STUDYING THE VOLATILE ORGANIC COMPOUND COMPOSITION IN THE FLOWERS OF *ACACIA MANGIUM* AND *ACACIA AURICULIFORMIS* USING THE HEADSPACE TECHNIQUE COMBINED WITH GC-MS

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Le Van Dung [1,2]*, Dao Van Nam[2], Le Thi Yen Nghi[2], Nguyen Le Kim Thuy[2], Mai Truc Vy[2], Nguyen Vinh Hien[2], Vo Duy Can[2], Nguyen Cao Hien[3], Ngo Le Ngoc Luong[4], Nguyen Thanh Danh[1,2], Dang Chi Hien[1,2]*

[1]*Graduated University of Science and Technology, VAST*

[2]*Institute of Chemical Technology, VAST*

[3]*Ho Chi Minh City University of Industry and Trade*

[4]*Cantho Economics and Technology College*

[*]E-mail: dangchihien@gmail.com, tohoahocctb@gmail.com

TÓM TẮT

NGHIÊN CỨU THÀNH PHẦN HỢP CHẤT HỮU CƠ DỄ BAY TRONG HOA KEO TAI TƯỢNG (*ACACIA MANGIUM*) VÀ KEO LÁ TRÀM (*ACACIA AURICULIFORMIS*) SỬ DỤNG KỸ THUẬT HEADSPACE KẾT HỢP GC-MS

Thành phần hóa học của các hợp chất hữu cơ dễ bay hơi từ hoa keo tai tượng (Acacia mangium) và hoa keo lá tràm (Acacia auriculiformis) thu thập vào các thời điểm khác nhau trong ngày được xác định bằng phương pháp phân tích Headspace kết hợp với GC-MS. Hoa keo tai tượng thu hái vào buổi sáng chứa 43 chất, trong đó chất chính là n-hexanal (9,19%), (Z)-3,7-dimethyl-1,3,6-octatriene (8,52%), n-nonanal (14,35%), heneicosane (17,38%) và Kaur-16-ene (18,8%). Hoa keo tai tượng vào buổi chiều chứa 48 chất, bao gồm styrene, benzaldehyde, linalool và tetradecanal ngoài các chất chính có trong buổi sáng. Hoa keo lá tràm thu hái vào buổi sáng chứa 47 chất, trong đó chất chính là n-hexanal (4,07%), (Z)-3,7-dimethyl-1,3,6-octatriene (12,86%),

n-nonanal (6,65%), heneicosane (35,24%) và Kaur-16-ene (16,68%). Hoa keo lá tràm hái vào buổi chiều chứa 40 chất, bao gồm styrene, n-heptanal, benzaldehyde, 1-octanol, (E)-9-eicosene và cis-9-hexadecenal, ngoài những chất chính được tìm thấy vào buổi sáng. Nhiều hợp chất tìm thấy trong hoa keo tai tượng và keo lá tràm có trong thành phần pheromone của ong, bướm và côn trùng như heptadecane (Xylocopa hirutissima - Ong thợ), cis-9-hexadecenal (Helicoverpa armigera - Sâu đục quả bông), 1-octanal (Bactrocera correcta - ruồi vàng đục ổi)), n-nonanal (Apis mellifera scutellata - ong mật vùng Đông Phi; Bactrocera oleae - Ruồi vàng đục quả ô liu).

Từ khoá: *Acacia mangium, Acacia auriculiformis, hợp chất hữu cơ dễ bay hơi, kỹ thuật Headspace, GC-MS.*

1. INTRODUCTION

Acacia mangium is a species of tree belonging to the subfamily Mimosoideae. It is native to Australia and Asia and is widely cultivated for environmental management and timber [1]. The flowers of *A. mangium* are pale yellow and arranged in branch-shaped inflorescences. Each inflorescence is 5-15 cm long, with small flowers alternating long flowers, single or 2-4 flowers in leaf axils. The tree can flower as early as 18-24 months old, but most often at the age of 4-5, usually in June-July. *Acacia auriculiformis* is another species of tree in the *Acacia* genus that was newly introduced into Vietnam during the 1960s and 70s. It is naturally distributed in Indonesia and Papua New Guinea, and is now widely grown in tropical countries. The flowers are squirrel-shaped and yellow. The fruit is a twisted bean with black seeds and a yellow hilum. The wood of *A. auriculiformis* is used in the manufacture of paper, household furniture, and tools. It also contains tannins, so it can be used in the leather tanning industry.

