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# Spatial variation of volatile composition in musk melon (*Cucumis melo* L.) flowers and their cues as bee attractant

**Praghadeesh Manivannan, Sonairajan Thangaraj, Selvaraju P and Mohankumar Subbarayalu**

**Abstract**
Flowering plants exhibits various morphological and olfactory cues to attract pollinators to visit and to promote pollination effectively. Among the signaling mechanisms, olfactory cues and honey substances play a crucial role for attraction of honeybees to flowers. The floral parts producing the volatile volatile compounds are considered as the chief source of olfactory cues. The presence of volatile compounds across the floral parts are diverse in nature. Thus the identification and spatial variation of volatile compounds across floral parts is important for better understanding of interaction between honeybees and flowering plants and also helpful to synthesis the effective pheromones to attract more honeybees and to enhance production. Hence, the present study was focused to assess the spatial variation of volatile compounds in muskmelon (*Cucumis melo* L.) and their influence on attraction of honeybees. Fully bloomed male and female flowers of muskmelon were collected from experimental plots and segregated in the sub groups of male (whole, petals and stamen) and female (whole, petals and pistils) for further processing. Volatile compounds were extracted using soxhlet based approach followed by analysis in Gas chromatography Mass Spectrometry (GC-MS). Around twenty-three volatile compounds were identified across the male and female floral parts of muskmelon. Among the compounds, tetradecanal was found only in male flowers and the compounds 2,6-Octadienal 3,7-dimethyl, β-Cyclocitral and 2-phenylethanol were observed in female flowers. Interestingly, the compound 1-Limonene was observed in the petals of both male and female flowers in trace quantity (0.4 percent peak area) and the same was not detected in whole flower extract and the reproductive structures of both the sexes. Methyl geranate and 1-Nonanol were the only compounds found specifically in reproductive structures. In addition to referencing the twenty-three identified compounds against Golms Metabolome Database, the biological activity of the volatile compounds identified from the present study were assessed using PASS (Prediction of activity spectra of substances) prediction.

**Keywords:** spatial variation, volatile compounds, muskmelon, honeybees, GCMS, AMDIS

## Introduction
Insect pollination of flowering plants is a process of significant importance in terrestrial environment and it provides vital ecosystem services for human well-being such as crop production [1]. Thus the attraction of pollinators towards flowering plants is essential for increasing the production of crops that are often facilitated by diverse signals *viz.*, olfactory, visual and nectarine vis-à-vis pollen rewards. The interaction promotes mutual benefits between partners wherein, flowers gets increased pollination and pollinators accumulates pollen and nectar for the survival of the colony. Among the different signals evinced by flowers, olfactory signals play a major role in endorsing the attraction of bees. Among different flowering plants, honeybees are found particularly attracted towards cucurbits crops owing to the floral anatomy and pollen cum nectar rewards. Musk melon (*Cucumis melo* L.) crop owing to their typical cucurbit flower anatomy and as ominously preferred choice by different bee species, can be used as a model system to study the spatial variation of volatile compounds in flower anatomy eg. Whole flower, petals and reproductive structures. In addition, cucurbits vis-à-vis musk melon has unique flower anatomy *ie.*, Imperfect flowers (flowers are either male or female), Monoecious (different sexes of flower on same plant), radially symmetric, epigynous, 5 petals (fused or free), 5 sepals, 5 stamens in staminate flower, Pistillate flower contains single style, 3 stigmas and inferior ovary [2], that qualifies them as perfect example to study spatial variations in volatile compounds. Improvements in floral scent

extraction procedures and employing appropriate solvents for extraction, has greatly increased the efficiency of capturing volatile compounds *in situ* without major variations. Over the past two decades, the technological advancement in identifying and quantifying metabolites and volatile compounds with higher degree of precision *viz.*, Gas Chromatography, head space sampling, solid phase micro extraction (SPME), help to deduce their role in plant pollinator interactions. Further, knowledge on ever increasing library of compounds coupled with mass spectrum database, enables precise identification and quantification of floral volatile compounds with greater degree of efficiency and accuracy. Floral biology, pollination, floral volatile compounds and flowering phenology in flowers were extensively studied in many crops species *viz.*, orchids [3–6]; *Brassica rapa* [7]; *Salix caprea* [8]; Butterfly bush (*Buddleja*, Scrophulariaceae) [9] and Lilium [10, 11]. The flower can be divided into components intrinsically based on their roles viz., calyx, corolla, sepals, petals, stamen, pistil etc. each having its contribution in terms of attracting pollinators. In this study, we attempted to study the localization of volatile cues present in different flower component and its diversity using musk melon flowers as a model system. Muskmelon flowers being imperfect and monoecious flowering nature, can be easily segregated into different experimental setup and presents opportunity to study localized volatile emission effectively. This study is aimed to enumerate and categorize different floral volatiles emanating from different parts of the musk melon flowers and cross validating across male and female flowers.

