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Aromatic profiling of *Murraya koenigii* leaves by Thermal Desorption Gas chromatography-Mass Spectroscopy (TD-GC-MS)

Santhanakrishnan V.P [a,*], Shoba N [b], Senthamizh Selvi B [b], Varun E [a], Mohankumar S [a], Raveendran M [a]

[a] *Department of Plant Biotechnology, Centre for Plant Molecular Biology & Biotechnology, Tamil Nadu Agricultural University, Coimbatore, 641003, Tamil Nadu, India*

[b] *Department of Spices and Plantation Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, 641003, Tamil Nadu, India*

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ABSTRACT

The germplasms of the *Murraya koenigii* were collected from Rajahmundry, Annur, Kollihills, Suvashini, Bhavanisagar, Karamadai (KMM5, KMM6, KMM7, KMM8 and KMM14) and the Kerala Agricultural University (KAU). The fresh leaves were analyzed for its volatile organic compounds by Thermal Desorption Gas chromatography–Mass Spectroscopy (TD-GC-MS) to obtain germplasm specific volatile fingerprinting. The correlation between genotypes based on volatile profiles has been analyzed using principal component analysis (PCA) and hierarchical cluster analysis (HCA). A wide variety of volatile compounds identified in the eleven *M. koenigii* genotypes belongs to terpenoids, monoterpenes, sesquiterpenes, aldehyde, ketones, benzenes, azulenes and other minor compounds. The α-pinene and β-pinene content is high in Suvashini and Bhavanisagar (BSR) genotypes respectively. The monoterpenes such as γ-terpinene, α-myrcene and terpinolene are high in Karamadai variety (KMMK8), whereas caryophyllene content is high in the Rajahmundry. The results of PCA revealed that significant variances with 45.47% (PC 1) and 21.40% (PC 2). In AHC, the α-pinene and chloral hydrate forms the one major cluster. Additionally, α-fenchene and α-caryophyllene has observed forming second major cluster with significant magnitude. The cluster formed by sesquiterpenes are observed high in Annur (65.34%), followed by KMMK8 (48.01%), Kollihills (39.89%) and Rajahmundry (39.27%). The PCA and AHC combined with the fingerprint of TD-GC-MS have discriminated qualitative volatile profile and indicated that major changes VOCs emitted are highly attributed to the genetic factors.

1. Introduction

The plants produce volatile organic compounds (VOCs) to defend themselves from insects and other herbivores. These volatiles from aromatic plants can be extracted as essential oils, or the parts producing volatiles can be directly trapped to enhance the odor and flavour of culinary preparations [1]. In recent decades, there has been increasing interest in the use of plant volatile material by the food, fragrance and cosmetic industries [2]. The plant volatiles are also used as alternative medicine in the form of aroma-therapy because they alter an individual's mood, behavior, cognitive function, and health; thus, they have applications in the

* Corresponding author.
E-mail address: santhanakrishnan.vp@tnau.ac.in (S. V.P).

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Abbreviation

TD-GC-MS	Thermal Desorption Gas chromatography–Mass Spectroscopy
MS	Mass Spectroscopy
Da	Dalton
eV	electron-Volt
RT	Retention Time
VOCs	Volatile Organic Compounds
MSL	Mean Sea Level
NIST	National Institute of Standards and Technology
KMM	Karamadai
BSR	Bhavanisagar
KAU	Kerala Agricultural University
TNAU	Tamil Nadu Agricultural University
AHC	Agglomerative Hierarchical Clustering
HCA	Hierarchical Cluster Analysis
PCA	Principal Component Analysis

pharmaceutical industry as well [3].

