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PHYTOCHEMICAL PROFILING OF BARK AND LEAF VOLATILE OIL OF TWO WILD CINNAMOMUM SPECIES FROM EVERGREEN FORESTS OF WESTERN GHATS

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Abstract

Cinnamonum riparium and *C. macrocarpum* are the two under exploited wild species those can potentially act as an alternative source for *Cinnamonum* oil. Present investigation was carried out to assess and compare species and organ wise yield of *Cinnamonum* oil and distribution of cinnamaldehyde, eugenol and cinnamyl acetate in the oil. Phytochemical profile of both species also was compared. Results indicated that the bark and leaf oil of *C. riparium* contained 26 and 20 constituents and those of *C. macrocarpum* contained 69 and 68 constituents respectively. Phytochemical profiling of volatile oils indicated that the major constituents of *C. riparium* bark oil were euginyl methyl ether (63.65%), Shikimole (15.76%), delta cadinene (2.24%), whereas that of leaf was euginyl methyl ether (83.38%) and shikimole (14.04%). The major compounds in bark oil of *C. macrocarpum* were benzyl benzoate (49.68%), linalool (6.66%), 4-teroinol (3.62%), cinnamyl acetate (3.09%). *C. macrocarpum* leaf oil showed a different chemical profile in which gamma terpinene (21.47%), azulene (7.23%), cinnamyl acetate (4.65%) were the major compounds. Distribution of minor compounds in the profile showed variation due to species and organ. Quantification of major commercially exploited chemical constituents *viz*. cinnamaldehyde, eugenol and cinnamyl acetate indicated that the concentration in bark and leaf volatile oils varied with species. Although, the oil recovery from these species were low compared to other commercially exploited species theses species can be used as an alternative for oil extraction.

Key words : Cinnamomum riparium, C. macrocarpum, cinnamaldehyde, eugenol, cinnamyl acetate.

Introduction

Non timber forest products play a major role income generation of local communities in and around forests. *Cinnamomum* is the largest genus of the family Lauraceae comprising 250 species, mainly evergreen trees in tropical and subtropical latitudes. This genus have a centre of diversity in Western Ghats and the adjoining regions of south India and it possesses considerable economic importance as several species are the source of '*Cinnamomum* of commerce'. *Cinnamomum cassia* and *C. zeylanicum* are the two widely cultivated species, which are over exploited because of high spice and medicinal value (Ravindran *et al.*, 2004). The most important *Cinnamomum* oils in world trade are obtained from *C. zeylanicum*, *C. cassia* and *C. camphora*. However, most of the species of *Cinnamomum* yield

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volatile oil on distillation, which can be utilized as sources for chemical isolates. But these are distilled on a much smaller scale and these oils either used locally or exported (Anonymous, 1992). A better understanding chemical constituent of wild species of Cinnamomum gives an edge as an alternative resource. Most of the wild species of Cinnamomum are in danger of extinction due to indiscriminate extraction of bark (Priya and Maridass, 2008). Cinnamaldehyde, eugenol and cinnamyl acetate are the most commercially exploited compounds in Cinnamomum oil. C. riparium and C. macrocarpum are the two under exploited wild species of *Cinnamomum*. C. riparium is a small tree usually found in the bank of streams in evergreen forest and is distributed in Southern Western Ghats. C. macrocarpum is a medium sized tree, up to 15 m tall and about 75 cm girth distributed in Southern Western Ghats. Present study was undertaken with the following objectives. 1- To quantify the species and organ wise yield of *Cinnamomum* oil, 2- Find out the distribution of most commercially exploited cinnamaldehyde, eugenol and cinnamyl acetate and 3- phytochemical profiling of both *C. riparium* and *C. macrocarpum*. This paper forms the first report on chemical composition of the volatile oils of two wild species of *Cinnamomum*.

