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Full Length Research Paper

Chemical composition of essential oil of *Psidium cattleianum* var. *lucidum* (Myrtaceae)

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The aim of this study was to investigate the essential oil composition of *Psidium cattleianum* var. *lucidum* from South Africa. The essential oils were extracted by hydrodistillation and the components were identified by gas chromatography coupled to mass spectrometry (GC-MS) to determine the chemical composition of the essential oil. A total of 53 chemical components were identified, accounting for 61% of the essential oil. The major component was caryophyllene oxide (12.43%), while other predominant constituents were identified as bicyclo(4.4.0)dec-l-ene (6.61%), 2,3-butanediol diacetate (4.84%) and patchoulene (4.73%). The presence of many terpenic and ester compounds is thought to contribute to the unique flavor of the *P. cattleianum* var. *lucidum* leaves.

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

INTRODUCTION

Global interest in biopreservation of food systems has recently been increased because of great economic costs of deterioration and poisoning of food products by food pathogens. Essential oils and extracts of various species of edible and medicinal plants consist of very potent natural biologically active agents (Nychas et al., 2003). However, until recently, very little significance was given to the natural, hidden and life-supporting services of the natural ecosystems. It is only when the disruption/loss of these natural resources poses/results in a severe threat to the very existence of human civilization that these intrinsic values have been highlighted. In fact, these services are ignored largely due to their non-marketable potential and a negligible role in modern trade economy. Nevertheless, during the last decade, the importance of these natural benefits has been highlighted and the perils linked to their loss realized. The phrase ‘ecosystem services’ has been widely used for these underpinned natural environmental benefits (Ehrlich and Ehrlich, 1981) and considered as ‘world’s natural capital’ (Costanza et al., 1997). As reported by the World Bank (2006), more than one billion people are directly dependent upon ecosystem services.

Research into plant essential oils have also gained momentum due to their fumigant and contact insecticidal activities and the less stringent regulatory approval mechanisms for their exploration due to long history of use (Isman, 2006). Of late, the essential oils are being tried as potential candidates for weed control (Singh et al., 2003; Batish et al., 2004, 2007), and pest and disease management (Isman, 2000; Pawar and Thaker, 2006; Abad et al., 2007). Furthermore, essential oils are easily extractable, eco-friendly in that they are bio-degradable and get easily catabolized in the environment (Zygadlo and Grosso, 1995), do not persist in soil and water (Misra and Pavlostathis, 1997; Isman, 2000, 2006), possess low or no toxicity against vertebrates, fishes, birds and mammals (Enan et al., 1998; Isman, 2000; Isman and Machial, 2006; Bakkali et al., 2008), and these enable the oils to have applications even in sensitive areas such as schools, restaurants, hospitals and homes.

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Looking at the importance of ecosystem services to mankind, it is worthwhile to explore environmental benefits of the natural products to mankind.

Psidium cattleianum Sabine var. *lucidum* (Degener) Fosb. (*P. cattleianum* var. *lucidum*) is known as 'Araca' in Brazil and strawberry guava in many parts of the world. It belongs to the family Myrtaceae; the majority of these species are essential oil bearing plants. The family consists of about 75 genera and nearly 3000 species of mainly tropical evergreen trees and shrubs. *Psidium* is native to the Neotropics and is widely cultivated and naturalized in the tropical and subtropical areas of the world (Wagner et al., 1990). The species has been introduced to Hawaii, Mauritius, Australasia – Norfolk, Tropical Polynesia (Cronk and Fuller, 1995). Plants belonging to the *Psidium* genus have been known to exhibit several therapeutic properties, including anti-bacterial, hypoglycemic, anti-inflammatory, analgesic, anti-pyretic, spasmolytic and central nervous system depressant activities, and are therefore used as a popular medicine (Begum et al., 2002).

