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Journal of Chemical and Pharmaceutical Research, 2015, 7(8):67-71



Research Article

ISSN : 0975-7384 CODEN(USA) : JCPRC5

Chemical polymorphism in *Cinnamomum tamala* (Buch.-Ham.) Nees. & Eberm. growing in Uttarakhand Himalaya (India)

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ABSTRACT

GC fingerprinting of the essential oil composition among various populations of Cinnamomum tamala (Lauraceae), collected from different forest ranges of Uttarakhand (India) was carried out to study chemical polymorphism and interpopulation variability. In most of the populations, cinnamaldehyde was found as the main constituent, followed by linalool and cinnamyl acetate. The results arise from the analysis of essential oils indicated that qualitative and quantitative differences are existed in various populations. In order to detect some distribution pattern and to identify the specific constituents which differentiate the groups of individuals, the essential oil components were subjected to cluster analysis and four chemotype groups obtained, these were I: cinnamaldehyde, II: cinnamyl acetate+cinnamaldehyde, III: linalool<cinnamaldehyde and IV: cinnamaldehyde</td>

Keywords: Cinnamomum tamala, essential oil, polymorphism, cinnamaldehyde, linalool

INTRODUCTION

The genus *Cinnamomum*, which belongs to the Lauraceae family, consists of 250 species of trees and shrubs [1] and distributed in South East Asia, China and Australia [2]. In India, the genus is represented by 26 species [3] of which some species have medicinal and spice value and commercially used. Among these, *Cinnamomum tamala* (Buch.-Ham.) Nees. & Eberm. (bay leaves), commonly known as 'Tejpat' (commercially as Indian Cassia) is widely used all over the globe and especially in India as spice, in flavoring and in pharmaceutical and ayurvedic preparations [4]. In India, *C. tamala* is distributed in North-East Himalaya, North-Western Himalaya and Southern parts of the country from sub tropical to tropical Himalayas at the altitudes of 900-2500 m [5]. *C. tamala* has carminative, hypoglycemic, anthelmintic, stimulant, stomachic properties and also used in Indian systems of traditional medicines for colic, diarrhea and rheumatism [6-8]. India is a leading consumer of *Cinnamomum tamala* leaves and imports about 86% of Tejpat leaves from Nepal. The market of tejpat leaves in Uttarakhand is also estimated at 1470 tons, with the spice industry as the main consumer [9]. In India, there are four chemotypes, namely, Cinnamaldehyde, eugenol, cinnamaldehyde-linalool and linalool rich type have been reported in the essential oil of *C. tamala* [10]. Eugenol is occurring more widely than cinnamaldehyde [11].

In continuation of our work on the chemical composition of the *C. tamala*, we now report the results obtained in a thorough investigation of the essential oils of various populations of the genus growing in Uttarakhand (India). The aim of this investigation was to study the chemical polymorphism through GC-fingerprinting and to find out pattern

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of distribution of compounds and the natural chemotypes among *C. tamala* populations growing in this particular phytogeographical region of Uttarakhand (India).

EXPERIMENTAL SECTION

Plant material

In order to study the chemical variability, samples (leaves) of *Cinnamomum tamala* were collected during October, 2011 from various forest ranges of different districts of Uttarakhand (India), viz., Nainital, Pithoragarh, Champawat, Almora, Tehri, Pauri, Rudraprayag and Chamoli.

Oil isolation

The volatile oils were isolated from shade-dried leaves (200g) by hydro-distillation using a Clevenger apparatus. The yield of the essential oils in various populations varied from 0.4 to 1.2% (v/w).

Gas-chromatography (GC)

GC analyses were carried out by an Agilent Technology 6890 N gas chromatograph data handling system equipped with a split/splitless injector and fitted with FID using N₂ as the carrier gas. The column was HP-5 capillary column ($30m \times 0.32mm$, $0.25\mu m$ film thickness) and temperature program was used as follows: initial temperature of 60°C (hold: 2 min) programmed at a rate of 3°C /min to a final temperature of 220°C (hold: 5 min). Temperatures of the injector and FID were maintained at 210°C and 250°C, respectively.

RESULTS AND DISCUSSION

To determine the chemical variability in Uttarakhand's tejpat, all the oils were subjected to GC-analysis gaschromatography (GC-analysis). The percentage composition of the major components of the essential oils was used to determine the relationship between the different populations of *C. tamala*. The data were processed by Cluster analysis using hierarchical clustering by Ward's technique and Euclidean distance measure (SPSS 13.0 ver.).

The percentage content of the individual components is summarized in Table 1. For chemical profiling of Uttarakhand Tejpat, all the populations had 3 homologous samples (mean \pm SD). In general, the populations were found rich in cinnamaldehyde followed by linalool. Some of the populations have shown an appreciable amount of cinnamyl acetate with cinnamaldehyde (Fig. 1). The other minor compounds in the essential oils from all the populations were found as benzaldehyde, β -pinene, 1,8-cineole and caryophyllene oxide.

