Study on volatile components of three *Curcuma* species by gas chromatography-mass spectrometry

Nungruthai Suphrom*, Surat Boonphong, Phitchaporn Sutamuang, Kamonluk Insumrong, Wannakul Meesuanthong and Phongsan Itsarangkool

Department of Chemistry, Faculty of Science and Center of Excellence for Innovation in Chemistry, Naresuan University, Phitsanulok, 65000, Thailand
*Corresponding author. E-mail: suphrom.n1@gmail.com

ABSTRACT

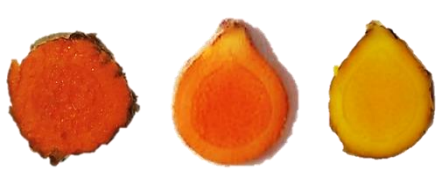
The rhizome of two *Curcuma* species (khamin lueang and khamin thong) have been widely used as ingredients in both folk medicines and foods instead of *Curcuma longa* in some areas of northern part of Thailand. In this study, the comparison of the volatile compounds in *C. longa* and two *Curcuma* spp. ethanolic extracts were conducted using gas chromatography–mass spectrometry (GC-MS). The results indicated that the main components were bisabolane-type sesquiterpene in both of ketone (i.e. ar-tumerone, α-tumerone and β-tumerone) and hydrocarbon skeletons (zingiberene, β-sesquiphellandrene, and ar-curcumene). Some unique compounds which might be used as markers for the discrimination of these *Curcuma* species were found. The structural relationship of main detected compounds were also proposed. It should be noted that farnesyl cation played pivotal role for the formation of compounds found in these plants. The cyclization and allylic oxidation of some compounds lead to the numerous of their analogs. This finding revealed that fingerprint analysis based on GC-MS could provide scientific data, which might be a useful supporting for further utilization as useful plant resources.

Keywords: Curcuma, GC-MS, volatile components, sesquiterpenes, tumerone

INTRODUCTION

Curcuma longa Linn. (turmeric), is belonging to Zingiberaceae family. It is a native plant of southern Asia and cultivated extensively throughout the tropical areas. It is commonly known as "khamin chan" in Thai. Its rhizome is used for the preparation of food, supplements and traditional medicine (World Health Organization 1999). It contains a number of monoterpenoids, sesquiterpenoids, and curcuminoids (Chen et al. 2010, Jayaprakasha, Jagan Mohan Rao, and Sakariah 2005). Various pharmacological effects of *C. longa* were reported such as antioxidation (Kutti Gounder and Lingamallu 2012, Singh et al. 2010, Gianni Sacchetti et al. 2005, Mohanty et al. 2004, Koo et al. 2004), anti-ulcer (Rafatullah et al. 1990), antiinflammation (Lantz et al. 2005, Gupta and Ghosh 1999), antiplatelet (Lee 2006), antidepressant (Yu, Kong, and Chen 2002), immune activation (Madan, Gade, and Ghosh 2001), antiatherosclerotic effects (Zahid Ashraf, Hussain, and Fahim 2005), antimicrobial effects (Gurdip Singh, Om Prakash Singh, and Maurya 2002), and antifungal effects (Apisariyakul, Vanittanakom, and Buddhasukh 1995, Khattak et al. 2005).

In Thailand, people in some provinces in the north of Thailand such as Phitsanulok, Sukhothai, Phetchabun, etc. commonly used the other two *Curcuma* species, which namely "khamin lueang" (*Curcuma* sp1) and "khamin thong" (*Curcuma* sp2) in Thai, as the ingredients both in folk medicines and food preparation instead of *C. longa*. From the naked eye observation, their fresh rhizomes in cross sections appeared the different shades of colors as following (Fig. 1); deep orange-yellow (*C. longa*), yellowish orange (*Curcum*a sp1) and bright yellow (*Curcum*a sp2). However, the comparative study of chemical constituents in these *Curcuma* rhizomes was not done. Therefore, the objective of this study was to compare the volatile components profiles of *C. longa* together with two *Curcuma* spp. rhizome extracts by gas chromatography-mass spectrometry (GC-MS). The structural relationships of detected compounds were also studied.