Figure 1a. Tree and flower of *Acacia mangium*

Figure 1b. Tree and flower of *Acacia auriculiformis*

Headspace (HS) analysis is the most effective gas chromatography technique for extracting volatile organic compounds. There are four types of such analysis (Static headspace analysis (SHA), Direct Headspace Analysis (DiHA), Headspace Solid Phase Microextraction (HS-SPME), and Dynamic Headspace Analysis (DyHA)). Using DiHA to determine volatile organic compounds has several advantages, such as short sample preparation and the ability to sample continuously without the need to change extraction heads between samples [2].

This article aims to identify the VOCs present in A. mangium and A. auriculiformis flowers using the HS-GC-MS (Headspace - Gas Chromatography - Mass Spectrometry) technique. The qualitative analysis of VOCs in flowers will help address the lack of information on aromas from natural plant resources. The proposed HS-GC-MS technique is a rapid, simple, and reliable approach for the extraction and determination of VOCs, which minimizes the loss of volatile aroma compounds. By determining the VOC content in *A. mangium* and *A. auriculiformis* flowers, information on odor/fragrance substances can be established, which may help attract insects like bees, butterflies, and pests to the flowers of these two species. The results of this study will improve our understanding of the source of fragrance from *A. mangium* and *A. auriculiformis* flowers, which would further help in the development of natural forests for these two timber tree species.

2. MATERIALS AND METHODS

Tree and flower samples from two species of the *Acacia* family, namely *A. mangium* (Fig. 1a) and *A. auriculiformis* (Fig. 1b), were identified by experts from the Institute of Southern Ecology, VAST. After harvesting, fresh flowers are processed by removing any pest-infested parts and then placed into vials of the Headspace device.

2.1 Equiptments

Gas chromatograph mass spectrometer: GC-2030 (Shimadzu) combined with MS-QP2020 (Shimadzu). Chromatography column: Rxi-5 Sil MS (Shimadzu) 30 m long, inner diameter 0.25 *mm*, film thickness: 0.25 μm - Institute of Chemical Technology, VAST. Headspace device: HS-20 (Shimadzu, Japan).

2.2 Methods

Harvest: flowers of *A. mangium* and *A. auriculiformis* are harvested in Thanh Loc Ward, District 12, Ho Chi Minh City. Flower samples of two types of Acacia plants were analyzed while still fresh.

Harvesting time: morning samples (8 a.m); afternoon sample (2 p.m).

Sample preparation: One gram of the flower sample was taken directly from the plant and placed in a 20 mL vial to analyze the headspace, then sealed with a headspace vial vlamp cap (20 mL), PTFE septa, and promptly analyzed [3].

Analysis: determine the chemical composition of VOCs collected on the headspace device combined with GC-MS. The VOC composition of *A. mangium* and *A. auriculiformis* flowers was analyzed using the Headspace GCMS technique with a Shimadzu Headspace HS-20 instrument. The sample was placed into the incubation chamber at 100 °C for an equilibration time of 20 min. Both sample line and transfer line temperatures were set at 160 °C, the gas pressure during pressurization was 50 kPa, with a pressurizing time of 0.5 min. The pressure equilibration time is 0.1 min, followed by a load time of 0.5 min. Additionally, the load equilibration time is 0.1 min, and the injection time is 0.5 min. Thermal programming of GC-MS was conducted using a Shimadzu gas chromatograph GC-2030 coupled to MS-QP2020. Analysis was performed using a Rxi-5 Sil MS capillary column (30 *m* length, 0.25 *mm* internal diameter, film thickness: 0.25 μm, Shimadzu). The column head temperature was initially set at 50 ^{o}C for 2 minutes, then increased to 100 ^{o}C at a rate of 5 ^{o}C/min, further to 200 ^{o}C at a rate of 15 ^{o}C/min, and finally to 280 ^{o}C at a rate of 20 ^{o}C/min, where it was held for 5 min.

The ion chamber temperature (Ion source Temp) and heatsink temperature (Interface Temp) were both set at 260 ^{o}C. Helium was used as the carrier gas with a flow rate of 1.69 *mL*/min. The stream was split at a ratio of 1:10, and the column head pressure was maintained at 100 Kpa.