The curry leaf is botanically known as *Murraya koenigii* and belongs to the family *Rutaceae*. The *M. koenigii* is a small perennial aromatic tree, but cultivated as a shrub and originated from the Tarai regions of Uttar Pradesh, India [4]. It is widely cultivated in Malaysia, India, Bangladesh, Nepal, Sri Lanka, Australia, China and Burma [5]. This species reported to grow up to an altitude of 1500 m MSL [6]. The *M. koenigii* mainly cultivated for its aromatic leaves (fresh and dry forms) and used as a spices and condiments, as well as culinary art. This species also considered as highly therapeutic due to its anti-fungal, anti-bacterial, anti-oxidant, anti-diabetic, anti-carcinogenic, *anti*-helmintic, anti-tumor and anti-inflammatory properties [7,8]. It is rich source of beta carotene, calcium and iron [9]. Additionally, the essential oil and oleoresin is extracted from curry leaves has its value in food, pharmaceutical and cosmetic industries [2]. The distillation of curry leaves produces a yellow-colored volatile oil with a pungent taste and a spicy odor [10]. Many reports are available on the chemical composition of *M. koenigii* oil [11]. However, there is no report on the aroma profiling of the volatiles present in *M. koenigii* leaves, thus the present research focuses on the trapping of the volatiles present in the *M. koenigii* leaves. The *M. koenigii* genotypes of Rajahmundry, Kollihills, Suvashini, Bhavanisagar, Kerala Agricultural University and local genotypes of the Coimbatore that includes genotypes from different locations in Karamadi (KMM5, KMM6, KMM7, KMM8 and KMM14) and Annurr were taken in this study. The VOCs were trapped from the 11 genotypes and the comparative metabolite profiling was performed utilizing Thermal Desorption Gas chromatography –Mass Spectroscopy.

The Thermal Desorption Gas chromatography –Mass Spectroscopy is an analytical technique that has been mainly used for the detection of the key pollutants in air and odorous compounds: hydrogen sulfide, methane thiol, dimethyl sulfide, and dimethyl disulphide near landfill sites and sewage treatment works. It also finds applications in food, flavor, fragrance, forensics, civil and defense etc. [12]. The above technique is applied to trap the volatiles in *M. koenigii* for its application in the food and fragrance industries.

2. Materials and methods

2.1. Raw material

The *M. koenigii* leaves were freshly harvested from the germplasm maintained at the college orchard in the Department of Spices and Plantation Crops, HC &RI, TNAU, Coimbatore and utilized for the present study. The germplasm involved the collection from Rajahmundry, Annur, Kollihills, Bhavanisagar, Suvashini and from the different locations in the Karamadai (KMM5, KMM6, KMM7, KMM8 and KMM14) and from the Kerala Agricultural University (KAU).

2.2. Sample preparation for GC-MS

Fresh curry leaves (2 g) were taken in a special conical flask connected to a tenax column (9 cm, PerkinElmer HO 244966), which is used for trapping the volatile and semi-volatile organic compounds. The volatile organic compounds (VOCs) that are present in the gas stream were efficiently trapped in this column. This tenax column is then removed from the conical flask and connected to the Gas chromatography chamber. The VOCs trapped in the tenax were thermally desorbed using Thermal Desorber turbomatrix 150 (PerkinElmer, USA) and analyzed with the following optimized instrument conditions.

2.3. Instrumentation

The Clarus SQ 8C Gas Chromatography - Mass Spectrometer from PerkinElmer, was engaged for analysis. The instrument was set as

Table 1
Characterization of volatile aroma compounds in Murraya koenigii leaf genotypes.

