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Aroma profiling of jasmine (*Jasminum sambac* Ait.) flowers using electronic nose technology

X. Alex Isac[1]*, **K.R. Rajadurai**[1], **M. Jawaharlal**[2], **K. Arul Mozhi Selvan**[3], D. Uma[4], Hena Roy[5] and Nabrun Bhattacharyya[5]

*[1]Department of Floriculture and Landscaping, Tamil Nadu Agricultural University, India. [2]Horticultural College & Research Institute for Women, Navalur Kutapattu, Trichy, India. [3]Department of Soil Science & Agricultural Chemistry, Tamil Nadu Agricultural University, India. [4]Department of Biochemistry, Centre for Plant Molecular Biology, Tamil Nadu Agricultural University, India. [5]Centre for Development of Advanced Computing (C-DAC), Kolkata, India. *E-mail: senthamil.alex@gmail.com*

Abstract

The Jasmine (*Jasminum sambac* Ait.) flowers are highly fragrant and used for extraction of essential oil, preparation of perfumes and scented water. Since there is a growing demand for the fresh flowers, there arises a need to develop a technique to identify the flower quality in non-destructive and quickest possible manner. A study was undertaken using hand held electronic nose technology (HEN) at the Department of Floriculture and Landscaping, Tamil Nadu Agricultural University, Coimbatore during the year 2013-2015. The result showed that, the HEN device generated Aroma Index (AI) score increased over the flower development stages and varied from 0.41 in immature bud (stage I) to 4.26 in matured bud (stage V). The comprehensive study on quantum of fragrance releasing pattern at different flower opening stages (physiologically matured bud to fully opened flower) over period of time interval showed that, minimum of 5.41 was recorded in an unopened closed bud stage which gradually increased upto 41.26 in the fully opened flowers. The biochemical constituents responsible for the unique jasmine flower fragrance were identified using Gas Chromatography –Mass Spectrometry (GC-MS).

Key words: *Jasminum sambac*, electronic nose, MOS, volatile emission

Introduction

Jasmine occurrences are distributed in tropical and subtropical ecologies around the world. Jasmines are grown commercially in India, Thailand, China, Sri Lanka and the Philippines for its fresh flowers. The genus *Jasminum* contains more than 200 species and is mostly tropical in distribution (Abdul Khader and Kumar, 1995). Though there are a large number of species and varieties in jasmine, commercial cultivation is confined to only a very few, *viz*., *Jasminum sambac*, *J. auriculatum* and *J. grandiflorum*, which are largely cultivated and *J. multiflorum* (Syn: *J. pubescence*) which is cultivated to a small extent. The flowers are highly fragrant and used for religious offerings in temples and highly preferred by ladies for adorning their hair. They are also used for extraction of essential oil which is used in the preparation of perfumes and scented water. The jasmine concrete is highly valuable for perfume, confectionaries, cosmetics and toiletry industries.

Advancement of chemical and instrumental research in revealing the fragrance compounds in nature and also in synthesizing those components in laboratory has been achieved to some extent. The natural odor of the flowers are perfect and unchanged, cannot be completely extracted and analysed. There are various methods of analyzing these natural extract from flowers. Till date these fragrance estimation were mostly by subjective methods performed by panel of skilled persons. The analysis had been done through instruments like chromatography, spectrography *etc*., by trained operator.

To simplify the fragrance estimation, portable and reliable tools are required. So far, a specially designed Electronic Nose has been successfully used to monitor volatile substances emission pattern over repeated measurements. Electronic Nose is a unique tool that is capable of sensing the volatile compounds of the given sample and reliably predicts scores with a high degree of accuracy. Neural Network based Soft Computing Techniques are used to tune near accurate correlation smell print of multi-sensor array. The software framework has been designed with adequate flexibility and openness, so that may train the system of scoring with reliable predictions of such smell print scores. Since there is a growing demand for the fresh flowers, there arises a need to develop a technique to identify the flower quality in non-destructive and quickest possible manner. These kind of technique would facilitate export of these flowers to both short and long distance overseas markets without much loss of the post harvest quality.