3. RESULTS AND DISCUSSION

Headspace analysis in GC has been considered by Snow and Slack [2], with automated headspace GC (static, dynamic, purge, and trap) being well-established, mature techniques that have been used since the 1980s for the analysis of disinfectants such as EDB in cereals and fruit, and methyl bromide in various products. The headspace technique is based on Henry's Law [4], which states that the ratio of the dissolved substance in an aqueous solution to the vaporous substance in a sealed glass vial is constant and depends on temperature. The concentration of volatile substances, such as alcohol in body fluids, can be determined by analyzing the vapor in equilibrium with the body fluids in a sealed glass vial at an elevated temperature. The headspace sample is then removed from the vial using an airtight syringe and injected into the GC.

Our analysis results using headspace combined with GC-MS on *A. mangium* and *A. auriculiformis* flowers indicate changes in the composition of VOCs in the flowers over time. *A. mangium* flowers collected in the morning contain 43 substances, including *n*-hexanal, (*Z*)-3,7-dimethyl-1,3,6-octatriene, *n*-nonanal, heneicosane, and Kaur-16-ene as the main substances. Aldehydes such as *n*-hexanal, *n*-heptanal, benzaldehyde, *n*-octanal, 3-cyclohexene-1-carboxaldehyde, *n*-nonanal, *n*-decanal, tridecanal, (*Z*)-7-tetradecenal, tetradecanal, *cis*-9-hexadecenal, (*Z*)-9,17-octadecadienal, and esters and fragrances like benzyl acetate, ethyl benzoate, methyl salicylate, propanoic acid phenylmethyl ester, 2-methyl-propanoic acid phenylmethyl ester, 2-methoxy-benzoic acid methyl ester, butanoic acid phenylmethyl ester, 2-methyl-butanoic acid phenylmethyl ester, pentanoic acid phenylmethyl ester, dihydro-*β*-ionone, and benzyl benzoate were also identified. In addition to the main substances from morning flowers, *A. mangium* flowers collected in the afternoon contain 48 substances, including styrene, benzaldehyde, linalool, and tetradecanal. The aldehydes are similar to the morning flowers but without *n*-nonanal, and there is a change in the composition of esters and fragrances. The presence of methyl benzoate, benzyl isovalerate, and *α*-ionone (essential oil fragrance) [5] is noted in addition to the substances already present in the morning flowers. Therefore, there is a clear difference in the composition of VOCs in *A. mangium* flowers between morning and afternoon.

Our results found that *A. auriculiformis* flowers collected in the morning contain 47 VOCs. The main substances identified were *n-n-hexanal*, (*Z*)-3,7-dimethyl-1,3,6-octatriene, *n*-nonanal, heneicosane, and Kaur-16-ene. The morning flowers also contain aldehyde groups such as *n*-hexanal, *n*-heptanal, benzaldehyde, *n*-octanal, *n*-nonanal, *n*-decanal, 2-aminobenzaldehyde, tetradecanal, and *cis*-9-hexadecenal. Additionally, esters, alcohols, ketones, and aromatic substances were present such as heptane-2-one, 4-ethylcyclohexanol, 1-octanol, linalool, benzyl acetate, methyl salicylate, and other acids. On the other hand, *A. auriculiformis* flowers collected in the afternoon contain 40 substances. These flowers had the same main substances as the morning flowers along with some additional substances such as styrene, *n*-heptanal, benzaldehyde, 1-octanol, (*E*)-9-eicosene, and *cis*-9-hexadecenal. The aldehyde group in the evening flowers was similar to that of the morning flowers. However, the ester groups, alcohols, ketones, and fragrances were different from the morning flowers. The afternoon flowers did not contain benzoic acid methyl ester, propanoic acid phenylmethyl ester, 2-methyl-propanoic acid phenylmethyl ester, succinic acid non-4-enyl nonyl ester, butanoic acid phenylmethyl ester, 4-methoxy-benzoic acid methyl ester, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butanone, carbonic acid ethyl octadecyl ester, and 6,10,14-trimethyl-2-pentadecanone. Instead, the afternoon flowers contained 6-methyl-5-hepten-2-one, *α*-terpineol, succinic acid dodec-2-en-1-yl non-3-en-1-yl ester, and dihydro-*β*-ionone. Therefore, the VOC composition of *A. auriculiformis* flowers significantly changed between the morning and afternoon.

