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CHEMICAL CONSTITUENTS OF ESSENTIAL OILS OF *BOESENBERGIA ARMENIACA* AND *B. STENOPHYLLA* (ZINGIBERACEAE) ENDEMIC TO BORNEO

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Abstract

Boesenbergia armeniaca and *B. stenophylla* are two endemic wild gingers from Borneo. Limited information are available on these wild species. The aim of this study was to identify the leaf essential oil constituents using hydrodistillation and analysed via GC-FID and GC-MS. The essential oil yields of leaves obtained from *B. armeniaca* and *B. stenophylla* were 0.01% and 0.08% respectively. The leaf oil of *B. armeniaca* was dominated by oxygenated sesquiterpenes (47.95%), while the leaf oil of *B. stenophylla* was dominated by oxygenated monoterpenes (85.12%). The main volatile constituents in the leaf oil of *B. armeniaca* were Nerolidol (42.55%), Linalool (11.63%) and β-caryophyllene (6.25%) while in the leaf oil of *B. stenophylla*, methyl cinnamate (83.17%) was most abundant. To date, this is the first report revealing the essential oil components of *Boesenbergia armeniaca* and *B. stenophylla* from Sabah, Borneo.

Key words: Methyl cinnamate, *Boesenbergia armeniaca*, *Boesenbergia stenophylla*, Essential oil.

Introduction

In recent years, interest in the utilization of essential oils from medicinal plants for scientific research are increasing especially for pharmaceutical, nutritional and cosmeceutical industry (Ksouri *et al*., 2009). This is due to the fact that the oils exhibit various potent biological activities including antimicrobial (Kamal & Dhruva, 2010), antioxidant (Bellik, 2014) and anti-inflammatory (Alina *et al.*, 2014) activities. Essential oils are secondary metabolites that plants produce for their defence mechanism against herbivory and disease infections (Suthisut *et al*., 2011). In general, among the important constituents of essential oils are monoterpene and sesquiterpene hydrocarbons, oxygenated monoterpenes and other derivatives (Kamaliroosta *et al.,* 2013).

Zingiberaceae is one of the largest families of the order Zingiberales found in tropical and subtropical forests, with approximately 50 genera and over 1500 species worldwide (Larsen *et al*., 1999). In the Southeast Asian region, uniqueness of the flavour and medicinal properties of these species lead to their uses as spices, medicines and flavouring agents (Yob *et al*., 2011). Members of Zingiberaceae are aromatic due to the presence of various essential oil components among others include β-zingiberene, linalool and 1,8-cineole (Joseph *et al*., 2001; Bickers *et al*., 2003; Tripathi *et al*., 2013).

Belonging to the tribe Zingibereae, the genus *Boesenbergia* comprises of approximately 80 extant species distributed throughout India to Southeast Asia (Saensouk & Larsen, 2001). The centre of diversity for the genus *Boesenbergia* is mainly in Borneo with more than 21 species followed by Thailand. The habitats of *Boesenbergia* species are mainly the undergrowth areas of tropical and subtropical forests, in particular, damp, humid and shady places in mixed deciduous and evergreen forests and also limestone hills (Gobilik & Limbawang, 2010; Jing *et al*., 2011). Growing *Boesenbergia* species outside their natural habitats such as a garden may be difficult but possible (Gobilik & Limbawang, 2010). To date, among the *Boesenbergia* species only *B. rotunda* is widely cultivated commercially (Techaprasan *et al*., 2006).

Rhizome of *B. rotunda* have been proven to contain panduratin A which was found to inhibit the growth of MCF-7 human breast cancer and HT-29 human colon adenocarcinoma cells, as well as for the treatment of rheumatism, gout and for culinary purposes (Techaprasan *et al*., 2006: Kirana *et al*., 2007: Chong *et al*., 2012). Several species of *Boesenbergia* such as *B. longiflora* and *B. pulchella* have been reported to contain bioactive components such as flavonoids and chalcones which have been reported to have antioxidant and anti-inflammatory properties (Salama *et al*., 2013; Sudsai *et al*., 2013; Yuliana *et al*., 2013).

