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Variation in the chemical composition of essential oils from *Mangifera indica* L. leaves by comprehensive two-dimensional gas chromatography

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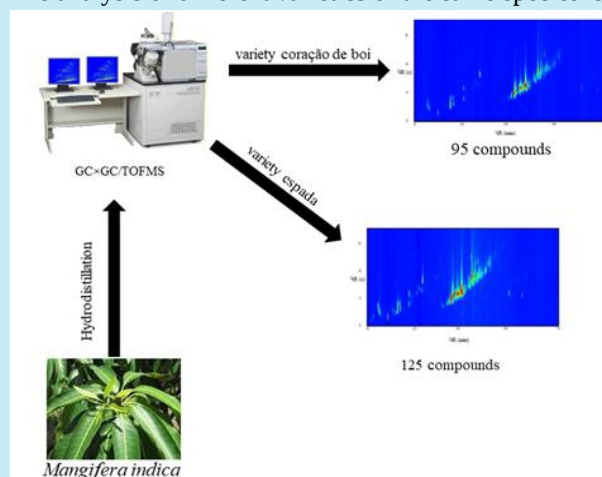
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ABSTRACT: The leaves of *Mangifera indica* L. have been used in the medical system of India to treat diseases such as asthma, dysentery, cough, leucorrhea, jaundice, pain, and malaria. The analysis of different varieties of the same species is intended to determine if the compounds have a differential distribution. The present study investigates the volatile compounds from the leaves of two *M. indica* varieties extracted by hydrodistillation and analyzed by comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC/TOFMS). The number of compounds identified by GC×GC/TOFMS was superior to that obtained by gas chromatography/mass spectrometry (GC/MS) for the same variety of *M. indica*. This study demonstrates the applicability of the GC×GC/TOFMS for the comprehensive profiling of essential oils from *M. indica*, in which 125 and 95 compounds were identified in the varieties 'espada' and 'coração de boi', respectively. These results show that the compositions of the two analyzed essential oils present differences concerning the GC×GC/TOFMS and conventional chromatography technique, the GC/MS.



1. Introduction

Plant-derived essential oils are known and primarily used for their biological properties (Mesquita *et al.*, 2015). Combined with this, the major interest of the pharmaceutical, food and cosmetics industries in the use of new oils as well as the consumer receptivity to new products of natural origin, transformed the evaluation methods of these plants into widely used tools in the search for new products (Aćimović *et al.*, 2022).

The proportion of individual compounds in the oil composition differs from trace levels to over 90% (Bassolé and Juliani, 2012). Then, the complete separation and the correct identification of the essential oil compounds appear to be very important to a better understanding of the mechanisms involved in those biological activities and the prospection of new active compounds (Cagliero *et al.*, 2022).

Mangifera indica L., belonging to the Anacardiaceae family, is one of the 40 species of the *Mangifera* genus that can be found in tropical and subtropical regions of Southeastern Asia, Africa, and Latin America (Nikhil and Mahajan, 2010). Its fruits are considered multifunctional foods. However, other parts of this plant, such as bark, flowers, branches, and leaves, have also bioactive compounds (Gupta *et al.*, 2022).

The leaves of *M. indica* have been used in the medical system of India to treat diseases such as asthma, dysentery, cough, leucorrhea, jaundice, pain, and malaria (Basha *et al.*, 2011). In Brazil, the leaves are used as analgesic, anti-inflammatory and the treat hepatitis (Oliveira *et al.*, 2022). The study of the aqueous extracts of the bark of a selected variety of *M. indica* resulted in a pharmaceutical formula, commercially named Vimang. The volatile constituents of *M. indica* fruits present a considerable variation in their chemical composition, which has been extensively investigated (Dzamić *et al.*, 2010). The variability of the volatile constituents can be influenced by factors such as the stage of development, variety, and extraction method (Pino *et al.*, 2005).

The essential oils of *M. indica* leaves from Egyptian varieties have antimicrobial activity (Ouf *et al.*, 2021). The latex essential oil of *M. indica* from the 'rosa' and 'espada' varieties showed cytotoxic activity against HL-60 human tumor cells (Ramos *et al.*, 2014). The volatile compounds in *M. indica* were usually obtained by hydrodistillation and analyzed by gas chromatography/mass spectrometry (GC/MS) (Ansari *et al.*, 2000; Berenbaum *et al.*, 1985; Dzamić *et al.*, 2010; Moreno *et al.*, 2010; Oliveira *et al.*, 2017; Pino *et al.*, 2005).

