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GC-MS aided phytochemical profiling of Jasmine (*Jasminum* spp.) stem extracts

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Abstract

Plants have long been a vital source of raw materials for pharmaceuticals, providing essential resources for medicine over the years. This study specifically investigated the bioactive compounds in the stems of three novel genotypes of *Jasminum*, namely, Double Flower type of *J. sambac* (L.) *Aiton* (DF), White Flower Bud type of *Jasmine grandiflorum* L. (WF), and a new cultivar CO.1 Winter Jasmine of *Jasmine multiflorum* (Burm.f.) Andrews (CO.1 WJ). The present study was initiated to explore the phytochemical profile of stems of the above mentioned three *Jasminum* genotypes, in view of the pharmacological utilization of *Jasminum* species in ancient as well as modern systems of medicine. Extraction was made using methanol solvents and the extracts were analysed using gas chromatography-mass spectrometry (GC-MS). The GC-MS analysis revealed the presence of 12 bioactive compoundsin *J. sambac*, 17 in *J. grandiflorum*, and 16 in *J. multiflorum*. These compounds have various properties in including antimicrobial, antibacterial, antioxidant, and anticancer effects, making them potentially valuable for medicinal applications. The identification of these compounds was done using standard protocols and comparison with the Willey and NIST libraries. Dr. Duke's Phytochemical and Ethnobotanical Databases were used to confirm the biological roles of the identified compounds. Overall, the results revealed that the three novel *Jasminum* genotypes studied are rich sources of bioactive compounds, many of which are proven as beneficial in preventing various disorders, highlighting the potential of these *Jasminum* genotypes in pharmaceutical applications and their importance in the ongoing search for natural medicinal resources.

1. Introduction

Gas chromatography-mass spectrometry (GC-MS) is a powerful tool for recognizing volatile compounds including alcohols, acids and esters; it also detects long- and branched-chain hydrocarbons. Ethnobotanical evidences have reported that out of 122 elements used in modern healthcare products, 80 per cent were found to possess similar activities to those found in their corresponding original herbal drugs (Parnami and Lakhawat, 2022; Pipon, 2010). It is believed that herbal remedy takes care of around 80 per cent of the overall global health demands, catering to millions residing in underdeveloped areas especially rural ones. Moreover, there is a dependence of over 65 per cent across continents on traditional medical systems when it comes to primary care (Kethamakka and Meena, 2014). Since time immemorial, numerous plants have been employed as medicine by humans as a means of fighting different diseases (Alam *et al.*, 2019; Moond *et al.*, 2023).

Jasminum is a genus with over 200 species, that includes small trees and vines and belongs to the Oleaceae family (Zhang *et al.*, 1995).

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Jasmine is considered one of the plants which has existed since ancient times and is known for its beautiful and fragrant flowers. It is mainly valued as a highly treasured ornamental plant since its fresh flowers are popularly used in making floral arrangements (Saripalle, 2016). Besides ornamental value, *Jasminum* species have been known to possess pharmaceutically important alkaloids, cardiac glycosides, phenols, sterols, tannins, and sesquiterpenes (Kumaresan *et al.*, 2019).

New drugs have been developed following identification of potential bioactive components in plants for effective protection as well as cure for several illnesses such as cancer and Alzheimer's disease (Mukherjee *et al.*, 2007; Sheeja and Kuttan, 2007). Plants are one of the most vital sources of medicine and have become very important in the field of nutraceuticals. Additionally, it has been proven that many medicinal plants with their various pure compounds possess significant healing capabilities (Devi *et al.*, 2023; Khalaf *et al.*, 2007). In this respect, it is crucial to undertake a comprehensive evaluation of plant composition and functions in order to determine their application as possible antimicrobial agents (Nair and Chanda, 2006; Packia Lincy *et al.*, 2013).

There are various new techniques to determine the number of substances found in plants and quantify them, thus enabling standardization of herbal medicines and their preparations. Of these techniques, GC-MS is the most efficient for identifying bioactive compounds such as alcohols, ethers or acids (Dhama *et al.*, 2022;

Muthulakshmi *et al.*, 2012; Sivakumar *et al.*, 2022). The emergence of methods like GC or LC-MS has made it easier and cheaper to analyse small amounts of substances. For instance, GC-MS analysis enables detection of less than one-gram pure compounds (Liebler *et al.*, 1996).

This study was conducted to determine the bioactive compounds present in the stems of three novel *Jasminum* genotypes using the GC-MS technique, providing insights into phytochemical compounds used in traditional medicine. These three genotypes have shown to possess higher consumer and market preferences for their flowers. The present study aimed at assessing the pharmaceutical values of stem extracts of these three genotypes so that it will serve as an additional source of income to the jasmine farmer.

