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Sequential ultrasound-microwave-assisted extraction of tuberose (*Polianthes tuberosa* L.) concrete: the effect of processing parameters on yield, volatile metabolite profiles, and functional groups

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Abstract

Sequential ultrasound-microwave assisted extraction (UMAE) technique was performed to investigate the effect of solvent type (n-hexane, methanol and petroleum ether), size [cut flower (1.75 ± 0.26 cm) and whole flower (Tepal dimensions: length: 5.73 ± 0.27 and breadth: 4.17 ± 0.23 cm)], and soaking durations (0, 30, and 60 min) on the extraction yield of concrete from tuberose flowers. The optimized conditions were n-hexane, cut samples, and 30-min soaking duration, with an extraction yield of 95.04%. The tuberose concrete extracted by all three extraction methods with n-hexane as a solvent contained aroma compounds such as 1,8-cineole, methyl benzoate, indole, α-terpineol, trans-methyl isoeugenol, trans-farnesol, and benzyl benzoate. The attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) spectra of concretes extracted by different extraction methods with n-hexane solvent were notably similar in terms of apparent composition. Principal component analysis (PCA) and sparse partial least-squares discriminant analysis (sPLS-DA) could differentiate and separate the volatile organic compounds for the classification; however, sPLS-DA performed better than PCA.

Keywords FTIR · PCA · sPLS-DA · Tuberose flowers · Ultrasound and microwave-assisted extraction · Volatile organic compounds

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1 Introduction

Essential and volatile oils are widely used in the food, cosmetic, and pharmaceutical industries, and their demand is expected to grow at a compound annual growth rate (CAGR) of 7.5% from 2020 to 2027 [1]. Essential oil is constituted of a complex mixture of terpenes, oxygenated terpenes, sesquiterpenes, oxygenated sesquiterpenes, and related compounds [2]. These are conventionally obtained from the leaves, bark, fruits, stem, peel, buds, flowers, roots, rhizomes, and seeds of plants [3, 4]. The tuberose (*Polianthes tuberosa* L.) is a tuberous perennial plant with a waxy, luminous white flower belonging to the family Agavaceae. Tuberose flowers have a sweet, fragrant, and honey-like odour that is mood-elevating, which is why they have been utilised in perfumery as a source of concretes and fragrance compounds for a long time [5]. Besides, the extracts from tuberose florets contain bioactive compounds, which show anti-inflammatory, antimicrobial, and insecticidal activities [6]. Essential oils are the purified form of concrete. Concretes are extracted from aromatic plant materials like flowers using volatile solvents such as n-hexane and petroleum ether. The plant extract

containing solvents is evaporated, leaving behind concretes, a wax-like substance used in cosmetics. Terpenes and sesquiterpenes, as well as fatty acids and their methyl esters, paraffin, and other high molecular weight compounds, are found in an essential oil [7].

Traditionally, extraction methods like hydro distillation, steam distillation, solvent extraction, maceration, percolation, enfleurage extraction, etc. were used to extract concrete or essential oil from natural materials. The traditional extraction techniques, such as maceration, percolation, solvent extraction, and enfleurage extraction, typically involve organic solvents and require a large amount of solvents as well as longer extraction duration. Moreover, there are a number of issues with extraction techniques like steam distillation and hydro distillation when it comes to extracting and maintaining the composition of natural fragrances because the high temperatures used in these methods cause water-sensitive compounds to hydrolyse and thermo labile compounds to degrade [8]. These shortcomings have led to the use of modern techniques for concrete extraction to shorten the extraction duration, reduce the organic solvent consumption, improve the extraction yield, and enhance the extract quality. Modern techniques include microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), and supercritical fluid extraction (SCFE) methods [9–13]. The supercritical fluids extraction technique has a number of advantages, but it is substantially more expensive to set up and operate than a conventional extraction method and consumes significantly more energy [8]. Using ultrasonic waves and microwave radiation to extract bioactive compounds has long been shown to speed up the extraction process and improve concrete extraction from flowers like Roses [14] and Jasmine [15]. The use of sonochemistry in natural product extraction has grown in popularity due to various benefits, including reduced extraction duration, energy savings, and higher yield. The chemical and food industries are well aware that microwave energy, with a frequency of 2.45 GHz, significantly influences the rate of various processes. Because of the reduced processing time, simpler manipulation and ease of scaling up-up, and higher purity of the final product, microwave dielectric heating has received much attention in analytical chemistry. Microwave-assisted extraction (MAE) technology is also considered eco-friendly [16].

There is very little information available regarding the methods used to maximise the efficiency of concrete extraction from tuberose flowers using different solvents. Therefore, the authors of this article hypothesised that sequential ultrasound and microwave irradiation would contribute to maximising the concrete yield. In addition, sequential ultrasound and microwave irradiation methods can be used to speed up the extraction process and release the target phytochemicals from the matrix in a relatively shorter time [17,

18]. Considering the merits of ultrasound and microwave, this study has aimed at assessing the effect of solvent type, size, and soaking duration on the yield and chemical composition of volatile organic compounds (VOCs) of concrete extracted by a sequential UMAE from tuberose flowers and also to standardise parameters for an effective UMAE approach for tuberose flowers. The UMAE process was also compared with conventional and organic extraction methods. This research aims to increase the effectiveness of concrete extraction from tuberose flowers. The results of this study may encourage and promote the use of UMAE technique for the extraction of tuberose flower concrete for the production of essential oil, which has substantial use in the fragrance industry.

2 Materials and methods

2.1 Materials, chemicals, and reagents

Bulbs of *P. tuberosa* cultivar Phule Rajani were collected from ICAR-Directorate of Floricultural Research, Pune, Maharashtra, India, and were planted at the experimental area of Precision Farming Development Centre (PFDC), ICAR-Central Institute of Agricultural Engineering (ICAR-CIAE), Bhopal, India during June 2019. During the experiment, fresh half-opened tuberose flowers were collected in the morning hours (8.00–9.00 am) since it is reported that the concentration of VOCs is maximum in half-opened tuberose flowers [19]. The harvested flowers were immediately packaged in airtight plastic bags and kept in a refrigerator (at 4 °C) until the start of the individual experiment. All solvents and chemicals (analytical and HPLC grade) used during the experiments were procured from M/s. Himedia Laboratories, LLC, India, and Sigma-Aldrich, Germany.

2.2 Experimental plan

Process protocol for sequential ultrasound-microwave-assisted concrete extraction from tuberose flowers was developed through a series of experiments. Various independent variables and their levels were selected based on rigorous preliminary trials. Since most aroma compounds found in tuberose flowers have non-polar terpenoids, benzonoids, and fatty acid derivatives and few polar compounds like phenylpropanoids [20], both non-polar solvents like n-hexane and petroleum ether and polar solvents like methanol were chosen for this investigation. Three independent parameters i.e. [1] solvent types (n-hexane, methanol and petroleum ether), [2] material size [cut flower (1.75 ± 0.26 cm), and whole flower (Tepal dimensions: length: 5.73 ± 0.27 and breadth: 4.17 ± 0.23 cm)], and [3] soaking durations (0, 30, and 60 min) were selected for investigating the sequential

ultrasound-microwave-assisted extraction of tuberose concrete. Ultrasound power (400 W), microwave power (480 W), solvent: material ratio (6:1), ultrasound treatment time (10 min), and microwave treatment time (3 min) were taken as the constant parameters, which were optimised in our earlier works (data not shown). In order to avoid splashes caused by quick heating, the microwave and ultrasound power levels were set to 480 W and 400 W, respectively, which are considered in the middle range of power [21]. The effect of this treatment on the concrete yield and the volatile profile was studied.

2.2.1 Sequential ultrasound-microwave-assisted extraction

A microprocessor-assisted ultrasonic probe sonicator (Labman Scientific Instruments Pvt. Ltd, India, Model-Pro-650) was used for the experiment. A microwave oven (Model-GMX 20GA3 M.K.Z., Godrej India Pvt. Ltd., India) was modified as per its suitability to accommodate oil extraction assembly [22]. The microwave oven has a multimode magnetron of 2.45 GHz with a maximum power delivery of 800 W. In the extraction assembly, the glass extraction flask (500 mL) was coupled with an overhead condenser. The microwave output was 480 W (60% of Maximum output power). When microwave treatment is given to the sample and solvent mixture, the solvent and volatile organic compounds (VOCs) start to evaporate from the extraction vessel when its temperature reaches its boiling point. Therefore, there is a need to convert that vapours into liquid form. To convert that vapour into liquid, a condenser is directly connected to the extraction vessel but kept outside the microwave. The condensers use a helical circulation cooling mechanism to cool the vapours that the solvents emit and transform them back into liquid form. The condenser holds water, which is pumped into and out of it through the cylinder's side arms. The water cools and condenses the vapour inside the tube. The condensed solvent and VOCs return back to the extraction/reaction flask through connecting pipe. As a result, the condensed solvents can be recovered, and there will be a maximum recovery of volatile organic compounds.

