Research Article

Alberto J. Núñez Sellés*, Juan Agüero Agüero, Lauro Nuevas Paz

GC-MS analysis of mango stem bark extracts (*Mangifera indica* L.), Haden variety. Possible contribution of volatile compounds to its health effects<https://doi.org/10.1515/chem-2021-0192>

received November 5, 2020; accepted December 23, 2020

Abstract: Mango stem bark extracts (MSBE) have been used as bioactive ingredients for nutraceutical, cosmeceutical, and pharmaceutical formulations due to their antioxidant, anti-inflammatory, and analgesic effects. We performed the MSBE preparative column liquid chromatography, which led to the resolution and identification by GC-MS of 64 volatile compounds: 7 hydrocarbons, 3 alcohols, 1 ether, 3 aldehydes/ketones, 7 phenols, 20 terpenoids (hydrocarbons and oxygenated derivatives), 9 steroids, 4 nitrogen compounds, and 1 sulphur compound. Major components were β -elemene, α -guaiene, aromadendrene, hinesol, 1-octadecene, β -eudesmol, methyl linoleate, juniper camphor, hinesol, 9-methyl ($3\beta,5\alpha$)-androstane-3-ol, γ -sitosterol, β -chamigrene, 2,5-dihydroxymethyl-phenethyl-alcohol, *N*-phenyl-2-naphthaleneamine, and several phenolic compounds. The analysis of MSBE, Haden variety, by GC-MS is reported for the first time, which gives an approach to understand the possible synergistic effect of volatile compounds on its antioxidant, analgesic, and anti-inflammatory effects. The identification of relevant bioactive volatile components from MSBE extracts, mainly terpenes from the eudesmane family, will contribute to correlate its chemical composition to previous determined pharmacological effects.

Keywords: *Mangifera indica*, mango stem bark, Haden variety, GC-MS, preparative column chromatography

1 Introduction

Mango stem bark extract (MSBE) has been developed as a bioactive ingredient for nutraceutical, cosmeceutical, and pharmaceutical formulations due to its antioxidant, anti-inflammatory, and analgesic effects [1]. The MSBE's major component is a xanthone (mangiferin, 2- β -D-glucopyranosyl-1,3,6,7-tetrahydroxyl-9H-xanthen-9-one, hereafter MF), which has been intensively studied as a promising candidate to be developed for neurodegenerative diseases treatment [2], besides its use as antioxidant in food formulations against lifestyle disorders [3]. Our first work about the chemical composition of the MSBE led to the isolation of seven phenolic components: gallic acid and its methyl and propyl esters, MF, (+)-catechin, (–)-epicatechin, benzoic acid and its propyl ester, and 3,4-dihydroxybenzoic acid; four sugars: glucose, galactose, arabinose, and fructose; and 3 polyols: sorbitol, myoinositol, and xylitol [4]. All previous reported components from the MSBE were analyzed by high performance liquid chromatography with photodiode detection coupled to mass spectrometry (HPLC-DAD-MS), but no report has been published about its volatile components, which are usually analyzed by high resolution gas chromatography coupled to mass spectrometry (GC-MS). The composition of polyphenol-rich extracts from mango by-products (stem bark and branch tree) on two varieties (Haden and Tommy Atkins) has been compared, and we concluded that the Haden variety would be the best choice for a future exploitation of mango by-products for the production of polyphenol-rich extracts [5].

We report in this manuscript the MSBE volatiles' composition from the Haden variety by GC-MS to contribute to its full chemical characterization. The possible influence of several volatile components on previous described MSBE health effects (antioxidant and anti-inflammatory)

* **Corresponding author: Alberto J. Núñez Sellés**, Universidad Nacional Evangélica (UNEV), Research Division, Paseo de los Periodistas 54, Ensanche Miraflores, Distrito Nacional, Santo Domingo, CP 10203, Dominican Republic, e-mail: anunez@unev.edu.do, nunez500412@gmail.com, tel: +1-809-481-6256
Juan Agüero Agüero: Phytomedicamenta S.A. de C.V., R&D Department, Isla st. 31, Colonia Ampliación Alpes, CP 01710 CDMX, México

Lauro Nuevas Paz: Universidad Nacional Evangélica (UNEV), Research Division, Paseo de los Periodistas 54, Ensanche Miraflores, Distrito Nacional, Santo Domingo, CP 10203, Dominican Republic; Laboratorios MAGNACHEM, Research Department, Av Jose F Peña Gómez & Calle K, Zona Industrial de Haina 9100, San Cristóbal, Dominican Republic

and their possible synergistic effects with nonvolatile components are discussed.

