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Nontargeted Metabolite Profiling of the Most Prominent Indian Mango (*Mangifera indica* L.) Cultivars Using Different Extraction Methods

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ABSTRACT: Aroma has a crucial role in assessing the quality of fresh fruit and its processed versions, which serve as reliable indications for advancing local cultivars in the mango industry. The aroma of mango is attributed to a complex of hundreds of volatile, polar, and nonpolar metabolites belonging to different chemical classes like monoterpenes, sesquiterpenes, nonterpene hydrocarbons (alkanes), alcohols, esters, fatty acids, aldehydes, lactones, amides, amines, ethers, and many more. This study looked at the volatile, nonpolar, and polar metabolites from 16 mango cultivars to determine their relative quantities and intervarietal changes using hexane, ethanol, and solid-phase microextraction (SPME), followed by gas chromatography−mass spectrometry (GC−MS) analysis. In total, 58 volatile compounds through SPME, 50 nonpolar metabolites from hexane extract, and 52 polar metabolites from ethanol extract were detected from all of the cultivars, belonging to various chemical classes. Through the SPME method, all 16 mango cultivars except Dashehari and Neelum exhibited abundant monoterpenes with maximum concentration in Kesar (91.00%) and minimum in Amrapali (60.66%). However, the abundance of fatty acids and sesquiterpenes was detected in Dashehari (37.91%) and Neelum (74.80%), respectively. In the hexane extract, 23 nonterpene hydrocarbons exhibited abundance in all 16 mango cultivars except Baneshan, with a higher concentration in Dashehari (95.45%) and lower in Ratna (77.63%). The ethanol extraction of 16 mango cultivars showed a higher concentration of esters, aldehydes, alcohols, and amides in Jamadar (52.16%), Dadamio (74.30%), Langra (64.38%), and Kesar (37.10%), respectively. There have been a lot of metabolite variations observed and analyzed using hierarchical cluster analysis (HCA) and principal component analysis (PCA) based on the similarity of various chemical compounds. Cluster analysis revealed the true similarity and pedigree of different mango cultivars, *viz.*, Neeleswari, Dashehari, Neelum, Alphonso, Baneshan, Sonpari, and Neeleshan. They occupied the same cluster during analysis.

INTRODUCTION

Mango (*Mangifera indica* L.) is the most well-known and most valued tropical fruit. It is a member of the Anacardiaceae family and originated in the Indo-Burma area. Due to its distinctively powerful, yet appealing flavor, it is regarded as the best fruit in the world.[1] India is the largest mango producer and contributes 40% of the total global mango production. The area and production of mango cultivation in India is 23.15 lakh hectare and 208.99 lakh MT, respectively, with a productivity of 9.03 MT ha^{-1}. In Gujarat, the area under mango cultivation is 1.66 lakh ha with a production of 12.19 lakh MT (Anon., 2021). Mango is a more or less compressed and fleshy drupe fruit. It is an excellent source of vitamin A and phenolic compounds.[2] It is a climacteric fruit in which ripening is accompanied by a peak in respiration and concomitant burst of ethylene production. In mango, during ripening, some physicochemical changes may occur that result in softening of the fruit, changes in color and flavor of the fruit, an increase in sugar content, a reduction in organic acids, and formation of pigments, especially carotenoids. Each mango variety on ripening has distinct characteristics and flavor. The stage of maturity can affect this process and affect the final flavor/aroma quality of the ripened fruit.[3] The biosynthesis of volatile compounds in fruit has been reported to occur mainly during the ripening phase. Most volatile compounds, such as terpene alcohols, nor-isoprenoid derivatives, and aromatic alcohols, are glycosidically bound and are liberated during ripening.[4]

Plant volatile organic compounds (VOCs) are secondary metabolites that play important roles in biotic interactions and abiotic stress responses. They are often found in high concentrations in certain plant tissues and organs such as luscious fruits. It may be feasible to understand more about the

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interactions of fleshy fruits with insects, bacteria, and pathogens by investigating the VOCs emitted during fruit development and the initiation of infection. Fruit VOC information might be used in agriculture to develop fruits that attract or repel pests.[5] Apart from their diverse ecological functions and sensory properties, many VOCs are significant to human health and are used as medicines; this is perhaps the least-studied aspect of these compounds.

Aroma is an important attribute, reflecting fruit quality, ripeness, and consumer acceptance, which is conferred by a range of diverse low-molecular-weight and volatile chemical compounds that are distributed widely in nature.[3] It plays a key role in the quality profiles of fruit, including mango, apple, grape, citrus, *etc*., and is an attribute considered in flavor and fragrance production not only in the food industry but also in the cosmetics industry. Sweetness, sourness, and aroma constitute the main components of fruit flavor, with aroma being the most important contributing factor. With the increasing requirement for quality in table fruit and processed products, fruit aroma has gained increasing research attention in recent years. Aroma components in fruits mainly consist of aldehydes, alcohols, esters, lactones, ketones, quinones, and terpenes.[3] Each of these volatile compounds has a distinct odor, and their combinations, concentrations, and ratio confer unique aroma characteristics to different fruits through cumulative, synergistic, and masking effects.[6] The concentrations and composition of volatile compounds in fruits, although influenced by climatic and cultivation conditions, are mainly determined by the genetic background of the plants.[7] Each mango cultivar has its own distinctive aroma, which is an important feature of the fruit quality. Liu et al.[8] reported that a wide range of volatile compounds (up to 435) have been identified in mango fruit. Among these, 3-carene was the predominant compound found in most cultivars and is primarily responsible for the typical mango aroma, while limonene, β-ocimene, myrcene, and α-terpinolene were prominent in Tainong and Jinmang cultivars. Alphonso became a unique mango cultivar due to the abundance of lactones and furanone in its ripe fruits.[9] Monoterpenes, sesquiterpenes, esters, lactones, alcohols, aldehydes, ketones, volatile fatty acids, some degradation products of phenols, and some carotenoids contribute to the aromatic volatile profile of mango fruit. They are also important to assess the quality of fresh mango and its processed products and are a good index for screening mango germplasm resources for the development of local cultivars.[10]

The complex profile of VOCs keeps changing during the life cycle of the plant. Factors like age, genetics, environmental conditions, postharvest handling, storage conditions, sunlight, irrigation, fertilization, chemical applications, and other human practices can alter the qualitative and quantitative composition of the VOCs among fruits.[1] To determine the aromatic components of mango, simultaneous solvent-assisted flavor evaporation (SAFE), solid-phase microextraction (SPME), and simultaneous distillation extraction (SDE) have been employed in food aroma extraction.[11] The procedures of SDE and SAFE isolate aromatic compounds from food matrices using organic solvents; however, these methods are highly laborious and time-consuming and entail preconcentration of extracts. Unlike well-established protocols, SPME has been extensively used in the preparation of volatile and semivolatile compounds from various types of samples.[8] This technique was developed more than two decades ago and is a rapid, simple, sensitive, and solvent-free technique for the analysis of volatile organic compounds (VOCs). Coupling of the methods of aroma extraction with the GC−MS technique, in particular, solid-phase microextraction, creates a highly reliable method of identifying potent odorants.

Although mango germplasm resources are relatively abundant throughout the world, mango breeding objectives largely focus on disease resistance, fruit size, sweetness, or color rather than aroma quality. Currently, conventional breeding that has ignored the properties of fruit aroma, making mango cultivars lack aroma diversification and resource superiority, has not been used to its full potential. The different classes of chemicals that contribute to the aroma of particular germplasm are still unclear. Evaluation of volatiles at the germplasm level is useful for future breeding efforts aimed at improving fruit quality. In the present investigation, gas chromatography−mass spectrometry (GC−MS) was used to analyze the variations and abundances of metabolites present in 16 mango cultivars extracted using hexane, ethanol, and solid-phase microextraction (SPME).

MATERIALS AND METHODS

Cultivars and Sampling Procedures. Uniform mature fruits of 16 mango cultivars, as shown in Table 1, were collected from the 10−15-year-old orchards grafted at the RHRS (Regional Horticulture Research Station), Navsari Agricultural University, Navsari. Four to five fruits were collected from the center and periphery of the plant and washed with running tap water for the removal of dirt and other impurities. The samples were stored at the Department of Fruit Science, Aspee College of Horticulture, Navsari Agricultural University, Navsari, to analyze changes in color and texture. Mature and fully ripe fruits were peeled off to remove the pulp from the fruits. The pulp was immediately transferred to a Supelco glass vial tightly capped with a tan PTFE/silicon septum (Supelco, Bellefonte, PA). The vial was initially kept at 40 °C for 5 min and then the SPME syringe with poly(dimethylsiloxane) (PDMS; 100 μm) fiber was inserted into the headspace of the vial. The extraction of volatile metabolites was carried out by keeping the vial at 40 °C with continuous agitation for 20 min; then after, the fiber holder was removed from the vial and injected into the inlet of GC−MS. Polar and nonpolar metabolites of the pulp were analyzed by mixing the 10.0 g pulp in 50 mL of ethanol and hexane separately. The suspension was incubated overnight under constant shaking at 4 °C. Overnight incubated samples were evaporated to dryness under a nitrogen evaporator (TurboVap) followed by addition of 1 mL of acetonitrile and stored at 4 °C for further use.

Instrumentation Parameters. GC−MS analysis was carried out with a Thermo Scientific Trace GC Ultra equipped with a tandem ITQ 900 mass spectrophotometer. The equipment contains an Rxi-5 MS fused-silica nonpolar column of 30 m × 0.25 mm ID and 0.25 μm film thickness (Restek). Chromatography parameters were helium as the carrier gas at a flow rate of 1.0 mL/min, GC inlet in the split-less mode, and injector temperature 280 °C. The initial temperature was 40 °C (2.0 min hold time), which was increased to 160 °C at the rate of 5 °C/min and then to 250 °C at the rate of 5 °C/min for 10 min. The total run time was 41 min. The ion source temperature was 290 °C in electron ionization mode (EI) with 70 eV ionization energy and a mass range of 35−500 m/z. Excaliber 1.0 was used for data acquisition and processing.

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Table 1. Metabolites Identified in Different Mango Cultivars Using SPME

No.	Ret. Time	Peak Name	Molecular Formula	CAS Number	Molecular weight	Dasheharri	Sonpari	Vanraj	Neeleswari	Dadamio	Amrapali	Alphonso	Kesar	Ratna	Jamadar	Neeleshan	Totapuri	Langra	Rajapuri	Neelum	Baneshan
Monoterpene																					
1	4.283	ß-Pinene	$C_{10}H_{16}$	127-91-3	136.23						60.66								50.9		
2	4.849	3-Carene	$C_{10}H_{16}$	13466-78-9	136.23					79.08								73.55			
3	5.269	Orthodene	$C_{10}H_{16}$	4889-83-2	136.23									27.96							
4	5.711	ß-Ocimene	$C_{10}H_{16}$	13877-91-3	136.2				70.27			44.59	30.68			84.59	66.79				82.6
5	6.798	Allo-ocimene	$C_{10}H_{16}$	3016-19-1	136.23		89.36	86.09	4.99			41.39	60.32	62.96	70.14		12.37		29.13		
Sesquiterpenes																					
1	8.877	Elixene	$C_{15}H_{24}$	3242-08-08	204.35													0.55			
2	8.981	α-Cubebene	$C_{15}H_{24}$	17699-14-8	204.35												0.47				
3	9.209	Copaene	$C_{15}H_{24}$	3856-25-5	204.35				1.46			0.27	0.64			0.55	2.84			7.72	
4	9.491	γ-Maaliene	$C_{15}H_{24}$	20071-49-2	204.35			0.79		7.29	1.45			0.15			7.72				
5	9.588	Caryophyllene	$C_{15}H_{24}$	87-44-5	204.35	21.40	5.74	7.84	8.42		11.04	7.88	4.21	5.46	7.81	6.5		11.35	10.67	40.45	2.5
6	9.863	Humulene	$C_{15}H_{24}$	6753-98-6	204.35																4.02
7	9.943	γ-Muurolene	$C_{15}H_{24}$	30021-74-0	204.35															6.08	
8	9.99	(+)-aristolochene	$C_{15}H_{24}$	823810-22-6	204.35					1.86					1.75						
9	10.08	Cis-β-copaene	$C_{15}H_{24}$	18252-44-3	204.35				0.96												
10	10.15	β-Selinene	$C_{15}H_{24}$	17066-67-0	204.35					9.58					11.59	3.19					1.48
11	10.21	β-chamigrene	$C_{15}H_{24}$	18431-82-8	204.35							0.19									
12	10.27	α-Guaiene	$C_{15}H_{24}$	3691-12-1	204.35				2.93								5.4				
13	10.27	α-Bulnesene	$C_{15}H_{24}$	3691-11-0	204.35													2.86		15.1	
14	10.38	α-Amorphene	$C_{15}H_{24}$	16729-01-4	204.35				0.78								0.91	1.07		4.94	
15	10.42	α-Maaliene	$C_{15}H_{24}$	489-28-1	204.35					0.64											
16	10.48	α-Bisabolene	$C_{15}H_{24}$	25532-79-0	204.35							0.09									
17	10.62	Nerolidol	$C_{15}H_{26}O$	142-50-7	222.37							0.04									
18	10.9	γ-Bisabolene	$C_{15}H_{24}$	53585-13-0	204.35							0.22					0.04				