Materials and Methods

The bark and leaves of *Cinnamomum riparium* were collected from Aralam, Kannur district, Kerala and those of *C. macrocarpum* were collected from Munnar, Idukki district, Kerala. The specimens were identified and voucher specimen was deposited in at Kerala Forest Research Institute Herbarium (Herbarium No: KFRI 11085 and KFRI 11083, respectively).

Bark and leaf oil of both species were extracted by hydro-distillation using Clevenger type apparatus. Sliced plant material was continuously distilled for six hours and the volume of oil was determined using graduated oil trap. Extracted oil samples were dissolved in GC grade Hexane and stored in sealed vial at low temperature till the analysis.

Phytochemical profiling of bark and leaf oil of both species were carried out using Shimadzu GC-2010.GC equipped with OP2010 MS and DB-5 column (30 m \times $0.25 \times 0.25 \ \mu$ m). Helium was used as the carrier gas at a flow rate of 1 ml/min. The injection port was maintained at 200°C; the detector temperature was 200°C; oven was programmed as follows. Initial temperature 80°C/min. then increased to 200°C at a rate of 4°C/min and held for 14 min, then to 280°C at 10°C/min at which the column was maintained for 2 min. The split ratio was 1:50; 1µL sample was injected. The constituents of the oil were identified by the comparison of retention indices by matching the mass spectrometer with those stored in the National Institute of Science and Technology library (Strenhagen et al., 1974). Quantification of vital compounds in oil was done by co-injection with authentic standard.

Results

Volatile oil from bark and leaf of *C. riparium* and *C. macrocarpum* was obtained through hydrodistillation and analyzed in GC-MS. Oil yield on hydrodistillation of leaf and bark of *C. riparium* was 2.0 and 1.6 ml/kg and that of *C. macrocarpum* was 3.0 and 5.0, respectively (fig. 1). Of the plant components, oil yield from bark was higher and of the two species, *C. macrocarpum* recorded a higher oil content. However, between species variation in oil content of the *Cinnamomum* species was not statistically significant.

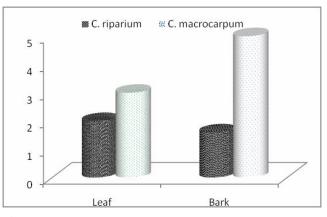


Fig. 1 : Oil yield (ml/kg) from bark and leaf of *Cinnamomum riparium* and *C. macrocarpum*.

Quantification of cinnamaldehyde, eugenol and cinnamyl acetate

Quantification of major commercially exploited chemical constituents viz. cinnamaldehyde, eugenol and cinnamyl acetate in the volatile oil of bark and leaf of the two species were carried out. Cinnamaldehyde, eugenol and cinnamylacetate concentration of bark and leaf volatile varied with species (table 1). Among the species, C. riparium showed highest quantity of cinnamaldehyde, eugenol and cinnamylacetate. The highest cinnamaldehyde content was observed in the leaf oil of C. riparium and the lowest was recorded in the bark oil of C. macrocarpum. On an average, cinnamaldehyde content of leaves and bark of C. riparium was 34 and 9.5 times more compared to that of C. macrocarpum. Eugenol content was the highest in C. riparium leaf oil and C. macrocapum bark oil showed the least quantity. With regards to cinnamylacetate content, C. riparium leaf showed the highest quantity of oil and the C. macrocarpum bark showed the least. Organ wise and species wise analysis of cinnamaldehyde, eugenol and cinnamylacetate revealed varying distribution of constituents which can be used as an indicator of oil quality. Significant difference in cinnamaldehyde and eugenol content of both C. riparium and C. macrocarpum due to main effect of species, plant part and the interaction effect of species x plant part at one per cent level. However, only the main effect of species and plant part was significant (p = 0.01 with respect to)cinnamylacetate content.

Phytochemical profiling

The volatile oil extracted from bark and leaf of *C. riparium* and *C. macrocarpum* through hydro-distillation was analyzed using GC-MS. Phytochemicals were identified by matching their fragmentation pattern in Mass Spectra with those stored in National Institute of

 Table 1 : Distribution of Cinnamaldehyde, eugenol and cinnamylacetate in bark and leaf of Cinnamomum riparium and C.

 macrocarpum.