2 Materials and methods

2.1 Stem bark collection

Stem Bark (SB) from mango, Haden variety, was collected from a farm located in Bani region (Dominican Republic) in the autumn season (2019), according to a Standardized Operational Procedure [6]. Briefly, bark was marked, with not more than a 2 cm depth, as a rectangle (10 × 50 cm approximately), depending on the tree size. The bark was collected without damaging the stem, with specially designed tools. Bark pieces were cleaned manually from dust, and residues, milled with a hammer mill (3–5 mm pieces), and dried at 60°C for 2 h. SB was dried until constant weight, and water content was determined with a humidity balance (Radwag, Poland, PMR-50).

2.2 Mango stem bark extract (MSBE)

SB (200 g) was Soxhlet-extracted with 1.5 L petroleum ether (boiling point: 40–60°C) for 12 h. Solvent was evaporated down to 10 mL with a dry nitrogen flux, and the residue brought into a stoppered vial and kept at 4–8°C until chromatographic analysis (extract A). The remaining SB was Soxhlet-extracted thereafter with 1.5 L chloroform for 2 h. Solvent was evaporated through vacuum rotary evaporation (ROVA-100, MRC Labs, Israel), and the residue dissolved in acetone, filtered through a 0.45 µm filter disc with a syringe, brought into a stoppered vial, and kept at 4–8°C until preparative chromatography.

Commercially available MSBE powder, obtained through a standardized industrial technology [7], was extracted by a simultaneous steam distillation-solvent extraction (SDE) procedure. The sample was suspended in 90 mL of sodium chloride saturated solution and heated at 140°C for 1 h. Condensed vapors were collected with 10 mL of diethyl ether. Cooling temperature in the condenser was fixed at 0°C. The extract was concentrated to 1 mL in a Kuderna-Danish apparatus with a Vigreux column, dried overnight (4–8°C) with anhydrous sodium sulfate, brought into a stoppered vial, and kept at 4–8°C until chromatographic analysis (extract B).

2.3 Preparative chromatography

Extract A was subjected to preparative chromatography using a Spectrum Labs, USA, Model CF-2, fraction collector equipped with a YL-9160 UV detector (Young Lin, Korea), and a silica gel (80–100 mesh) column, 25 × 5 cm i.d., with a solvent gradient of *n*-hexane:ethyl acetate as follows: 0–30 min (*n*-hexane), 30–60 min (1:1 hexane–ethyl acetate), and 60–90 min (ethyl acetate) at a flow rate of 2.5 mL/min, and eluent detection at 254 nm, to yield extracts C, D, and E, respectively. Fractions were concentrated by vacuum rotary evaporation; the residue dissolved in 5 mL acetone, dried overnight (4–8°C) with anhydrous sodium sulfate, brought into a stoppered vial, and kept at the same temperature until chromatographic analysis.

2.3.1 High resolution gas chromatography mass spectrometry (HR-GC-MS)

HR-GC-MS was performed on a Carlo Erba (Italy), model MEGA 2, coupled to a VG (UK) mass detector, model TRIO 1000 with splitless injection. Chromatographic analyses were performed on a SPB-1 column (Supelco, USA, 30 × 0.32 mm i.d., df = 1 µm) with helium as carrier gas (1 mL/min). Experimental conditions were as follows: injection volume, 1 µL; splitless time, 30 or 60 s; injector temperature, 260–290°C; detector (FID) temperature, 300°C. Oven heating was programmed from 30 to 60°C to 250–300°C at 4–10°C/min according to extract polarity.