2. Materials and Methods

2.1 *Jasminum* genotypes

The *Jasminum* genotypes studied included a Double Flower type of *J. sambac* (DF), White Flower Bud type of *J. grandiflorum* (WF), and a new cultivar CO.1 Winter Jasmine of *J. multiflorum* (CO.1 WJ) evolved at TNAU (Figure 1).

J. sambac (DF) | *J. grandiflorum* (WF) | *J. multiflorum* (CO.1 WJ)

Figure 1: Novel *Jasminum* genotypes.

The double flowered genotype of *J. sambac* (DF) is high yielding with bolder buds. The white flower bud type of *J. grandiflorum* (WF) is a unique type in jasmine, since the commercial varieties available in the market produce pink tinged buds. CO.1 Winter Jasmine of *J. multiflorum* is an off-season flowering type producing high yield. Fresh stem extracts of the three *Jasminum* genotypes were collected from the jasmine germplasm being maintained at the Department of Floriculture and Landscaping, Tamil Nadu Agricultural University, Coimbatore district, Tamil Nadu, India during 2023-2024.

2.2 Synthesis of methanolic stem extract

The collected stem was cleaned thoroughly and cut into small pieces and washed two times with de-ionized water and then allowed to dry naturally in a cool environment. After complete drying, a blender was used to crush the stem into fine powder which was then kept in an air-tight container for future use (Guha *et al.*, 2010; Sultana *et al.*, 2009). The active constituents were extracted by maceration using 70% methanol and water. Fifty grams of the macerated plant material was weighed in to 500 ml conical flask (Salisu and Garba, 2008) and 300 ml of methanol was added and left to stand for three days while stirring at intervals. The extracts were then filtered and dried with a rotary evaporator (Brinkmann, R110). The concentrates were dispensed into labelled sample vials and stored in the fridge at 4°C.

2.3 GC-MS analysis

The volatile components of the methanolic extract of *Jasminum* stems were characterized using GC-MS, employing an Agilent Technologies model 7890A gas chromatograph equipped with a Mass Selective Detector model 5975C (MSD) operating under electron ionization (70 V) with ion source temperature set at 250ºC. For analysis of this extract, a capillary column Agilent DB5MS (30 mm × 0.25 mm × 0.25 µm) was used. Helium (99.9%) was the high-purity carrier gas used, with a ml/min flow rate. The injector mode was split (1:60), with an injection volume of 1 µl. The oven temperature program began at 100ºC and maintained for 0.5 min before increasing to 140ºC at 20ºC/min, keeping it there for a minute and then finally going up to 280ºC at 11°C/min over 20 min. Mass Hunter software was used for peak area measurement and data processing. The identification of components was based on a comparison of their mass spectra with those contained in the NIST Wiley 2008 library.

2.4 Identification of bioactive compounds

Peaks were identified using the databases namely, Wiley mass spectral library (W9N11) and the National Institute of Standards and Technology (NIST) on GC-MS for determination of volatile compounds that are unknown. Dr. Duke's Phytochemical and Ethnobotanical databases were consulted for gathering information pertaining to the biological activities of these compounds. Molecular weight and molecular formula were confirmed using PubChem.

3. Results

GC-MS is renowned for its application in the determination of volatile organic compounds such as alcohols or esters. It also has the capacity of distinguishing between long chain aliphatic hydrocarbons and olefins as well as aromatics like naphthenes or alkyl benzenes. GC-MS has proved highly efficient in aroma profiling of various *Jasminum* species (Ranchana *et al.*, 2017a, 2017b, 2017c, 2017d) as well as for identifying the metabolites and their respective biosynthetic pathways in putative mutants of *J. grandiflorum* (Soundarya *et al.*, 2022). In the present study, various phytochemical components were observed when the stem extracts of jasmine were subjected to GC-MS analysis. Analysis of peak area, retention time and molecular formulae confirmed these phytochemicals. Figures 2, 3 and 4 explain the results of the present study.

