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Short Report

Zizyphus mauritiana Lam. (Rhamnaceae) and the Chemical Composition of its Floral Fecal Odor

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As flores de *Zizyphus mauritiana* Lam. (Rhamnaceae) exalam um forte odor fecal, e sua análise por micro-extração em fase sólida (SPME) associada a cromatografia gasosa- espectrometria de massas (CG-EM), co-injeção de padrões e índices de retenção revelou o benzaldeído como constituinte majoritário. Foram observados ainda benzenóides, ácidos carboxílicos, aldeídos, hidrocarbonetos alifáticos e monoterpenóides. A avaliação olfatométrica do SPME realizada por CG- olfatometria em *sniffing port* indicou o escatol (3-metil-indol) como o responsável pelo odor fecal das flores e a vanilina pelo seu odor adocicado.

When in blossom, jujube (*Zizyphus mauritiana* Lam., Rhamnaceae) emanates a strong fecal odor. The substances responsible for this scent were analysed by headspace solid-phase microextraction and gas chromatography-mass spectrometry (SPME-GC-MS) associated to standard co-injection and retention indexes, which showed benzaldehyde as major constituent. Minor benzenoids, aliphatic carboxylic acids, aldehydes, hydrocarbons and oxygenated monoterpenes were also observed. Olfactometric evaluation of the SPME using GC-sniffing port indicated skatole (3-methyl-indole) as responsible for the fecal odor and vanillin for the sweety odor.

Keywords: *Zizyphus mauritiana*, Rhamnaceae, aroma, skatole, headspace solid-phase microextraction

Introduction

Around 170 tropical and subtropical species of *Zizyphus* Mill. are known. *Z. mauritiana* Lam. is popularly known as Aprin, Ber, Dunks or Indian jujube and belongs to the family Rhamnaceae. The plants vary from bushy shrubs 1.2-1.8 m tall to trees up to 12 m and about five yellowish-green flowers with 4-5 petals are borne on short inflorescences at the leaf axils. During the week of blossom, the thousands of flowers emanate a strong scent reminiscent of human excrement, which is detectable over 30 m from the tree, attracting hundreds of flies. We have seen small green dung-beetles visiting the flowers as well.

Many publications deal with the varieties, cultivars, cultivation, propagation and analyses of the constituents present on *Z. mauritiana*,[1] and more rarely with pollination of the Indian jujube.[2] However, we were unable to find any mention of the strong fecal odor of the flowers and vegetative parts of any *Zizyphus* species, and decided to study the chemical constituents responsible for this notable character.

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Materials and Methods

Plant material

Flowers were collected from a single specimen of *Zizyphus mauritiana* Lam. cultivated at the Botanical Orchard of the Museu Nacional, Rio de Janeiro, during 2 successive blossoms. A voucher specimen (R. J. V. Alves, 6990), was deposited in the herbarium of Museu Nacional.

Instrumentation

An Agilent 6890 gas chromatograph was used coupled to an Agilent 5973 quadrupole mass spectrometer with electron ionization mode (EI) generated at 70 eV (ion source at 230 °C and transfer line at 280 °C). GC was performed on

a J&W DB-5 capillary column (30 m x 0.25 mm i.d. x 0.25 μm film) from an oven temperature of 35 °C (initial temperature) to 270 °C at a rate of 5 °C min^{-1}, where it was held for 20 min. At the end of the column, the effluent was split 1:1 (by volume) into the mass detector and the sniffing port, a stainless steel tube held at 200 °C. Hydrogen was the carrier gas at a flow rate of 1.0 mL min^{-1}. The split/splitless injector was operated in splitless mode (2 min) at 250 °C. Linear retention indices were calculated with reference to *n*-alkanes. Compounds were identified by comparison with linear retention indices from the literature,[3] EI mass spectra (Wiley 275 library) or from the co-injection of standards.

Headspace solid-phase microextraction

The floral scent of *Z. mauritiana* was trapped on a 100 µm polydimethylsiloxane (PDMS) fibre of a SPME holder just after the flowers were cut. The flowers were placed in a 250 mL erlenmeyer flask immersed in water at 40 °C, trapped for 1h and the SPME fiber was exposed to this atmosphere for 30 min, subsequently removed and introduced into the GC injector standing for 2 min at 250 °C.

Sensory studies

Sniffing analyses of the SPME on GC were divided in parts of 10 min. Four judges, previously trained on olfactory sensations, participated in evaluating at 10 min intervals, during different sections. An odor description for each odorant was assigned.

Odors detected by fewer than three panelists were classified as noise.

