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Evaluation of coumarin content and essential oil constituents in *Cinnamomum cassia* (Nees & T. Nees) J. Presl.

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Abstract

The use of cinnamon bark from commercial sources has raised concerns globally, as it is widely adulterated with *Cinnamomum cassia* which possesses coumarin that is established as a hepatotoxic chemical in animal trials. The current study reveals the availability of *C. cassia* trees with high levels of cinnamaldehyde but low in coumarin. The estimated level of coumarin in three trees by HPLC analysis was found to be <100 mg kg^{-1} of dry bark which is below the stipulated upper levels put forth by the European Food Safety Authority and Food Safety Standards Authority of India. Considering the internationally accepted flavor of cassia oleoresin and the low coumarin levels, the *C. cassia* of Indian origin can be widely propagated and may be popularized along with *C. verum.*

Keywords: *C. cassia*, coumarin, HPLC, GC-MS

Introduction

The bark of cinnamon is popular as an aromatic spice in food industry, perfumery as well as in traditional medicine. The quality of cinnamon used is very important as any adulteration can have adverse effect on its efficacy and safety (Ding *et al.* 2011). True cinnamon or Ceylon cinnamon (*Cinnamomum verum* J. S. Presl/ *Cinnamomum zeylanicum* Blume) (Lauraceae) which is cultivated in Sri Lanka and south India is often adulterated with other cheaper cinnamon varieties especially *Cinnamomum cassia* (Nees & T. Nees) J. Presl. / *Cinnamomum aromaticum* Nees) popularly known as Chinese cassia found in Burma, China, and Vietnam, as well as *Cinnamomum burmannii* (Nees & T. Nees) Blume, known as Indonesian cassia native to Indonesia and Sumatra (WHO monographs 1999; He *et al.* 2005; Tainter & Grenis 2001). Internationally, the adulteration of cinnamon with other species is generally considered illegal.

Cassia cinnamon has a strong and spicy taste whereas true cinnamon is sweeter. Cassia bark is usually dark brown in color and rolled to the centre resembling scrolls and possess an average thickness of 1.5 mm. True cinnamon is light reddish-brown rolled into quills of less than 0.08 mm thickness and usually debarked. However,

in the case of cinnamon powder, this differentiation fails and reports prove that 85% of the ground cinnamon is Chinese cassia (Lungarini *et al.* 2008).

Chemically, cassia possess up to 1% 'coumarin'; while *Cinnamomum verum* has only trace (*i.e.* 0.004%) (WHO monographs 1999; He *et al.* 2005) which is major factor that differentiate cassia and cinnamon. Although *Cinnamomum cassia* essential oil is found to possess anti-tyrosinase activity (Chang *et al.* 2013), the coumarin present in it is said to have many drawbacks. Clinical studies using laboratory animals suggested that coumarin is hepatotoxic to rat and mice (IARC monographs Vol. 778). Liver tumors in rats and mice as well as lung tumors and Clara cell toxicity in mice were reported after clinical studies with coumarin (Felter *et al.* 2006). According to the German Federal Institute for Risk Assessment (BfR), very high level of coumarin administered over longer period can trigger cancer in rats and mice (Abraham 2007).

Although coumarin was suggested to be a genotoxic and carcinogenic in 1980's; subsequent studies suggest that it is not a genotoxic agent (Opinion 2004; Scientific opinion 2008, Lake *et al.* 1999). Adverse effects of coumarin exposure are rarely reported in humans and that too, only at high dosage and for long duration during clinical therapies. The International Agency for Research on Cancer (IARC) has categorized coumarin under group 3 ("not classifiable as to its carcinogenicity in humans") (IARC monographs Vol. 778). But based on the hepatotoxicity on laboratory animals, the US FDA prohibited synthetic coumarin being used as a food additive (Blahov´a & Svobodov´a 2012).

