

PHYSICO-CHEMICAL ANALYSIS AND DETERMINATION OF VARIOUS CHEMICAL CONSTITUENTS OF ESSENTIAL OIL IN *ROSA CENTIFOLIA*

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Abstract

This paper reports the isolation and characterization of essential oil of *Rosa centifolia*. The oil was extracted from freshly collected flower petals of *R. centifolia* with petroleum ether using Soxhlet apparatus. *Rosa centifolia* showed 0.225% concrete oil and 0.128% yield of absolute oil. Some physico-chemical properties of the extracted oil like colour, refractive index, congealing point, optical rotation, specific gravity, acid number and ester number were determined and were found to be yellowish brown, 1.454 at 34°C, 15°C, -32.50 to +54.21, 0.823 at 30°C, 14.10245 and 28.1876. High-resolution gas liquid chromatographic (HR-GLC) analysis of the essential oil of *R. centifolia*, showed the phenethyl alcohol, geranyl acetate, geraniol and linalool as major components whereas, benzyl alcohol, benzaldehyde and citronellyl acetate were detected at trace levels.

Introduction

A variety of roses are generally cultivated for home and garden beautification in many parts of the world. Its rich fragrance has perfumed human history for generations (Rose, 1999). Roses contain very little essential oil but it has a wide range of applications in many industries for the scenting and flavouring purposes. It is used as perfumer in soap and cosmetics and as a flavor in tea and liquors. Rose oil is also used in creams, lotions and soaps for its mild anti-viral and bactericidal properties as well as for its fragrance (Nenov *et al.*, 1995). Rose oil is cooling, relaxing and toning. Its effects are similar to Bergamot, Geranium and Jasmine, decreasing sympathetic nervous system activity, while at the same time strengthening the parasympathetic nervous system. Rose oil therefore increases feeling of vitality and creating a sense of well-being (Sheppard-Hanger, 1995). It is also considered as an asthma remedy (Berkowsky & Bruce, 2000).

At present different extraction techniques like distillation, effleurage, CO₂ extraction, expression and solvent extraction are applied for oil extraction from rose. But commonly steam distillation and solvent extraction methods are used (Boutekedjiret *et al.*, 2003). Rose essential oil comprises a number of different types of complex constituents. So their separation and analysis is performed by gas chromatography. During the last few years, gas chromatography has been established as a fast efficient and relatively simple technique for separation and analysis of mixture of volatile substances and is being extensively used by the perfumers (Lin *et al.*, 2003).

Rosa centifolia, a native of Asia and Middle East, belongs to botanical group Chinesis. This rose cultivar has medium sized flowers which are rich in fragrance. Rose oil extracted from *R. centifolia* is antiseptic, balancing, antidepressant, astringent, digestive, anti-inflammatory and aphrodisiac (Allardice, 1994). As a part of our systematic studies of essential oils of rose, the present study was aimed to extract

essential oil of *R. centifolia* by solvent extraction method and investigate its physico-chemical characteristics and constituents profile.

Materials and Methods

Collection and preparation of samples: *R. centifolia* flowers were collected from the Experimental Floriculture Area, University of Agriculture, Faisalabad in paper boxes. Flowers were weighed before and after the removal of unwanted material (sepals, pollens and anthers) and petals were kept under shade at room temperature for the removal of extra moisture and were then subjected to oil extraction.

Extraction of oil: For the extraction of essential oil from *R. centifolia*, solvent extraction was performed and concrete & absolute oils were obtained. 20kg flowers of the cultivar *R. centifolia* were used for the extraction of oil. The extraction process was carried out using petroleum ether as organic solvent. When the entire aroma was taken out by solvent, then the process of distillation was carried out. The apparatus to be used in each process was thoroughly washed and dried. Distillation was done in rotary evaporator.