Materials and methods

The experiment was undertaken at the Department of Floriculture and Landscaping, Tamil Nadu Agricultural University, Coimbatore in collaboration with Centre for Development and Advanced Computing (C-DAC), Kolkata during the year 2013-2014. The experimental material consisted of flowers of major species of Jasmine *i.e., J. sambac* was used. The experiment was laid out in Completely Radomized Block Design (CRD) with three replications.

Fresh flowers (unopened fully matured flowers) from *J. sambac* were collected from randomly selected plants in the early morning hours around 5 am to 6 am during the entire period of study. The harvesting stages were classified based on the visual appearance of the flower bud. The different stages of harvesting are given in Table 1. Observations of E-nose generated Aroma Index (AI), ethylene emission rate and respiration rate was recorded for each stage of harvesting.

Table 1. Different harvesting stages of *J. sambac* flowers

Stage of harvest	Age of flower bud (days)
Stage I	5
Stage II	8
Stage III	10
Stage IV	12
Stage V	15

The matured unopened buds were harvested from the randomly selected plants. The flower buds were continuously observed using E-nose till it opened fully (*i.e.,* from morning to late evening). Postharvest physiological parameters such as E-nose generated Aroma Index (AI), CO_2 rate and ethylene emission rate were recorded at 10.00 am, 4.00 pm, 6.00 pm, 7.00 pm and 8.00 pm. The E-nose generated Aroma Index (AI) values for the above parameters were analysed statistically and least significant difference was applied to compare the differences among different time intervals at 5% as critical level of probability (α). The flower opening index was categorized based on the values in flower opening index chart (Table 2).

Identification of different fragrances was performed by using electronic nose, equipped with a metal oxide semiconductor sensor (MOS). E-nose has a great potential to discriminate fragrances and would be a useful tool for the fragrance of ornamentals.

Electronic Nose system for gradation of jasmine based on aroma characteristics comprised of two main components (i) The Sniffing Unit and (ii) Data Processing Unit. The sniffing unit consists of the sensory and sensing unit. The sniffing unit is the odor capture and delivery system to the sensor array and the data processing unit is responsible for data acquisition from the sensor array through a proper signal conditioning circuit and the acquired data is processed to generate and display the Fragrance Index.

The experimental sniffing cycle consists of automated sequence of internal operations: (i) headspace generation, (ii) sampling, (iii) purging before the start of the next sniffing cycle. Initially these MOS sensors require heating for at least one hour to be stable. Heating is done by supplying 5 Volts to the heater coils of the sensors. This heating phase of sensors is referred to as Pre-heating. The MOS sensors react to volatile compounds on contact; the adsorption of volatile compounds on the sensor surface causes a physical change of the sensor.

Table 2. Flower Opening Index chart of jasmine flowers (*J. sambac*) at different flower opening stages

Flower opening index	Flower opening pattern
0	Un-open bud
0.5	Nearly slight open
1.0	Slightly open
1.5	Nearly half open
2.0	Half open
2.5	Nearly full open
3.0	Fully open

Principle of operation: The samples to be tested were placed in a sample holder (be specific) in the E-Nose set-up. Data was recorded separately for flowers and buds. It has been observed that the system was able to identify the bud and also the blossoming state of Jasmine.

Air flow was blown into the sample container as pressure applied in time scale of second in order to ensure adequate concentration of volatiles in the air within the container. In the same time scale, output voltage was recorded with sensor when exposed to volatile substance influences. The time specified in seconds, for which the sensor array is exposed to fresh air in order to reestablish baseline values of the sensors. Due to the strong scent of jasmine, it is recommended that a purging time of at-least 15 minutes be used.

The sensor array was exposed to a constant flow of volatiles emanated from Jasmine flower at time duration of 50 seconds. Data from all the sensors were stored all through this sampling operation, but the steady-state value for each sensor is considered for computation purposes. During the purging operation of 100 secs, sensor heads were cleared through the blow of air so that the sensors can go back to their baseline values.

Data acquisition module: Sensor outputs were fed to this module. After signal conditioning, the channels were multiplexed and were fed to an data acquisition card. The DAQ output was fed to the processor for analysis and storage.

Flower opening index (FOI): The fragrance emission was determined during fresh flowers opening period which is under influence of their development stage varying from Stage I to stage V. There were significant differences in opening of flower from tight bud stage to fully opened condition as in V stage. The various flower opening indices recorded at different time intervals were categorized (Table 3).