Flowers were soaked in n-hexane, methanol, and petroleum ether solvents (1:6) for different time intervals as per the experimental design. The solvent containing the flowers was then subjected to sonication in pulse mode (pulse ratio: 4 s ON and 4 s OFF) for 10 min with an output power of 400 W. This ultrasound pre-treated sample was immediately transferred into a modified MAE assembly, where the sample was exposed to microwave radiation with 480W output power for 3 min. After 3 min of microwave radiation (480 W) exposure, the sample was taken out, appropriately shaken, and kept at room temperature for 20 min

in a sealed condition to reach a steady state for the leaching of the fragrance compounds. Further, the tuberose flowers were removed from the solvent-material mixture and filtered with Whatman No. 1 filter paper. The solvent-material mixture obtained as filtrate was evaporated in a rotary vacuum evaporator to obtain the concrete. The solvent recovered from the concrete was recycled. The concrete was stored in an airtight amber-coloured bottle at a temperature of 4 °C until further analysis. All the extraction procedures were performed in triplicates.

2.2.2 Organic solvent extraction

In the organic solvent extraction process, an infusion was made using the 30 g of tuberose flowers and the 180 mL n-hexane solvent and kept at room temperature for 24 h without any pre-treatment. After 24 h, the solvent containing concrete was separated by filtration from the infusion. Finally, the concrete and solvents were separated by a rotary vacuum evaporator [19]. In the organic solvent extraction process, we did not give any heat treatment to the infusion of flowers and organic solvent (n-hexane). It is mainly leaching-based extraction. The extraction of concrete from the tuberose was conducted in triplicates.

2.2.3 Soxhlet extraction

The theoretical maximum concrete present in the tuberose flowers used for the experiments was determined using the soxhlet extraction method [23]. About 30 g of fresh tuberose flowers were extracted in 200 mL of n-hexane (at 63–65 °C) for 10 h in a soxhlet apparatus. The weight of crude essential oil, i.e. concrete, after separation of solvent, was estimated and expressed as the overall recoverable concrete yield [24]. Experiments were carried out in triplicate.

2.2.4 Conventional solvent extraction

Batch extractions were performed in a cylindrical reactor of 600 mL volume equipped with a mechanical agitator. The concrete was extracted by soaking 30 g of tuberose flowers in 180 mL of n-hexane at 50 °C for 30 min without any pre-treatments [19, 22]. The concrete from the sample matrix was distilled using a rotary evaporator, as explained earlier. In the case of conventional solvent extraction, we gave the heat treatment. The conventional solvent extraction (CSE) extractions were performed in a cylindrical reactor equipped with a mechanical agitator and a water bath for temperature regulation. The conventional solvent extraction method has been used extensively in the industry as an alternative to solvent extraction to increase the convective bulk flow of solute into the solvent, lower the mass transfer barrier, and increase the extraction efficiency and reduce the processing

time. Therefore, the extraction experiment was conducted using both organic solvent extraction (non-thermal extraction) and conventional solvent extraction method (thermal extraction) to compare with our methods [25–27]. Extraction was performed in triplicates.

The amount of extracted concrete was determined gravimetrically after collection, and then the extraction yield was expressed as the per cent ratio of the mass of extracted concrete to the mass of total concrete present in the given mass of tuberose flowers, i.e. overall concrete yield, using Eq. (1), as follows.

$$Extraction\ yield\ (\%) = \frac{mass\ of\ extracted\ concrete(g)}{overall\ concrete\ yield(g)} \times 100$$

(1)

2.3 Volatile profile of tuberose concrete through GC–MS analysis

The volatile profile of each concrete was carried out using a GC–MS (Shimadzu, GC 2010 Plus, GCMS-OP2020). The system is equipped with a DB-5 column (30 m (Length) × 0.25 mm (Internal Diameter), 0.25 μm (film thickness). The operating conditions were a carrier gas flow rate of 1.1 mL helium/min in splitless injection mode, injector temperature of 250 °C, and detector temperature of 280 °C. The stepwise temperature profile of the oven was 60 °C for 5 min, rising to 250 °C at the rate of 4 °C/min, holding isothermally for 10 min. The ionisation voltage was set at 70 eV in the 45–456 a.m.u. range with a scanning speed of 0.4 s. Ion source and interface temperatures were 200 and 250 °C, respectively. The compounds were identified by comparing the mass spectrum of the components to that of the mass spectral library from NIST 05 (National Institute of Standards and Technology, Gaithersburg, USA) and Wiley 8.0 (Wiley, New York, NY, USA). The compounds were quantified through their relative area percentages derived from the spectral data. Two replications were performed for each treatment.

2.4 ATR-FTIR-Spectroscopy analysis

The FTIR spectra were acquired using an IR Affinity-1S Fourier Transform Infrared (FTIR) Spectrophotometer (Shimadzu Corporation Pvt Ltd., Kyoto, Japan) interfaced with an Attenuated Total Reflectance (ATR) sampling accessory. In the absorbance mode, Spectra were measured from 4000 to 400 cm^{-1} by the accumulation of 64 scans at a spectral resolution of 4 cm^{-1}. A reference (background spectrum of air) was scanned under the same instrumental conditions before each sample measurement. Spectra were processed with Lab Solutions IR control software (Shimadzu Corporation Pvt Ltd., Kyoto, Japan). The concrete sample (1 mL)

was placed on the surface of the ATR crystal, and the sample spectrum was collected. The analysis was conducted in triplicates for each sample.

2.5 Multivariate data analysis

Statistical analysis, such as Duncan's multiple range tests of concrete yield data, was carried out using SAS software (SAS 9.3, USA) at a significance level of 0.05. The analysis of variance (ANOVA) of concrete yield data obtained from the individual experimental condition was carried out to interpret the significance of the effect of selected operating variables on response. Principal component analysis (PCA) was used to dimensionally reduce and categorise the GC–MS data obtained under various experimental settings. PCA qualitatively expressed and interpreted the similarity and differences among the compounds through their spatial distance among themselves. Similarly, the sparse partial least-squares discriminant analysis (sPLS-DA) has been employed for the same data for classifying the groups based on discriminant features of fragrance compounds. It is a supervised chemometrics technique used for distinct separation and classification between different groups among the two major components. The data analysis was carried out using the MetaboAnalyst software (Open access software package). The entire data set was normalised at the first stage of the data analysis, and it was then plotted against the sPLS-DA score plot of the first two principal components, which were chosen based on their eigenvalues and scree plots.

Further, the hierarchical cluster analysis (HCA) was employed to illustrate the similarity among the fragrance compounds and possible discrimination among the sample groups i.e. n-hexane, methanol, and petroleum ether. This hierarchical cluster analysis was performed using Ward's method. The clustering process considers every storage condition as an independent cluster and then selects and combines these clusters among themselves. The cluster heat map of the different VOCs against the different conditions was drawn using TBtool software (version v1.075) with two-way hierarchical cluster dendograms. These heat maps revealed the row and column hierarchical cluster structure of the data matrix [28]. The cluster heat map of the fragrance data matrix visualised the dominant fragrance compounds obtained using n-hexane, methanol, and petroleum ether as solvents for extraction.

3 Results and Discussions

The effect of different processing parameters on the sequential ultrasound-microwave-assisted extraction of tuberose concrete quality was investigated, and the results are delineated as follows.

3.1 The effect of extraction parameters on yield

3.1.1 The effect of solvent types on extraction yield

Selecting a suitable solvent for extracting the analytes of interest from the sample is the most crucial first step in developing any extraction process. The extraction yield (%) was estimated by using Eq. (1) which is mentioned in Sect. 2.2.4. The maximum yield of concrete (95.41%) was obtained with methanol (Table 1). The extraction yield of concrete obtained from n-hexane and methanol was significantly ($P < 0.05$) different than that obtained with petroleum ether as a solvent for both the cut and whole flowers and soaking durations (0, 30, 60 min). The extraction yields of n-hexane and methanol are not significantly different ($P < 0.05$). Table 1 presents the extraction yield of three solvents for various sizes and soaking durations. The maximum yield of concrete was non-significantly ($P < 0.05$) different from the conventional solvent extraction (30 min) (control). In the microwave extraction process, the polarity of the solvent plays a significant role. In general, microwave energy absorption increases with the molecule's dielectric constant, dissipating power within the solvent and plant material, resulting in more efficient molecular movement and heating. When extracting thermolabile analytes and concrete from high-water-content vegetable matrices, the sample can be extracted using a microwave-transparent solvent viz. n-hexane. Methanol is a good microwave absorber but not a suitable extraction solvent for concrete; on the other hand, n-hexane is a suitable extraction solvent but not a good microwave absorber. This is because most volatile organic compounds, such as terpenes, are non-polar in nature and insoluble in polar solvents but soluble in non-polar solvents like n-hexane [29]. Besides, n-hexane has properties such as easy recovery, non-polarity, low latent heat of vaporisation (330 kJ/kg), and excellent solvent selectivity [30]. As a result, combining the n-hexane solvent with another solvent with a higher microwave absorption capacity (like water) is a requirement. As fresh tuberose flowers contain 91.2%

moisture, this water might have helped in absorbing more amount of microwave power, which in turn aided in extraction yield when combined with n-hexane. This hypothesis is supported by [31], who also suggested that a small amount of water (e.g. 10%), when incorporated in non-polar solvents such as hexane, xylene, or toluene, improved the heating rate and hence extraction yield of solanesol from tobacco leaves. A group of researchers observed that the mixture of solvent and water increased the extraction yields during MAE [32].