Due to the volatility and polarity of essential oils components, capillary gas chromatography is the

preferable technique for their analysis because essential oils are generally complex mixtures of components with similar physicochemical characteristics (Aspromonte *et al.*, 2019; Rubiolo *et al.*, 2010). However, the satisfactory separation of a complex sample requires a higher peak capacity. In this case, comprehensive two-dimensional gas chromatography (GC×GC), a relatively new technique, can be the best alternative (Keppler *et al.*, 2018).

Comprehensive GC×GC, idealized by Liu and Phillips (1991), has since emerged as the most powerful separation technique for analyzing volatile compounds. The satisfactory separation in complex samples, such as some essential oil, requires a higher peak capacity, achieved using GC×GC. In this technique, two independent separation mechanisms are used to resolve the compounds of complex samples within a single analysis, based on applying two GC columns with different stationary phases connected in series, with a transfer device defined as a modulator. The modulator's function is continuously isolating, reconcentrating, and introducing small portions of the first (1D) effluent onto a second column (2D). The time required to complete this process is defined as the modulation period. Each 1D peak is modulated several times, preserving the 1D separation (Adahchour *et al.*, 2008; Stefanuto *et al.*, 2021).

The GC×GC has the advantage of increasing the resolution and sensitivity of the analysis due to the concentration of the sample fraction through the modulation process allowing the detection of compounds in trace levels as well as the separation of related compounds in the second dimension (Baharum *et al.*, 2010). GC×GC is the most powerful separation system now available when combined with mass spectrometry (MS).

The time-of-flight mass spectrometer (TOFMS) can obtain high spectra acquisition rates for the correct peak assignment and quantification in GC×GC. However, its high cost limits its laboratory utilization. Some studies used GC×GC with a time-of-flight mass spectrometry detector (GC×GC/TOFMS) to analyze essential oils (Eyres *et al.*, 2007; Ieri *et al.*, 2019; Jalali *et al.*, 2012; Rubiolo *et al.*, 2010; Wang *et al.*, 2012). The results obtained by these studies showed an important improvement in the characterization of these samples by GC×GC.

In the present study, the volatile compounds of two *M. indica* were analyzed by GC×GC/TOFMS to evaluate the difference between the compounds in the varieties, allowing an adequate selection for medicinal and industrial purposes.

2. Experimental

2.1 Samples

The leaves of the *M. indica* variety were collected in Campo Grande/MS, Brazil. The ‘espada’ variety (collected at 20°30’7” S and 54°37’17” W) and the ‘coração de boi’ variety (20°30’13” S and 54°37’14” W) were identified by Dr. Ronaldo Posella Zaccaro (Centro Universitário Moura Lacerda, Ribeirão Preto/SP, Brazil) and deposited with voucher specimens’ numbers CM105 and CM 107, respectively. All the used solvents and reference standards (linear alkanes) were HPLC grade (JT Baker and Sigma Aldrich). The collection was recorded in the SisGen, number AF9B3C3.

2.2 Essential oil

Each essential oil was isolated from a 400 g sample of fresh leaves of *M. indica* by hydrodistillation using a Clevenger-type apparatus. The essential oils were recovered, dried with anhydrous sodium sulfate, transferred to dark vials, and finally stored at –4 °C for further analysis. Before gas chromatographic analysis, the essential oils (1 mg) were diluted in 1 mL n-hexane. The essential oil yield calculated based on fresh leaves was 0.2% for ‘coração de boi’ and 0.3% for ‘espada’.

2.3 Chromatographic analysis

A GC×GC/TOFMS Pegasus-IV system (LECO, St. Joseph, USA) was equipped with a liquid nitrogen quadrupole modulator and CTC Combi Pal autosampler (CTC Analytics, Carrboro, NC, USA). Electron ionization was 70 eV, the mass acquisition was performed in the range of 50 to 550 amu at 100 Hz, and the detector voltage was –1,706 V. The injector, transfer line and detector temperature were maintained at 250 °C. A conventional column set was employed: DB-5 (5% phenyl–95% dimethylpolysiloxane) with 60 m length, an internal diameter of 0.25 mm, and 0.10 µm of film thickness in the first dimension and a DB-17ms (50% phenyl–50% dimethylpolysiloxane) with 2.15 m length, the internal diameter of 0.18 mm and 0.18 µm of film thickness. Both columns were acquired from Agilent Technologies – J&W Scientific (Palo Alto, CA, USA). The temperature program of the first column started at 50 °C for 5 min, heating at 3 °C min^{–1} till 250 °C. The second column temperature was maintained 10 °C above the temperature of the first column. The modulation period was 10 s, and the Hot pulse was 40% of the modulation period. ChromaTOF software version 3.32 was employed for

