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# Study on the Effect of Dual Solvent Proportions on Composition of *Rosa x damascena* Concrete Oil Obtained using Soxhlet Extraction Method

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Concrete oil was extracted from *Rosa x damascena* using different percentage ratios of solvents (petroleum ether and ethanol) by the Soxhlet extraction method. The extraction was carried out using petroleum ether and ethanol in five different percentage ratios of (v/v) (100:0, 75:25, 50:50, 25:75, 0:100) (petroleum ether:ethanol). The rotary vacuum evaporator was used to separate concrete oil and the solvents. The extracted concrete oil was analyzed using gas chromatography-mass spectrometry (GC-MS) technique. The obtained results show that many new compounds were identified, at two different solvents and its ratios. Phenylethyl alcohol in the percentages of (61.71%), (10.07%) and (25.92%) was obtained as a major compound with the solvent percentages of (100:0), (50:50) and (75:25) (PE:E), respectively. Hexacosane (37.2%) was identified as a major compound when pure ethanol is used as a solvent. The highest number of components were identified (totally 93 components) when an equal percentage (50:50) of petroleum ether and ethanol were mixed. The usual monoterpenes components, *e.g.* geraniol, nerol, citronellol and linalool, were not found in the present extraction study. This study concludes that the compositions of concrete oil were mainly influenced by the type of solvents and its ratios used for the extraction.

**Keywords: *Rosa x damascena*, Concrete oil, Dual solvent, GC-MS.**

## INTRODUCTION

Various products obtained from *Rosa x damascena* have broad applications in aroma, medicinal, food, cosmetics industries, *etc*. [1,2]. The components of *Rosa x damascena* products vary concerning the climate condition, soil condition and extraction methods [3,4]. Compared to the other parts of the *Rosa x damascena*, the flower parts contains several fragrance compounds and a mixtures of organic compounds with wide applications [5]. The chemical compounds obtained from rose flowers in concrete, absolute, rose water and rose oil have a great demand in the world market [6]. It is also one of the costly oils; (liquid gold) to get one kilogram oil, three thousand kilograms of petals have been used [6-8]. Therefore, the method of obtaining products should balance both cost and quality. The *Rosa x damascena* is famous in Damascus, capital of Syria, Bulgaria [3], Iran [8-11], Turkey [2,12], with significant contribution from France and India [9,13,14]. In India, especially in Tamil Nadu, farmers started to cultivate rose plants because of their Indian medical system usage like anti-agent for the virus, bacterial, diabetic, ageing, hiatus, cancer, HIV, depressant, inflammatory, *etc*. [1]. Further use in mild laxative, sedative, cardiac disease, eye problems, gall stones, the main symptom of covid-19, *i.e.* sore throat treatment, blood cholesterol, antioxidant [15], *etc*. It is also used as a vehicle for other medicines [16].

The rose products were obtained by different methods [17], traditionally distillation [18], extraction [19] and Clevenger's apparatus were used [3-5]. Several modern methods were also proposed such as super critical fluid extraction [6,7,9], hydrodistillation [11-15], microwave [3], headspace techniques [17, 18], membrane process [4], direct thermal desorption, superheated water extraction [20], *etc*. Solvent extraction using an organic solvent is one of the effective method to obtain the natural products, because of the higher yield [2]. The efficiency of extraction mainly depends on the type of solvent used [6]. The oil produced by the solvent extraction is known as concrete or absolute, which cannot be used directly in cosmetic and perfumery products [7,9,10]. The concrete has many chemical compositions it can be convert to absolute for further usage.

Both polar and non-polar solvents have been used to extract components from *Rosa x damascena*. For the extraction of aroma compounds, alcohols are used as a solvent in the fragrance industry. Many other solvents have also been used like *n*-hexane [12,13], petroleum ether [7], diethyl ether, chloroform, cyclohexane [7], dichloromethane [9], *etc*. Hexane or petroleum ether is a suitable solvent used in many processes due to its non-polar nature. The solvents of two or more are also added to get more effective extraction like *n*-hexane and ethanol [4].