(a) (b) (c)

Fig. 1 The cross sections and naked eye observation of (a) *C. longa*, (b) *Curcum*a sp1, and (c) *Curcum*a sp2 fresh rhizomes

METHODOLOGY

Plant materials and Extraction

Fresh rhizomes of *C. longa*, khamin lueang (*Curcuma* sp1) and khamin thong (*Curcum*a sp2) were cultivated and collected from Department of Chemistry, Faculty of Science, Naresuan University, Phitsanulok province. The plant materials were authenticated by Dr. Pranee Nangngam and deposited at Department of Biology, Faculty of Science, Naresuan University, Phitsanulok, Thailand.

The powdered rhizomes of three *Curcuma* samples were extracted with 95% ethanol (RCI Labscan Ltd., Thailand) at a ratio of 1 : 10 of plant powder to solvent, for

3 days at room temperature and filtered. The maceration procedure was repeated 3 times. The filtrates were evaporated under reduced pressure and pooled to produce the ethanolic extracts.

Gas Chromatography-mass spectrometry analysis of volatile components in *Curcuma* rhizome extracts

The analysis for volatile components in three extracts were performed by Gas chromatography-mass spectrometry (GC-MS). An instrument was performed on a Hewlett Packard (Agilent Technologies, Palo Alto, CA, USA) model 6890 gas chromatograph equipped with a mass selective detector (MS). A fused silica capillary Hewlett Packard HP-5 (5% phenyl methyl siloxane) column (30 m x 0.25 mm i.d., 0.25 µm film thickness) was used for the GC separation. High purity helium was used as carrier gas with constant flow rate 1.0 mL/min. The injector was set at 250°C and performed by split mode with a split ratio of 10:1 v/v. The initial oven temperature was held at 70°C for 3 min, then programmed at 5°C/min to 280°C and finally held for 10 min. The temperature of transfer line heater was set at 280°C. The mass scanning range was set from 50–550 amu in full scan. The sample solutions were prepared by dissolving 50 mg extract of each into 1 mL of dichloromethane (RCI Labscan Ltd, Thailand) and filtered prior to injection. Then, 1.0 µL of each was injected into above GC-MS system. The identification of volatile components was performed by matching their recorded mass spectra with that of the standard libraries; wiley7n and the National Institute of Standards and Technology (NIST) Chemistry WebBook (Babushok et al. 2007, Linstrom and Mallard 2016), or with the literature data. The results were also confirmed base on their retention indices (RIs) on HP5-MS capillary column. RIs were determined by analyzing a solution containing the homologous series of *n*-alkanes (C_8–C_{20}, Fluka analytical, Germany) and then calculated as described by van Den Dool and Kratz (van Den Dool and Dec. Kratz 1963). The relative contents of each component in all samples were also calculated by normalization of peak areas as the percentage of total detected volatile components.

RESULTS AND DISCUSSION

The volatile compositions of three ethanolic *Curcuma* rhizome extracts were determined using GC-MS. Their total ion chromatograms are shown in Fig. 2. Their different chemical profiles were observed. The number of chemical constituents of individual extracts ranged from 13-24. Maximum number of compounds was detected in *C. longa* (Table 1). The majority of identified compounds belong to sesquiterpene hydrocarbons and oxygenated sesquiterpenes. The relative amount (%) of the compositions was calculated by peak-area normalization. As listed in Table 1, the volatile components of *C. longa* contained zingiberene (13.18%), α-tumerone (10.94%), ar-tumerone (10.57%), α-santalene (8.77%), β-sesquiphellandrene (7.45%) as major compounds. Other constituents such as β-bisabolene (4.15%), ar-curcumene (3.06%), α-bergamotene (2.12%), β-farnesene (2.05%) were also found. The detected compounds were corresponded to that of the previous reports (Jayaprakasha, Jagan Mohan Rao, and Sakariah 2005, Singh et al. 2010, Hu et al. 2014, Shiyou Li et al. 2011). A total of 14 compounds were detected in *Curcuma* sp1, of which the principal compounds were zingiberene (18.98%), β-sesquiphellandrene (11.83%) α-tumerone (12.73%), ar-tumerone (9.54%), β-tumerone (5.57%) and ar-curcumene (4.57%). Analysis of *Curcuma* sp2 revealed that the main constituents were ar-tumerone