Soxhlet extraction: The compounds responsible for fragrance emission in fully opened jasmine flowers were detected through the concrete extracted using Soxhlet extractor. The fresh samples of about 20 g were taken in the extraction chamber placed in a tube above the extraction solvent. The solvent used was food grade hexane (analytical reagent) to wash the sample using a reflux apparatus. When heated, the solvent evaporates into a gas, and then cools into a liquid in a condenser. It then refluxes back into the sample tube. This continuous cyclic process takes around 45 to 60 minutes per cycle until the concrete is separated from the sample. The solvent was evaporated off, by keeping it in the water bath and the amount of concrete was determined.

Gas chromatography: Later the extracted concrete samples at different interval of time were subjected to Gas Chromatography –Mass Spectrometry analysis. About one micro litre of sample concentrate was injected into a Thermo GC - Trace Ultra Ver: 5.0,Thermo MS DSQ II gas chromatograph equipped with a flame ionization detector. The column used was DB 35 - MS Capillary Standard Non - Polar Column. The specifications of Gas Chromatography used for analysis; Column: 50m × 0.25mm internal diameter (i.d.) coated with PEG 20 M, film thickness: 0.15 pm, Carrier gas: N, with a flow rate at 1.2 mL/min, oven temperature: 60°C (4 min) + 220°C, injection and detector temperature: 200°C Split ratio: 10: 1

Results and discussion

Fragrance parameters estimation at various development stages of flowers were recorded and given in the Table 3 and Table 4. Time taken for flower opening is an important character, which signifies the earliness or late flowering habit of the genotype. Both the habits are helpful in determining the availability of flowers for longer period (Khader and Kumar, 1995).

It was observed that there was no ethylene evolution observed till stage III while it was triggered up to 1 ppm during the stage IV. Then after, ethylene was released up to 3.2 ppm after Stage V of harvest. Under ambient conditions, ethylene evolution rate with the range of 2.0 ppm and 22.8 ppm after harvest of fresh flowers at different time intervals were observed in fully matured opened flower (Table 4). The results indicated that the rate of ethylene evolution increased rapidly after harvest upto 16 hours, after which the senescence of flower starts (Fig. 2). Similar results were observed by Mayak and Halevy (1974), Suttle and Kende (1978, 1980) and Borochov *et al*. (1997). Also it was observed that as the flowers started showing symptoms of wilting, there was a rise in level of ethylene emission rate.

The E-nose generated Aroma Index (AI) of jasmine flowers during different flower developmental stages were observed and it varied from 0.41 in I stage, 0.83 in II stage, 1.58 in III stage, 2.53 in IV stage to 4.26 in V stage. It clearly indicates that the Metal oxide Sensor (MoS) used in the E-nose instrument, sniffed the compounds responsible for fragrance and generated the Aroma Index (AI) as the stage of harvesting progressed. Fully matured harvested jasmine flowers were continuously observed from its closed unopened stage to fully opened condition. Based on our observation the E-nose generated Aroma Index (AI), gradually increased from 5.41 in 10.00 am (unopend closed bud stage) to 16.83 in 4.00 pm (nearly slight open stage), 24.58 in 6.00 pm (slightly opened stage), 32.53 in 6.00 pm (half opened) and 41.26 in 8.00 pm (fully opened). As the flower opens, the fragrance emission is higher and the senescence of flower takes place.

Table 3. E-nose generated aroma index (AI) of *J. sambac* at different flower developmental stages

Harvesting stages	E-nose Value (Aroma Index)	CO_2 Rate (ppm)	Ethylene Emission Rate (ppm)
Stage I	0.41	0	0
Stage II	0.83	0	0
Stage III	1.58	1.2	0
Stage IV	2.53	2.3	1.0
Stage V	4.26	8.4	3.2
SEm ±	0.02	0.1	0.1
LSD (*P*=0.05)	0.07	0.1	0.1

Table 4. Ethylene, CO_2 and Aroma Index at different Flower Opening Index (FOI) of *J. sambac*

Time	*Flower opening index (FOP)	E-nose value (Aroma Index)	CO_2 Rate (ppm)	Ethylene emission rate (ppm)
10.00 am	0	5.41	8.4	2.0
04.00 pm	0.5	16.83	27.8	9.5
06.00 pm	1	24.58	36.3	14.9
07.00 pm	2	32.53	42.6	18.6
08.00 pm	3	41.26	56.8	22.8
SEm ±		0.01	0.1	0.1
LSD (*P*=0.05)		0.21	0.3	0.4

Ethylene hormone has been known to play a crucial role in senescence of flowers, the sensitivity of which varies depending on the flower species (Redman *et al*., 2002). Ethylene reduces the longevity of some flowers causing rapid wilting of petals (*e.g.*, carnations), shedding or shattering of petals, or other changes to petal tissues, such as loss or change of colour.