data processing the total ion current chromatogram, including tools such as peak finder and mass spectra deconvolution. Data processing was performed using a signal-to-noise ratio equal to three. The criterium for accepting a detected compound was a minimum of 80% similarity with the library.

Temperature-programmed retention indices (Mota *et al.*, 2013) were calculated using a mixture of linear alkane (C6–C30), which was analyzed under the same conditions as the chromatographic analysis of the samples. The volatile components’ identification was based on comparing their mass spectra with those of the database NIST 2.0, the comparison of their retention index and mass spectrum (Adams, 2007) and the interpretation of the mass spectrum.

The results obtained in this study were compared with the data obtained using GC/MS by Oliveira *et al.* (2017).

3. Results and discussion

Some studies show that the main compounds of the essential oils obtained from mango leaves are sesquiterpenes and monoterpenes (Dzamić *et al.*, 2010; Gerbara *et al.*, 2011; Moreno *et al.*, 2010; Pino *et al.*, 2005). These results of this study corroborate the data on the composition of *M. indica* leaves in the ‘espada’ and ‘coração de boi’ varieties.

The essential oil from mango contains constituents such as α -gurjunene, trans-caryophyllene, α -humulene, α -selinene, and camphor (Kumar *et al.*, 2021).

Ramos *et al.* (2014) identified 25 compounds in the essential oil of *M. indica* leaves using gas chromatography with flame ionization detection (GC-FID) and GC/MS. Fontenelle *et al.* (2017) studied the essential oil from different *M. indica* leaves by GC/MS, obtaining 20 compounds for the ‘Tommy Atkins’ variety, 13 for the ‘rosa’ variety, 6 for the ‘muscat’ variety and 15 for the ‘jasmine’ variety. Ouf *et al.* (2021) identified 31 compounds in the ‘Alphonso’ variety, 33 compounds in the ‘Sidik’ variety, 29 compounds in the ‘waste’ variety, 26 compounds in the ‘zebda’ variety and 31 compounds in the ‘fagri-kalan’ variety and trans-caryophyllene (8.06–18.88%), α -selinene (4.33–16.92%), and α -humulene (8.48–25.98%) were found in the higher concentrations. For the ‘coração de boi’ variety, cyperene, (*E*)-caryophyllene and α -humulene are the predominant compounds. Those compounds were also reported to be the most important in the leaves of the ‘coquinho’ variety by GC/MS analysis (Gerbara *et al.*, 2011).

The study performed by Oliveira *et al.* (2017) identified 23 volatile compounds such as monoterpenes

and sesquiterpenes from the leaves of *M. indica* 'espada' and 'coração de boi' varieties extracted by hydrodistillation and analyzed by GC/MS. In the essential oil of leaves obtained from the 'espada' variety, the major compounds were β -selinene (34.90%), cyperene (22.40%), (*E*)-caryophyllene (16.39%), α -humulene (10.84%), terpinolene (2.31%), and α -selinene (2.31%), while in 'coração de boi' variety, the major compounds were cyperene (32.62%), (*E*)-caryophyllene (26.91%), α -humulene (17.12%), β -selinene (5.70%), myrcene (2.80%), and β -phellandrene (2.70%) [Oliveira et al. \(2017\)](#).

The 'espada' and 'coração de boi' varieties generated essential oils of leaves with 125 and 95 tentatively identified compounds using the GC $\times$ GC/TOFMS technique. Due to its superior performance over the GC/MS, the GC $\times$ GC/TOFMS increased the number of identified peaks in *M. indica* essential oils.