## Materials and Methods

### Sampling of floral parts

Fully bloomed male and female flowers of musk melon was collected from the experimental plots located at the orchard, Tamil Nadu Agricultural University, Coimbatore. The sampling of flowers was carried out during 5.30 AM to 6.30 AM. Caution was excised in collecting and segregating male and females flowers without any admixtures and cross exposure, that would potentially impact the volatile compounds in different subgroups. Field collected male and female flowers were preserved separately in plastic sample covers and transferred to laboratory for further processing. Flowers were segregated into six experimental categories *viz*., Female flower (whole flower, petals, pistils) and Male flowers (whole flower, petals and stamens). Samples and volatile compound extraction was performed on five consecutive days to obtain true representation of the volatile compounds.

### Extraction of volatile compounds

To assess the spatial variation of volatile compounds from the floral parts of male and female flowers, the floral components were divided into three categories in male and female flowers of muskmelon. To ensure optimal representation of volatile compounds in all the experimental setup 10 gm of each sample units which amounts, five whole flowers, petals from five flowers and reproductive parts from ten flowers were manually dissected and sorted in both male and female flowers. The floral parts were transferred to Whatman® high performance cellulose extraction thimbles and subjected to solvent extraction using SOCS PLUS Two Phase automatic solvent extraction system (Model SCS8AS; Make: Pelican Equipments, India) with anhydrous Hexane HPLC grade (Merck) as solvent at the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore. The samples in the thimbles were subjected to 50 cycles of extraction at 70ºC resulted in 30 ml of the solvent containing the volatile compounds. The extracts were further concentrated to 5 ml using Rota Vac (Model- R150; Make: Superfit Laboratory Instruments) and the samples were preserved in air-tight amber glass vials at 4ºC until GCMS analysis.

### GC-MS data acquisition for volatile analysis

The identification of volatile compounds was performed in Gas Chromatography combined with mass spectroscopy at the Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore. The processed samples were injected into GC injection port (AI3000 II, Thermo Fischer Scientific, USA) connected with a GC/MS (TRACE™ GC Ultra) with DSQII Single Quadrupole mass spectrometer equipped with non-polar capillary column of 30 cm in length, 0.25 mm diameter and 0.25 µm film thickness. The injection port temperature was maintained at 260 °C, the oven temperature was programmed at 80 °C (2 min hold) and increased at the rate of 10 °C/min to 200°C (1 min hold) and then to 260ºC at 5ºC/min rate (10 min hold). Helium (99.99%) was used as a carrier gas at a flow rate of 1 ml/s. One microlitre of the extract was injected using auto sampler into the GC-MS system for analysis. Injections were done in split 10:1 mode. The electron ionization source temperature was maintained at 260 °C and the abundances of ions in the range of 50–650 were scanned at a rate of 1.1 scans/s.

### Processing of chromatographic data

Raw data was generated using X-Caliber m/z software package. Injected samples were separated into various constituents with different retention time and detected by mass spectrophotometer. The compounds of interest were identified using standard NIST MS 2 (National Institute of Standards and Technology - Mass Spectral) library with more than 62,000 patterns [12]. From the GC/MS output, deconvolution of peaks, extraction of the baseline corrected mass spectra and identification of the retention time of every component was carried out using AMDIS software (Automated Mass Spectral Deconvolution and Identification System) [13, 14]. The major mass spectral fragments of each peak across the samples were manually verified for their consistency using retention time as a reference. The mass spectral tags (MSTs) in each of the replicate were compared with the five best matches and Golms Metabolome Database (http://csbdb.mpimp-olm.mpg.de/csbdb/gmd/gmd.html) and the best spectral match was selected and metabolite name was assigned. The low probability matches were designated as unidentified and not inducted in further analysis. The chromatogram of the six samples were processed for visual representation using MZmine2 GUI version [15, 16]. The probability and percent peak area of the compounds in each sample set was compared and inference were drawn on the abundance of volatile compounds in each sample set.

### Prediction of biological activity of phytochemicals

In order to assess the biological activity of volatile compounds identified in the present study, the molecular structures (MOL format) of the compounds were retrieved from the chemical book database (www.chemcialbook.com) and the structures were fed to biological activity predication tool, PASS ver.9.1 (Prediction of activity spectra of substances) [17, 18] as well as from the published literatures. PASS prediction estimates the predicted activity spectrum of

a compound as probable activity (Pa) and probable inactivity (Pi). Prediction of this spectrum by PASS is based on qualitative structure-activity relationships (SAR) analysis of the training set containing more than 205,000 compounds exhibiting more than 3750 kinds of biological activities [19, 20].

