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GAS CHROMATOGRAPHY-MASS SPECTROMETRY OF *Murraya exotica* ESSENTIAL OIL EXTRACTED THROUGH DIFFERENT EXTRACTION TECHNIQUES

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ABSTRACT

The trial was conducted to explore the essential oil production potential of *Murraya exotica* under Faisalabad conditions through various extraction methods viz. solvent extraction, steam distillation, and supercritical fluid extraction (SCFE). SCFE was found to be the most efficient method for the extraction of good quality essential oil from *M. exotica* flowers. Gas chromatography-mass spectrometry (GC-MS) was performed for the characterization of chemical constituents contained in the essential oil of *M. exotica*. It was observed that 41 components representing 99.94% of the oil were identified from supercritical fluid extraction method (SCFE) while 46 and 49 components that constitute 96.4% and 88.7% of the oils were analyzed from solvent and steam distillation methods respectively. It was noted that manool, E-nerolidol, germacrene D, benzyl benzoate, palmitic acid, methyl palmitate δ-cadinene and anthranilic acid, were found as the major components in essential oil. The values of other physio-chemical parameters viz. refractive index, optical rotation, specific gravity, congealing point, acid and ester number were variable in oil extracted through different methods.

Key words: physiochemical, solvent extraction, steam distillation, supercritical fluid extraction.

INTRODUCTION

Murraya exotica, commonly known as orange Jasmine, belongs to the family Rutaceae. It is used as a hedge plant in tropics and used in landscape due to its beautiful shiny green leaves and highly fragrant white flowers. *M. exotica* flowers are highly aromatic and contain a sufficient amount of essential oil. Essential oils are distilled volatiles of planting materials which have strong aromatic compounds that occur naturally in plants i.e. alcohol, phenols, hydrocarbons, aldehydes and esters etc. are some of the constituents of most essential oils (Younis *et al*., 2008).

Different methods can be used to extract essential oil from aromatic plants. Steam distillation is one of the traditional methods for the extraction of the essential oil from aromatic plants, but the oil yield is less compared to other recent methods (Younis, 2006). Solvent extraction method produces a considerable yield of essential oil at very cheap inputs and is mostly now being utilized in routine life to extract perfume from the flowers. Extraction of the volatile oils by means of the supercritical fluid extraction method (SCFE) has been the subject of substantial interest to extract sweet scented volatile oil from various plants like jasmine (Younis *et al*., 2011). In this procedure a supercritical fluid mainly the CO_2 is used to extract the aromas of plants. Carbon dioxide has many distinct characteristics as it is non-hazardous, inert gas with low critical values of temperature (31.1°C) and pressure (7.38MPa). As compared to conventional methods, SCFE is more convenient and superior because it is more selective and causes no thermal degradation of components and extracts obtained have no added solvent, which gives the oil of superior quality (Wood *et al*., 2006). The essential oil components can be recognized by using the gas chromatography-mass spectrometry (GC-MS). It is a specific analytical tool meant for identification, characterization, qualitative and quantitative analysis of oil (Niemann *et al*., 2005; Farooq *et al*., 2012).

This study was planned to exploit various extraction methods viz. solvent extraction, steam distillation, and supercritical fluid extraction (SCFE) to extract essential oil from *M. exotica* flowers under Faisalabad conditions. GC-MS analysis was also carried out for quantitative and qualitative analysis of extracted essential oil.

MATERIALS AND METHODS

Collection and preparation of flowers: Flowers were collected from the *Murraya exotica* plants from the field area, Institute of Horticultural Sciences, University of Agriculture, Faisalabad (latitude 31º30 N, altitude 213 m and longitude 73º10 E), during 2013-2014. Flowers were picked in the early morning hours to prevent loss of aromatic from tiny flowers. Flowers were collected, cleaned and were spread in a perforated tray under shade conditions overnight at room temperature to remove excessive moisture.

Essential oil extraction methods

Steam distillation: Steam distillation was done in a still and 20kg flowers were put in the distillation chamber and steam was generated in another chamber and forced to pass through the petals. The steam facilitates the tiny inter-cellular pockets containing the essential oil to open and release essential oil. The steam having essential oil passes through a condensation chamber at low temperature. All the vapors condensed and collected in the receiver, the liquid called as hydrosols (water + oil). Recovery evaporator was used for separation of oil extracted through distillation. The process was followed by as adopted by (Younis *et al*., 2007).