Since it is reported to have potential hepatotoxic effect in humans, the Codex alimentarius (Codex Alimentarius 1985) suggested a Tolerable Daily Intake (TDI) limit for the consumption of coumarin in food. According to this, the amount of coumarin in food and beverages should be limited to 2 mg kg^{-1} food/day; while the limit for caramels and alcoholic beverages is 10 mg kg^{-1}. This was later adopted by the European Commission (European 1988). Based on data of No-Observed-Adverse-Effect Level (NOAEL) on the hepatotoxicity during animal trials, the 'Scientific Panel on Food Additives, Flavorings, Processing Aids and Materials in Contact with Food (AFC)' and the 'European Food Safety Authority' stipulated a tolerable daily intake of 0.1 mg/kg of body weight (Opinion 2004; Scientific opinion 2008). The German Federal Institute for Risk Assessment (BfR) also proposed a similar guideline for the clinical use of coumarin (Abraham 2007). The BfR also gives a warning against consuming of high quantities of cinnamon powder sold as nutritional supplements or as diebetic foods to reduce blood sugar especially in the form of capsules.

The current research is undertaken to evaluate the coumarin content of the *Cinnamomum cassia* maintained at the ICAR-Indian Institute of Spices Research Experimental Farm germplasm collection and compare the same with market samples.

Materials and methods

Plant materials and chemicals

Dried bark from authentic samples of *Cinnamomum cassia* Nees were collected from 23 trees from the Experimental Farms at ICAR-Indian Institute of Spices Research, Chelavoor, Kozhikode (Kerala), All India Coordinated Research Project on Spices centres at Dapoli (Maharashtra) and Pechiparai (Tamil Nadu) (Fig. 1). The accessions tested include IC Nos. IC370401, IC370408, IC370410, IC370418, IC370419, IC370424, IC370428, IC370429, IC370423, IC370425, IC370427, IC370415 and IC370423. Two market samples of cassia were also analyzed for coumarin content (Fig. 1).

Coumarin (>99%), ammonium acetate and HPLC grade solvents (methanol, ethanol, acetonitrile and water) were purchased from Merck. Syringe filters with a pore size of 0.45 µm were obtained from Millipore.

Instrumentation and HPLC

The HPLC system consisted of a Shimadzu Model SCL-10AVP liquid chromatograph connected with a binary pump and a UV detector (Shimadzu SPD-10AVP). Separation was attained using a reversed phase column (Purospher Star RP-18 endcapped, Merck, Germany, Hibar RT 250 mm – 4.6 mm i.d., 5 µm pore size); using mobile phase A (water, 5 mM