3.1 Compare the main chemical composition of VOC from *A. mangium* flowers collected by time of day

The analysis in *Table 1* indicates that the composition of volatile organic compounds in *A. mangium* flowers varies between those collected in the morning and those collected in the afternoon. Flowers collected in the morning contain fewer compounds compared to those collected in the afternoon.

Table 1. Main chemical composition of VOC of *A. mangium* flowers collected at by times of the day - Analysis on headspace combined with GC-MS

No.	RT	Chemical		Content %(*)		SI
		Morning	**Afternoon**	**Morning**	**Afternoon**	
1	3.295	*n*-hexanal	*n*-hexanal	9.19 ± 1.63	9.69 ± 0.84	95
2	5.136	-	Styrene	-	2.82 ± 0.41	97
3	8.183	*n*-Octanal	*n*-Octanal	2.60 ± 0.66	3.49 ± 0.55	96
4	9.503	(*Z*)-3,7-Dimethyl-1,3,6-octatriene	(*Z*)-3,7-Dimethyl-1,3,6-octatriene	8.52 ± 0.82	9.78 ± 1.22	96
5	11.264	*n*-Nonanal	*n*-Nonanal	14.35 ± 2.68	18.81 ± 2.57	97
6	14.85	3,5-Dimethoxytoluene	3,5-Dimethoxytoluene	6.80 ± 1.69	2.64 ± 0.54	95
7	20.105	Benzyl benzoate	Benzyl benzoate	2.03 ± 0.23	2.83 ± 0.44	98
8	20.216	*cis*-9-Hexadecenal	*cis*-9-Hexadecenal	3.06 ± 0.14	3.77 ± 0.28	96
9	20.907	Heneicosane	Heneicosane	17.38 ± 1.97	9.50 ± 2.41	96
10	22.079	Kaur-16-ene	Kaur-16-ene	18.8 ± 1.58	19.34 ± 3.68	88

(*)Content of VOCs ≥ 2%

Additionally, flowers harvested in the afternoon not only contain all the main substances present in the morning flowers, but also have 4 others: styrene, benzaldehyde, linalool, and tetradecanal. This suggests that the composition of VOCs in *A. mangium* flowers changes according to the time of day, with afternoon-collected flowers exhibiting greater diversity. Some main substances, such as *n*-hexanal, (*Z*)-3,7-dimethyl-1,3,6-octatriene, *n*-nonanal, heneicosane, and Kaur-16-ene, have relatively high concentrations, with minimal difference between morning and afternoon. However, heneicosane in afternoon flowers (9.5%) decreased to just over half compared to that in the morning (17.38%).

3.2 Compare the main chemical composition of VOC from *A. auriculiformis* flowers collected by time of day

The analysis results in *Table* 2 show differences in the composition of VOCs in *A. auriculiformis* flowers collected in the morning and afternoon. Morning flowers have fewer compounds compared to afternoon flowers, which not only contain all the main substances found in morning flowers but also have the presence of 6 others: styrene, *n*-heptanal, benzaldehyde, 1-octanol, (*E*)-9-eicosene, and *cis*-9-hexadecenal.

Some main substances, such as *n*-hexanal, (*Z*)-3,7-dimethyl-1,3,6-octatriene, *n*-nonanal, heneicosane, and Kaur-16-ene, have relatively high concentrations in flowers, especially heneicosane which is up to 35.24% in the morning. However, the contents of heneicosane and (*Z*)-3,7-dimethyl-1,3,6-octatriene were reduced to only about one-third in the afternoon, while *n*-nonanal, 3,5-dimethoxytoluene and Kaur-16-ene doubled or increased by one-and-a-half times more. Thus, not only the composition but also the content of volatile organic compounds in *A. auriculiformis* flowers changed significantly between morning and afternoon.