Solvent extraction: For solvent extraction, n-hexane was used using Soxhelt's apparatus. Flowers were placed in a flask and then n-hexane was added and heated to boil. Solvent vaporized through the side tube into the condenser, where vapors condensed and fell into the thimble. This organic solvent extracted all the volatile compounds from the petals. This process was revised for three to four times. Concrete oil was further processed to remove any remaining solvent from the oil. Distillation process was performed to recover solvent from concrete oil (organic solvent and essential oil) and this was done by rotary evaporator.

Supercritical fluid extraction (SCFE): The *M. exotica* petals were filled in the extraction vessel after weighing of 20kg weight. The pressure was maintained through a gas booster, censored by pressure-transductor and a pneumatic control-valve. The samples were placed in a separation-vessel at specific temperature and pressure range maintained through a connected computer having SCFE software. The oil was separated by changing the CO_2 phase and absolute oil was collected in a flask.

The percentage of concrete oil was calculated by using equation;

$$\text{Percentage of concrete oil} = \frac{\text{Weight of concrete oil}}{\text{Weight of petals}} \times 100$$

The absolute oil percentage was calculated by using formula;

$$\text{Percentage of absolute oil} = \frac{\text{Weight of absolute oil}}{\text{Weight of petals}} \times 100$$

Physio-chemical analysis of *Murraya exotica* essential oil: This part of study was carried out at Department of Chemistry, University of Agriculture, Faisalabad and Department of Chemistry, Forman Christian College Lahore. Color of absolute oil was determined by spectrophotometric method (Paquot, 1986a). The refractive index of the essential oil was measured at 25°C by using Abbe's refractometer (Paquot, 1986b). Congealing point of the oil was determined by placing minute amount of oil in the capillary tube that was further suspended into a larger tube having a thermometer. The maximum temperature at which solidification occurred was noted as the congealing point of the oil. To get value for optical rotation, polarimeter-tube (10 ml) was used. The essential oil's specific gravity was determined by using gravity-bottle.

The acid number was determined through indicator method (Paquot, 1986b). Then,the acid number was calculated by the formula,

$$\text{Acid number} = \frac{56.1(\text{Number of c c of 0.1 KOH})}{\text{Weight of the sample (g)}}$$

The ester content was calculated by:

$$\text{Ester number} = \frac{28.05\ (a)}{s}$$

, Where, a = number of c.c. of 0.5 normal HCI used in the saponification; s = Weight of sample in g

GC-MS analysis: For the qualitative analysis of essential oil gas chromatography-mass spectrometry (GC-MS) was performed by using agilent technology (6890N-5975B system), with data acquisition parameters: carrier gas was helium with a flow rate of 1mL/min, at constant flow; injection volume 0.1µL, with inlet temperature 250°C; Agilent Technology (HP-5MS 30 m 0.25 µm column). Temperature settings: 50°C for 1 min, 5°C/min to 100°C, 9°C /min to 200°C, hold 7.89 min; transfer-line temperature 280°C; electron ionization, electron energy 70 eV, scan mode, mass range 35-400 Da, quadrupole temperature 150°C, source temperature 230°C. The compound characterization was confirmed by comparing relative retention time value with literature values (Raina *et al*., 2006; Rout *et al*., 2010).

RESULTS

Effect of the extraction methods on properties of *M. exotica* essential oil: The properties of essential oil of *M. exotica* was compared and presented in Table 1. It can be observed from the results that variations in the properties of essential oil extracted through different extraction methods have remarkable effects on the properties of essential oil. The color of *M. exotica* oil obtained by SCFE was clear yellow, whereas, yellowish brown oil from steam distillation and light brown color of essential oil was obtained by the solvent extraction method. Refractive index of oil obtained by SCFE was 1.48 at 25°C. The oil obtained by solvent extraction had a refractive index of 1.43, whereas the refractive index of oil yielded by steam distillation was 1.41 at the same temperature (Table 1). It was observed that the super critical fluid extraction method produced oil with the highest congealing point (22°C), whereas the oil obtained by solvent extraction method gave the second highest value (18°C) for this property. The steam distillation method yielded oil with a congealing of 17°C (Table 1).The optical rotation of *M. exotica* essential oil was highly influenced by various extraction methods. It was observed that essential oil extracted using SCFE method exhibited a value of +4.4, whereas the oil obtained by solvent extraction and steam distillation methods had an optical rotation of +3.9 and +3.8, respectively. The various extraction methods differed significantly for specific gravity and it was noted that SCFE method yielded oil with a specific gravity of 0.871. The specific gravity of the oil obtained by solvent extraction was found to be 0.858 and it was 0.851 for the oil extracted by steam distillation (Table 1).