3.1.2 The effect of size on extraction yield

The maximum yield of concrete obtained in the case of the cut samples (smaller size) was 95.41%, while the minimum yield was obtained from the whole flower samples (81.12%) (Table 1). A significant increase in the yield was observed for the cut material with an average size of 1.75 ± 0.26 cm compared to the whole flowers with an average dimension of the length of 5.73 ± 0.27 and; breadth of 4.17 ± 0.23 cm. This increased extraction yield could be attributed to the increased extraction rate and contact surface area between the solvent and material in the cut samples [33]. Besides, smaller-sized particles improved the deeper penetration of the microwaves into the product and aided the spontaneous leaching of concrete into the solvent [34]. In ultrasound-assisted extraction, a smaller size means a shorter mass transfer distance and a larger surface area [31]. In cut materials, due to damage to the original cell wall structure, leaching of the cellular material would occur, culminating in a higher yield of the bioactive fragrance compounds.

3.1.3 The effect of soaking duration on extraction yield

The extraction yield increased significantly with the soaking duration ranging from 0 to 30 min; however, no significant change in the extraction yield was observed on further increasing the soaking duration from 30 to 60 min (Table 1). This may have happened because mass transfer equilibrium was attained during the initial 30 min of soaking when the

Table 1 The effect of independent variables on the concrete essential oil yield (%) extracted from tuberose florets

Parameters	Concrete essential oil yield, %					
Material size	Cut florets			Whole florets		
Soaking duration, (min)	0	30	60	0	30	60
Solvent type						
n-Hexane	91.12 ± 0.11^a	95.34 ± 0.12^b	95.38 ± 0.11^b	84.14 ± 0.09^a	86.23 ± 0.10^b	86.26 ± 0.06^b
Petroleum ether	88.65 ± 0.10^b	91.93 ± 0.09^c	91.98 ± 0.10^c	81.12 ± 0.12^b	83.66 ± 0.41^c	84.05 ± 0.20^c
Methanol	91.50 ± 0.14^a	95.41 ± 0.13^b	95.46 ± 0.08^b	84.44 ± 0.09^a	86.31 ± 0.10^b	86.39 ± 0.06^b

(i) All values are mean of three replicates ± SD, (ii) Means ± SD followed by same superscript letter in the same row and column are not significantly different at the 0.05 probability level

gradient was zero. Once the equilibrium was reached, no further mass transfer could be observed. A soaking duration of 30 min resulted in the highest extraction yield, i.e. 95.41%, with methanol as a solvent for cut samples. Therefore, 30 min was chosen as the optimal soaking duration. The increased extraction efficiency of the concrete after soaking for 30 min in the solvent might be due to increased solvent diffusion into the cellular structure, allowing for enhanced concrete solubilisation. During soaking, the solvent diffuses into the floret cell, resulting in a higher moisture concentration in the floret cell. This additional moisture helps absorb the maximum amount of microwave energy as water is a polar solvent with higher dielectric properties than any other solvents, which in turn improves the dielectric constant of the materials during soaking, inevitably increasing its ability to absorb sufficient microwave energy during the extraction process and improving the solubilisation of the fragrance compounds in the solvent. A similar result was also observed when MAE was used to extract the polyphenols and caffeine from green tea leaves [35]. Researchers reported that adding moisture to the ultrasound-assisted extraction (UAE) process improves the recovery of natural products [36]. This may be clarified by the fact that the ultrasound extraction method could allow oil transfer from the flowers into the solvent at higher moisture levels due to wave propagation in the liquid medium and subsequent cellular disintegration. Therefore, when flowers were soaked in the solvent after the ultrasound treatment, the solvent penetrated readily into the flowers' tissues due to their improved permeability and mass diffusivity. The increased soaking duration softened the flower tissues and improved diffusion, allowing the concrete to be leached into the solvent.

3.2 Volatile profiling of tuberose concrete under different extraction methods

The concrete compositions from tuberose were determined by comparing the relative retention duration and the mass spectra of concrete components with standards and mass spectra from the data library. A list of identified compounds in different extraction methods is presented in Tables 2 and 3. All three methods succeeded in extracting the fragrance compounds from tuberose flowers. Monoterpene, sesquiterpene, and other oxygenated compounds were among the volatile organic compounds found in all the extracted samples of tuberose concrete (Fig. 1). Different extraction methods showed differences in the chemical composition of the concrete obtained. From Tables 2 and 3, it was clear that the total number of 32, 30, and 33 VOCs compounds were identified by GC–MS analysis in extracts of tuberose flowers obtained by sequential UMAE with solvent n-hexane, methanol, and petroleum ether, respectively. The type and percentage of volatile organic compounds varied

in sequential UMAE with the type of solvents. Comparing the proportions of the different compound categories, the sample obtained by sequential UMAE with n-hexane as a solvent (CH0) contained the highest percentage of monoterpenes (18.3%) and phenylpropanoids (7.07%) compounds than the extract obtained by sequential UMAE with methanol and petroleum ether as solvents (Fig. 1C). The lowest amounts of monoterpenes (1.23%) and phenylpropanoids (1.1%) were extracted from cut (CH30) and whole flower (UCH0) samples, respectively, in the methanol extracted concrete sample (Tables 2 and 3; Fig. 1A). The reason behind this is that terpenes are relatively less polar compounds and can be effectively extracted with a non-polar solvent like n-hexane than the polar solvent like methanol [37]. However, plants often produce less polar terpenes, and many monoterpenes and sesquiterpenes have the potential to be volatile as they have an extremely low boiling point [37]. The n-hexane extracted concrete sample from the cut samples (CH30) had the highest percentage of sesquiterpenes compounds (12.21%), whereas the petroleum ether extracted concrete sample from the cut samples (CH0) had the lowest percentage (2.77%) of sesquiterpenes compounds (Fig. 1C). While using methanol as solvent, the quantity of benzoid derivatives compounds detected was the maximum (39.24%) in the whole samples and the minimum (23.64%) in the cut (CH0) flowers (Fig. 1A). This happens as each class of metabolites has a degree of polarity so; we need the same strength of solvent to extract it. Methanol also has the benefit of having a low boiling point, which makes it easier to extract both volatile and non-volatile compounds. Methanol is the most effective polar solvent for the extraction, giving the highest content of benzoid derivatives compounds. It is necessary to mention that there have been no reports on GC–MS analysis of the concrete extracted by MAE or UMAE. Compounds such as 1,8-cineole, methyl benzoate, methyl salicylate, α-terpineol, indole, methyl anthranilate, acetyl eugenol, trans-iso-eugenol alpha-farnesene, cis-beta-farnesene, benzyl benzoate, benzyl salicylate, geranyl benzoate, and 7-decen-5-olide were isolated and identified by three extraction methods, namely sequential UMAE with n-hexane as a solvent, organic solvent extraction (leaching based), and conventional solvent extraction. All these compounds were also identified and reported earlier [5, 6, 19].