Species .	Leaf (µg/ml of extract)		Bark(µg/ml of extract)			
	Cinnamaldehyde	Eugenol	Cinnamyl acetate	Cinnamaldehyde	Eugenol	Cinnamyl acetate
C. riparium	143.64	43.53	27.85	19.29	1.28	13.64
C. macrocarpum	4.25	0.41	18.73	2.04	3.25	5.28

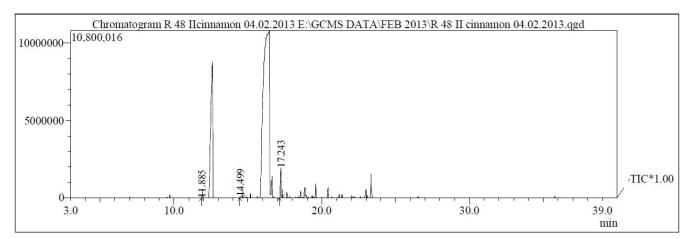
Table 2 : Phytocompounds identified in *Cinnamomum riparium* bark volatile oil.

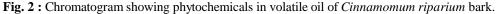
Sl. no.	Compounds	Retention time (min)	Peak area (%)
1.	Camphor	8.306	1.43
2.	Cinnamylaldehyde	11.805	0.37
3.	Rhyuno oil, Shikimole	12.464	15.76
4.	Eugenol	14.469	1.2
5.	Copaene	15.166	2.64
6.	BETAELEMENE	15.626	0.07
7.	Eugenyl methyl ether	16.058	63.65
8.	2-NORPINENE	16.353	0.13
9.	betaCaryophyllen	16.544	2.9
10.	.ALPHATRANS-BERGAMOTENE,	16.945	0.21
11.	Cinnamyl acetate	17.104	1.02
12.	ALPHACARYOPHYLLENE \$\$. ALPHAHUMULENE	17.542	0.68
13.	GAMMA-MUUROLEN	18.182	0.29
14.	alphaCurcumene	18.307	0.21
15.	Ledene \$\$ Varidiflorene	18.765	0.82
16.	Naphthalene, ALPHA-MUUROLEN	18.882	1
17.	DELTACADINENE	19.558	2.24
18.	AROMADENDREN	21.359	1.47
19.	5-AZULENEMETHANOL, GUAIOL	21.639	0.47
20.	betaEudesmol	21.851	0.17
21.	Myristic aldehyde	22.024	1.13
22.	Aromadendrene	22.967	0.98
23.	deltaCadinol	23.074	0.42
24.	Gamma Gurjunene	23.316	0.43
25.	ASCABIN, Benzyl Benzoate	26.185	0.12
26.	Palmitic Acid	31.197	0.19

Standards and Technology library. The *C. riparium* bark oil contained 26 constituents (table 2) and the leaf oil contained 20 constituents (table 3). The major constituents of *C. riparium* bark were euginyl methyl ether (63.65%), Shikimole (15.76%), delta cadinene (2.24%), whereas leaf contained euginyl methyl ether (83.38%) and shikimole (14.04%). Minor compounds showed variation in distribution (figs. 2 & 3). *C. macrocarpum* bark oil contained 69 constituents (table 4) and that from leaf contained 68 constituents (table 5). The major compounds in bark oil were benzyl benzoate (49.68%), linalool (6.66%), 4-teroinol (3.62%), cinnamyl acetate (3.09%). While, *C. macrocarpum* leaf oil showed a different profile in which gamma terpinene (21.47%), azulene (7.23%), cinnamylacetate (4.65%) were the major compounds (figs. 4 and 5).