In current study, the mainly focusing on the influence of the type of solvent and its proportions in the constituents of the rose products. *Rosa x damascena* mixed solvent extraction was carried out in the Soxhlet apparatus using two different solvent petroleum ether and ethanol. The reflux and temperature conditions are the main advantage of Soxhlet apparatus. The solvent which are chosen for the current study has a different polarity in nature. The combined effect may give a new type of compound. To the best of the our knowledge, the literature has not reported any performance data on mixed solvents for *Rosa x damascena*. In the present study, solvents are taken at five different percentage ratios (v/v) (100:0, 75:25, 50:50, 25:75, 0:100) (petroleum ether:ethanol) and placed along with the rose petals in the Soxhlet extractor. The Soxhlet apparatus is run for all five percentage ratios and the oil is concentrated in a rotary vacuum evaporator (Heidolph). It is analyzed in GC-MS (Shimadzu QP2010-Plus), the components were then identified and reported. The GC-MS results are helpful in identifying some new types of components, which are different from those already reported in literature.

## EXPERIMENTAL

The raw material used for this investigation is *Rosa x damascena* obtained from Tuticorin, located in Southern India. The rose samples contain a mixture of local varieties. After collecting the flowers, the petals are segregated from sepals and air-dried for 24 h to remove extra moisture. The dried petals are directly used for the extraction using the desig-nated solvents.

**Solvents:** Analytical grade solvents are used in this study. Ethanol is purchased from M/s Rasayan Trading Co, Chennai, India and petroleum ether is sourced from M/s Siscon India Pvt Ltd, Chennai, India, they are used without any purification.

**Soxhlet extraction:** First, 200 mL of the extracting solvent (petroleum ether/ethanol/mixture of petroleum ether and ethanol) was taken in the round-bottomed flask of the Soxhlet apparatus. Different percentage ratios of the solvents were taken, 50:50 (100 mL of ethanol and 100 mL of petroleum ether), 25:75 (50 mL of ethanol and 150 mL of petroleum ether), 75:25 (150 mL ethanol and 50 mL of petroleum ether), 100:0 (100 mL of petroleum ether and 0 mL of ethanol) and 0:100 (0 mL of petroleum ether and 100 mL of ethanol) for the extraction. The rose petals (15 g) were taken in a filter paper and inserted in the Soxhlet apparatus thimble and then the Soxhlet was attached to the condenser. When the extraction chamber gets filled, the vapour solvent was removed by the siphon arm and the liquid solvent reaches the boiling flask by gravity. This is considered a single-stage process. This process continues for 2 h and after 2 h, the solvents were removed using a rotary vacuum evaporator and added to the solvent bottles [9].

**Rotary vacuum evaporator:** Heidolph rotary vacuum evaporator was used to separate the solvents. Initially, the water bath was heated to reach the solvent boiling temperature. The water bath temperature was measured and maintained near the solvent's boiling point and the condenser temperature reaches 15 ºC. The collected rose extract was placed in a round-bottomed flask. When the solvent reached the boiling temperature, the round-bottomed flask with the mixture was fixed to the rotor part. Then the rotor attached flask was moved towards the water bath and the speed of the rotation was fixed and it begins to rotate. The solvent gets vaporized, condensed and was collected in another round-bottomed flask. The obtained concrete oil was analyzed in GC-MS.

**GC-MS analysis:** Shimadzu QP2010-Plus was used for the GC-MS analysis. The samples were analyzed using HP-5 MS capillary column operating at an initial temperature of 65 ºC for 2-3 min and later at 225 ºC. The length of the column was 30 cm and the internal diameter was 0.25 cm. Helium was used as carrier gas with a flow rate of 1 mL/min, with a flame ionization detector (FID). Based on the retention time, different species were identified and the area of the peaks was used to estimate the relative proportions.

## RESULTS AND DISCUSSION

**Total components:** The chemical compositions of *Rosa x damascena* were identified from the GC-MS analysis using Soxhlet apparatus at five different solvent percentage ratios (v/v) and the results are reported in Table-1. GC-MS spectra (Fig. 1) shows the five different solvent percentage ratios (v/v) (100:0, 75:25, 50:50, 25:75, 0:100) (petroleum ether:ethanol), respectively. It clearly shows that the *Rosa x damascena* chemical components vary depending on the type of solvents used and its proportion of mixing. A total of 47, 56, 93, 61 and 63 components were identified at five different solvent percentage ratios (100:0, 75:25, 50:50, 25:75, 0:100) (petroleum ether:ethanol), respectively. The highest number of components, 93, was obtained with the solvents petroleum ether and ethanol mixed in equal percentage ratio (50:50). The identified components using five different solvent percentages are given in Table-1, along with their retention time values.