The monoterpenes, sesquiterpenes, and benzenoids compounds, viz. benzyl alcohol, methyl benzoate, methyl salicylate, 2-hydroxy-1,8-cineole, 3β-hydroxy-1,8-cineole, indole, farnesane, methyl anthranilate, eugenol acetate, eso-eugenol, diethyl ehthalate, farnesol, cis-beta-farnesene, alpha-farnesene, benzyl benzoate, and benzyl salicylate, were isolated and identified through sequential UMAE method with methanol as solvent. These compounds were also reported in tuberose extract [5, 19, 20]. The compounds, namely methyl linolenate, 7-decen-5-olide, methyl linoleate, hexacosanal,

Table 2 The relative contents of each component in *P. tuberosa* flower concrete essential oil extracted by UMAE (n-hexane solvent), OSE, CSE, and control (Soxhlet) methods

Sr. no	Compounds	RI	Cut florets 0	Cut florets 30	Cut florets 60	Whole florets 0	Whole florets 30	Whole florets 60	OSE*	CSE**	Control***
1.	Bicyclo [4.2.0]octa-1,3,5-triene	1263	3.15±0.16	2.54±0.11	2.55±0.16	3.51±0.09	4.52±0.14	4.56±0.19	3.18±0.14	3.14±0.17	3.34±0.22
2.	Decane	1000	1.36±0.10	0.00	00.00	2.20±0.10	1.23±0.08	1.21±0.07	2.14±0.09	0.00	0.00
3.	1,8-Cineole	1232	7.87±0.08	7.70±0.16	7.97±0.14	6.81±0.18	7.84±0.16	6.87±0.30	6.90±0.15	6.74±0.13	6.93±0.18
4.	Decane, 2,3,5,8-tetramethyl-	1318	2.20±0.08	2.22±0.09	2.34±0.12	1.21±0.18	1.42±0.18	1.28±0.2	1.04±0.12	2.10±0.06	2.16±0.12
5.	Decane, 5-ethyl-5-methyl-	1001	0.00	0.00	0.00	1.15±0.08	3.25±0.05	3.26±0.14	1.39±0.21	1.14±0.07	1.12±0.08
6.	hexa-hydro-Farnesol	2351	0.00	1.27±0.11	0.00	1.13±0.06	1.18±0.03	1.17±0.09	1.17±0.10	1.15±0.08	1.21±0.09
7.	Methyl benzoate	1091	6.53±0.15	3.18±0.19	3.62±0.13	3.29±0.19	3.84±0.15	3.79±0.17	3.07±0.14	3.05±0.16	3.19±0.22
8.	Terpineol	1197	5.60±0.19	3.15±0.11	3.75±0.30	2.35±0.15	0.00	2.48±0.15	2.50±0.15	2.54±0.14	2.16±0.13
9.	Dodecane	1200	0.00±	0.00	0.00	2.22±0.12	2.59±0.12	2.57±0.22	1.32±0.22	1.20±0.08	1.11±0.07
10.	2-acetoxy-1,8-Cineole	1219	2.30±0.11	2.17±0.08	2.17±0.09	2.17±0.09	2.24±0.10	2.21±0.11	2.17±0.09	2.13±0.05	2.10±0.07
11.	3-Hydroxy-1,8-cineole	1259	2.53±0.09	2.21±0.13	2.27±0.14	2.26±0.08	2.37±0.10	2.33±0.15	2.26±0.10	2.30±0.16	2.45±0.40
12.	Indole	1304	4.73±0.27	5.27±0.11	5.84±0.13	4.39±0.14	5.06±0.11	4.95±0.12	3.76±0.18	4.75±0.14	4.15±0.29
13.	Methyl anthranilate	1363	2.35±0.09	2.84±0.12	2.67±0.24	2.51±0.11	2.63±0.19	2.56±0.14	2.73±0.14	2.76±0.12	2.84±0.13
14.	Acetyl eugenol	1525	3.23±0.08	3.23±0.11	3.19±0.06	1.00±0.16	4.39±0.09	4.27±0.21	3.11±0.04	3.09±0.04	3.15±0.09
15.	Tridecane, 3-methyl-	1373	1.42±0.11	1.22±0.09	1.19±0.08	1.14±0.07	1.16±0.08	1.15±0.09	1.65±0.19	1.12±0.07	1.14±0.10
16.	alpha-Bourbonene	1374	1.00±0.08	1.14±0.05	1.16±0.08	1.14±0.06	1.18±0.15	1.17±0.10	0.00	1.10±0.05	1.13±0.08
17.	Tetradecane	1400	2.66±0.14	2.45±0.09	2.27±0.13	2.32±0.10	2.50±0.20	2.51±0.16	2.01±0.19	2.29±0.13	2.35±0.14
18.	Trans-iso-eugenol	1451	3.84±0.11	3.32±0.17	3.18±0.10	0.00	0.00	0.00	3.04±0.14	3.06±0.13	3.17±0.16
19.	7-Decen-5-olide	1181	11.37±0.15	9.47±0.24	10.57±0.12	11.22±0.12	11.50±0.85	11.73±0.60	10.33±0.25	10.40±0.30	11.53±0.26
20.	alpha-Farnesene	1512	3.16±0.09	3.18±0.10	3.11±0.08	2.43±0.13	2.51±0.24	2.56±0.12	3.03±0.15	3.05±0.19	3.14±0.14
21.	Eicosane	2000	3.35±0.09	2.46±0.22	2.27±0.11	3.33±0.11	3.86±0.25	3.64±0.16	2.19±0.11	2.19±0.09	2.27±0.10
22.	Diethyl phthalate	1585	04.07±0.04	4.32±0.08	5.45±0.13	9.72±0.15	7.44±0.27	8.44±0.24	7.05±0.16	5.88±0.14	5.35±0.17
23.	cis-beta-Farnesene	1674	4.12±0.10	5.29±0.11	5.27±0.09	4.43±0.16	4.73±0.28	4.75±0.21	4.85±0.13	4.95±0.17	5.20±0.23
24.	Benzyl benzoate	2655	10.92±0.07	12.77±0.15	11.85±0.11	8.24±0.13	8.48±0.20	8.75±0.24	9.98±0.23	9.94±0.16	10.10±0.30
25.	Benzyl salicylate	2810	3.79±0.15	3.54±0.08	3.52±0.11	3.27±0.12	3.40±0.14	3.39±0.08	3.47±0.20	3.44±0.18	3.35±0.14
26.	n- Hexadecanoic acid	1969	2.35±0.16	1.65±0.15	2.67±0.08	4.48±0.13	2.67±0.18	2.68±0.07	2.58±0.19	2.54±0.14	2.84±0.24
27.	Geranyl benzoate	1951	2.26±0.12	2.45±0.09	2.37±0.09	2.12±0.04	2.20±0.11	1.16±0.08	2.28±0.10	2.31±0.11	2.34±0.12
28.	Octacosane	2800	0.00	1.59±0.19	1.62±0.11	0.00	1.13±0.06	1.47±0.09	1.51±0.05	1.69±0.14	1.53±0.15
29.	Heneicosane	2100	2.00±0.15	1.50±0.12	2.86±0.10	3.24±0.07	2.29±0.11	1.21±0.15	2.49±0.12	2.67±0.14	2.38±0.15
30.	Octacosanal	3014	0.00	1.41±0.11	1.57±0.10	0.00	0.00	0.00	0.00	2.50±0.23	2.15±0.06
31.	Tetracosane	2400	1.47±0.12	0.00	0.00	3.24±0.07	2.33±0.17	1.27±0.17	3.18±0.17	0.00 0.00	2.10±0.05
32.	Hexacosane	2600	0.00	2.68±0.16	2.33±0.11	2.27±0.16	0.00	0.00	2.48±0.19	2.36±0.17	1.53±0.18

Table 2 (continued)

Sr. no	Solvent type	n-Hexake (% Area)						OSE*	CSE**	Control***
	Material size	Cut florets			Whole florets					
	Soaking duration (min)	0	30	60	0	30	60			
	Compounds (RI)									
	Monoterpens	18.3	15.23	16.16	13.59	12.45	13.89	13.83	13.71	13.64
	Sesquiterpenes	09.9	12.21	10.99	11.02	11.84	11.76	11.2	11.23	10.98
	Benzenoids	27.3	26.63	26.87	27.47	29.47	29.48	27.43	26.3	26.74
	Phenylpropanoids	7.07	6.55	6.37	1.00	4.39	4.27	6.15	6.15	6.32
	Fatty acid derivatives	14.46	15.53	16.45	22.32	18.34	16.29	17.82	19.26	19.84
	Other Compounds	22.6	23.37	22.79	23.53	22.32	23.70	23.65	20.45	21.99
	Total	99.63	99.52	99.63	98.93	98.81	99.39	98.86	97.58	99.51

(i) All oil constituents were identified by (a) mass spectral database match, (a) RI—Kovats Retention indices based on NIST on non- polar HP5MSTM or DB5 capillary column. (ii) All values are mean of two replicates ±SD. (iii) OSE*—organic solvent extraction method, CSE**—conventional solvent extraction method, control ***—Soxhlet extraction method

linolenic acid, octacosanol, and hydroquinone, were present in more significant amounts in methanol solvent extract, and these compounds were also identified and reported in tuberose flower extracts [38].

Xylene, 1, 8-cineole, methyl benzoate, alpha-terpineol, indole, methyl anthranilate, iso-eugenol, farnesol, cis-beta-farnesene alpha-farnesene, benzyl benzoate, and benzyl salicylate were isolated and identified by sequential UMAE with petroleum ether as solvent.