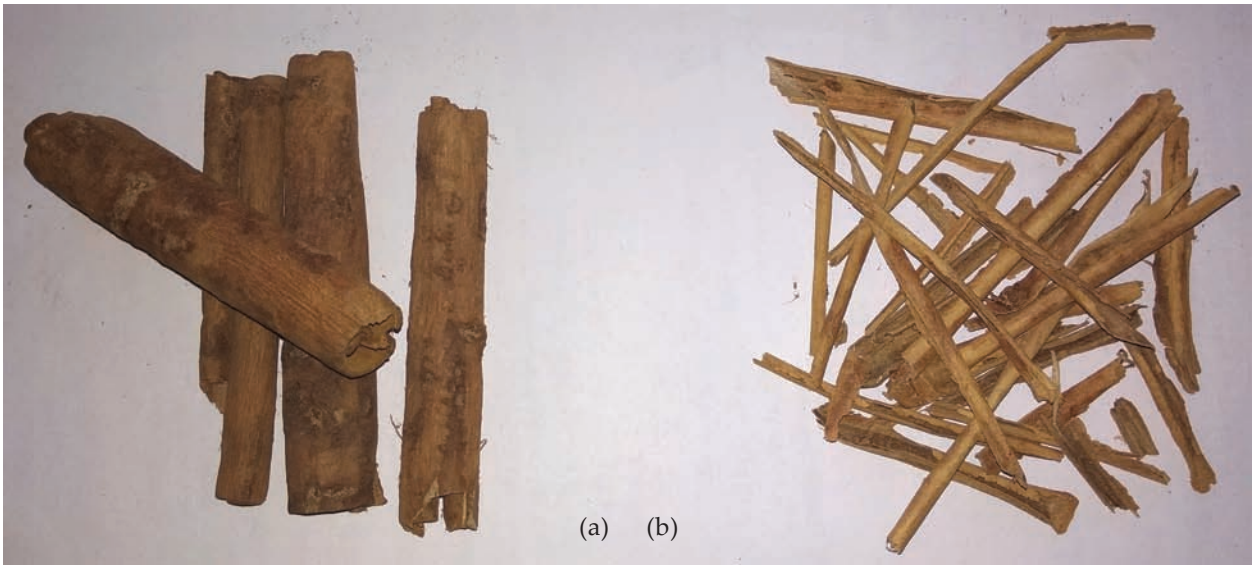

Fig. 1. (a) *Cinnamomum cassia* bark and (b) *Cinnamomum verum* bark

ammonium acetate buffer, 0.1% (v/v) acetic acid) and mobile phase B (acetonitrile/methanol 1:1 (v/v)) with gradient elution as follows: 0–14 min: 0–22% B; 14–16 min: 22–70% B; 16–22 min: 70% B, 22–25 min: 70-30% B; 30 min: stop. All solvents were filtered using Whatman No.1 filter and degassed before use. The rate of flow of the solvent was set to be 0.8 mL min^{-1}. The wave length was set to be 275 nm for quantitative analysis.

Preparation of standard coumarin

From a stock solution of coumarin (1 mg mL^{-1}) working standard of varying concentration *viz*., 10, 20, 40 and 100 μg mL^{-1} were prepared.

Extraction of coumarin

Coumarin was extracted from cassia using solvent extraction method (Sproll *et al.* 2008). About 0.5 g of powdered sample was taken, 25 mL of 90% HPLC methanol was added and agitated at room temperature for 30 min. It was filtered and injected to the HPLC system. Quantification of coumarin in the sample was done by comparing the peak area of the sample with that of standard coumarin.

Isolation of volatile components and GCMS analysis

Fresh leaf and bark samples of *C. cassia* were air-dried, ground into powder (60 mesh) and appropriate quantity (25 g bark 100 g^{-1} of leaves) was subjected to hydro-distillation using a Clevenger trap for 3.5 h. The collected essential oil was dried over anhydrous sodium sulfate and stored at 4°C. The yields of the essential oils were determined in duplicate, and mean values are reported.

The essential oil was analyzed (Lozhkin & Sakanyan 2006) using a Shimadzu GC-2010 gas chromatograph equipped with QP 2010 mass spectrometer and RTX-5 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Helium was used as the carrier gas with a flow rate of 1 ml/min. The temperature of the injection port was 250°C and of the detector was 220 °C. The temperature programming of the oven was as follows: 60°C for 5 min, then ramped to 110°C at a rate of 5°C min^{-1}, then to 200 °C at 3 °C min^{-1}, again to 220 °C at 5 °C min^{-1}, at which the column was maintained for 5 min. The ionization energy was 70 eV and split ratio was 1:40. Injection volume was 0.1 μL. The essential oil constituents were identified by matching the mass spectral data with those in NIST and Wiley libraries and by manual matching to the library of mass spectra of essential oils (Adams *et al.* 1989).

