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The Chemical Composition of Leaf Essential Oils of Psidium guajava L. (White and Pink fruit forms) from South Africa

Raju K. Chalannavar[a], Katharigatta N. Venugopala[a], Himansu Baijnath[a] & Bharti Odhav[a]
[a] Department of Biotechnology and Food Technology, Durban University of Technology, Steve Biko Campus, Durban - 4001, South Africa

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Raju K. Chalannavar, Katharigatta N. Venugopala, Himansu Baijnath and Bharti Odhav*
Department of Biotechnology and Food Technology, Durban University of Technology, Steve Biko Campus, Durban - 4001, South Africa

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Abstract: The leaf oils of *Psidium guajava* (white fruit) and *Psidium guajava* (pink fruit) collected in KwaZulu-Natal province of South Africa has been examined by Gas chromatography-Mass spectrometry (GC-MS), and the apparent concentrations were determined by gas chromatography with a flame ionization detector. A total of twenty compounds of 88.9 % from white fruit and forty eight compounds representing 97.5 % from pink fruit of the oils were identified. *P. guajava* (white fruit) produced oil that was much richer in hydrocarbons (38.8 %), sesquiterpenes hydrocarbons (24.0 %), oxygenated sesquiterpenes (19.1 %) and alcohol (6.8 %). The major constituents of the essential oil were caryophyllene oxide (14.0 %), caryophyllene (13.9 %), 1H-cycloprop[e]azulene (11.6 %), adamantane (9.4 %), 3,7,11-trimethyl-1,6,10-dodecatrien-3-ol (6.8 %), α-cubebene (6.7 %), 1,2,3,4-tetrahydronaphthalene (3.9 %), β-humulene (3.5 %), 1,2,4a,5,6,8a-hexahydronaphthalene (3.2 %) and α-caryophyllene (3.0 %). The leaf oil of *P. guajava* (pink fruit) contained a mixture of hydrocarbons (30.5 %), sesquiterpene hydrocarbons (25.4 %), alcohol (24.4 %) and oxygenated sesquiterpenes (15.0 %). The major constituents of the essential oil were caryophyllene oxide (13.0 %), tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol (12.9 %), caryophyllene (9.5 %), 3,7,11-trimethyl-1,6,10-dodecatrien-3-ol (9.5 %), 1*H*-cycloprop[e]azulene (8.1 %), *Z*-3-hexadecen-7-yne (4.6 %) and eudesma-4(14),11-diene (4.1 %). High concentration of caryophyllene oxide and caryophyllene in both the oils suggests its usefulness as natural preservatives in the food industry. The terpenic and ester compounds could contribute to the unique flavor of *P. guajava* leaves.

Key words: *P. guajava* (white & pink fruit), essential oil, caryophyllene oxide.

Introduction

Psidium guajava L. (family Myrtaceae), commonly called guava, is considered a native to Mexico [1] extends throughout South America, Africa and Asia. Based on archaeological evidence, it has been used widely and known in Peru since pre-Columbian times. It grows in all the tropical and subtropical areas of the world, adapts to different climatic conditions but prefers dry climates [2]. More recent ethnopharmacological studies show that *P. guajava* is used in many parts of the world for the treatment of a number of diseases; in Mexico it is widely used to treat gastrointestinal and respiratory disturbances and is used as an anti-inflammatory medicine [3]. Leaves are applied on wounds, ulcers and for rheumatic pain, while they are chewed to relieve toothache [4]. A decoction of the new shoots is taken as a febrifuge. A combined decoction of leaves and bark is given to expel the placenta after childbirth [5]. A water leaf extract is used to reduce blood glucose level in diabetics. This hot tea was very common among the local people of Veracruz [3].

The tea made from the leaves is well known,

*Corresponding author (Bharti Odhav)
E-mail: < odhavb@dut.ac.za >

being used for cramps and diarrhoea; many studies have been performed in this respect [6]. The leaves were observed to contain amino acids, triterpenes, steroids, acids, phenols, saponins and carotenes. Volatile acids [(*E*)-cinnamic acid and (*Z*)-3-hexenoic acid], fatty acids, and the essential oil was also encountered [7]. The extracts of the leaves from *P. guajava* present numerous antimicrobial activities against fungi such as *Candida albicans* and bacteria such as *Staphylococcus aureus, Salmonella enteritidis,* and *Bacillus cereus*. They also possess antioxidant activity as a result of the presence of vitamins, carotenoids, polyphenols and, principally, ascorbic acid [8]. The essential oil from the guava leaves have been found to possess many compounds, 1,8-cineole and *trans*-caryophyllene being the most frequently encountered [9]. Pharmacological studies reported important anti-proliferation, anti-oxidant and antimicrobial activities [10].