Among all these compounds, methyl benzoate, primarily responsible for the distinctive fragrance of tuberose, was identified. According to Tables 2 and 3, the percentage area of these monoterpenes, sesquiterpenes compounds extracted by methanol, and petroleum ether was less than n-hexane solvent extract (Fig. 1). An essential aspect of this fact is that the maximum amount of volatile organic compounds which are mainly responsible to typical tuberose fragrance was soluble in n-hexane compared to the methanol solvent. According to this study's findings, n-hexane was inferred as a better solvent than methanol in terms of the fragrance quality of the extracted concrete.

3.3 FTIR Analysis of tuberose concrete

In this study, the FTIR analysis of tuberose concrete was carried out to know the effect of different extraction methods on the quality (functional group changes) of extracted tuberose concrete while keeping solvent as a constant parameter. The organic solvent extraction process is a non-thermal process, while both conventional and UMAE methods employ thermal treatment. Since some of the VOCs are thermo sensitive, the study aimed to find out if there would be any change in the quality of the concrete if different extraction procedures were followed. Our hypothesis is confirmed through the FTIR analysis. The ATR-FTIR spectrum of the concrete extracted by different methods in Fig. 2 shows the absorption bands at different wavelengths reflecting their functional groups. The spectrum properties of all the concrete samples extracted by various methods were almost identical. The IR spectra displayed a complete overlap of each component's absorption spectrum. Infrared absorption peaks occur in 1800–490 cm^{-1} spectra of all samples, as shown in Fig. 2, implying that all samples nearly contain molecules of the same functional groups.

In most cases, prominent spectral bands were observed at 723, 763, 1379, 1452, and 1471 cm^{-1}. The C–C–C ring vibrations of volatile compounds are responsible for the band with a peak at 2983 cm^{-1} [39]. The band at 2839 cm^{-1} was assigned to the methylene C–H symmetric stretching vibrations [40]. The presence of volatile and aroma compounds was indicated by bending and stretching vibrations of C-O and C = O groups of aldehydes, acids, and alcohols in the

Table 3 The relative contents of each component in *P. tuberosa* flower concrete essential oil extracted by UMAE (Methanol and Petroleum ether) methods

Sr. No	Compounds	RI	Methanol						Petroleum ether					
			Cut florets			Whole florets			Cut florets			Whole florets		
			0	30	60	0	30	60	0	30	60	0	30	60
1.	o-Xylene	876	0.00	0.00	0.00	0.00	0.00	0.00	7.49±0.21	14.49±0.32	13.7±0.16	14.1±0.21	14.6±0.38	14.12±0.27
2.	p-Xylene	888	0.00	0.00	0.00	0.00	0.00	0.00	8.48±0.25	10.88±0.18	9.3±0.22	11.2±0.14	11.1±0.17	09.32±0.16
3.	Benzene, 1,2,3-trimethyl-	1012	0.00	0.00	0.00	0.00	0.00	0.00	7.35±0.23	7.60±0.11	7.3±0.14	8.79±0.19	7.89±0.21	6.96±0.11
4.	Benzene, 1-ethyl-2-methyl-	970	0.00	0.00	0.00	0.00	0.00	0.00	2.40±0.17	4.13±	4.89±0.09	4.5±0.08	5.2±0.12	4.16±0.11
5.	Benzene, 1,2,4-trimethyl-	1016	0.00	0.00	0.00	0.00	0.00	0.00	3.10±0.25	4.77±0.15	5.4±0.16	6.4±0.19	5.7±0.08	5.56±0.12
6.	1,8-Cineole	1232	0.00	0.00	0.00	0.00	0.00	0.00	7.97±0.22	4.68±0.16	4.61±0.26	4.43±0.29	3.38±0.15	2.93±0.17
7.	Benzyl alcohol	1052	3.23±0.1	3.4±0.2	5.28±0.2	7.25±0.11	5.58±0.20	4.34±0.19	0.00	0.00	0.00	0.00	0.00	0.00
8.	Methyl benzoate	1091	2.24±0.11	2.49±0.13	2.35±0.15	2.44±0.21	2.61±0.16	2.75±0.19	2.66±0.18	1.35±0.24	2.51±0.08	2.54±0.08	2.26±0.05	3.19±0.18
9.	alpha-Terpineol	1197	0.00	0.00	0.00	0.00	0.00	0.00	2.55±0.20	1.46±	0.00	1.29±0.11	1.38±0.16	1.49±0.17
10.	2-hydroxy-1,8-cineole	1219	1.28±0.12	1.23±0.19	1.24±0.12	1.37±0.20	1.22±0.17	1.20±0.13	0.00	0.00	0.00	0.00	0.00	0.00
11.	3-hydroxy-1,8-cineole	1259	0.00	0.00	2.48±0.12	2.74±0.20	2.33±0.25	2.57±0.20	1.13±0.04	0.00	2.57±0.20	0.00	0.00	0.00
12.	Hydroquinone	1334	6.7±0.6	7.1±0.5	8.65±1.05	10.76±0.22	1.20±0.14	4.88±0.23	0.00	2.29±0.16	2.98±0.27	3.23±0.21	0.00	3.11±0.14
13.	Indole	1304	2.35±0.1	2.45±0.15	4.11±0.4	1.10±0.18	0.00	2.96±0.20	1.45±0.22	1.78±	1.79±0.15	1.47±0.18	1.73±0.13	1.32±0.09
14.	Farnesene	1482	3.17±0.04	0.00	0.00	1.29±0.12	0.00	3.52±0.17	0.00	1.04±0.07	0.00	0.00	0.00	1.08±0.05
15.	Methyl anthranilate	1363	2.12±0.05	2.14±0.06	2.67±0.32	1.09±0.35	1.00±0.23	2.35±0.16	1.09±0.21	1.23±0.09	1.24±0.08	1.32±0.07	1.56±0.11	1.81±0.09
16.	Acetyl eugenol	1525	2.09±0.65	0.98±0.25	2.18±0.21	0.00	0.00	2.17±0.11	1.07±0.11	1.06±	0.00	0.00	0.00	0.00
17.	Tetradecane	1400	00±00	0.00	0.00	1.29±0.17	0.00	2.33±0.16	1.20±0.16	0.00	0.00	0.00	0.00	3.05±0.04
18.	Iso-eugenol	1453	2.21±0.04	2.27±0.1	3.05±0.4	1.10±0.19	2.39±0.28	2.00±0.17	1.47±0.22	1.33±0.05	1.43±0.03	1.26±0.06	1.34±0.05	1.36±0.14
19.	7-Decen-5-olide	1181	5.83±0.1	4.11±0.3	7.15±0.85	6.18±0.23	5.39±0.28	5.69±0.28	5.81±0.17	4.86±0.18	5.25±0.18	3.45±0.17	5.74±0.19	6.81±0.15
20.	Diethyl phthalate	1585	5.23±0.11	6.32±0.19	6.84±0.17	11.21±0.37	12.20±0.66	7.38±0.20	3.98±0.14	4.11±0.18	7.12±0.16	7.34±0.27	8.54±0.25	4.69±0.27
21.	Hexadecane	2023	00±00	2.09±00	1.68±0.23	2.13±0.09	3.67±0.21	2.47±0.21	4.25±0.16	4.14±0.15	2.21±0.13	00±00	2.13±0.08	2.17±0.06
22.	Eicosane	2000	5.13±0.13	2.98±00	5.74±0.1	4.84±0.15	7.68±0.18	6.19±0.18	8.27±0.32	0.00	0.00	0.00	0.00	0.00
23.	Farnesol	1722	2.43±0.2	2.87±0.12	4.85±0.4	0.00	1.01±0.11	4.77±0.20	0.00	2.63±0.14	1.87±0.09	1.90±0.07	2.21±0.16	2.91±0.18
24.	alpha-Farnesene	1512	2.23±0.14	0	0	3.64±0.16	3.82±0.18	0.00	2.77±0.19	1.18±	1.23±0.16	1.12±0.15	1.21±0.06	1.11±0.16
25.	Benzyl benzoate	2655	2.82±0.4	2.93±0.18	5.19±0.13	3.19±0.27	4.64±0.22	4.43±0.17	5.59±0.28	3.39±	4.45±0.15	2.97±0.18	2.89±0.21	3.00±0.25
26.	Benzyl salicylate	2810	2.25±0.1	2.29±0.1	2.91±0.1	2.95±0.17	0.40±0.17	2.19±0.23	1.43±0.18	1.13±	1.39±0.25	1.28±0.18	1.38±0.23	1.41±0.19

Table 3 (continued)