In the column setup used, nonpolar in the 1D and medium polar in the 2D, the compounds are separated in the first dimension based on their different volatilities. In the second dimension, the separation is governed by polarity. Consequently, compounds with similar volatility had similar or even exact retention times in the 1D and will be resolved in the 2D. The GC $\times$ GC/TOFMS analyses revealed a complex organic compound mixture ([Figs. 1 and 2](#)). Combining a low polar 5% phenyl phase in the first dimension with a medium polar 50% phenyl phase in the second dimension allowed efficient use of the available chromatographic space. [Figures 1 and 2](#) highlight the complexity of the *M. indica* essential oil and the efficiency of GC $\times$ GC to reduce the peak coelution, obtaining pure MS spectra and increasing peak detectability.

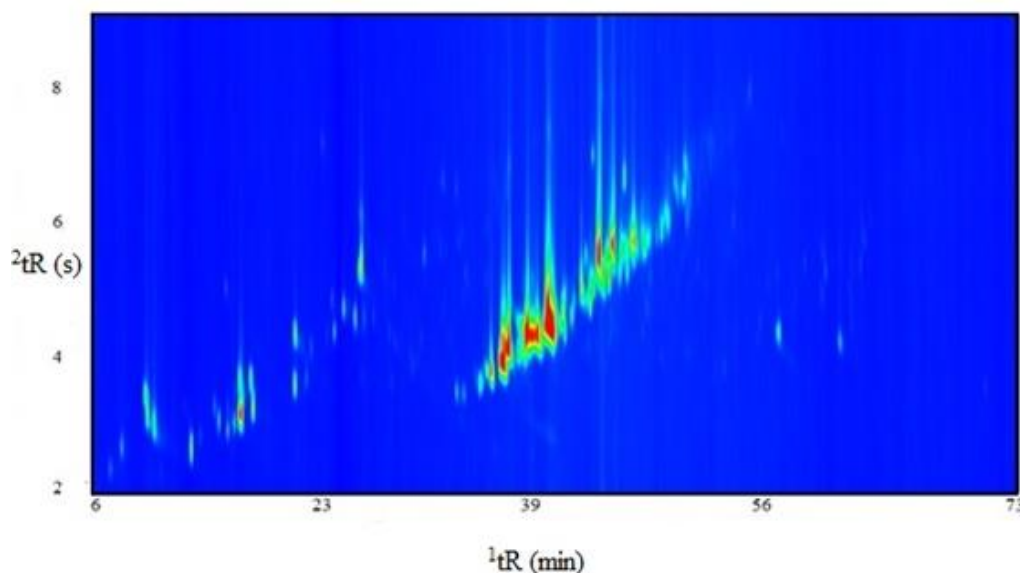


Figure 1. Color diagram of the essential oils of *M. indica* 'espada' variety obtained by comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometric detector.

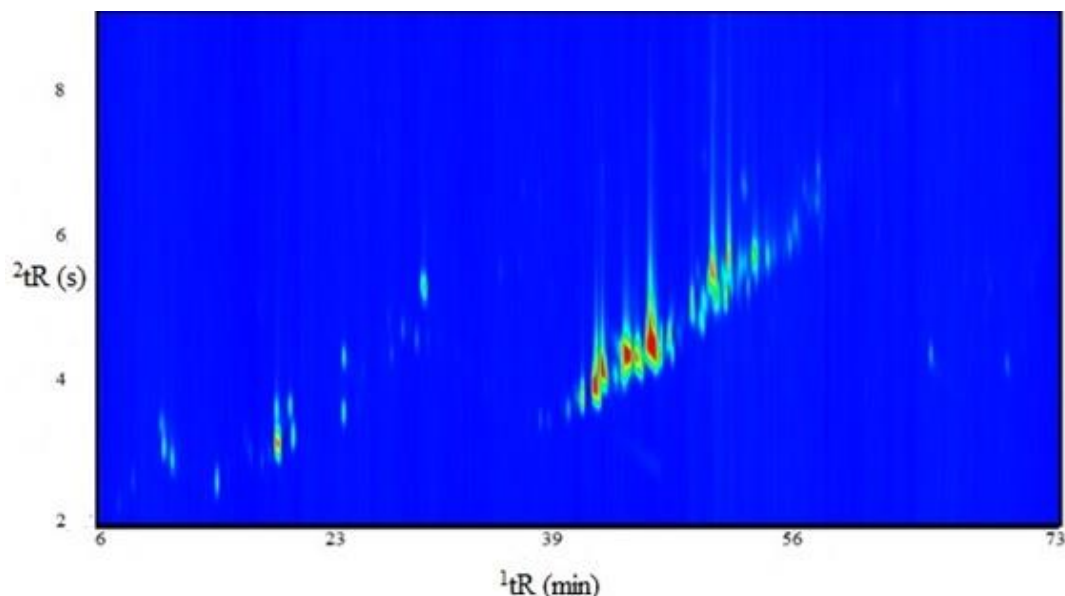


Figure 2. Color diagram of the essential oils of *M. indica* 'coração de boi' variety obtained by comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometric detector.