Earlier reports (Naidu and Reid, 1989) have indicated that the flowers are ethylene sensitive based on the fact that though the flowers produce moderate ethylene during opening and senescence, they do not respond to exogenously applied ethylene (Veen, 1983) indicating that this hormone is not involved in their senescence. No report is available on *Jasminum* spp. with respect to ethylene evolution, however some records on wilting of flowers other than jasmine caused by ethylene have been discussed. Involvement of ethylene in wilting of flowers (Borochov *et al.*, 1997) has been observed in carnation (Ten Have and Woltering, 1997) and in *Gypsophila paniculata* (Vandoorn and Reid, 1992). It has also been noticed that the flower parts including petals, sepals, the ovary and labellum were the major site of ethylene production (Chao Chia *et al.*, 1991) and that ethylene promoted the accumulation of sugars and inorganic materials in the ovary, with a simultaneous loss of fresh and dry weight of the petals. These are some evidences of ethylene sensitive species where in, ethylene is the major cause of wilting of flowers.

Respiration rate: The minimum respiration rate of 8.4 ppm was observed at 10 am. It steadily increases and reached maximum of 56.8 ppm at 8.0 pm in the evening. The respiration of flowers started from III stage upto 1.2 ppm, followed by 2.3 ppm in IV stage and gradually increased in V stage upto 8.4 ppm (Table 3). All the flowers showed a climacteric rise in respiration rate after harvest. The lowest respiration rates were recorded immediately after harvest. This may be due to short supply of readily respirable substrates in the flowers due to onset of senescence. Similar results were reported by Coorts (1973) in cut flowers and Maxie *et al*. (1973) in carnation. Increased respiration leads to formation of free radicals with high oxidation potential. Free radicals promote senescence in tissues which in turn increases sensitivity to ethylene (Fig. 2). Respiration is the central process in living cells that mediates the release of energy through the oxidative breakdown of carbon compounds (starch, sugar and organic acids) and the formation of carbon skeletons necessary for maintenance and synthetic reactions after harvest (Wills *et al*., 1998). In the present study, a respiratory climacteric rise from the initial level and a decline thereafter was noticed with all the treatments.

With regard to *J. sambac* under ambient conditions a similar trend was noticed, recording minimum respiration rate and sufficient amount of carbohydrate levels. These significant levels of carbohydrates might have served as the substrate for respiration for a longer duration. Evidences supporting this fact have been reported in case of flowers supplied with exogenous sugar, wherein pool of dry matter and respirable substrates were maintained at favourable levels thus promoting respiration (Coorts, 1973) and in turn extending the longevity (Rogers, 1973). The

observation of Maxie *et al.* (1973) that the respiratory activity in flowers and the production of carbon-di-oxide by flowers was similar to the pattern in climacteric fruits, characterized by a rise in level of respiration with senescence also supports the present study.

Kaltaler and Steponkus (1976) have associated the decline in respiratory activity of aging rose petals with their inability to metabolise substrates consequent to decline in activity of mitochondria in the aging petals. Moreover, increased respiratory activity leads to the formation of free radicals with high oxidation potential and these free radicals have been found to promote senescence in the tissues, associated with an increased sensitivity to ethylene (Baker *et al*., 1977; Mishra *et al*., 1976). The typical climacteric respiratory rise reported in carnation *cv.* White Sim (Burger *et al*., 1986) and day-lily (Lukaszewski and Reid, 1989) is consistent with the present result. In contrary, Trippi and Paulin (1984) had reported a decrease in respiratory activity in carnation *cv.* White Sim.