The acid number varied in *M. exotica* essential oil extracted by different methods. It was found that supercritical fluid extracted essential oil possessed more acid number with a value of 1.55 as compared to other two methods i.e. solvent extraction (1.34) and steam distillation (1.26) (Table 1). The essential oil of *M. exotica* extracted by using different methods showed variation in ester number. It was observed that SCFE method gave oil with maximum number of esters (143). The oil extracted by steam distillation and solvent extraction methods gave the values of 119 and 128 for ester number, respectively (Table 1).

Essential oil yield by different extraction methods: Results depicted that steam distillation yielded 0.09% concrete and 0.002% absolute oil on petals fresh weight basis (Table 2). In solvent extraction, hexane was used for the extraction of essential oil by employing soxhlet's apparatus. The petals from fresh flowers were used for solvent extraction and 0.15% on petal weight basis concrete oil was produced by this method. Whereas, absolute oil separated from concrete was 0.005% on petals fresh weight basis. The determined optimized condition for essential oil extraction of *M. exotica* from super critical fluid extraction experiment was used for comparison with the other two methods. The results obtained showed that the concrete oil (0.18%) and absolute oil of 0.008% on petal weight basis was obtained by employing 40°C and 80 bar pressure (Table 2).

Gas chromatography-mass spectrometry analysis: In total 49 components were identified that represents 99.94% of the oil extracted from SCFE method (Table 3). Manool, E-nerolidol, germacrene d, benzyl benzoate, palmitic acid, methyl palmitate δ-cadinene and anthranilic acid were found to be the major components in the oils extracted from SCFE, solvent extraction and steam distillation but with varying concentrations. Manool was found to be 17.27% of the oil extracted through SCFE, 15.45% of the oil of solvent extraction method, whereas steam distilled oil gave 12.21% of the concentration of manool. While, the relative concentrations of other major constituents i.e. E-nerolidol (13.52%, 12.36%, 11.38%), germacrene d (8.54%, 6.90%, 5.65%), benzyl benzoate (8.19%, 7.63%, 6.86%), palmitic acid (6.23%, 5.23%, 4.53%), methyl palmitate (5.31%, 4.68%, 3.45%), δ-cadinene(5.13%, 4.32%, 4.61%) and anthranilic acid (3.32%, 2.68%, 2.94%) were found in the oils extracted from SCFE, solvent extraction and steam distillation respectively (Table 3).

Table 1. Physiochemical properties of *Murraya exotica* essential oil extracted through different extraction methods.

Treatments	SCFE	Solvent extraction	Steam distillation
Color	Clear yellow	Light brown	Yellowish brown
Refractive index	1.48 at 25°C	1.43 at 25°C	1.41 at 25°C
Congealing point	22°C	18°C	17°C
Optical rotation	+4.4 at 25°C	+3.9 at 25°C	+3.8 at 25°C
Specific gravity	0.871	0.858	0.851
Acid number	1.55	1.34	1.26
Ester number	143	128	119

Table 2. Concrete and absolute oil percentage of *Murraya exotica* essential oil extracted through different extraction methods.

Treatments	Concrete oil %	Absolute oil %
SCFE	0.18a	0.008a
Solvent extraction	0.15b	0.005b
Steam distillation	0.091c	0.0023c

Table 3. GC-MS analysis of the percentage of different constituents of essential oils of *Murraya exotica* extracted through different extraction methods.