Concrete oil recovery: Dissolved organic residue in the petroleum ether was collected in a flask and dried over by adding anhydrous NaSO₄. The last traces of petroleum ether were removed by bubbling nitrogen gas through the oil. Concrete oil was taken in pre-weighed 100ml flask and the weight of concrete oil was determined by again weighing the flask. Percentage yield of concrete oil was calculated on the basis of petal weight.

Absolute oil recovery: Concrete oil was dissolved in minimum volume of absolute alcohol to remove the natural waxes present in the essential oil and was then filtered through a Whatmann filter paper # 43. Alcohol was removed by distillation and by passing nitrogen gas through the oil. Percent yield of absolute oil was also calculated on the basis of petal weight.

Physico-chemical analysis: Physical and chemical properties such as colour, refractive index, specific gravity, congealing point, optical rotation, acid number and ester number were observed. Colour of absolute oil was noted from physical appearance. Refractive index of the oil was determined at 34°C using Abbe's refractometer. For congealing point determination, a small amount of oil was placed in a capillary tube which was then suspended inside a large tube along with a standard thermometer. Cooled the inner and outer tube together in water to a temperature 5°C below the expected congealing point, gently stirred the liquid until it started to solidify. The highest temperature recorded during solidification was considered as the congealing point. The specific gravity of the oil was determined by using the specific gravity bottle. In case of optical rotation determination, 10ml polarimeter tube containing oil was placed in the trough of the instrument between polarizer and analyzer. Care was taken in filling the tube to avoid the entrance of air bubble which could disturb the rotation of light. Analyzer was slowly turned until both the halves of the field were viewed through the telescope. The direction of rotation was determined, if the analyzer was turned counter clock wise from the zero position to obtain the final reading, the rotation is levo (-) if clock wise and dextro (+) if anti clockwise.

For the determination of acid number, 1.5 gm of absolute oil was weighed accurately into a 100 c.c. saponification flask. 15 c.c of neutral 95% alcohol and 3 drops of 1% phenolphthalein solution was added. Titration of the free acid with a slandered 0.1 normal aqueous solution of NaOH was done by adding the alkali drop wise at a uniform rate. The contents of the flask were continuously agitated. The first appearance of red colour was considered as an end point. Process was repeated 3 times to get mean value and the acid number was calculated.

Another sample of 1.5 gram of absolute oil was taken and same procedure was followed and 10 c.c of 0.5N alcoholic solution of NaOH was added. A glass air cooled condenser was attached to the flask and contents were refluxed for one hour on a water bath. The apparatus was removed and allowed to cool at room temperature for 15 minutes. Excess alkali was titrated against standardized 0.5N aqueous HCl and the ester contents were calculated.

All the experiments were carried out in triplicate and the data is reported as mean \pm standard deviation (SD) (Steel *et al.*, 1997). During all the experiments, calibrated glassware and chemicals of analytical grade were used.

Gas chromatographic analysis: Gas chromatographic analysis of absolute oil of *R. centifolia* was carried out using SHIMADZU Gas Chromatograph, Model 17A, equipped with FID. The other analysis conditions were as, column SP2330 (30m \times 0.32mm), initial oven temperature 50°C, ramp rate 5°C/minute, final oven temperature 150°C; initial holdup time was 3 minutes while final holdup time was 9 minutes. The initial Injector and detector temperature were 175 and 200°C respectively. Moisture free pure nitrogen at a flow rate of 5ml /minute was used as carrier gas. The constituents of essential oil of *R. centifolia* were identified by comparing their relative and absolute retention times with those of authentic standards. The quantification was done by a Chromatography Station for Windows (CSW32) data handling software (Data APEX LTD., The Czech Republic). The Essential Oil composition was reported as a relative percentage of the total peak area.

Results and Discussion

Concrete oil yield: The concrete oil recovered from 20 kg petals of *R. centifolia* showed a yield of 45.8 gm which is equivalent of 0.225% oil content on petal weight basis. This yield showed that *R. centifolia* had concrete oil yield comparable to that of *R. gallica* which has been reported as 0.2065% and 0.29% (Naves and Mazuyer, 1983; Nofal, *et al.*, 1982) while lesser than 0.6851% of *R. Gruss-an-teplitz* (Mumtaz, *et al.*, 2007).