Herbal medicines are an important part of the culture and traditions of African people. Today, most of the populations in urban South Africa, traditional healers, as well as smaller rural communities are reliant on herbal medicines for their health care needs. The leaf of *P. guajava* is used traditionally in South African folk medicine to manage, control, and/or treat a plethora of human ailments, including diabetes mellitus and hypertension [11]. It is also a remedy for diarrhoea, ulcers, boils, and wounds [12].

Apart from their cultural significance, this is because herbal medicines are more accessible and affordable [13]. As a consequence, there is an increasing trend, worldwide, to integrate traditional medicine with primary health care. Renewed interest in traditional pharma-copoeias has meant that researchers are concerned not only with determining the scientific rationale for the plant's usage, also with the discovery of novel compounds of the pharmaceutical value. Instead of relying on trial and error, as in random screening procedures, traditional knowledge helps scientists to target plants that may be medicinally useful [14].

The use of essential oils as functional ingredients in foods, drinks, toiletries and cosmetics is gaining momentum, both for the growing interest of consumers in ingredients from natural sources and also because of increasing concern about potentially harmful synthetic additives [15]. Within the wide range of the above-mentioned products, a common need is availability of natural extracts with a pleasant taste or smell combined with a preservative action, aimed to avoid lipid deterioration, oxidation and spoilage by microorganisms. Those undesired phenomena are not an exclusive concern of the food industry but a common risk wherever a lipid or perishable organic substrate is present. In fact, they induce the development of undesirable off-flavors', create toxicity and severely affect the shelf-life of many goods [16].

Until recently, essential oils have been studied most from the viewpoint of their flavor and fragrance chemistry only for flavoring foods, drinks and other goods. However, essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use [17]. Many authors, in fact, have reported antimicrobial, antifungal, antioxidant and radical-scavenging properties [16b].

The chemical composition of the essential oils can vary widely in different regions, principally because of environmental factors, as well as genetic factors that can induce modifications in the secondary metabolism of the plant [18]. In continuation of our ongoing research on extraction and characterization of essential oil constituents of natural plants [19], the present study sought to identify and compare the contents of the essential oils from the leaves of *P. guajava* (white and pink fruit). Hopefully, this will lead to new information on this plant application and new perspective on the potential use of guava essential oils in South Africa.

Materials and methods

Plant material

Leaves of *P. guajava* (white and pink fruit) were collected in October 2010 in the KwaZulu-Natal province of South Africa. The species was identified by Prof. Baijnath and a voucher specimen has been deposited in the Ward Herbarium at University of KwaZulu-Natal, Westville Campus, Durban, South Africa. KwaZulu-Natal (Durban) lies at an altitude of ~40 m at latitude (29°48'S) and at longitude (30°56'E). *P. guajava* (white variety) collected from Durban, Reservoir Hills (Baijnath *s.n.,* October 16th 2010) and *P. guajava* (pink variety) collected from Durban, Chatsworth (Baijnath *s.n.,* October 24th 2010).

Extraction of the essential oil

The essential oil from dried leaves of *P. guajava* (white and pink fruit) was extracted using a modification of an established procedure [20]. 100 g of milled leaves were hydrodistilled in a Clevenger apparatus. After 5 h of distillation, the essential oil was removed from the water surface. The oil was dried over anhydrous sodium sulphate and filtered. The solvent from the filtrate was removed by distillation under reduced pressure in a rotary evaporator at 35°C and the pure oil samples were sealed and kept in an amber colored bottle at 4°C in the refrigerator. The resulting pale yellow oil (40 μL) was dissolved in 1 mL of methyl ethyl ketone before the injection. 1 μL of this solution was directly used for GC-MS analysis.

Gas chromatography-flame ionization detector (GC-FID)

Capillary gas chromatography was performed using an Agilent system consisting of a model 6820 gas chromatograph (Agilent, USA), using a fused silica capillary column DB-5, 30 m x 0.35 mm, 0.1 μm film thickness (J & W Scientific, USA). The temperature program was set from 80-280°C in 1-20 min at 15°C/min. The injection temp-erature was 250°C and the injection volume was 1.0 μL. The inlet pressure was 100 kPa. Nitrogen was used as a carrier gas. Sampling rate was 2 Hz (0.01 min) and flow ionization detector temperature was set at 280°C.

Gas chromatography-mass spectrometry (GC-MS)

The GC-MS analysis of the essential oil was performed on an Agilent GC 6890 model gas chromatograph-5973N model mass spectrometer equipped with a 7683 series auto-injector (Agilent, USA). A DB-5MS column (30 m x 0.25 mm x 0.25 μm film thickness) was used. Temperature program was set from 80-280°C in 1-20 min. Injection volume was 1 μL and inlet pressure was 38.5 kPa. Helium was used as carrier gas. Linear velocity (u) was 31 cm/sec. Injection mode was split (75:5). MS interface temperature was 230°C. MS mode was EI, detector voltage was 1.66 Kv, mass range was 10-700 u, scan speed was 2.86 scan/s and interval was 0.01 min (20 Hz).