**Results and Discussion**

It is important to study the volatile compounds, their quality and quantity in floral components. These factors determine the foraging behavior of pollinators and which results in mutual benefit to both plants in terms of pollination and to insects in terms of food reward. Schiestl and Johnson [21] reviewed that, quantity of floral rewards in terms of olfactory signals of plant species has important consequences for pollinator mediated foraging activities and such signals would help to establish constancy and fidelity to source among the pollinators. Further, many studies have been conducted demonstrating that honeybees use honest floral signals (quality and quantity) as a yardstick for pollen reward during foraging [7].

**GC-MS raw data processing for metabolome analysis**

The spatial variation of volatile compounds in the floral parts of muskmelon was analysed through GCMS. The resulting chromatogram consisting of overlapping peak from six samples derived using MZmine2 showed peaks (Fig.1a). The overlay of individual chromatogram depicting the GCMS output from female and male flower components were presented in Fig 1b and 1c. There were many peaks that are overlapping across the whole flowers, petals and reproductive structures in both female and male flowers. The base peak chromatogram was created encompassing the maximum possible m/z range of 50 to 650 using MZmine2. The female floral parts, particularly whole flowers and petals resulted high noise level that masked the comparative outcome of samples against male flowers. Subjecting the samples to derivatization using N,O-bis-(trimethyl silyl) tri fluoro acetamide (BSTFA) or n-trimethylsilylimi-dazole (TMSI), Pentafluorobenzyl bromide (PFBBr) and Pentafluorobenzyl-hydroxylamine hydrochloride (PFBHA) may have decreased the noise ratio and increase the resolution of identified compounds. Appropriate derivatization process would render samples containing highly polar or containing functional groups with active hydrogens such as –NH, SH, -COOH, -OH to be sufficiently volatile and in turn can be eluted at reasonable temperatures without losing the compounds in thermal decomposition [22].

The raw data generated from Xcalibur (preloaded with NIST Library) resulted in a total of 45, 40 and 13 compounds in Whole flower, petals and pistils of female respectively. Similarly, whole flower, petals and stamens of male flowers resulted 39, 30 and 10 volatile compounds respectively. The raw data was subjected to spectral deconvolution in AMDIS using the parameters (First scan: 20, Last scan: 88 with maximum range of deconvolution of 64 scans) [23] and the output was referenced with open source GOLM's Metabolome library (available at: http://csbdb.mpimp-olm.mpg.de/csbdb/gmd/gmd.html). Deconvolution is a crucial step in GC-MS data processing, which involves computationally separating compounds that have similar mass spectrum that would have co-eluted in normal conditions into pure spectrum of individual compounds [24].

**Qualitative comparison**

Qualitative analysis on spatial variation of volatile compounds in female vs male flowers and their individual flower component with reference to GOLM library were depicted in venn diagram (Fig.2). A total of 23 volatile metabolites identified from the current study. The final dataset with targeted search from GOLM library, identified 21 and 19 volatile compounds from the whole female and male flowers of muskmelon respectively, of which 18 volatile metabolites were found in both male and female flowers. Tetradecanal was found in male flowers alone with a per cent peak area of 0.678 and probability of 21.0 per cent. Similarly, the metabolites 2,6-Octadienal 3,7-dimethyl, β-cyclocitral and 2-phenylethanol were found only in female flowers with a per cent peak area of 0.522, 0.410 and 0.624 respectively. The number of volatile metabolites that are found separated spatially in individual flower components in female and male flowers were also represented in the form of venn diagram. The characteristics features of compounds including chemical structure, molecular formula, molecular weight, Kovats retention index were enumerated and presented in table 1.

**Diversity in localization of volatile metabolites in six floral components**

The comparative qualitative analysis was performed across the female flower (whole flower, petals, pistils) and male flowers (whole flower, petals and stamens). The per cent peak area of the twenty-three compounds in all sample types ranged from 0 to 2. Datasets obtained by targeted searching in GOLM library and their percent area was represented in the form of heat-map (Fig. 3). The volatile metabolites with most prominence vis-à-vis highest per cent area are denoted by green and the least prominence vis-à-vis lowest per cent area are denoted by red. The volatile metabolites profile observed between whole flowers and petals of female flowers are almost similar and with minor degree of variation in per cent area. Compounds *Cis*-3-Hexenol, 2,6-Octadienal 3,7-dimethyl, 2-phenylethanol and docosanoin were found only in female whole flowers and were absent in female petals. Alternatively, 1-Limonene was found only in female petals in trace quantity based on 0.4 per cent peak area, which indicates that it is exhibited only in petals. Similar floral volatile compound localization study conducted by Knauer and Schiestl [7], in *Brassica rapa* inflorescence demonstrated that limonene was found to be predominantly exhibited by filament (Male flower) and inflorescence and reportedly absent in female reproductive organs like pistils and receptacle. It is evident from our findings that, the reproductive structures (pistils and stamens) does not contribute significantly towards volatile compound emission. Further, their study demonstrated that phenylacetaldehyde, acetophenone, p-anisaldehyde and α-farnesene play major role in eliciting increased response from pollinators.