Results and discussion

HPLC analysis of C. cassia

The coumarin content in *C. cassia* determined by HPLC analysis (Fig. 2) is given in Table 1. In general, the coumarin content in the leaf was

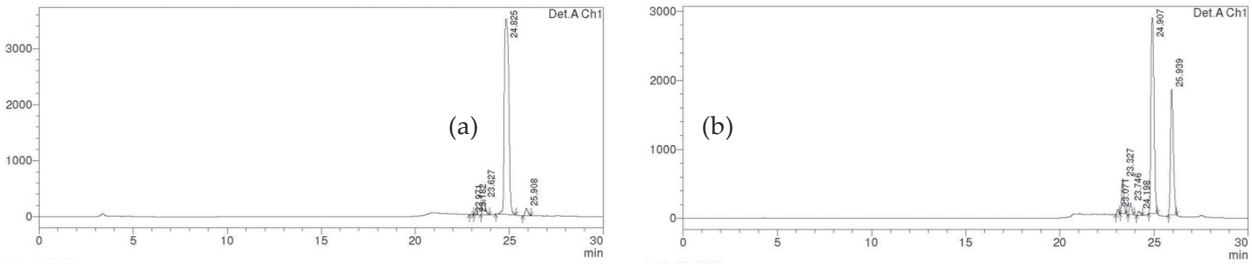

Fig. 2. HPLC chromatogram of (a) *C. cassia* bark, (b) *C. cassia* leaf

found to be much higher than that of the bark. Among the barks the coumarin level was found to be less than 100 mg kg^{-1} (3–86 mg kg^{-1}); whereas the reported values of market samples are in the range of 3000–5000 mg kg^{-1} (Shimna *et al.* 2017). The coumarin level varied slightly among trees of same accession. The coumarin level in the leaf was in the range of 481–2462 mg kg^{-1}. Earlier reports indicate higher coumarin content in leaf than bark (Shimna *et al.* 2017).

To analyze the variation in the coumarin content with respect to location, samples from two experiential stations were also analyzed (Table 2). Samples from cassia grown in Pechiparai (Tamil Nadu) as well as the trees from AICRP centre at Dapoli (Maharashtra) were tested and found to possess low coumarin levels. Coumarin content in *C. verum* bark was found to be 53 mg kg^{-1} and notably, the leaves possessed significantly lower levels of coumarin (13 mg kg^{-1}) in comparison with *C. cassia* leaves; which may be used as a distinguishing factor among the species of *C. verum* and *C. cassia*.

Table 1. Coumarin content of *C. cassia* accessions from ICAR-Indian Institute of Spices Research Farm

IC no.	Sample code	Coumarin (mg kg^{-1})			
		Bark		Leaf	
IC370401	C1	17	Range:17–31	1410	Range:957–1410
	C2	24	Mean:24	1408	Mean:1195
	C3	31		957	
	C4	25		1005	
IC370408	C5	45	Range: 45–51	949	Range:949–1345
	C6	51	Mean: 48	1345	Mean:1147
IC370410	C7	12		1092	
	C8	15	Range: 3–17	789	Range: 679–789
IC370418	C9	3	Mean:12	679	Mean:722
	C10	17		699	
	C11	16	Range: 16–57	1722	Range: 682–2462
IC370419	C12	47	Mean:40	682	Mean:1622
	C13	57		2462	
IC370424	C14	21	Range: 21–22	1887	Range: 481–1887
	C15	22	Mean:21	481	Mean:1184
IC370428	C16	70		1183	
IC370429	C17	79	Range: 79–86	1584	Range: 822–1584
	C18	86	Mean:82	822	Mean:1203

Table 2. Coumarin content of *Cinnamomum cassia* from Experimental Farms of Dapoli and Pechiparai

AICRPS - Dapoli		Coumarin (mg kg^{-1})	
		Bark	Leaf
IC370423	C19	20	1307
IC370425	C20	44	718
IC370427	C21	30	601
IC370415	C22	68	894
Pechiparai			
IC370423	C23	57	763
C. verum		53	13
Cassia-Market Sample-a		1373	
Cassia-Market Sample-b		3355	
Indonesian cassia		3357	