The column was connected through a direct inlet interface (280°C) to the quadrupole ionic source (EI⁺) fixed at 70 eV at a temperature of 230 or 270°C. Mass spectra were recorded from 10 to 600 Da, scan rate 0.8 s, and stored until data processing. Experimental data were processed with Lab-Base™ software (Fisons, UK) and chromatographic peak identification was done for direct comparison with a library search program and/or retention times and spectra from standard compounds when available. Peaks identification by the library search program should have a match value higher than 0.9.

2.4 Chemicals

All reagents and solvents for extraction and preparative chromatography were purchased from JT Baker, USA. Solvents were Pure for Analysis quality. The following standards were purchased from Sigma-Aldrich Co.

(Missouri, USA): (+)-aromadendrene (97% purity, MW: 204.35, colorless liquid), dodecanal (92% purity, MW: 184.32, colorless liquid), β -eudesmol (98% purity, MW: 222.37, colorless liquid), α -humulene (96% purity, MW: 204.35, colorless liquid), hinesol (98% purity, MW: 194.27, colorless liquid), 3-methyldibenzothiophene (96% purity, MW: 198.29, colorless liquid), 6-methyl-3-heptanol (99% purity, MW: 130.23, colorless liquid), methyl linoleate (>98% purity, MW: 294.47, colorless liquid), (+)-nootkatone (>99% purity, MW: 218.33, colorless liquid), octanal (99% purity, MW: 128.21, colorless liquid), 1-octadecene (95% purity, MW: 252.48, colorless liquid), myristic acid (>99% purity, MW: 228.37, amorphous white solid), palmitic acid (>99% purity, MW: 256.42, amorphous white solid), and heptadecanenitrile (95% purity, MW 251.45, colorless liquid).

The following standards were purchased from Supelco Inc. (USA): β -amyrin (98% purity, MW: 426.72, colorless liquid), *trans*-(-)-caryophyllene (98% purity, MW: 204.35, colorless liquid), β (-)-elemene (98% purity, MW: 204.35, colorless liquid), *n*-hexadecane (>99% purity, MW: 226.44, colorless liquid), *n*-heptadecane (>99% purity, MW: 240.47, colorless liquid), and guaiol (2 mg/mL solution, MW: 222.37, colorless liquid).

The following pure compounds were available in the laboratory (JT Baker, USA) and used as standards for identification: phenol (>99%, MW: 94.11, colorless hygroscopic solid), hexanoic acid (>99%, MW 116.16, colorless liquid), methyl 4-hydroxymethylbenzoate (>99%, MW: 166.17, white amorphous solid), and 3,4,5-trimethoxyphenol (97% purity, MW: 184.19, colorless amorphous solid).

Ethical approval: The conducted research is not related to either human or animal use.

3 Results

Figure 1 shows the chromatograms from extracts A to E, respectively. Ten compounds from 14 (71%) in extract A, nine compounds from 11 (82%) in extract B, 29 compounds from 53 (55%) in extract C, 20 compounds from 31 (65%) in extract D, and seven compounds from 10 (70%) in extract E could be identified by their mass spectra either by library search software or by comparison with mass spectra of pure standard compounds when available.

Major components on extract A (Figure 1a) were peaks 1, 5, 6, 8, and 12, identified as β -elemene, β -selinene, 3,7(11)-selinadiene, bulnesol, and palmitic acid,

respectively. Peaks 10 and 14 could be identified as a saturated hydrocarbon, with more than 17 carbons, and a glyceride from 9-octadecenoic acid, respectively, but fragmentation patterns were not conclusive. β -elemene (peak 1) and palmitic acid (peak 12) were identified by comparison of their chromatographic behavior and identical mass spectra of pure standards. β -selinene (peak 5), 3,7(11)-selinadiene (peak 6), and bulnesol (peak 8) matched library spectra with values of 0.99, 0.96, and 0.98, respectively, and had identical molecular ions as compared to published mass spectra data of pure compounds. Other identified minor components in extract A by comparison with authentic standards were *trans*-(-)-caryophyllene (peak 2), α -humulene (peak 3), *n*-hexadecane (peak 7), *n*-heptadecane (peak 9), and palmitic acid (peak 12).

