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Quantitative and Qualitative Analysis of Essential Oil of Arabian Jasmine (*Jasminum sambac*) Flowers Harvested from Pothohar Region of Pakistan

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Abstract

The essential oil extracted by n-Hexane solvent extraction and hydro-distillation of Arabian jasmine flowers collected from Pothohar region was analyzed by GC/MS. Concrete oil recovery on fresh petal weight basis was 1.78 g (0.17%) and absolute oil recovery from this concrete was 0.09%. Essential oil recovery in hydro-distillation was 0.08 g (0.008%). The colour of absolute oil was very light brown. The readings for specific gravity, congealing point of concrete and refractive index of essential oil were 0.89, 57°C and 1.37, respectively. Overall 35 compounds were identified. Main components (>5%) were benzyl benzoate (15.63%), jasmine (9.90%), linalool (8.58%), isophytol (7.56%), geranyl linalool (6.07%), phytol (5.75%) and palmitic acid (5.01%). Other constituents varied from less than 1% to 5%.

Keywords: GC-MS analysis, Jasmine, n-Hexane, Oil extraction, Pothohar.

INTRODUCTION

Essential oils are extensively used in cosmetics, pharmaceuticals, aerated water, confectionary, perfumery, disinfectants, ice-creams, sweets, candies, snacks, chewing gums, tobacco and a number of other related products (Skaria *et al*., 2007). An increasing popularity of essential oils and extracts of numerous flowers like rose, jasmine, tuberose, champa etc. can be viewed in high grade perfumes and aroma therapy (Sahoo, 2001). Since the middle ages, these oils had been widely used for bactericidal, virucidal, fungicidal, anti-parasitical, insecticidal, medicinal and cosmetic applications (Bakkali *et al*., 2008). The extraction of essential oils has remained one of the main purposes behind the cultivation of aromatic plants.

The genus *Jasminum* belongs to the Oleaceae family (the olive family) and comprises an important group of plants being used in the whole world for the extraction of its precious essential oil. The genus contains about 200 species which are mostly native to Southeast Asia and the Pacific into Australia and Africa (Heywood and Chant, 1982; Arnold, 1979; Anonymous, 1997). The flowers of Arabian jasmine are used to make jasmine tea (Fang *et al*. 2003), while the other plant parts - the flowers, leaves and roots are used in traditional medicines to combat a variety of ailments (Zhang *et al.,* 1995). Arabian jasmine is frost tender and is the most abundant plant of this genus in the country which is cultivated for making garlands and for its use in various kinds of social and religious gatherings. The plant is an evergreen climber which bears clusters of white flowers that change their shade to purplish as they fade. There are about 400,000 odorants which include both pleasant and unpleasant odors. Among these odorants *Jasmine* is a principal perfume with a beautiful scent containing more than 200 components ranging from pleasant jasmine lactone to extremely unpleasant indole (Mitsuri, 1997).

In Pakistan the total cost of imports of essential oils, perfumes and flavouring materials was Rs. 10158.35 million in the year 2003-04 (Pakistan Statistical Yearbook, 2010). Data about the exports of such materials are silent. The climate of Pakistan is favourable for the cultivation of jasmine plant. This is already under commercial cultivation in various parts of the country, but only for making garlands and wreaths and not for essential oil extraction. The present study was initiated to extract the essential oil from Arabian jasmine and its physico-chemical analyses for making further recommendations for growers and manufacturers.

MATERIALS AND METHODS

Plant materials

Fully open, fresh flowers were collected from Potohar region (33°36'N; 73°4'E) before sunrise to ensure the minimum loss of volatile aromatic substances with maximum oil recovery. The flowers were then shifted immediately to the laboratory for extraction of essential oil. Sepals, leaves and other undesirable plant parts were separated from the flowers to avoid contamination in the final product. Flowers were weighed after cleaning.