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Table 1. continued

No.	Ret. Time	Peak Name	Molecular Formula	CAS Number	Molecular weight	Dasheharri	Sonpari	Vanraj	Neeleswari	Dadamio	Amrapali	Alphonso	Kesar	Ratna	Jamadar	Neeleshan	Totapuri	Langra	Rajapuri	Neelum	Baneshan
Sesquiterpenes																					
19	11.68	(-)-Globulol	$C_{15}H_{26}O$	489-41-8	222.37					0.05											
20	13.57	Isolongifolene, 4,5,9,10-dehydro-	$C_{15}H_{20}$	156747-45-4	200.32	2.36															
Diterpenes																					
1	15.03	m-Camphorene	$C_{20}H_{32}$	20016-73-3	272.5											0.48	0.53		0.22	1.45	
2	15.51	p-Camphorene	$C_{20}H_{32}$	20016-72-2	272.5											0.18	0.3		0.06	0.36	
Triterpenes																					
1	27.65	Squalene	$C_{30}H_{50}$	111-02-4	410.73															0.15	
Non-terpene hydrocarbons (alkane)																					
1	9.866	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	$C_{15}H_{24}$	0	204.35	12.67	2.64	4.01	4.20	1	4.66	4.91	2.95	2.65	2.91	3.34	2.44	4.92	6.3	16.32	
2	9.967	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	107-50-6	519.07		0.34				1.29		0.37					3.66			
3	11.47	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	556-68-3	593.23		0.3	0.29	0.11	0.28	1.23	0.08	0.38	0.27	0.48	0.72	0.06	1.30	1.40	2.72	
4	13.24	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	556-71-8	667.39			0.09	0.02	0.03	0.22		0.10	0.11	0.20	0.25		0.21	0.72	1.33	
5	15.28	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	$C_{16}H_{50}O_7Si_8$	19095-24-0	577.2									0.05				0.03		0.22	
6	15.28	Heptasiloxane, hexadecamethyl-	$C_{16}H_{48}O_6Si_7$	541-01-5	533.15										0.06						
7	15.28	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$	18772-36-6	741.5											0.05		0.05	0.2	0.45	
8	17.47	Tetracosamethyl-cyclododecasiloxane	$C_{24}H_{72}O_{12}Si_{12}$	18919-94-3	889.8										0.11	0.02		0.08	0.04	0.5	
9	19.94	Eicosane, 2-methyl-	$C_{21}H_{44}$	1560-84-5	296.57	0.67															
10	21.45	Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$	54833-48-6	296.57	0.59															
11	22.94	Octadecane, 2-methyl-	$C_{19}H_{40}$	1560-88-9	268.52	0.62															
Fatty acids																					
1	8.927	3-Hydroxy-2,6,6-trimethyl-hept-4-enoic acid	$C_{10}H_{18}O_3$		186.13																1.97
2	11.45	Ethylene glycol - Adipate - Diethylene glycol	$C_{10}H_{18}O_6$	0	234.25	35.68															
3	14.96	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	57-10-3	256.42				0.09												
4	16.21	Palmitic Acid, TMS derivative	$C_{19}H_{40}O_2Si$	55520-89-3	328.61	2.23			0.09	0.02			0.07			0.04		0.07		0.37	
5	18.77	12-Hydroxyoctadeca	$C_{24}H_{52}O_3Si$	0	444.84													0.01			

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Table 1. continued

No.	Ret. Time	Peak Name	Molecular Formula	CAS Number	Molecular weight	Dasheharri	Sonpari	Vanraj	Neeleswari	Dadamio	Amrapali	Alphonso	Kesar	Ratna	Jamadar	Neeleshan	Totapuri	Langra	Rajapuri	Neelum	Baneshan
Fatty acids																					
		noic acid, TMS derivative																			
Esters																					
1	7.415	Butanoic acid,4-hexen-1-yl ester	$C_{10}H_{18}O_2$	0	170.25						14.26										
2	7.914	cis-3-Hexenyl crotonate	$C_{10}H_{16}O_2$	0	168.23						3.19										
3	8.682	Hexenyl tiglate, 4Z-	$C_{11}H_{18}O_2$	0	182.26						0.91										
4	16.2	Hexadecanoic acid, dimethyl(isopropyl)silyl ester	$C_{21}H_{44}O_2Si$	0	356.7		0.11	0.14			0.13			0.06	0.15		0.05				
Aldehyde																					
1	7.888	5-Hydroxymethylfurfural	$C_6H_6O_3$	67-47-0	126.11				5.26												6.78
2	12.02	Pentadecanal-	$C_{15}H_{30}O$	2765-11-9	226.4														0.06		
Phenol																					
1	11.33	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	$C_{17}H_{26}O_2$	14035-34-8	262.4	2.40															
2	18.22	3,4'-Isopropylidenediphenol	$C_{15}H_{16}O_2$	46765-25-7	228.29	3.02	0.66	0.75	0.42	0.17	0.96	0.12	0.24	0.33	2.74	0.09	0.08	0.29	0.3	1.84	0.65
Ketones																					
1	11.85	6,8-Nonadien-2-one, 6-methyl-5-(1-methylethylidene)-	$C_{13}H_{20}O$	60714-16-1	192.29							0.05	0.04								
2	12	Tropolone	$C_7H_6O_2$	533-75-5	122.12	4.77															
Lactone																					
1	8.159	γ-Octalactone	$C_8H_{14}O_2$	104-50-7	142.19		0.85					0.17			2.06						
Amide																					
1	20.91	Palmitoleamide	$C_{16}H_{31}NO$	106010-22-4	253.42	1.22															
Amine																					
1	14.26	Dimethyl palmitamine	$C_{18}H_{39}N$	112-69-6	269.51	3.03															
Other																					
1	11.81	2,6,6-Trimethylcyclohex-1-enylmethanesulfonyl)benzene	$C_{16}H_{22}O_2S$	56691-74-8	278.4	9.35															

Identification of Metabolites and Statistical Analysis. Identification of metabolites was done by comparing the mass spectra obtained from the experiment with those available in the National Institute of Standards and Technology (NIST) database with greater than 75% match. The concentration of individual metabolites was considered as a percentage of the total peak area. The raw data thus obtained were analyzed using Metaboanalytes 5.0 online tool.[12] The data were normalized and clustered afterward. PCA and heat maps were used to profile the differential volatiles present in cultivars.

RESULTS AND DISCUSSION

The diversity of metabolites, their relative abundance, and their combination are significant factors for determining consumers' preference for a particular fruit. A variety of metabolites were detected from mango pulp using polar and nonpolar solvents, and SPME. Fruit aroma is one such trait that is attributed to a

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combination of diverse metabolites. The Supporting File shows a representative GC−MS chromatogram of all 16 mango cultivars identified using SPME, hexane, and ethanol extracts.

Analysis of Metabolites through SPME. The name, chemical class, CAS number, molecular formula, and relative peak area of the metabolites are listed in Table 1. Sesquiterpenes, nonterpene hydrocarbons, and phenols were detected in all cultivars, whereas amide (palmitoleamide), amine (dimethyl palmitamine), and (2,6,6- trimethylcyclohex-1-enylmethanesulfonyl)benzene were detected only in Dashehari cultivar. The cultivar Neelum had the maximum number of volatile components (16), whereas cultivar Baneshan had the minimum number of volatile components (7).

Terpenoid Hydrocarbons. Except for Dashehari and Neelum, where sesquiterpenes contributed to the aroma, monoterpenes were the primary contributors to the mango aroma in all cultivars. β-Ocimene was the dominant compound in five cultivars, *viz*., Neeleshan, Baneshan, Neeleswari, Totapuri, and Alphonso, whereas allo-ocimene was found to be prominent in Sonpari, Vanraj, Jamadar, Ratna, and Kesar. Two of the cultivars, Amrapali and Rajapuri, were shown to have β-pinene as a major monoterpne; on the other hand, 3-carene was reported to be present only in Dadamio and Langra with a higher peak area. Orthodene was reported only in Ratna, whereas m-camphorene was found in relatively low peak area percentages in Neeleshan, Totapuri, Rajapuri, and Neelum. This is in agreement with previous reports that monoterpenes, sesquiterpenes, and diterpenes dominate the aroma of mango fruits.[13,14] Allo-ocimene and β-ocimene contribute to the mango flavors and fragrance.[15] In a similar study, β-ocimene accounted for the major components of ripe Alphonso.[14,16] On the other hand, myrcene, p-cymene, limonene, and 3-carene were detected at higher concentrations in Alphonso.[16] Depending on its abundance, orthodene can impart turpentine flavor to ripe mango cultivars.[3] According to the experiment, the combination of different terpenes resulted in different aroma profiles of mangos, resulting in a wide range of aroma profiles. It is the combination and uniqueness of different volatiles that give that cultivar its unique flavor. An abundance of any one of these metabolites provides a differential flavor to each cultivar, as each monoterpene has its own blend and aroma. The presence and abundance of 3-carene were reported by several researchers in diverse mango cultivars that are major aroma-active compounds.[8,17,18] The assessment, which was done using pulp, intends to analyze the aroma-active metabolites of pulp rather than the fruit as a whole.[19] This method was therefore used to measure the actual volatiles emitted by pulp. The description of the fragrance of several ripe mangoes sheds light on certain fundamental concepts of pest preference for particular crops. Monoterpenes and sesquiterpenes are independently produced in the cytosol and chloroplast, respectively.[14] Thereby, the female parent is crucial in defining the cultivar's aroma value.

In the context of the present study, sesquiterpenes represent the second most abundant class of metabolites found in the mango cultivars. β-Caryophyllene was the dominant compound in 12 cultivars, *viz*., Neelum, Dashehari, Langra, Amrapali, Rajapuri, Neeleswari, Alphonso, Vanraj, Neeleshan, Sonpari, Ratna, and Kesar. Among them, Neelum and Dashehari appeared to contain a significant amount of β-caryophyllene in comparison with all other cultivars that are characterized by their abundance of monoterpenes. The cultivars Totapuri and Dadamio were found to be rich in γ-maaliene; Neelum in α-amorphene and α-bulnesene; and Totapuri and Jamadar in α-guaiene and β-selinene. Volatile components, namely, elixene, α-cubebene, humulene, iso-longifolene, and 4,5,9,10-dehydro- and cis-β-copaene were reported only in cultivars Langra, Totapuri, Baneshan, Dashehari, and Neeleswari, respectively. The compounds nerolidol, β-chamigrene, and α-bisabolene were found only in Alphonso; α-malliene and (−)-globulol were reported only in Dadamio, whereas γ-muurolene and squalene were reported only in Neelum cultivars. Caryophyllene contributed to sweet, woody, and spicy aroma, which was most abundantly found in the pulp and peel of "Nam Dok Mai" mangoes.[20] A similar finding of abundance of sesquiterpenes was reported for Espada and Tommy Atkins.[19] Lalel et al.[21] reported on two compounds, namely (−)-spathulenol (flavoring agent in food) and γ-maaliene (woody odor), in ripe mango. Sesquiterpenes like β-chamigrene, α-bulnesene, α-bisabolene, γ- bisabolene, elixene, nerolidol, aristolochene, globulol, α-guaiene, and squalene (triterpene) were also reported in various mango cultivars at lower concentrations.[3,15,20]

Nonterpene Hydrocarbon Alkanes. Out of the 11 alkanes, the compounds 1,4,7-cycloundecatriene, 1,5,9,9-tetramethyl-, *Z,Z,Z*- (1.00 to 12.67%) and cyclooctasiloxane, and hexadecamethyl- (0.06 to 2.72%) were noted in all of the studied mango cultivars except Baneshan and Dashehari. Three alkanes, *viz*., eicosane, 2-methyl-heptadecane, 2,6,10,15-tetramethyl- and octadecane, and 2-methyl- were registered only in Dashehari, whereas heptasiloxane and hexadecamethyl- were registered only in Jamadar mango. Langra was rich in cycloheptasiloxane and tetradecamethyl- (3.66%) alkane as compared to other mango cultivars, *viz*., Sonpari, Amrapali, and Kesar. Surprisingly, there were no alkanes identified in Baneshan cultivar, which may be due to the relatively low abundance of such compounds. Nonterpene hydrocarbons (hexadecane, tetradecane, octadecane, tridecane, dodecane, and so on) were found in several mango cultivars; this class contributed to the volatile blend, as reported by several researchers.[3,16] A total of 29 distinct nonterpene hydrocarbons, such as 1,4,7,-cycloundecatriene, 1,5,9,9-tetramethyl-, *Z,Z,Z*-, heptadecane, 2,6,10,15-tetramethyl, eicosane, *etc*., have been found in several mango cultivars.[22] Li et al.[3] detected various alkanes in eight mango cultivars, including octamethyl-cyclotetrasiloxane, hexamethyl-cyclotrisiloxane, eicosane, *etc*. Hexamethyl-cyclotrisiloxane was the most dominant chemical in the Magovar cultivar, accounting for 46.66% of the total volatile chemicals. Alkanes may not be involved in imparting aroma, but may play a diverse role in metabolic processes.