The components were identified by comparing the mass spectra with MS library. The NIST 98 spectrometer data bank was used for identification of the chemical composition.

Results and discussion

The volatile oils of the dried leaves from *P. guajava* (white and pink fruit) were a light yellowish liquid with a strong aromatic fragrance, with yields of 0.92 and 0.66 % (v/w), respectively. A distribution of the different chemical groups to which the compounds belong is shown in Figure 1. GC-MS analyses of *P. guajava* oils resulted in the identification of twenty compounds of 88.9 % and forty-eight compounds of 97.5 % respectively (Table 1 and 2). Terpenes and their derivatives predominated, the major compounds of both (white and pink fruit) *P. guajava* essential oil were caryophyllene oxide, caryophyllene and 1*H*-cycloprop[e]azulene.

The major groups of *P. guajava* (white fruit) compounds were hydrocarbons (38.8 %), sesquiterpene hydrocarbons (24.0 %), oxygenated sesquiterpenes (19.1 %) and alcohol (6.8 %) as major groups. The most abundant components of hydrocarbons were 1*H*-cycloprop[e]azulene (11.6 %), adamantane (9.4 %), 1,2,3,4-tetrahydronaphthalene (3.9 %), β-humulene (3.5 %), 1,2,4a,5,6,8a-hexahydronaphthalene (3.2 %), 1*H*-cyclopropa[a]naphthalene (2.2 %), 12-oxabicyclo [9.1.0]dodeca-3,7-diene (2.0 %) and 1,2,3,4,4a,5,6,8a-octahydronaphthalene (2.0 %). In case of sesquiterpene hydrocarbons, the major compounds were caryophyllene (13.9 %), α-cubebene (6.7 %) and α-caryophyllene (3.0 %). Among oxygenated sesquiterpenes the major groups were

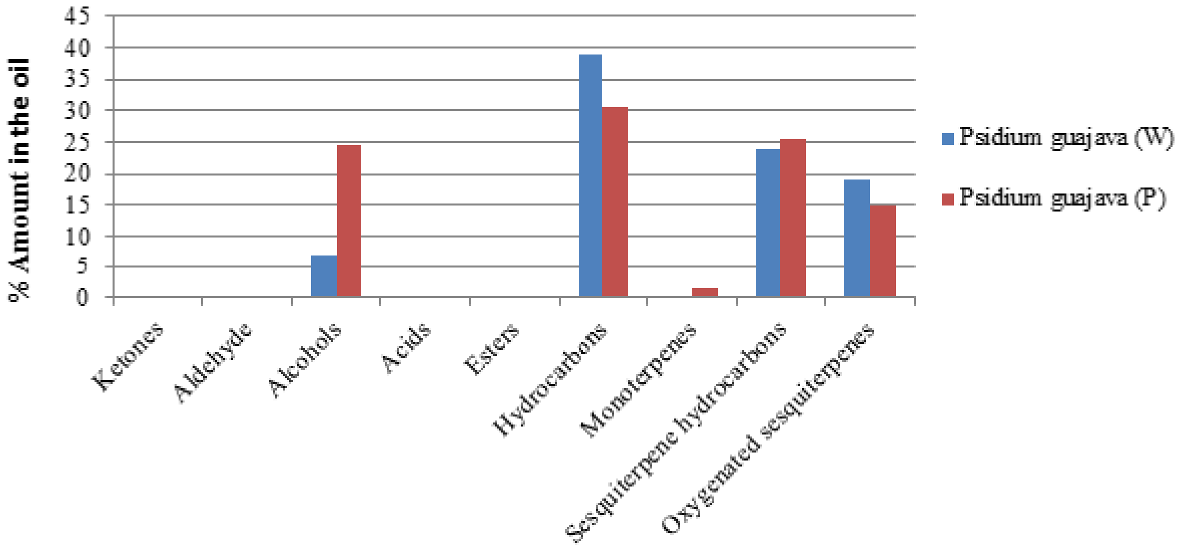

Figure 1. Essential oil constituents in *Psidium guajava* (white and pink fruit forms)

caryophyllene oxide (14.0 %), epiglobulol (2.4 %), and isoaromadendrene epoxide (2.1 %). In case of alcohols and monoterpenes major compounds were 3,7,11-trimethyl-1,6,10-dodecatrien-3-ol (6.8 %) and eucalyptol (0.08%).