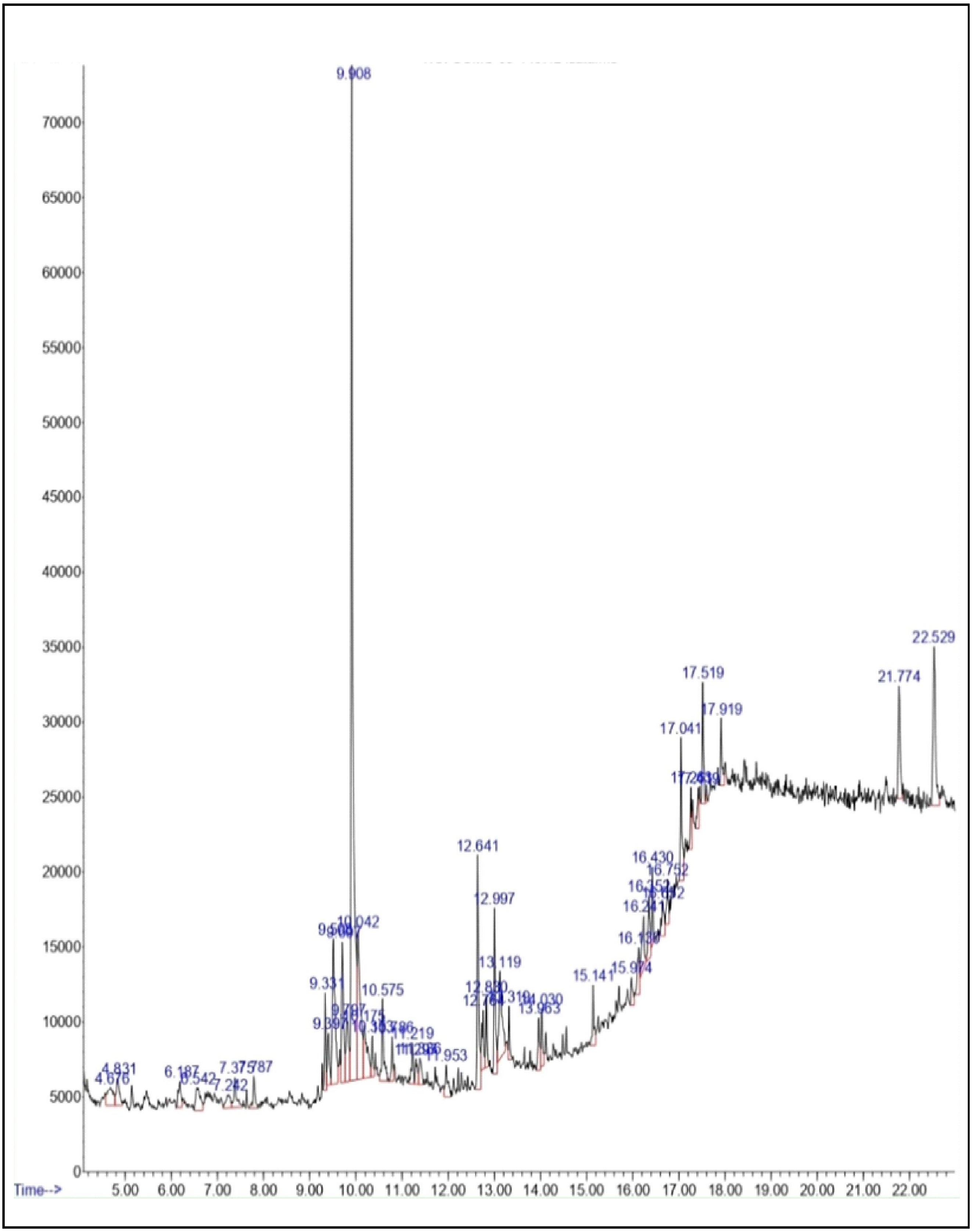

Figure 2: **GC-MS chromatogram of methanol extract of *J. sambac* (DF) stem.**