GC-MS analysis: The concrete obtained from soxhlet extractor was injected into Gas Chromatography–Mass Spectrometry instrument and constituents were identified by comparison of both mass spectra and retention indices, strictly measured on the same instrument with those of authentic jasmine samples. The identified constituents listed in Table 5 and Table 6 with their respective chromatogram obtained shown in Fig. 1 and 2 exhibited significant compounds identified at specific time intervals (10.00 am and 8.00 pm). The GC-MS chromatogram at 10 am shows, the preliminary indication about the composition of some major volatile components. However, the quantitative composition of these compounds differs considerably from the other samples. The jasmine flower possesses maximum composition and recorded peak during this time. They are Eicosanoic acid, phenylmethyl ester (Benzyl icosanoate) (26.47%), 9-Octadecenoic acid (Z), phenylmethyl ester (Benzyl oleate) (24.04%), Nonadecane (17.41%) and 2,6-Octadien-1-ol, 3,7 dimethyl-(Z)- (cis-Geraniol) (14.24%).

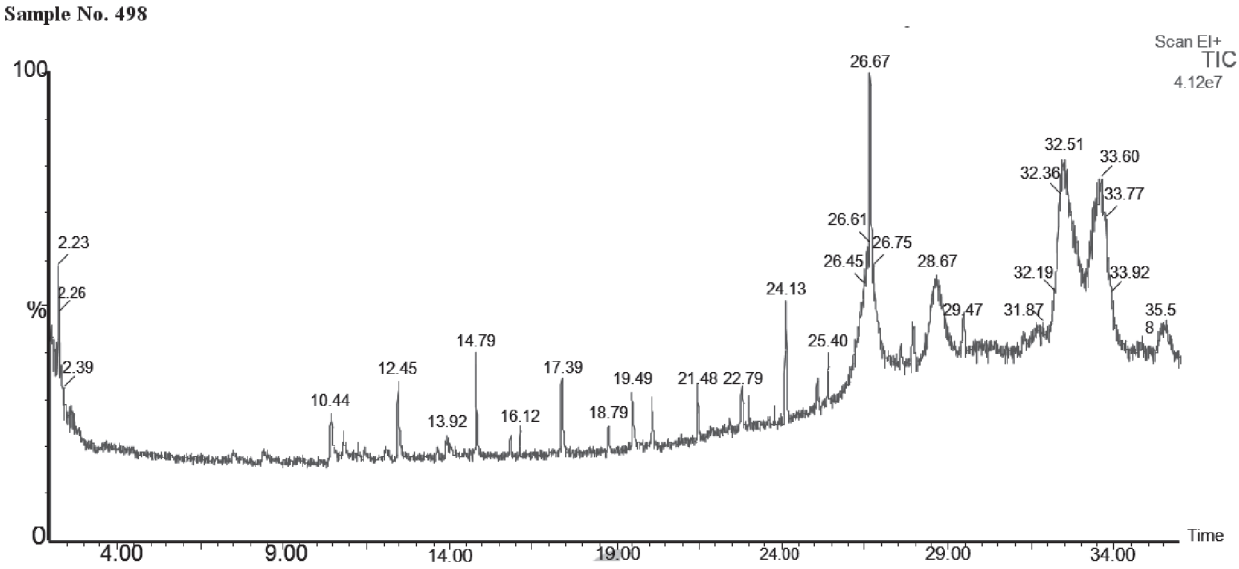

Fig. 1. GC-MS Chromatogram of *J. sambac* extract (soxhlet extraction) at 10.00 am