The major groups of *P. guajava* (pink fruit) compounds were hydrocarbons (30.4 %), sesquiterpene hydrocarbons (25.4 %), alcohol (24.4 %), oxygenated sesquiterpenes (15.0 %) and monoterpenes (1.4 %). The major compounds of hydrocarbons were 1*H*-cycloprop[e]azulene (7.9 %), *Z*-3-hexadecen-7-yne (4.6 %), 1*H*-benzocycloheptene (4.0 %), 1*H*-cyclopropa[a]naphthalene (3.8 %), 1*H*-indene,1-ethylideneoctahydro-7a-methyl (2.7 %), 1,2,3,4,4a,5,6,8a-octahydronaphthalene (2.1 %), 12-oxabicyclo[9.1.0] dodeca-3,7-diene (2.0 %) and 1*H*-indene (1.2 %). In case of sesquiterpene hydrocarbons major compounds were caryophyllene (9.5 %), eudesma-4(14),11-diene (4.1 %), cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-(S)-(2.9 %), cyclohexene-4-(1,5-dimethyl-1,4-hexadienyl)-1-methyl-(2.5 %), α-caryophyllene (2.3 %), α-cubebene (1.4 %), copaene (1.2 %) and cycloisolongifolene (1.0 %). Among alcohols, tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol,4,4-dimethyl (12.9 %) and 3,7,11-trimethyl-1,6,10-dodecatrien-3-ol (9.5 %) were the major compounds. In case of oxygenated sesquiterpenes and monoterpenes the major compounds were caryophyllene oxide (13.0 %), epiglubulol (1.8 %) and eucalyptol (1.2 %).

Caryophyllene oxide (Figure 2) is the main component in both oils. Caryophyllene oxide, caryophyllene, adamantane, α-cubebene, α-caryophyllene, 1*H*-cycloprop[e]azulene, epiglobulol, 1*H*-cyclopropa[a]naphthalene, 3,7,11-trimethyl-1,6,10-dodecatrien-3-ol and 1,2,3,4,4a,5,6,8a-octahydronaphthalene were observed as the ten versatile common components present in both the oils with variations in percent content, the total percentage of compounds concentration of *P. guajava* (pink fruit) is higher than the *P. guajava* (white fruit) Table 1 and 2. The bulk of both leaf essential oils were made up of hydrocarbons, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and alcohols.

Caryophyllene oxide, an oxygenated terpenoid is one of the main constituents of the essential oil from guava leaves from various countries such as China (18.8 %), Cuba (21.6 %), Nigeria (21.3

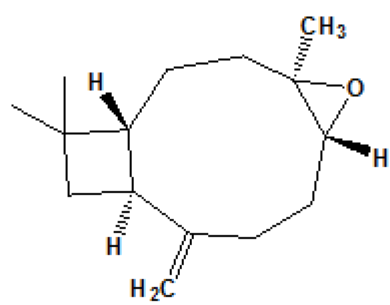

Figure 2. Chemical structure of caryophyllene oxide

Table 1. Chemical composition of *Psidium guajava* (white fruit)

No.	Constituents	Mol. Formula	Mol Weight	Rt (min)*	(%)
Alcohols					
1	3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	$C_{15}H_{26}O$	222	14.843	6.808
2	1-Naphthalenol	$C_{10}H_8O$	144	17.787	0.069
	Hydrocarbons				
3	1*H*-Cycloprop[e]azulene	$C_{11}H_8$	140	13.433	11.654
4	1,2,3,4,4a,5,6,8a-Octahydronaphthalene	$C_{10}H_{16}$	136	14.044	2.005
5	1,2,4a,5,6,8a-Hexahydronaphthalene	$C_{10}H_{14}$	134	14.103	3.246
6	Cyclohexene	C_6H_{10}	82	14.156	0.798
7	1,2,3,4-Tetrahydronaphthalene	$C_{10}H_{12}$	132	14.420	3.922
8	1*H*-Cyclopropa[a]naphthalene	$C_{11}H_8$	140	15.402	2.238
9	12-Oxabicyclo[9.1.0]dodeca-3,7-diene	$C_{11}H_{16}O$	164	15.572	2.018
10	β-Humulene	$C_{14}H_{22}$	190	15.707	3.502
11	Adamantane	$C_{10}H_{16}$	136	15.913	9.484
	Monoterpenes				
12	Eucalyptol	$C_{10}H_{18}O$	154	7.364	0.088
	Sesquiterpene hydrocarbons				
13	α-Cubebene	$C_{15}H_{24}$	204	12.534	6.708
14	(+)-Cyclosativene	$C_{15}H_{24}$	204	12.440	0.313
15	Caryophyllene	$C_{15}H_{24}$	204	13.204	13.923
16	α-Caryophyllene	$C_{15}H_{24}$	204	13.627	3.060
	Oxygenated sesquiterpenes				
17	Epiglobulol	$C_{15}H_{26}O$	222	14.996	2.463
18	Caryophyllene oxide	$C_{15}H_{24}O$	220	15.313	14.019
19	Isoaromadendrene epoxide	$C_{15}H_{24}O$	220	16.248	2.170
20	Ledene oxide-(II)	$C_{15}H_{24}O$	220	16.365	0.455
	Total				88.943

* Retention time

Table 2. Chemical composition of *Psidium guajava* (pink fruit)

No.	Constituents	Mol. Formula	Mol Weight	Rt (min)*	(%)
	Ketones				
1	+/-,4-Acetyl-1-methylcyclohexene	$C_9H_{14}O$	138	8.974	0.094
	Aldehyde				
2	2,6-Octadienal, 3,7-dimethyl-, (*Z*)-	$C_{10}H_{16}O$	152	16.853	0.103
	Alcohols				
3	3-Cyclohexene-1-methanol	$C_7H_{12}O$	112	9.955	0.318
4	8-Quinolinol, 7-methyl	$C_{10}H_9NO$	159	14.403	0.641
5	1,5,7-Octatrien-3-ol, 3,7-dimethyl-	$C_{10}H_{16}O$	152	14.450	0.182
6	3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	$C_{15}H_{26}O$	222	14.873	9.565

table 2. (continued).