Boesenbergia stenophylla, a perennial rhizomatous wild ginger is known as 'Jerangau merah' (Malay) and Kaburo adak (Kelabit/Lun Bawang) which is endemic in the highlands of Borneo with altitude of not less than 3000 ft. (Ahmad & Jantan, 2003; Toyat *et al*., 2015). Ethnobotanically, *B. stenophylla*, has been utilised for various purposes by many ethnic groups, among others include protection against convulsions, prevention of intoxication, cough relief, increase libido, treatment of food poisoning etc. (Ahmad & Jantan 2003; Jing *et al*., 2010).

Boesenbergia armeniaca is also endemic to Borneo distributed from western Sabah to Brunei. It is not commonly used as a medicinal plant by the local people in Sabah probably due to lack of information with regards to its medicinal properties. However, Jing *et al*., (2010) and Sarwar Muhammad (2012) reported that the major flavones of luteolin present in the crude extract of *B. armeniaca* might act as a catalyst in contributing to the anticancer activities, especially against MCF-7 cell lines (breast cancer). In addition, the presence of polyphenols (naringin and hesperidin) in the methanol extract of stem and rhizome of *B. armeniaca*, may contribute to the antioxidant activities (Jing *et al*., 2010). To the knowledge of the authors, there has been no studies on the essential oil of *B. armeniaca*.

Many researchers have demonstrated several pharmacological properties of Zingiberaceae species but studies on biological activities of *Boesenbergia* species are very few including research on the essential oils. Hence, the aim of this study is to analyse and identify the chemical constituents of hydrodistilled leaf oils of *B. armeniaca* and *B. stenophylla* collected from Sabah. Identification of the volatile compounds can provide useful information with regard to their potential applications.

Material and Methods

Plant material: The leaves of *B. armeniaca* and *B. stenophylla*, collected from Serinsim sub-station and Long Pasia, Sabah, were processed at the Institute for Tropical and Conservation Biology of University of Malaysia Sabah, Malaysia (UMS). The leaves were rinsed, cut into small pieces and ground in a blender. All the plants were identified by a taxonomist at the institute and voucher specimens were deposited at BORNEENSIS, Universiti Malaysia Sabah (BORH).

Isolation of essential oils: The fresh samples of ground leaves (1000g) of *B. armeniaca* and *B. stenophylla* were added with distilled water (5L) and subjected to hydrodistillation using modified Clavenger apparatus for about 8 hours. About 5-10 ml of pentane was added through the top of the condenser to trap the condensed oil. Later, the mixtures of water and pentane were dried over anhydrous sodium sulphate and the pentane solution was then evaporated by nitrogen blower to give yellowish essential oils. The oils were stored in dark vials at 4°C until further use.

GC-MS analyses: GC analyses of the volatile constituents were carried out with an Agilent GC model 7890 A, equipped with flame ionization detector (FID) and a CBP-5 capillary column (30 m length x 250 µm internal diameter x 0.25 µm film coating). Helium was used as a carrier gas at the flow rate of 1.3mL/minute. The oven temperature was programmed from 50°C to 250°C at 3°C/min with an initial hold time of 1 minute at 50°C, followed by final hold time of 3 minutes at 230°C. Detector temperature was maintained at 250°C. The sample (1 µL) was injected in split ratio (20:1) at 250°C. GC/MS analyses were performed on Agilent MSD detector 5975C and a CBP-5 capillary column (30 m length x 250 µm internal diameter x 0.25 µm film coating). The operating conditions were as follows: injection and detector temperatures were set at 250°C respectively. A series of n-alkanes, C_8-C_{20} and C_{21}-C_{40} was subjected to GC-FID to calculate the retention indices (RIs) of samples.

Identification of components: Identification of essential oil constituents was done on the basis of their retention indices (RI) determined with reference to homologous series of *n*-alkanes, matching spectra with MS library search (NIST/Wiley/Adams) and by comparing with the MS literature data (Sivasothy *et al*., 2011).

