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CHEMO VARIATIONS OF WILD CURRY LEAF (*Murraya koenigii* Spreng.) FROM WESTERN GHATS OF INDIA

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Abstract- Wild curry leaf (*Murraya koenigii)* samples collected from seven locations of three provinces from Western Ghats, one of the 34 biodiversity hot spots of the world were examined for differences in their essential oil profiles and yields. Forty components accounting for 89.53-97.24 % of the essentials were identified by GC-FID-LRI and GC-MS analysis. The essential oil yields varied from 1.6-3.70 mL/kg with Bisalakoppa, Serisi recording the highest yield. Essential oil chemical profiles exhibited significant variations with respect to monoterpene hydrocarbons α-pinene (1.93-63.66%), β-phellandrene (1.39-45.89%), sabinene (6.90-40.59%), and sesquiterpene hydrocarbon β-caryophyllene (6.68-18.46%). Essential oils of all the locations exhibited predominance of monoterpene hydrocarbons (58.04-81.14%) with two of them having significant amounts of sesquiterpene hydrocarbons (27.9-28.46%). The essential oils were categorised into four chemotypes with α-pinene and β-caryophyllene predominant is proposed as new chemotype. Chemical diversity is useful for the flavour industry in selecting curry leaf plants with desirable essential oil composition.
Keywords- *Murraya koenigii*, Rutaceae, curry leaf, essential oil composition, Western Ghats, chemotypes.

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Introduction

Murraya koenigii (L) Spreng. commonly known as curry leaf or curry neem is an important Indian culinary and medicinal plant belonging to the Rutaceae family. It is a small tree occurring throughout India up to an altitude of 1500 m. It adorns every house yard of southern India and is also widely cultivated in Burma, Ceylon, Nigeria, China, Australia and Pacific Islands [15,39,42]. The leaves are aromatic, slightly bitter, acrid, cooling, weakly acidic in taste**.** The leaves are widely used for flavoring food items like buttermilk, egg, and meat and for seasoning the south Indian preparations like curries, chutneys, sambar, and rasam. Curry leaf powder and essential oil are exported regularly from India. Leaves are rich source of β-carotene, calcium and Iron [11]. Padmavathi et al. in 1992 reported the changes of β-carotene after cooking. The leaf, bark, and root are reported to have medicinal properties. Curry leaf is used in Indian system of medicine as analgesic, digestive, appetiser, and as a tonic [13,19,43,45]. The anti-diabetic property of the leaves was widely quoted [13,28]. Leaves are also used to treat piles, inflammation, itching, fresh cuts, dysentery, diaorrehea, vomiting, burses and dropsy. The high protective anti-carcinogenic activity of the leaves was also reported [9]. Roots are used for body aches, while the bark is used to treat snakebite [2,4,5,14]. The antioxidant activity of this spice was also wildly discussed [10,16,17,26,27,31,37,40, 41]. The volatile oil of the leaves is reported to possess antimicrobial, antifungal, and pesticide activities [7,12,33]. Koenigine an alkaloid from this plant has shown radical scavenging activity [17]. Chemical diversity was observed in curry leaf essential oils of diverse origins. Essential oils from China, Malaysia, and northern India were rich in α- or β-pinenes [39]. Essential oils from Nigeria, Sri Lanka, and southern part of India were rich in sesquiterpenes with β-caryophyllene as the major constituent [23,29,33]. Western Ghats of India is rich in biodiversity. Changing climatic patterns and anthropological pressures have eroded the rich diversity of the species and genetic diversity leading to labeling Western Ghats as one of the 34 biodiversity hot spots of the World. (http://www.biodiversityhotspots.org/xp/hotspots/hotspotsscience/Pages/default.aspx accessed on 29 June 2011). Central Institute of Medicinal and Aromatic Plants is an Indian research institute collecting, conserving the germplasm of the medicinal and aromatic plants, and utilizing them for developing high yielding varieties for large-scale cultivation to meet the needs of the flavour industry. To the best of our knowledge no earlier attempts were made to assess the chemical diversity of *Murray koenigii* plants of Western Ghats. Therefore, samples were collected from several locations of Western Ghats to examine quantitative and qualitative differences in essential oil chemical profiles and yields, and to identify plants with desirable chemical composition for consideration and development of new varieties for the flavour Industry.