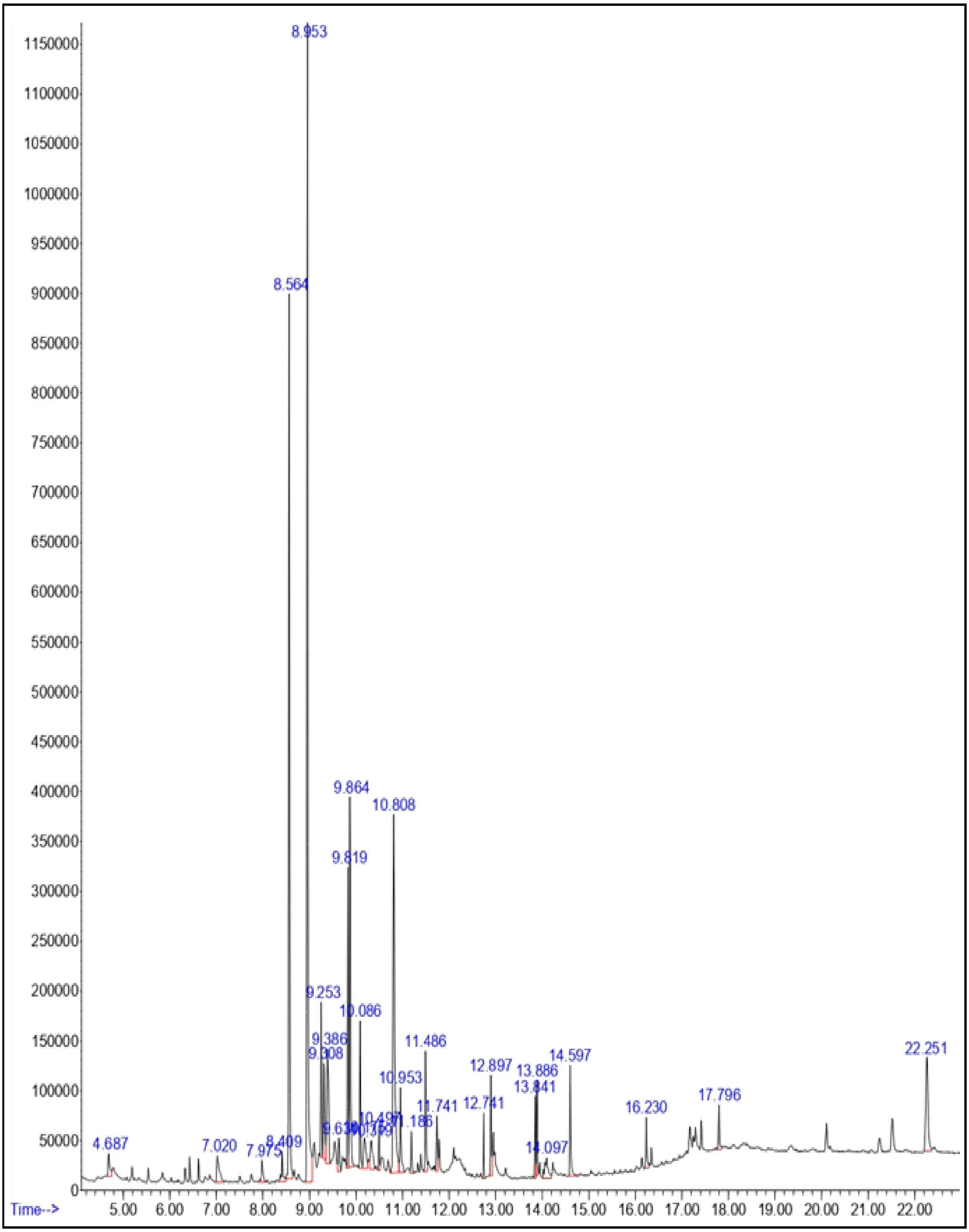

Figure 3: GC-MS chromatogram of *J. grandiflorum* (WF) stem.