Sr. No	Compounds	RI	% Area — Methanol						% Area — Petroleum ether					
			Cut florets			Whole florets			Cut florets			Whole florets		
			0	30	60	0	30	60	0	30	60	0	30	60
27.	n-Hexadecanoic acid	1969	00±00	5.99±0.3	5.1±1.0	3.33±0.15	3.34±0.24	6.76±0.19	4.27±0.18	3.21±0.14	00±0.00	1.25±0.18	2.29±0.16	0.00
28.	Geranyl benzoate	1951	1.17±0.17	0	0.78±0.19	1.44±0.19	1.47±0.21	0.00	1.13±0.09	1.06±0.18	1.12±0.17	1.21±0.11	0.00	0.00
29.	Methyl linoleate	2078	5.25±0.48	7.15±0.19	0.68±0.11	5.53±0.26	4.24±0.19	4.43±0.35	0.00	0.00	0.00	0.00	0.00	1.07±0.05
30.	Methyl linolenate	2084	8.89±0.11	11.7±0.2	0.79±0.14	8.37±0.23	8.74±0.24	6.50±0.30	0.00	1±0.19	0.00	0.00	0.00	1.20±0.07
31.	Methyl stearate	2128	2.12±0.05	4.14±0.05	2.98±0.21	2.10±0.05	2.17±0.11	2.67±0.20	0.00	1.76±0,18	0.00	0.00	0.00	0.00
32.	Octadecanoic acid	2170	4.76±0.23	4.81±0.15	4.87±0.10	4.65±0.14	2.11±0.14	6.22±0.27	4.11±0.07	4.11±0.19	0.00	0.00	0.00	2.97±0.17
33.	Octacosane	2800	4.21±0.1	4.15±0.05	4.39±0.2	4.28±0.18	3.22±0.21	0.00	3.68±0.12	0.00	4.32±0.07	0.00	2.34±1.18	0.00
34.	Squalene	2666	4.24±0.16	0	0.99±0.19	0.00	4.04±0.15	2.55±0.20	2.47±0.21	1.21±0.18	2.26±0.14	3.89±0.12	2.67±0.19	3.64±0.17
35.	Heneicosane	2100	0	5.63±0.17	5.16±0.32	4.54±0.33	4.16±0.35	4.24±0.23	2.19±0.17	1.20±	3.80±0.13	4.86±0.25	3.80±	3.32±0.13
36.	n-Octadecane	1800	6.26±0.05	0	5.19±0.1	0.00	4.81±0.17	2.43±0.21	0.00	0.00	2.73±0.27	3.33±0.22	2.73±0.27	3.36±0.23
37.	Octacosanol	3120	6.11±0.2	8.66±0.3	0.94±0.17	0.00	6.24±0.26	0.00	0.00	6.14±0.29	5.12±0.24	6.22±0.27	5.12±0.24	2.22±0.18
	Monoterpens		1.28	1.23	3.72	4.11	3.55	3.77	8.65	6.14	4.61	5.72	4.76	4.42
	Sesquiterpenes		7.83	2.87	4.85	4.93	4.83	8.29	2.77	4.85	3.1	3.02	3.42	5.1
	Benzenoids		23.64	24.53	32	39.24	28.1	25.97	27.64	29.83	37.16	38.26	33.86	32.08
	Phenylpropanoids		4.3	3.25	5.23	1.1	2.39	4.17	2.54	2.39	1.43	1.26	1.34	1.36
	Fatty acid derivatives		20.36	25.65	32.13	25.06	28.99	30.64	27.97	12.66	13.06	9.44	13.29	14.87
	Other Compounds		39.31	40.35	20.31	25.36	31.83	27.15	29.79	43.35	38.73	41.35	42.52	41.51
	Total		96.72	97.88	98.24	99.80	99.69	99.99	99.36	99.22	98.09	99.35	99.19	99.34

(i) All oil constituents were identified by (a) mass spectral database match, (b) RI—Kovats Retention indices based on NIST on non- polar HP5MSTM or DB5 capillary column. (ii) All values are mean of two replicates ±SD
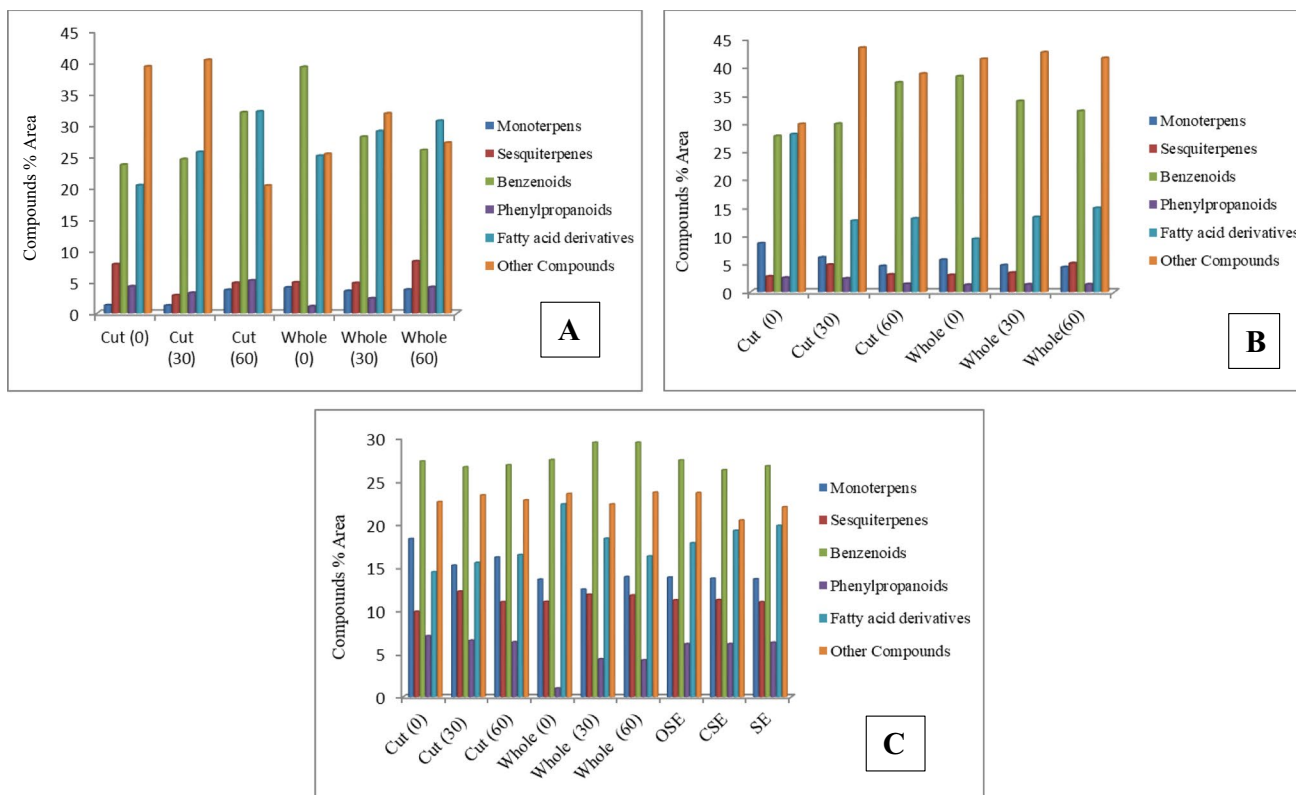
Fig. 1 Percentage area of different group of compounds for **A** methanol, **B** petroleum ether, and **C** n-hexane, OSE, CSE, & Soxhlet extraction (SE) methods
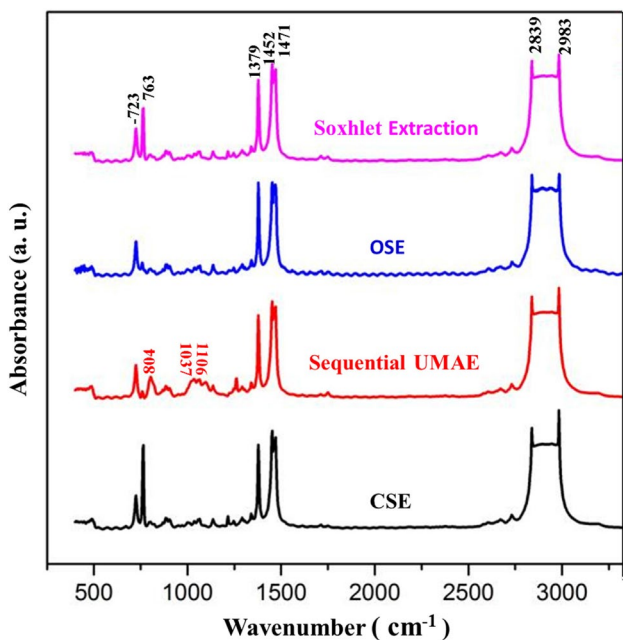
Fig. 2 FTIR Spectra of concrete essential oil of tuberose flower extracted by **a** conventional solvent extraction (CSE), **b** organic solvent extraction (OSE), **c** Soxhlet extraction, **d** n-hexane sequential UMAE (optimized condition)