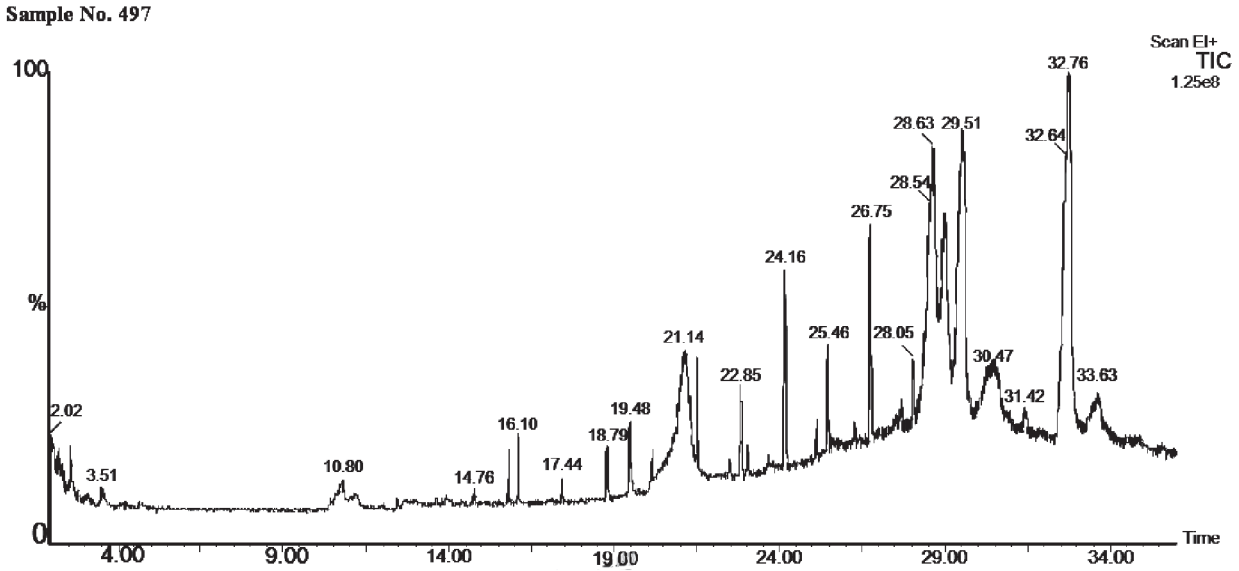

Fig. 2. GC-MS Chromatogram of *J. sambac* extract (soxhlet extraction) at 08.00 pm

Table 5. GC-MS analysis of *J. sambac* extract (soxhlet extraction) at 10.00 AM

RT	Name of the compound	Molecular Formula	MW	Peak Area (%)
2.23	2,6-Octadien-1-ol, 3,7 dimethyl-(Z)- (cis-Geraniol)	$C_{10}H_{18}O$	154	14.24
10.44	2-Aminononadecane	$C_{19}H_{41}N$	283	1.23
10.82	Cyclooctyl alcohol	$C_8H_{16}O$	128	0.57
12.45	1-Tetracosanol	$C_{24}H_{50}O$	354	1.51
13.92	1-Methyldodecylamine	$C_{13}H_{29}N$	199	0.38
14.76	2,4,6,8-Tetramethyl-1-undecene	$C_{15}H_{30}$	210	1.49
15.80	Octodrine	$C_8H_{19}N$	129	0.26
16.10	Heptadecane, 2-methyl-	$C_{18}H_{38}$	254	0.35
17.39	1-Hexacosanol	$C_{26}H_{54}O$	382	1.06
18.79	Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$	296	0.31
19.49	Didodecyl phthalate	$C_{32}H_{54}O_4$	502	1.07
20.09	Decane, 2,3,5,8-tetramethyl-	$C_{14}H_30$	198	0.76
21.48	6H-Pyrazolo[1,2 a][1,2,4,5] tetrazine, hexahydro-2,3-dimethyl-	$C_7H_{16}N_4$	156	0.67
22.44	2-Nonen-1-ol	$C_9H_{18}O$	142	0.17
22.79	Octadecane, 6-methyl-	$C_{19}H_{40}$	268	0.76
23.01	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl- (Farnesol)	$C_{15}H_26O$	222	0.37
23.79	1-Eicosanol	$C_{20}H_{42}O$	298	0.09
24.13	Nonadecane, 2-methyl-	$C_{20}H_{42}$	282	1.54
25.40	Tetracontane, 3,5,24-trimethyl-	$C_{43}H_{88}$	604	0.86
26.67	Nonadecane	$C_{19}H_{40}$	268	17.41
27.95	Octadecane, 1-(ethenyloxy)-	$C_{20}H_{40}O$	296	0.94
28.67	1-Octadecyne	$C_{18}H_{34}$	250	2.53
29.47	Z,Z-2,5-Pentadecadien-1-ol	$C_{15}H_{28}O$	224	0.92
32.51	9-Octadecenoic acid (Z)-, phenylmethyl ester (Benzyl oleate)	$C_{25}H_{40}O_2$	372	24.04
33.60	Eicosanoic acid, phenylmethyl ester (Benzyl icosanoate)	$C_{27}H_{46}O_2$	402	26.47

Twenty eight constituents were identified as active principles in the jasmine samples taken at 8 pm. Major are, 9-Octadecenoic acid (Z)-, phenylmethyl ester (Benzyl oleate) (21.01%), 1-Octadecyne (15.35%) and Octadecanoic acid, phenylmethyl ester (Benzyl stearate) (14.04%). The jasmine volatile compounds responsible for its unique fragrance were released during this time.