Absolute oil yield: Absolute oil recovered from concrete oil of *R. centifolia* showed a yield of 0.128% on the petal weight basis. This yield was lesser than that of *R. gruss-an-teplitz*, which was 0.3425% (Mumtaz *et al.*, 2007).

Physical properties

Colour: Colour of the essential oil of *R. centifolia* was yellowish brown which was somewhat different to the colour of Rose oil which is pale yellow (Allen, 1991). However, this oil was similar to that reported from Soviet Union which is yellowish and yellowish brown (Lozzi, 1983; Hardy & Pollard, 1960).

Refractive index: Abbe's Refractometer was used to measure the refractive index (RI) of the essential oil of *R. centifolia*. The measured refractive index of *R. centifolia* oil was 1.454 at 34°C which is higher than 1.451 at 34°C of *R. Gruss-an-teplitz* (Mumtaz *et al.*, 2007).

Congealing point: The essential oil of *R. centifolia* was also subjected to congealing point analysis which was found to be 15°C. This value is little lesser than 15.9°C for the oil of *Rosa Gruss-an-teplitz* (John *et al.*, 1980; Poucher, 1974).

Optical rotation: The optical rotation of essential oil of *R. centifolia* was found to range from -32.50 to +54.21. These results are quite greater than the reported values of -1 to -5, -7 to +4 and -0.4 to -3 for the essential oils of different rose varieties (Poucher, 1974; John *et al.*, 1980; Martin, 1990).

Specific gravity: The specific gravity of the essential oil of *R. centifolia* was 0.823 at 30°C. These results are comparable with the early reported results of 0.809 to 0.810 at 30°C and 0.848 to 0.861 at 30°C (Allen, 1981; John *et al.*, 1980), while less than that of 0.953 to 0.986 at 20°C (Lozzi, 1983).

Chemical properties

Acid number: Acid number of the Essential oil of *R. centifolia* was calculated as 14.10245 which was greater than that of 11.59 for *R. Gruss-an-teplitz*¹¹ while much greater than 1.5-3.8 for Ku Shui Rose (Chen, *et al.*, 1985).

Ester number: Ester number of Essential oil of *R. centifolia* was found to be 28.1876 which was lesser than that of *R. gruss-an-teplitz* which is reported to be 29.92 (Mumtaz *et al.*, 2007) while greater than that of Ku Shui rose which is 3.7-17.5 (Chen, *et al.*, 1985).

Gas chromatographic analysis: Gas Chromatographic analysis was performed under some specific conditions and results thus obtained were analyzed statistically. The compounds are listed along with corresponding peak No. and their percent constituents (Table 4). The amount of Geraniol (peak 6) was 10.5% which was lesser than the amount reported in *R. bourbonica* i.e., 12.7% (Nigam *et al.*, 1959) and greater than 3.6% in *R. gruss-an-teplitz* (Mumtaz. *et al.*, 2007). Peak 7 refers to Linalool showing an amount of 6.9%, which is greater than 0.222% Linalool for *R. centifolia* (Mumtaz *et al.*, 2007) but is lesser than 12.682% for *R. abyssinica* (Eckart, 1991; Al-Rehaily *et al.*, 2003).