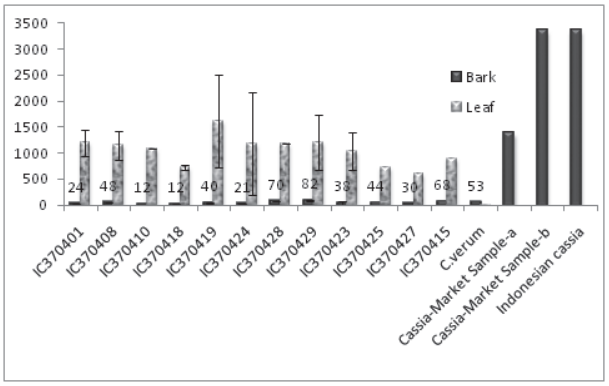

Fig. 3. Variation in Coumarin content among leaf and bark of *C. cassia*

It has been earlier reported that the cinnamon available in the market contains varying levels of coumarin (Ananthakrishnan *et al.* 2018) and usually very high compared to the 0.3% stipulated by the Food Safety and Standards Authority of India (FSSAI 2017). Frequently, true cinnamon is adulterated with *C. cassia* which is the main cause of high coumarin content in the market samples. Commercial samples that were analyzed in the present study were also found to have high levels of coumarin.

The variations within the trees of the same IC numbers are depicted in Fig. 3 and it indicates a higher variation in coumarin content among the leaves than in barks. This is because coumarin is synthesized in leaf and then transported to the bark through phloem (Shimna *et al.* 2017). Earlier reports from the literature also reveal a wide variation in the coumarin content of bark samples of *C. cassia, C. burmann* and *C. loureirii.* Studies by Archer *et al.* (1988) and Sagara *et al.* (1987) revealed that coumarin levels in cassia bark ranged from 40 mg kg^{-1} to 11180 mg kg^{-1}. Further studies of He *et al.* (2005) reported coumarin in the range of 40–850 mg kg^{-1} in authentic cassia samples, whereas it ranged from 130 to 12180 mg kg^{-1} in unspecified market samples. Two different groups of German researchers reported coumarin levels from 130-10900 mg kg^{-1} (Woehrlin *et al.* 2010) and 2880–4820 mg kg^{-1} (Sproll *et al.* 2008) in cassia bark. Wang *et al.* (2013) reported very low levels of coumarin in cassia samples; *viz.*, 85–310 mg kg^{-1} where as *C. burman* bark; it was 2140–9300 mg kg^{-1} and in *C. loureirii* bark possessed 1060–6970 mg kg^{-1} of coumarin.

Essential oil composition of C. cassia

The leaf and bark of cinnamon cassia yielded light yellowish essential oil and the yield is tabulated in Table 3. The yield of oil from leaf samples was much lower (0.27%–0.7%) compared to that of the bark (2%–4%) (Krishnamoorthy *et al.* 1999). Earlier reports points out that the cells containing essential oil are primarily distributed in the bark and are plenty in the phloem of thick barks (Li *et al.* 2013). The chemical constituents identified in the GCMS analysis are listed in Tables 4 & 5. As outlined in Tables 4 & 5, there are 15 compounds common among the bark samples and a total of 20 components in leaf samples; which represents 89.95-99.64% of the essential oil constituents. Most of the constituents in oil are terpenes, consisting of monoterpenes, sesquitepenes and phenyl propanoids (Bongiovanni *et al.* 2017) . The major compound in the bark oil was t-cinnamaldehyde (75–97%) and minor compounds such as delta-cadinene (0.09-3.07%) and o-methoxy cinnamaldehyde (0.44–5.49%) were also observed. The presence of delta-cadinene helps to differentiate *C. cassia* from *C. verum* (He *et al.* 2005). A typical GCMS chromatogram of *C. cassia* bark and *C. cassia* leaf are given in Fig. 4.