Results and Discussion

The flowers of *Z. mauritiana* were submitted to SPME headspace analysis with a 100 μm polydimethylsiloxane fiber.[4] By GC-MS, 46 substances were identified of which data are supported by retention indices, mass spectrometry data and standard co-injection. The complete list of volatiles identifed in the SPME headspace of the flowers of *Z. mauritiana* is presented in Table 1, including many benzenoid compounds, monoterpenoids, carboxylic acids, minor aldehydes and *n*-hydrocarbons. The predominating volatiles were benzaldehyde and nonanal in 26.5 and 12.6 %, respectively. From the 46 substances identified, 55 % are benzenoids present as phenolics or their methylated derivatives, in different oxidative states, as methyl and benzyl benzoates, benzyl alcohol, *p*-anisic acid (4-methoxy benzoic acid), *p*-anisaldehyde, *p*-anisyl alcohol, methyl anisate, vanillic acid (3-methoxy-4-hydroxy-benzoic acid), vanillin and methyl vanillate. All these methoxylated benzenoids suggest the presence *O*-methyltransferases, well-known enzymes which catalyse the transfer of methyl groups from the methyl donor *S*-adenosyl-L-methionine to hydroxyl or carboxyl groups on a wide range of acceptor molecules. They are involved in the biosynthesis of floral scent substances exemplified by snapdragon (*Antirrhinum sp.*)[5] and roses (*Rosa spp.*).[6]

The monoterpenes found were *trans*-β-ocimene, linalool, *cis*-linalool oxide, α-terpineol, myrtenol and *trans*-carveol, common chemicals in floral scents. Monoterpenes are recognized as important mediators in pollinization syndromes.[7]

Table 1. Substances identified in the headspace SPME from *Zizyphus mauritiana* flowers

Compounds	Retention index (DB-5)	Relative area (%)
Cis-3-hexenol *	857	0.6
Heptanal	899	1.1
Benzaldehyde *	961	26.5
Octanal *	1001	0.7
4-Methylanisole *	1024	1.2
Benzyl alcohol *	1032	4.5
Trans-β-ocimene *	1040	0.4
Cis-linalool oxide	1074	0.4
Guaiacol *	1084	1.1
Methyl benzoate *, [a]	1095	1.0
Nonanal	1102	12.6
Linalool *	1098	1.5
4-ketoisophorone	1139	0.2
Benzoic acid *	1170	0.3
α-Terpineol *	1189	1.0
Myrtenol	1194	0.4
Trans-carveol	1217	0.7
3,4-Dimethoxytoluene *	1230	2.0
p-Anisaldehyde *	1252	3.0
Phenylacetic acid *	1263	0.2
p-Anisyl alcohol	1279	2.0
Nonanoic acid *	1293	3.0
Undecanal	1310	0.5
Methyl anisate *,[a]	1340	0.4
Eugenol *	1356	5.0
Methyl cinnamate *,[a]	1373	0.2
Skatole *	1381	2.4
Vanillin *	1396	4.3
Methyeugenol	1405	0.4
p-Anisic acid *	1489	0.1
Methyl vanillate *,[a]	1525	0.1
Vanillic acid *,[b]	1592	0.1
Veratrylic acid *	1670	0.1
Benzyl benzoate	1762	2.5
Palmitic acid *	2150	0.1
Linoleic acid *	2173	0.1
Stearic acid *	2180	0.1
n-Hydrocarbons (from $C_{21}H_{44}$ to $C_{29}H_{60}$) *		1.2
Total identified		82.0

*standard co-injection; [a] obtained from the methylation with CH_2N_2/Et_2O of the respective carboxylic acids; [b] obtained as cited in ref. 18.

Cis-3-hexenol, heptanal,octanal, nonanal and undecanal are derived from the lipooxygenase pathway from fatty acids. Among them, nonanal is the most representative and was previously detected in Cruciferae (flowers of *Roripa nasturtium-aquaticum* as well as the aroma of cauliflower and broccoli which are cultivars of *Brassica oleracea*), is also a major component of the epicuticular wax of plums.[8]

GC-Olfatometry (GC-O) of the SPME headspace from the flowers of *Z. mauritiana* showed an intense fecal odor, which by comparison with the mass spectra library and after standard co-injection, confirmed the presence of skatole (3-methyl-indole). Skatole is a tryptophane metabolite and considered an off-flavor in food, posing a serious problem to the processing of pork. It has also been identified in *Sauromatum guttatum* inflorescence, which produces a strong putrid odor during anthesis, associated to the presence of amines and organic sulphides.[9] In *Piper nigrum* L. the fecal off-flavour of pepper was attributed to the presence of skatole enhanced by *p*-cresol.[10] However, in low concentrations (< 0.1 ppm) it becomes useful by introducing ripe-fruity and warm flavors, being commonly added to jelly, pudding, chewing-gum and some drinks. Skatole is indicated to attract the common housefly, *Musca domestica* L., as cited by previous studies.[11,12] However, electrophysiologic research indicates that the attraction is not a response to a single substance but to a matrix with several aromatic constituents.[13]

The sweet odor also perceived from the flowers of *Z. mauritiana* was associated by GC-O and GC-MS to vanillin (4-hydroxy-3-methoxybenzaldehyde), one of the most widely used flavorizers, with applications in foods, fragrances and pharmaceuticals, in preparations or as intermediate for synthetic products.[14]

The last characteristic odor of Z. *mauritiana* flower, the phenolic aroma, was detected in various regions of the chromatogram, and no substance alone is responsible for the phenolic component in the odor.

This paper describes the volatile chemical constituents responsible for the fecal scent detected in flowers of *Zizyphus mauritiana* Lam., adding the Rhamnaceae to the list of families with fecal-scented flowers.[15-17] From the 46 substances identified in the SPME headspace analysis of *Z. mauritiana,* 80 % was confirmed by co-injection of standards, improving confiability in the identification of the compounds and in the characterization of the floral scent.

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