Extraction of essential oils

Essential oil extraction was carried out by two methods, solvent extraction and hydro-distillation. The extraction of oil in solvent extraction method was carried out using n-Hexane as organic solvent (hydrocarbon solvent) with Soxhlet apparatus. Essential oil was extracted in batches of 1000 g every time. Thimbles of Whatmann filter paper No. 40 were filled with flowers tightly and kept in jacket of Soxhlet apparatus, n-Hexane was added in the flask and boiled at a temperature of 55 to 60 oC. On boiling vaporized n-Hexane passed through flower-containing thimbles in the jacket and condensed by the condenser. The whole material came back in the flask after condensation. This process was repeated 8 to 10 times to ensure maximum collection of essential oil extract. When approximately the entire aroma of flowers was taken out by the solvent, the process of distillation was carried out in rotary evaporator leaving a resionid or concrete. As a result, concrete oil was obtained which included non-aromatic waxes, pigments and highly volatile aromatic molecules. This concrete required further processing to make it pure. High yield of jasmine essential oil can be best extracted from the fragile jasmine flowers by the solvent extraction method. Steam distillation is not suitable for the delicate jasmine flowers because the sheer amount of flowers needed to produce the essential oil of jasmine would make it one of the most expensive oil. In hydro-distillation the plant material was immersed in water in the still and heated to boiling point using external heat source. The boiling hot water drew out the oil. The vapours were allowed to condense and the oil was then separated from the aqueous phase (Houghton and Raman, 1998). This method produces a finer, more complete product, and shocks the plant material less (Ackerman, 2001). The left-over concrete and absolute oil, kept in tightly packed Falcon tubes and HPLC vials, were stored in refrigerator at 2-4°C to assure the minimum loss of volatile substances from the oil.

Color and specific gravity

Color of absolute oil was determined by using spectrophotometer. The instrument recorded transmittance measurement between 400 and 700 nm. Specific gravity acts as important criteria for the determination of quality and purity of an essential oil. The pre-weighed 10 ml specific gravity bottle was filled with absolute oil leaving no air bubbles, and then weighed. The density of oil was computed with the following formula.

Density = Weight / Volume

SG = Density of liquid at 20°C / Density of water at same temp.

Congealing point

In case of mixtures, such as essential oils, congealing point is determined instead of melting point. In its determination, the oil is super cooled so that upon congealation liberation of heat occurs with immediate crystallization (Gunkel *et al.*, 2010). For its determination about 10 ml of absolute oil was placed in a dry test tube of 20 mm diameter. This was cooled in water the temperature of which was about 5°C lower than the supposed congealing point of the essential oil. The inner walls of the tube were rubbed with a thermometer quickly up and down in the oil. The temperature was noted constantly. There was a rise in temperature which soon approached a constant value. That value was taken as congealing point of the oil. The process was repeated many times in order to ensure accuracy. The refractive index of the oil was determined at room temperature of 25°C by using Abbe's Refractometer.

Gas chromatography-mass spectrometry

Gas chromatography-mass spectrometry (GC-MS) analysis was performed by Agilent Technologies 6890N-5975B system. Carrier gas was Helium with a flow rate 1.0 mL/min, constant flow mode; injection volume 0.1 µL, inlet temperature 250°C; Agilent Technologies HP-5MS 30 m 0.25 µm column. Temperature program: 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. Acquired data were analyzed by Agilent Technologies MSD Chem Station software in conjunction with AMDIS (Automated Mass Spectral Deconvolution and Identification System) and NIST MS Search software. Two different mass spectra libraries were used for mass spectra identification: Wiley Registry of Mass Spectral Data 7th Edition (338000 spectra, 289000 unique compounds), NIST/EPA/NIH Mass Spectral Library 05 with 190825 spectra, 163198 unique compounds. The compound identification was finally confirmed by comparison of their relative retention indices with literature values (Adams, 1995; Adam, 2001; Mimica-Dukic *et al*., 2003; Rout *et al*., 2007; Vagionas *et al*., 2007; Grbović, *et al.,* 2010; NIST Standard Reference Data No. 69, 2011). Adams (1991) suggested that although a large library of mass spectra were readily available from reliable sources like NBS (National Bureau of Standards), but only the major components could be easily and unequivocally identified. However, since then large improvements have been made in the library of mass spectra. On-line available literature was also of consulted at large in this regard.

Statistical analyses

Each experiment was carried out in triplicate (except GC-MS analysis). The data were statistically analyzed using Statistix 8.1. A Least Significant Difference (LSD 0.05) was used to test the effects through a general linear model. The test was statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Physical properties of essential oil

Concrete oil recovery on fresh petal weight basis was 1.78 g. The percentage of this concrete oil recovery came to be 0.178%. Concrete contained components of essential oil, waxes and other materials which were then separated by special techniques to make it absolute. Absolute oil recovery on fresh petal weight basis came to be 0.094% which was almost half or slightly more than half from concrete oil. The essential oil obtained from hydro-distillation was almost pure and was not subjected to any other process for purification. Thus, the quantity obtained was less as compared to solvent extraction. The essential oil recovery was 0.088 g (Table 1).