Sl. no.	RT (min.)	Compounds	ANNUR	KARAMADAI (KMM5)	KAU	KMMK7	KMMK6	KMMK8	KMMK14	KOLLIHILLS	RAJAHMUNDRY	SUVASHINI	BSR	Odor note
			Con. (Area %)											
1	1.674	Ethyl Acetate	0.433	–	–	–	–	–	–	1.506	1.986	–	0.992	fruity smell
2	1.684	Chloral Hydrate	–	–	29.238	35.733	48.774	13.052	22.768	–	–	–	–	acrid odor
3	1.904	Benzene	0.136	–	–	–	–	–	–	–	1.695	0.77	0.395	sweet, aromatic, gasoline-like
4	2.544	Isopropylamine	–	3.473	1.22	1.629	–	–	–	–	0.479	2.751	0.164	ammonia-like
5	3.134	Bestatin	–	–	0.377	0.609	–	–	0.274	–	–	–	1.649	green floral
6	3.845	o-Xylene	–	0.847	–	0.163	–	0.133	0.079	–	–	0.293	–	sweet odor
7	4.03	Acetic acid	–	–	0.102	–	–	0.241		2.44	–	–	–	pungent vinegar
8	4.545	1-(*p*-Tolyl)butan-1-one	–	0.212	–	–	–	–	0.115	0.89	–	–	–	sweet fruity floral
9	4.66	α- Phellandrene	–	1.01	0.243	0.674	0.279	0.34	0.787	–	–	0.375	–	black pepper odor
10	4.77	α- pinene	6.447	24.438	37.206	23.521	11.502	6.892	32.124	24.067	7.53	39.963	–	turpentine odor
11	5.05	Camphene	–	0.73	2.814	0.744	0.377	0.193	2.424	0.659	–	1.954	3.746	pungent smell.
12	5.47	sabinene	1.31	1.731	0.507	0.166	–	–	–	–	–	1.711	0.921	Woody
13	5.115	β- pinene	–	–	7.543	0.368	0.175	0.12	8.536	0.715	–	0.936	13.017	woody, piney
14	5.76	á-Myrcene	1.985	–	–	–	–	4.845	–	–	0.825	–	0.217	citrusy
15	6.155	Benzene, 1-methyl-4-propyl-	–	9.262	0.176	–	–	–	–	0.493	3.684	14.152	–	floral, green, fruity
16	6.165	γ-Terpinene	9.846	–	0.126	0.934	–	11.046	–	–	–	1.533	0.131	terpenic type
17	6.385	terpinolene	0.444	–	–	–	–	0.504	–	–	–	–	0.239	herbal type
18	6.461	*o*-Cymene	–	0.7	0.077	–	–	–	0.228	–	–	–	0.246	terpenic type
19	6.566	Limonene	–	–	–	–	–	–	–	–	0.294	–	3.399	citrus note
20	6.796	alpha-fenchene	42.904	7.157	–	3.35	–	38.302	–	–	–	–	–	camphoreous type
21	6.941	á-Ocimene	1.18	–	–	–	–	1.506	–	–	8.299	–	–	fruity type
22	7.681	2-Carene	0.171	–	–	–	–	0.103	–	–	–	–	0.161	sweet and pungent
23	8.046	Nonanal	0.162	0.223	0.108	0.173	–	–	0.099	–	–	–	0.181	fatty odor
24	12.138	à-Cubebene	0.958	0.761	0.075	0.533	0.247	0.678	0.135	0.385	3.042	–	0.186	citrus type
25	12.493	Ylangene	0.225	0.276	0.067	0.147		0.182	0.077		0.641	–	0.156	–
26	12.588	alfa.-Copaene	2.01	2.073	0.521	0.864	0.348	1.588	0.67	1.63	4.344	0.635	0.941	–
27	12.868	azulene	0.173	0.218	0.08	0.137	–	0.234	0.093		0.669	–	0.108	–
28	13.058	Isocaryophillene	0.882	2.269	0.444	–	–	–	0.3	5.781	–	2.455	2.083	–
29	13.398	α- caryophyllene	15.907	19.695	–	2.578	–	6.848	3.269	28.048	28.991	9.415	11.366	woody, clove
30	13.513	*cis*-à-Bergamotene	1.185	–	–	–	–	–	0.271	1.155	–	–	0.536	woody type
31	13.663	Aromandendrene	0.639	0.539	0.145	–	–	0.241	0.135	1.41	0.985	0.329	0.371	–
32	13.953	Humulene	1.817	0.991	0.125	–	–	0.173	0.088	2.638	1.276	0.47	0.552	Woody, Oceanic-watery

follows: the interface temperature was set as 250 °C, while the source temperature remained at 220 °C. The oven temperature programmed as available: 50 °C for 1 min, 80 °C at 10 °C/min, up to 240 °C at 10 °C/min. The DB-5 MS capillary standard non - polar column, whose dimensions were 0.25 mm OD x 0.25 μm ID x 30 m length was procured from Agilent Co., USA. Helium was used as the carrier gas at 1 ml/min. The MS was set to scan from 50 to 550 Da. The source was maintained at 220 °C and $4.5e^{-6}$ motor vacuum pressure. The ionization energy was −70eV. The MS also had an inbuilt pre-filter which reduced the neutral particles. The data system has inbuilt libraries for searching and matching the spectrum. NIST MS Search 2.2v contains more than five lakh references [13].