The dendrogram, obtained by the application of cluster analysis represents the relationships among the populations and the groups on the basis of their essential oil components and divided the populations into four groups (Fig. 2). The first group encompassed 13 populations and characterized by cinnamaldehyde (60.1-82.0%) as the main component. The group shared 37.14% of the total populations. Cinnamaldehyde is having sweet and warm spicy taste and extensively used in flavor composition and also a powerful spicy ingredient [12]. The second group comprised of 3 populations, which contained high concentration of cinnamyl acetate (18.4-42.3%), along with cinnamaldehyde (28.2-55.0%) and shared 8.57% of the populations. Cinnamyl acetate is used in flavor composition for imitation cinnamon, apricot, apple, vanilla etc [12]. The third group exhibited 6 populations and characterized by high amount of linalool (46.4-62.0%), followed by cinnamaldehyde (7.7-30.8%). This group shared 17.14% of the total populations. Linalool is used very extensively in perfume compositions [12]. The group four, which included 13 populations, displayed high cinnamaldehyde content (44.4-61.7%), followed by linalool (22.7-30.2%) and shared 37.14% of total populations.

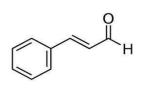
Generally, chemical polymorphism in the essential oil of a plant species occurs due to various factors including difference in altitudes and geographical factors [13,14], genotype and genetic variation [15,16] and exogenous and endogenous factors [17]. The endogenous factors are related to anatomical and physiological characteristics of the plants and to the biosynthetic pathways of the volatiles and the exogenous factors might affect some of the genes responsible for volatiles formation [17]. Those factors lead to ecotypes or chemotypes in the same plant species.

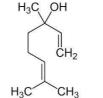
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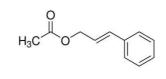
Table1. Peak area (%) of investigated compounds in Cinnamomum tamala collected from different regions of Uttarakhand (India)

Populations*	Content (%)							
	benzaldehyde	β-pinene	1,8- cineole	linalool	cinnamaldehyde	cinnamyl acetate	caryophyllene oxide	total
CT1	0.88±0.13	1.44 ± 0.14	0.53±0.02	33.45±0.24	38.07±0.33	1.30±0.03	1.06±0.06	76.71
CT2	0.55±0.01	1.09 ± 0.11	0.33 ± 0.01	41.97±0.61	39.65±0.54	0.59±0.02	0.63±0.09	84.81
CT3	1.03±0.05	0.46 ± 0.65	1.83±0.23	40.23±1.25	38.80±2.39	1.52±0.54	0.78±0.12	84.64
CT4	0.49 ± 0.05	1.23±0.19	0.39 ± 0.07	49.32±2.36	30.83±2.22	0.65±0.13	0.70 ± 0.02	83.61
CT5	0.55±0.23	0.25±0.14	0.24±0.13	48.77±1.45	25.11±1.41	5.17±0.93	1.48 ± 0.43	81.57
CT6	2.23±0.23	0.65 ± 0.08	0.82 ± 0.24	2.87±0.42	81.97±2.83	1.47±0.91	0.70 ± 0.01	90.70
CT7	2.34±0.05	0.30±0.11	0.20 ± 0.03	8.15±2.28	70.34±0.45	11.05±0.16	0.39±0.33	92.77
CT8	2.26±0.40	0.68 ± 0.21	1.29±0.86	9.63±1.95	61.85±0.21	2.04±0.40	0.97±0.21	78.72
СТ9	2.09±0.14	1.11±0.09	0.80 ± 0.02	17.94±0.18	60.07±0.68	2.56±0.94	0.46±0.24	85.02
CT10	2.14±0.17	0.76 ± 0.09	2.50 ± 0.28	18.52 ± 0.88	62.78±0.80	2.22±0.15	0.66±0.01	89.59
CT11	1.98±0.16	0.80 ± 0.50	1.30 ± 0.15	16.80±1.50	62.05±3.30	3.00±1.96	1.08 ± 0.38	87.00
CT12	2.38±0.36	0.73 ± 0.07	1.60 ± 0.49	14.96±0.31	63.43±1.79	7.41±0.64	0.63 ± 0.08	91.13
CT13	2.35±0.20	0.46 ± 0.06	0.72 ± 0.75	10.14±0.33	73.98±0.53	4.22±1.15	0.59±0.04	92.46
CT14	2.67±0.31	0.56 ± 0.05	0.30 ± 0.25	9.05±1.05	75.80±0.80	2.83±0.38	0.54±0.21	91.75
CT15	2.66±0.04	0.52 ± 0.03	0.81±0.43	13.43±0.74	67.66±0.38	4.13±0.47	0.82±0.03	90.03
CT16	2.60±0.29	0.65±0.23	0.79 ± 0.84	11.66±0.13	67.90±2.59	4.12±0.57	0.68 ± 0.05	88.41
CT17	3.07±0.01	0.52 ± 0.09	0.34 ± 0.05	13.30 ± 1.82	68.62±1.57	7.55±0.79	0.52 ± 0.01	93.92
CT18	3.23±0.92	1.01 ± 0.14	1.15 ± 0.72	7.73±1.93	70.62±2.46	1.26±1.09	1.20±0.32	86.19
CT19	1.29±0.20	0.96 ± 0.40	1.63 ± 0.87	30.25±1.03	51.71±0.39	1.24±0.75	0.75 ± 0.07	87.83
CT20	1.60 ± 0.27	1.07 ± 0.02	2.33 ± 0.55	28.72±0.57	45.45±0.39	1.19±0.13	0.91±0.59	81.27
CT21	0.99±0.27	1.08 ± 0.19	2.16±1.68	29.94±1.02	47.85±0.44	1.04 ± 0.47	0.87±0.02	83.93
CT22	1.80 ± 0.68	0.69±0.19	1.81±1.31	26.56±1.03	57.31±0.66	1.00±0.92	0.69±0.33	89.86
CT23	0.90±0.45	0.59±0.31	0.24 ± 0.14	30.06±1.06	58.49±0.61	1.75±0.35	0.47±0.43	92.51
CT24	1.99±0.32	0.78±0.13	2.56 ± 0.85	22.74±0.28	49.76±1.12	3.00±1.25	1.20±0.16	82.03
CT25	1.83±0.42	1.41 ± 0.01	2.45±1.56	24.16±0.80	52.29±1.50	4.26±2.00	0.67±0.16	87.06
CT26	1.37±0.55	0.52 ± 0.43	0.21±0.17	25.78 ± 0.98	61.67±1.53	2.20±0.50	0.54±0.20	92.30
CT27	2.02±0.43	0.73 ± 0.47	2.09 ± 0.72	22.89±0.94	44.79±0.84	3.69±0.81	1.68±0.23	77.90
CT28	0.59±0.30	0.61 ± 0.04	2.13±0.50	61.99±1.54	19.10±2.31	0.74±0.32	0.85±0.56	86.01
СТ29	0.67±0.26	0.65 ± 0.47	2.55 ± 0.45	53.67±0.83	20.71±0.54	0.87±0.48	1.59±0.61	80.72
CT30	0.50±0.30	1.80 ± 0.90	1.23±0.33	58.94±1.07	7.66±1.00	1.86±0.91	1.54 ± 0.64	73.52
CT31	1.70±0.65	2.40 ± 0.60	3.74 ± 0.84	46.39±1.39	11.24±0.76	3.50±0.50	1.14 ± 0.14	70.11
CT32	0.75±0.57	0.19 ± 0.09	0.15 ± 0.05	35.30±1.30	38.30±1.70	13.74±0.26	1.00 ± 0.20	89.43
CT33	1.30±0.45	0.13±0.09	0.10 ± 0.05	13.49±0.51	54.98±1.73	22.22±1.22	0.67 ± 0.48	92.89
CT34	1.83±0.37	0.86 ± 0.40	4.40 ± 0.60	0.28±0.20	53.75±1.37	18.43±0.57	0.52±0.38	80.07
CT35	3.74±2.84	0.44±0.35	3.52±0.63	6.52±0.54	28.20±1.00	42.33±0.90	0.68 ± 0.05	85.43

