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Biosynthetic schema of volatile organic compounds produced in the flowers of *Jasminum sambac* (L.) Aiton

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Abstract
The fragrance of *Jasminum sambac*, 'Gundumalli', is specific to this species and it is characterised by various volatile organic compounds (VOCs). The construction of a biosynthetic pathway of these specific VOCs helps to comprehend the role of various precursors, enzymes and energy transfer. The article showcases the formation of the important VOCs in the flowers of *J. sambac* based on biosynthetic principles of metabolite production and the KEGG pathway database.

Keywords: *Jasminum sambac*, volatile organic compounds, fragrance, KEGG database

1. Introduction
Jasmine is a perennial flowering shrub, well-known for its fragrant and beautiful flowers. Of the many species of Jasmine, *Jasminum sambac* is widely cultivated for its unique fragrant essential oil. *J. sambac* L., belonging to Oleaceae, is known as Arabian Jasmine, Tuscan Jasmine, Gundumalli or Mogra, and is native to the regions of Himalayas and widely distributed across India. It produces bold, greenish-white to white flower buds, which are highly fragrant when fully opened. Jasmine is a late-blooming flower, where whole flower opening is observed in the late evening to midnight. *J. sambac* flowers fully bloom from 6 to 8 in the evening (De and Bhattacharjee, 2011) [1].
The fragrance emitted from flowers is attributed to a range of volatile organic compounds (VOCs) released from the flower petals. Opening of the petals is the principal activity, following which the floral scent is released. The VOCs are the secondary metabolites aiding in plant protection and pollination (Vivaldo *et al.*, 2017) [2]. Whereas to man-kind, they are industrially and economically relevant for perfumery, food additive and pharmacology needs.
Experimental studies where VOCs from *J. sambac* flowers are extracted through various methods like solid-phase micro-extraction (SPME) fibres (Issa *et al.*, 2020) [3], or by using solvents, followed by gas chromatography and mass-spectroscopy (GC-MS) (Yu *et al.*, 2017) [4] has helped to analyse and identify the VOCs discharged from the flower petals.
As the name suggests, VOCs are highly volatile (*i.e.*) these compounds possess vapour pressures higher than the room temperatures, along with very low boiling points (Muhlemann *et al.*, 2014) [5]. Once released, VOCs vaporize rapidly and the fragrance is soon lost. For commercial uses, VOCs are used in various formats wherein the VOCs are locked in or adjunct with compounds of lower vapour pressure to ensure that the aroma lasts longer.
A floral scent is not credited to one VOC alone; instead, it is a combination of many compounds in varying amounts (Piechulla *et al.*, 2010) [6]. The abundance and recurrence of certain VOCs within the species and related genera help determine the most characteristic VOCs for a particular flower.
In the flowers of *J. sambac*, the sweet and heady fragrance is characterised by the occurrence of terpenoids, benzenoids, esters, phenols and alcohols.

2. Materials and Methods
Floral volatiles (volatile organic compounds - VOCs) are the secondary metabolites of the plants. The VOCs of *J. sambac* have been collected from previous studies and reports. They widely consist of terpenoids, phenols, alcohols, and benzenoid esters. About 7 VOCs constitute the most vital and abundant compounds responsible for the characteristic jasmine fragrance and flavour. Based on the resulting VOCs, a biosynthetic pathway has been created utilizing the KEGG pathway database (Kanehisa, 2016) [7].

3. Results and Discussion

The anabolism of various metabolites involved in plant growth and development and defense mechanism in the presence of aiding enzymes and catalysts using energy is biosynthesis. The structure of the pathway of these metabolites formed from their precursor is the biosynthetic pathway. It is a vital tool to follow and demarcate the origin and progress or conversion of metabolites through various stages of growth. The biosynthetic pathway showcasing the formation of the VOCs in *J. sambac* was constructed using KEGG pathway database (Fig 1).