No.	Constituents	Mol. Formula	Mol Weight	Rt (min)*	(%)
7	Tetracyclo[6.3.2.0(2,5).0(1,8)] tridecan-9-ol,4,4-dimethyl	$C_{13}H_{20}O$	192	15.942	12.975
8	2,6,10-Dodecatrien-1-ol	$C_{12}H_{20}O$	180	16.600	0.559
9	2,6,10,14-Hexadecatetraen-1-ol	$C_{16}H_{26}O$	234	17.058	0.169
	Acids				
10	6-Heptenoic acid	$C_7H_{12}O_2$	128	17.511	0.049
	Esters				
11	Butanoic acid, 3-hexenyl ester, (*Z*)-	$C_{10}H_{18}O_2$	170	9.732	0.125
12	(-)-trans-Pinocarvyl acetate	$C_{12}H_{18}O_2$	194	10.971	0.080
13	Benzyl Benzoate	$C_{14}H_{12}O_2$	212	17.340	0.126
	Hydrocarbons				
14	Benzene, 1-methyl-4-(1-methylethyl)	$C_{10}H_{14}$	134	7.234	0.033
15	Cyclopentane,1,2-dimethyl-3-methylene,*trans*	C_8H_{14}	110	8.750	0.038
16	3-Ethylidenecycloheptene	C_9H_{14}	122	11.212	0.096
17	Adamantane	$C_{10}H_{16}$	136	11.606	0.062
18	1,3-Cyclohexadiene	C_6H_8	80	12.047	0.099
20	4,7-Methanoazulene	$C_{11}H_8$	140	13.245	0.532
21					
22	1*H*-Cycloprop[e]azulene	$C_{11}H_8$	140	13.421	8.108
23	1,2,3,4,4a,5,6,8a-Octahydronaphthalene	$C_{10}H_{16}$	136	13.809	2.187
24	Bicyclo[7.2.0]undec-4-ene	$C_{11}H_{18}$	150	14.250	0.086
25	Cycloheptane	C_7H_{14}	98	14.497	0.151
26	1*H*-Cyclopropa[a]naphthalene	$C_{11}H_8$	140	15.413	3.897
27	1*H*-Indene	C_9H_8	116	15.519	1.283
28	12-Oxabicyclo[9.1.0]dodeca-3,7-diene	$C_{11}H_{16}O$	164	15.584	2.085
29	Bicyclo[4.1.0]heptane	C_7H_{12}	96	15.642	0.209
30	1*H*-Indene, 1-ethylideneoctahydro-7a-methyl	$C_{12}H_{20}$	164	15.707	2.784
31	*Z*-3-Hexadecen-7-yne	$C_{16}H_{28}$	220	16.265	4.621
32	1*H*-Benzocycloheptene	$C_{11}H_{10}$	142	16.353	4.002
33	1,2,3,4,5,6,7,8-Octahydroazulene	$C_{10}H_{16}$	136	16.806	0.215
	Monoterpenes				
34	D-Limonene	$C_{10}H_{16}$	136	7.299	0.089
35	Eucalyptol	$C_{10}H_{18}O$	154	7.364	1.235
36	(+)-4-Carene	$C_{10}H_{16}$	136	12.417	0.160
	Sesquiterpene hydrocarbons				
37	α-Cubebene	$C_{15}H_{24}$	204	12.505	1.447
38	Caryophyllene	$C_{15}H_{24}$	204	13.198	9.597
39	α-Caryophyllene	$C_{15}H_{24}$	204	13.621	2.364
40	Copaene	$C_{15}H_{24}$	204	14.309	1.285
41	Cyclohexene, 4-(1,5-dimethyl-1,4-hexadienyl) -1-methyl	$C_{15}H_{24}$	204	14.044	2.512
42	Cycloisolongifolene	$C_{15}H_{24}$	204	14.115	1.024
43	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-(S)	$C_{15}H_{24}$	204	14.173	2.969

table 2. (continued).