absorption spectra of 1000 to 1500 cm^{-1}. The peaks around 1452 and 1471 cm^{-1} represented the aromatic ring C = C skeleton vibrations of aromatic substances [41]. The strong band at 1379 cm^{-1} was assigned to the C–H asymmetric plus symmetric bending vibrations [42]. A variation in the case of UMAE-extracted concrete was noticed near the band regions of 804, 1037, and 1106 cm^{-1}. The other functional group of compounds may be produced due to the synergistic effect of ultrasonic and microwave energy, which allows the tuberose flowers to be fully exposed and subjected to oxidation, hydrolysis, and maybe some other reactions. The FTIR bands identified at 804 and 1037 cm^{-1} in UMAE tuberose extract could be related to C–H stretching vibrations and hydroxyl group vibrations, respectively [43, 44]. A significant peak at 1106 cm^{-1} indicates the C-O stretching vibration of terpenoid components [40]. The band regions of 804, 1037, and 1106 cm^{-1} were absent in the concrete extracted by other than UMAE extraction method. The C-H vibration absorption of benzene rings is attributed to the peak at 763 cm^{-1}, and the vibration absorption of alkenes is represented by the peak at 723 cm^{-1}. All significant bands observed in the primary components

were found in the tuberose concrete, enabling the main components of the tuberose concrete to be identified using FTIR.

Sharp absorption peaks were observed for all samples of tuberose concrete extracted by different methods (Fig. 2). In their IR spectra, all four tuberose concrete samples showed approximately the same common peaks. Therefore, their spectral patterns were nearly comparable, as seen in Fig. 2. The favourable impact of microwave and ultrasonic intensity on the quality of extracted concrete was seen in the case of UMAE tuberose extract because it contains more VOCs compounds having hydroxyl and methylene groups, which have antimicrobial properties. This explains the VOCs profile obtained through GC–MS analysis of the tuberose concrete, as discussed in the previous section. Therefore, based on the previous analysis, it is possible to infer that there is no adverse effect of microwave and ultrasound intensity on the fragrance quality of extracted concrete. Table 2 also showed no difference in the overall compounds and their % area coverage based on data obtained from GC–MS analysis of four tuberose concrete samples extracted by n-hexane solvent. Therefore, it can be deduced from the GC–MS and FTIR analyses that all four tuberose concrete samples extracted by four different extraction procedures using n-hexane as a solvent had approximately similar fragrance quality and constituents.

3.4 Chemometric analysis

3.4.1 Cluster heat map

PCA was used to get a sense of how well GC–MS data may be used to differentiate concrete components. HCA separates a collection of objects into clusters or groups, with objects in one cluster being more similar to those in other clusters. By emphasising their similarity, HCA can divide the number of tested samples into several groups based on the chemical composition of concretes. The results of the cluster analysis revealed that the concrete of tuberose has high variability. HCA was used to distinguish three distinct groups of concrete from the eighteen extraction samples that were submitted to multivariate analysis. Based on the data, three sub clusters can be observed.

3.4.2 PCA and sPLS-DA of GC–MS data

The PCA of the fragrance compounds present in the concrete obtained from ultrasound-microwave-assisted extraction was carried out using three different solvents like n-hexane, methanol, and petroleum ether. Accordingly, the classification of normalised GC–MS data has been performed under three distinct classes and is represented in Fig. 3A. In the PCA score plot, the first two components of the plot of fragrance compounds obtained from the selected concrete sample (Fig. 3A) accounted for 94.7% variance, with
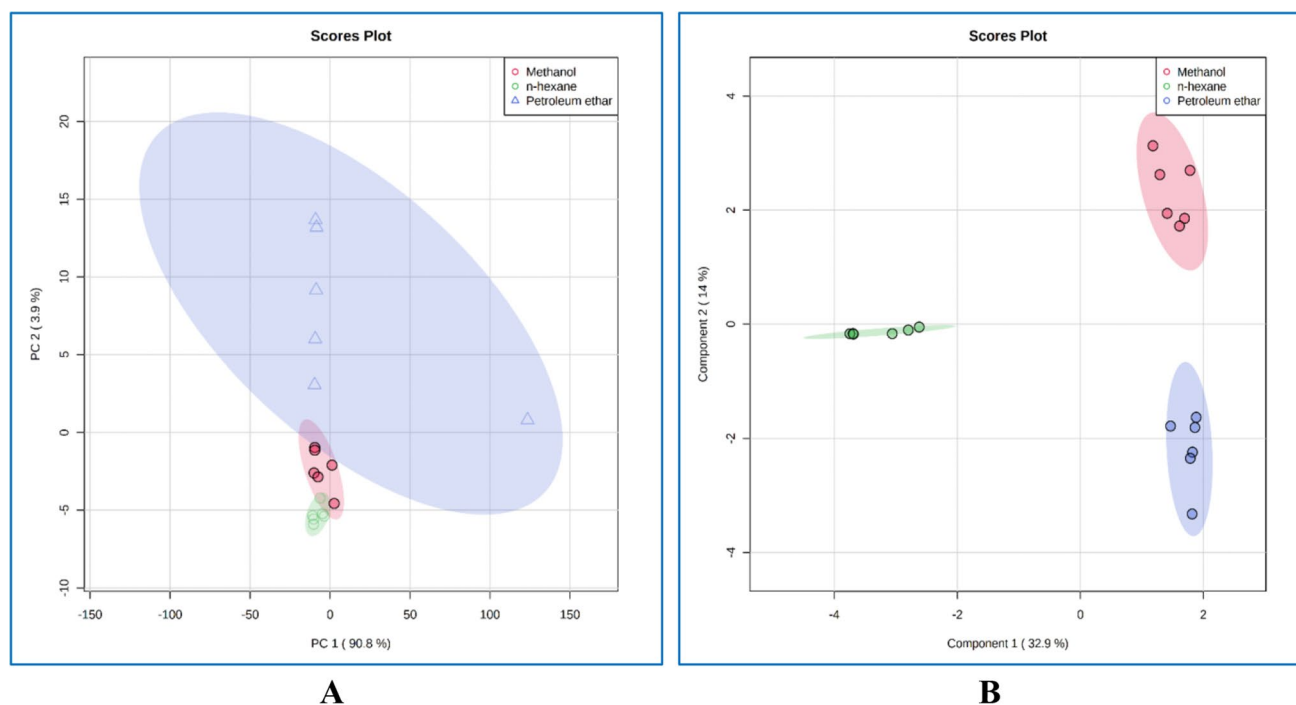
Fig. 3 Chemometric analysis of GC–MS spectra of tuberose concrete **A** PCA, **B** s-PSLDA

PC1 explaining a maximum of 90.8% and PC2 explaining 3.9% variance. The system could distinguish between the concrete based on the fragrance compounds obtained using three different solvents used for extraction. Slight overlapping of methanol was observed with the clusters of n-hexane and petroleum ether compounds.

On the other hand, there was a distinct separation among the clusters of compounds of n-hexane and petroleum ether. Therefore, smaller clusters of n-hexane and methanol over petroleum ether may be due to fewer compounds participating in the cluster formation. At the same time, clear separation of fragrance compounds was obtained under selected chopping conditions and soaking duration in the cluster of petroleum ether, while it was not so evident in the clusters of n-hexane and methanol.

The cluster formed for the separation of concrete obtained using three selected solvents (i.e. n-hexane, methanol, and petroleum ether) during PCA showed a slight overlap among the groups. Hence, the solvent groups' better distinct separation and classification based on the discriminant feature approach were carried out using sparse partial least-squares discriminant analysis (sPLS-DA) of the same fragrance

compound data. Figure 3 B represented the sPLS-DA score plot of the concrete samples obtained using three selected solvents which displayed about 46.9% variance. Here, the discriminant component 1 and 2 accounted for 32.9% and 14.0% of the variance, respectively. The score plot also represented a clear and distinct separation among the concrete samples obtained through n-hexane, methanol, and petroleum ether as solvents in three separate clusters. In addition, a distinct separation among the other extraction conditions for sample preparation, like cut and whole flowers and soaking duration, was also observed in each of the clusters.