Essential oil from *C. cassia* leaf (Table 5) yielded 33.5–69.3% t-cinnamaldehyde along with o-

Table 3. Yield (%) of essential oil derived from the *C. cassia* leaf and bark

No.	Sample code	Essential oil (%)	
		Leaf	Bark
1	C1	0.30	2.4
2	C2	0.27	2.8
3	C3	0.30	2.8
4	C4	0.27	2.5
5	C5	0.27	2.0
6	C6	0.35	2.4
7	C7	0.55	2.5
8	C8	0.55	2.4
9	C9	0.40	2.0
10	C10	0.65	4.0
11	C11	0.60	2.4
12	C12	0.50	3.3
13	C13	0.35	4.0
14	C14	0.45	3.2
15	C15	0.55	3.6
16	C16	0.70	4.0
17	C17	0.55	2.4
18	C18	0.50	2.4
	C. verum	4.0	2.0
	Market Sample		2.1

methoxy cinnamaldehyde (11.29–23.37%) as major compounds. Presence of coumarin was prominent in all leaf samples and varied from 0.2–1.2%. However, it was not detected in the bark oil due to the very low concentration in the bark. Eugenol, which is the most prominent compound in the leaf oil of *C. verum* was not detected in the essential oil leaf of *C. cassia* (He *et al.* 2005).

C. verum is often adulterated with cheap *C. cassia* in commercial samples. Although there are conflicting reports on the medicinal values of *C. cassia* (Dinesh *et al.* 2015), the consumption of cassia with high coumarin content needs to be discouraged due to its hepatotoxicity in laboratory animals. In this context, the current study opens up the possibility of using cassia bark with less amount of coumarin that is maintained at the ICAR-Indian Institute of Spices Research germplasm collection as well as at the experimental farms of AICRPS centers. It also indicates that growing *C. cassia* in India may be beneficial to obtain barks with low coumarin. The bark of these samples were found to possess less than 100 mg kg^{-1} of coumarin which is well within the limits stipulated by FSSAI; whereas the market samples contain more than 3000 mg kg^{-1} of coumarin. These accessions of cassia also contain high levels of t-cinnamaldehyde which is responsible for the sweet flavor to the spice. Hence it is highly recommended that these low coumarin cassia accessions can be propagated along with *C. verum* to meet the global demand of cinnamon and must be made popular in the international market.

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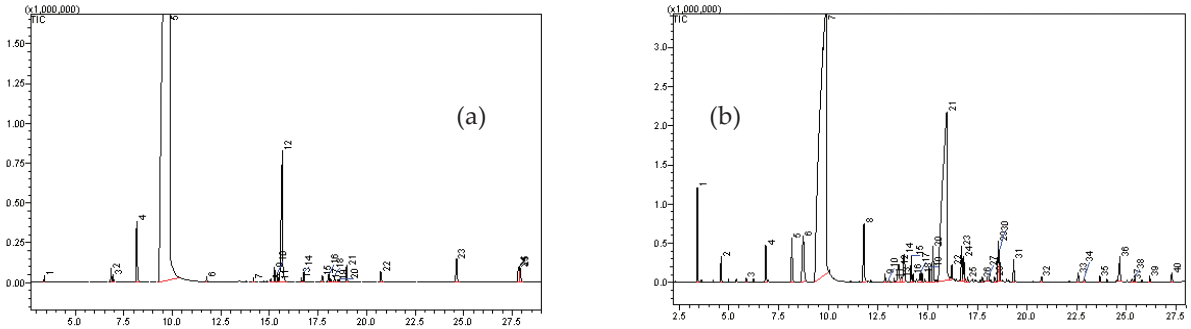