In contrast to volatile metabolite profile of female flowers, the number of differentially localized compounds between male whole flowers and male petals are high with 9 unique compounds (Tetradecanal, *Cis*-3-Hexenol, Dihydro-β-ionone, 2,6-Octadienal 3,7-dimethyl, β-Cyclocitral, 3-Hexanone 2,5-dimethyl-4-nitro, Methyl octaonate, Methyl benzoate, α-Terpinolene, 1-Butene-4-isothiocyanate, 1-Ethoxy-pentane and Docosanoin) present in whole flower extract only. Strikingly, similar to the female flowers, volatile metabolite 1-Limonene is found only in male petals (per cent area: 0.390) and absent male whole flowers (Table 2). The results are similar to the floral volatile study carried out in *Xanthosoma sp*. (Araceae), that demonstrated that compounds like Benzenepropyl acetate, Methyl geranate, β-Cyclocitral,

Benzyl acetate, 2-phenylethanol, 1-Nonanol, Methyl benzoate, α-Terpinolene, Phenyl acetaldehyde, Benzaldehyde are found in floral structures [25]. In general, the number of volatile metabolites emanating reproductive structures viz. pistils and stamens are relatively less when compared to that of the whole flower and petal counter parts. Methyl geranate and 1-Nonanol were the only compounds found in pistils and similarly, stamens showed the presence of 1-Nonanol only.

**Prediction of biological activity of volatile compounds using PASS**

The potential biological activity at *in vivo* conditions of the twenty-three compounds were predicted using PASS. The structural formula for the compounds were retrieved in MOL format and are queried in against PASS spectral database. The predicted biological functions of the volatile metabolites majorly relates to Sugar- Phosphate inhibitor and other functions like glutarate-semialdehyde dehydrogenase inhibitor, prenyl-diphosphatase inhibitor, gluconate 2-dehydrogenase inhibitor, muco-membranous protector, G-protein-coupled receptor kinase inhibitor, benzoate 4-monooxygenase inhibitor, glutamate-5-semialdehyde dehydrogenase inhibitor, alcohol dehydrogenase substrate, aspartate–phenylpyruvate transaminase inhibitor (Table 1). GC-MS analysis coupled with PASS prediction has been widely used tool to predict the function of compounds sourced from various plants and to exploit the information in chemical ecology, pharmaceuticals and medicinal crop research [26, 27].

**Conclusion**

This study is the first detailed analysis of spatial variation of volatile compounds across different floral parts of muskmelon and their influence on attraction of honeybees towards muskmelon. Interactions between flowers and pollinators are dynamic and happens in relatively short time span. Provided, pollinators at any given point of time are presented with multiple types and varied quality of reward signals constituting colour, shape, nectarine and volatile compounds, of which volatile compounds emanating from flowers plays paramount role in positively influencing the pollination process. Among the floral parts of muskmelon, petals of both the sexes were identified as the chief source of volatile compounds in comparison to the reproductive structures in both male and female flowers. Around twenty-three compounds were identified across the sexes. However, the variation of volatile compounds was also observed between male and female flowers. In addition, the compounds identified through this study will serve as a repository of reference for future studies and can be effectively incorporated in producing commercial lures and attractants. The result of the present study could help to synthesis the effective pheromones to attract honeybees to enhance the production of muskmelon. In addition, it would be also helpful for better understanding the interactions between honeybees and muskmelon.