The study on ideal harvesting stage for *Jasminum* sp. fresh flowers revealed that the E-nose instrument aids in identifying the ideal harvesting stage for fresh flower stage and suitable time for concrete extraction. This ultimately helps in industrial utility to identify the perfect stage and time for higher concrete recovery. Considering the bulk of the components eluted under these chromatographic conditions, and assuming these compounds possesses a response for fragrance exclusive for jasmine species. The identified constituents from jasmine concrete were responsible for their unique fragrance.

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Table 6. GC-MS analysis of *J. sambac* extract (soxhlet extraction) at 08.00 PM

RT	Name of the compound	Molecular Formula	MW	Peak Area (%)
2.21	2,6-Octadien-1-ol, 3,7 dimethyl-(Z)- (cis-Geraniol)	$C_{10}H_{18}O$	154	3.50
2.58	1-Octanol, 2,7-dimethyl-	$C_{10}H_{22}O$	158	3.01
3.51	Cyclopropyl carbinol	C_4H_8O	72	3.83
10.80	Cyclooctyl alcohol	$C_8H_{16}O$	128	1.44
14.76	2,4,6,8-Tetramethyl-1-undecene	$C_{15}H_{30}$	210	0.15
15.80	Octodrine	$C_8H_{19}N$	129	0.42
16.10	Heptadecane, 2-methyl-	$C_{18}H_{38}$	254	0.54
17.44	1-Hexacosanol	$C_{26}H_{54}O$	382	0.22
18.79	Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$	296	0.42
19.48	Didodecyl phthalate	$C_{32}H_{54}O_4$	502	0.96
20.16	Decane, 2,3,5,8-tetramethyl-	$C_{14}H_30$	198	0.62
21.14	Octadecanoic acid, phenylmethyl ester (Benzyl stearate)	$C_{25}H_{42}O_2$	374	14.04
21.51	6H-Pyrazolo[1,2 a][1,2,4,5] tetrazine, hexahydro-2,3-dimethyl-	$C_7H_{16}N_4$	156	1.37
22.48	2-Nonen-1-ol	$C_9H_{18}O$	142	0.22
22.85	Octadecane, 6-methyl-	$C_{19}H_{40}$	268	0.93
23.03	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl- (Farnesol)	$C_{15}H_26O$	222	0.31
23.66	1-Eicosanol	$C_{20}H_{42}O$	298	0.33
24.16	Nonadecane, 2-methyl-	$C_{20}H_{42}$	282	2.00
25.46	Tetracontane, 3,5,24-trimethyl-	$C_{43}H_{88}$	604	1.08
26.75	Nonadecane	$C_{19}H_{40}$	268	2.41
28.05	Octadecane, 1-(ethenyloxy)-	$C_{20}H_{40}O$	296	0.86
28.63	1-Octadecyne	$C_{18}H_{34}$	250	15.35
28.99	Dodeca-1,6-dien-12-ol, 6,10-dimethyl-	$C_{14}H_{26}O$	210	7.83
29.51	Z,Z-2,5-Pentadecadien-1-ol	$C_{15}H_{28}O$	224	13.22
30.47	Heptadecanoic acid, heptadecyl ester	$C_{34}H_{68}O_2$	508	2.41
31.42	1,4-Dioxaspiro[4.5]decane, 8-(methylthio)-	$C_9H_{16}O_2S$	188	0.39
32.76	9-Octadecenoic acid (Z)-, phenylmethyl ester (Benzyl oleate)	$C_{25}H_{40}O_2$	372	21.01
33.63	Eicosanoic acid, phenylmethyl ester (Benzyl icosanoate)	$C_{27}H_{46}O_2$	402	1.13

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