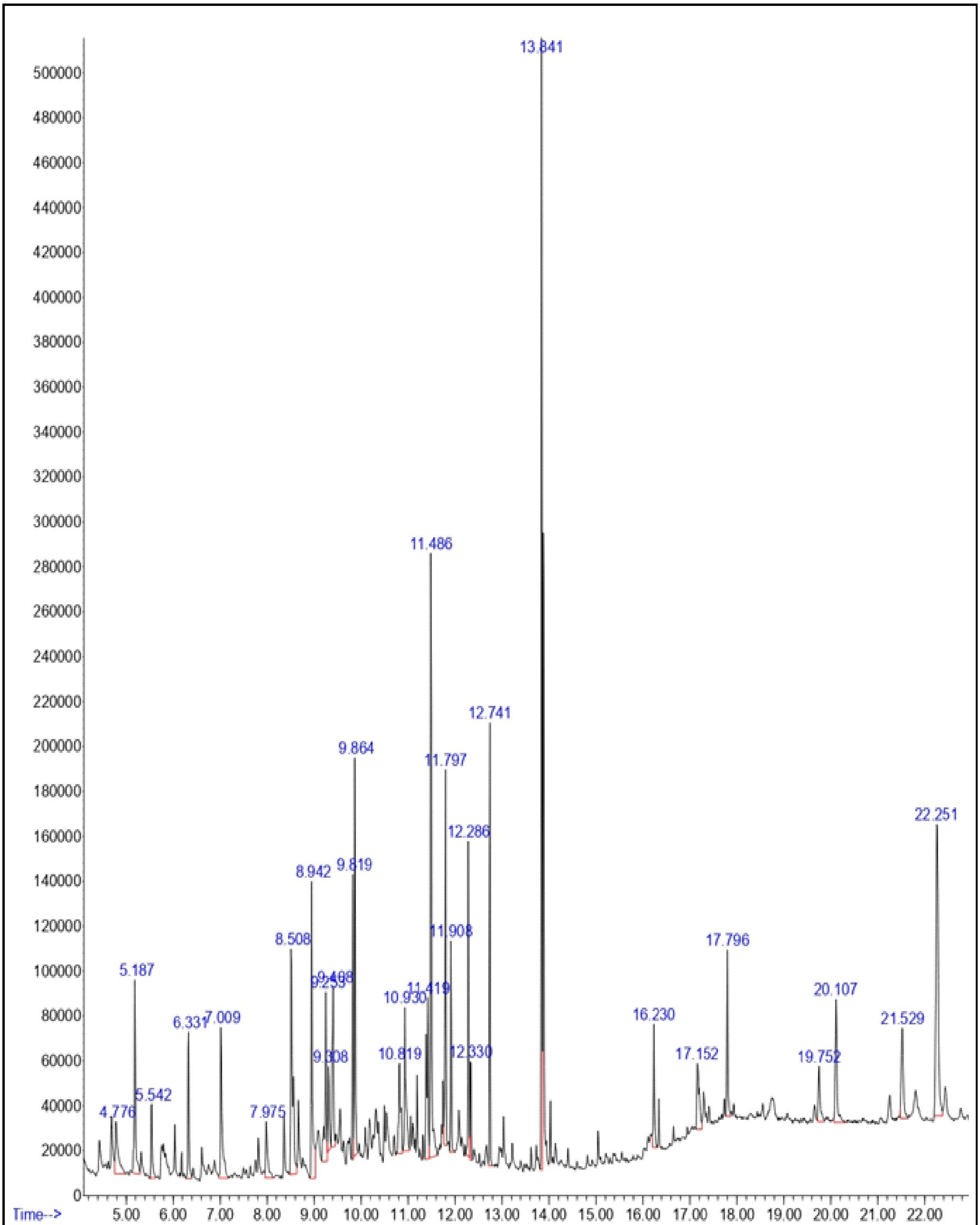

Figure 4: GC-MS chromatogram of *J. multiflorum* (CO.1 WJ) stem.

The compounds were predicted using the NIST Database. The twelve compounds identified in *J. sambac* (Table 1) are 3-isopropoxy-4-methoxybenzamide (4.89%), benzene acetic acid (3.37%), silane [(1,1-dimethyl-2-propenyl) oxy]dimethyl (2.43%), benzoic acid 4-ethoxy ethyl ester (23.64%), ethyl paraben (2.43%), eicosapentaenoic acid methyl ester (4.93%), diphenyl sulfone (3.21%), carbamic acid 3-methylphenyl butyl ester (3.35%), 2-methyl-7-phenylindole (2.64%), hexamethylcyclotrisiloxane (1.89%), silane, trimethyl[5-methyl-2-(1-methylethyl) phenoxy (2.76%) and 1H-indole, 1-methyl-2-phenyl (4.87%).

Table 1: Phytochemical compounds identified in the methanol extract of *J. sambac* (DF) stem

S.No.	RT time	Chemical compound	Molecular formula	Molecular wt. (g/mol)	Area %	Function	Reference
1	9.508	3-Isopropoxy-4-methoxybenzamide	$C_{11}H_{15}NO_3$	209.24	4.89	Antifungal potential	Khan and Javaid, 2020
2	9.697	Benzene acetic acid	$C_8H_8O_2$	136.15	3.37	Natural auxin property	Wightman and Lighty, 1982
3	9.797	Silane [(1,1-dimethyl-2-propenyl) oxy] dimethyl	$C_8H_{18}OSi$	158.31	2.43	Cytotoxic activity	Ahmad *et al*., 2016
4	9.908	Benzoic acid 4-ethoxy ethyl ester	$C_{11}H_{14}O_3$	194.230	23.64	Antimicrobial property	Sheela and Uthayakumari, 2013
5	10.175	Ethyl paraben	$C_9H_{10}O_3$	166.17	2.43	Anti-microbial activity	Jianmei *et al*., 2015
6	12.641	Eicosapentaenoic acid methyl ester	$C_{26}H_{44}O_2Si$	416.71	4.93	Potential for synthesis in transgenic plants	Cheng *et al*., 2010
7	12.997	Diphenyl sulfone	$C_{12}H_{10}O_2S$	218.27	3.21	Strong radical scavenging property	Barabasz-Krasny *et al*., 2024
8	13.119	Carbamic acid, 3-methylphenyl butyl ester	$C_9H_{11}NO_2$	165.19	3.35	Phytotoxicity activity	Shaw and Swanson, 1953
9	17.041	2-Methyl-7-phenylindole	$C_{15}H_{13}N$	207.27	2.64	Anticancer activity	Yousif, 2019
10	17.519	Hexamethylcyclotrisiloxane	$C_6H_{18}O_3Si_3$	222.4618	1.89	Antioxidant and anti-Inflammatory activity	Shrivastava, 2023
11	21.774	Silane, trimethyl[5-methyl-2-(1-methylethyl) phenoxy	$C_{13}H_{22}OSi$	222.40	2.76	Antifungal activity	Kubinec *et al*., 2020
12	22.529	1H-Indole, 1-methyl-2-phenyl	$C_{15}H_{13}N$	207.27	4.87	Free radical scavenging activity	Oloyede, 2016