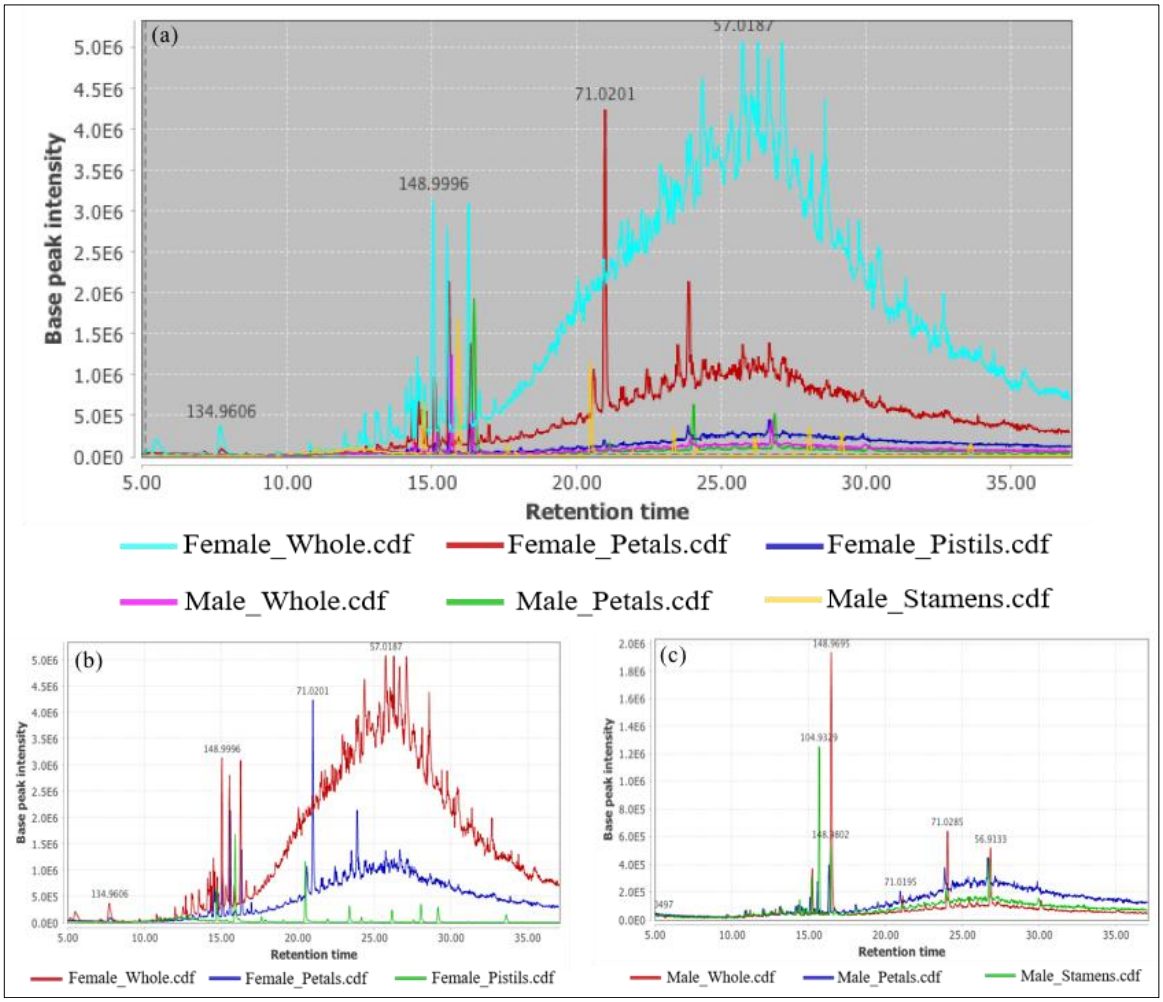



**Fig 1:** Base peak chromatogram (m/z range: 50 to 650) generated through MZmine2. (a) Combined chromatogram of six samples. (b) Female flower components. (c) Male flower components