Table 2. Main chemical composition of VOC of *A. auriculiformis* flowers collected at by times of the day - Analysis on headspace combined with GC-MS

No.	RT	Chemicals		Content %(*)		SI
		Morning	**Afternoon**	**Morning**	**Afternoon**	
1	3.295	*n*-hexanal	*n*-hexanal	4.07 ± 0.55	6.43 ± 0.48	95
2	5.136	-	Styrene	-	5.25 ± 2.11	97
3	9.503	(*Z*)-3,7-Dimethyl-1,3,6-octatriene	(*Z*)-3,7-Dimethyl-1,3,6-octatriene	12.86 ± 4.03	4.22 ± 2.16	96
4	11.264	*n*-Nonanal	*n*-Nonanal	6.65 ± 1.23	11.98 ± 0.32	97
5	14.85	3,5-Dimethoxytoluene	3,5-Dimethoxytoluene	3.49 ± 0.97	7.523 ± 2.75	95
6	20.907	Heneicosane	Heneicosane	35.24 ± 7.39	11.51 ± 2.36	96
7	22.079	Kaur-16-ene	Kaur-16-ene	16.68 ± 1.38	24.77 ± 4.30	88

(*)Content of VOCs ≥ 2%

3.3 Comparison of the primary chemical composition of VOC from flowers of *A. mangium* and *A. auriculiformis* collected in the morning

According to *Table 3*, the composition and content of compounds in *A. mangium* and *A. auriculiformis* flowers collected in the morning show significant differences. Both species share six main substances, including *n*-hexanal, *n*-octanal, (*Z*)-3,7-dimethyl-1,3,6-octatriene, *n*-nonanal, 3,5-dimethoxytoluene, heneicosane, and Kaur-16-ene. However, some substances are unique to each species. For example, *A. mangium* flowers contain *n*-heptanal, 1-octanol, benzyl benzoate, and *cis*-9-hexadecenal, while *A. auriculiformis* flowers contain linalool, methyl salicylate, (*E*)-9-octadecene, and heptadecane. The content of some substances also varies significantly between the two species. For instance, the heneicosane content in *A. auriculiformis* flowers (35.24%) is twice as high as in *A. mangium* flowers (17.38%). On the other hand, aldehydes such as *n*-hexanal, *n*-octanal, and *n*-nonanal are twice as concentrated in *A. mangium* flowers compared to *A. auriculiformis* flowers. Therefore, despite sharing some main substances, the composition and content of compounds in the flowers of the two *Acacia* species exhibit significant difference.

Table 3. Main chemical composition of VOC from flowers of *A. mangium* and *A. auriculiformis* collected at in the morning - Analysis on Headspace combined with GC-MS

No.	RT	Chemicals		Content %(*)		SI
		A. mangium	*A. auriculiformis*	*A. mangium*	*A. auriculiformis*	
1	3.295	*n*-hexanal	*n*-hexanal	9.19 ± 1.63	4.07 ± 0.55	95
2	9.503	(*Z*)-3,7-Dimethyl-1,3,6-octatriene	(*Z*)-3,7-Dimethyl-1,3,6-octatriene	8.52 ± 0.82	12.86 ± 4.03	96
3	11.124	-	Linalool	-	2.55 ± 1.35	94
4	11.264	*n*-Nonanal	*n*-Nonanal	14.35 ± 2.68	6.65 ± 1.23	97
5	14.85	3,5-Dimethoxytoluene	3,5-Dimethoxytoluene	6.80 ± 1.69	3.49 ± 0.97	95
6	20.105	Benzyl benzoate	-	2.03 ± 0.23	-	98
7	20.216	*cis*-9-Hexadecenal	-	3.06 ± 0.14	-	96
8	20.907	Heneicosane	Heneicosane	17.38 ± 1.97	35.24 ± 7.39	96
0	22.079	Kaur-16-ene	Kaur-16-ene	18.8 ± 1.58	16.68 ± 1.38	88

(*)Content of VOCs ≥ 2%

3.4 Comparison of the primary chemical composition of VOC from flowers of *A. mangium* and *A. auriculiformis* collected in the afternoon

According to *Table 4,* there are differences in the composition and content of compounds in *A. mangium* and *A. auriculiformis* flowers collected in the afternoon. Both species share 13 main substances, including *n*-hexanal, styrene, *n*-heptanal, benzaldehyde, (*Z*)-3,7-dimethyl-1,3,6-octatriene, linalool, *n*-nonanal, 3,5-dimethoxytoluene, *cis*-9-hexadecenal, heneicosane, and Kaur-16-ene. However, each species also has unique substances. For example, tetradecanal and benzyl benzoate is found in *A. mangium* flowers, while methyl salicylate, (*E*)-9-octadecene, (*E*)-9-eicosene, and heptadecane are found in *A. auriculiformis* flowers. Kaur-16-ene is the highest concentrated compound in both species. In terms of levels, some substances, such as styrene and 3,5-dimethoxytoluene, are twice as concentrated in *A. auriculiformis* flowers compared to *A. mangium*, while (*Z*)-3,7-dimethyl-1,3,6-octatriene is twice as abundant in *A. mangium* flowers. Aldehydes like *n*-hexanal, *n*-octanal, and *n*-nonanal are present in concentrations one and a half times higher in *A. mangium* flowers. Therefore, the composition and content of compounds in the flowers of these two *Acacia* species collected in the afternoon exhibit significant differences.