Table 2: Phytochemical compounds identified in the methanol extract of *J. grandiflorum* (WF) stem

S.No.	RT time	Chemical compound	Molecular formula	Molecular wt. (g/mol)	Area %	Function	Reference
1	5.187	Phenol 2-methyl	C_7H_8O	108.13	2.98	Antioxidant and a anti-diabetic potential	Mohamed *et al*., 2022
2	7.009	N-Benzyl-2-phenethylamine	$C_{15}H_{17}N$	211.30	3.05	Antioxidant and anti-bacterial Potential	Hansen *et al*., 2014; Kishore *et al*., 2020
3	8.508	Benzoic acid 4-ethoxy-	$C_{11}H_{14}O_3$	194.23	5.37	Antimicrobial property ethyl ester	Sheela and Uthaya Kumari, 2013
4	8.942	Benzene ethanol 4-hydroxy	$C_8H_{10}O_2$	138.16	4.54	Natural effective nematicide	Li *et al*., 2018
5	9.253	Butane dioic acid methoxydimethyl ester	$C_7H_{12}O_5$	176.17	3.07	Enhance the P availability	Khorassani *et al*., 2011
6	9.408	Ethanone 1-(4-hydroxy -methoxyphenyl)	$C_9H_{10}O_3$	166.17	2.82	Anticancer property	Gangadharan *et al*., 2024

7	9.819	Ethyl paraben	$C_9H_{10}O_3$	166.17	2.69	Antimicrobial activity	Jianmei *et al.*, 2015
8	9.864	Homo vanillyl alcohol	$C_9H_{12}O_3$	168.19	4.45	Antioxidant agent	Bernini *et al.*, 2019
9	10.808	Benzene acetic acid	$C_8H_8O_2$	136.15	10.92	Natural auxin property	Wightman and Lighty, 1982
10	11.419	Bicyclohept-2-en-7-ol	$C_7H_{10}O$	110.15	3.48	Antibacterial activity	Bouhouia *et al.*, 2020
11	11.486	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxy-phenol	$C_{10}H_{12}O_3$	180.20	8.35	Antioxidant, antibacterial and anti-inflammatory activity	Ravikumar *et al.*, 2012
12	11.797	Ethyl 2-octynoate	$C_{10}H_{16}O_2$	168.23	4.14	Phytotoxicity activity	Yadav and Chandra, 2018
13	12.741	Hexadecenoic acid methyl ester	$C_{17}H_{34}O_2$	270.5	4.18	Antibacterial activity	Shaaban, 2021
14	13.841	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.5	10.79	Antidiarrheal activity	Shoge and Amusan, 2020
15	17.796	1H-Indole1-methyl-2-phenyl	$C_{15}H_{13}N$	207.27	0.84	Free radical scavenging activity	Oloyede, 2016
16	20.107	dl-alpha-tocopherol	$C_{29}H_{50}O_2$	430.7	2.78	Antioxidant activity	Slavova and Kancheva, 2018
17	22.251	Gamma sitosterol	$C_{29}H_{50}O$	414.7	8.33	Exerts potential anti-cancer activity through the growth inhibition	Sundarraj *et al.*, 2012

Table 3: Phytochemical compounds identified in the methanol extract of *J.multiflorum* (CO.1 WJ) stem