The experimental linear retention indices show a good agreement between the identified compounds and the linear retention indices reported by literature for 1D-

GC (Bogusz Junior *et al.*, 2011). The list of identified compounds is shown in Table 1.

Table 1. Percentage composition of essential oils of leaves of *M. indica* by GC×GC/TOFMS.

KI ^a	KI ^b	¹ tR	² tR	Compound	'Espada' (%)	'Coração de boi' (%)
769	771	7.83	3.29	2-pentenol	0.05 ± 0.01	-
800	800	8.50	2.02	octane	0.03 ± 0.01	0.05 ± 0.01
800	802	8.50	3.54	caproaldehyde	0.16 ± 0.01	0.16 ± 0.01
848	854	10.17	4.3	2-hexenal	2.10 ± 0.10	1.00 ± 0.10
852	851	10.33	3.97	3-hexen-1-ol	0.44 ± 0.02	0.40 ± 0.03
867	867	10.83	3.85	hexen-1-ol	0.86 ± 0.03	0.81 ± 0.09
867	869	10.83	3.79	santene	1.75 ± 0.09	1.50 ± 0.20
904	902	12.17	4.11	heptanal	0.10 ± 0.01	-
929	930	13.33	3.93	α-thujene	0.06 ± 0.01	0.10 ± 0.01
932	939	13.50	3.5	α-pinene	1.20 ± 0.10	1.01 ± 0.07
946	944	14.17	2.71	valeric acid 3-methyl	0.07 ± 0.01	-
975	975	15.50	3.91	sabinene	0.30 ± 0.01	0.34 ± 0.01
989	991	16.17	3.79	myrcene	0.21 ± 0.01	1.60 ± 0.10
1000	1000	16.67	3.89	<i>m</i> - mentha-1(7).8-diene	0.21 ± 0.01	0.22 ± 0.01
1006	1005	17.00	4.51	α- phellandrene	0.33 ± 0.01	0.23 ± 0.01
1010	1011	17.17	4.01	γ-carene	4.20 ± 0.10	2.70 ± 0.20
1027	1031	18.00	4.14	limonene	0.99 ± 0.05	1.04 ± 0.08
1033	1031	18.33	5.01	β-phellandrene	0.02 ± 0.01	2.80 ± 0.20
1087	1087	21.00	5.22	terpinolene	0.02 ± 0.01	-
1093	1089	21.30	3.21	p-cimenene	0.05 ± 0.01	0.04 ± 0.01
1110	1108	22.17	5.03	maltrol	0.09 ± 0.01	-
1128	1128	23.00	8.03	allo-ocimene	0.09 ± 0.01	-
1134	1134	23.33	5.22	1-terpineol	0.03 ± 0.01	-
1141	1141	23.67	5.73	cis-verbeneol	0.12 ± 0.01	0.22 ± 0.01
1159	1159	24.50	5.61	karahanaenone	0.41 ± 0.03	0.38 ± 0.02
1176	1177	25.33	5.45	terpinen-4-ol	0.35 ± 0.02	0.37 ± 0.01