Major components on extract B (SDE extraction, Figure 1b) were peaks 2, 3, 4 and 7, identified as β -elemene, β -selinene, 3,7(11)-selinadiene, and juniper camphor, respectively.

Peak 8 could be identified as a saturated aldehyde with more than 10 carbons, but mass spectrum was not conclusive. β -elemene (peak 2) was identified by comparison of its chromatographic behavior and identical mass spectrum of an authentic standard. β -selinene, 3,7(11)-selinadiene, and juniper camphor matched library spectra with values of 0.99, 0.96, and 0.99, respectively, and had identical molecular ions as compared to published mass spectra data of pure compounds. Other identified minor components in extract B by comparison with authentic standards were guaiol (peak 5), myristic acid (peak 9), palmitic acid (peak 10), and heptadecanenitrile (peak 11). Peak 6 was identified as α -eudesmol with a matched library spectrum of 0.94 and identical molecular ion as compared to published mass spectrum data of the pure compound.

Major components on extract C (nonpolar fraction, Figure 1c) were peaks 8, 10, 11, 19, and 29, identified as β -elemene, α -guaiene, (+)-aromadendrene, hinesol, and 1-octadecene, respectively. Peak 42 was identified as squalene, a common compound from stationary phase column bleeding. β -elemene (peak 8), (+)-aromadendrene (peak 11), hinesol (peak 19), and 1-octadecene (peak 29) were identified by comparison of their chromatographic behavior and identical mass spectra of pure standards. Peak 10 was identified as α -guaiene with a matched library spectrum of 0.95 and identical molecular ion as compared to published mass spectrum data of the pure compound. Other identified minor components in extract C by comparison with authentic standards were octanal (peak 1), dodecanal (peak 2), (+)-nootkatone

Figure 1: Gas chromatograms (FID) of mango stem bark extracts, Haden variety. (a) Fresh mango stem bark extract by Soxhlet extraction. (b) Industrial spray-dried mango stem bark extract (MSBE) by simultaneous steam distillation-solvent extraction. (c) Hexane eluate by preparative chromatography from extract A. (d) Hexane-ethyl acetate (1:1) eluate by preparative chromatography from extract A. (e) Ethyl acetate eluate by preparative chromatography from extract A.

(peak 27), 3-methyldibenzothiophene (peak 31), and β -amyrin (peak 49). Another 21 compounds were identified with matched library spectra values between 0.92 and 0.99 and had identical molecular ions as compared to published mass spectra data of pure compounds (see Table 1).

Major components on extract D (medium-polarity fraction, Figure 1d) were peaks 10, 15, 19, 23, and 31, identified as β -selinene, β -eudesmol, methyl linoleate,

9-methyl-($3\beta,5\alpha$)-androst-3-ol, and γ -sitoesterol, respectively. Peaks 24–30 were identified as alkyl substituted phenols, but identification was not possible due to their low concentrations.

β -eudesmol (peak 15) and methyl linoleate (peak 19) were identified by comparison of their chromatographic behavior and identical mass spectra of pure standards. β -selinene (peak 10), 9-methyl-($3\beta,5\alpha$)-androst-3-ol (peak 23), and γ -sitoesterol (peak 31) matched library

Table 1: Identified components in mango stem bark extracts, Haden variety. Extract A: Soxhlet extraction from dried stem bark; extract B: simultaneous steam distillation-solvent extraction of industrial spray-dried stem bark extract (MSBE); extract C: hexane eluate by preparative chromatography from extract A; extract D: hexane-ethyl acetate (1:1) eluate by preparative chromatography from extract A; extract E: ethyl acetate eluate by preparative chromatography from extract A (Match = 1.00 means mass spectra comparison with authentic pure standard)