(20.58%), α-tumerone (16.06%) and β-tumerone (15.46%). As detailed in Table 1, they could be seen that although there were considerable differences in the compositions of three samples, as well as the responses (contents) of detected compounds, the main constituents found in ethanolic rhizome extract of three *Curcuma* species were almost the same. Their main components were sesquiterpene ketones (ar-tumerone, α-tumerone and β-tumerone), zingiberene, β-sesquiphellandrene, and ar-curcumene. Interestingly, a total of 9 compounds found in *C. longa* (i.e. α-bergamotene, β-santalene, α-humulene, α-farnesene, β-himachalene, α-bisabolene, santalol, *p*-hydroxybenzalacetone, α-bisabol-1-one) and only one compound (germacrene B) found in *Curcuma* sp1 were the unique compounds which might be used as markers for the discrimination of these *Curcuma* species. For *Curcuma* sp2, it was no unique compound. However, it should be noted that its composition profile was similar to that of both *C. longa* and *Curcuma* sp1. The identification of volatile components in these *Curcuma* extracts provided the chemical fingerprints and scientific supporting data that might be helpful in their utilization as useful plant resources.

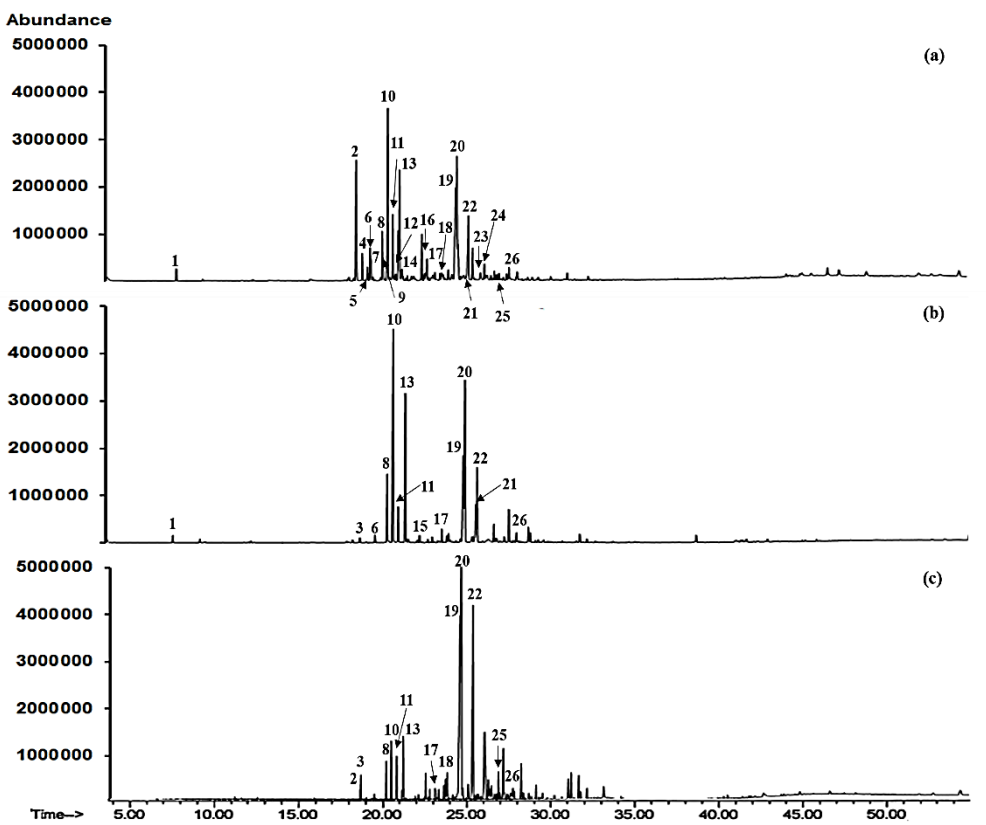


Fig. 2 GC-MS total ion chromatograms of (a) *C. longa*, (b) *Curcuma* sp1, and (c) *Curcuma* sp2

Table 1 Volatile compounds of three ethanolic *Curcuma* rhizome extracts identified by GC-MS

No.	RT (min)	RI[a]	Identified compounds	Relative amount (%)[b]		
				C. longa	*Curcuma* sp1	*Curcuma* sp2
1	7.83	1034	1,8-cineole	0.69	0.43	-
2	18.57	1427	α-santalene	8.77	-	0.55
3	18.59	1427	β-caryophyllene	-	0.36	1.47
4	18.93	1441	α-bergamotene	2.12	-	-
5	19.25	1454	β-santalene	1.07	-	-