Discussion

C. zeylanicum, *C. cassia* and *C. camphora* are the most important source of cinnamon oil in world trade. However, many species of cinnamon yield a volatile oil on distillation which can be utilized as sources for chemical isolates. The oil yielding potential of such species required study because it is known that some species are differing in oil compositions due to the existence of different chemotypes (Coppen, 1995). Although, most parts of the





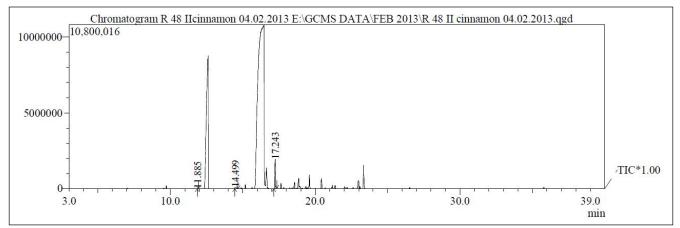


Fig. 3 : Chromatogram showing phytochemicals in volatile oil of *Cinnamomum riparium* leaf.

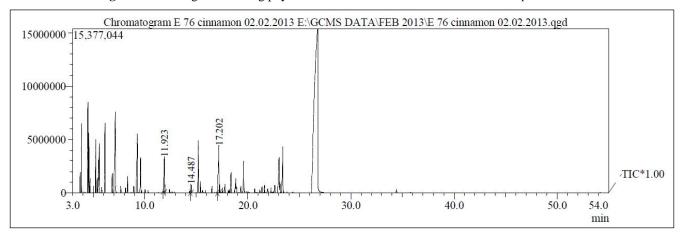


Fig. 4 : Chromatogram showing phytochemicals in volatile oil of *Cinnamomum macrocarpum* bark.

tree like leaf, bark, flower buds yield oil, the recovery varies from part to part. The oil recovery from the leaves of *Cinnamomum zeylanicum* is about 1.6 - 1.8% and that from bark is 0.5 - 1.00% oil which was higher compared to both the species in the present study. Of the plant parts, oil recovery from bark was 25 and 66% higher compared to leaf in *C. riparium* and *C. macrocarpum*, respectively.

Results of the presenty study revealed that the major commercially exploited chemical constituents *viz*. cinnamaldehyde, eugenol and cinnamylacetate in bark and leaf volatile varied between species. The flavour and aroma of cinnamon is due to an essential oil which contains many different compounds, but the most abundant (65 to 75% of the oil) is cinnamaldehyde (Newall *et al.*, 1996). In the present investigation, *C. riparium* showed

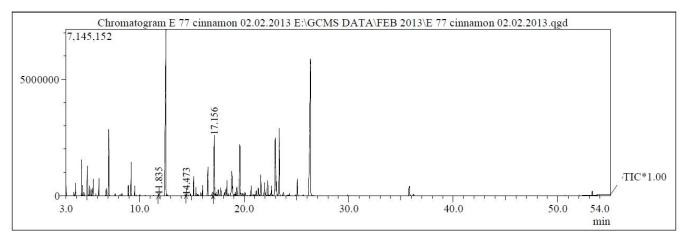


Fig. 5: Chromatogram showing phytochemicals in volatile oil of Cinnamonum macrocarpum leaf.

Sl. no.	Compounds	Retention time (min)	Peak area (%)
1.	ALPHAPINENE	3.854	0.06
2.	BETAMYRCENE	4.550	0.02
3.	Camphor	8.307	0.06
4.	Cinnamylaldehyde	11.805	0.12
5.	Rhyuno oil, Shikimole	12.513	14.04
6.	Eugenol	14.474	0.28
7.	Copaene	15.164	0.11
8.	Eugenyl methyl ether	16.231	83.39
9.	betaCaryophyllene	16.573	0.52
10.	Cinnamyl acetate	17.104	0.52
11.	ALPHACARYOPHYLLENE, ALPHAHUMULENE	17.573	0.09
12.	1,6-CYCLODECADIENE	18.207	0.01
13.	GERMACRENE B	18.823	0.1
14.	NAPHTHALENE	18.898	0.01
15.	1,3-BENZODIOXOLE	19.425	0.03
16.	DELTA-CADINENE	19.566	0.21
17.	BETAASARONE, Isoelemecin	20.413	0.45
18.	deltaCadinol,	21.356	0.11
19.	Aromadendrene	22.957	0.17
20.	gammaGurjunene	23.319	0.32