3.4.3 Cluster analysis and heat map of volatile organic compounds

Ward's method was used to perform a cluster analysis and heat map of the aroma compounds of the concrete with a hierarchical cluster. The cluster analysis dendograms with Mahalnobis distance are given in Fig. 4. The colour of each rectangle in the heat map (Fig. 4) represented the concentration of the matching fragrance/concrete compounds. Prior to performing concentration-based colour scaling, the actual concrete

Fig. 4 Clustered heat map of VOCs present in concrete essential oil of tuberose flower extracted in different extraction treatments
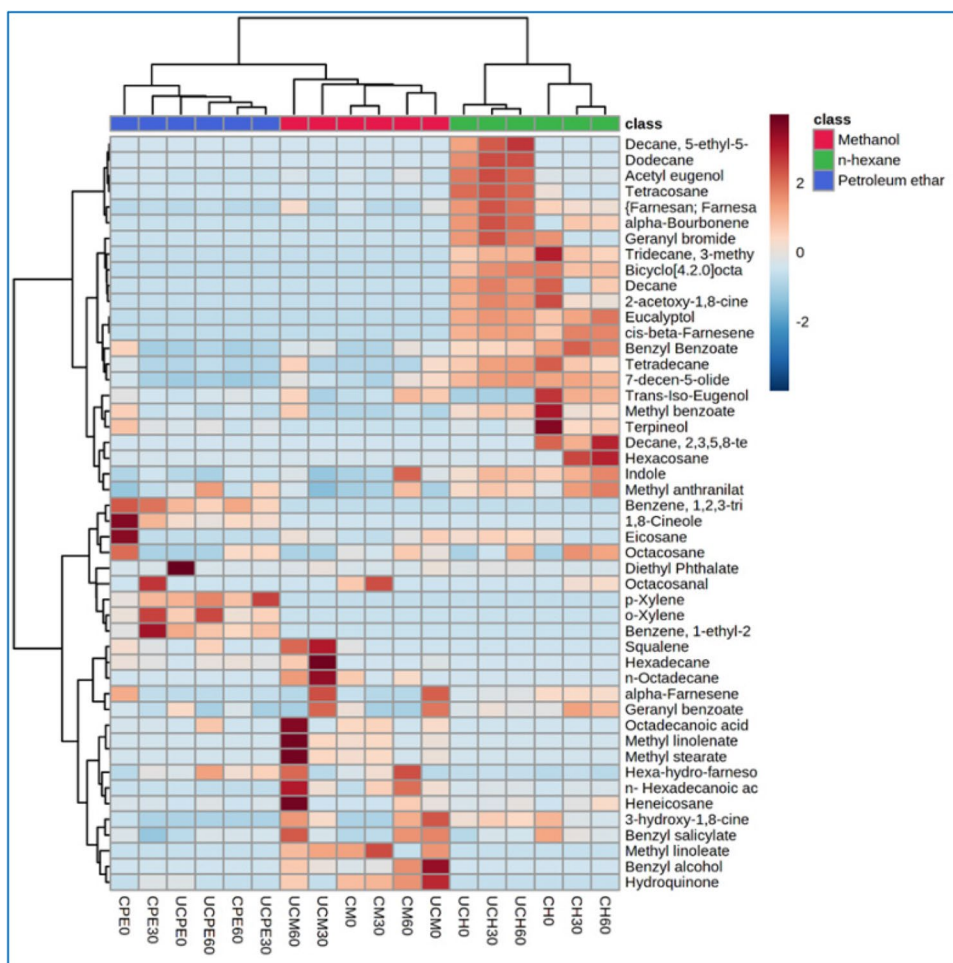
Table 4 Comparative analysis of different extraction methods for cut florets

Extraction methods	Extraction duration (min)	Concrete yield (%)
Organic solvent extraction (leaching based)	1440[a]	94.32 ± 0.34[a]
Conventional solvent extraction	30[b]	93.47 ± 0.18[a]
Hybrid ultrasound-microwave-assisted extraction (at optimized condition)	12[c]	95.04 ± 0.12

(i) Values followed by the same superscript letter in the same column are not significantly different ($P < 0.05$). (ii) All values are mean of three replicates ± SD

compounds data acquired from GC–MS were normalised. The maximum concentration of the compound was indicated by dark brown colour on the colour scale, which gradually altered with changes in concentration until it reached its lowest value, which was represented by a dark blue colour. As a result, Fig. 4 depicted a clustered heat map of the VOCs of concrete obtained under various extraction conditions of UMAE. In the case of the n-hexane cut samples (CH0, CH30, and CH60), trans-iso-eugenol, methyl benzoate, terpineol, indole, methyl anthranilate, benzyl benzoate, and eucalyptol were the most dominant VOCs obtained for varied soaking durations. The dominance of these VOCs was found to be changed and shifted to the different compounds for whole tuberose flowers with varied soaking durations. The VOCs like decane-5-ehyle-5, dodecane, acetyl eugenol, alpha-bourbonene, farnesene, and tetracosane were dominant for n-hexane extracted whole samples (UCH0, UCH30, and UCH60). Because of the non-polar characteristics and ease of recovery of n-hexane, it has been used as an effective extraction solvent.

On the other hand, the VOCs obtained from the other two solvent extracts were not the same as the VOCs obtained from the n-hexane solvent extract. The most dominant VOCs obtained from the whole flowers for different soaking durations in the methanol (UCM0, UCM30, and UCM60) were methyl linolenate, methyl stearate, octadecanoic acid, hexadecane, benzyl alcohol, hydroquinone, and n-octadecane; whereas, using methanol as solvent from the cut samples, dominating VOCs obtained were indole, octacosanal, hydrofernesol, methyl linoleate and hydroquinone.

Similarly, in the petroleum ether extracted concrete from the cut samples (CPE0, CPE30, and CPE60) for various soaking periods, dominant compounds were 1, 8 cineole, eicosane, octacosane, o-xylene, and p-xylene, while diethyl phthalate, o-xylene, benzene 1-ethyl-2, methyl anthranilate, 1, 8 cineole, and-xylene were found in petroleum ether extracted whole samples (UCPE0, UCPE30, and UCPE60) for different soaking durations.

3.5 Comparison of UMAE and conventional extraction methods

A comparative analysis of the extraction of concrete from tuberose flowers by using conventional solvent extraction, organic solvent extraction (leaching-based), and sequential UMAE is shown in Table 4. A 24-h solvent extraction experiment was conducted in accordance with the previous studies [19]. The processing time for conventional solvent extraction has been carried out as a preliminary study (Unpublished data). The 30-min extraction time in the case of conventional solvent extraction methods is according to the method followed by [19], which is considered the overall concrete yield [22]. Therefore, only the optimised extraction duration and yields of the conventional solvent extraction method were considered and compared with the UMAE. There was a significant ($P < 0.05$) reduction in processing time for concrete extraction from 1440 min (24 h) and 30 min to 13 min in sequential UMAE than organic solvent extraction (leaching based) and conventional solvent method, respectively. It also showed that concrete yield through sequential UMAE was 95.43% in 13 min treatment, which was non-significantly ($P < 0.05$) different for both organic solvent extraction (leaching based) (24 h) and conventional solvent method (30 min). The concrete extracted by all three extraction methods contained different classes of compounds viz. benzenoids, phenylpropanoids, nitrogen-containing compounds, monoterpenes, sesquiterpenes, and fatty acid derivatives. Table 3 shows the chemical composition of P. tuberosa flower extracts at different extraction methods by organic solvent extraction (leaching-based), conventional solvent method, and sequential UMAE. The extracted tuberose concrete by all three extraction methods with n-hexane as a solvent contained a significant amount of aroma compounds like 1,8-cineole, methyl benzoate, indole, α-terpineol, trans-methyl isoeugenol, trans-farnesol, and benzyl benzoate.

4 Conclusions

The study showed how the most effective extraction yield and fragrance quality could be achieved by sequential ultrasound-microwave-assisted concrete extraction from the tuberose flowers. The concrete yield extracted through the sequential UMAE method with n-hexane as a solvent was at par with methanol and of the highest quality. Due to the intensive disruption of tissue structure, cut samples have a higher extraction yield. The pre-treatment soaking duration

(30 min) is the most effective, as higher solvent diffusion into the cellular structure and concrete solubilisation is improved. The maximum amount of fragrance compounds present in the concrete extracted by n-hexane as a solvent is an added advantage from the fragrance quality perspective. Compared with traditional extraction methods, UMAE with n-hexane solvent is more effective in extracting the maximum amount of concrete from tuberose flowers. Taking everything into account, it can be summarised that UMAE is an efficient extraction method for obtaining high-quality concrete extracts and reducing extraction duration. The UMAE extraction technique had the potential to be applied to other plant materials, particularly other flowers. Further studies will be focused on optimising other extraction parameters of UMAE in the future.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication All authors agreed on the publication of this research work.

Competing interests The authors declare no competing interests.

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