S.No.	RT time	Chemical compound	Molecular formula	Molecular wt. (g/mol)	Area %	Function	Reference
1.	8.564	Benzene 1,2-dimethoxy-4-(1-propenyl)	$C_{12}H_{16}O_3$	208.25	16.22	Pesticidal activity	Kumar *et al.*, 2016
2	8.942	Benzene acetic acid	$C_8H_8O_2$	136.15	4.54	Natural auxin property	Wightman and Lighty, 1982
2	8.953	Benzene ethanol 4 -hydroxy	$C_8H_{10}O_2$	138.1638	23.72	Natural effective nematicide	Li *et al.*, 2018
3	9.253	Silane[(1,1-dimethyl-2-propenyl) oxy]trimethyl	$C_{10}H_{22}OSi$	186.3666	2.53	Cytotoxic activity	Ahmad *et al.*, 2016
4	9.308	Silane trimethyl(2-pen-tenyloxy)	$C_8H_{20}OSi$	160.33	2.28	Cytotoxic activity	Ahmad *et al.*, 2016
5	9.386	Linoleic acid trimet-hylsilyl ester	$C_{21}H_{40}O_2Si$	352.6	3.52	Plant antioxidant and Jasmonic acid (JA) bio-synthesis	Zi *et al.*, 2022
6	9.819	Benzoic acid 4-ethoxy-ethyl ester	$C_{11}H_{14}O_3$	194.23	4.06	Antimicrobial property	Sheela and Uthaya kumari, 2013
7	9.864	Homovanillyl alcohol	$C_9H_{12}O_3$	168.19	6.80	Antioxidant property	Bernini *et al.*, 2019
8	10.086	N-Acetyl tyramine	$C_{10}H_{13}NO_2$	179.22	2.40	Anti-free radical activity	Pan *et al.*, 2023
9	10.175	Methyl beta-D-gluco-pyranoside	$C_7H_{14}O_6$	194.18	1.36	Antimicrobial glycoside	Olawumi *et al.*, 2020
10	10.808	Methyl 4-fluorobenzoate	$C_8H_7FO_2$	154.14	10.92	Metabolite of the bac-terial degradation	Schlomann *et al.*, 1990
11	10.953	Quinazolin-4(3H)-one, 3 -(2-methoxy phenyl)-2 -thiol	$C_{17}H_{16}N_2O_2S$	312.4	1.69	a-glucosidase inhibitors	Moheb *et al.*, 2022
12	11.486	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxy phenol	$C_{10}H_{12}O_3$	180.20	2.09	Antioxidant compounds	Mahatheeranont, 2020

13	12.897	Diphenyl sulfone	$C_{12}H_{10}O_2S$	218.27	1.76	Strong radical scavenging property	Barabasz-Krasny *et al.*, 2024
14	13.886	Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	296.5	1.62	Antioxidant and anti-cancer activities	Yu *et al.*, 2005
15	14.597	Benzaldehyde 3,4-dimethoxy	$C_9H_{10}O_3$	166.17	2.06	*In vitro* antioxidant	Puja *et al.*, 2020
16	22.251	1H-Indole1-methyl-2 -phenyl	$C_{15}H_{13}N$	207.27	3.82	Free radical scavenging	Oloyede, 2016

The seventeen compounds identified in *J. grandiflorum* (Table 2) are phenol, 2-methyl (2.98%), N-benzyl-2-phenethylamine (3.05%), benzoic acid 4-ethoxy-ethyl ester (5.37%), benzene ethanol, 4-hydroxy (4.54%), butane dioic acid methoxy-dimethyl ester (3.07%), ethanone 1-(4-hydroxy-3-methoxyphenyl) (2.82%), ethyl paraben (2.69%), homovanillyl alcohol (4.45%), benzene acetic acid (10.92%), bicyclo[2.2.1]hept-2-en-7-ol (3.48%), 4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol (8.35%), ethyl 2-octynoate (4.14%), hexadecenoic acid methyl ester (4.18%), 9,12-octadecadienoic acid, methyl ester (10.79%), 1H-indole,1-methyl-2-phenyl (0.91%), dl-alpha-tocopherol (2.78%) and gamma sitosterol (8.33%).

The sixteen compounds identified in *J. multiflorum* (Table 3) are benzene 1,2-dimethoxy-4-(1-propenyl) (16.22%), benzene acetic acid (4.54%), benzene ethanol 4-hydroxy (23.72%), silane[(1,1-dimethyl-2-propenyl)oxy]trimethyl (2.53%), silane trimethyl(2-pentenyl oxy) (2.28%), linoleic acid trimethylsilyl ester (3.52%), benzoic acid 4-ethoxy- ethyl ester (4.06%), homovanillyl alcohol (6.80%), N-acetyl tyramine (2.40%), methyl beta-D-glucopyranoside (1.36%), methyl 4-fluorobenzoate (10.92%), quinazolin-4(3H)-one 3-(2-methoxy phenyl)-2-thiol (1.69%), 4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol (2.09%), diphenyl sulfone (1.76%), octadecenoic acid, methyl ester (1.62%), benzaldehyde 3,4-dimethoxy (2.06%) and 1H-indole,1-methyl-2-pheny (3.82%).