**Major components:** The major components identified are presented in Fig. 2. The highest percentage of phenylethyl alcohol (61.71%) is obtained when petroleum ether alone was used as a solvent. These results were in agreement with the reported results of an earlier study [14,16], which used other non-polar solvents like dichloromethane, hexane and benzene. The percentage of phenylethyl alcohol decreases when the quantity of petroleum ether is decreased, especially when ethanol content was higher in the solvent mixture, the percentage of phenylethyl alcohol is minimum (4.35%). Hexacosane (37.2%) and heneicosane (15.99%) were the major components when pure ethanol was used as a solvent.

The components hexacosane and heneicosane were not identified when petroleum ether portion dominates the solvent

TABLE-1
CHEMICAL COMPOSITION OF *Rosa x damascena* FOR FIVE DIFFERENT SOLVENT RATIOS

| RT | Name of the component | % |
|---|---|---|
| | PE:ETH (100:0) | |
| 10.46 | Phenylethyl alcohol | 61.71 |
| 3.057 | *tert*-Butyl dimethyl silane | 9.59 |
| 3.37 | Silane | 3.48 |
| 30.49 | 13-(1-Phenylethylimino)methyl)tricyclo(8.2 | 2.04 |
| 26.66 | 9-Octadecenoic acid (z)- methyl ester | 1.78 |
| 9.5 | Formic acid | 1.5 |
| 24.29 | Hexadecanoic acid, methyl ester | 1.27 |
| 29.59 | Propanoic acid | 1.02 |
| | Components which are less than 1% | 17.61 |
| | PE:ETH (25:75) | |
| 34.53 | Hexacosane | 27.31 |
| 26.6 | Heneicosane | 13.88 |
| 37.57 | Tetratetracontane | 8.84 |
| 10.46 | Phenylethyl alcohol | 8.55 |
| 35.26 | Octacosane | 5.25 |
| 13.5 | 5-Hydroxymethylfurfural | 4.37 |
| 13.1 | Acetic acid | 2.62 |
| 11.4 | 1,5-Anhydro-6-deoxyhexo-2,3-diul | 1.68 |
| 30.84 | Bicyclo[4.2.0]Octa-1,3,5-triene | 1.3 |
| 4.06 | 2-Furancarboxaldehyde | 1.22 |
| | Components which are less than 1% | 24.98 |
| | PE:ETH (75:25) | |
| 10.46 | Phenylethyl alcohol | 25.92 |
| 3.08 | Ammonium acetate | 8.6 |
| 3.06 | 2-Amino-1,3-propanediol | 8.03 |
| 13.13 | Acetic acid | 4.82 |
| 3.28 | *tert*-Butyl dimethyl silylamine | 3.62 |
| 9.5 | Formic acid | 3.51 |
| 3.414 | Methylbenzene | 2.93 |
| 3.37 | Silane | 2.8 |
| 11.32 | 4*H*-pyran-4-one 2 3-dihydro-3 5-dihydroxy | 2.76 |
| 5.39 | 2-Furanmethanol | 2.61 |
| 9.58 | 2,5-Anhydro-1,6-dideoxyhexo-3,4-D | 1.91 |