Another important constituent, phenyl ethyl alcohol (peak 8) was also found in the oil the amount of which was 43%. This amount was much greater than 4.23% found in *R. rugosa* and 17.983% for *R. abyssinica* (Eckart, 1991; Al-Rehaily *et al.*, 2003). Benzaldehyde (peak 9) with an amount of 1.5% was also found to be present in the oil, which had not been reported by any worker reviewed in this manuscript. Citronellyl acetate (peak 10) was another important constituent of the oil, whose concentration was 0.3% which is smaller than 0.4% of *R. gruss-an-teplitz* (Mumtaz *et al.*, 2007). 3.3% Benzyl alcohol (peak 11) was also present in low amount and has not been reported previously by any worker. Another important constituent Geranyl acetate (peak 12) was also found in the oil. Its amount was 15.6% which is greater than 9.5% of *R. gruss-an-teplitz* (Mumtaz *et al.*, 2007). Peaks from 1 to 7 remain unidentified due to the unavailability of pure standards.

Table 1. Yield of essential oil of *Rosa centifolia*.

Parameters	Mean values \pm S.D
Concrete oil yield (%)	0.225 \pm 0.013
Absolute oil yield (%)	0.128 \pm 0.010

Table 2. Physico-chemical constants of essential oil of *Rosa centifolia*.

Sr. No.	Parameters	Mean Values \pm S.D
1.	Colour	Yellowish brown
2.	Refractive index (34°C)	1.454 \pm 0.46
3.	Congealing point (°C)	15 \pm 0.12
4.	Optical rotation	-32 \pm 0.76 to + 54.21 \pm 0.93
5.	Specific gravity (30°C)	0.823 \pm 0.045
6.	Acid number	14.10245 \pm 0.436
7.	Ester number	28.1876 \pm 0.781

Table 3. Chemical constituents of essential oil of *Rosa centifolia* as revealed by Gas Chromatography.

Sr. No.	Retention time (Min)	Chemical constituents	Percentage composition (%)
1.	4.900	Unidentified	3.92 \pm 0.180
2.	4.580	Unidentified	2.80 \pm 0.120
3.	5.660	Unidentified	0.30 \pm 0.015
4.	6.370	Unidentified	1.10 \pm 0.065
5.	8.190	Unidentified	8.80 \pm 0.290
6.	21.620	Geraniol	10.5 \pm 0.380
7.	22.531	Linalool	6.90 \pm 0.270
8.	24.110	Phenylethyl alcohol	43.0 \pm 0.870
9.	24.760	Benzaldehyde	1.50 \pm 0.078
10.	25.922	Citronellyl acetate	0.30 \pm 0.015
11.	26.590	Benzyl alcohol	3.30 \pm 0.160
12.	28.710	Geranyl acetate	15.6 \pm 0.480
13.	30.120	Unidentified	2.00 \pm 0.097

The values given are mean of three readings. \pm indicates the standard deviation from the mean.

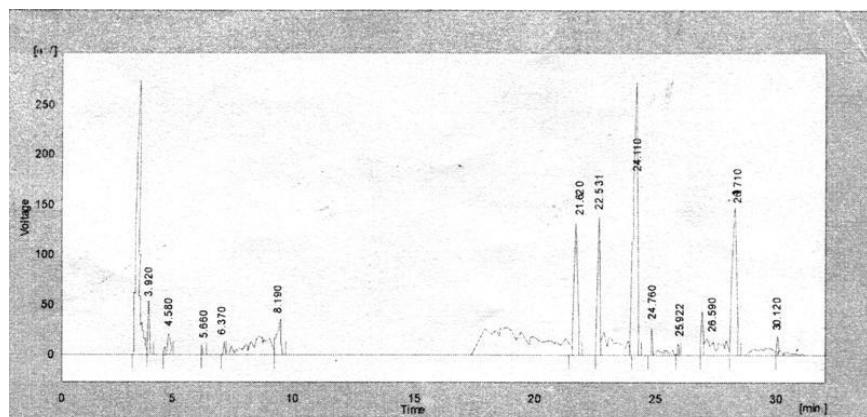


Fig. 1. Gas chromatogram of essential oil of *Rosa centifolia*.

Conclusion

These results indicated that *R. centifolia* contains important chemical constituents and is suitable for the extraction of essential oil for medicinal and perfumery purposes on commercial scale.

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