4. Discussion

The compounds identified in the three species are known to possess numerous potential pharmacological properties. Benzoic acid 4-ethoxy ethyl ester, benzene acetic acid and 1H-indole1-methyl-2-phenyl were the unique compounds detected in the three plant species studied. Ethyl paraben was the common compound detected in *J. sambac* and *J. grandiflorum* and homovanillyl alcohol was the compound detected in *J. grandiflorum* and *J. multiflorum*. The compounds identified in this study which have been reported to possess antioxidant activity include tetrahydro-1,3-oxazine-2-thione (Zinad *et al.*, 2020), homovanillyl alcohol (Benincasa *et al.*, 2024; Bernini *et al.*, 2019), dl-alpha-tocopherol (Slavova and Kancheva, 2018)**,** 4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol (Mahathe-eranont, 2020), octadecenoic acid, methyl ester (Yu *et al.*, 2005) and clionasterol (Liyanage *et al.* 2022). Similarly, antifungal activity identified in the stem extracts are 3-isopropxy-4-methoxyben-zamide (Khan and Javaid, 2020), silane trimethyl[5-methyl-2-(1-methylethyl) phenoxy(Kubinec *et al.*, 2020) and hexadecenoic acid methyl ester (Sharma *et al.*, 2021).

The compounds with antimicrobial activity include benzoic acid, 4-ethoxy ethyl ester (Sheela and Uthayakumari, 2013), ethyl paraben (Jianmei *et al.*, 2015) and tetrasiloxane deca methyl (Momin and Thomas, 2020)**.** Compounds with antibacterial activity are bicyclo-hept-2-en-7-ol (Bouhouia *et al.*, 2020), hexadecenoic acid methyl ester (Shaaban, 2021), 9,12-octadecadienoic acid, methyl ester (Shoge and Amusan, 2020). Generally, phenylacetic acid (Wightman and Lighty, 1982) and 1H-indole,1-methyl-2-phenyl (Oloyede, 2016) function as natural auxins synthesized by the plant. The compound with cytotoxic activity is silane [(1,1-dimethyl-2-propenyl) oxy] trimethyl (Ahmad *et al.*, 2016). The study also identified several compounds, including carbamic acid, 3-methylphenyl, butyl ester (Shaw and Swanson, 1953), and ethyl 2-octynoate (Yadav and Chandra, 2018), which are known for their phytotoxic properties.

The compound diphenyl sulfone demonstrates strong radical scavenging properties, making it potentially effective in neutralizing free radicals and reducing oxidative stress (Barabasz-Krasny *et al.*, 2024). Methyl 4-fluorobenzoate acts as a metabolite in the process of bacterial degradation, playing a role in the breakdown and transformation of compounds within bacterial systems (Schlomann *et al.*, 1990). Dual function of linoleic acid trimethylsilyl ester (Zi *et al.*, 2022) in protecting plants and supporting major biochemical pathways includes being an antioxidant in plants and being a precursor to jasmonic acid biosynthesis. Gamma sitosterol (Sundarraj *et al.*, 2012) a plant sterol, has health benefits including potential in reducing inflammation and cholesterol. It may also help with cardiovascular health, metabolic disorders, and potentially inhibit cancer cell growth.

Potential for eicosapentaenoic acid methyl ester (Cheng *et al.*, 2010) synthesis in transgenic plants suggests its effectiveness in the field of genetic engineering aimed at boosting plant production of the compound. 1H-indole, 1-methyl-2-phenyl exhibits free radical scavenging activity, highlighting its potential for neutralizing harmful free radicals and contributing to oxidative stress reduction (Oloyede, 2016). N-Acetyl tyramine exhibits anti-free radical activity, indicating its potential to combat oxidative stress in plants by neutralizing free radicals and thereby reducing damage caused by oxidative processes (Pan *et al.*, 2023). Additionally, the compound benzene ethanol, 4-hydroxy (Li *et al.*, 2018) was identified for its nematicidal activity.

5. Conclusion

The GC-MS analysis of stem extracts of the three novel *Jasminum* genotypes led to the identification of 45 bioactive compounds with significant pharmacological activities. The study has opened up newer and unexplored avenues for the jasmine crop, which till date has remained only as an ornamental plant. The observations made in the present study have thrown light on the alternate potentials of *Jasminum* species as useful plants with immense pharmaceutical significance. These findings emphasize the significance of the identified compounds in traditional medicine and lay thrust on further research in this line.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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