Results and Discussion

The leaf samples collected from their natural habitat in Western Ghats of India on hydro distillation resulted, essential oil in the range of 1.6-3.7 mL/kg of biomass. The yields corroborate the findings of earlier researchers [35,36]. The samples collected from different elevations from of Bisslakopa, Sersi had markedly higher essential oil yields (3.2-3.7 mL/kg) relative to other locations. The oils obtained were analysed and compared by capillary gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) in order of their elution from a CP-Sil-5CB column. The analysis led to the identification of forty compounds accounting for 89.53-97.24% of the essential oils and was listed in Table 1. Significant chemical diversity is evident from the essential oil profiles in respect of monoterpene hydrocarbons α-pinene (1.93-63.66%), β-phellandrene (1.39-45.89%), sabinene (6.90-40.59%), and sesquiterpene hydrocarbon β-caryophyllene (6.68-18.46%). Differences in the presence or absence of specific compounds and their percentages are also clearly apparent among the locations. Chemical diversity among populations of species growing in different agro-climatic locations is known owing to anthropological, climatological, and ecological factors. This variability offers opportunity for selecting genotypes with desirable flavor characteristics. In India it is common experience that curry leaf plants of different regions possess diverse flavor characteristics [35,36]. Differences in the chemical composition of curry leaf plants of different regions were also reported earlier [23,29,34].

In the present study the leaves of *M. koenigii* collected from forest region of Papanasam Hills, Tamil Nadu State yielded 0.29% (v/w) volatile oil and is rich in β-phellandrene (45.89%) and β-caryophyllene (18.46%). The other significant minor components were α-pinene (1.93%), myrcene (2.43%), α-phellandrene (4.95%), *E*-β-ocimene (2.08%), linalool (1.13%), bornyl acetate (1.28%), α-humulene (3.68%), and γ-cadinene (2.75%). Sabinene and β-pinene were totally absent in this oil. α-phellandrene (4.95%) was found to be more in this sample when compared with other samples. In other collected oils it was not found except a small amount (0.17%) in sample collected from Goa. The samples collected from the Central part of Western Ghats areas in Karnataka state yielded the oil content (0.21-0.37%) and showed α-pinene as the major component ranging from 31.89-66.6% and β-pinene and β-caryophyllene as the other major compounds. The samples collected from the forest areas in northern Karnataka also showed α-pinene (36.68-66.6%), sabinene (6.90-9.85%) and β-caryophyllene (6.68-17.15%) as the major components. These samples showed variations from the samples collected earlier from the plains of Karnataka State [24]. From another wild collection from Shorla Hills in Goa, sabinene (40.59%) is the major component along with α-pinene (11.83%), β-pinene (3.14%), γ-terpinene (4.09%), terpinene-4-ol (5.37%) and β-caryophyllene (11.16%). γ-terpinene was observed in this sample and not in the other samples in the present study. These results on the oils from Western Ghats from south to north showed variations in the composition in the major components. It is interesting that β-pinene, γ-terpinene and *t*-sabinene hydrate were present only in the sample collected from Goa region among all the samples. Terpinene-4-ol was one of the major components in Goa sample than other collections. Sabinene was totally absent in the sample collected from Papanasam hill area. Bornyl acetate was totally absent in samples collected from Bisalkoppa in Karnataka State. Further it is observed that the concentrations of monoterpenes are more than the sesquiterpenes in the Western Ghats areas. The maximum amount of β-caryophyllene was 18.46%, sample from Papanasam Hills. The concentration of α-pinene varied from 66.66% Bisalkoppa, Karnataka region to 1.93% Papanasam Hills in Tamil nadu.

Based on chemical composition, the seven samples are categorised into four chemotypes (Table 1). α-pinene-β-caryophyllene rich chemotype is proposed as a new chemotype. Other chemotypes are known [35]. Reviewing the diversity in curry leaf essential oils Rajeswara Rao et al 2011b, hypothesised the existence of several new chemotypes in addition to fourteen chemotypes known earlier. Due to the use of curry leaf plants from centuries, their free transportation and domestication in diverse agro-climatic zones provide ample opportunity for crossing among existing chemotypes to produce new chemotypes. Our results corroborate these views on the existence of new chemotypes. Samples (5) collected from Karnataka Province (Central part of Western Ghats) were dominant in α-pinene, those from Goa and Tamil Nadu Provinces were predominated by sabinene and β-phellandrene, respectively. In monoterpene chemotypes α-pinene [21,22,24,25,32,34,37,39,44,46] rich chemotypes were reported. In the present investigation the essential oil of Dandeli sample possessed 10.86% globulol. This is the highest percentage of globulol observed so far in curry leaf oils.