2.4. Identification of volatile components

The components were identified based on their retention indices. The resulted component's spectrum was compared to the known component's spectrum stored in the inbuilt library. The values obtained were compared with the NIST library database (http://www.nist.gov) [13] and reported literature [14–16]. The relative peak area (%) of volatile compounds were compared within the germplasm and utilized for further analysis.

2.5. Data analysis

The experimental data was analyzed statistically using the XLSTAT plugin v. 2009.3.02 for Microsoft Excel (Microsoft Corporation, Redmond, Washington, USA). The raw data obtained by GC-MS was processed using Mass Hunter Qualitative Analysis software (Version B 07.00, Agilent Technologies). The correlation between the volatile profiles of various germplasms has been analyzed using principal component analysis (PCA). Agglomerative Hierarchical Clustering (AHC) was used to identify the variables contributing to group classification.

3. Results and discussion

3.1. Volatile profiling of curry leaves

A wide variety of volatile compounds were identified in the 11 curry leaf genotypes including terpenoids, monoterpenes, sesquiterpenes, aldehyde, ketones, benzenes, azulenes and other minor compounds (Xylenes, Carboxylic acids, Amines, Ethyl Acetate and Bestatin). The TD-GC-MS results indicated the presence of the 25–30 major volatile organic compounds that included pinene, caryophyllene, phellandrene and others which are responsible for the aroma of curry leaves. The minor compounds such as δ-limonene, α-myrcene, α-copaene, α-ocimeme were observed in some of the accessions. The presence of the major volatile organic compounds (i.e α- and β-pinene) were confirmed by recording the TD-GC-MS of the reference standard purchased from Sigma Aldrich. The α-pinene

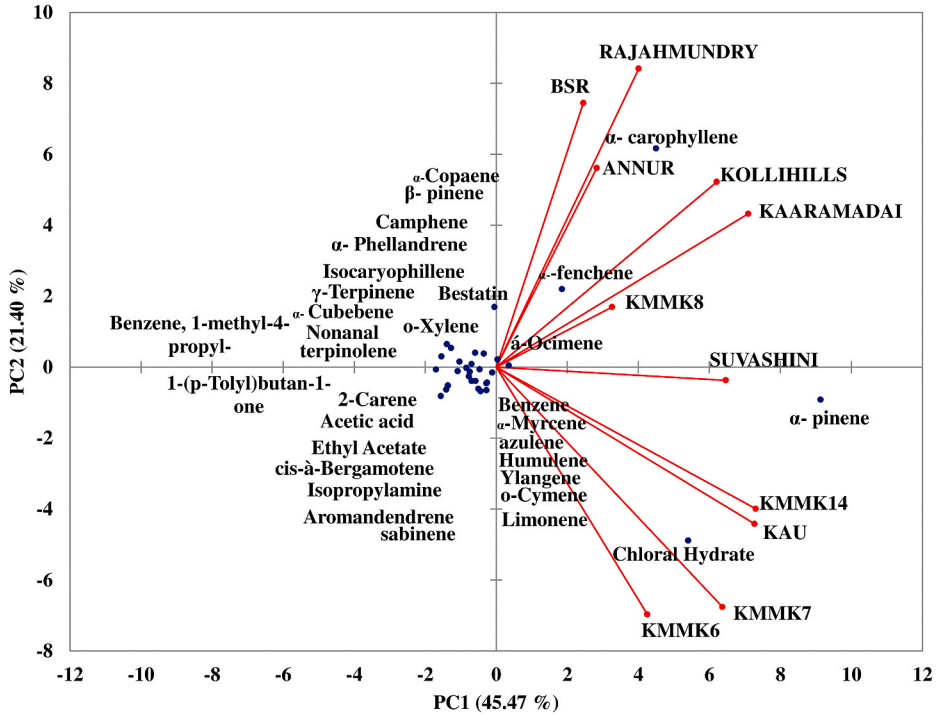

Fig. 1. Biplot of PC1 (45.47%) and PC2 (21.40%) of *Murraya koenigii* leaf volatiles.

content was high in Suvashini. The content of chloral hydrate, isopropylamine, o-Xylene, α-phellandrene and γ-terpinene were high in Karamadai genotypes. The KMMK8 genotype has a high amount of γ-terpinene and α-myrcene, whereas KMM5 contains a high amount of isopropylamine (Table 1).