highest quantity of cinnamaldehyde, eugenol and cinnamylacetate. Highest concentration of cinnamaldehyde (14.3%) was present in C. riparium bark oil. Senanayake et al. (1978) stated that the major compounds present in stem-bark oil and root bark oil of *Cinnamomum zeylanicum* were cinnamaldehyde (75%) and camphor (56%), respectively. The cinnamadehyde content in the volatile oils from bark and leaf of two species were lower compared to earlier reports. Our results are inconsistent with the finding of Simic et al. (2004) that the bark oil is rich in transcinnamaldehyde and a leaf oil is rich in eugenol. Organ wise and species wise analysis of cinnamaldehyde, eugenol and

cinnamylacetate revealed varying distribution, which can be used as an indicator of oil quality, which helps to develop international standards.

Perusal of literature on phytochemical profiling of *Cinnamomum* species indicated that the plants from different localities and different plant parts yield oil with varying chemical composition (Kaul *et al.*, 2003). The number of constituents in the present investigation also varied with species and plant part. The major constituents also showed variation among the species. The major compounds in of *C. macrocarpum* leaf were different from that of bark oil with benzyl benzoate (49.68%),

Table 4 : Phytocompounds identified in *Cinnamomum macrocarpum* bark volatile oil.

Sl. no.	Compounds	Retention time (min)	Peak area (%)
1.	alphaPhellandrene	3.741	0.45
2.	alphaPinene	3.88	1.67
3.	Camphene	4.117	0.03
4.	Benzaldehyde, Artificial Almond Oil	4.232	0.02
5.	SABINENE	4.489	2.65
6.	BICYCLOHEPTANE,BETA-PINEN	4.583	1.47
7.	1,6-OCTADIENE,BETAMYRCENE	4.691	0.33
8.	1,3-CYCLOHEXADIENE, ALPHA-HELLANDRENE	5.02	0.18
9.	BICYCLOHEPT-3-ENE,	5.144	0.01
10.	1,3-CYCLOHEXADIENE,ALPHA. TERPINENE	5.26	1.47
11.	CYMENE, CYMOL	5.4	0.41
12.	BICYCLO[3.1.0]HEXANE,SABINENE	5.541	1.4
13.	Eucalyptol, Cineole	5.597	1.27
14.	1,3,6-Octatriene	5.825	0.15
15.	1,4-CYCLOHEXADIENE	6.151	2.27
16.	CYCLOHEXENE, ALPHA TERPINOLEN	6.839	0.6
17.	1,6-OCTADIEN-3-OL, LINALOOL	7.165	6.66
18.	2-NORBORNANOL, ALPHAFENCHOL	7.505	0.01
19.	5-ISOPROPYL-2-METHYLBICYCLOHEXAN-2-OL	8.144	0.16
20	Bicyclo heptan-2-one,Camphor	8.339	0.55
21.	Hydrocinnamaldehyde	8.693	0.02
22.	Benzoic acid	8.946	0.29
23.	4-Terpineol	9.289	3.62
24.	(+)-ALPHA-TERPINEOL	9.614	1.6
25.	3-Carene,Bicyclo[4.1.0]hept-3-ene	11.333	0.02
26.	Cinnamaldehyde	11.923	2.49
27.	1,3-Benzodioxole, methylene ether	12.416	0.12
28.	Propanoic acid	12.655	0.04
29.	Butanoic acid	14.11	0.01
30.	alphaCubebene	14.343	0.06
31.	Eugenol	14.487	0.36
32.	BICYCLO HEPT-3-ENE,-3-CARE	14.68	0.02
33.	1,6-CYCLODECADIENE	14.883	0.01
34.	Copaene	15.213	2.31
35.	Pentanoic acid	15.398	0.43
36.	1,6-CYCLODECADIENE	15.597	0.12
37.	Eugenol methyl ether	15.878	0.11
38.	Caryophyllene	16.523	0.26
39.	transalphaBergamotene	16.938	0.05
40.	CINNAMYLACETATE	17.202	3.09
41.	alphaCubebene	17.433	0.05
42.	alphaCaryophyllene alphaHumulene	17.553	0.2
43.	1H-Cycloprop[e]azulene	17.651	0.01
44.	1H-CYCLOPROP[E]AZULENE	17.777	0.45
45.	Naphthalene.delta.Cadinene	18.115	0.1
46.	Naphthalene, gammaMurrolene	18.204	0.14