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1186	1183	25.83	6.15	p-cimen-8-ol	2.00 ± 0.20	2.90 ± 0.20
1190	1190	26.00	3.36	neo isoverbenol	0.25 ± 0.01	-
1211	1212	27.00	3.21	2.4-nonadienal	0.25 ± 0.01	-
1278	1278	30.00	3.27	isopulegyl acetate	0.30 ± 0.01	-
1300	1300	31.00	3.42	tridecane	0.33 ± 0.02	-
1331	1330	32.50	4.85	acetate cis-piperitol	0.02 ± 0.01	-
1334	1333	32.67	7.38	hexyl tiglate	0.08 ± 0.01	0.40 ± 0.02
1338	1339	32.83	6.59	γ-elemene	0.04 ± 0.01	-
1345	1344	33.17	5.77	verbenol acetate	0.08 ± 0.01	0.17 ± 0.01
1348	1348	33.33	6.12	7-epi -silphiperfol-5-one	0.05 ± 0.01	-
1369	1368	34.33	4.51	cyclosativene	0.42 ± 0.02	0.42 ± 0.02
1372	1373	34.50	3.36	isolekene	0.39 ± 0.01	0.58 ± 0.02
1376	1376	34.67	4.73	α-copaene	0.02 ± 0.01	0.04 ± 0.01
1379	1379	34.83	6.02	silphiperfol-6-one	0.02 ± 0.01	-
1379	1379	34.83	6.85	methyl cinnamate	0.02 ± 0.01	-
1386	1391	35.17	4.67	β-elemene	2.10 ± 0.20	3.10 ± 0.20
1393	1393	35.50	3.55	jasmone	0.35 ± 0.01	0.47 ± 0.02
1397	1398	35.67	4.53	cyperene	5.00 ± 0.30	7.00 ± 0.30
1405	1404	36.01	4.75	methyl eugenol	0.84 ± 0.04	0.84 ± 0.02
1405	1406	36.01	4.85	italicene	3.50 ± 0.30	2.50 ± 0.10
1409	1409	36.17	5.89	α-gurjunene	0.02 ± 0.01	-
1414	1417	36.33	6.61	4.8-α-epoxy caryophyllane	0.02 ± 0.01	-
1414	1417	36.33	5.22	sesquiterpene	0.02 ± 0.01	-
1414	1418	36.33	5.10	(e)-caryophyllene	5.20 ± 0.20	6.50 ± 0.30
1423	1423	36.67	5.69	β-duprezianene	0.06 ± 0.01	0.06 ± 0.01
1427	1429	36.83	4.81	cis thujopsene	0.06 ± 0.01	0.05 ± 0.01
1436	1432	37.17	4.95	β-gurjunene	0.47 ± 0.01	0.52 ± 0.02
1445	1444	37.50	5.30	cedrene	1.04 ± 0.07	1.30 ± 0.10
1450	1450	37.67	4.98	epicedrene	0.13 ± 0.01	0.19 ± 0.01
1450	1450	37.67	5.36	cis-muurolo-3.5-diene	0.16 ± 0.01	0.15 ± 0.01
1455	1454	37.83	5.23	α -humulene	6.90 ± 0.30	7.20 ± 0.30
1459	1460	38.00	3.46	β-santalene	0.03 ± 0.01	0.02 ± 0.01
1459	1460	38.00	5.23	allo-aromadendrene	2.10 ± 0.20	2.00 ± 0.10
1473	1473	38.50	5.40	α -terpinyl isobutanoate	0.57 ± 0.02	0.50 ± 0.02
1473	1473	38.50	5.22	drima-7.9(11)-diene	4.80 ± 0.20	4.90 ± 0.20
1477	1477	38.67	5.06	β-gurjunene	0.48 ± 0.02	0.45 ± 0.01
1482	1480	38.83	5.25	γ-murolene	0.30 ± 0.01	0.32 ± 0.01
1486	1480	39.00	5.48	germacrene d	0.72 ± 0.02	0.64 ± 0.02
1486	1485	39.00	5.40	β-selinene	10.20 ± 0.20	10.40 ± 0.20
1495	1496	39.33	3.71	asaricinae	0.28 ± 0.01	0.25 ± 0.01
1495	1498	39.33	5.38	α-selinene	6.70 ± 0.10	5.00 ± 0.20
1500	1500	39.50	5.42	biciclogermacrene	0.87 ± 0.01	0.87 ± 0.03
1509	1509	39.83	5.39	farenol	0.22 ± 0.01	0.21 ± 0.01
1509	1509	39.83	5.24	germacrene a	0.60 ± 0.02	0.62 ± 0.01
1517	1518	40.17	5.34	menthyl isovalerate	0.24 ± 0.01	0.24 ± 0.01
1522	1523	40.33	5.51	eugenol acetate	0.85 ± 0.03	0.83 ± 0.02
1526	1526	40.50	5.80	1-phenyl heptan-3-one	0.18 ± 0.01	0.19 ± 0.01
1526	1527	40.50	5.26	vanillin acetate	0.21 ± 0.01	0.21 ± 0.01
1535	1535	40.83	5.58	10-epi-cubenol	0.33 ± 0.01	0.36 ± 0.02
1539	1539	41.00	5.40	α-cadinene	0.13 ± 0.01	0.17 ± 0.01
1543	1543	41.17	5.75	8.14-cedranoxide	0.43 ± 0.02	0.41 ± 0.01
1548	1548	41.33	6.14	silphiperfolan-6-beta-ol	0.02 ± 0.01	-
1557	1557	41.67	6.93	elemicine	0.03 ± 0.01	0.01 ± 0.01
1561	1561	41.83	5.91	germacrene b	1.31 ± 0.08	2.01 ± 0.12