Fatty Acids and Esters. The results showed that ethylene glycol−adipate−diethylene glycol (hexanoic acid derivatives) (35.68%) was the dominant metabolite in the Dashehari cultivar, while the rest of the fatty acids appeared to be the least in the other mango cultivars. In the present study, 4 esters were observed, *viz*., butanoic acid, 4-hexen-1-yl ester; *cis*-3-hexenyl crotonate; hexenyl tiglate, 4Z-, and hexadecanoic acid, dimethyl (isopropyl) silyl ester, only in Amrapali cultivar at noticeable amounts compared to all cultivars. Furthermore, in the current experiment, which analyzes metabolites using the SPME extraction technique, the majority of the cultivars exhibited no trace of esters. Fatty acids are considered to be precursors for the synthesis of various plants volatiles, which are synthesized through β-oxidation and the lipoxygenase (LOX) pathway.[15,23] The results from Malgoa, Totapuri, Alphonso, Pairi, Kent, Benishan, Sundari, and Neelam

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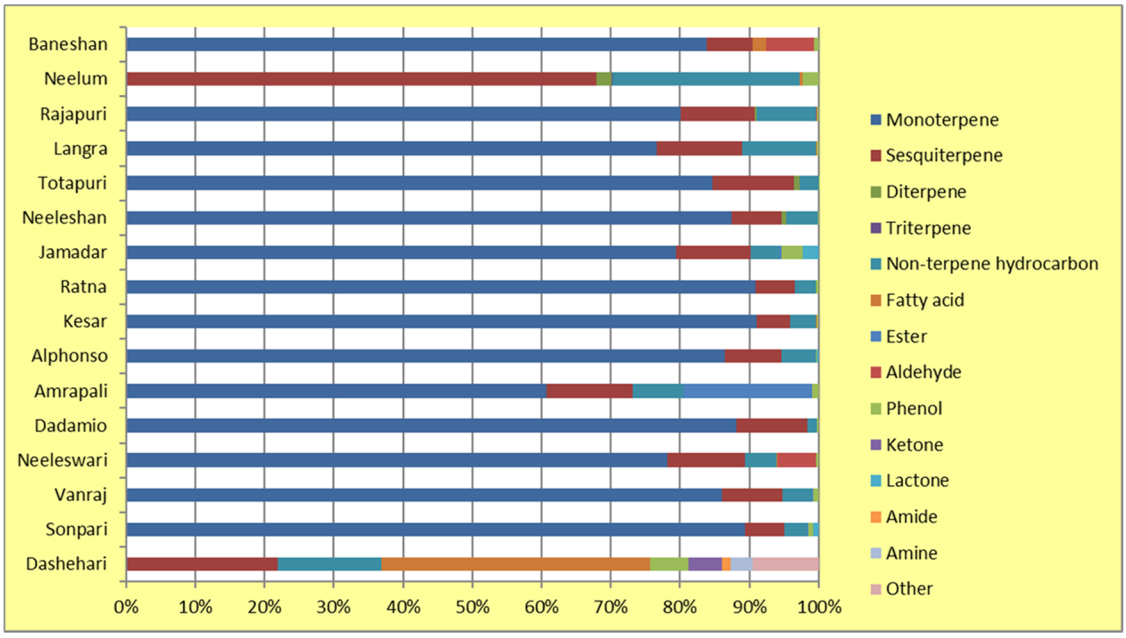

Figure 1. Relative abundance of different classes of metabolites extracted using SPME.

corroborated the fact that pulp had a low content of fatty acids.[23] Octadecadienoic acid, hydroxy acid derivatives, hexanoic acid derivatives, methyldihydromalvalate, and palmitic acid are presumed to have a significant metabolic role in the ripening of mango. The volatiles of mature mango fruits are greatly influenced by palmitic-palmitoleic acid and its derivatives.[24−26] The formation of the distinctive aroma in the ripe mango fruit has been attributed to a rise in fatty acids in Alphonso, Langra, Neelum, Totapuri, and Rajapuri mangoes, among others.[13] There is always perplexity regarding the role of esters for their contribution to the flavor and aroma of ripe mangoes.[19,27,28] There were some reports suggesting the significance of some of the esters, *viz*., butanoic acid, hexyl ester; butanoic acid,octyl ester; 4 hydroxymandelic, ethyl ester, cis-3- hexenyl crotonate, and dimethyl(isopropyl)silyl ester in different mangoes.[3,7,8]

Phenols, Ketones, Lactones, and Others. Of the two detected phenols, 3,4′-isopropylidenediphenol was reported in all 16 mango cultivars, whereas 2,6-bis(1,1-dimethylethyl)-4-(1-oxopropyl) phenol was recorded in the Dashehari cultivar only. Alphonso and Kesar were reported to have 6,8-nonadien-2-one and 6-methyl-5-(1-methylethylidene)- in a relatively low peak area percentage, while Dashehari cultivar had only Tropolone in a considerable amount. γ-Octalactone was the only lactone detected among the total volatile components in the present study, with its concentration of 0.19 to 2.06% in Sonpari, Alphonso, and Jamadar cultivars, respectively. Moreover, Dashehari cultivar was represented by unique metabolites such as palmitoleamide (amide), dimethyl palmitamine (amine), and (2,6,6-trimethylcyclohex-1-enylmethanesulfonyl)benzene. Two aldehydes, namely, 5-hydroxymethylfurfural was found in Neeleswari and Baneshan, whereas pentadecanal was noted in Rajapuri cultivar only. Similarly, aldehydes alone cannot contribute to the mango flavor, as it has a high odor active value (OAV). Several scientists have reported aldehydes from different parts of fruit.[3,11] Aldehyde concentrations were high in mature fruits in Alphonso, suggesting that they may be important aroma components in raw mango products and may also contribute to aroma.[13,16] In Riesling and Cabernet Sauvignon grapes, pentadecanal was the most abundant volatile compound.[29] The pulp of the ripe Kasturi mango was found to contain a variety of volatile compounds, including 5-hydroxymethylfurfural with the highest relative abundance (21.96%). These results are consistent with earlier findings for ketones in mango pulp.[33,30] Li et al.[3] observed 2,6-bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol and 4−4′-(1-methylethylidene)bis-phenol in different mango cultivars. De Santana Silva et al.[31] identified 8 individual phenolic compounds in fresh and processed mango pulp samples. Guiya mangoes contain γ-octalactone, a lactone, in measurable but low amounts.[3,17] Remarkably, a substantial percentage of this substance was also detected in peach and papaya.[32,33]

As the contribution of volatile compounds to mango aroma and flavor is complex, a more thorough subjective research must be conducted to ascertain the precise role of volatile compounds in mango. There may be a variety of factors that influence the volatile profile of mango fruit, including environmental factors, metabolites' availability,[16] fruit-ripening stages,[21] season and harvesting time, extraction solvents, chromatographic conditions, sophistication, and column properties.[11,28]

Relative Abundance of Different Classes of Volatile Compounds through the SPME Method. In the present study, Kesar mango had the maximum monoterpene content (91.00%), whereas cultivar Neelum had the minimum content (1.45%). However, monoterpenes were not detected in the Dashehari cultivar (Figure 1). Neelum had a high amount of total sesquiterpene (74.80%) mainly due to the high contents of caryophyllene, α-bulnesene, and copaene. However, Kesar showed the lowest sesquiterpene content (4.85%). Differences of terpene constituents were also found among the cultivars. For instance, Sonpari (89.36%) and Vanraj (86.09%) cultivars had a very high amount of allo-ocemene, but β-pinene, 3-carene, orthodene, β-ocimene, and *m*-camphorene were not detected. Among 16 studied mango cultivars, Neelum (21.54%), Dashehari (14.54%), and Langra (10.25%) were

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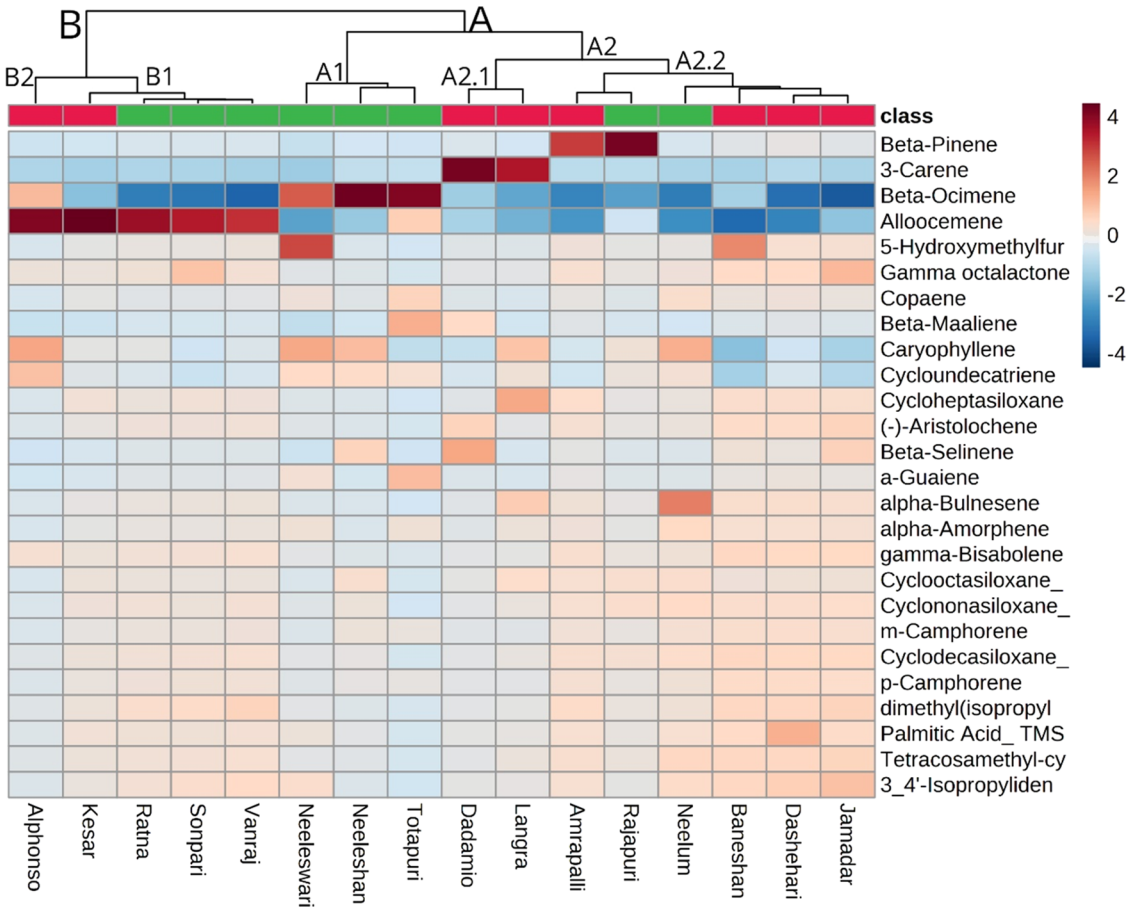

Figure 2. Hierarchical cluster analysis (HCA) and heat map visualization of the metabolite profiles of 16 mango cultivars using SPME.

found to be rich in nonterpene hydrocarbons as compared to other cultivars.