6	19.40	1460	β-farnesene	2.05	0.58	-
7	19.45	1462	α-humulene	1.35	-	-
8	20.13	1489	ar-curcumene	3.06	4.57	2.24
9	20.18	1491	α-farnesene	1.29	-	-
10	20.45	1502	zingiberene	13.18	18.98	3.02
11	20.75	1515	β-bisabolene	4.15	2.50	2.21
12	20.92	1522	β-himachalene	0.47	-	-
13	21.16	1532	β-sesquiphellandrene	7.45	11.83	3.36
14	21.24	1535	α-bisabolene	0.60	-	-
15	22.00	1567	germacrene B	-	0.51	-
16	22.64	1594	santalol	0.47	-	-
17	23.26	1622	zingiberenol	0.52	0.97	0.59
18	23.70	1641	propylparaben	0.40	-	1.88
19	24.51	1677	ar-tumerone	10.57	9.54	20.58
20	24.58	1680	α-tumerone	10.94	12.73	16.06
21	25.21	1708	germacrone	1.92	2.89	-
22	25.27	1711	β-tumerone	4.29	5.57	15.46
23	25.99	1745	*p*-hydroxybenzalacetone	0.51	-	-
24	26.22	1756	α-bisabol-1-one	0.93	-	-
25	26.82	1784	α-atlantone	0.55	-	1.52
26	27.70	1826	dehydrozingerone	0.77	0.19	0.47

Remark: [a]Retention indices were calculated using a homologous series of *n*-alkanes (C_8–C_{20})
[b]Results obtained by peak-area normalization

The sesquiterpenes C_{15} compounds are derived by the assembly of three isoprene units. They are widely used in folk medicines, health-supporting preparations and cosmetics due to their numerous biological activities (Afzal et al. 2013, Bartikova et al. 2014). From the above mentioned results, numerous different sesquiterpenes were found in extracts. These detected compounds could be grouped based on their skeletons and were divided into four major categories including bisabolane-type, germacrane-

type, humulane-type and santalane-type sesquiterpenes. The structural relationships of main compositions were also proposed based on their possible mechanisms including various modes of cyclization, skeletal rearrangement, and oxidation together with comparing to that of previous reports.