Table 1. Physical characteristics of essential oil of Arabian jasmine

Physical Characteristics	Reading
Specific gravity	0.8950 at 30°C ± 0.0001
Congealing point of concrete	57°C ± 1.00
Refractive index	1.375 ± 0.002

The findings support the results of Younis *et al*. (2011) who observed about half absolute oil recovery from the concrete of *J. sambac*. The results are also in confirmatory with those of Weiss (1997) who reported the concrete yield of 0.17% for *J. sambac*. The results of present study do not support the findings of Waheed-ur-Rehman (2006) who reported much higher percentage of 0.30% of absolute oil recovery in Arabian jasmine from n-Hexane solvent extraction and 0.23% absolute oil recovery when methyl alcohol was used for oil extraction. However, he also recorded low essential oil recovery of 0.207% by hydro-distillation as compared to solvent extraction. Researchers have gained quite variable results with respect to oil production. This is so because many factors may affect the oil yield from flowers. These factors include the variety itself, topography, time of harvest, month of harvest, skill of labor engaged in harvesting of flowers, climatic conditions, cultural practices and administration control over the whole process of extraction.

The color of absolute oil of Arabian jasmine was very light brown. The readings for specific gravity of essential oil were taken. The results showed that the specific gravity of Arabian jasmine was 0.89 at 30°C. The results for observations on congealing point of the concrete oil revealed that it was 57°C. The refractive index of essential oil was 1.375 (Table 2).

Table 2. Essential oil yield of Arabian jasmine from solvent extraction (SE) and hydro-distillation (HD)

No.	Quantity of petals (g)	Concrete oil yield (g) from SE	% of concrete yield from SE	Absolute oil yield (g) from SE	% of absolute yield from SE	Absolute oil yield (g) from HD	% of absolute yield from HD
1	1000	1.80	0.180	0.918	0.0918	0.090	0.009
2	1000	1.77	0.177	0.938	0.0938	0.088	0.0088
3	1000	1.79	0.179	0.984	0.0984	0.087	0.0087
Total	3000	5.36	0.536	2.840	0.2840	0.265	0.0268
Average	1000	1.786	0.178	0.946	0.0946	0.088	0.0088

Guenther (1952) reported the colour of jasmine absolute as a clear yellow-brown liquid. Our results are more or less similar to those who have already reported their findings. Weiss (1997) reported the colour of concrete of *J. sambac* to be deep red. Younis *et al.* (2011) reported the colour of oil of *J. sambac* to be off-whitish yellow. Guenther (1952) reported specific gravity at 15°C (without giving the name of jasmine variety) to be 0.93 to 0.97; Weiss (1997) reported specific gravity of concrete of *J. sambac* as 0.8794 at 30°C; Younis *et al.* (2011) reported specific gravity of absolute of *J. sambac* to be 0.9850 at 20°C. Thus, it can be seen that the results of various researchers differ from one another to some extent. Varying results have been reported for the determination of congealing point which vary from one another to some extent, and vary to large extent for concrete and absolute. The congealing point of concrete is always higher because it contains waxes and other odourless substances. Congealing point of absolute of *J. samba*c was 22°C and 17.25°C as reported by Rehman (2006) and Younis *et al*. (2011), respectively. Measurements of refractive index showed that this index for *J. sambac* was 1.375. Guenther (1952) observed refractive index of jasmine to be 1.4822 at 2 °C; Weiss (1997) reported refractive index of concrete of J. sambac to be 1.4665, and Rehman (2006) observed this for absolute of *J. sambac* to be 1.4192 at 20°C. The varying results may be seen in the context of different climatic and geographical conditions as concluded by early research workers.

Chemical properties of essential oil

Peaks obtained as a result of chemical analysis of essential oil of Arabian jasmine were identified separately. Chemical constituents were determined by GC-MS analysis. The chromatogram has been presented in Fig. 1. The results (Table 3) revealed that the main components of *J. sambac* (> 5%) were benzyl benzoate (15.63%), jasmine (9.908%), linalool (8.58%), isophytol (7.56%), geranyl linalool (6.07%), phytol (5.75%) and palmitic acid (5.01%). Other constituents varied from less than 1% to 5% (Table 3).

The jasmonate family consists of cis-jasmone (CJ), jasmonic acid (JA), and methyl jasmonate (MJ) which are fatty acid-derived cyclo pentanones and were first isolated from the jasmine plant. They are a class of plant stress hormones which are similar to the salicylates. Jasmonates suppress the proliferation of various cancer cells and induce their death (Cohen and Flescher, 2009). When categorized according to percentage ranges, the compounds with <1%, between 1 to 5% and more than 5% were 16, 10 and 9, respectively.