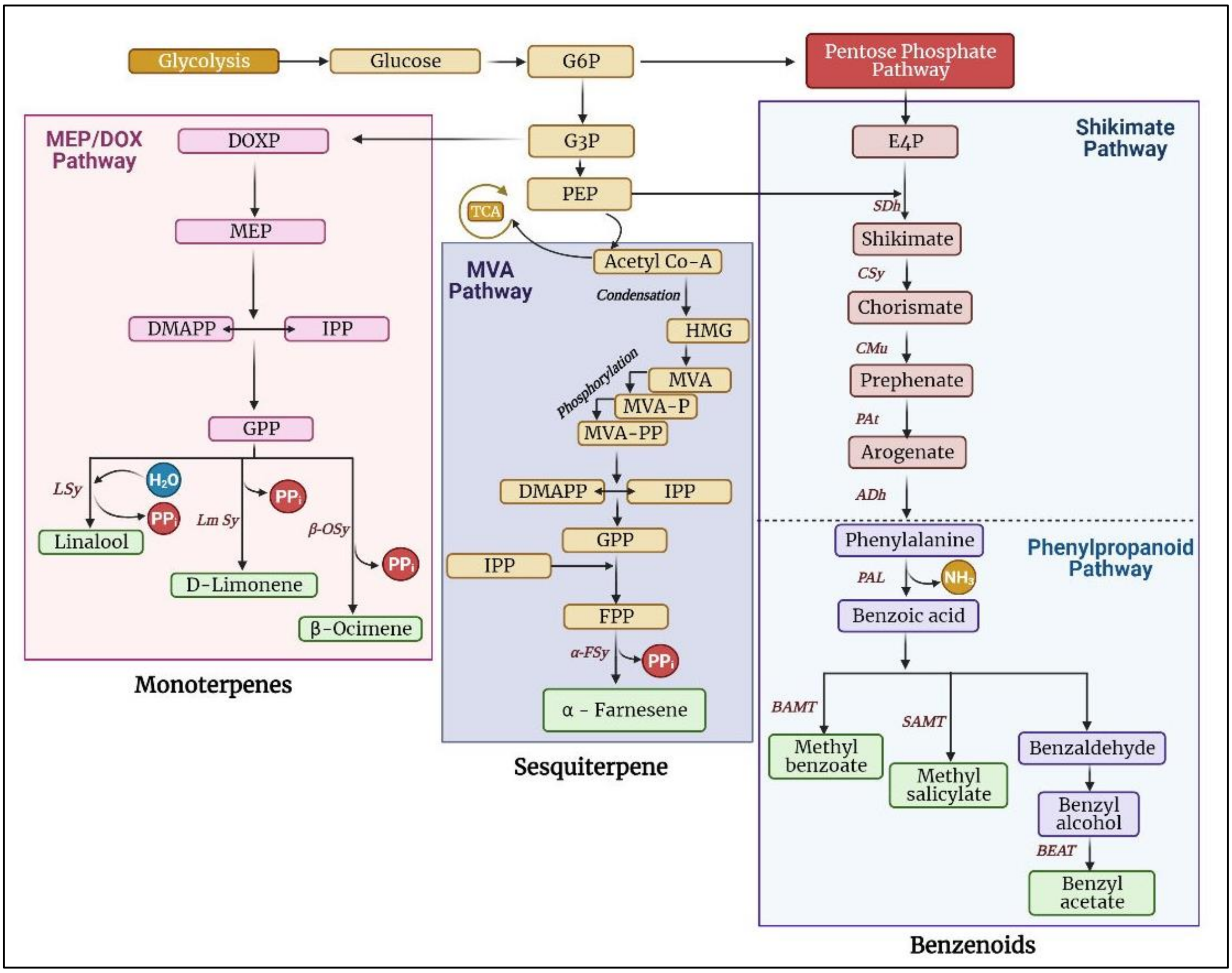
Glycolysis is the critical breakdown mechanism, where the stored assimilates from photosynthesis is catabolized to produces energy and various precursors for secondary metabolite production. Bera *et al.*, (2017) [8] had reported b -Ocimene, Linalool, D-Limonene and a-Farnesene as the major terpenoids in the VOCs of *J. sambac*. Terpenoids or isoprenoids are hydrocarbons, derived from iroprenes (five-carbon compounds). They are synthesized through the non-mevalonate [9] and mevalonate pathways [10].

The non-mevalonate pathway or the mevalonate-independent pathway, or the MEP pathway takes place in the plastids. Glyceraldehyde 3-phosphate from glycolysis reacts with pyruvate to form 1-deoxy-D-xylulose 5- phosphate (DOXP), and reduced to form 2-C-methyl-D-erthritol 4- phosphate (MEP). MEP on cyclic transformation and phosohorylation forms the isomers Isopentyl pyrophosohate (IPP) and Dimethyl ally pyrophosphate (DMAPP). IPP and DMAPP are the first formed isoprenes. The mevalonate pathway occurs in the cyotosol, where three molecules of Acetyl CoA formed at the end of glycolysis enter the tricarboxylic acid (TCA) cycle and condenses to form 3-hydroxy-3-methyl-glutaryl-CoA (HMG CoA). On reduction, HMG CoA forms mevalonate (MVA). MVA is phosphorylated twice to form mevalonate pyrophosphate (MVP-PP). By decarboxylation, MVA-PP forms the isomers IPP and DMAPP as seen in the MEP pathway. The polymerization of isoprenes forms successive classes of terpenoids with multiples of five carbon compounds. In *J. sambac*, monoterpenes and sesquiterpenes are among the significant VOCs.