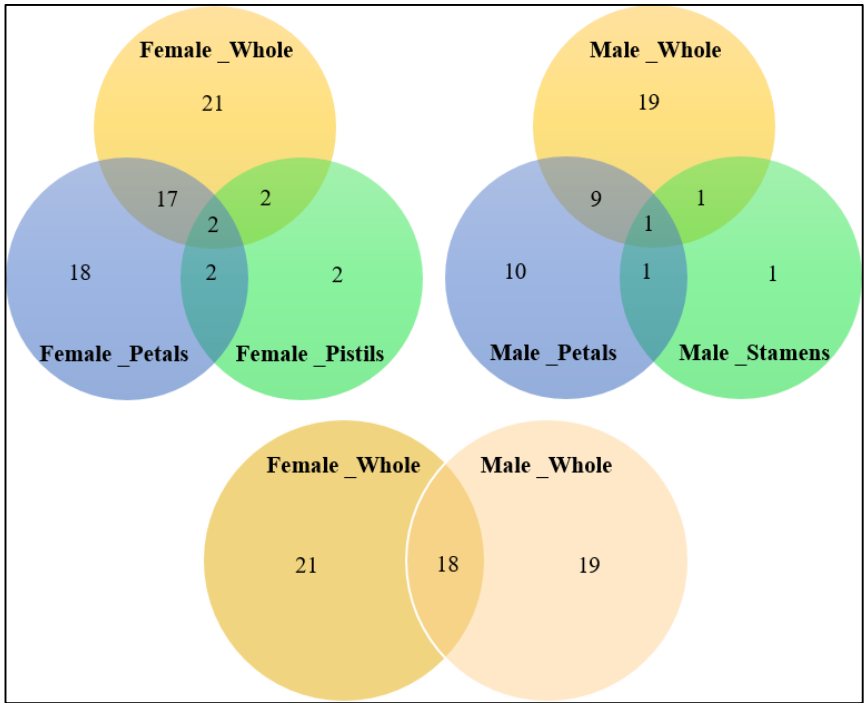



**Fig 2:** Venn diagrammatic representation of number of overlapping compounds across test samples

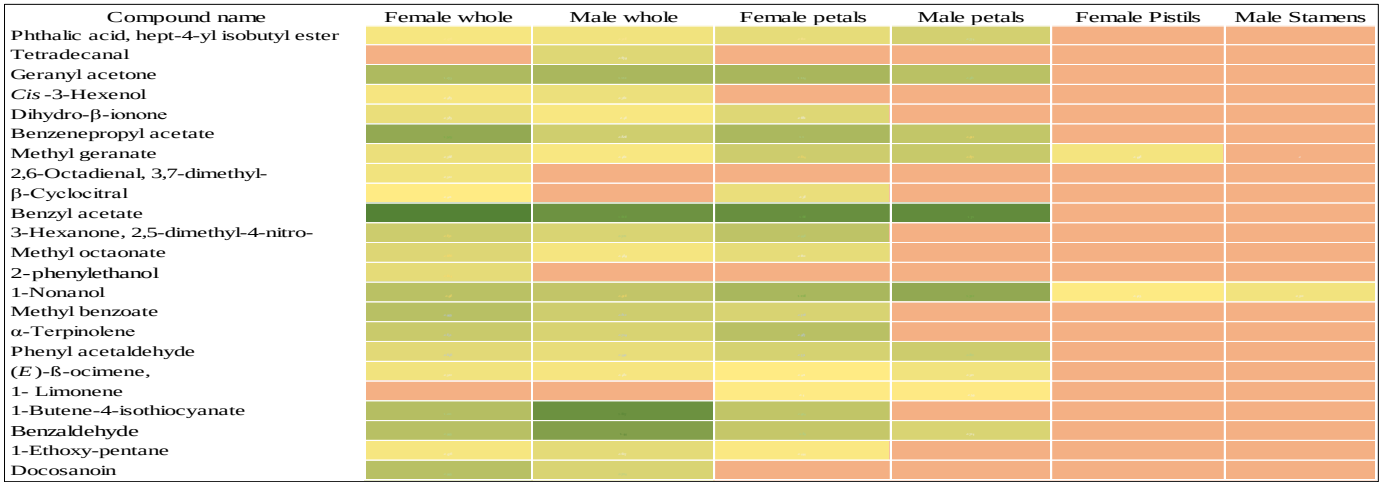
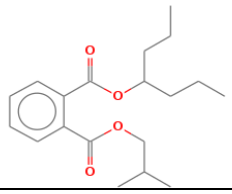



**Fig 3:** Heatmap diagram representation of area percent of compounds identified from GOLM library across test samples

**Table 1:** List of compounds from the GCMS datasets referenced against GOLM library and their biological activity prediction using PASS

| S. No. | Compound Name | Chemical Structure | Molecular Formula | Molecular Weight | Kovats Retention Index | PASS Prediction |
|---|---|---|---|---|---|---|
| 1. | Phthalic acid, hept-4-yl isobutyl ester |  | $C_{19}H_{28}O_4$ | 320.4232 | 2085 | Sugar- Phosphatase inhibitor |
| 2. | Tetradecanal |  | $C_{14}H_{28}O$ | 212.3715 | 1601 | Glutarate-semialdehyde dehydrogenase inhibitor |
| 3. | Geranyl acetone |  | $C_{13}H_{22}O$ | 194.3132 | 1447 | Prenyl-diphosphatase inhibitor |