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 (Taiz and Ziger, 1991). There are reports of the presence of isoflavonoids and volatile compounds from the leaves and the fruit oil of *P. cattleianum* Sabine (Pino et al., 2001; Lapcik et al., 2005.) The chemical compositions of the leaf oils of *P. cattleianum* Sabine from different geographical areas were assessed *via* gas chromatography (GC) and gas chromatography coupled to mass spectrometry (GC/MS). Depending on the location from which the samples were collected, the oil was composed of different percentages of the following primary components: β-caryophyllene, α-pinene, myrcene, α-thujene, 1,8-cineole, epi-α-murolol, α-cadinol, epi-α-cadinol and caryophyllene oxide, at different percentages (Marques et al., 2008; Chen et al., 2007; Pino et al., 2004). The leaves of *Psidium guajava* a related species has been found to possess 1, 8-cineole and trans-caryophyllene (Li et al., 1999; Chen et al., 2007; Cole and Setzer 2007). Pharmacological studies by recent workers (Manosroi, 2006; Sacchetti et al., 2005) show that these compounds have anti-proliferative, anti-oxidant and anti-microbial activities.

No previous work on the chemical composition of the leaf oils of *P. cattleianum* var. *lucidum* has been reported, whereas three papers about *P. cattleianum* leaf oil composition has been published (Tucker et al., 1995; Pino et al., 2004; Limberger et al., 2001). In continuation of our ongoing research on extraction and characterization of essential oil constituents from natural plants (Chalannavar et al., 2011), herein we made an attempt to isolate and characterize the contents of the essential oils from leaves of *P. cattleianum* var. *lucidum* from KwaZulu-Natal province of South Africa.

MATERIALS AND METHODS

Leaves of *P. cattleianum* var. *lucidum* were collected in September 2010 in the KwaZulu-Natal province of South Africa. The species was identified 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. *lucidum* was extracted using a modification of an established procedure (Denny, 1989). Briefly, 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 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. 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.

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 × 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, with a linear velocity (*u*) of 31 cm/s. 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).

Identification of components

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 and also compared on the basis of comparison of their retention indices and mass spectra with those given in the literature (Julian and Konig, 1988; Adams, 2007). Retention Indices (RI) were determined with reference to a homologous series of *n*-alkanes, by using the following formula (Kovats, 1958):

$$KI = 100\,[n+(N-n)\ X\ \frac{\log t^1_R\,(\text{unknown}) - \log t^1_R\,(C_n)}{\log t^1_R\,(C_N) - \log t^1_R\,(C_n)}$$

Where, t^1_R is the net retention time (t_R-t_0), t_0 is the retention time of solvent (dead time), t_R is the retention time of the compound, C_N is the number of carbons in longer chain of alkane; C_n is the number of carbons in shorter chain of alkane; n is the number of carbon atoms in the smaller alkane and N is the number of carbon atoms in the larger alkane.

RESULTS AND DISCUSSION

The average percentage of the essential oils of the dried leaves from *P. cattleianum* var. *lucidum* was light yellow with yields of 1.24% (v/w). A distribution of the different chemical groups of the compounds is shown in Figure 2. The compounds from essential oils are grouped in Table 1 based on their chemical structures in which they are classified. The GC-MS analysis of the oils of *P. cattleianum* var. *lucidum* resulted in 61% from 53 compounds (Table 1). The highest percentage of compounds (Figure 2) were oxygenated sesquiterpenes (17.53%), followed by hydrocarbons (16.70%), sesquiterpene hydrocarbons (5.87%), esters (5.34%), ketones (5.17%), acids (4.28%), alcohols (3.49%), oxygenated hydrocarbons (1.19%), amines (0.67%), amide (0.42%), aldehyde (0.35%) and diterpenes (0.33%).

In the oxygenated sesquiterpenes, the major compounds of this populations are caryophyllene oxide made up (12.43%). Caryophyllene oxide is an important constituent of most of the Psidium species. Bicyclo(4.4.0)dec-l-ene (6.61%), 2,3-butanediol diacetate (4.84%), patchoulene (4.73%), butanone (2.71%), dodecatrien-3-ol (2.66%), alpha cadinol (2.24%), naphthalene (2.27%), azulene (2.22%), butonoic acid (1.97%), spiro(4.4)nonan-2-one (1.75%), naphthalene-1,6-dimethyl (1.50%), 1*H*-cyclopropa (a) naphthalene (1.33%), *cis*-z-alpha-bisabolene epoxide (1.23%), ledol (1.08%) and acetic acid (1.07%) were found as other major compounds. The molecular structure of the major constituent of essential oil from *P. cattleianum* var. *lucidum* is depicted in Figure 1.