| 6.67 | 2(3*H*)-Furanone,-5-methyl- | 1.69 |
| 5.59 | 1,3-Cyclopentenedione | 1.59 |
| 13.4 | 1,2-Benzenediol | 1.58 |
| 4.135 | Propanoic acid | 1.32 |
| 4.63 | 2,5-Furandione | 1.31 |
| 16.06 | 2-(4'-Methoxyphenyl)-2-(2'methoxyphenyl)pr | 1.24 |
| 3.99 | 2,2'-Bioxirane | 1.11 |
| 6.36 | α,β-Crotonolactone | 1.07 |
| 20.07 | α-D-Glucopyranoside | 1.07 |
| 12.6 | 2*H*-Pyran | 1.04 |
| | Components which are less than 1% | 19.47 |
| | PE:ETH (50:50) | |
| 10.46 | Phenylethyl alcohol | 10.5 |
| 8.33 | Propenyl alcohol | 3.7 |
| 5.25 | 2-Furanmethanol | 3.66 |
| 3.02 | Urea | 3.52 |
| 11.28 | 4*H*-Pyran-4-one,3,5-dihydroxy-2-methyl | 3.38 |
| 3.37 | Silane | 3 |
| 6.57 | 2(3*H*)-Furanone, 5-methyl | 3 |
| 20.05 | β-D-Glucopyranoside, methyl | 2.8 |
| 10.14 | Diazene | 2.42 |
| 13.50 | 5-Hydroxymethylfurfural | 2.26 |
| 13.03 | Phosphonic acid | 2.17 |
| 16.03 | Androstane-3,17-diol,(3β,5β,17β | 2.1 |
| 5.57 | Protoanimonine | 1.99 |
| 3.93 | Propanoic acid | 1.91 |
| 6.27 | α,β-Crotonolactone | 1.89 |
| 1.65 | Cyclohexasiloxane | 1.65 |
| 9.49 | 2,5-Anhydro-1,6-dideoxyhexo-3,4-D | 1.53 |
| 17.73 | Xanthosine | 1.47 |
| 17.85 | 3-Cyclohexen-1-ol,1-methyl-4-(1-M | 1.47 |
| 20.64 | 1,6,10-Dodecatrien-3-ol,3,7,11-trime | 1.46 |
| 4.10 | 1-Alamine | 1.45 |
| 26.61 | 9-Octadecenoic acid | 1.42 |
| 4.62 | 1*H*-Imidazole | 1.36 |
| 8.62 | 3-Methylcyclopentane-1,2-dione | 1.34 |
| 21.02 | 1,3,4,5-Tetrahydroxycyclohexane | 1.27 |
| 19.67 | 1-Methyl-1-(3-methyl butyl)oxy-1-silacyclobut | 1.2 |
| 36.31 | Dotriacontane | 1.15 |
| 30.47 | 13-[1-Phenylethylimino)methyl]tricyclo[8,2., | 1.14 |
| 9.85 | 5-Ethyl-2-furaldehyde | 1.11 |
| 3.78 | Butane | 1.1 |
| 13.90 | Resorcinol | 1.07 |
| 14.50 | 3-(4-*tert*-Butylphenoxy)benzalde | 1.02 |
| 35.20 | Heneicosane | 1.01 |
| | Components which are less than 1% | 28.9 |
| | PE:ETH (0:100) | |
| 34.53 | Hexacosane | 37.2 |
| 26.6 | Heneicosane | 15.9 |
| 38.94 | Tetratetracontane | 8.02 |
| 13.5 | 5-Hydroxymethylfurfural | 7.05 |
| 38.52 | Tetracontane | 4.57 |
| 10.46 | Phenylethyl alcohol | 4.35 |
| 12.62 | 2*H*-Pyran | 2.43 |
| 23.89 | Nonadecane | 1.86 |
| 5.15 | Furanmethanol | 1.12 |
| 4.58 | 2-Furancarboxaldedyde | 1.1 |
| | Components which are less than 1% | 16.3 |