Table 4 continued....

47.	1,6-CYCLODECADIENE	18.376	0.9
48.	NAPHTHALENE	18.688	0.1
49.	gammaElemene	18.832	0.8
50.	alphaMuurolene	18.899	0.21
51.	Naphthalene	19.089	0.03
52.	Naphthalene, gammaCadinene	19.318	0.32
53.	NAPHTHALENE	19.593	1.47
54.	alphaMuurolene	19.992	0.04
55.	NEROLIDOLA	20.676	0.16
56.	SPATHULENOL	21.172	0.13
57.	1H-CYCLOPROP[E]AZULENE	21.376	0.28
58.	Cubenol	22.237	0.2
59.	Azulene	22.462	0.03
60.	(+)-BETA-GUAJEN	22.533	0.04
61.	alphaCubebene	22.608	0.33
62.	1-Naphthalenol	23.027	2.69
63.	tauMuurolol	23.398	2.57
64.	ALPHASELINENE, EUDESMA-3	23.495	0.04
65.	Isovaleric acid, cinnamyl ester	23.8	0.04
66.	Naphthalene.gammaMurrolene	24.354	0.03
67.	BENZYLBENZOATE	26.771	49.68
68.	PALMITIC ACID	31.173	0.01
69.	1,4-BUTANEDIONE	34.411	0.2

Table 4 continued...

 Table 5 : Phytocompounds identified in Cinnamomum macrocarpum leaf volatile oil.

Sl. no.	Compunds	Retention time (min)	Peak area (%)
1.	BICYCLO HEXANE, Thujene	3.742	0.11
2.	LINALOOL	3.876	0.41
3.	3-Cyclohexen-1-ol, 4-Terpineol	4.475	1.33
4.	1,3-Benzodioxole	4.572	0.41
5.	Caryophyllene	4.688	0.14
6.	NAPHTHALENE	5.144	0.06
7.	1-Naphthalenol	5.252	0.39
8.	1-NAPHTHALENOL	5.396	0.25
9.	Benzyl Benzoate Ascabin	5.53	0.47
10.	BICYCLO .ALPHATHUJENE	5.584	0.7
11.	BICYCLO HEPT-2-ENE .ALPHA(+)-PINENE	5.825	0.09
12.	BICYCLO HEXANE, SABINENE	6.13	0.75
13.	betaPinene,Bicyclo heptane	6.767	0.02
14.	1,6-OCTADIENE,BETAMYRCENE	6.834	0.32
15.	alphaPhellandrene	7.071	4.07
16.	3-Carene	7.142	0.02
17.	1,3-CYCLOHEXADIENE,ALPHA. TERPINENE	7.676	0.08
18.	BENZENE, CYMENE, CYMOL	8.135	0.06
19.	Eucalyptol, Cineole	8.939	0.54
20.	1,3,6-Octatriene,etaOcimene	9.214	1.9
21.	1,4-CYCLOHEXADIENE.GAMMA. TERPINENE	9.554	0.55

Table 5 continued...