Multivariate Data Analysis. Hierarchical cluster analysis (HCA) was used to catalog the 16 cultivars into various clusters as per the abundance, similarity, and distribution of 58 metabolites identified through SPME (Figure 2). The analysis resulted in a dendrogram and heat map that assisted in the understanding of relationship among metabolites and cultivars. The clustering results were measured using Euclidean and war. D algorithm. The sixteen mango cultivars were divided into two major clusters (A and B). Cluster A was further divided into 2 subgroups (A1 and A2). Cultivars Neeleswari, Totapuri, and Neeleshan occupied the A1 group, which was characterized as having β-ocimene, copaene, caryophyllene, 1,4,7,-cycloundecatriene, 1,5,9,9-tetramethyl-, *Z*,*Z*,*Z*-; cyclooctasiloxane, hexadecamethyl-, cyclononasiloxane, octadecamethyl-, and cyclodecasiloxane, eicosamethyl-; however, this group contained a higher concentration of 5-hydroxymethylfurfural particularly in Neeleswari. Meanwhile, Jamadar, Dashehari, Baneshan, Neelum, Rajapuri, and Amrapali were classified as an A2.2 subcluster with high concentrations of palmitic acid, TMS derivative, octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,-15,15-hexadecamethyl-, α-bulnesene, tetracosamethyl- cyclododecasiloxane, and β-pinene. Langra and Dadamio were grouped into the A2.1 subgroup with maximum concentrations of 3-carene, (−)-aristolochene, β-selinene, and cycloheptasiloxane, tetradecamethyl-. Subsequently, cluster B was also divided into 2 subgroups (B1 and B2); among them, Vanraj, Sonpari, Ratna, and Kesar were included in B1, which were characterized as having hexadecanoic acid, dimethyl-(isopropyl)silyl ester, γ-octalactone, allo-ocimene, and 6,8-nonadien-2-one, 6-methyl-5-(1-methylethylidene)-. However, Alphonso was the only cultivar in subgroup B2 with high concentrations of γ-bisabolene and 6,8-nonadien-2-one, 6-methyl-5-(1-methylethylidene)-. Neeleswari and Neeleshan were developed by crossing of Neelum with Dashehari and Baneshan, respectively, while Dashehari and Neelum were crossed to create Amrapali. This could be one of the reasons of assigning one cluster to these six cultivars.

Principal component analysis (PCA) and biplot were used to observe the significant variables that represent the maximum variance. The biplot of PCA indicated that the first five components contributed to 96% of the total variance and among them, PC1 and PC2 represented 58.6 and 19.1% of the variance, respectively, which accounts for 77.7% of the total variance (Figure 3). The PCA value tends to move in either direction from zero, and if it is farthest away from zero, it indicates their positive influence on varietal characteristics. The volatiles represented in PC1 and PC2 could be used to discriminate different cultivars as they showed higher variance within them. This indicates that varieties with high aroma and flavor tend to have a lot of terpenoid hydrocarbons.

Hexane Extract Method. *Identification and Concentration of Volatile Compounds (Nonpolar Metabolites) in Mango Cultivars through the Hexane Extraction Method.* The results of GC−MS using the nonpolar solvent suggested that mango pulp contains 50 nonpolar metabolites belonging to 16 cultivars (Table 2). The cultivar Neelum had the most

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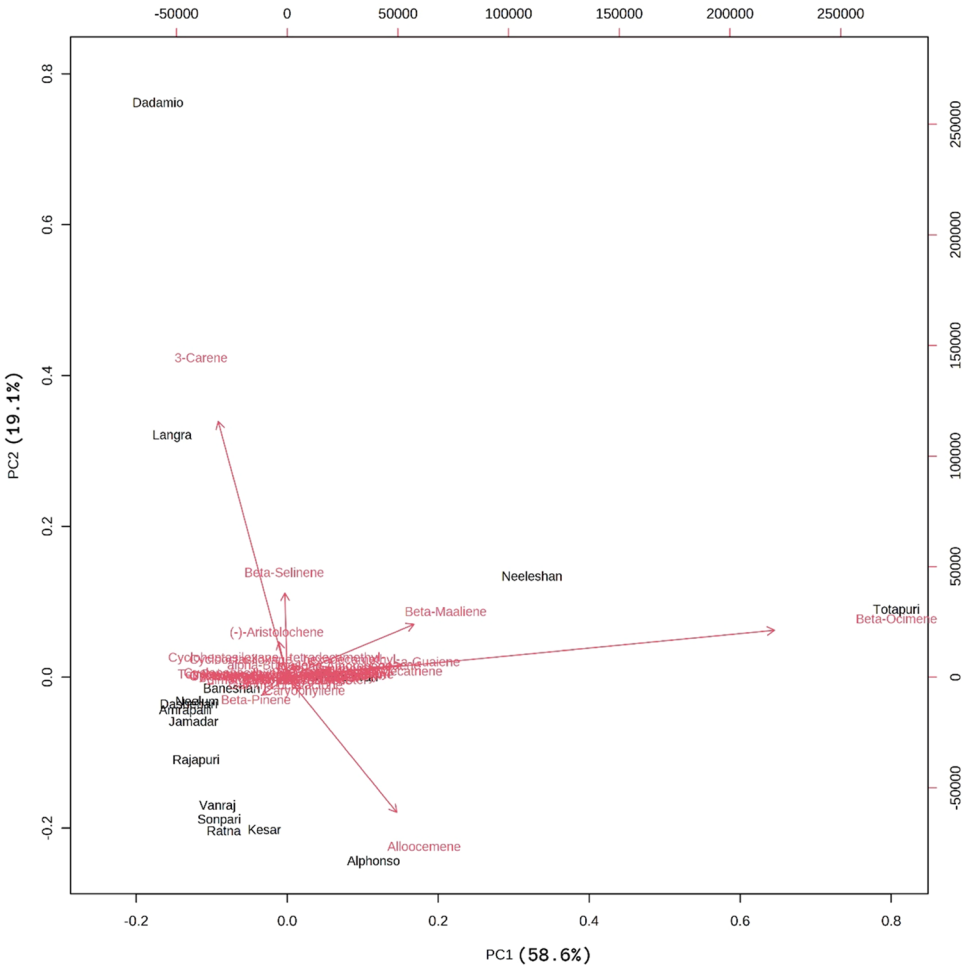

Figure 3. PCA biplot between the selected PCs. The left and bottom axes describe PC, and the top and right axes belong to the loading plot score; the angles formed by vectors of distinct variables describe their correlation.

nonpolar metabolites (24 compounds), ranking first among all of the cultivars analyzed. In contrast, the cultivar Dashehari had the least nonpolar metabolites (11 compounds).

Nonterpene Hydrocarbons (Alkanes). The nonpolar metabolite components decane, 2,6,8-trimethyl-; decane, 2,3,5,8-tetramethyl-; eicosane, 7-hexyl-; decane, 2,4,6-trimethyl-; and octacosane, 2-methyl- were detected only from Dashehari; Baneshan; Alphonso; Amrapali; and Totapuri, respectively. Compound tridecane, 7-cyclohexyl- was found only in Rajapuri and Neelum; heptadecane, 2,3-dimethyl- was observed in Alphonso and Rajapuri mangoes. Heptadecane, 2,6-dimethyl-; nonadecane, 2-methyl-; heptadecane, 2,6,10,14-tetramethyl-; and tetracontane, 3,5,24-trimethyl- were reported to be higher in Sonpari; Neelum; Dashehari; and Dadamio, respectively. In the present study, heptadecane, 2,6,10,15-tetramethyl-; and 1- Iodo-2-methylundecane and eicosane, 2-methyl- appeared at two different retention times, indicating stereoisomers of the same mass, but diverse compounds of the same mass are not present in the MS library. Since nonterpene hydrocarbons of different lengths are not the only components that make up aroma, a considerable mixture of aroma-active compounds is needed to make it more reflective. In addition, their odor detection thresholds are higher, and there is little discussion in the literature of their relative significance for aroma.[34] Alkanes of different chain lengths, such as heptadecane, 2,6,10,15-tetramethyl-; decane; nonadecane; tetradecane; decane, 2,3,5-trimethyl; hexadecane; heptadecane; eicosane; pentadecane; and octacosan have been detected in several mango cultivars.[7]

Esters. During the present experiment, pentanoic acid, 5-hydroxy-2,4-di-*t*-butylphenyl esters were reported only in Alphonso, while trichloroacetic acid, hexadecyl ester was found to be the highest among all nonpolar metabolites in Baneshan. There was a smaller but significant contribution by all other esters to metabolite variation among cultivars. All studied mango cultivars except Dashehari and Alphonso were found to have carbonic acid, eicosyl vinyl ester, and *i*-propyl 12-methyl-tridecanoate, but Baneshan did not have the latter one. Various researchers have produced similar results where different esters such as propanoic acid, 2-methyl-3-methylbutyl ester, hexanoic acid, ethyl ester, dodecanoic acid, and ethyl ester have been found to be present in different mango cultivars.[7,35] GC−MS analysis revealed important medicinal biomolecules, including *i*-propyl 12-methyl-tridecanoate, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester, and acetic acid ester, which were found in siddha medicine and have antioxidant properties.[36]

Alcohols, Ketones, Aldehydes, Phenols, and Ethers. In this study, in total 3 alcohols were detected; among them, 2-hexyl-1-octanol and 1-dodecanol, 2-hexyl- were present only in Neelum and Baneshan cultivars, respectively, while propan-2-ol, 1-(2-isopropyl-5-methylcyclohexyloxy)-3-(4- morpholyl)-

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Table 2. Metabolites Identified in Different Mango Cultivars Using the Hexane Extraction Method

No.	Ret. Time	Peak Name	Molecular Formula	CAS Number	Molecular weight	Dasheharri	Sonpari	Vanraj	Neeleswari	Dadamio	Amrapali	Alphonso	Kesar	Ratna	Jamadar	Neeleshan	Totapuri	Langra	Rajapuri	Neelum	Baneshan
Alkane																					
1	17.23	Decane, 2,6,8-trimethyl-	$C_{13}H_{28}$	62108-26-3	184.36	0.51															
2	18.082	Decane, 2,3,5,8-tetramethyl-	$C_{14}H_{30}$	192823-15-7	198.39																4.18
3	18.36	Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$	54833-48-6	296.60	9.86	15.16	10.39	19.81	11.56	11.25		12.25	11.27	11.23	13.86	6.03	6.75	6.99	9.23	12.53
4	18.364	Eicosane, 7-hexyl-	$C_{26}H_{54}$	55333-99-8	366.7							2.63									
5	18.468	1-Iodo-2-methylundecane	$C_{12}H_{25}I$	73105-67-6	296.23		1.65		1.41	0.47	0.37	1.96	0.48				0.41				
6	18.739	Heptadecane, 2,6-dimethyl-	$C_{19}H_{40}$	54105-67-8	268.52		3.85	1.54			0.44	1.95		0.48					0.58	0.55	
7	18.743	Decane, 2,4,6-trimethyl-	$C_{13}H_{28}$	62108-27-4	184.36						0.43										
8	18.847	Nonadecane, 2-methyl-	$C_{20}H_{42}$	1560-86-7	282.55					1.41	0.45		1.50				0.71			19.92	
9	19.427	Heptadecane, 2,6,10,14-tetramethyl-	$C_{21}H_{44}$	18344-37-1	296.60	3.16	0.74	2.95		1.33		3.18		1.56	1.74		2.33	1.97	1.55	2.03	1.56
10	20.188	Dodecane, 2,6,10-trimethyl-	$C_{15}H_{32}$	3891-98-3	212.41		3.05	3	3.95		2.52		3.09	4.36	2.37	2.15	3.91	3.27	1.68	0.83	7.64
11	20.413	Tetradecane, 2,6,10-trimethyl-	$C_{17}H_{36}$	14905-56-7	240.5	10.28	25.88	15.89	11.49	31.85	29.74		10.43	23.55	29.20	26.86	32.38	25.77	28.71	20.35	
12	20.564	Octacosane, 2-methyl-	$C_{29}H_{60}$	1560-98-1	408.80												5.19				
13	20.705	Tetracontane, 3,5,24-trimethyl-	$C_{43}H_{88}$	55162-61-3	605.2		1.55	2.14	1.24	4.01	1.91		2.47	0.77	2.77		2.20	5.28	2.10		
14	21.16	Tridecane, 7-cyclohexyl-	$C_{19}H_{38}$	13151-92-3	266.5														0.83	0.23	
16	23.444	Eicosane, 2-methyl-	$C_{21}H_{44}$	1560-84-5	296.6	5.35	5.52	3.83		4.82			5.32	4.27	5.8	4.37	6.88		4.22	5.19	
17	30.933	Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$	54833-48-6	296.6	14.69	4.9			2.85	7.59	12.02	2.07			3.11			2.16		
18	30.936	1-Iodo-2-methylundecane	$C_{12}H_{25}I$	73105-67-6	296.23				3.01			2.68									
19	35.93	Octadecane, 2-methyl-	$C_{19}H_{40}$	1560-88-9	268.52			10.09	5.85	3.16	1.98	11.03	9.87	10.68	2.97	9.45	3.85	15.8	3.69		
20	46.316	Eicosane, 2-methyl-	$C_{21}H_{44}$	1560-84-5	296.6	28.09			9.17	20.23	12.38	48.91	12.95	20.69	24.2	19.05	14.83	13.96	30.46	10.88	
21	49.153	Pentacosane	$C_{25}H_{52}$	629-99-2	352.70	19.42		26.48	20.90		14.06		19.56			8.08		6.34		7.36	
22	51.43	Heptadecane, 2,3-dimethyl-	$C_{19}H_{40}$	61868-03-9	268.52							5.12							2.34		
23	53.358	Eicosane, 2-methyl-	$C_{21}H_{44}$	1560-84-5	296.6	4.08	20.2	7.21	8.40				5.75							3.62	
Ester																					
1	18.552	Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	$C_{19}H_{30}O_3$	166273-38-7	306.4							6.53									
2	19.645	Sulfurous acid, pentyl tetradecyl ester	$C_{18}H_{38}O_3S$	0	334.6						0.27										