RT-Retention time; ETH-Ethanol; PE-Petroleum ether

mixture. The component 5-hydroxymethylfurfural was identified only with three proportions, *i.e.* 7.05%, 4.37% and 2.26% at 0:100, 25:75, 50:50 (PE:E) respectively. *tert*-Butyldimethyl silylamine of 9.59% and 3.62% concentration was identified at the solvent percentage ratio of 100:0, 75:25 (PE:E) when petroleum ether proportion dominates the solvent mixture. It indicates that the solvents used for extraction play an important role in deciding the components. Tetratetracontane (8.02% and 8.84%) was obtained as another component, especially when ethanol dominates as a solvent. The toxic component silane was identified (3-2%) especially when petroleum ether used as a dominant solvent. It decreases when the solvent proportions of petroleum ether were decreased, *i.e.* 3.48%, 2.8% and 0.27% at the solvent percentage ratios of 100:0, 75:25, 50:50 (PE:E), respectively. Several studies concluded that the presence of silane in the oil might due to the soil condition and climatic conditions of the area in which the flowers are cultivated. Urea (3.52%) was obtained as a new component at the equal percentage proportions of the solvent mixture (50:50) (PE:E). Tetracontane was obtained only when 100% of ethanol was used as

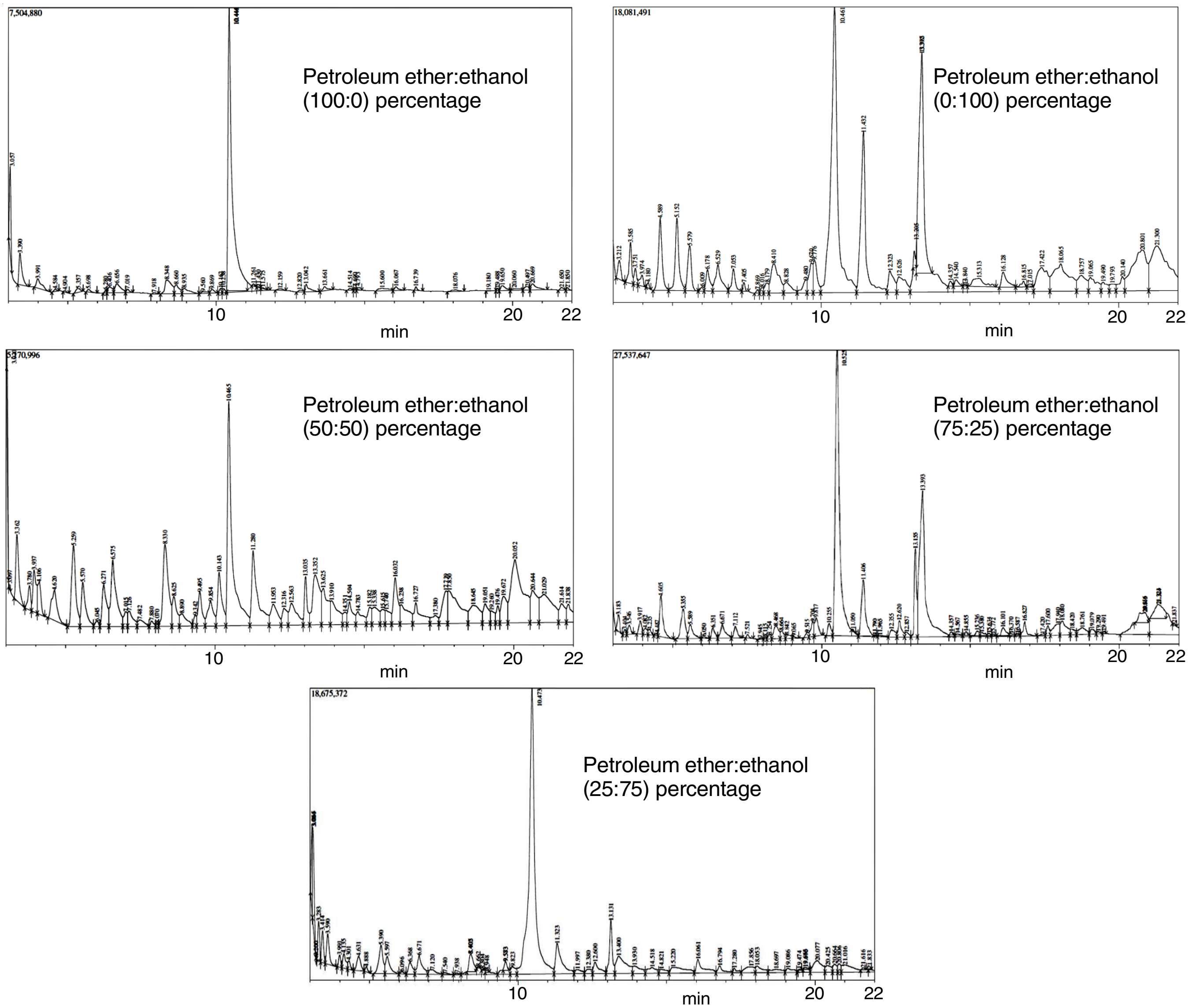


Fig. 1. GC-MS spectrum of *Rosa x damascena* concrete oil at five different solvent percentage ratios