No	Compound	M ⁺	Calc. mass	Match*	Type of extract				
					A	B	C	D	E
Hydrocarbons (7 compounds)									
1	α -Terpinolen[3,8-dimethyl-4-(1-methylidene)-(8S-cis)-2,4,6,7,8,8 α hexahydro-5 (1H)-azulenone]	136	136.23	0.91			x		
2	Pentamethylethylbenzene	176	176.40	0.97			x		
3	Hexadecane	225	226.44	1.00	x				
4	Heptadecane	240	240.47	1.00	x				
5	1-Ethyldecylbenzene	246	246.40	0.97			x		
6	1-Octadecene	252	252.48	1.00			x		
7	3-Eicosene	280	280.50	0.99			x		
Alcohols/ethers (4 compounds)									
8	6-Methyl-3-heptanol	130	130.23	1.00				x	
9	1,1-Ethoxypropoxyethane	132	132.20	0.99					x
10	2-Butyloctanol	186	186.33	0.98			x		
11	3,4,5-Trimethoxybenzenemethanol	243	243.21	0.99				x	
Aldehydes/ketones (3 compounds)									
12	Octanal	128	128.21	1.00			x		
13	Dodecanal	184	184.32	1.00			x		
14	4-Hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl)-2-cyclohexen-1-one	222	222.28	0.91				x	
Carboxylic/fatty acids/esters (9 compounds)									
15	Hexanoic acid	116	116.16	1.00					x
16	4-Hydroxymethylbenzoate	151	151.14	1.00				x	
17	Myristic acid	228	228.37	1.00		x			
18	Palmitic acid	256	256.42	1.00	x	x		x	
19	Methyl 13-methylpentadecanoate	270	270.50	0.98			x		
20	Methyl 2-methylhexadecanoate	284	284.50	0.98			x		
21	Methyl 2-oxohexadecanoate	284	284.40	0.99			x		
22	Methyl linoleate	294	294.47	1.00				x	
23	Methyl 10-octadecenoate	296	296.50	0.99			x		
Phenols (7 compounds)									
24	Phenol	94	94.11	1.00				x	
25	<i>o</i> -Cathecol (1,2-benzenediol)	110	110.11	0.99				x	
26	1-(2-Hydroxy-5-methylphenyl)ethanone	150	150.07	0.91				x	
27	2,5-Dihydroxy- α -methylphenetyl alcohol	168	168.19	0.91					x
28	3,4,5-Trimethoxyphenol	184	184.19	1.00				x	
29	3-Octylphenol	206	206.32	0.98				x	
30	3-Pentadecylphenol	304	304.50	0.98				x	
Terpenes/sesquiterpenes (20 compounds)									
31	β -Elemene	204	204.35	1.00	x	x	x		
32	α -elemene	204	204.35	0.91			x		
33	<i>trans</i> -Caryophyllene	204	204.35	1.00	x				
34	α -Humulene	204	204.35	1.00	x				
35	α -Guaiene	204	204.35	0.95			x		
36	β -Chamigrene	204	204.35	0.96	x				x
37	γ -Selinene	204	204.35	0.95			x	x	
38	β -Selinene	204	204.35	0.99	x	x		x	
39	α -Selinene	204	204.35	0.96				x	x
40	3,7(11)-Selinadiene (naphthalene-1,2,3,4,4 α ,5,6,8 α -octahydro-4,8-dimethyl-2-(1-methylethenyl)-[2 <i>R</i> -(2 α ,4 α 8 β)])	204	204.35	0.96	x	x			
41	(+) Aromadendrene	204	204.35	1.00			x		
42	(+) Nootkatone	218	218.33	1.00			x		

