academicJournals

Vol. 7(13), pp. 783-789, 3 April, 2013
DOI: 10.5897/JMPR12.929
ISSN 1996-0875 ©2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Chemical constituents of the essential oil from leaves of *Psidium cattleianum* var. *cattleianum*

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Accepted 21 December, 2012

The essential oil obtained by hydrodistillation of dried leaves of *Psidium cattleianum* var. *cattleianum* was analysed by gas chromatography (GC) and combined gas chromatography-mass spectrometry (GC-MS). A total of seventy six compounds, representing 97.5% of the oil were identified. The major constituents of the essential oil were caryophyllene oxide (29.56%), alloaromadendrene oxide-(1) (6.82%), 12-oxabicyclo [9.1.0] dodeca-3,7-diene (5.85%) and 1*H*-cycloprop[e]azulene (3.49%). High concentration of caryophyllene oxide in the oil suggests its usefulness as natural preservative in food industry. The terpenic and ester compounds could contribute to the aroma of P. *cattleianum* var. *cattleianum*.

Key words: *Psidium cattleianum* var. *cattleianum*, essential oil, caryophyllene oxide.

INTRODUCTION

The strawberry guava, *Psidium cattleianum* Sabine var. *cattleianum* is a species native to Brazil (Fiaschi and Pirani, 2009; Souza et al., 2008). *P. cattleianum* var. *cattleianum* belongs to family Myrtaceae (myrtle family) which consists of at least 133 genera and more than 3800 species (Wilson et al., 2001). Many fruits of this family have a history of being used in traditional medicines in ethnobotanical practices in the tropical and subtropical regions (Marin et al., 2008). Members of the family include the *Eugenia*, *Myrcianthes*, *Campomanesia* and *Psidium* genera (Marin et al., 2008). *Psidium* has approximately 120 to 150 species and may be found throughout the tropics and subtropics of America and Australia (Pino et al., 2004). In Brazil, *P. cattleianum* is known by various popular names, including “arac¸´a, arac¸´a-rosa, arac¸´a-de-comer, and arac¸´a-da-praia”. Corrˆea (1926) cites *P. cattleianum* as a producer of yellow and red fruits. There is no consensus, however, among specialists about this species and a recent study showed differences in the structural organization of the stems of the plant (Rocha et al., 2008). Thus, two botanical varieties can be considered according to Popenoe (1920); *P. cattleianum* (also named as *P. cattleianum* var. *cattleianum*), which produces red fruits, and *P. cattleianum* var. *lucidum* Hort., producing yellow fruits. The fruit is 2 to 3 cm in diameter and contains numerous seeds, and its weight can exceed 20 g in some cases. The pulp is translucent, very juicy and has an excellent strawberry-like flavor with a spicy touch. It is rich in vitamin C with a content that is 3 to 4 times that of citrus fruits (Raseira and Raseira, 1994). It blooms from May to July and the fruit ripens between September and November in South Africa. Currently, the plant is cultivated in many countries, where it has easily adapted to a variety of climates. In tropical climates, it is often found growing at greater heights, where the mean temperature is not too cold. The yellow variety grows at slightly lower elevations. The constituents of some of the species have been studied with special focus being placed on the flavour components of the fruits. Antioxidant activities of

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some members have also been examined. The most popular *Psidium* species, *Psidium guajava*, simply called guava, has been extensively examined and even characterized anticancer potential and identified the parts of the fruit involved in its anti-neoplastic action (Bontempo et al., 2012). The round-oval fruit is green-yellow and shows a light yellow, pink (Shreier and Idstein, 1985), white or salmon pulp depending on the variety (Mercadante et al., 1999). World production of guavas is estimated at approximately 500,000 metric tonnes with Brazil, Colombia, Mexico and Venezuela producing significant quantities from the South American regions.

The industry provides a range of processed products such as beverages, syrup, ice cream, jams and jellies, to name a few (Jiménez-Escrig et al., 2001). The major producers of guava products are South Africa, India, Hawaii, Colombia, Puerto Rico, Jamaica, Brazil and Israel (Mercadante et al., 1999).