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Table 2. continued

No.	Ret. Time	Peak Name	Molecular Formula	CAS Number	Molecular weight	Dashehari	Sonpari	Vanraj	Neeleswari	Dadamio	Amrapali	Alphonso	Kesar	Ratna	Jamadar	Neeleshan	Totapuri	Langra	Rajapuri	Neelum	Baneshan
Ester																					
3	20.047	Carbonic acid, eicosyl vinyl ester	$C_{23}H_{44}O_3$	22437 91-78-6	368.60		2.43	3.13	3.28	3.2	2.75		1.89	2.89	2.45	1.79	4.49	6.28	3.16	1.16	1.54
4	20.55	Trichloroacetic acid, pentadecyl ester	$C_{17}H_{31}Cl_3O_2$	74339 -53-0	373.80																1.35
5	21.08	Trichloroacetic acid, hexadecyl ester	$C_{17}H_{31}Cl_3O_2$	74339 -53-0	373.79																38.26
6	21.308	Acetic acid, 3,7,11,15-tetramethyl-hexadecyl ester	$C_{22}H_{44}O_2$	0	340.6																5.58
7	21.872	i-Propyl 12-methyl-tridecanoate	$C_{17}H_{34}O_2$	0	270.50		0.98	1.03	1.07	0.95	1.02		1.06	1.21	1.63	1.29	1.57	2.04	0.97	0.86	
8	22.029	3-Trifluoroacetoxypentadecane	$C_{17}H_{31}F_3O_2$	0	324.42															0.34	
9	22.801	Dodecyl angelate	$C_{17}H_{32}O_2$	0	268.43												2.95		2.24	2.68	
10	23.86	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	$C_{18}H_{28}O_3$	6386-38-5	292.41					0.53	0.52			0.53			0.44			0.56	
11	24.206	Di-3,7-dimethyl-1-octyl phthalate	$C_{24}H_{38}O_4$	31680 8-86-3	446.66									2.2							
12	24.3	E-11-Hexadecenoic acid, ethyl ester	$C_{18}H_{34}O_2$	0	282.46									1.83							
13	24.712	Ethyl 13-methyl-tetradecanoate	$C_{17}H_{34}O_2$	64317 -36-1	270.5									1.98		1.04					
Alcohol																					
1	18.676	2-Hexyl-1-octanol	$C_{14}H_{30}O$	19780 -79-1	214.39															0.19	
2	21.61	1-Dodecanol, 2-hexyl-	$C_{18}H_{38}O$	11022 5-00-8	270.5																3.95
3	22.804	Propan-2-ol, 1-(2-isopropyl-5-methylcyclohexyloxy)-3-(4-morpholyl)-	$C_{17}H_{33}NO_3$	32078 4-52-9	299.4			2.12	2.16	2.26			1.89	2.61				2.59			
Ketone																					
1	22.797	Pentacosane-2,4-dione	$C_{25}H_{48}O_2$	65351 -32-8	380.65		2.66														
2	22.801	Pentadecane-2,4-dione	$C_{15}H_{28}O_2$	53759 -23-2	240.38						2.41				3.14						
3	23.495	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	82304 -66-3	276.37		3.85	2.58	3.13	3.17	2.58	3.99	1.84	2.94	4.24	3.27	5	2.7	4.59	4.18	1.92
Other																					
1	18.37	Silane, trichlorodocosyl-	$C_{22}H_{45}Cl_3Si$	7325-84-0	444																0.53
2	19.514	2-Piperidinone, N-[4-bromo-n-butyl]-	$C_9H_{16}BrNO$	19519 4-80-0	234.13			1.69		1.76	2.14		1.73		0.76	0.83	1.26	2.96		2.36	
3	21.157	Heptacos-1-ene	$C_{27}H_{54}$	15306 -27-1	378.72																1.55

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Table 2. continued

No.	Ret. Time	Peak Name	Molecular Formula	CAS Number	Molecular weight	Dasheharri	Sonpari	Vanraj	Neeleswari	Dadamio	Amrapali	Alphonso	Kesar	Ratna	Jamadar	Neeleshan	Totapuri	Langra	Rajapuri	Neelum	Baneshan
Other																					
4	21.204	Pentacos-1-ene	$C_{25}H_{50}$	16980-85-1	350.7																9.37
5	22.036	Clonitazene	$C_{20}H_{23}ClN_4O_2$	3861-76-5	386.9					0.56									0.53		
Ether																					
1	20.882	Docosyl octyl ether	$C_{30}H_{62}O$	0	438.8															1.78	
Aldehyde																					
1	17.354	Dodecanal	$C_{12}H_{24}O$	112-54-9	184.32	0.54	0.72	0.67		0.63	0.50		0.62	0.55	0.65	0.48	0.49	0.54		0.36	
Phenol																					
1	18.548	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	96-76-4	206.32	4.00	6.86	5.26	5.13	5.25	4.69		5.23	5.63	6.85	4.37	5.08	3.75	3.20	5.34	9.68

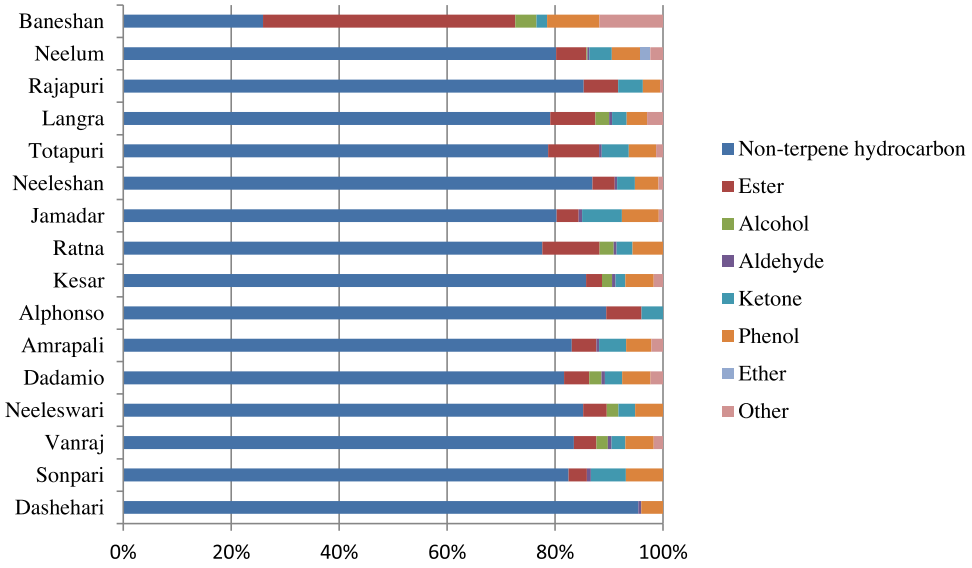

Figure 4. Relative abundance of different classes of metabolites extracted using the hexane extraction method.

compound was identified in Ratna, Langra, Dadamio, Neeleswari, Vanraj, and Kesar. Among the three ketones, 7,9-di*tert*-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione was observed in all of the studied mango cultivars except Baneshan. In the same way, one of each aldehyde, phenol, and ether were detected in different mango cultivars. Phenol (2,4-di*tert*-butylphenol) was identified in all of the studied mango cultivars except Alphonso and recorded a maximum relative peak area percentage in Neelum and minimum in Rajapuri. Aldehydes are usually converted into alcohols and do not contribute significantly to odor or flavor due to their higher odor detection threshold.[3,7] Five alcohols, *viz.*, 2-propyl-1-heptanol, 2-butyl-1-octanol, 1-nonanol, 4-ethyl-1-hexyn-3-ol, and fluoren-9-ol, were detected from the different mango cultivars.[7] Several alcohols have been reported from the Banginapalli and Alphonso mangoes.[35,37] It was reported that the pulp and peel of Kensington Pride mango contain several ketones, *viz.*, 7,9-di*tert*-butyl-1-oxaspiro(4,5) you deca-6,9-diene-2,8-dione (antimicrobial and antioxidant property); 2,4- pentanedione.[28] Dodecanal, with its woody odor, was identified as one of the odor active compounds present in the Ataulfo mango.[38]

Relative Abundance of Different Classes of Nonpolar Metabolites Identified in Hexane Extract. Except for Baneshan, none of the 16 mango cultivars contained nonterpene hydrocarbons (Figure 4). The maximum concentration of nonterpene hydrocarbons was recorded in Dashehari (95.45%), while minimum was found in Baneshan (25.91%). Esters were recorded in all of the studied mango cultivars except Dashehari; the total relative abundance of ester compounds varied widely, ranging from 2.95% (Kesar) to 46.73% (Baneshan). Alcohols were detected in 8 mango cultivars ranging from 0.19 to 3.95%. Aldehyde occurs at a low level, but plays a key role in mango flavor.[3] Similar to this trend, in the present study, aldehydes were noted in 12 mango cultivars with lower concentrations (0.36 to 0.72%). Ketones were detected in all of the studied mango cultivars except