Among the oxygenated sesquiterpenes, caryophyllene oxide (12.43%) was the major predominated compound. This compound 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%), Taiwan (27.7%) (Lie et al., 1999; Pino et al., 2001; Ogunwande et al., 2003; Chen et al., 2007). It is also found in *Psidium myrsinoides* as 19.7% (Freitas et al., 2002), *Psidium salutare* as 39.8% (Pino et al., 2003), *Psidium striatulum* as 7.6% (da Silva et al., 2003) and *Psidium guajava* fruit as 5.1% (Paniandy et al., 2000). This compound has been associated with antifungal activity against dermatophytes (Yang et al., 2000), has antimicrobial (de Souza et al., 2004; Brighteni et al., 2008) analgesic and anti-inflammatory activity (Chavan et al., 2010) and shows anti caries activity in rats (Menezes et al., 2010). It is also well known as a preservative in food, drugs and cosmetics (Yang et al., 2000). Despite its large array of biological activities, the other major compounds of this species are alpha cadinol, *cis*-Z-alpha-bisabolene epoxide and ledol.

Within the sesquiterpene hydrocarbons, the major compounds of *P. cattleianum* var. *lucidum* were patchoulene, alpha-cubebene and caryophyllene. In the case of hydrocarbons and oxygenated hydrocarbons, the major compounds of *P. cattleianum* var. *lucidum* were bicyclo(4.4.0)dec-1-ene, azulene, naphthalene 1,2,3,4,4a,5,6,8 a-octahydro (1,2,3,4,4a,5,6,8a-octahydronapthalene), 1*H*-cyclopropa(a)naphthalene and ethoxy (methyl) chlorosilane, oxazole and oxirane. In the case of esters, alcohols, acids and ketones, the major compounds of *P. cattleianum* var. *lucidum* were 2,3-butanediol diacetate, triacetin, dodecatrien-3-ol, 3-heptanol, butanoic acid, acetic acid, 2-butanone and spiro(4.4)nonan-2-one. Among the diterpenes, the major compounds of *P. cattleianum* var. *lucidum* were hexadeca-2, 6, 10, 14-tetraen-1-ol, 3, 7, 11, 16-tetramethyl (E, E, E). The compounds dioxolane-4-carboxaldehyde (aldehyde), and 1-hexanamine, tetraacetylethylenediamine, tetradecanamine (amines) and butanamide (amide) 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* species. Although most of these compounds are well documented 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. *lucidum* from South Africa. Interestingly, there were significant differences between the main components of the essential oil of *P. cattleianum* var. *lucidum* and those previously determined in other species of Psidium. 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). Our results were generally different according to literature findings. The observed differences may be probably due to different environmental and genetic factors, different chemotypes and the nutritional status of the plants as well as other factors that can influence the oil composition. These results show that *P. cattleianum* var. *lucidum* are remarkably variable species. Actually, the high quantities of caryophyllene oxide make them a most interesting species from the economic point of view.

The GC and GC-MS study of the essential from *P. cattleianum* var. *lucidum* led to the identification of 53 compounds, representing 61% of the total mass. The major components were terpenes and their derivatives and the most prominent one was caryophyllene oxide (12.43%). Essential oils are extensively used as flavor ingredient in a wide variety of food, beverage and confectionary products. The dominant presence of caryophyllene oxide in essential oils makes them

Table 1. Chemical composition of essential oil from *Psidium cattleianum* var. *lucidum.*