In Jamaica, *P. guajava* is commonly used in the manufacture of jams, jellies, ice cream and juices, among other products. *P. cattleianum* (strawberry guava) is often described as being more aromatic than *P. guajava* (common guava). This species is a shrub or tree which can grow 3 to 10 m high and is very common to Southern Brazil (Marin et al., 2008). Other names synonymous with strawberry guava include *Psidium littorale* Raddi or purple guava.

The fruit is widely cultivated in Central and South America and has been naturalized in Jamaica in the parishes of Clarendon, Manchester and St. Ann (Adams, 1972). In Hawaii, the plant is actually considered as a forest weed and is able to propagate quite easily from the seed. It has not been as extensively studied as the guajava species; however, the volatile constituents of the fruit have been quantified and characterized (Pino et al., 2001).

Despite the amount of attention being devoted to their production, few studies have been made concerning the chemical composition of strawberry guava fruits (Biegelmeyer et al., 2011). Galho et al. (2007) studied the composition of primary metabolites, whereas Miriam et al. (2006) suggest essential oils are important sources of antimicrobial and anti-inflammatory. Moreover, two other articles describe the chemical composition of the essential oil. One of these studies studied the species with red skin collected in Cuba (Pino et al., 2001) and the other one worked with both yellow (*P. cattleianum* var. *lucidum*) and red-skinned fruit (*P. cattleianum* var. *cattleianum*) from the Reunion Island (Vernin et al., 1998). In both studies, β-caryophyllene was found to be the major compound.

There are several publications on the genus *Psidium* (Chalannavar et al., 2012; Chen et al., 2007; Ogunwande et al., 2003; Pino et al., 2004; Adam et al., 2011; Marques et al., 2008; Limberger et al., 2001; Tucker et al., 1995), but so far no analysis of the volatile compounds in *P. cattleianum* var. *cattleianum* is available from Durban, KwaZulu-Natal Province of South Africa. An exploration of the natural products from South African guava leaves would shed light on these essentially unknown properties which could be potentially exploited to improve the country's economy via commercialization.

MATERIALS AND METHODS

Plant

Leaves of *P. cattleianum* var. *cattleianum* were collected in October, 2010 in the KwaZulu-Natal province of South Africa. The species was identified by co-author, Professor Himansu 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 on latitude (29°48'S) and longitude (30° 56'E).

Extraction of the essential oil

The essential oil from dried leaves of *P. cattleianum* var. *cattleianum* was extracted using a modification of an established procedure (Denny, 1989). 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 was 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 average percentage of the essential oils was light yellow with yields of 0.76% (v/w). The resulting pale yellow oil (40 µL) was dissolved in 1 mL of methyl ethyl ketone before the injection. One microliter of this solution was directly used for gas chromatography-mass spectrometry (GC-MS) analysis.

Gas chromatography-flame ionization detector (GC-FID)

Capillary gas chromatography was performed using a Agilent system consisting of a model 6820 gas chromatograph (Agilent, USA), using a fused silica capillary column DB-5, 30 m × 0.35 mm, 0.1 µm film thickness (J & W Scientific, USA). The temperature program was set from 80 to 280 °C in 1 to 20 min at 15 °C min^{-1}. The injection temperature 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.

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 × 0.25 mm × 0.25 µm film thickness) was used. Temperature program was set from 80 to 280 °C in 1 to 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/s.

Injection mode was split (75:5). MS interface temperature was 230 °C. MS mode was electron ionization (EI), detector voltage was 1.66 Kv, mass range was 10 to 700 m/z, scan speed was 2.86 scan/s and interval was 0.01 min (20 Hz).