Table 4. Main chemical composition of VOC from flowers of *A. mangium* and *A. auriculiformis* collected at in the afternoon - Analysis on Headspace combined with GC-MS

No.	**RT**	**Chemicals**		**Content %**(*)		**SI**
		A. mangium	*A. auriculiformis*	*A. mangium*	*A. auriculiformis*	
1	3.292	*n*-hexanal	*n*-hexanal	9.69 ± 0.84	6.43 ± 0.48	96
2	5.136	Styrene	Styrene	2.82 ± 0.41	5.25 ± 2.11	97
3	8.184	*n*-Octanal	*n*-Octanal	3.49 ± 0.55	2.05 ± 0.28	96
4	9.505	(*Z*)-3,7-Dimethyl-1,3,6-octatriene	(*Z*)-3,7-Dimethyl-1,3,6-octatriene	9.78 ± 1.22	4.22 ± 2.16	96
5	11.264	*n*-Nonanal	*n*-Nonanal	18.81 ± 2.57	11.98 ± 0.32	97
6	14.848	3,5-Dimethoxytoluene	3,5-Dimethoxytoluene	2.64 ± 0.54	7.523 ± 2.75	96
7	20.105	Benzyl benzoate	-	2.83 ± 0.44	-	97
8	20.215	*cis*-9-Hexadecenal	*cis*-9-Hexadecenal	3.77 ± 0.28	0.89 ± 0.38	96
9	20.905	Heneicosane	Heneicosane	9.50 ± 2.41	11.51 ± 2.36	95
10	22.078	Kaur-16-ene	Kaur-16-ene	19.34 ± 3.68	24.77 ± 4.30	88

(*)Content of VOCs ≥ 2%

Many compounds found in the flowers of *A. mangium* and *A. auriculiformis* serve as important pheromones for bees, butterflies, and insects. For example, heptadecane is a pheromone for the Carpenter bee (*Xylocopa hirutissima*) [6], while *cis*-9-hexadecenal serves as a pheromone for the Cotton bollworm (*Helicoverpa armigera*) [7]. Similarly, 1-octanal attracts the Guava fruit fly (*Bactrocera correcta*) [8], and *n*-nonanal is important for the East African lowland honey bee and the Guava fruit fly (*Apis mellifera scutellata* and *Bactrocera oleae*) [9, 10]. (*Z*)-7-tetradecenal is a sex pheromone for the Citrus flower moth (*Prays citri* M.) [11, 12]. These compounds explain why certain species of bees, butterflies, and pests are likely to be present and attracted to the flowers of *A. mangium* and *A. auriculiformis*. Pheromone compounds in flowers play a crucial role in mating activities and finding food sources for these insects.

(Note: *The compounds are mentioned in this article's text but not in the tables, as their contents are all less than 2%.*).

4. CONCLUSION

The combination of the headspace technique and GC-MS has proven to be effective and superior in analyzing VOCs composition from plant resources. Using this technique to analyze the VOC composition from the flowers of *A. mangium* and *A. auriculiformis* has several advantages, including rapid analysis without complicated extraction processes, a simple sample preparation process, and quicker results than the solvent extraction method. The analysis results show that both *Acacia* species' flowers contain high levels of substances, such as *n*-octanal, *n*-nonanal, heneicosane, and Kaur-16-ene. Additionally, the content of VOCs in the flowers of both species varied significantly between morning and afternoon. Some substances were identified as pheromones and attractants for bees, butterflies, and insects, including heptadecane, *cis*-9-hexadecenal, 1-octanal, *n*-nonanal, and (*Z*)-7-tetradecenal. This result provides an overview of the natural VOCs present in *Acacia spp*. and shows the potential for using the headspace technique combined with GC-MS to analyze natural flowers and plants in various fields, such as medicine, the food industry, cosmetics, and agriculture.

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