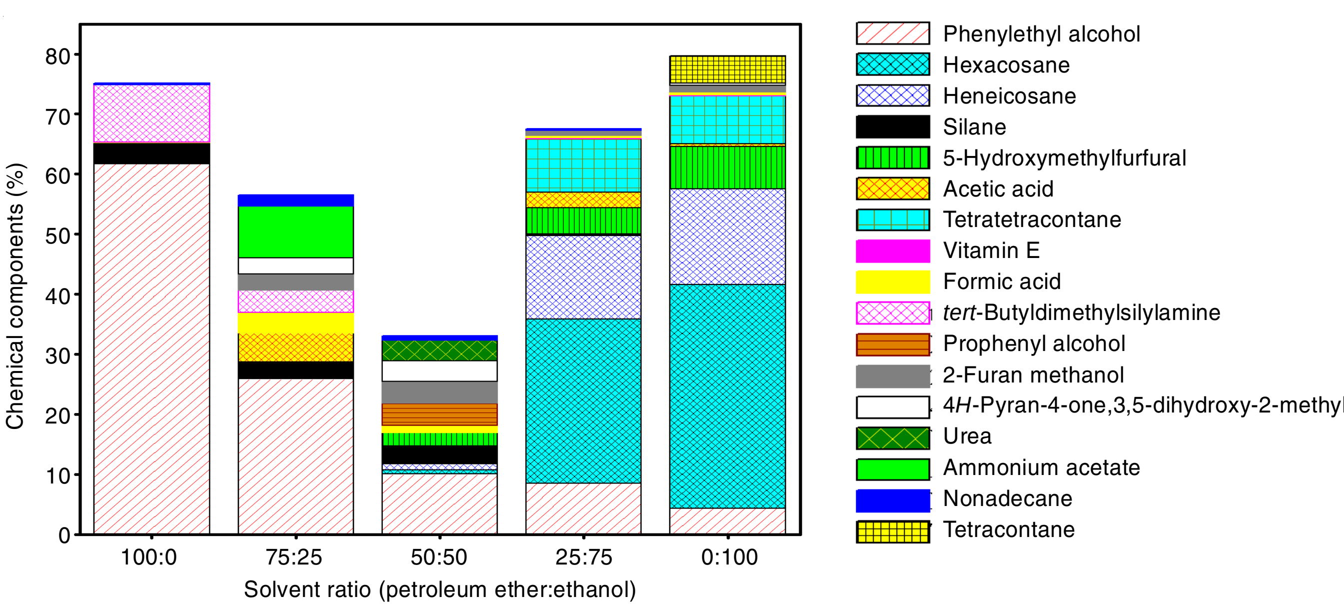


Fig. 2. Major components identified in *Rosa x damascena* concrete oil at different solvent percentage ratios

a solvent. Carboxylic acids like formic and acetic acid were obtained when using the solvent mixture at different percentage ratios. The component nonadecane 0.27%, 1.86%, 0.67%, 0.27% was obtained at the solvent percentage ratios of 100:0, 75:25, 50:50 (PE:E) respectively. It is also noted that this component is available in the presence of petroleum ether but not found with pure ethanol solvent. The component ammonium acetate (8.6%) was identified, especially at the ratio of (75:25) of petroleum ether and ethanol, which may be due to side reactions.

**Comparison studies:** Table-2 showed the different species of *Rosa x damascena* used for the study, the method of extraction, place of cultivation and principal components identified. The results of the present study are compared with those available in literature. Earlier studies show that fragrance components such as geraniol, citronellol, nerol, *etc*. were obtained using solvents with hydrodistillation, pressurized $CO_2$ extraction and solid-phase micro extraction methods. As these volatile components, they could escape if the Soxhlet apparatus is used. Most of the studies obtained phenylethyl alcohol, nonacosane, heneicosane and citronellal as major components [14,17].

The phenylethyl alcohol (61.7%) percentage reported in present study is comparatively good compared to the values available in literature. Some studies reported the presence of hydrocarbons like nonacosane, heneicosane, nonadecane and hexatricontane as the major components, especially when hydrodistillation was used. The value of heneicosane (15.97%) identified in the current study also shows good agreement with literature [9,19]. In present dual solvent study, the major components identified include both alcohol and hydrocarbons; this may be due to the solvent's presence at different solvent percentage ratios. Phenylethyl alcohol, which is used as a common ingredient in the flavour and perfumery industry, was found to be more when using the solvent extraction method. The results of the current study and the findings of earlier research studies available clearly show that the type of solvent has a significant influence on the type of component.