Sr. #	Compounds	SCFE (%)	Steam distillation (%)	Solvent extraction (%)	RT (Min)
1	Benzyldehyde	0.32	0.18	0.25	6.92
2	β-Myrcene	0.43	0.27	0.36	7.67
3	Limonene	0.62	0.32	0.53	7.89
4	β – Ocimene	0.39	0. 24	0.35	8.40
5	Methyl benzoate	0.71	0.45	0.54	8.93
6	Linalool	1.34	0.94	1.17	9.82
7	Phenyl ethyl alcohol	2.31	0.86	1.56	11.30
8	Ethyl benzoate	0.16	0.26	0.42	11.62
9	Nerol	----	0.23	0.18	12.15
10	Methyl salicylate	0.53	0.31	0.48	15.16
11	Indole	1.10	0.45	0.61	16.42
12	2-Phenyl ethyl acetate	0.47	0.29	0.36	17.31
13	δ-Elemene	0.26	1.36	1.17	17.70
14	Methyl anthranilate	1.75	1.38	2.78	18.60
15	α-Cubebene	-----	1.21	0.18	19.37
16	α-Copaene	0.48	0.32	0.15	20.09
17	β-Cubebene	0.17	0.92	0.76	20.36
18	β-Elemene	0.68	0.41	0.51	20.61
19	Z-Jasmone	0.23	0.35	0.67	21.12
20	E-Carophyllene	0.84	3.36	1.64	21.99
21	γ-Elemen	1.54	0.73	1.36	22.14
22	β- Farnesene	2.48	1.82	2.23	12.90
23	Germacrene D	8.54	5.65	6.90	23.03
24	α- bergamotene	1.45	1.38	1.12	23.10
25	δ- Gurjunene	0.39	0.12	0.25	23.78
26	(E,E)-α-Farnesene	3.43	4.45	3.82	24.98
27	α- Carophyllene	-----	1.65	1.44	25.08
28	Muurolol	0.11	0.42	0.26	26.18
29	δ-Cadinene	5.13	4.61	4.32	26.07
30	Cadine-1,4-diene	----	0.45	----	26.18
31	E-Nerolidol	13.52	11.38	12.36	26.70
32	Spathulenol	0.13	0.62	1.74	26.90
33	Caryophyllene oxide	-----	3.14	0.29	27.28
34	Viridiflorol	0.78	0.56	0.98	27.34
35	Tau-cadinol	---	0.37	----	27.70
36	Benzyl benzoate	8.19	6.86	7.63	28.80
37	Anthranilic acid	3.32	2.94	2.68	29.34
38	Phenyl ethyl benzoate	2.51	0.72	1.28	29.84
39	Benzyl salicylate	1.64	1.22	1.45	30.17
40	Methyl palmitate	5.31	3.45	4.68	30.32
41	Phytol	1.62	1.21	0.86	30.54
42	Palmitic acid	6.23	4.53	5.23	30.70
43	Manool	17.27	12.21	15.45	31.05
44	Geranyl linalool	-----	1.42	1.19	31.24
45	Methyl linoleate	0.63	0.28	1.34	31.80
46	Methyl linolenate	1.56	1.92	1.13	31.92
47	Methyl stearate	0.16	0.22	0.32	32.28
48	Osthole	1.21	0.29	0.42	32.52
49	n-Eicosane	---	0.97	---	32.80
	Total	99.94%	88.7%	96.4%	

RT= retention time

DISCUSSION

Essential oil extraction of *M. exotica* had been studied by several authors, Raina *et al*. (2006); Matasyoh *et al*. (2007); Li *et al*. (2010). However, none of investigations have examined in detail the comparison of essential oil between oil obtained by SCFE and the other conventional extraction techniques. Qualitative analysis was made by observing the chemical and physical properties of the essential oil. Essential oils extracted through different methods have varied physio-chemical properties (Joy *et al*., 2001). Color is an important physical parameter used for the qualitative determination of the essential oil. The change in color of the essential oil can be the result of burning of chemical constituents of the oil due to high temperature of steam and the presence of undesired impurities and organic solvent residues in the essential oil while extracting through solvent extraction method. Effect of extraction methods on the oil's color was also confirmed by researchers (Assis *et al*., 2000; Wenqiang *et al*., 2007). Physiochemical analysis for the quality assessment of the essential oils was also carried by Essien *et al.* (2008) and they observed the values of specific gravity (0.884), specific rotation (21.58), refractive index (1.47), ester number (31.16) and acid value (1.99) in the essential oil of *Citrus madica*. It was observed that the physical and chemical characteristics are the measure of the purity and quality of the essential oils. Supercritical fluid method was found the best method in producing high quality oil. Variation in the physio-chemical characters of the can be the result of composition of the chemical constituents of the essential oils (Vijayalakshmi *et al*., 2010).