*each population having 3 homologous samples (mean ±SD)







cinnamaldehyde linalool cinnamyl acetate Figure 1. Chemical structure of the marker compounds (major constituents) of the essential oil of *C. tamala*

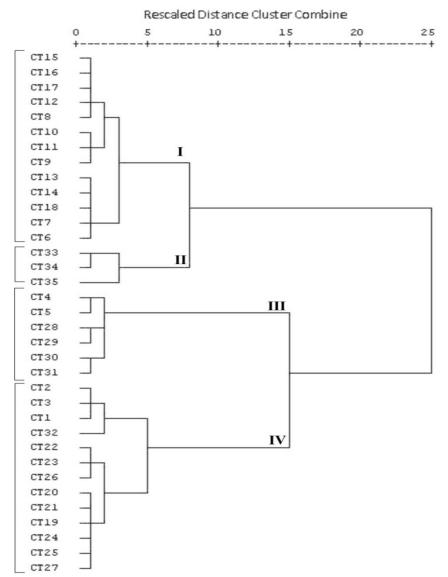


Figure 2. Dendrogram obtained by Hierarchical cluster analysis of *Cinnamomum tamala* components (cinnamaldehyde, linalool and cinnamyl acetate), clustering based on Ward Method (Euclidean distance)

CONCLUSION

The study has shown a significant chemical polymorphism in Uttarakhand Tejpat, collected from different regions and also identified populations with natural existence of unique aroma chemicals mainly cinnamaldehyde, linalool and also cinnamyl acetate, since each chemotype may have a unique application as oils, in spices and in ayurvedic and pharmaceutical industries. The quality also differs with North-East states, because of absence of eugenol in Uttarakhand Tejpat. Due to this reason Uttarakhand Tejpat is known as 'mitha tejpatta' and mainly consumed by ayurvedic pharmacies instead of raw spices in foods. In the state, local inhabitants are also being benefitted through commercial distillation of essential oil of Tejpat. The work in this study will allow the quality production of Tejpat for development of trade.

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