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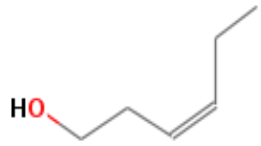
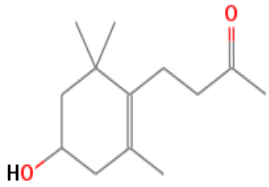
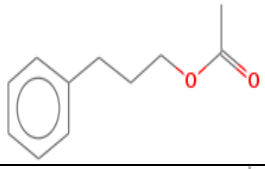
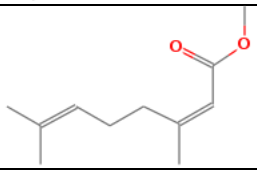
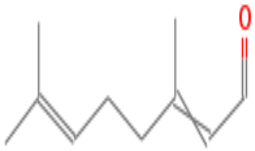
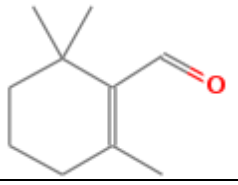
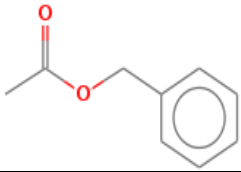
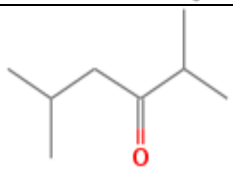
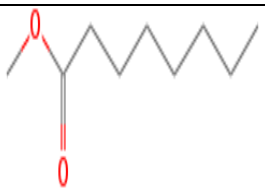
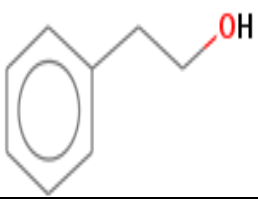
|---|---|---|---|---|---|---|
| 4. | *Cis*-3-Hexenol |  | $C_6H_{12}O$ | 100.1589 | 1389 | Sugar-phosphatase inhibitor |
| 5. | Dihydro-β-ionone |  | $C_{13}H_{22}O_2$ | 210.3126 | 1373 | Gluconate 2-dehydrogenase inhibitor |
| 6. | Benzenepropyl acetate |  | $C_{11}H_{14}O_2$ | 178.2277 | 1359 | Sugar – Phosphate inhibitor |
| 7. | Methyl geranate |  | $C_{11}H_{18}O_2$ | 182.2594 | 1302 | Muco-membranous protector |
| 8. | 2,6-Octadienal, 3,7-dimethyl- |  | $C_{10}H_{16}O$ | 152.2334 | 1239 | G-Protein-coupled receptor kinase inhibitor |
| 9. | β-Cyclocitral |  | $C_{10}H_{16}O$ | 152.2334 | 1221 | Benzoate 4-monooxygenase inhibitor |
| 10 | Benzyl acetate |  | $C_9H_{10}O_2$ | 150.1745 | 1160 | Sugar-phosphatase inhibitor |
| 11. | 3-Hexanone, 2,5-dimethyl-4-nitro- |  | $C_8H_{16}O$ | 128.2120 | 1145 | Gluconate 2-dehydrogenase inhibitor |
| 12. | Methyl octaonate |  | $C_9H_{18}O_2$ | 158.2380 | 1126 | Prediction unavailable |
| 13. | 2-phenylethanol |  | $C_8H_{10}O$ | 122.1644 | 1120 | Sugar-phosphate inhibitor |
| 14. | 1-Nonanol |  | $C_9H_{20}O$ | 144.2545 | 1173 | Sugar-phosphatase inhibitor |

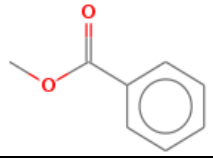
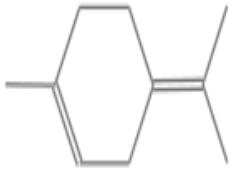
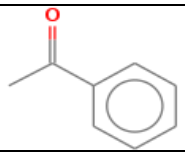
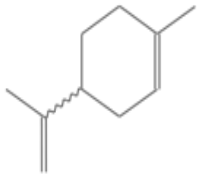
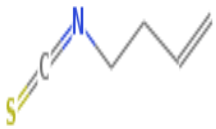
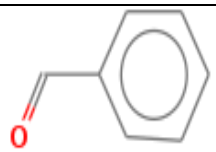
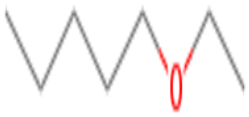
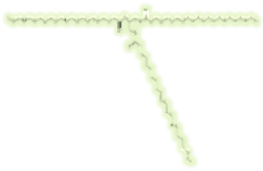
| | | | | | | |
|---|---|---|---|---|---|---|
| 15. | Methyl benzoate |  | $C_8H_8O_2$ | 136.1479 | 1088 | Sugar-phosphatase inhibitor |
| 16. | α-Terpinolene |  | $C_{10}H_{16}$ | 136.2340 | 1079 | Glutamate-5-semialdehyde dehydrogenase inhibitor |
| 17. | Phenyl acetaldehyde |  | $C_8H_8O$ | 120.1485 | 1065 | Sugar-phosphatase inhibitor |
| 18. | (*E*)-ß-ocimene, |  | $C_{10}H_{16}$ | 136.2340 | 1050 | Prediction unavailable |
| 19. | 1- Limonene |  | $C_{10}H_{16}$ | 136.2340 | 1009 | Alcohol dehydrogenase substrate |
| 20. | 1-Butene-4-isothiocyanate |  | $C_5H_7NS$ | 113.181 | 973 | Mucomembranous protector |
| 21. | Benzaldehyde |  | $C_7H_6O$ | 106.1219 | 957 | Aspartate–phenylpyruvate transaminase inhibitor |
| 22. | 1-Ethoxy-pentane |  | $C_7H_{16}O$ | 116.2013 | 751 | Sugar- Phosphatase inhibitor |
| 23. | Docosanoin |  | $C_{69}H_{134}O_6$ | 1059.7987 | NA | Sugar- Phosphatase inhibitor |