Table 1: Continued

No	Compound	M ⁺	Calc. mass	Match*	Type of extract				
					A	B	C	D	E
43	Spathulenol	220	220.35	0.93			x		
44	Ledol	222	222.37	0.99			x		
45	Hinesol	222	222.37	1.00			x		
46	Bulnesol	222	222.37	0.98	x		x		
47	α -Eudesmol	222	222.37	0.94		x			
48	β -Eudesmol	222	222.37	1.00				x	x
49	Guaiol	222	222.37	1.00		x			
50	Juniper camphor	222	222.37	0.99		x			
Steroids (9 compounds)									
51	9-Methyl-(3 β ,5 α)-androstan-3-ol	290	290.50	0.96				x	
52	3 β -Campesterol	400	400.68	0.98			x		
53	3 β ,5 α -4,4-Dimethylcholesta-8,14-dien-3-ol acetate	412	412.70	0.93			x		
54	Stigmast-4-en-3-one	412	412.70	0.99			x		
55	γ -Sitoesterol	414	414.70	0.98				x	
56	β -Amyrin (olean-12-en-3 β -ol)	426	426.72	1.00			x		
57	D:C-Friedoolean-3-one (multifluorenone)	426	426.72	0.91			x		
58	24-Methylencycloartanol	440	440.70	0.92			x		
59	3 β -Cycloartane-3,25-diol	444	444.70	0.93			x		
Nitrogen compounds (4 compounds)									
60	N-Phenyl-1-naphthalenamine	219	219.28	0.91			x		
61	N-phenyl-2-naphthalenamine	219	219.28	0.91					x
62	Heptadecanenitrile	251	251.45	1.00		x			
63	9-Octadecenamide	281	281.50	0.99				x	
Sulphur compound (1 compound)									
64	3-Methyldibenzothiophene	198	198.29	1.00			x		

spectra with values of 0.99, 0.96, and 0.98, respectively, and had identical molecular ions as compared to published mass spectra data of pure compounds. Other identified minor components in extract D by comparison with authentic standards were 6-methyl-3-heptanol (peak 1), phenol (peak 2), and palmitic acid (peak 16). Another 11 compounds were identified with matched library spectra values between 0.91 and 0.99 and had identical molecular ions as compared to published mass spectra data of pure compounds (see Table 1).

The major component on extract E (polar fraction, Figure 1e) was peak 8, identified as an oxygenated sesquiterpenoid (C₁₅H₂₆O), probably a naphthalenemethanol derivative, but fragmentation pattern was not conclusive. Hexanoic acid (peak 1) and β -eudesmol (peak 6) were identified by comparison of their chromatographic behavior and identical mass spectra of pure standards. β -chamigrene (peak 3), α -selinene (peak 5), 2,5-dihydroxy- α -methylphenetyl alcohol (peak 7), and N-phenyl-2-naphthalenamine (peak 9) were identified with matched library spectra values of 0.96, 0.96, 0.91, and 0.92, respectively, and had identical molecular ions as compared to

published mass spectra data of pure compounds. Summarizing, 64 compounds from 96 detected chromatographic peaks (67%) could be identified either by comparison with pure authentic standards (23 compounds, 24%) or by library mass spectra matches between 0.91 and 0.99 (41 compounds, 43%). Results are shown in Table 1. Relevant chemical structures of phenols (7 compounds), terpenoids (20 compounds), and steroids (9 compounds), regarding their possible biological significance, are shown in Figures 2–4, respectively.

4 Discussion

Volatile compounds in fruits and vegetables usually have been analyzed in terms of their contribution to aroma [8] and flavor [9] properties of fresh or processed products, as part of their contribution to organoleptic properties in terms of product acceptance by the consumer. Fresh or processed fruits, fruit juices, fruit residues after industrial processing (peel and seeds), flowers, pollen, and roots

Figure 2: Chemical structures of identified phenols: (1) phenol, (2) 3o-catechol (1,2-benzenediol), (3) 1-(2-hydroxy-5-methylphenyl) ethanone, (4) 2,5-dihydroxy- α -methyl-phenetyl alcohol, (5) 3,4,5-trimethoxyphenol, (6) 3-octylphenol, (7) 3-pentadecyl-phenol.

and leaves (fresh or dried) are the most frequently studied parts for the determination of volatiles [10]. Volatile essential oil components from stem, or SB, have been studied in order to determine possible insect attractants or pheromones [11], insect repellents [12], and compounds with certain pharmacological activities [13].