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1565	1566	42.00	6.32	β -calacorene	0.03 $\pm$ 0.01	0.03 $\pm$ 0.01
1565	1566	42.00	6.42	davanone b	0.23 $\pm$ 0.01	0.32 $\pm$ 0.01
1574	1575	42.33	5.67	α -cedrene epoxy	0.28 $\pm$ 0.01	0.25 $\pm$ 0.01
1574	1575	42.33	6.01	silphiperfol-5-em-3-one a	0.38 $\pm$ 0.01	0.35 $\pm$ 0.01
1583	1583	42.67	6.26	turmerol	0.75 $\pm$ 0.06	0.34 $\pm$ 0.01
1583	1583	42.67	6.43	caryophyllene oxide	3.95 $\pm$ 0.08	2.15 $\pm$ 0.06
1591	1591	43.00	6.36	β -copaen-4- α -ol	0.14 $\pm$ 0.01	0.19 $\pm$ 0.01
1596	1596	43.17	6.04	turmerone-ar-dihydro	0.60 $\pm$ 0.01	0.64 $\pm$ 0.02
1600	1600	43.33	3.69	hexadecane	0.01 $\pm$ 0.01	-
1600	1601	43.33	5.15	cedrol	0.02 $\pm$ 0.01	-
1600	1601	43.33	5.92	guaiacol	0.04 $\pm$ 0.01	0.04 $\pm$ 0.01
1600	1601	43.33	6.25	β -elemenone	0.05 $\pm$ 0.01	0.05 $\pm$ 0.01
1605	1605	43.50	6.40	sesquilavandulol	0.88 $\pm$ 0.03	0.94 $\pm$ 0.03
1609	1608	43.67	6.21	platyphyllol	0.17 $\pm$ 0.01	0.15 $\pm$ 0.01
1609	1608	43.67	6.06	β -atlantol	0.92 $\pm$ 0.03	1.12 $\pm$ 0.05
1614	1614	43.83	6.42	ethyl chromone 2	0.44 $\pm$ 0.02	0.40 $\pm$ 0.01
1614	1614	43.83	6.59	β - biotol	3.40 $\pm$ 0.10	4.00 $\pm$ 0.20
1618	1618	44.00	6.16	butyl anthranilate	0.12 $\pm$ 0.01	-
1632	1631	44.50	6.31	eremoligenol	0.23 $\pm$ 0.01	0.27 $\pm$ 0.01
1632	1632	44.50	6.55	γ -eudesmol	0.82 $\pm$ 0.04	0.89 $\pm$ 0.02
1641	1641	44.83	7.52	epoxy allo alloaromadendrene	0.34 $\pm$ 0.01	0.31 $\pm$ 0.01
1645	1646	45.00	6.15	α -murolol	0.16 $\pm$ 0.01	0.13 $\pm$ 0.01
1655	1655	45.33	6.55	dihydromyrcene. 1.6 –diol. e	0.14 $\pm$ 0.01	0.15 $\pm$ 0.01
1655	1655	45.33	6.61	3- tujopsanone	1.64 $\pm$ 0.08	1.4 $\pm$ 0.1
1659	1659	45.50	6.62	atractilone	0.61 $\pm$ 0.02	0.63 $\pm$ 0.03
1668	1668	45.83	6.46	citronellyl tiglate e	0.15 $\pm$ 0.01	0.12 $\pm$ 0.01
1677	1677	46.17	6.6	cadelene	1.04 $\pm$ 0.06	0.90 $\pm$ 0.10
1686	1686	46.50	6.65	α -bisabolol	0.15 $\pm$ 0.01	0.13 $\pm$ 0.01
1695	1694	46.83	3.7	germacrone	0.20 $\pm$ 0.01	0.22 $\pm$ 0.01
1700	1700	47.00	3.79	heptadecane	0.41 $\pm$ 0.01	0.43 $\pm$ 0.02
1705	1705	47.17	6.68	δ -dodecalactone	0.11 $\pm$ 0.01	0.14 $\pm$ 0.01
1714	1714	47.50	4.91	cedroxide	0.03 $\pm$ 0.01	-
1714	1714	47.50	6.76	α -humulene. 14hydrixy	0.43 $\pm$ 0.01	0.52 $\pm$ 0.03
1724	1723	47.83	7.03	crisolide	0.44 $\pm$ 0.02	0.48 $\pm$ 0.03
1738	1740	48.33	7.53	oplopanone	0.29 $\pm$ 0.01	0.34 $\pm$ 0.01
1748	1748	48.67	7.29	α - oxobisabolone	0.20 $\pm$ 0.01	-
1800	1800	50.50	3.85	octadecane	0.23 $\pm$ 0.01	0.31 $\pm$ 0.01
1897	1898	53.83	4.70	seseline	0.26 $\pm$ 0.01	-
1901	1902	54.00	3.91	laurencene	0.26 $\pm$ 0.01	-
1963	1962	55.83	5.25	tetrahydro rimuene	1.40 $\pm$ 0.10	0.75 $\pm$ 0.03
2000	2000	57.00	3.94	eicosane	0.34 $\pm$ 0.01	-
2088	2088	59.33	6.16	abietadiene	0.56 $\pm$ 0.02	-
2100	2100	60.00	4.03	heneicosane	0.35 $\pm$ 0.01	-
2115	2116	60.67	5.14	laurensen-2-one	0.64 $\pm$ 0.02	0.73 $\pm$ 0.03
2176	2175	62.17	6.63	grandiflorene	0.12 $\pm$ 0.01	-