Fig. 4. GCMS chromatogram of (a) *C. cassia* bark, (b) *C. cassia* leaf

Table 4. Essential oil composition of *Cinnamomum cassia* bark

Compound	C 1	C2	C3	C4	C5	C6	C7	C9	C10	Market
Benzaldehyde	0.46	0.27	0.38	0.43	0.99	0.57	0.11	0.10	0.12	0.19
Hydrocinnamaldehyde	0.30	0.41	0.38	0.80	0.21	0.15	0.30	0.33	0.22	0.71
Borneol	0.16	0.15	0.16	-	-	0.07	0.11	0.28	0.07	-
Cinnamaldehyde	1.11	1.61	1.48	1.39	0.95	0.92	1.88	1.93	1.29	1.03
t-Cinnamaldehyde	86.90	89.50	89.70	93.70	77.30	87.3	87.7	75.1	96.7	79.94
α-Copaene	0.71	2.24	1.51	0.41	-	0.28	0.11	3.88	0.37	2.04
t-Cinnamic acid	4.47	0.84	1.91	1.63	16.10	3.43	-	-	0.09	-
γ-Muurolene	0.09	0.29	0.28	-	-	0.12	-	1.46	0.08	0.58
α-Muurolene	0.09	0.14	0.12	-	-	-	-	0.67	0.03	3.41
δ-Cadinene	0.23	0.58	0.41	0.09	-	0.23	0.32	3.07	0.18	4.51
o-Methoxycinnamaldehyde	3.32	1.43	1.53	1.11	1.95	4.47	5.49	1.75	0.44	1.03
Caryophyllene oxide	0.25	0.14	0.16	-	0.11	0.14	0.20	0.36	-	-
δ-Cadinol	0.17	-	-	-	-	-	0.23	0.83	-	1.16
α-Cadinol	0.21	0.15	0.11	-	-	-	0.15	0.56	-	-
Benzyl benzoate	0.26	0.16	0.14	0.10	0.24	0.14	0.31	0.26	0.05	-

Table 5. Essential oil composition of *Cinnamomum cassia* leaf

Compound	C 1	C2	C3	C4	C5	C6	C7	C9	C10
Benzaldehyde	2.10	1.49	2.12	1.14	1.58	1.33	1.35	1.67	1.80
Hydrocinnamaldehyde	1.46	2.57	1.29	1.54	0.93	1.78	1.33	0.76	1.06
L-Borneol	1.04	1.05	0.73	0.36	0.39	0.39	0.58	0.36	0.41
Cinnamaldehyde	0.43	0.03	0.07	0.72	1.47	1.22	1.82	1.74	0.45
o-Anisaldehyde	2.77	2.13	3.29	1.99	2.64	4.39	3.13	2.40	2.08
t-Cinnamaldehyde	38.97	33.50	57.45	51.43	54.82	39.68	43.80	69.30	67.86
2-Methoxyphenylacetone	1.80	2.40	2.57	1.94	1.96	4.67	3.68	1.03	1.27
Coumarin	1.25	0.46	0.70	0.69	0.90	0.78	0.27	0.47	0.46
2-Methoxycinnamaldehyde	0.69	-	-	0.38	1.21	1.38	0.86	0.58	0.28
Alloaromadendrene	0.96	1.85	1.71	1.2	0.27	1.05	0.89	0.22	0.79
γ-Muurolene	1.72	1.10	0.53	0.67	0.57	1.49	1.39	0.06	0.23
δ-Cadinene	0.59	1.20	0.47	1.32	1.09	2.09	2.31	0.33	0.44
o-Methoxycinnamaldehyde	13.32	11.29	18.55	15.00	22.53	23.37	21.69	15.23	15.91
Nerolidol	0.95	2.68	1.11	1.02	0.40	0.64	0.87	0.09	0.66
Spathulenol	1.60	2.48	0.75	1.18	0.96	1.48	1.00	0.55	0.62
Caryophyllene oxide	1.75	2.69	0.85	1.14	0.78	1.51	1.02	0.28	0.39
τ-Cadinol	0.43	1.38	0.29	0.60	0.30	0.83	0.56	0.06	-
α-Cadinol	0.59	1.24	0.15	0.14	0.18	0.16	0.11	-	-
Benzyl benzoate	0.93	1.27	0.13	0.70	0.18	0.83	0.46	0.19	0.16

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