Weiss (1997) also found the major constituents of isophytol (7.41 to 8.37), phytol (9.17 to 24.76%) and linalool (5.74 to 6.54%). Other major compounds reported by him as benzyl acetate and phytol acetate could not be found in our results. Waheed-ur-Rehman (2006) also identified linalool as a major component of *J. sambac*. Younis *et al*. (2011) found geraniol as a major component in absolute of *J. sambac.* The components, the percentage range of which was 1-5%, were

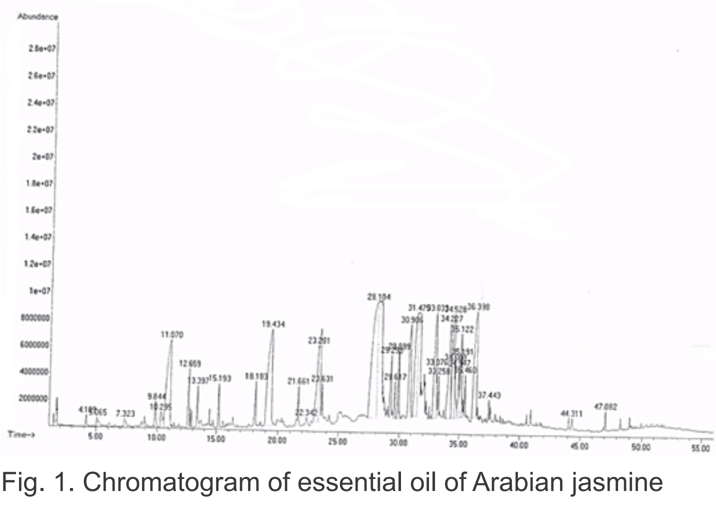

Fig. 1. Chromatogram of essential oil of Arabian jasmine

Table 3. Chemical constituents of essential oil of Arabian jasmine as identified by GC- MS.

No.	Retention time (min)	Compound	Common name	%
1.	4.18	5,9-Dodecadien-2-one, 6,10-dimethyl (E.E)		0.34
2.	5.06	Ethanaone , 1-[2-(1-hydroxyl-1-methylethyl) cyclopropyl]	Novel N-(Aryl-,aryloxy-,arylthio-,aryl-sulfinyl-and arylsulfonyl-) alkyl-N,N'-(or N',N') alkylaminoalkyl ureas, thio ureas and cyanoguanidines	0.54
3.	7.32	5-Hepten-2-one, 6-methyl	Methyl heptenone	0.37
4.	9.84	Alpha,-methyl-alpha,-[4-methyl-3-pentenyl]oxiraneme thanol	Linalool, epoxydihydro	0.90
5.	10.29	-do-		0.82
6.	11.07	1,6-octadien-3-ol,3,7 dimethyl	Linalool	8.58
7.	12.65	Acetic acid, phenyl methyl ester	Acetic acid, phenyl methyl ester	1.72
8.	13.39	3-cyclohexene-1-methanol,alpha,alpha trimethyl	Terpineol	1.08
9.	15.19	2,6-octadien-1-ol, 3,7 dimethyl(E)	Geraniol	1.09
10.	18.18	Eugenol	Eugenol	1.19
11.	19.43	2-cyclopenten-1-one, 3 –methyl-2- (pentenyl)z	Jasmone	9.90
12.	21.66	Alpha- farnesene	Alpha-farnesene	0.85
13.	22.34	9,12-octadecadienoyl chloride z	Linoleic acid chloride	0.33
14.	23.28	1,6,10 dodecatrien-3-ol 3,7,11-trimethyl	Nerolidol	4.19
15.	23.63	E-2 hexenyl benzoate		0.82
16.	28.10	Benzyl benzoate	Benzyl benzoate	15.63
17.	29.29	2-pentadecanone 6,10,14-trimethyl	Per hydro farnesyl acetone	1.81
18.	29.61	Benzoic acid, 2- phenylethyl ester	Phenylethyl benzoate	0.88
19.	29.89	Benzoic acid, 2-hydroxy, phenyl methyl ester	Salicylic acid	1.55
20.	30.90	Hexadecanoic acid, methyl ester	Palmitic acid	5.01
21.	31.47	Isophytol	Isophytol	7.57
22.	33.03	1,6,10,14-Hexadecatetraen-3-ol, 3,7,11,15 tetra methyl E	Geranyl linalool	6.07
23.	33.07	Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3 yl ester		0.44
24.	33.25	-do-		
25.	34.22	9,12,15 Octadecatrienoic acid, methyl ester ZZZ	Methyl linolenate	0.64
26.	34.52	Phytol	Phytol	5.83
27.	34.60	Heptadecanoic acid,16 methyl-methyl ester	Methyl isostearate	5.75
28.	34.94	Cholestan-3-ol, 2-methylene (3 beta 5 alpha)		1.53
				1.64
29.	35.12	3,7,11,15 tetramethyl-2-hexadecen-1-ol	Phytol	2.88
30.	35.19	Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3 yl ester		0.55
31.	35.46	22-tricosenoic acid	22-tricosenoic acid	0.85
32.	36.39	3,7,11,15 tetramethyl-2-hexadecen-1-ol	Phytol	7.43
33.	37.44	Heptacosane	Heptacosane	0.36
34.	44.31	Pregn-5-en-20-one,3(acetyloxy)-16,17-epoxy-6-mrthyl (3 beta 6 alpha)		0.32
35.	47.08	2,6,10,14,18,22-tetracasahexaene, 2,6,10,15,19,23-hexamethyl(E)		0.37
Total				99.99