The discoveries of quinine in the *Chinchona* bark in the XVII century, which subsequently led to chloroquine and hydroxychloroquine for malaria treatment, and of salicylic acid in the willow SB extract (*Salix alba*), the precursor of the worldwide known aspirin (acetylsalicylic acid) in the XIX century, are examples of nonvolatile

blockbuster bioactive components that gave a sound contribution and discovery of new drugs from natural products chemistry. The possible contributions of volatiles from, i.e., willow SB extracts [14], different parts of pomegranate including SB [15], and cinnamon bark extract [16], as bioactive components have been studied. Our interest was to determine the presence of possible bioactive volatile components in the MSBE, which may contribute to its antioxidant, anti-inflammatory, and analgesic effects through a possible synergy mechanism [17]. The antioxidant, anti-inflammatory, analgesic, and immune-regulatory effects of the MSBE have been tested both *in vitro* and *in vivo* [1]. The concentrations at which MSBE exhibited its antioxidant effect were extremely low, no prooxidant effect were observed, and protection to oxidative damage was highly significant [18–22].

Haden mango SB extract components were mainly of nonpolar nature, and compounds found in higher relative amounts were sesquiterpene hydrocarbons as β -elemene, β -selinene, α -guaiene, and aromandendrene, in that order. A report has indicated the high anti-proliferative activity of β -elemene on glioma cells, and it was also an inducer of apoptosis in these cell lines [23]. It has shown to inhibit atherosclerotic lesions by reducing vascular oxidative stress and maintaining the endothelial function by improving plasma nitrite levels [24]. Several studies have shown that β -elemene may be a mediator in cancer prevention through an autoimmune mechanism [25]. Therefore, the possible contribution of β -elemene to the MSBE health effects is considerably high according to these previous reports.

Aromandendrene has been reported as a component of *Eucaliptus* sp. essential oils, and has shown to have synergistic properties with 1,8-cineole against antibiotic-resistant pathogens [26], but no other biological effect has been reported. β -selinene and α -guaiene have been found in several essential oils [27], and therefore, have no distinctive contributions to MSBE pharmacological effects. β -selinene-rich essential oils (between 37 and 57%) have shown to have strong reducing power as compared to gallic acid and catechin; good ability to chelate iron II; to moderate free radical scavenging activity; and have acceptable anti-inflammatory and antipyretic activities [28]. The presence of several terpenes from the eudesmane family, like β -selinene, in the MSBE volatiles composition, highly related to the antioxidant activity, must be considered in further studies in the attempt to correlate this health effect with eudesmane-type terpenes.

Major polar components on MSBE extracts were juniper camphor, hinesol, β -eudesmol, 9-methyl-(3 β ,5 α)-androstan-3-ol, γ -sitosterol, and 2,5-dihydroxy-methylphenetylalcohol.

Figure 3: Chemical structures of identified terpenoid compounds: (1) β -elemene, (2) α -elemene, (3) *trans*-(–)-caryophyllene, (4) α -humulene, (5) α -guaiene, (6) β -chamigrene, (7) γ -selinene, (8) β -selinene, (9) α -selinene, (10) 3,7(11)-selinadiene, (11) (+) aromadendrene, (12) (+) nootkatone, (13) spathulenol, (14) ledol, (15) hinesol, (16) bulnesol, (17) α -eudesmol, (18) β -eudesmol, (19) guaiaol, (20) juniper camphor.

Figure 4: Chemical structures of identified steroid compounds: (1) 9-methyl-(3 β ,5 α)-androstane-3-ol, (2) 3 β -campesterol, (3) 3 β ,5 α -4,4-dimethylcholesta-8,14-dien-3-ol acetate, (4) stigmast-4-en-3-one, (5) γ -sitosterol, (6) β -amyrin (olean-12-en-3 β -ol), (7) D:C-friedoolean-3-one (multifluorenone), (8) 24-methylencycloartanol, (9) 3 β -cycloartane-3,25-diol.