The olfactory note of the samples from Karnataka area is observed as inferior when compared with other samples collected from Tamil nadu and Goa areas. This present study reveals that there is a considerable difference in chemical composition of wild *Murraya koenigii* in Western Ghats region. Since this plant is widely considered for commercial exploitation, proper genetic material needs to be selected before starting cultivation in large area.

Experimental

Plant materials

The aerial parts of the *M. koenigii* samples were collected from several locations of three provinces from Western Ghats of India. Western Ghats is situated along the western coastal zone of India.

Western Ghats constitutes the Malabar province of the Oriental realm, running parallel to the west coast of India (8°N to 21°N latitudes) for 1600 km. The hills reach up to a height of 2800 m before they merge to the east with the Deccan Plateau at an altitude 550 to 750 m. The voucher specimens were identified by one of the authors and deposited (Nos. DST-88, 215, 342, 347, 354, 356 and 357) at the herbarium in CIMAP, Research Centre, Bangalore.

Extraction of essential oil

The leaf samples were hydro-distilled in triplicate in Clevenger type [3] apparatus for 5 hrs. The essential oil yields obtained in samples are shown in the Table 1. The oil samples after drying over anhydrous Na_2SO_4 were stored at 5°C till the samples were subjected to GC and GC/MS analyses. Essential oil yields varied from 1.6-3.7 mL/kg of biomass (Table 1).

Analysis of essential oils

GC: Gas Chromatographic analysis of the essential oil samples was done on a Varian CP-3800 gas chromatograph (GC) fitted with two Flame Ionization Detectors (FID), split/split less capillary injectors, and Star workstation software. Dimethylpolysiloxane (100%) column (CP-Sil-5CB: 50m x 0.25mm i.d. film thickness 0.4μ) and CP-Wax column: (60m x 0.25mm i.d. film thickness 0.4μ) were used with Nitrogen as the carrier gas with pressures of 16 and 17 *psi,* respectively. Samples 0.2μL were injected in split mode with a ratio 1:100. The column was initially held at 60º C for 5 min., then heated to 220º C at 5º C per min., ramp rate, and was held for 3 min. It was further raised to 250º C at 5º C per min ramp rate and was held at this temperature for 4 min. Injector and detector temperatures were kept at 250º C and 300º C, respectively.

GC/MS: Analysis was carried out on a Perkin Elmer Turbo mass Auto XL Instrument at 70eV ionization energy, employing PE-5 column (5% phenyl 95% dimethylpolysiloxane) of 50m length, 0.32 mm i.d with a film thickness of 0.33 mm. Oven temperature was programmed from 100-280º C at 3º C /min rising rate with an initial hold time of 2 min. Helium was used as the carrier gas at 10 *psi* inlet pressure.

Identification of compounds

Linear retention indices (LRI) were obtained from the gas chromatograms by comparing with natural homologous series of hydrocarbons. Compounds were identified by comparison of retention indices of the peaks on CP-Sil-5CB and CP-Wax columns with literature values [6,18,38], computer matching against library spectra built up using pure substances and components of known essential oil, and finally confirmed by matching their mass spectra with those recorded in the MS library data already available in NIST library and literature [1]. The identity of the components was further confirmed by co-injection of the oil samples with standard compounds and component-rich known essential oil fractions by peak enrichment. Relative amounts of individual components are based on peak areas obtained without FID response factor correction. Identified compounds are listed in Table 1.

Conclusions

Curry leaf plants collected from seven locations of three provinces in Western Ghats revealed significant chemical diversity in their essential oils providing an opportunity to flavorists to select essential oils with preferential chemical profiles. Essential oils of all the locations exhibited predominance of monoterpene hydrocarbons with two of them having significant amounts of sesquiterpene hydrocarbons (27.9-28.46%). Curry leaf essential oils collected were categorised into four chemotypes, and the one with α-pinene and β-caryophyllene predominant is proposed as new chemotype

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Table 1-Percentage composition of volatile components of essential oils of curry leaf plants collected from seven locations of Western Ghats. (n = 3)