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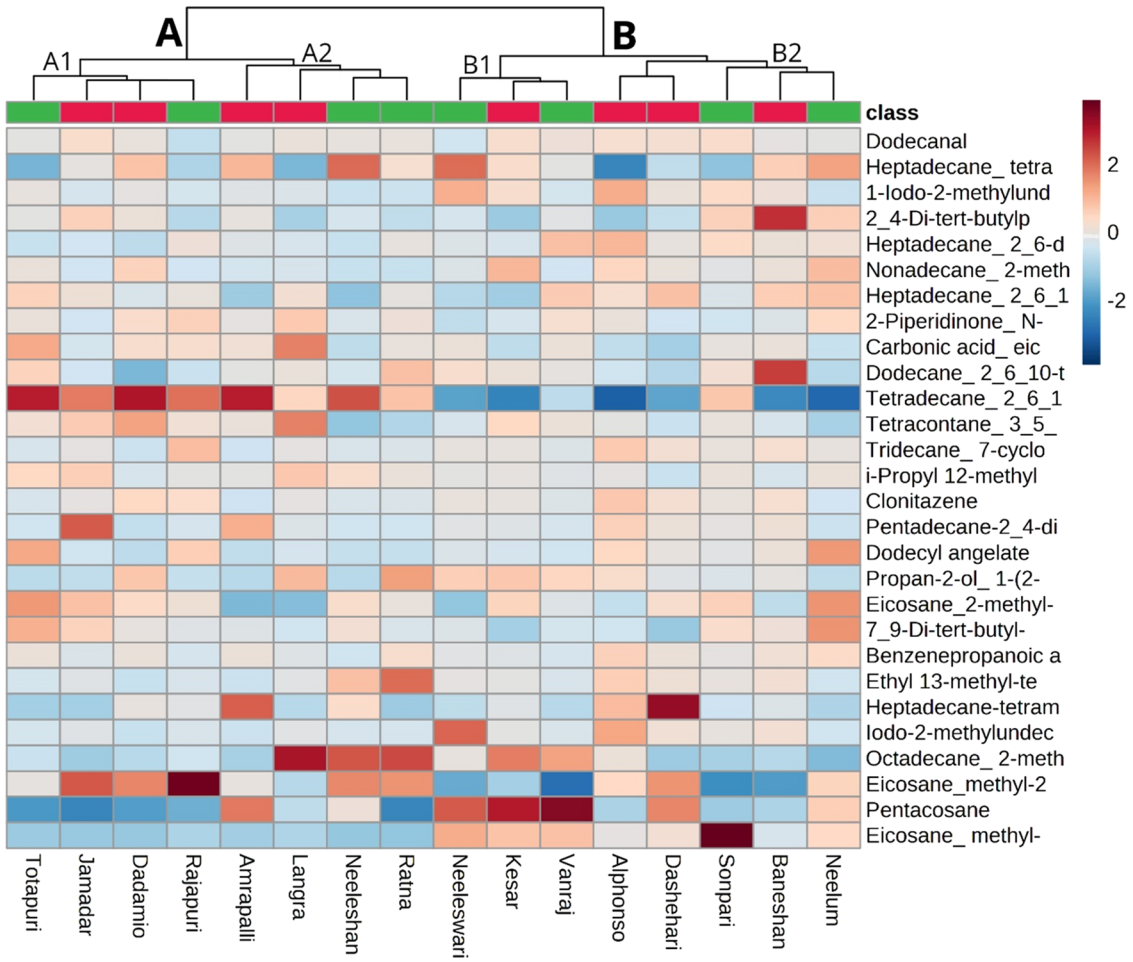

Figure 5. Hierarchical cluster analysis (HCA) and heat map visualization of the metabolite profiles of 16 mango cultivars using the hexane extraction method.

Dashehari with concentrations ranging from 1.84% (Kesar) to 7.38% (Jamadar).

Multivariate Data Analysis of Mango Cultivars Using Hexane. HCA was utilized to assess nonpolar metabolites belonging to 16 mango cultivars and cluster them using the Euclidean and war. D algorithm (Figure 5). As per the results, Sonpari, Neelum, and Baneshan were assigned to B2 due to the abundance of 2,4-di*tert*-butylphenol; dodecane, 2,6,10-trimethyl-, and eicosane, 2-methyl- (isomer). However, Dashehari and Alphonso were located in the same cluster, which were characterized by having an abundance of heptadecane, 2,6,10,15-tetramethyl- (isomer) especially in Dashehari. The cultivars Neeleswari, Kesar, and Vanraj were included in B1 subcluster, which were distinguished by esters, alcohol, ketone, aldehyde, and phenol. Cluster A was subsequently separated into two subgroups (A1 and A2). Ratna, Neeleshan, Amrapalli, and Langra were assigned to A2, with the maximum concentration of ethyl 13-methyl-tetradecanoate particularly in Ratna. Rajapuri, Dadamio, Totapuri, and Jamadar were placed in A1 subcluster with high concentrations of tridecane, 7-cyclohexyl, and clonitazene. Sonpari and Neeleswari were developed by crossing of Alphonso with Baneshan and Neelum with Dashehari, respectively. These six cultivars are therefore represented in the same cluster (cluster B).

By using PCA and biplot, reduction in the information contents of a large data set was achieved, interpretations were improved, and information loss was minimized (Figure 6). Consequently, it created new uncorrelated variables that maximized the variance one after another. PCs 1 and 2 each represented the variance by 30.1 and 15.7%, respectively (Figure 6), which corresponds to a variation of 45.8% overall. This suggested that the composition and concentration of nonpolar metabolites in various mango cultivars vary considerably.

Ethanol Extract Method. *Identification and Concentration of Volatile Compounds (Polar Metabolites) in Mango Cultivars through the Ethanol Extraction Method.* A total of 52 polar metabolites belonging to 16 mango cultivars were identified, and the results revealed that the polar metabolite content of mango pulp was relatively high (Table 3).

Esters. Diethoxymethyl acetate was predominantly found in four cultivars, *viz.*, Amrapali, Jamadar, Alphonso, and Rajapuri, while D-alanine, *N*-propargyloxycarbonyl-, isohexyl ester was found higher in Sonpari, Ratna, Neeleswari, Neeleshan, Totapuri, and Kesar. Formic acid phenacyl ester, ethyl 13-methyl- tetradecanoate, and butyl 4,7,10,13,16,19-docosahexaenoate were identified only in Jamadar, while acetic acid, trifluoro-, hexadecyl ester and 1-ethyldodecyl 2,2,3,3,4,4,4-heptafluorobutanoate were found only in Neeleshan mango. Ethyl acetoxycarbamate was observed in considerable amount in Langra, Baneshan, Neeleshan, Jamadar, Ratna, and Totapuri. Several researchers reported esters, *viz.*, ethyl 2-methylpropanoate, ethyl butanoate, methyl benzoate, propyl acetate,

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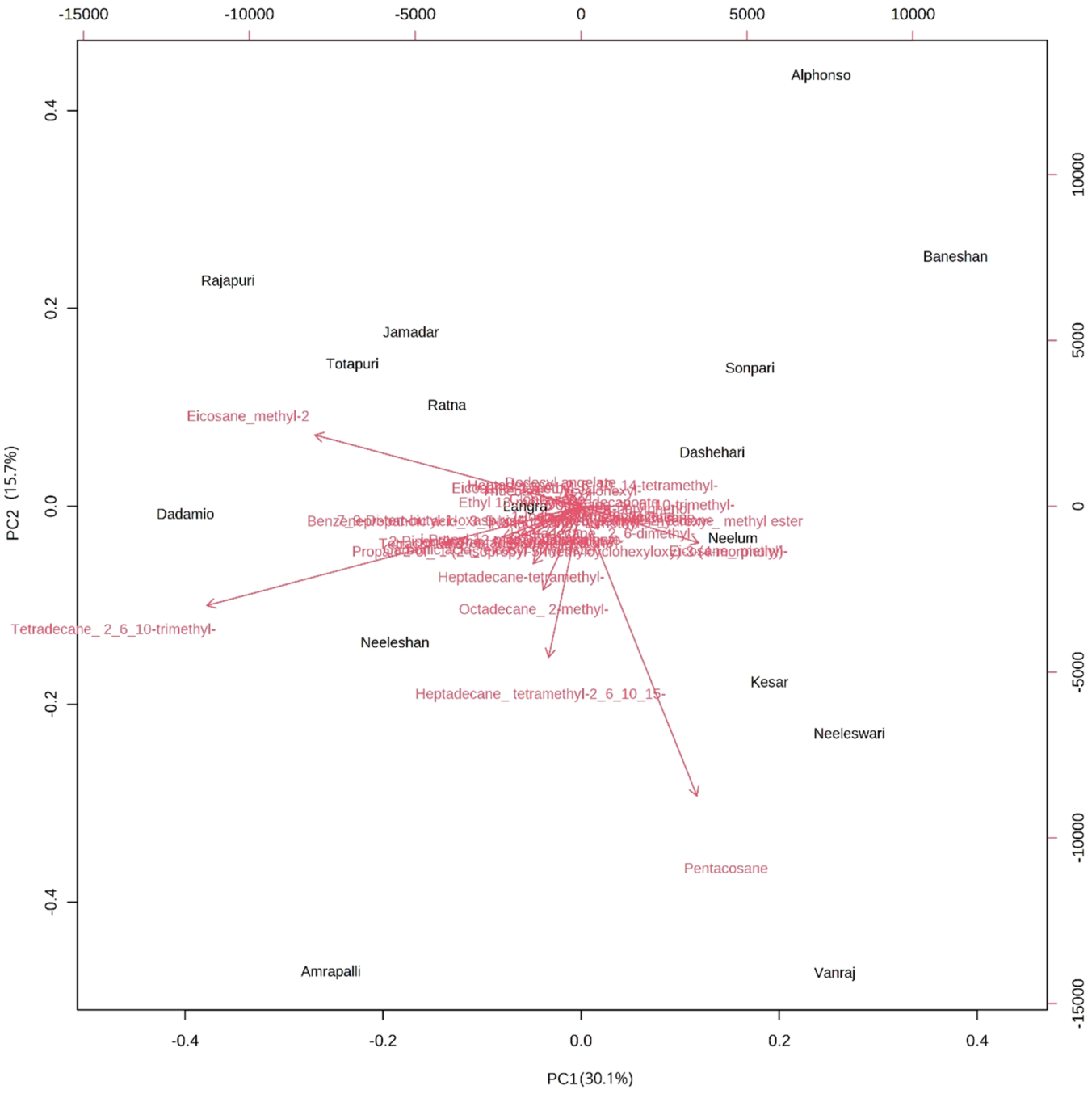

Figure 6. PCA biplot between the selected PCs. The left and bottom axes describe PC, and the top and right axes denote the loading plot score; the angles formed by vectors of distinct variables describe their correlation.

diethoxymethyl acetate, methyl-2-methyl propanoate, ethyl-methyl-tetradecanoate, butyl-hexanoate, butyl-docosahexanoate, and ethyl-dodecylacetate, from various fruits, which give a fruity aroma.[27,39−42] Components ethanone, 2-(formyloxy)-1-phenyl-; propanoic acid, 2-oxo-, methyl ester; pentanoic acid, heptyl ester; and ethyl 13-methyl-tetradecanoate were recorded in 13 different Egyptian mango cultivars; these compounds are responsible for the general characteristic fragrance of mango fruit.[35] The esters have higher odor threshold values; as a result, they impart least to the aroma of particular blends.[43]

Alcohols. It was found that 1,3-butanediol, a phenol, is an important polar metabolite of various mangos, *viz.*, Dashehari, Vanraj, Sonpari, Kesar, Ratna, Langra, Neeleshan, Baneshan, and Totapuri. In addition to that, 1-butanol, 2-nitro- was found in Jamadar only, while 2-furanmethanol and β-sitosterol were identified in Amrapali cultivar in a relatively higher peak area compared to other cultivars. Several alcohols including 2-furanmethanol, 1-butanol, 2-nitro-1-buten-3-ol, 6-oxa-bicyclo[3.1.0]hexan-3-ol *etc.* were found in the pulp of different mango cultivars such as Alphonso and Banginapalli.[37,44] Similarly, (*E*)-3-hexen-1-ol and hexadecanol were found more abundantly in the pulp of yellow "Thai" mango.[45]

Ketones. A total of 9 ketones were detected in this study; among them, 2-propanone, 1-hydroxy- compound was present exclusively in Neeleswari cultivar at a higher concentration,

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Table 3. Metabolites Identified in Different Mango Cultivars Using the Ethanol Extraction Method