Peak number	Chemical constituent	Molecular formulae	Molecular weight	RI[a]	Percentage (%)
	Aldehyde				
1	Dioxolane-4-carboxaldehyde	$C_4H_6O_3$	102	579	0.35
	Subtotal				0.35
	Ketones				
2	2-Butanone	C_4H_8O	72	236	2.71
3	Ethylene maleic anhydride	$C_6H_4O_3$	124	401	0.29
4	2*H*-Pyran-2-one	$C_5H_4O_2$	96	446	0.13
5	Pentanedione	$C_5H_8O_2$	100	535	0.25
6	(4-Fluorophenyl) acetone	C_9H_9FO	152	871	0.04
7	Spiro(4.4)nonan-2-one	$C_9H_{14}O$	138	1004	1.75
	Subtotal				5.17
	Alcohols				
8	3-Heptanol	$C_7H_{16}O$	116	464	0.30
9	Pyrrolidinol	C_4H_9NO	87	495	0.04
10	Dodecatrien-3-ol	$C_{12}H_{20}O$	180	1478	2.66
11	2,6-Octadien-1-ol	$C_8H_{14}O$	126	965	0.27
12	Dodecatrien-1-ol	$C_{12}H_{20}O$	180	1133	0.22
	Subtotal				3.49
	Acids				
13	Propanoic acid	$C_3H_6O_2$	74	262	0.57
14	alpha-Chloroacrylic acid	$C_3H_3ClO_2$	105	298	0.06
15	Hexanoic acid	$C_6H_{12}O_2$	116	468	0.10
16	Butanoic acid	$C_4H_8O_2$	88	932	1.97
17	Acetic acid	$C_2H_4O_2$	60	960	1.07
18	Phenylacetic acid	$C_8H_8O_2$	136	974	0.51
	Subtotal				4.28
	Esters				
19	4-Trifluoroacetoxyoctane	$C_{10}H_{17}F_3O_2$	226	383	0.11
20	2,3-Butanediol diacetate	$C_8H_{14}O_4$	174	414	4.84
21	Triacetin	$C_9H_{14}O_6$	218	520	0.39
	Subtotal				5.34
	Amines				
22	1-Hexanamine	$C_6H_{15}N$	101	481	0.02
23	Tetraacetylethylenediamine	$C_{10}H_{16}N_2O_4$	228	547	0.59
24	Tetradecanamine	$C_{14}H_{31}N$	213	605	0.06
	Subtotal				0.67
	Amides				
25	Butanamide	C_4H_9NO	87	575	0.42
	Subtotal				0.42
	Hydrocarbons				
26	Toluene	C_7H_8	92	755	0.59
27	2-Pentene	C_5H_{10}	70	512	0.13
28	Neopentyl isothiocyanate	$C_6H_{11}NS$	129	559	0.32

Table 1. Contd.

29	Butane	C_4H_{10}	58	588	0.64
30	1*H*-Cycloprop(e)azulene	$C_{11}H_8$	140	866	0.19
31	Cycloundecatriene	$C_{11}H_{16}$	148	878	0.04
32	Cyclohexene	C_6H_{10}	82	890	0.14
33	1*H*-Cyclopropa(a)naphthalene	$C_{11}H_8$	140	904	1.33
34	1,2,3,4,4a,5,6,8a-octahydronaphthalene	$C_{10}H_{16}$	136	916	2.27
35	Naphthalene, decahydro-4a	$C_{10}H_{18}$	138	991	0.15
36	Bicyclo(4.4.0)dec-1-ene	$C_{10}H_{16}$	2033	1027	6.61
37	Azulene	$C_{10}H_8$	128	1299	2.22
38	Naphthalene 1,6-dimethyl	$C_{12}H_{12}$	156	1763	1.50
39	Cyclohexane	C_6H_{12}	84	1100	0.57
	Subtotal				16.70
	Oxygenated hydrocarbons				
40	Oxirane	C_2H_4O	44	347	0.13
41	*sec*-Butyl nitrite	$C_4H_9NO_2$	103	367	0.05
42	Oxazole	C_3H_3NO	69	730	0.38
43	Ethoxy(methyl)chlorosilane	C_3H_9ClOSi	124	1192	0.63
	Subtotal				1.19
	Sesquiterpene hydrocarbons				
44	alpha-Cubebene	$C_{15}H_{24}$	204	1353	0.89
45	Caryophyllene	$C_{15}H_{24}$	204	1423	0.20
46	alpha-Caryophyllene	$C_{15}H_{24}$	204	1454	0.05
47	Patchoulene	$C_{15}H_{24}$	204	1360	4.73
	Subtotal				5.87
	Oxygenated sesquiterpenes				
48	Caryophyllene oxide	$C_{15}H_{24}O$	220	1581	12.43
49	Ledol	$C_{15}H_{26}O$	222	1602	1.08
50	alpha-Cadinol	$C_{15}H_{26}O$	222	1610	2.24
51	*trans*-*Z*-alpha-Bisabolene epoxide	$C_{15}H_{24}O$	220	1495	0.55
52	*cis*-*Z*-alpha-Bisabolene epoxide	$C_{15}H_{24}O$	220	1680	1.23
	Subtotal				17.53
	Diterpenes				
53	Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tetramethyl(E,E,E)-	$C_{20}H_{34}O$	290	1084	0.33
	Subtotal				0.33
	Total				61