3.2. Principal component analysis (PCA)

A total of 32 variables (volatile compounds) of 11 *Murraya koenigii* germplasm were used for PCA analysis and observed variance of 45.47% (PC 1) and 21.40% (PC 2). The α-pinene, α-fenchene, β-pinene, chloral hydrate and α-caryophyllene are distributed distinctly and are found to be prominent components for distribution of these 11 accessions (Fig. 1). All the 11 accessions were divided into two quartiles, with major varieties having an acute angle and being positively correlated. The varieties KMMK6 and KMMK7 have observed obtuse angles with Bhavanisagar, Annur and Rajahmundry, resulting in negatively correlation. The compounds α-pinene and chloral hydrate were found in the same quartile where the varieties Suvashini, KMMK6, KMMK7, KMMK14 and KAU were mapped. Similarly, the α-fenchene, α-caryophyllene, β-pinene falls in the quartile consisting of varieties Bhavanisagar, Annur, Rajahmundry, Kollihills, KMM5 and KMMK8. Interestingly, the accessions were clearly grouped in two quadrants supported by key variable compounds. The sesquiterpenes and few monoterpenes are a few variables that influence genotype differentiation and may be chemotaxonomic markers [17,18].

3.3. Agglomerative Hierarchical Clustering (AHC)

The Agglomerative Hierarchical Clustering (AHC) has been analyzed for volatiles of 11 germplasm of *M. koenigii* leaves. AHC has provided another source of additional support for PCA by forming clusters that are comparable in terms of the distinct distribution of components in PCA. AHC has observed two major clusters, which are divided into 9 and 2 sub clusters respectively. The α-pinene and chloral hydrate form one major cluster. Additionally, α-fenchene and α-caryophyllene have observed another major contribution with significant magnitude in the second major cluster (Fig. 2).

3.4. Odor characteristics of VOCs

Monoterpenes, sesquiterpenes, aldehydes and ketones are the major class of aromatic compounds present in the curry leaf (Fig. 3). The odor was more pronounced in leaves. The sesquiterpenes are high in Annur (65.34%), followed by KMMK8 (48.01%), Kollihills (39.89%) and Rajahmundry (39.27%). The major sesquiterpenes such as, α-fenchene, α-caryophyllene and α-copaene imparting characteristic camphoreous, spicy-clove odors respectively. The other minor sesquiterpenes include α-cubebene, ylangene, aromandendrene and humulene, which imparts characteristic woody and earthy notes. The aldehydes and ketones are also found to have significant positive odor notes, which consist of chloral hydrate, 1-(*p*-Tolyl)butan-1-one and nonanal. These compounds impart a characteristic acrid, sweet-fruity-floral and rose-orange odors respectively. The benzene and azulene classes have also been observed which include benzene, p-propyl toluene and azulene, which impart sweet-aromatic, floral-green-fruity odors respectively. Benzene was found in only a few accessions (Annur, Rajahmundry, Suvashini and Bhavanisagar), whereas *p*-propyltoluene was observed high in Suvashini (14.15%). The azulene observed in almost all genotypes in low concentration (0.08–0.66%).