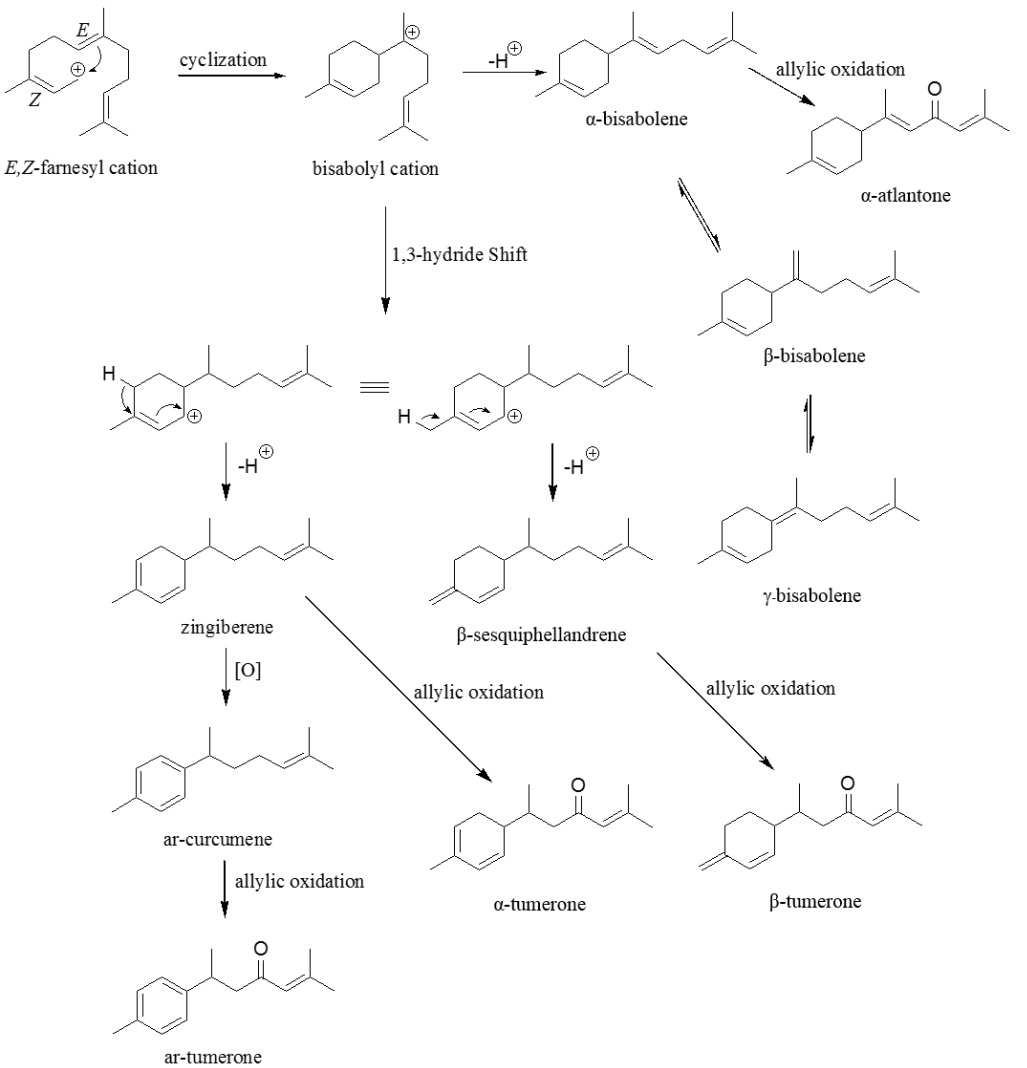


Fig 3. Proposed structural relationships of bisabolane-type sesquiterpenes

The structural relationships of bisabolane-type sesquiterpenes (i.e. bisabolene, curcumene, zingiberene, sesquiphellandrene, tumerone) are shown in Fig. 3. Their formations related to the cyclization of a fundamental sesquiterpenes intermediate, *E*,*Z*-farnesyl cation, which lead to the formation of bisabolyl cation. The bisabolyl cation can be further lost proton to give bisabolene compounds (α-bisabolene, β-bisoblene, and γ-bisbolene). Moreover, when 1,3-hydride shift was occurred in bisabolyl cation, the related compounds including ar-curcumene, zingiberene and β-sesquiphellandrene

could be found in extracts. In addition, the allylic oxidation of them lead to formation of tumurone analogs (ar-tumerone, α-tumerone and β-tumerone) (Dewick 2009).