b-Ocimene, Linalool, D-Limonene are monoterpenes formed by the addition of two units of isoprenes. They are ten carbon compounds, present in the floral scent of many plants including *J. sambac*. IPP and DMAPP from the MEP pathway in the plastid condenses to form the first monoterpene, the precursor, Geranyl pyrophosphate (GPP). *b-Ocimene* is an acyclic monoterpene, formed by the elimination of a pyrophosphate group from GPP in the presence of b -Ocimene synthase. Pragadeesh *et al.*, (2011) [11] first reported the presence of b -Ocimene in volatiles extraction of *J. sambac* through SPME technique. *Linalool* is the characteristic VOC of Jasminum. It is an acyclic monoterpenoid derived from GPP by depyrophosphorylation in the presence of water and Linalool synthase. *D-Limonene* is a cyclic monoterpene obtained from GPP through neryl carbocation cyclization after removal of a pyrophosphate group in the presence of D-Limonene synthase. The anti-inflammatory, antimicrobial and anti-cancerous properties of linalool and D-limonene were stated by Jiang *et al.*, (2015) [12] and Mukhtar *et al.*, (2018) [13]. a -Farnesene is a sesquiterpene comprising of three units of isoprenes, making it a fifteen-carbon compound. Most of the sesquiterpenes are formed in the cytosol through the MVA pathway. The IPP and DMAPP formed through the MVA pathway condense to form GPP in the cytosol. GPP joins with another molecule of IPP to form the three-isoprene compound Farnesyl pyrophosphate (FPP). FPP in the presence of a -Farnesene synthase loses a pyrophosphate group to form a-Farnesene. It is the most common and abundant sesquiterpene found in floral scent of *J. sambac*.

Bera *et al.*, (2017) [8] also reported that benzenoids and their esters, *viz.*, Methyl benzoate, Methyl salicylate and Benzyl acetate are also comprised in the VOCs emitted from *J. sambac*. Benzenoids, also known as Phenylpropanoid, are VOCs derived from the shikimate pathway [14], especially from the phenylalanine amino acid in the case of *J. sambac* floral scent. The shikimate pathway follows the reactions between Erythrose 4-phosphate (E4P) from pentosephosphate pathway and Phosphoenol pyruvate (PEP) from glycolysis. They undergo various reactions and in the presence of shikimate dehydrogenase enzyme, shikimate is formed. Chorsimate is synthesised from shikimate by the enzyme Chorsimate synthase. Chorsimate mutase converts Chorsimate into Prephenate, which is the presence of aminotransferase forms Arogenate. It dehydrates to form Phenylalanine. Further following the phenylpropanoid pathway, phenyalanine ammonia-lyase removes a molecule of ammonia from the amino acid to form benzoic acid. Three vital enzymes, Benzoic acid carboxyl methyl transferase (BAMT), Salicylic acid carboxyl methyl transferase (SAMT), and Benzyl alcohol acetyl transferase (BEAT) forms benzoic acid derivatives Methyl benzoate, Methyl salicylate and Benzyl acetate, respectively.

Bera *et al.*, (2015) [15] accounted that the fragrance of *J. sambac* is majorly contributed by the VOCs Linalool and Benzyl acetate



Compounds		Enzymes	
G6P	Glucose 6-phosphate	***LSy***	Linalool synthase
G2P	Glyceraldehyde 3-phosphate	***LmSy***	Limonene synthase
E4P	Erythrose 4-phosphate	***b-OSy***	b-Ocimene synthase
PEP	Phosphoenolpyruvate	***a-FSy***	a-Farnesene synthase
HMG	Hydroxyl methyl glutaryl CoA	***SDh***	Shikimate dehydrogenase
MVA	Mevalonic acid	***CSy***	Chorismate synthase
MVA-P	Mevalonic acid phosphate	***CMu***	Chorismate mutase
MVA-PP	Mevalonic acid pyrophosphate	***Pat***	Prephenate aminotransferase
DOXP	1-deoxy-D-xylulose 5-phosphate	***ADh***	Arogenate dehydratase
MEP	2-C-methyl-D-erythritol 4-phosphate	***PAL***	Phenylalanine ammonia lyase
DMAPP	Dimethyl allyl pyrophosphate	***BAMT***	Benzoic acid carboxyl methyl transferase
IPP	Isopentyl pyrophosphate	***SAMT***	Salicylic acid carboxyl methyl transferase
GPP	Geranyl pyrophosphate	***BEAT***	Benzyl alcohol acetyl transferase
FPP	Farnesol pyrophosphate		
TCA	Tricarboxylic acid cycle		
H_2O	Water		
NH_3	Ammonia		
PP_i	Pyrophosphate		

Fig 1: Biosynthetic pathway representing the formation of volatile organic compounds (VOCs) in *Jasminum sambac* flowers

4. Conclusion
In summary, the construction of a biosynthetic pathway elucidating the formation of chief VOCs in the flowers of *J. sambac* has revealed the essential precursors and enzymes involved in the biosynthesis. The VOCs showing pharmaceutical and aromatic properties are industrially important. A deeper understanding of its biosynthesis in flowers may help to establish processes of their production *in vitro*.

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