M. exotica produce very delicate flowers and it is a well-known fact that extraction methods and time of harvesting of flowers greatly influenced the yield and chemical composition of the volatile oils (Mostafa *et al.*, 2004; Younis *et al.*, 2009). It was obvious from the results that oil yield (%) extracted by supercritical fluid extraction (SCFE) was the highest among the yields obtained from other extraction methods. Concrete and absolute oils were 0.18% and 0.008% respectively, when extraction was carried out with supercritical fluid extraction whereas, solvent extraction gave 0.16% concrete and 0.0048% absolute oil and 0.09% concrete and 0.002% absolute oil was produced from steam distillation. These results are in contrast with the findings of Raina *et al*. (2006) who extracted 0.05% of oil from the flowers of *M. exotica* and EL-Sakhawy *et al*. (1998) where oil yield of 0.02% was obtained while using the hydro-distillation method.

It was observed that the lowest yields of the oil were obtained from steam distillation which can be the effect of long distillation time, and high temperature of steam that cause the loss of volatile compounds, degradation or chemical modification of essential oil constituents. The high temperature effect of steam on low yields of essential oils was also reported by Assis *et al.* (2000). Oil yield was found better in solvent extraction compared to steam distillation that can be the result of high diffusivity of the solvent than steam. The lower yields than SCFE might be due to the long extraction times and thermal degradation of chemical constituents as extraction happens at high boiling points (Wang, 2000). Secondly, the main drawback of this method is the use of organic solvents in large amounts that causes the contamination of the extracts and can be dangerous, as solvent can cause harmful effects to environment and human health. Therefore, oils obtained through solvent extraction are restricted in different industries like pharmaceutical, food and cosmetic industries (Yeddes *et al.*, 2012).

Supercritical fluid extraction was found superior than the other conventional methods in extracting good yield of the oil. It was found that SCFE have many advantages like shorter extraction time, no thermal degradation of chemical constituents, high percentage of active ingredients and higher yield etc. Therefore, SCFE can be considered as the best possible method for obtaining *M. exotica* essential with quality and quantity and it is in line with the work of Mostafa *et al*. (2004) and Biljana *et al*. (2005).

Gas-Chromatography-Mass-Spectrometery (GC-MS) results indicated that total of 41, 46 and 49 components were identified from the oils extracted through supercritical fluid extraction (SCFE), solvent extraction and steam distillation methods respectively. These results are closely related to the findings of El-Sakhawy *et al*. (1998) where 44 components were identified and are in contrast to the observations of Raina *et al*. (2006) who found 56 and 72 components from the essential oils of leaves and flowers of *M. exotica*. It was observed that the composition of the major constituents extracted from various methods was similar, whereas the difference was in the relative concentrations of the identified compounds contained in the essential oil of the *M. exotica*. Moreover, manool, E-nerolidol, germacrene D, benzyl benzoate, palmitic acid, methyl palmitate δ-Cadinene and anthranilic acid, were identified as the major components.

The variability in *M. exotica* essential oil's composition reported by various researchers was observed compared with our results and it is because of the fact that the composition of essential oils are significantly influenced by the locality, plant population and the type of extraction method employed (Raina *et al*., 2006; Rout *et al*., 2010). More number of components with low percentages from the solvent extraction and steam distillation methods would be the result of long extraction time and high temperature that caused the thermal degradation and ultimately change in the chemical nature of the constituents (Assis *et al.*, 2000).

Higher percentages of the identified chemical constituents suggested no thermal degradation and more selectivity of the supercritical fluid extraction. It can be concluded that supercritical fluid extraction (SCFE) was best method for the extraction of good quality and quantity of essential oil from *M. exotica* flowers.

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