22.	CYCLOHEXENE ALPHA TERPINOLEN	10.032	0.06
23.	1,6-OCTADIEN-3-OL, LINALOOL	10.545	0.04
24.	Cinnamaldehyde	11.923	5.2
25.	GAMMA. TERPINENE	12.543	21.47
26.	BICYCLO[2.2.1]HEPTAN-2-ONE, (-)-BORNANone	12.67	0.05
27.	BENZOIC ACID,	14.343	0.04
28.	3-Cyclohexen-1-ol, 4-Carvomenthenol, 4-Terpineol	14.473	0.03
29.	Eugenol	14.594	0.03
30.	3-CYCLOHEXENE-1-METHANOL	14.677	0.03
31.	Copaene	15.205	0.14
32.	6-OCTEN-1-OL, BETACITRONELLYLACETATE	15.389	0.57
33.	3-Carene, Bicyclo[4.1.0]hept-3-ene	15.574	0.16
34.	1,3-Benzodioxole	15.883	0.18
35.	Propanoic acid	16.035	0.6
36.	Caryophyllene	16.523	1.05
37.	Cinnamyl acetate	17.156	4.65
38.	Butanoicacid,Benzylisovalerate	17.432	0.08
39.	CYCLOHEXANE	17.77	0.8
40.	Eugenol methyl ether	18.114	0.19
41.	DODECANAL, LAURALDEHYDE	18.201	0.49
42.	Caryophyllene	18.364	1.05
43.	transalphaBergamotene	18.687	0.16
44.	Cinnamyl acetate	18.828	2.13
45.	alphaCubebene	19.101	0.15
46.	alphaCaryophyllene.alpha.Humulene	19.195	0.21
47.	1H-CYCLOPROP[E]AZULENE	19.315	0.72
48.	Naphthalene,eltaCadinene	19.442	0.02
49.	Naphthalene,GammaMurrolene	19.586	3.45
50.	1,6-Cyclodecadiene,Germacrene D	19.657	0.13
51.	Naphthalene, Eudesma	19.792	0.12
52.	gammaElemene	19.993	0.1
53.	alphaMuurolene	20.088	0.24
54.	1H-Benzocycloheptene	20.283	0.03
55.	CEDR-8-ENE	20.563	0.04
56.	NAPHTHALENE,	20.973	0.08
57.	BETACADINENE	21.108	0.13
58.	alphaMuurolene	21.501	0.07
59.	cisalphaBisabolene	21.607	1.46
60.	3-Hexen-1-ol, Benzoic acid,	22.333	0.09
61.	SPATHULENOL	22.605	0.64
62.	1H-CYCLOPROP[E]AZULENE	22.989	7.23
63.	1,6-Cyclodecadiene, Germacrene D	23.104	0.89
64.	Myristaldehyde	23.717	0.13
65.	Caryophyllene	24.142	0.03
66.	(+)-BETA-GUAJEN	24.353	0.15
67.	alphaCubebene	24.906	0.05
68.	1-Naphthalenol	25.093	1.16

Table 5 continued...

linalool (6.66%), 4-teroinol (3.62%), cinnamyl acetate (3.09%) constituting the major share in bark and gamma terpinene (21.47%), azulene (7.23%), cinnamylacetate (4.65%) major constituents in leaf oil. Vernin et al. (1994) after GC-MS analyses on Cinnamomum and Cassia essential oils could separate a total of 142 compounds, and they could identify 90 compounds definitely and 11 compounds tentatively, while 41 compounds remained unidentified. Among the 90 identified compounds, 25 were reported for the first time in Cinnamomum oil. Many authors had reported varying composition of different constituents in other species of Cinnamomum (Upadhyaya et al., 1994 and Koketsu et al., 1997; Mallavarapu & Ramesh, 2000; Jirovetz et al., 2001; Jayaprakasha et al., 2002). There are no international standards for volatile oil of Cinnamommum species, the higher the cinnamaldehyde content the higher will be the price. Since, there was drastic variation in their chemical constituents, phytochemical profiling of species will help to set up the international standards.

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