For the germacrane-type and humulane-type sesquiterpenes, their formations might concerned with the same key intermediate, *E*,*E*-farnesyl cation, as bisabolane-type. (Fig. 4). Its pattern of cyclization by electrophilic attack on to an appropriate double bond might lead to ring system larger than six carbons. The 10-memberred ring compound (germacrene) and 11-membered ring compounds (α-humulene (also called α-caryophyllene) and β-caryophyllene) were formed via germacryl cation and humulyl cation, respectively. For germacrone, the structural relationship suggested that the oxidation at allylic position was still needed to transform sesquiterpene hydrocarbons to ketone sesquiterpene. In addition, farnesyl cation was converted to its analog, β-fanesene, by losing proton (Koo and Gang 2012).

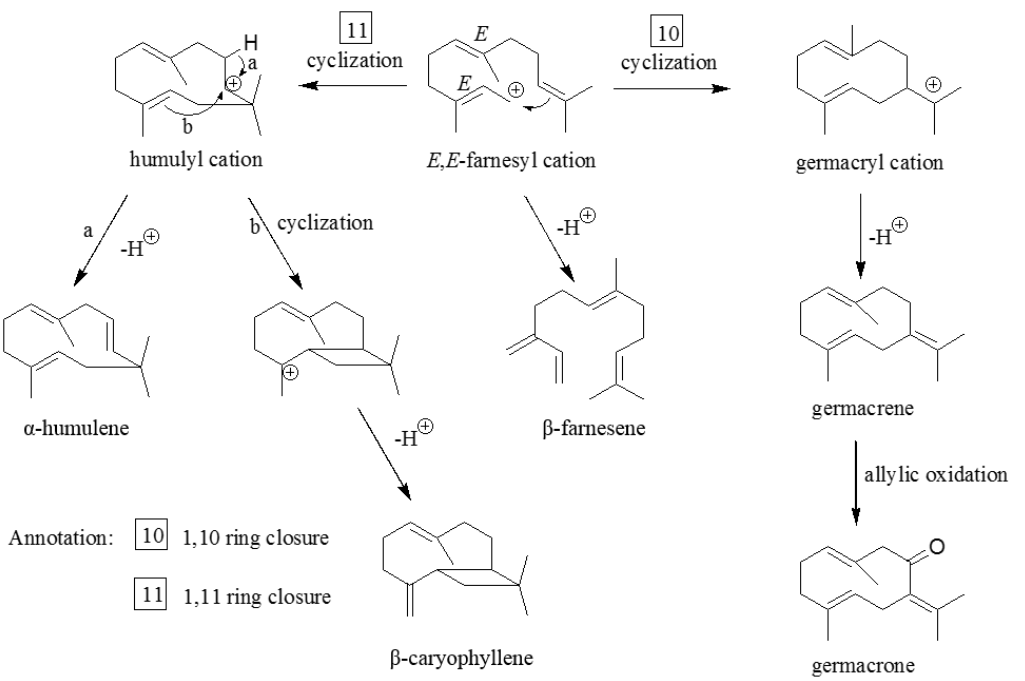


Fig 4. Proposed structural relationships of germacrane-type and humulane-type sesquiterpenes

The proposed structural relationships of santalane-type sesquiterpenes (i.e. α-santalene, β-santalene, santalol, α-bergamotene) are illustrated in Fig. 5. Their relationships were also compared with that of previously report data (Christophe Sallaud et al. 2009). Briefly, the *Z*,*Z*-farnesyl cation was converted to either the (*R*)- or the (*S*)-bisabolyl cations depending on the stereochemistry of ring closure. The (*R*)-bisbolyl cation was then lead to the formation of α-santalene and β-santalene, while the (*S*)-bisabolyl cation was further cyclization and lost proton to give α-bergamotene. Moreover, the allylic oxidation of β-santalene lead to another analog, β-santalol. From obtained structural relationship, it should be noted that farnesyl cation played important role for the formation of detected compound in these *Curcuma* species.