Kovats retention index formula:

$$\text{Kovats Index} = \frac{100\,[\text{Log}\,(Tx - Tm) - \text{Log}\,(Tn - Tm)]}{[\text{Log}\,(Tn + 1 - Tm) - \text{Log}\,(Tn - Tm)]} + 100\,(N)$$

where:
Tm = Mobile phase retention time
Tx = Sample component retention time
Tn = Standard hydrocarbon containing carbon retention time
N = Lowest carbon value of *n*-alkane

Results and Discussion

The leaf oils of *B. armeniaca* and *B. stenophylla* were obtained by hydrodistillation and characterized using GC-FID and GC-MS analyses. Upon hydrodistillation, the yield oil of *B. armeniaca* and *B. stenophylla* were 0.01% and 0.08%, respectively. The yield of the essential oils depends on the extraction type, the duration of the extraction as well as the age of the plants during harvesting (Mejdoub & Katsiotis, 1998; Onyenekwe & Hashimoto, 1999). The identified components of the oil with their Kovat indices (KI) are presented in Table 1 while the chromatograms of *B. armeniaca* and *B. stenophylla* are shown in Figs. 1 and 2, respectively.

The yellowish-coloured oil of *B. armeniaca* revealed thirty-eight components which represented 99.24% of the total oil composition. Analysis of the oil revealed that the oxygenated sesquiterpenes (47.95%) were found to be the major group in the leaf oil of *B. armeniaca*, followed by sesquiterpene hydrocarbons (20.69%), oxygenated monoterpenes (14.49%) and monoterpene hydrocarbons 5.99%). The oil mainly comprises of nerolidol (42.55%), linalool (11.63%), and β-caryophyllene (6.25%). Their chemical structures and economic importance are shown in Fig. 3 and Table 2, respectively. Other quantitatively significant constituents include β-pinene (4.83%), β-bisabolene (3.96%), phytol (3.15%), α-gurjunene (3.13%), elemol (2.67%) and α-caryophyllene (1.58%). To the best of our knowledge, the chemical profile of the essential oil of *B. armeniaca* has not been reported before.

A total of twenty-three volatile constituents were identified in the oil of *B. stenophylla* representing 100% of the total oil composition. Oxygenated monoterpenes constitute the major group with 85.12% of the total oils. This is followed by monoterpene hydrocarbons (6.78%), sesquiterpene hydrocarbons (6.44%) and oxygenated sesquiterpenes (0.51%). This oil is highly rich in methyl cinnamate (83.17%). The chemical structure and economic importance of methyl cinnamate are shown in Fig. 4 and Table 2, respectively. Other constituents that are present in appreciable amounts include β-pinene (4.84%), α-caryophyllene (3.59%). (R)-α-pinene (1.22%), and linalool (1.18%). A similarly high percentage of methyl cinnamate was reported to be present in *Alpinia malaccensis* var. *nobilis* (60.26%) collected from Pahang (Vejayan *et al*., 2017).

Ahmad & Jantan (2003) reported that the leaf oil of *B. stenophylla* collected from Sarawak was dominated by (E)-methyl cinnamate (49.9%). Several similar constituents were identified in both studies namely β-pinene, borneol, α-terpineol, and δ-eIemene but present in different compositions. The variations in some of the essential oil constituents may be due to the difference in geographical region, genetic makeup, growth stage and harvest time (Figueiredo *et al*., 2008; Okut *et al*., 2017).

Table 1. Chemical composition of the leaf oils of *B. armeniaca* and *B. stenophylla*.