**Other components:** Other functional group components identified using different solvent percentage ratios are plotted in Fig. 3. It can be inferred that when petroleum ether dominates the solvent portion, a greater percentage of alcohols was found and decreases when the ratio of petroleum ether is decre-

TABLE-2
COMPONENTS IDENTIFIED IN *Rosa x damascena* IN EARLIER STUDIES AVAILABLE IN LITERATURE WITH SPECIFICATIONS OF THE SPECIES' NAME, CULTIVATION PLACE, METHODS AND COMPONENTS IDENTIFIED

| Name of the species | Method of obtaining the component | Name of the product | Place of cultivation | Major component identified | Ref. |
|---|---|---|---|---|---|
| *Rosa damascena Mill* | Hydrodistillation | RO | New Delhi, India | *n*-Nonacosane (26.31%) | [12] |
| *Rosa damascena* | Hydrodistillation | EO | Rasht, Iran | Hexatriacontane (26.34%), 1-nonadecene(18.56%) | [13] |
| *Rosa damascena Mill* | Clevenger's, Hydro distillation, fermentation | EO | Isparta, Turkey | Geraniol (44.44%), Citronellol (33.94%) | [14] |
| *Rosa damascena Mill* | Solventextraction (hexane), Modern techniques | Con, Abs, Hyd | Turkey | Phenylethyl alcohol (40-60%) | [6] |
| *Rosa damascena* | Solvent extraction (dichloromethane, benzene, hexane, chloroform) | RO, RW | Kashmir, India | Phenylethyl alcohol (69.7-81-6%) | [15] |
| Rose petals | Solvent extraction (hexane), Steam distillation, High-pressure $CO_2$ extraction | RO, ROtt, Abs, Con | England | Phenylethyl Phenyl ethyl alcohol (17.29%) by high pressure $CO_2$ extraction | [16] |
| *Rosa damascena* | Headspace-solid phase microextraction | FRW FRO | Isparta, Turkey | Citronellol (43-44%) | [17] |
| *Rosa damascena* | Different pressure distillation and extraction (dichloromethane) | RO, RW, EO | Himalayas, India | Phenylethyl Phenyl ethyl alcohol (55, 63%) | [9] |
| *Rosa damascena Mill* | Hydrodistillation | EO | Bucharest, Romania | Heneicosane (8.3%) | [18] |
| *Rosa damascena Mill* | Hydrodistillation | RO | Seven countries | Nonacosane (26.31%) | [19] |
| *Rosa damascena* | DNA extraction, ANOVA statistical study | RO | Rose valley in Bulgaria | – | [20] |
| *Rosa damascena* | Hydrodistillation, Clevenger apparatus | RO, RW | Uttarakhand, India | Heneicosane rose oil (19.7%), rose water (15.7%) | [21] |
| *Rosa damascena Mill* | Hydrodistillation | EO | Fars province, Iran | Nonadecane (39.73%) | [10] |
| *Rosa damascena* | DTD, SHWE, WD | RO | Twenty locations in Iran | Phenylethyl alcohol (36%) | [22] |
| *Rosa damascena* | Solvent extraction (hexane, ether) | EO | Faisalabad, Pakistan | Citronellol (62.1%) | [23] |
| *Rosa damascene Mill* | Hydrodistillation | EO | Vidin, Bulgaria | β-Citronellol (30.24) | [10] |
| *Rosa x damascena* | Dual solvent extraction (petroleum ether and ethanol) | Con | Tamil Nadu, India | Phenylethyl alcohol (61.7%) & Hexacosane (37.2%) | Current study |

RO = Rose oil, EO = Essential oil, Con = Concrete oil, Abs = Absolute, Hyd = Hydrosol, RW = Rose water; Rott = Rose Otto, FRW = First rose water, FRO = First rose oil, DTD = Direct thermal desorption, SWE = Superheated water extraction, WD = Water distillation ased. Results showed the presence of alcohols like phenyl ethyl alcohol, propenyl alcohol, furan, methanol, *etc*. The components with medicinal value like resorcinol (1.07%), sorbitol (0.89%) and orcinol (0.87%) are mainly obtained at the solvent percentage ratio of (50:50) (petroleum ether: ethanol). The hydrocarbons are found to be more, when ethanol dominates the solvent mixture, it decreases with a decrease in the quantity of ethanol solvent. Hydrocarbons like hexacosane, heneicosane, tetracontane, tetratetracontane, nonadecane, heptadecane, pentadecane, octacosane and dotriacontane were identified when ethanol dominates the solvent portion. Carboxylic acids were found in the mixture of solvent petroleum ether and ethanol used in different percentage ratios. Carboxylic acids like formic (3.51%), acetic (4.82%), propionic (1.32%), butanoic (0.23%) and pentanoic acid (0.16%) we re obtained at different solvent percentage ratios. High molecular weight carboxylic acids like undecanoic acid ($C_{11}$), dodecanoic acid ($C_{12}$), tetra decanoic acid ($C_{14}$), hexadecanoic acid ($C_{16}$) and octadecanoic acid ($C_{18}$) were found in the percentage of 0.1 to 0.4 of PE and E. Other ketones, esters, aldehydes and ethers were found to be significantly less.