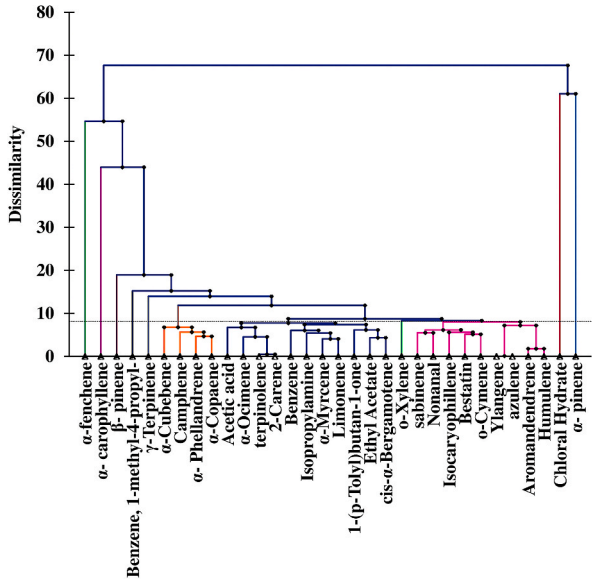

Fig. 2. Dendrogram of Agglomerative hierarchical clustering (AHC) of *Murraya koenigii* leaf Volatiles.

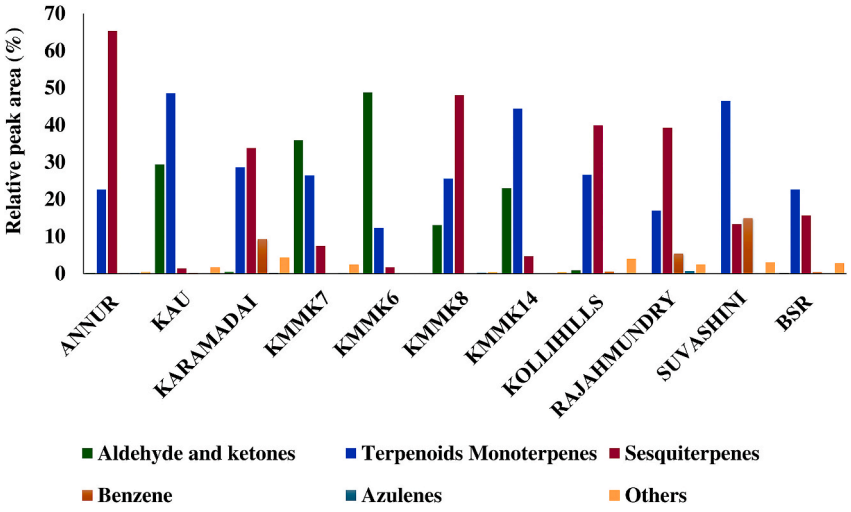

Fig. 3. Classification of Volatile compounds of *Murraya koenigii* leaf.

The intense characteristic aroma of curry leaf is due to the presence of the terpene hydrocarbons caryophyllene and phellandrene along with other terpenes [19]. The KMM5 genotype contains a high concentration of α-phellandrene (1.01%), which has a distinct black pepper odor. Similarly, α-caryophyllene is high in Rajahmundry (28.99%) and Kollihills (28.04%), which imparts a spicy, clove odor. The caryophyllene has a fixative property that may be responsible for the long-lasting aroma [20,21]. A combination of major and trace compounds is responsible for the intense aromatic nature of curry leaves.

4. Conclusions

The TD-GC-MS has been done for leaves collected from 11 different genotypes. Among the various accessions, the Karamadai variety (KMM8) contains a high amount of γ-terpinene and α-myrcene. The chloral hydrate, isopropylamine, *o*-wylene, α-phellandrene, γ-terpinene and α-myrcene content are high in Karamadai genotypes. Suvashini has high α-pinene content as well. The PCA has clearly distinguished between the accessions. These volatile database of curry leaves makes a versatile spice, offering an opportunity for choose plants rich in components desired by the flavour industry. Further analysis on the different accessions of the Karamadi variety and the extraction of the oil in yield is in progress to promote the above variety for multi-local field trials.

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Author contribution statement

V.P. Santhanakrishnan and E. Varun: Conceived and designed the experiments; Analyzed and interpreted the data and Wrote the paper. N. Shoba and B. Senthamizh Selvi: Performed the experiments and Wrote the paper. S. Mohankumar and M. Raveendran: Contributed reagents, materials, analysis tools or data and Wrote the paper.

Data availability statement

Data included in article/supp. material/referenced in article.

Additional information

No additional information is available for this paper

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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