Identification of components

The components were identified by comparing the mass spectra

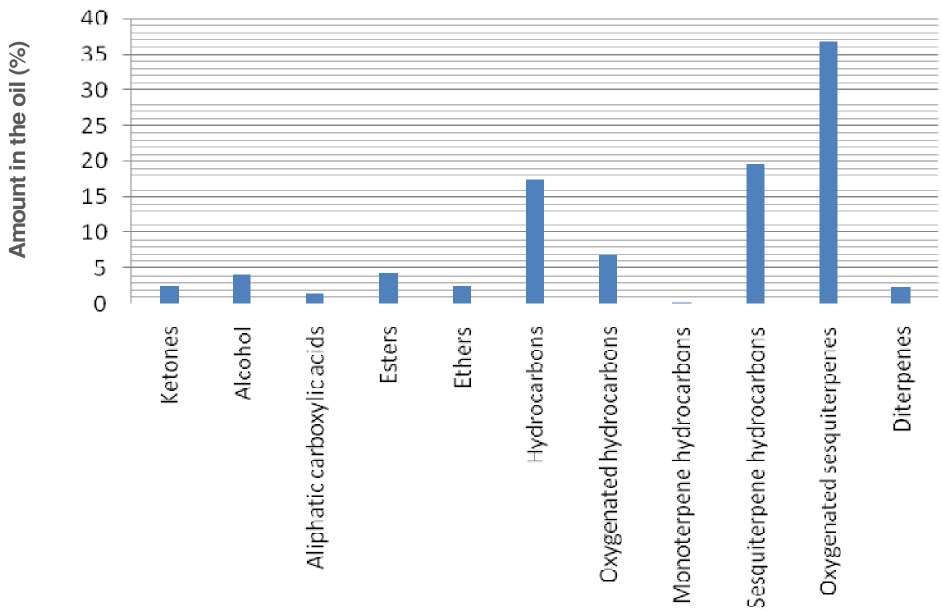

Figure 1. Essential oil constituents in *P. cattleianum* var. *cattleianum*.

with MS library. The NIST 98 spectrometer data bank was used for identification of the chemical composition.

RESULTS AND DISCUSSION

Volatile compounds in *P. cattleianum* var. *cattleianum* were isolated by simultaneous hydrodistillation and analyzed by GC and GC-MS. The compounds identified in the oil are shown in Table 1, and distribution of the different chemical groups to which the compounds belongs is as shown in Figure 1.

The GC-MS analysis of the oils of *P. cattleianum* var. *cattleianum* resulted in 97.5% from 76 compounds (Table 1). The highest percentages of compounds (Figure 1) were oxygenated sesquiterpenes (36.78%), followed by sesquiterpene hydrocarbons (19.57%), hydrocarbons (17.32%), oxygenated hydrocarbons (6.85%), esters (4.24%) and alcohols (4.006%).

The major compounds of *P. cattleianum* var. *cattleianum* were caryophyllene oxide (29.56%) which has been found to be major constituent in other *Psidium* spp. Alloaromadendrene oxide-(1) (6.82%) and 12-oxabicyclo[9.1.0]dodeca-3,7-diene (5.85%), were found as other major compounds.

In case of oxygenated sesquiterpenes, caryophyllene oxide is the most abundant compound (29.56%). This com-pound 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%), and Taiwan (27.7%) (Chen et al., 2007; Li et al., 1999; Ogunwande et al., 2003; Pino et al., 2001). It is also found in *Psidium myrsinoides* (19.7%) (Freitas et al., 2002), *Psidium salutare* (39.8%) (Pino et al., 2003), *Psidium striatulum* (7.6%) (da Silva et al., 2003) and *P. guajava* fruit (5.1%) (Paniandy et al., 2000). This compound has been associated with antifungal activity against dermatophytes (Yang et al., 2000), antimicrobial activity (Brighenti et al., 2008; de Souza et al., 2004), analgesic and anti-inflammatory activity (Chavan et al., 2010) and shows anti caries activity in rats (de Menezes et al., 2010). It is also well known as a preservative in food, drugs and cosmetics (Yang et al., 2000). Other major compound of this group was alloaromadendrene oxide-(1).