No.	Constituents	Mol. Formula	Mol Weight	Rt (min)*	(%)
44	Eudesma-4(14),11-diene	$C_{15}H_{24}$	204	16.101	4.167
45	2,6,10,14,18,22-Tetracosahexaene	$C_{24}H_{38}$	326	26.900	0.120
	Oxygenated Sesquiterpenes				
46	*cis*-*Z*-α-bisabolene epoxide	$C_{15}H_{24}O$	220	13.903	0.174
47	Epiglobulol	$C_{15}H_{26}O$	222	14.996	1.863
48	Caryophyllene oxide	$C_{15}H_{24}O$	220	15.325	13.045
	Total				97.525

* Retention time

%), Taiwan (27.7 %) [9a,b,21]. It is also found in *Psidium myrsinoides* as 19.7 % [22], *Psidium salutare* as 39.8 % [23], *Psidium striatulum* as 7.6 % [24] and *Psidium cattleianum* var. *lucidum* as 12.4 % [25]. This compound is well known as a preservative in food, drugs and cosmetics, has been tested *in vitro* as an antifungal agent against dermatophytes [26]. It also has antimicrobial [27], analgesic and anti-inflammatory activity [28] and shows anti caries activity in rats [29]. The caryophyllene oxide , which exists in many plant essential oils [30], has been approved by the FDA as a food and cosmetic preservative [31] and has been included by the European Council in the list of natural and synthetic flavoring substances. It appears to be tolerable, safe and toxic-free [32].

Conclusion

The volatile compounds of *P. guajava* L. (white and pink fruit) were identified by GC-MS. A total of twenty compounds, (88.9 %) of *P. guajava* (white fruit) and forty eight compounds representing (97.5 %) of the oils were identified in *P. guajava* (pink fruit). There was a greater variation in the common constituents of the essential oils of both from guava leaves of white fruit and pink fruit. The major constituents identified in the guava leaves of both essential oils were caryophyllene oxide, caryophyllene, tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 1*H*-cycloprop[e]azulene, adamantine, 3,7,11-trimethyl-1,6,10-dodecatrien-3-ol, α-cubebene, 1,2,3,4-tetrahydronaphthalene, β-humulene, 1,2,4a,5,6,8a-hexahydronaphthalene, *Z*-3-hexadecen-7-yne, 1*H*-benzocycloheptane, eudesma-4(14),11-diene, α-caryophyllene, and epiglobulol. The presence of major groups of hydrocarbons, sesquiterpene hydrocarbons and oxygenated sesquiterpenes in essential oils makes it potential useful in the medicines because they exhibit antibacterial [10b], antifungal activity [33], anticancer activity [34], anti-inflammatory activity [35], antimalarial activity [36] and are also used traditionally as flavoring agent and antimicrobials in food. The higher concentration of caryophyllene oxide in both essential oils has been thought to contribute to the unique flavor of the guava leaves.

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References

1. **Rios, C. D., Salazar, C. R., Cardona, C., Victoria, K., Torres, M., Guayaba, En. (1997).** Instituto Colombiano Agropecuario. Bogot´a (Colombia), second ed. Frutales. Manual de Asistencia T´ecnica. No 4: 221-248.
2. **Stone, B. (1970).** The flora of Guam. Micronesica. 6: 454-455.
3. **Aguilar, A., Argueta, A., Cano, L. (1940).** Flora Medicinal Indigena de Mexico. Treinta Y Cinco

Monografias del Atlas de las Plantas de la Medicina Tradicional Mexicana. INI, Mexico, 245.

4. **Heinrich, M., Ankli, A., Frei, B., Weimann, C., Sticher, O. (1998).** Medicinal plants in Mexico: healers consensus and cultural importance. Social Science Medicine. 47: 1859-1871.
5. **Mart´inez, G.M., Barajas, B. (1991).** Estudio etnobot´anico de las plantas medicinales en el mercado Libertad del ´area metropolitana de Guadalajara, Jal. Tesis Licenciatura. Facultad de Agronom´ia. Universidad de Guadalajara. 100.
6. **(a) Lutterodt, G.D. (1989).** Inhibition of gastrointestinal release of acetylcholine by quercetin as a possible mode of action of *Psidium guajava* leaf extracts in the treatment of acutediarrhoeal disease. Journal of Ethnopharmacol. 25: 235-247.

(b) **Almeida, C.E., Karnikowski, M.G., Foleto, R., Baldisserotto, B. (1995).** Analysis of anti-diarrhoeic effect of plants used in popular medicine. Revista de Saude Publica. 29, 428-33;

(c) **Lozoya, X., Reyes-Morales, H., Chávez-Soto, M. A., Martínez-García, M.C., Soto-González, Y., Doubova, S.V. (2002).** Intestinal anti-spasmodic effect of a phytodrug of *Psidium guajava* folia in the treatment of acute diarrheic disease. Journal of Ethnopharmacology. 83: 19-24.

7. (a) **Opute, F.I. (1978).** The component fatty acids of *Psidium guajava* seed fats. Journal of the Science of Food and Agriculture. 29: 737-738.

(b) **Cuellar, A.C., Lara, R.A., Zayas, J.P. (1984).** *Psidium guajava* L. Tamizaje fitoquímico y estudio del aceite esencial. Rev Cubana Farm. 18: 92-9.