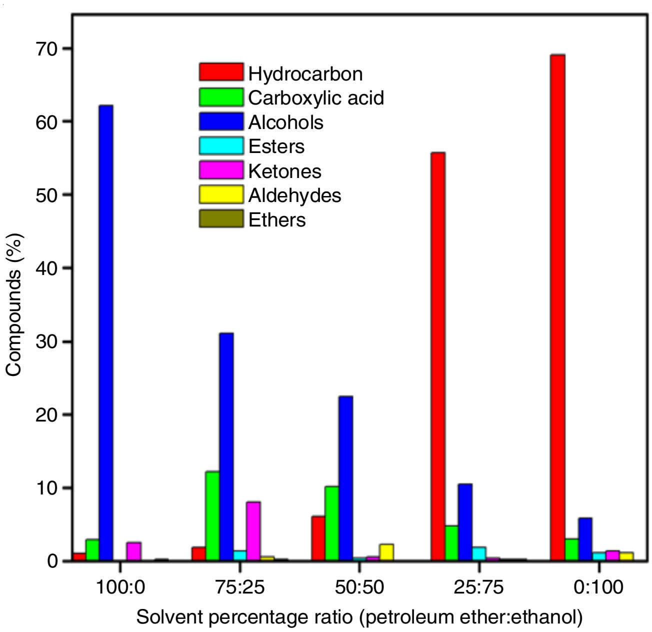


Fig. 3. Other functional groups identified in *Rosa x damascena* concrete oil at different solvent percentage ratios

**Yield:** The yield of rose concrete oil obtained after the Soxhlet extraction at different solvent percentage ratios was determined and given in Fig. 4. The yield was calculated using the following formula:

$$\text{Yield (\%)} = \frac{\text{Amount of concrete oil obtained after extraction}}{\text{Amount of rose petals taken}}$$

Fig. 4 showed the yield obtained for different solvent percentage ratios by Soxhlet extraction. The result is higher with the mixed solvent than the pure solvent. It is noted that with the solvent percentage ratio (25:75), the yield was 20%. It can therefore be concluded that the yield can be increased by mixing solvents in different ratios.

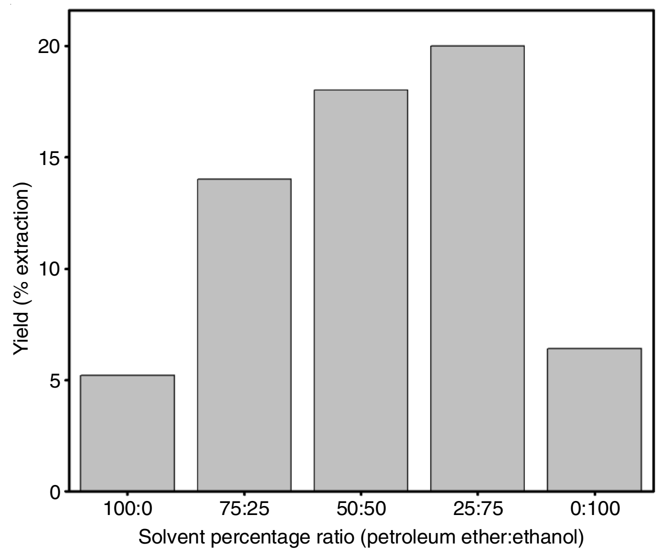


Fig. 4. Effect of mixing solvent at different percentage ratios on the yield of *Rosa x damascena* concrete oil

## Conclusion

The dual solvent extraction is found to be an effective method, as it leads to the identification of many new compounds that have not been reported. Maximum number of components is identified when the solvents were mixed in an equal percentage. The yield was higher with the mixed solvent than the single solvent. Phenylethyl alcohol was obtained as a major component when pure petroleum was used as a solvent. Hexacosane was identified as a major component when ethanol is used as a solvent. This study also concludes that phenylethyl alcohol identified is more when the solvent extraction method was used.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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