No.	Ret. Time	Peak Name	Molecular Formula	CAS Number	Molecular weight	Dasheharri	Sonpari	Vanraj	Neeleswari	Dadamio	Amrapali	Alphonso	Kesar	Ratna	Jamadar	Neeleshan	Totapuri	Langra	Rajapuri	Neelum	Baneshan
Ester																					
1	4.39	Ethanone, 2-(formyloxy)-1-phenyl-(formic acid phenacyl ester)	$C_9H_8O_3$	55153-12-3	164.16										5.42						
2	4.81	sec-Butyl nitrite (Nitrous acid, 1 methylpropyl ester)	$C_4H_9NO_2$	924-43-6	103.12												1.4				
3	5.03	Butanenitrile, 2,3-dioxo-, dioxime, O,O'-diacetyl-(Acetic acid [(E)-[(1Z)-1-acetyloxyimino-1-cyanopropan-2-ylidene]amino] ester)	$C_8H_9N_3O_4$	339246-62-7	211.18		8.34	10.6		0.45									1.01	
4	5.06	Ethyl acetoxycarbamate (Carbamic acid, (acetyloxy)-, ethyl ester)	$C_7H_{11}NO_5$	2193-93-7	189.17									5.23	6.48	7.05	4.72	8.68	2.52		7.36
5	5.08	2-Epoxy-3-propyl acetate	$C_5H_8O_3$	6387-89-9	116.11				6.94				2.3								
6	5.084	Propanoic acid, 2-oxo-, methyl ester	C_4H_6O	600-22-6	102.09							4.93	5.6				3.42				
7	5.281	Diethoxymethyl acetate	$C_7H_{14}O_4$	14036-06-7	162.18					2.79	27.35	22.7			26.68				15.32	5.74	
8	12.005	D-Alanine, N-propargyloxycarbonyl-, isohexyl ester	$C_{13}H_{21}NO_4$	0	255.31		13.06	9.46	9.37			9.17	6.39	10.75	7.76	7.15	6.53				
9	13.005	Pentanoic acid, heptyl ester	$C_{12}H_{24}O$	5451-80-9	200.32															0.42	
10	13.149	N-(2,6-dimethylphenyl)-N-(methoxyacetyl)alanine	$C_{14}H_{19}NO_4$	75596-99-5	265.30												1.38	2.18			
11	17.136	4-Trifluoroacetoxytetradecane (1-Propylundecyl trifluoroacetate #)	$C_{16}H_{29}F_3O_2$	0	310.39					0.3					1.31	1.11					
12	19.35	4-Trifluoroacetoxyhexadecane (Acetic acid, trifluoro-, hexadecyl ester)	$C_{18}H_{33}F_3O_2$	0	338.4											1.46					
13	21.456	3-Trifluoroacetoxytetradecane (1-Ethyldodecyl trifluoroacetate #)	$C_{16}H_{29}F_3O_2$	0	310.39										1.03	0.83	1.43				0.67
14	24.699	3-Heptafluorobutyroxytetradecane (1-Ethyldodecyl 2,2,3,3,4,4,4-heptafluorobutanoate #)	$C_{18}H_{29}F_7O_2$	0	410.4											0.56					
15	24.702	Ethyl 13-methyl-tetradecanoate	$C_{17}H_{34}O_2$	64317-63-1	270.5										1.18						
16	63.302	Butyl 4,7,10,13,16,19-docosahexaenoate	$C_{26}H_{40}O_2$	0	384.6										2.3						

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Table 3. continued

No.	Ret. Time	Peak Name	Molecular Formula	CAS Number	Molecular weight	Dashehari	Sonpari	Vanraj	Neeleswari	Dadamio	Amrapali	Alphonso	Kesar	Ratna	Jamadar	Neeleshan	Totapuri	Langra	Rajapuri	Neelum	Baneshan
Alcohol																					
1	4.621	1-Butanol, 2-nitro-	$C_4H_9NO_3$	609-31-4	119.12										5.44						
2	5.305	1,3-Butanediol	$C_4H_{10}O_2$	107-88-0	90.121	52.96	30.65	48.52					24.7	21.04		33.4	19.34	52.3			31.23
3	6.167	2-Furanmethanol (Furfuryl alcohol)	$C_5H_6O_2$	98-00-0	98.1						5.21						1.6	2.13	2.3	1.32	
4	10.959	2-Nitro-1-buten-3-ol	$C_4H_7NO_3$	37442-53-8	117.1															0.79	
5	12.217	6-Oxa-bicyclo[3.1.0]hexan-3-ol	$C_5H_6O_2$	74017-10-0	98.10										1.76	1.69		4.09			
6	22.66	1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$	2490-48-4	256.5													0.67			0.75
7	56.528	Mangol II [5-(Heptadec-12-enyl)resorcinol]	$C_{23}H_{38}O_2$	103462-06-2	346.56						0.54	0.94						2.52			
8	61.021	Ergost-5-en-3-ol, acetate, (3ß,24R)-	$C_{28}H_{48}O$	0	400.7						0.86					1.72	1.14				
9	63.305	Stigmastan-3-ol, 5-chloro-, acetate, (3ß,5a)-	$C_{31}H_{53}ClO_2$	55724-19-1	493.2							2.02								1.25	2.74
10	63.319	ß-Sitosterol	$C_{29}H_{50}O$	83-46-5	414.71						4.55							2.67	1.41		
Ketone																					
1	4.805	2-Propanone, 1-hydroxy-	$C_3H_6O_2$	116-09-6	74.07				28.15											1.08	
2	5.493	6-Oxa-bicyclo[3.1.0]hexan-3-one	$C_5H_6O_2$	74017-10-0	98.10															0.28	
3	6.459	3-Amino-2-oxazolidinone	$C_3H_6N_2O_2$	80-65-9	102.09															2.66	
4	7.807	2,5-Furandione, 3-methyl-	$C_5H_4O_3$	616-02-4	112.08					11.37							2.23			9.64	
5	7.341	2-Cyclopenten-1-one, 2-hydroxy-	C_5H_6O	10493-98-8	98.09												1.45				
6	8.719	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	10230-62-3	144.12					1.86							1.99		3.04	2.59	
7	12.029	3H-Pyrazol-3-one, 2,4-dihydro-2,4,5-trimethyl-	$C_6H_{10}N_2O$	17826-82-3	126.16	8.68															
8	13.555	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	28564-83-2	144.12	9.75				5.71				6.38	3.42	5.69	4.63	5.15	12.69	7.71	5.93
9	23.491	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	82304-66-3	276.4					0.89						0.55		0.48			0.39
Alkane																					
1	13.045	Cyclopropane, trimethyl(2-methyl-1-propenylidene)-	$C_{10}H_{16}$	14803-30-6	136.23							1.34			0.95						
2	17.257	4-Methyl(trimethylene)silyloxyoctane	$C_{12}H_{26}OSi$	0	214.42										2.62	0.66				1.02	2.16
3	19.35	8-Heptadecene	$C_{17}H_{34}$	16369-12-3	238.5										1.64						

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Table 3. continued

No.	Ret. Time	Peak Name	Molecular Formula	CAS Number	Molecular weight	Dashehari	Sonpari	Vanraj	Neeleswari	Dadamio	Amrapali	Alphonso	Kesar	Ratna	Jamadar	Neeleshan	Totapuri	Langra	Rajapuri	Neelum	Baneshan
Fatty acids																					
1	23.693	(E)-Hexadec-9-enoic acid	$C_{16}H_{30}O_2$	10030-73-6	254.41						1.89							0.93			0.69
2	23.988	n-Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	57-10-3	256.4					0.53	2.8		1.17	1.26	1.5	0.81		2.89		0.56	2.32
3	24.011	Octadecanoic acid	$C_{18}H_{36}O_2$	57-11-4	284.48	1.40															
Aldehyde																					
1	5.691	3-Furaldehyde	$C_5H_4O_2$	498-60-2	96.08					7.72										10.81	
2	15.037	5-Hydroxymethylfurfural	$C_6H_6O_3$	67-47-0	126.11	24.48	13.96	25.14	19.4	66.58	56.8	26.38	20.14	26.18	5.09	4.14	20.2	11.23	62.72	49.76	13.78
Amine																					
1	5.181	Pyrrolidine, 2-butyl-1-methyl-	$C_9H_{19}N$	3447-03-8	141.25					0.51											
2	13.112	2-Propanamine, N-methyl-N-nitroso-	$C_4H_{10}N_2O$	30533-08-5	102.14										0.6					1.86	2.12
Amide																					
1	5.198	N'-(Diaminomethylidene)formohydrazide	$C_2H_6N_4O$	4472-03-1	102.1															0.42	
2	5.838	Formamide, N-methoxy-	C_2H_5NO	34005-41-9	75.07		31.56		33.44			31.23	37.1	24.76	24.82	29.61	26.78				28.31
Triterpenes																					
1	53.865	Squalene	$C_{30}H_{50}$	111-02-4	410.73									1.47				4.08			
Monoterpene																					
1	13.035	Allo-ocimene	$C_{10}H_{16}$	3016-19-1	136.23			3.11													
other																					
1	4.436	Ethoxy(methoxy) methylsilane	$C_4H_{11}O_2Si$	0	119.21							1.29	1.16	1.35		3.57	1.76			1.08	1.55
2	63.322	Androst-5-ene,1-acetoxy-16,17-dimethyl-20-oxo-	$C_{25}H_{38}O_3$	29486-91-4	386.6		2.43		2.7												
3	63.369	Stigmasta-3,5-diene	$C_{29}H_{48}$	4970-37-0	396.7	2.73		3.17		1.29			1.44	1.58							

while 3*H*-pyrazol-3-one, 2,4- dihydro-2,4,5-trimethyl- was found in Dashehari only. 4*H*-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- was reported to have the maximum relative peak area percentage in Rajapuri, Dashehari, Neelum, Ratna, Baneshan, Dadamio, Neeleshan, Langra, Totapuri, and Jamadar. Compound 2,5-furandione, 3-methyl- was observed in considerable amounts in 3 cultivars, namely, Dadamio, Neelum, and Totapuri. Lalel et al.[46] detected 6 ketones including 7,9-di*tert*-butyl-1- oxaspiro(4,5)deca-6,9-diene-2,8-dione from the skin and pulp of 'Kensington Pride' mango. Bishr et al.[35] identified 4*H*-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- from the 'Fuss' mango, and 2-propanone, 1-hydroxy- and 2- cyclopenten-1-one, 2-hydroxy- from the 'Langary' cultivars. Silva et al.[31] identified different volatile compounds including 3-amino-2-oxazolidinone with its antioxidant activity in fresh and processed mango pulp. The ethanolic extract of the fruit of *Ficus carica* contained sitosterol; 3,5-dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one; and 2,4,5-trimethyl-2,4-dihydro-3*H*-pyrazol-3-one, which may have potential antidiabetic and antiobesogenic agents.[47]

Nonterpene Hydrocarbons (Alkanes), Fatty Acids, and Aldehydes. A total of 3 alkenes were identified at relatively low concentrations from the five mango cultivars, *viz.*, Alphonso, Jamadar, Neeleshan, Neelum, and Baneshan. Similarly, three fatty acids were detected with relative peak area percentages ranging from 0.53 to 2.89% in different mango cultivars. Similarly, Lalel et al.[28] and Pino et al.[39] also noted different fatty acids including palmitic acid, octadecanoic acid, hexadecenoic acids *etc.* in Alphonso, Kent, and Pairi mango cultivars. 5-Hydroxymethylfurfural was found to be prominent in all of the studied mango cultivars, with the relative peak area percentage ranging from 4.15% (Neeleshan) to 66.58% (Dadamio). However, 3-furaldehyde was reported only in Dadamio and Neelum. 5-Hydroxymethylfurfural is largely utilized in the food industry as a food additive, as a biomarker, and as a flavoring ingredient for food items. It was classified as a food enhancement agent.[34] According to Bishr et al.,[35] 3-furaldehyde, which has an almond-like odor and serves as a flavoring ingredient, is present in 12 cultivars in concentrations ranging from 1.46 to 32.06%.

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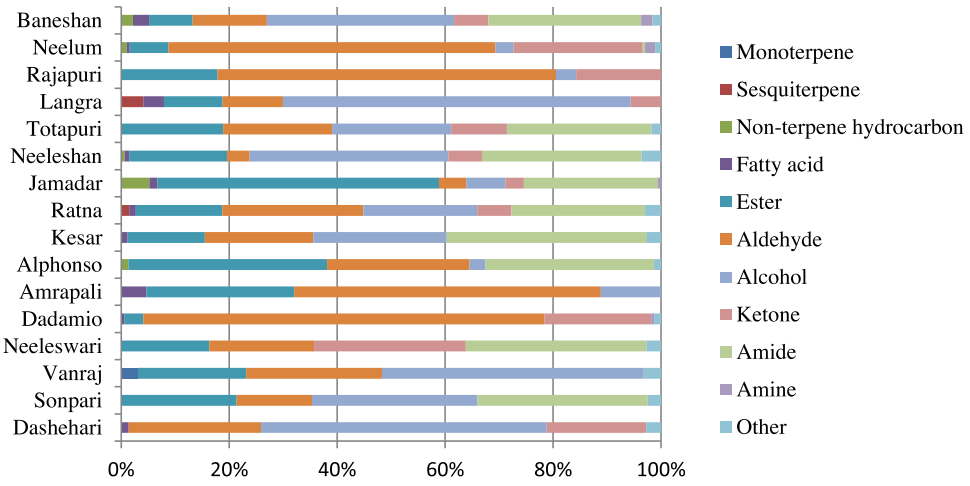

Figure 7. Relative abundance of different classes of metabolites extracted using the ethanol extraction method.