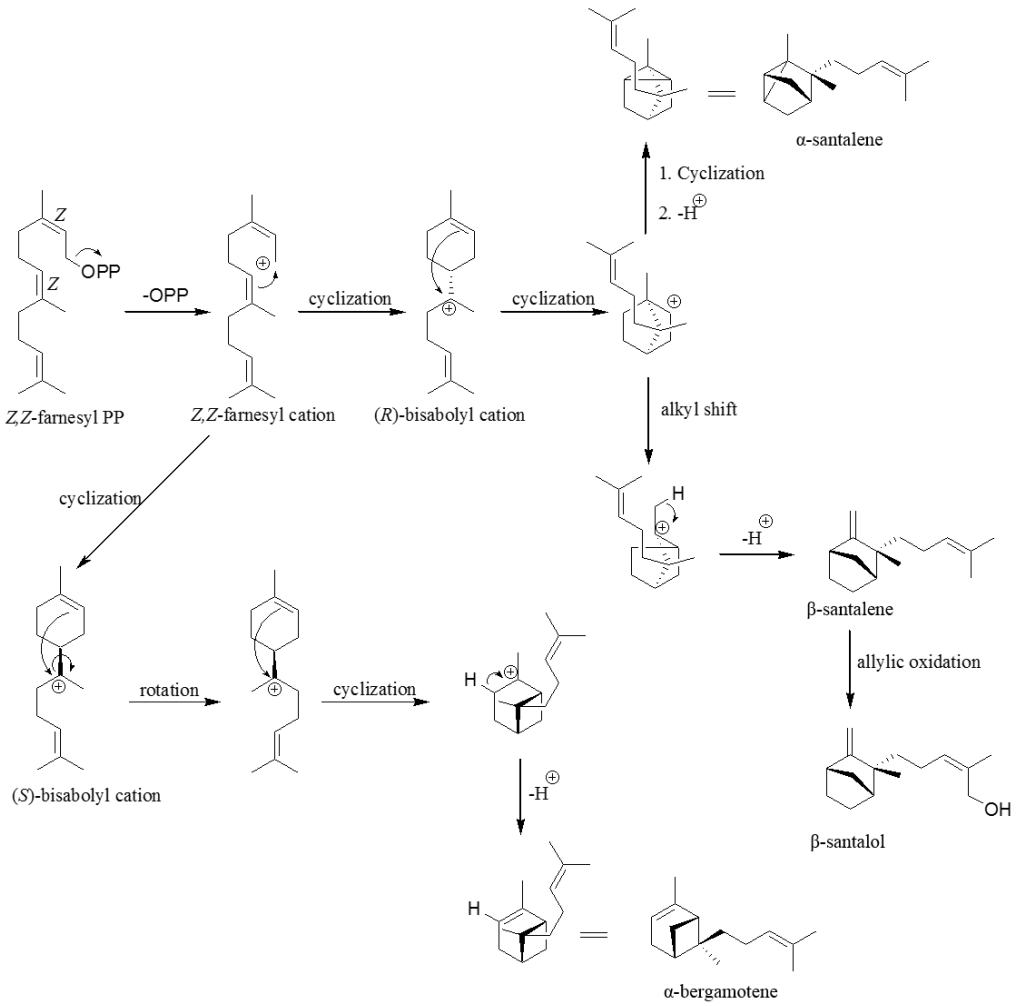


Fig 5. Proposed structural relationships of santalane-type sesquiterpenes

CONCULSION

In conclusion, the similar chemical profiles of three *Curcuma* species were found. The major volatile components were bisabolane-type sesquiterpene. The numerous related sesquiterpenes were observed due to their variety of cyclization, rearrangement and allylic oxidation. This finding might be a useful supporting for further application of these plants on pharmaceutical, food supplement and cosmetic proposes.

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