No.	Components	KI[a]	KI[b]	*B. a*	*B. s*
1.	Methylcyclohexane	800	740	0.22	0.14
2.	(R)-α-pinene	937	-	0.84	1.22
3.	Camphene	952	954	-	0.23
4.	β-Pinene	979	979	4.83	4.84
5.	β-Phellandrene	993	1029	-	0.11
6.	Limonene	1032	1029	-	0.11
7.	1,8-Cineole	1035	1031	0.78	-
8.	β-Ocimene	1053	-	0.32	0.27
9.	Linalool	1103	1100	11.63	1.18
10.	(E)-2-Butenoic acid, 2-(methylenecyclopropyl)prop-2-yl ester	1120	-	1.15	-
11.	Borneol	1170	1169	0.22	0.55
12.	L-4-terpineol	1181	-	0.42	-
13.	α-Terpineol	1194	1189	0.91	0.22
14.	δ-EIemene	1341	1335	-	0.31
15.	α-Copaene	1378	1377	0.95	0.21
16.	β-Caryophyllene	1394	1417	6.25	0.82
17.	Methyl cinnamate	1392	1379	-	83.17
18.	β-Elemene	1402	1393	0.21	-
19.	α-Gurjunene	1418	1410	3.13	-
20.	γ-Elemene	1426	1434	-	0.21
21.	α-Bergamotene	1439	-	0.66	-
22.	α-Elemene	1446	-	0.32	-
23.	epi-β-Santalen	1451	-	0.48	-
24.	α-caryophyllene	1457	-	1.58	3.59
25.	Isocaryophyllene	1464	-	0.32	-
26.	Germacrene D	1484	1487	0.51	0.58
27.	β-Bisabolene	1507	1505	3.96	-
28.	β-Sesquiphellandrene	1521	1523	1.46	-
29.	δ-Cadinene	1536	1514	0.46	0.22
30.	Elemol	1555	1550	2.67	-
31.	Hotrienol	1539	-	0.53	-
32.	Elixene	1568	-	-	0.5
33.	Nerolidol	1570	1539	42.55	0.19
34.	4-Methyl-1,5-Heptadiene	1582	-	1.54	-
35.	β-Gurjunene	1586	-	0.40	-
36.	Isobutyl cinnamate	1612	1623	0.85	-
37.	Humulene epoxide II	1612	1608	-	0.32
38.	1, 7, 7-Trimethyl-2-vinylbicyclo[2.2.1]hept-2-ene	1634	-	-	0.61
39.	γ-eudesmol	1637	1652	0.70	-
40.	β-eudesmol	1655	1651	0.64	-
41.	α-eudesmol	1658	1645	0.85	-
42.	cis-α-Santalol	1701	-	0.54	-
43.	Hexadecanal	1718	-	1.07	-
44.	Phytol	1915	1943	3.15	-
45.	Isophytol	1952	1948	1.03	-
46.	Kaurene	2033	2042	0.56	0.4
47.	1, 6, 10, 14, 18, 22-Tetracosahexaen-3-ol, 2, 6, 10, 15, 19, 23-hexamethyl-, (all-E)-	2039	-	0.55	-
	Total	**99.24**	**100**		
	Oil yield	**0.01**	**0.08**		
	Monoterpene hydrocarbons	**5.99**	**6.78**		
	Oxygenated monoterpenes	**14.49**	**85.12**		
	Sesquiterpene hydrocarbons	**20.69**	**6.44**		
	Oxygenated sesquiterpenes	**47.95**	**0.51**		
	Others	**10.12**	**1.15**		

[a]Kovats indices determined on a CBP-5 capillary column
[b]Kovats retention indices taken from Adams (2001) and/or literature
B.a: Boesenbergia armeniaca
B.s: Boesenbergia stenophylla