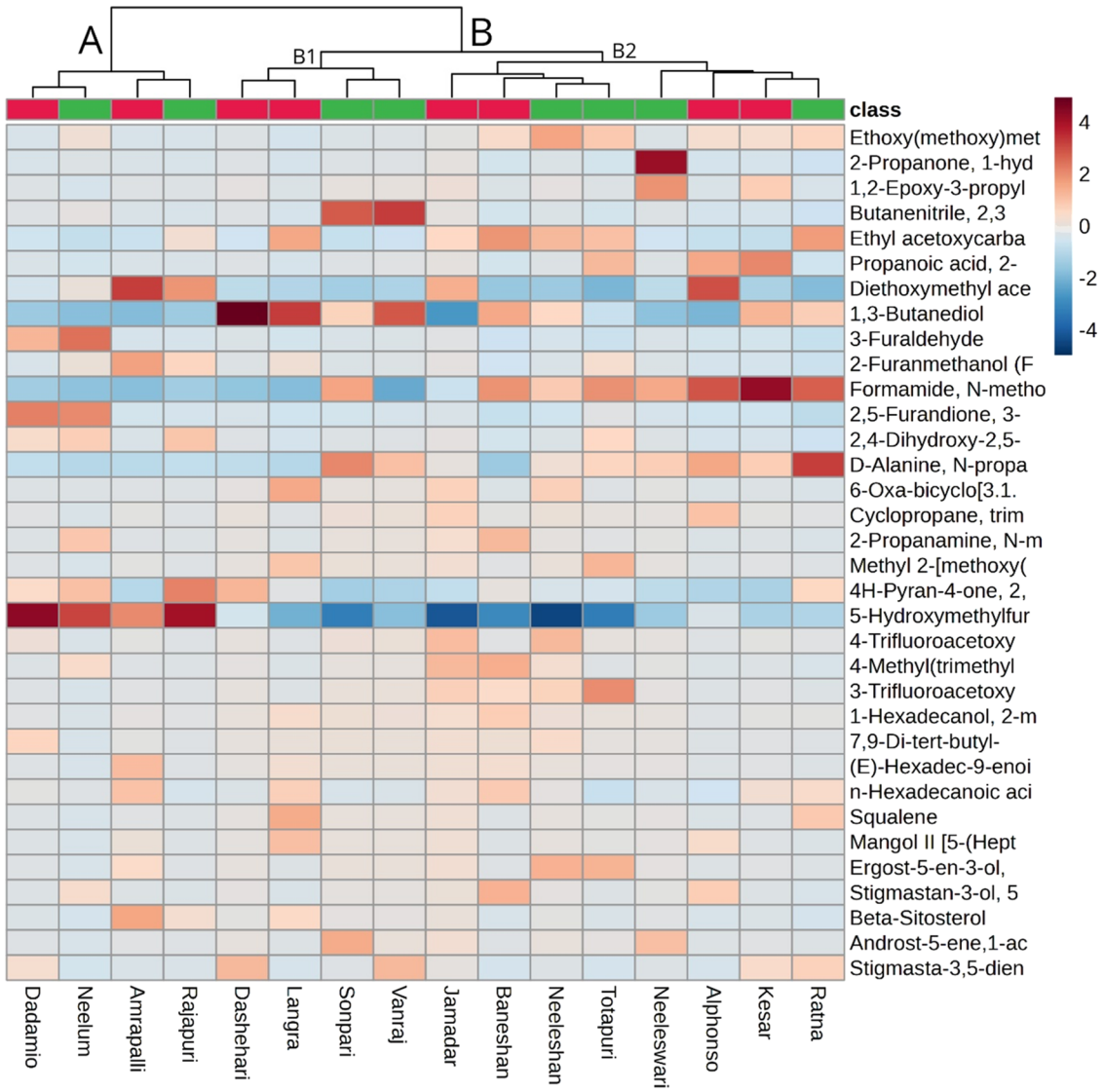

Figure 8. Hierarchical cluster analysis (HCA) and heat map visualization of the metabolite profiles of 16 mango cultivars using the ethanol extraction method.

Amides, Amines, and Others. Among the two amides, formamide, *N*-methoxy- was found as a major component in Kesar, Neeleswari, Sonpari, Alphonso, Neeleshan, Baneshan, Totapuri, Jamadar, and Ratna. The polar extracts of different

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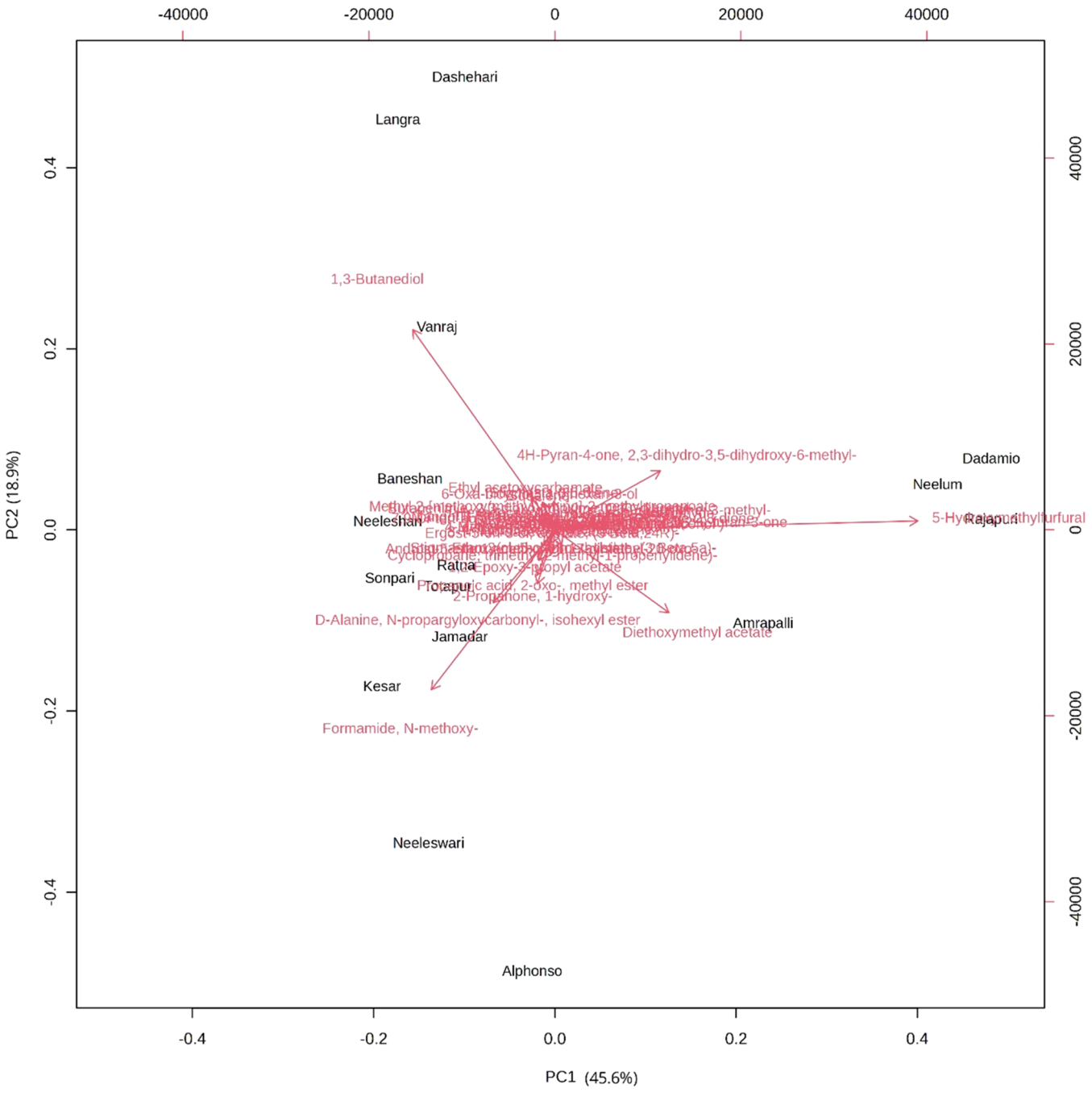

Figure 9. PCA biplot between the selected PCs. The left and bottom axes describe PC, and top and right axes denote the loading plot score; the angles formed by vectors of distinct variables describe their correlation.

mangoes contained little amines. Several polar metabolites belonging to other classes were also noted in the studied mangoes, *viz.*, ethoxy(methoxy)methylsilane (3.57%); androst-5-ene,1-acetoxy-16,17- dimethyl-20-oxo- (2.69%), and stigmasta-3,5-diene (3.17%).

Relative Abundance of Different Classes of Polar Metabolites Identified through the Ethanol Extraction Method. The highest concentrations of esters were recorded in Jamadar (52.16%) and Alphonso (36.78%); the highest amounts of aldehydes were found in Dadamio (74.30%), Rajapuri (62.72%), Neelum (60.57%), Amrapali (65.68%), and Ratna (26.18%) (Figure 7). Nevertheless, alcohols were also dominant in five cultivars: Langra (64.38%), Dashehari (52.96%), Vanraj (48.52%), Neeleshan (36.81%), and Baneshan (34.72%). The amides were detected dominantly in Kesar (37.11%), Neeleswari (33.44%), Sonpari (31.56%), and Totapuri (26.78%).

Multivariate Data Analysis through Ethanol Extract. Based on the similarities and abundance of polar metabolites in cultivars, HCA was used to differentiate cultivars and group them accordingly. The results of grouping were calculated based on the Euclidean and war. D algorithm (Figure 8). The sixteen mango cultivars were developed into two substantial clusters (A and B). In addition, Cluster B was divided into two groups (B1 and B2). Kesar, Ratna, Alphonso, and Neeleswari were assigned to the B 2.1 subcluster with high concentrations

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of cyclopropane, trimethyl(2-methyl-1-propenylidene); androst-5-ene,1-acetoxy-16,17-dimethyl-20-oxo-; 2-propanone, 1-hydroxy- and 1,2-epoxy-3-propyl acetate, while Totapuri, Neeleshan, Baneshan, Ratna, and Jamadar were positioned in the B 2.2 subcluster, which were characterized as having high contents of 3-trifluoroacetoxytetradecane; 1-hexadecanol, 2-methyl-; 4-methyl(trimethylene) silyloxyoctane and butanenitrile, 2,3-dioxo-, dioxime, *O*,*O*′-diacetyl-. Vanraj, Sonapari, Langra, and Dashehari were placed in the B1 group, which were distinguished with very high concentrations of mangol-II; methyl-2-[methoxy(methyl)amino]-2-methylpropanoate; 6-oxa-bicyclo[3.1.0]hexan-3-one, and squalene. Cluster A was then further separated into two groups, with Rajapuri and Amrapali being assigned to one group with high concentrations of (*E*)-hexadec-9-enoic acid, 2-furanmethanol, and *β*-sitosterol. Meanwhile, Neelum and Dadamio were clubbed together due to high contents of 2-propanamine, *N*-methyl-*N*-nitroso-; 3-furaldehyde and 7,9-di*tert*-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione.

Neelum was crossed with Dashehari and Baneshan to generate Neeleswari and Neeleshan, whereas Alphonso and Baneshan were crossed to produce Sonpari. These five cultivars were therefore represented by the same cluster. However, there was some incongruence in the grouping of some cultivars, such as those of Amrapali, Ratna, and Neelum, which were clubbed together into separate clusters.

PCA is a chemometric tool assisting in evaluating the metabolite profile of each mango cultivar and reducing the number of original variables for a better understanding of sample variations. PC1 and PC2 represented 45.6% and 18.9 of the variance, for a total variance of 64.5% (Figure 9).

CONCLUSIONS

Every cultivar of mangoes has a vast number of metabolites, and because these metabolites are so diverse, they all differ from one another. Because of the chemical composition of the metabolites, a single extraction solvent cannot identify and detect multiple groups of metabolites. As a result, in the current investigation, three distinct extraction procedures were used to sort and group 16 mango cultivars that are mostly farmed in India before being analyzed to determine the relationships between them. A variety of chemicals were examined using the SPME, hexane, and ethanol extraction processes, followed by GC−MS analysis, which was utilized to analyze multivariate data to distinguish several mango cultivars. The abundance of metabolites and their relative concentrations describe a unique blend of these mango cultivars. The metabolites identified in this study might be useful in quality assessment of mango-derived nutritional products and hence enhancement of the export. The cluster formed using each extraction method supports their parental relationship.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c03670.

GC−MS chromatograms of samples analyzed through SPME; GC−MS chromatograms of samples analyzed through Hexane; and GC−MS chromatograms of samples analyzed through ethanol (PDF)

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Notes

The authors declare no competing financial interest.

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