine, N-Benzoyl-2-Cyano	6.426	4.431	27.43
7	1-Cyclohexyl-1-(4-Ethyl cyclohexyl) Ethane	6.651	4.422	27.37
8	Benzoic Acid, 4-(1,3-Dioxo-3-Phenylpropylamino)-	6.806	1.796	11.12
9	(E, Z, Z)-2,4,7-Tridecatrienal	7.121	5.253	32.52
10	2-Cyclohexen-1-Ol, 1-Methyl-4-(1-Methylethyl)	7.496	1.987	12.30
11	1-Methyl-1h-Imidazole-2-Carboxylic Acid, Ethyl Ester	8.446	7.485	46.33
12	3-Cyclohexene-1-Methanol, Alpha., Alpha.4-Trimethyl	8.611	2.644	16.37
13	Bicyclo [3.1.0] Hexan-3-Ol, 4-Methyl-1-(1-Methylethyl) and Isobornyl Acetate	9.747	1.550	9.59
14	3-Cyclohexene-1-Methanol, Alpha., Alpha.4-Trimethylethylidene	10.387	1.321	8.18
15	Cyclohexane	11.217	1.550	9.59
16	Not identified	11.793	16.15	100.00
17	Azulene	12.173	5.332	33.01
18	Naphthalene	12.708	9.417	58.29
s19	2'-Ethylacetoacetanilide	13.688	2.675	16.56
20	Globulol	13.769	1.344	8.32
21	1-Naphthalenol, 1,2,3,4,4a,7,8,8a-Octahydro-1,6-Dimethyl-4-(1-Methylethyl) and Cadinol	14.369	1.387	8.59
22	1,10-Decanediol	19.011	3.669	22.71
			100%	

Table 2: Chemical composition of essential oil of leaf of *M. Koenigii*

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CITATION OF THIS ARTICLE

Mownika P R, Srikantha S N, Sudhakara B V, Chethan B M, Prajwala M and Sahana K M. GC-MS Analysis of essential oils extracted from the leaves of *Pelargonium graveolens* L. Herit and *Murraya koenigii* (L.) sprengel. Bull. Env. Pharmacol. Life Sci., Vol 13 [10] September 2024: 42-47.