**Table 2:** List of compounds from the GCMS datasets referenced against GOLM library and their biological activity prediction using PASS

| S. No | Compound Name | Molecular Formula | Molecular Weight | Kovats Retention Index | PASS Prediction |
|---|---|---|---|---|---|
| 1. | Phthalic acid, hept-4-yl isobutyl ester | $C_{19}H_{28}O_4$ | 320.4232 | 2085 | Sugar- Phosphatase inhibitor |
| 2. | Tetradecanal | $C_{14}H_{28}O$ | 212.3715 | 1601 | Glutarate-semialdehyde dehydrogenase inhibitor |
| 3. | Geranyl acetone | $C_{13}H_{22}O$ | 194.3132 | 1447 | Prenyl-diphosphatase inhibitor |
| 4. | *Cis*-3-Hexenol | $C_6H_{12}O$ | 100.1589 | 1389 | Sugar-phosphatase inhibitor |
| 5. | Dihydro-β-ionone | $C_{13}H_{22}O_2$ | 210.3126 | 1373 | Gluconate 2-dehydrogenase inhibitor |
| 6. | Benzenepropyl acetate | $C_{11}H_{14}O_2$ | 178.2277 | 1359 | Sugar – Phosphate inhibitor |
| 7. | Methyl geranate | $C_{11}H_{18}O_2$ | 182.2594 | 1302 | Muco-membranous protector |
| 8. | 2,6-Octadienal, 3,7-dimethyl | $C_{10}H_{16}O$ | 152.2334 | 1239 | G-Protein-coupled receptor kinase inhibitor |
| 9. | β-Cyclocitral | $C_{10}H_{16}O$ | 152.2334 | 1221 | Benzoate 4-monooxygenase inhibitor |
| 10 | Benzyl acetate | $C_9H_{10}O_2$ | 150.1745 | 1160 | Sugar-phosphatase inhibitor |
| 11. | 3-Hexanone, 2,5-dimethyl-4-nitro | $C_8H_{16}O$ | 128.2120 | 1145 | Gluconate 2-dehydrogenase inhibitor |
| 12. | Methyl octaonate | $C_9H_{18}O_2$ | 158.2380 | 1126 | Prediction unavailable |
| 13. | 2-phenylethanol | $C_8H_{10}O$ | 122.1644 | 1120 | Sugar-phosphate inhibitor |
| 14. | 1-Nonanol | $C_9H_{20}O$ | 144.2545 | 1173 | Sugar-phosphatase inhibitor |
| 15. | Methyl benzoate | $C_8H_8O_2$ | 136.1479 | 1088 | Sugar-phosphatase inhibitor |

| | | | | | |
|---|---|---|---|---|---|
| 16. | α-Terpinolene | $C_{10}H_{16}$ | 136.2340 | 1079 | Glutamate-5-semialdehyde dehydrogenase inhibitor |
| 17. | Phenyl acetaldehyde | $C_8H_8O$ | 120.1485 | 1065 | Sugar-phosphatase inhibitor |
| 18. | (*E*)-ß-ocimene, | $C_{10}H_{16}$ | 136.2340 | 1050 | Prediction unavailable |
| 19. | 1- Limonene | $C_{10}H_{16}$ | 136.2340 | 1009 | Alcohol dehydrogenase substrate |
| 20. | 1-Butene-4-isothiocyanate | $C_5H_7NS$ | 113.181 | 973 | Mucomembranous protector |
| 21. | Benzaldehyde | $C_7H_6O$ | 106.1219 | 957 | Aspartate –phenylpyruvate transaminase inhibitor |
| 22. | 1-Ethoxy-pentane | $C_7H_{16}O$ | 116.2013 | 751 | Sugar- Phosphatase inhibitor |
| 23. | Docosanoin | $C_{69}H_{134}O_6$ | 1059.7987 | NA | Sugar phosphatase inhibitor |

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