Within the sesquiterpene hydrocarbons, the major compounds of *P. cattleianum* var. *cattleianum* were naphthalene decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-[4ar-(4aα, 7α, 8aβ)], β-humulene, selina-6-en-4-ol, solavetivone, longifolenaldehyde and 8, 9-dehydro neoisolongifolene. Among hydrocarbons, major compounds of *P. cattleianum* var. *cattleianum* were 1*H*-cycloprop[e]azulene, (1,2,3,4,4a,5,6,8a-octahydronaphthalene), spiro[5.6]dodecane, bicyclo[2.2.1]heptanes and 1,2,3,4,4a,7-hexahydro naphthalene. In case of oxygenated hydrocarbons, esters, alcohols, ethers and ketones, the major compounds of *P. cattleianum* var. *cattleianum* were 12-oxabicyclo[9.1.0]dodeca-3,7-diene, methyl (Z)-5,11,14,17-eicosatetraenoate, 3-buten-2-ol, 1-benzoxirene and 1,4-methanoazulen-7(1*H*)-one. The compounds, 4-β-kauren-18-ol-acetate and globulol (diterpenes), acetic acid (aliphatic carboxylic acid) and (+)-carene (monoterpene hydrocarbons) were present in smaller quantities. Caryophyllene oxide, the main constituent of the essential oil in this study, is an important constituent of most of the *Psidium* spp. Although most of these compounds are well documented

Table 1. Chemical composition of *P. cattleianum* var. *cattleianum*.

Peak number	Compounds	Rt (min)*	Area peak (%)
	Ketones		
1	3-Buten-2-one	11.858	0.237
2	1,4-Methanoazulen-7(1*H*)-one	17.910	1.974
3	2(1*H*)-Naphthalenone	17.098	0.204
	Subtotal	-	2.415
	Alcohols		
4	α,α-4-Trimethyl-benzenemethanol	9.943	0.430
5	3-Nonanol	11.089	0.307
6	3-Cyclohexene-1-methanol,	12.722	0.303
7	3-Buten-2-ol	17.346	2.966
	Subtotal	-	4.006
	Aliphatic carboxylic acids		
8	Acetic acid	10.019	1.244
9	Subtotal	-	1.244
	Esters		
10	Linalyl butyrate	10.619	0.220
11	Bornyl acetate	11.206	0.345
12	Isopulegol acetate	11.612	0.340
13	Methyl (*Z*)-5,11,14,17-eicosatetraenoate	17.646	3.336
	Subtotal	-	4.241
	Ethers		
14	Oxirane, decyl	5.266	0.213
15	7-Oxabicyclo[4.1.0]heptane	11.529	0.226
16	4,7-Methanobenzofuran	12.246	0.213
17	1-Benzoxirene	16.853	1.815
	Subtotal	-	2.467
	Hydrocarbons		
18	1-Dodecyne	12.187	0.511
19	Cyclobuta[1,2:3,4]dicyclopentene	12.634	0.543
20	1,4-Methano-1H-indene	13.310	0.343
21	Cyclohexane	14.091	0.342
22	1,2,3,4-Tetrahydronaphthalene	14.397	0.592
23	1,2,3,3a,4,5,6,7-Octahydro-1-azulene,	14.567	0.333
24	2,6-Dimethyl-quinoline	14.673	0.668
25	1,2,3,4,4a,7-Hexahydronaphthalene	15.713	1.695
26	1,2,3,4,4a,5,6,8a-octahydronaphthalene	15.871	3.153
27	1*H*-Indene	16.007	0.180
28	1*H*-Cycloprop[e]azulene	16.059	3.491
29	Spiro[5.6]dodecane	16.400	3.025
30	1,4-Methanoazulene	16.735	0.267
31	Bicyclo[2.2.1]heptane	17.411	2.186
	Subtotal	-	17.329

Table 1. Contd.