(c) **Idstein, H., Bauer, C., Schreier, P. (1985).** Volatile acids in tropical fruits: cherimoya (*Annona cherimolia* Mill.), guava (*Psidium guajava* L.), mango (*Mangifera indica* L., var. Alphonso), papaya (*Carica papaya* L.). Zeitschrift für Lebensmittel-Untersuchung und -Forschung. 180: 394-397.

(d) **Mercadante, A.Z., Steck, A., Pfander, H. (1999).** Carotenoids from guava (*Psidium guajava* L.): isolation and structure elucidation. Journal of Agricultural Food Chemistry. 47: 145-151.

8. (a) **Nogueira, J.N., Soybihe, S.J., Vencosvsk, Y.R., Fonseca, H. (1978).** Effect of storage on the levels of ascorbic acid and beta-carotene in freeze dried red guava (*Psidium guajava* L.). Archivos Latinoamericanos de Nutricion. 28: 363-377.

(b) **Nascimento, G.G.F., Locatelli, J., Freitas, P.C., Silva, G.L. (2000).** Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bactéria. Brazillian Journla of Microbiology. 31: 247-256.

(c) **Qian, H., Nihorimbere, V. (2004).** Antioxidant power of phytochemicals from *Psidium guajava* leaf. Journal of Zhejiang Univversity-Science. 5: 676-683.

9. (a) **Li, J., Chen, F., Luo, J. (1999).** GC-MS analysis of essential oil from the leaves of *Psidium guajava*. Zhong Yao Cai. 22: 78-80.

(b) **Chen, H.C., Sheu, M.J., Lin, L.Y., Wu, C.M. (2007).** Chemical composition of the leaf essential oil of *Psidium guajava* L. from Taiwan. Journal of Essential Oil Research. 19: 345-347.

(c) **Cole, R.A., Setzer, W.N. (2007).** Chemical composition of the leaf essential oil of *Psidium guajava* from Monteverde, Costa Rica. Journal of Essential Oil Bearing Plants. 10: 365-373.

10. (a) **Manosroi, J., Dhumtanom, P., Manosroi, A. (2006).** Anti-proliferative activity of essential oil extracted from Thai medicinal plants on KB and P388 cell lines. Cancer Letters. 235: 114-120.

(b) **Sacchetti, G., Maietti, S., Muzzoli, M.V., Scaglianti, M., Manfredini, S., Radice, M., Bruni, R. (2005).** Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food and Chemical Toxicology. 91: 621-632.

11. (a) **Ojewole, J.A. (2005).** Hypoglycemic and hypotensive effects of *Psidium guajava* Linn. (Myrtaceae) leaf aqueous extract. Methods and Findings in Experimental and Clinical Pharmacology. 27: 689-695.

(b) **Oh, W.K., Lee, C.H., Lee, M.S., Bae, E.Y., Sohn, C.B., Oh, H., Kim, B.Y., Ahn, J.S. (2005).** Antidiabetic effects of extracts from *Psidium guajava*. Journal of Ethnopharmacology. 96: 411-

415.

12. **Wyk, B.E., Oudtshoorn, B., Gericke, N. (1997).** Medicinal Plants of South Africa. Briza Publications, Pretoria, South Africa. First edition. ISBN No. 1-875093-09-5.
13. **Mander, M. (1998).** A case study in KwaZulu-Natal. FAO, Rome. Marketing of indigenous medicinal plants in South Africa.
14. **Cox, P.A., Balick, M.J. (1994).** The ethnobotanical approach to drug discovery. Scientific American. 270: 60-65.
15. **Reische, D.W., Lillard, D.A., Eitenmiller, R.R. (1998).** Antioxidants in food lipids. In C.C. Ahoh & D.B. Min (Eds.), Chemistry, nutrition and biotechnology, New York: Marcel Dekker. 423-448.
16. (a) **Farag, R.S., Ali, M.N., Taha, S.H. (1990).** Use of some essential oils as natural preservatives for butter. Journal of the American Oil Chemists Society. 67: 188-191.

(b) **Hirasa, K., Takemasa, M. (1998).** Spice science and technology. New York: Dekker Inc.

17. (a) **Ormancey, X., Sisalli, S., Coutiere, P. (2001).** Formulation of essential oils in functional perfumery. Parfums, Cosmetiques, Actualites. 157: 30-40.

(b) **Sawamura, M. (2000).** Aroma and functional properties of Japanese yuzu (*Citrus junos* Tanaka) essential oil. Aroma Research. 1(1): 14-19.