^aKI: retention index calculated; ^bKI: retention index literature from Adams (2007); (-): not identified; ¹tR: retention time in the first dimension; ²tR: retention time in the second dimension.

Furthermore, the GC $\times$ GC increased the detectability of the compounds due to using the modulator (Baharum *et al.*, 2010), as can be observed in the increase in the number of compounds. These peaks were present at low concentrations, but the improvement of their signal by GC $\times$ GC achieved better mass spectra and separation than in the 1D-GC. In several cases, it was found that,

despite using two chromatographic separation columns, some compounds were still coeluting. The essential oils have many isomers with similar retention times and mass spectra, especially sesquiterpenes and oxygenated sesquiterpenes. However, peak deconvolution algorithms allowed for resolving chromatographic solutions and extracting the mass spectrum of each

compound, even in such situations. GC×GC promoted the identification of fivefold more compounds in the two essential oils than GC/MS.

The major constituents of 'espada' variety essential oil identified by GC×GC/TOFMS were β -selinene (10.2%), α -humulene (6.9%), α -selinene (6.7%), (*E*)-caryophyllene (5.2%), ciperene (5.0%), Drima-7.9(11)-diene (4.8%), γ -carene (4.2%), caryophyllene oxide (3.95%), italicene (3.5%) and β -biotol (3.4%). Ninety-five compounds were identified in essential oil of the 'coração de boi' variety by GC×GC/TOFMS and the major constituents were β -selinene (10.4%), α -humulene (7.2%), ciperene (7.0%), (*E*)-caryophyllene (6.5%), α -selinene (5.0%), Drima-7.9(11)-diene (4.9%), β -biotol (4.0%) and β -elemene (3.1%). A total of 31 compounds were identified exclusively in the 'espada' variety. The two essential oils were characterized by the predominance of β -selinene, (*E*)-caryophyllene and α -humulene. Also, monoterpenes were found in small concentrations in the two oils.

The use of GC×GC provided enhanced efficiency, mainly for minor compounds. The results showed a considerable increase in the number of separated compounds. In addition, the analysis of mass spectra data together with the retention index allowed the identification of three times more compounds which reflected a differentiation between the essential oils studied.

4. Conclusions

This study demonstrates the applicability of the GC×GC/TOFMS for the comprehensive profiling of *M. indica* essential oils. It also indicated that two-dimensional gas chromatography had a superior resolution, making it possible to identify more compounds. One hundred and twenty-five and ninety-five compounds were tentatively identified in the two studied essential oils of the 'espada' and 'coração de boi' varieties, respectively. These results showed that the compositions of the two analyzed essential oils showed differences in relation to the GC×GC/TOFMS and conventional chromatography technique, the GC/MS.

Authors' contribution

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Data availability statement

All data sets were generated or analyzed in the current study.

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