	Oxygenated hydrocarbons		
32	Eucalyptol	7.387	0.322
33	12-Oxabicyclo[9.1.0]dodeca-3,7-diene	15.578	5.855
34	2-(2-Furanylmethylene)-6-methyl- cyclohexanone	16.577	0.680
	Subtotal	-	6.857
	Monoterpene hydrocarbons		
35	(+)-4-Carene	12.058	0.233
	Subtotal	-	0.233
	Sesquiterpene hydrocarbons		
36	Ylangene	12.417	0.417
37	α-Cubebene	12.505	0.505
38	Isocaryophillene((1*R*,9*S*,*Z*)-4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene)	12.916	0.206
39	Caryophyllene	13.128	0.128
40	Germacrene D((3*R*,6*S*,9*R*,*Z*)-6-isopropyl-3,9-dimethylcyclodec-1-ene)	13.245	0.245
41	Humulen-(v1)	13.498	0.128
42	α-Caryophyllene	13.598	0.145
43	(+)-Cyclosativene	13.844	0.411
44	Naphthalene decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-[4ar-(4aα,7α,8aβ)]	14.038	2.790
45	Cadala-1(10),3,8-triene	14.732	0.234
46	Eudesma-4(14),11-diene	15.636	0.544
47	Longifolenaldehyde	15.807	1.511
48	Copaene	15.924	0.167
49	Selina-6-en-4-ol	16.124	1.782
50	Cedrol	16.500	0.544
51	8,9-Dehydro-neoisolongifolene	16.641	1.464
52	Isoaromadendrene epoxide	16.682	0.433
53	cis-*Z*-α-Bisabolene epoxide	16.800	0.280
54	Solavetivone	17.094	1.666
55	Ledol	17.252	0.511
56	β-Humulene	17.481	2.162
57	Murolan-3,9(11)-diene-10-peroxy	17.734	0.309
58	trans-Z-α-Bisabolene epoxide	17.816	0.199
59	Corymbolone	18.257	0.189
60	trans-Longipinocarveol	19.209	0.297
61	Calarene epoxide	19.620	0.423
62	1,2-Longidione	20.202	0.431
63	Culmorin	20.748	0.232
64	Diepicedrene-1-oxide	22.734	0.401
65	8*S*,14-Cedran-diol	23.504	0.317
66	Ledene oxide-(II)	26.653	0.505
	Subtotal	-	19.576
	Oxygenated sesquiterpenes		
67	α-Santalol	14.502	0.174
68	Caryophyllene oxide	15.319	29.568
69	Alloaromadendrene oxide-(1)	16.247	6.826
70	Viridiflorol	17.587	0.221
	Subtotal	-	36.789

Table 1. Contd.

	Diterpenes		
71	*Z*-2-Octadecen-1-ol acetate	8.715	0.243
72	Phytol(3,7,11,15-tetramethylhexadecan-1-ol)	10.554	0.533
73	Thunbergol	19.949	0.127
74	4-β-Kauren-18-ol-acetate	22.658	0.621
75	Demecolcine	25.748	0.229
76	Globulol	28.022	0.599
	Subtotal	-	2.352
	Total	-	97.509

*Retention time.

as essential oil components in various plant species (Zhu et al., 1995) to our knowledge this is the first report of their occurrence in the essential oil of *P. cattleianum* var. *cattleianum* from South Africa.

It is interesting to record that the values of the main components of the essential oil in *P. cattleianum* var. *cattleianum* are higher than those published in other species (Chalannavar et al., 2012; Chen et al., 2007; Li et al., 1999; Ogunwande et al., 2003; Pino et al., 2001). Existing variations in oil content and composition may be attributed to factors related to ecotype, phenophases and the environment including temperature, relative humidity, irradiance and photoperiod (Fahlen et al., 1997).

The high level of caryophyllene in the leaves of *P. cattleianum* var. *cattleianum* provides opportunities for manufacturing industry. Caryophyllene has been shown to have preservative values in the food, drug and cosmetic industry.

ACKNOWLEDGEMENTS

This work was supported by National Research Foundation and Durban University of Technology, South Africa for financial support.

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