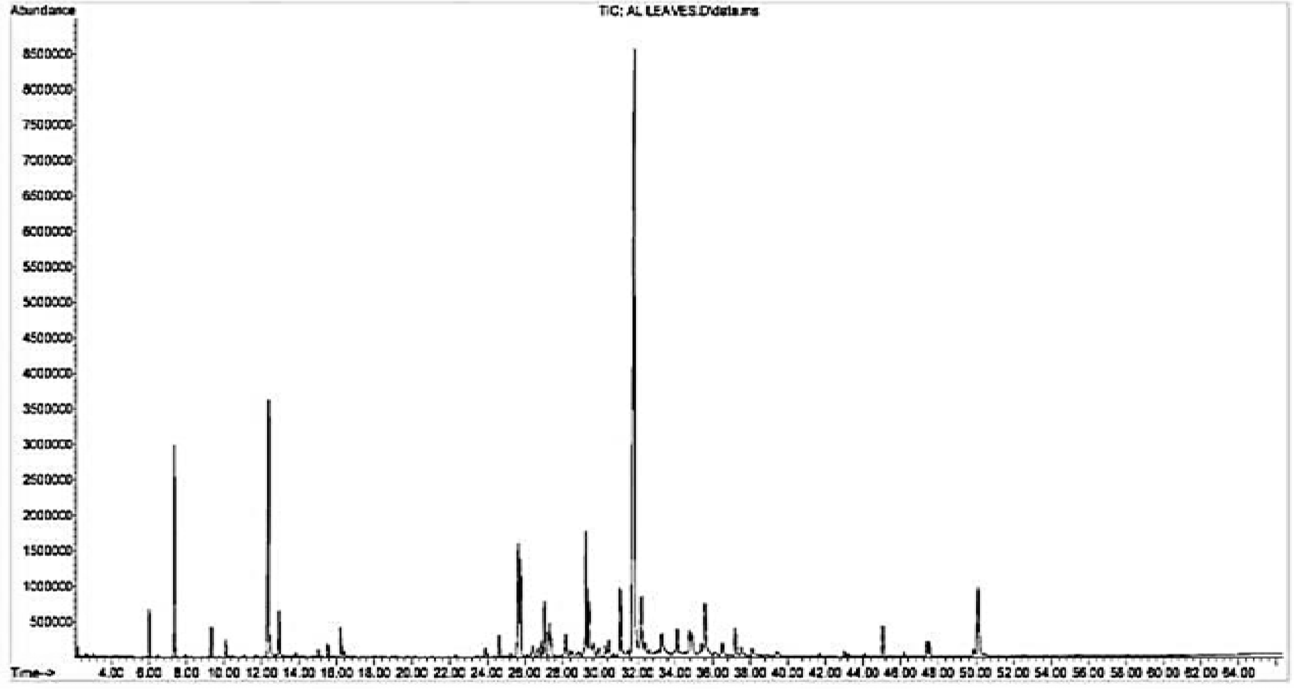


Fig. 1. GC-MS peaks of hydro-distilled leaf oil of *Boesenbergia armeniaca* collected from Sabah.

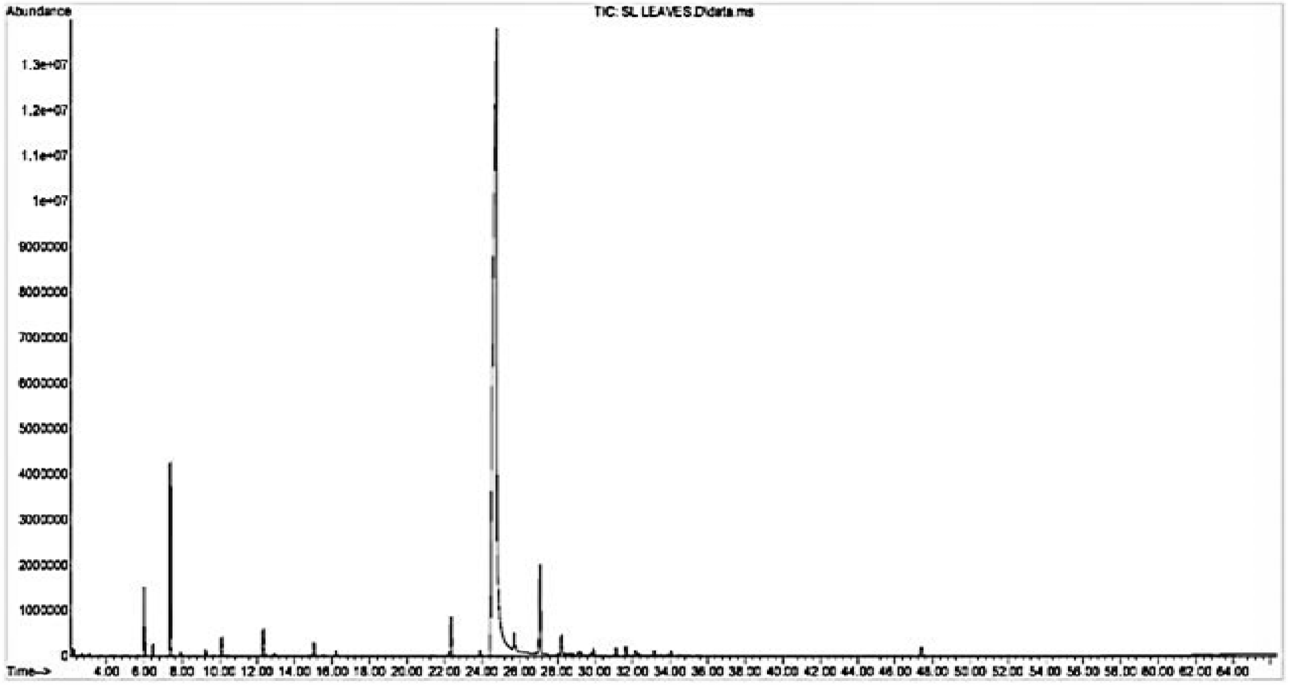
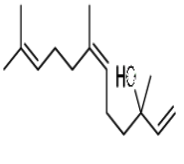
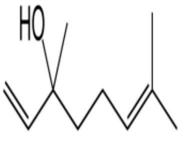


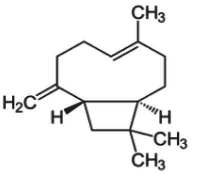
Fig. 2. GC-MS peaks of hydro-distilled leaf oil of *Boesenbergia stenophylla* collected from Sabah.