			Papanasam Hills	Bisalakoppa, Sersi, Karnataka state	Chorla, Goa	Near Ram Nagar, Dandeli, Karnataka	Dooor villlage Near Sersi, Karnataka	Bisalakoppa, Sersi, Karnataka state	Bisalakoppa, Sersi, Karnataka state
Essential oil yield mL/kg			2.9	3.7	3	1.6	2.3	3.2	3.6
Name of the compound	**CP-Sil-5CB**	**CP-Wax**	**Percentage composition of components on CP-Sil-5CB Column**						
α-thujene	931	1025	-	0.1	1.65	-	-	-	0.11
α-pinene	935	1025	1.93	63.66	11.83	45.78	36.68	49.51	59.11
camphene	947	1070	-	0.76	-	0.5	-	0.6	0.69
sabinene	970	1112	-	9.85	40.59	6.9	7.49	8.95	8.69
β- pinene	976	1124	-	-	3.14	-	-	-	-
myrcene	984	1161	2.43	0.57	1.51	0.43	-	0.65	0.98
α- phellandrene	998	1070	4.95	-	0.17	-	-	-	-
α-terpinene	1012	1168	0.76	0.43	2.16	0.49	-	-	-
p-cymene	1014	1274	-	-	1.06	-	-	0.34	0.22
β- phellandrene	1024	1205	45.89	5.11	1.39	4.23	4.72	5.53	4.83
Z-β-ocimene	1024	1212	-	-	0.93	-	-	-	-
E-β-ocimene	1040	1251	2.08	0.46	1.44	-	5.31	5.83	2.86
γ–terpinene	1052	1249	-	-	4.09	-	-	-	0.2
t-sabinene hydrate	1057	1461	-	-	1.57	-	-	-	-
terpinolene	1080	1285	-	0.2	0.93	0.66	-	0.32	0.26
linalool	1085	1547	1.13	0.21	1.55	0.58	-	0.66	0.42
cis-sabinene hydrate	1091	1537	-	0.21	-	1.1	0.65	-	-
nonanol	1109	1382	0.72	0.12	0.74	0.33	-	-	-
cis-Sabanol	1125	1683	-	0.2	0.38	0.68	-	-	-
Z-β-terpineol	1127	1616	0.6	0.33	-	1.16	1.03	-	-
terpinene -4-ol	1164	1604	0.58	0.23	5.37	0.59	-	-	-
α-terpineol	1176	1731	0.98	0.27	0.69	0.39	0.78	0.26	0.12
bornyl acetate	1272	1602	1.28	-	0.29	2.21		-	0.14
β- cubebene	1387	-	-	0.1	-	-	0.96	1.36	0.15
β-elemene	1395	1584	0.92	-	-	-	1.4	-	0.3
β- caryophyllene	1431	1618	18.46	6.88	11.16	6.68	17.15	12.58	8.12
β-gurjuenene	1439	1600	0.46	0.14	0.17	-		-	0.17
α-humulene	1464	1690	3.68	1.86	2.49	2.11	3.63	2.77	1.73
germacrene D	1480	1712	-	-	-	-	-	-	0.25
α-selinene	1495	1712	1.21	0.11	0.41	0.2	1.03	2.12	0.26
γ- muurolene	1499	-	0.46	0.4	-	0.32	0.57	-	1.09
γ-cadinene	1504	-	2.75		-	0.29	2.23	-	0.39
δ-cadinene	1519	1737	0.52	0.15	-	-	0.93	-	0.35
spathulenol	1572	2089	-	0.43	-	0.57	-	0.23	0.46
caryophyllene oxide	1583	1989	0.44	0.43	0.2	2.5	0.94	0.98	0.39
globulol	1586	2056	-	1.75	-	10.86	2.7	0.29	0.28
cederol	1617	-	0.77	0.1	0.51	2.54	0.52	0.43	0.18
t-cadinol	1621	-	-	-	0.22	0.48	-	0.3	0.17
β-eduesmol	1628	2235	0.46	0.37	0.22	0.25	0.81	0.38	0.38
α-cadinol	1647	-	0.49	0.18	0.38	0.52	-	1.46	0.37
Grouped components									
Monoterpene hydrocarbons			58.04	81.14	72.46	58.99	54.2	71.73	77.95
oxygenated monoterpene hydrocarbons			5.29	1.57	9.02	7.04	2.46	0.92	0.68
Sesquiterpene hydrocarbons			28.46	9.64	14.23	9.6	27.9	18.83	12.81
oxygenated sesquiterpene hydrocarbons			2.16	3.26	1.53	17.72	4.97	4.07	2.23
Total % of compounds identified			93.95	95.61	97.24	93.35	89.53	95.55	93.67
Chemotype characterization			β-phellandrene, β-caryophyllene	α-pinene	sabinene, α-pinene, β-caryophyllene	α-pinene	α-pinene, β-caryophyllene	α-pinene, β-caryophyllene	α-pinene