Although these oxygenated compounds were determined in relatively low amounts, as compared to sesquiterpene hydrocarbons, they have higher water solubility, and therefore, higher bioavailability in terms of their possible contribution to MSBE pharmacological effects. These components are commonly found in essential oils from several plants with antioxidant [28] or antimicrobial [29] effects. Hinesol has shown to inhibit H^+ , K^+ -ATPase [30], and this result may explain the observed benefits of MSBE on gastric disorders. It also enhanced the inhibitory effect of omeprazole on the hydrogen pump. On the other hand, β -eudesmol stimulates an increase in appetite through Transient Receptor Potential Ankyrin (TRPA1), and therefore body weight gain [31]. Again, the presence of eudesmane-type sesquiterpenic alcohols adds a new insight into their possible contribution to the MSBE antioxidant effect.

We conducted the analysis on an industrial MSBE (extract B), which has been used as an antioxidant bioactive ingredient on nutritional supplement formulations (tablets and capsules), and a cosmeceutical cream [7], in order to determine differences on volatiles composition as compared to fresh mango SB (extract A). Both extracts had similar content of β -elemene, β -selinene, and a naphthalene derivative (eudesmane type), but it could not be determined that juniper camphor was present in extract B, which was not present in extract A. Therefore, it might be assumed that these three nonpolar components would contribute to synergize the antioxidant and anti-inflammatory effects of other less volatile components of MSBE bioactive ingredients, like polyphenols and flavonoids, in commercial formulations. Juniper camphor (eudesmane family) is a common available component from plant extracts [32], but its possible contribution to biological effects of essential oils or plant extracts is not clear [33]. It has been found as the main component of *Pulicaris* sp. essential oils, which have shown high antioxidant activity [34], but reports about biological effects as pure compound are not available.

Studies about mango volatiles have shown that their composition may differ significantly among varieties [35]. The main research focus on mango volatiles has been to identify the major contributing compounds to fruit aroma and flavor [36,37]. The influence of germplasm on volatiles composition of mango fruit cultivated in seven countries has been studied [38]. A study on the volatile composition of the gum-resin exudated by the bark trunk of mango (non-specified variety) showed the presence of selinenes (α - and β -) as the major components, followed by β -caryophyllene, β -elemene, and β -chamigrene [39]. Our findings are consistent with these results in terms

of sesquiterpene hydrocarbons, but oxygenated components were not found in gum-resin, probably due to the sampling technique (headspace). Nevertheless, reports about composition on the MSBE for any variety, and its possible health effects, are only few.

We reported previously 14 components, mainly non-volatile polyphenols, sugars, and polyols, form an industrial MSBE [4] by HPLC, MS, and NMR techniques, and eight main minerals: Na, K, Ca, Mg, Mn, Cu, Zn, and Se by ICP-MS [40]. We report now a list of 64 volatile components in the MSBE, identified by GC-MS, which will give a sound basis in the attempts to correlate observed MSBE pharmacological effects to its chemical composition and their possible synergy effects with nonvolatile components.

5 Conclusions

The analysis of MSBE, Haden variety, by GC-MS is reported for the first time, which gives an approach in order to understand the possible synergistic effect of volatile compounds on its antioxidant, analgesic, and anti-inflammatory effects. The identification of relevant bioactive components in MSBE extracts, mainly terpenes and sesquiterpenes from the eudesmane family, will contribute to correlate their chemical composition to previous reported pharmacological effects.

Acknowledgments: Thanks to Dr. John Caccavale for English grammar revision.

Funding information: The financial support from the National Fund of Science and Technology (FONDOCYT), Ministry of Higher Education, Science and Technology (MESCYT), Dominican Republic, and the National Evangelic University (UNEV) through Project 2015-2A3-062 is gratefully acknowledged.

Conflict of interest: AJNS and JAA hold a patent about compositions containing mango extracts. LNP declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors contributions: All authors have participated in the work and have reviewed and agreed with the content of the article as follows: conceptualization, original draft preparation, supervision, and funding acquisition, AJNS;

investigation, data curation, and manuscript reviewing, JAA and LNP.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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