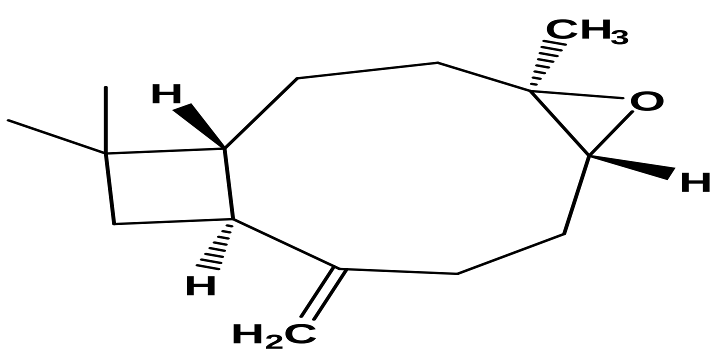

Figure 1. Structure of caryophyllene oxide.

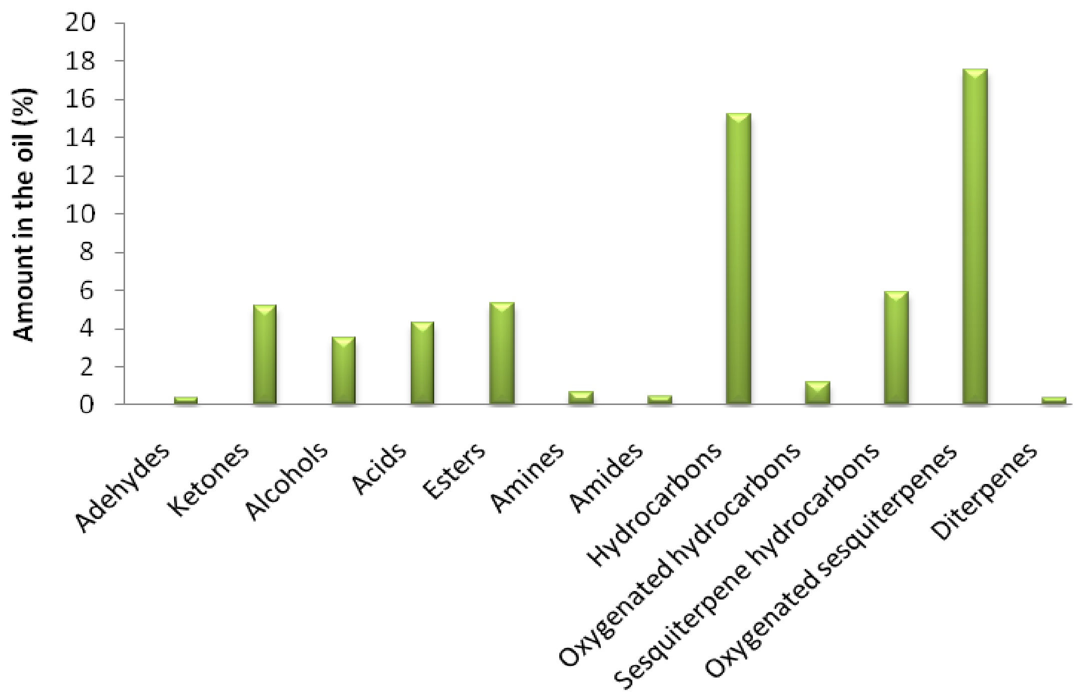

Figure 2. Essential oil constituents in *Psidium cattleianum* var. *lucidum*

potential natural preservatives in the food industry.

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