considered as minor components. The minor components observed in *J. sambac* were acetic acid phenyl methyl ester (1.72%), geraniol (1.09%), eugenol (1.19%), nerolidol (4.19%), per hydro farnesyl acetate (1.81%), salicylic acid(1.55%) , methyl isostearate (1.53%) and phytol (2.88%). Similarly, findings of other researchers have given variable results. For example, linalool has been found a major component in the present study with more than 5% share, but Waheed-ur-Rehman (2006) has reported this to be 4.13% in *J. sambac*. Edris *et al.* (2008) studied the composition of the volatile fraction of Egyptian *J. sambac* (L.) flowers picked in July using GC/MS and found the main volatile constituents of the concrete headspace and the absolute as benzyl acetate (23.7 and 14.2%), indole (13.1 and 13.4%), E-E-α-farnesene (15.9 and 13.1%), Z-3-hexenyl benzoate (4.9 and 9.4%), benzyl alcohol (7.7 and 8.4%), linalool (10.6 and 6.3%), and methyl anthranilate (5.0 and 4.7%). Eugenol which is a minor component in our results was a major component of *J. sambac* as reported by Younis *et al.* (2011). The compounds having less than 1% value were designated as trace components. Some of these components in *J. sambac* were methyl heptenone, alpha farnesene and heptacosane. Some components could not be identified by their common names. However, their chemical names have been given. Similarly, sometimes two compounds have been identified by the same name. Some components could not be identified by their common names. However, their chemical names have been given. Variable results have been reported by different researchers which could be attributed to the variety chosen, method of essential oil extraction, analysis techniques, identification of compounds, application of fertilizers and other inputs to the soil and other cultural practices. Retention time for essential oil of *J. sambac* was about 47 minutes. It means that if the run time of GC-MS apparatus is about 50 minutes, then maximum compounds of the mixture of essential oil of *J. sambac* can be identified. However, the more pure the oil is, the more number of compounds will be identified.

The findings of present study suggest that the production of essential oil of jasmine can be of high value in future for fulfilling domestic and foreign demands, because the consumption of aromatic substances is on increase. The world production of aromatic and medicinal plants' raw material is likely to touch $ 5 trillion by 2050 (Skaria *et al*. 2007). In this scenario, sincere and sound efforts of researchers and industrialists can pave the way of establishment of essential oil industry in the country. Jasmine oil is of particular importance in the whole family of essential oils. The flowers are light in weight as compared to other flowers. Thus, a large quantity of flowers yields a low percentage of high quality and high valued essential oil. Other plant parts also exhibit many medicinal properties. Thus, the whole plant can be used for human use in various forms. It seems meaningless to perceive that its cultivation for the purpose of essential oil extraction would involve some financial risks. Many countries are actively engaged in cultivation of jasmine and other aromatic plants for the ultimate use of their oil in medicine, cosmetics, perfumery etc. In the present study two methods were used for the extraction of essential oil from jasmine flowers. Hydro-distillation is still being used as the principal method for the preparation of essential oils. This is a cheap method and does not involve costly apparatus. The units of hydro-distillation can be set up in close proximity to the production fields.

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