Nerolidol ($C_{15}H_{26}O$)

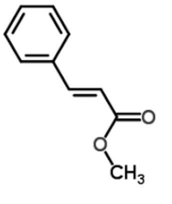


Linalool ($C_{10}H_{18}O$)



β-caryophyllene ($C_{15}H_{24}$)

Fig. 3. Chemical structures of some economically important volatile constituents found in the leaf oil of *B. armeniaca*.



Methyl cinnamate ($C_{10}H_{10}O_2$)

Fig. 4. Chemical structure of methyl cinnamate found in the leaf oil of *B. stenophylla*.

Table 2. Some economically important constituents identified in the leaf oils of *B. armeniaca* and *B. stenophylla* obtained from Sabah.

Compounds	Species	Properties	References
Nerolidol	*B. armeniaca*	1. Act as fragrance ingredient in decorative cosmetics, shampoos and non-cosmetic products such as detergents.	Lapczynski *et al*., 2008
		2. Exhibited anti-inflammatory, antinociceptive and anti-schistomotal activities.	Silva *et al*., 2014 & Fonseca *et al*., 2015
		3. Nerolidol displayed gastroprotective activity by inhibiting the formation of ulcers induced by physical and chemical agents in dose-dependent manner (50, 250, 500 mg/kg)	Klopell, 2007
Linalool	*B. armeniaca*	1. Showed anti-spasmodic activity with 65% of inhibition or stimulation and 70% of anti-inflammatory activity.	Rekha *et al.,* 2014
		2. Linalool at the IC_{50} values f 224 µM, 222 µM, and 290 µM exhibited cytotoxic effects against cancer cell of SW 620, T-47D and Hep G2, respectively using WST-1 analysis.	Chang & Shen, 2014
		3. Linalool gives pleasant odour and also known to elicit physiological effects such as inducing calmness and enhancing sleep.	Elsharif *et al*., 2015
β-caryophyllene	*B. armeniaca*	1. Showed significant anti-inflammatory effect on rats at 0.1 ml/kg dosage of β-caryophyllene.	Bakir *et al.,* 2008
		2. β-caryophyllene demonstrated selective antibacterial activity against *Staphylococcus aureus* (MIC 3 ± 1.0 µM) and also exhibited selective anti-proliferative effects against colorectal cancer cells (IC50 19 µM).	Dahham *et al*., 2015
Methyl cinnamate	*B. stenophylla*	1. E-isomer of methyl cinnamate from essential oil of *Ocimum micranthum* exhibit vasorelaxant effects which tested on rat with IC_{50} value of 877.6 µmol/L.	Vasconcelos-Silva *et al*., 2014
		2. Act as a source of fragrant ingredient that can be found in fine fragrances, shampoos and detergents since methyl cinnamate is reported to have a pleasant fragrance.	Sharma & Kanwar, 2012
		3. Exhibited antioxidant activity with IC_{50} values of 32.67mg/ml using DPPH free radical scavenging assay.	Vejayan *et al*., 2017

Conclusion

This is the first report on the chemical profiling of the leaf oil of *B. armeniaca* from Borneo. In the case of *B. stenophylla*, apart from the study on the essential oil constituents from Sarawak, this is the first report for *B. stenophylla* leaf oil constituents from Sabah. This study reveals that the leaf oil of *B. armeniaca* constitute three main components namely nerolidol (42.55%), linalool (11.63%), and β-caryophyllene (6.25%). *Boesenbergia stenophylla* from Sabah is mainly composed of methyl cinnamate (83.17%). The presence of high concentration of nerolidol (42.55%) in the leaf oil of *B. armeniaca* suggest that it can be potentially utilized as a natural source for nerolidol. This can be a good sample for further studies on biological activities, particularly for the pharmaceutical industry. As for the leaf oil of *B. stenophylla*, the high percentage composition of methyl cinnamate (83.17%) will be particularly useful for the perfume industry.

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