18. **Taiz, L., Zeiger, E. (1991).** Surface protection and secondary defense compound. In: Plant Physiology. California: Benjamin/Cumming. 318-345.
19. **Chalannavar, R.K., Baijnath, H., Odhav, B. (2011).** Chemical constituents of the essential oil from *Syzygium cordatum* (Myrtaceae). African Journal of Biotechnology. 10(14): 2741-2745.
20. **Denny, E.F.K. (1989).** Hydrodistillation of oils from aromatic herbs. Perfumer & Flavorist. 14(4): 57-63.
21. (a) **Pino, J. A., Aguero, J., Marbot, R., Fuentes, V. (2001).** Leaf oil of *Psidium guajava* L. from Cuba. Journal of Essential Oil Research. 13(1): 61-62.

(b) **Ogunwande, I.A., Olawore, N.O., Adeleke, K.A., Ekundayo, O., Koenig, W.A. (2003).** Chemical composition of the leaf volatile oil of *Psidium guajava* L. growing in Nigeria. Flavour and Fragrance Journal. 18(2): 136-138.

22. **Freitas, M.O., De Morais, S.M., Silveira, E.R. (2002).** Volatile constituents of *Psidium myrsinoides* O. Berg. Journal of Essential Oil Research. 14(5): 364-365.
23. **Pino, J., Bello, A., Urquiola, A., Aguero, J., Marbot, R. (2003).** Leaf oils of *Psidium cymosum* Urb. and *Psidium sartorianum* Niedz. from Cuba. Journal of Essential Oil Research. 15: 187-188.
24. **da Silva, J.D., Luz, A.I.R., da Silva, M.H.L., Andrade, E.H.A., Zoghibi, M.G.B. (2003).** Essential oils of the leaves and stems of four *Psidium* spp. Flavour and Fragrance Journal. 18(3): 240-243.
25. **Chalannavar, R.K., Venugopala, K.N., Baijnath, H., Odhav, B. (2012).** Chemical composition of essential oil of *Psidium cattleianum* var. *lucidum* (Myrtaceae). African Journal of Biotechnology. 11(33): 8341-8347.
26. **Yang, D., Michel, L., Chaumont, J.P., Clerc, J.M. (2000).** Use of caryophyllene oxide as an antifungal agent in an *in vitro* experimental model of onychomycosis. Mycopathologia. 148(2): 79-82.
27. (a) **de Souza, G.C., Haas, A.P., von Poser, G.L., Schapoval, E.E., Elisabetsky, E. (2004).** Ethnopharmacological studies of antimicrobial remedies in the south of Brazil. Ethnopharmacology. 90: 135-143.

(b) **Brighenti, F.L., Luppens, S.B., Delbem, A.C., Deng, D.M., Hoogenkamp, M.A., Gaetti-Jardim, E., Dekker, H.L., Crielaard, W., Ten, C.J.M. (2008).** Effect of *Psidium cattleianum* leaf extract on *Streptococcus mutans* viability, protein expression and acid production. Caries Research. 42: 148-154.

28. **Chavan, M.J., Wakte, P.S., Shinde, D.B. (2010).** Analgesic and anti-inflammtory activity of caryophyllene oxide from *Annona squamosa* L. bark. Phytomedicine. 17(2): 149-151.

29. **de Menezes, T.E.C., Delbem, A.C.B., Brighenti, F.L., Okamoto, A.C., Gaetti-Jardim Jr. E. (2010).** Protective efficacy of *Psidium cattleianum* and *Myracrodruon urundeuva* aqueous extracts against caries development in rats. Pharmaceutical Biology. 48: 300-305.

30. (a) **Connoly, J.D., Hill, R.A. (1991).** Di and higher terpenoids. Chapman and Hall, London. UK II: 655-1460.

(b) **Connoly, J.D., Hill, R.A. (1991).** Dictionary of terpenoids: Mono and sesquiterpenoids. Chapman and Hall. London. UK I: 1-654.

31. **Food Drug Administration. Washington, D.C., USA. (1973).** Rules and Regulations : title 21- Food and drugs, food additives, synthetic flavoring substances and adjuvants. Federal Register. 95(38): 12913-12914.

32. **Opdycke, D.L.J., Letizia, C. (1983).** Monographs on fragrance raw materials, Caryophyllene oxide. Food and Chemical Toxicology. 21(5): 661-662.

33. **Khan, M.R., Kihara, M., Omoloso, A.D. (2000).** Antimicrobial activity of *Evodia elleryana*. Fitoterapia. 71: 72-74.

34. **Angelis, L.D. (2001).** Brain tumors. The New England Journal of Medicine 344: 114-123.

35. **Olajide, O.A., Awe, S.O., Makinde, J.M. (1999).** Pharmacological studies on the leaf of *Psidium guajava*. Fitoterapia. 70: 25-31.

36. (a) **Lahlou, M., Berrada, R., Hmamouchi, M., Lyagoubi, M. (2001b).** Effect of some Moroccan medicinal plants on mosquito larvae. Therapie. 56: 193-196.

(b) **Lahlou, M., Berrada, R., Hmamouchi, M. (2001a).** Molluscicidal activity of thirty essential oils on *Bulinus truncatus.* Therapie. 56: 71-72.