

# Chemotypic Variation in Clove Oil and Lemongrass Oil Obtained from Three Different Geographical Locations of India

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## Abstract

Clove buds and lemongrass leaves were collected from three different locations of India and the essential oil was separated by hydrodistillation procedure. The essential oils were studied for its variation in physical properties and chemical composition. Chemical analysis by GC-MS evidenced that the main components of clove oil (CLV) were eugenol (78.5-87.7%) and caryophyllene (12.3-20.2%) and the main components of lemongrass oil (LGR) were citral in the form of neral and geranial (24.3-83.9%) and (+) camphor (12.07-30.62%). The lemongrass oil collected from Ootacamund hills, Tamilnadu have shown components like Limonene (2.7%), isocamphene (5.1%), endo isocamphene (5.3%), p-cymene (2.4%) and citronellol (1.73%) among which isocamphene and endo isocamphene were found to be new chemotypes. (+) fenchone was present in the range 5.6-7.76% in lemongrass oil collected from two different locations. Isopiperitone (6.23%) and geranyl butyrate (3.37%) were found to be new chemotypes in the essential oil obtained from Bhopal, Madhya Pradesh. Thus the results have shown variation in chemical composition and identification of new chemotypes in both clove oil as well as lemongrass oil collected from three different locations of India which may be due to the change in geographical conditions.

**Keywords:** Essential oil; Clove oil; Lemongrass oil; Eugenol; Caryophyllene; Citral

**Abbreviations:** CLV:Clove Oil; LGR:Lemongrass Oil; GC-MS:Gas Chromatography–Mass Spectrometry

## Introduction

Essential oils characterized by wonderful scents are concentrated volatile aromatic compounds produced by various plant sources. Each of these complex precious liquids is extracted from a particular species of plant type. Each plant species originates in certain regions of the world, with particular environmental conditions and neighboring fauna and flora. The result is a very diverse library of aromatic compounds, with some essential oils being made up of more than one hundred distinct organic chemicals. More than the commercial importance of the variability in yield and composition of essential oils, it also becomes highly essential when the volatiles are used as chemotaxonomic tools. Thus the knowledge of the factors that determine the chemical variability and yield for essential oils remains very important. Factors that influence the production and composition of essential oils include (a) physiological variations (b) environmental conditions (c) geographic variations (d) genetic factors and evolution (e) political/social conditions and also (f) amount of plant material/space and manual labour needs. There are innumerable examples of the occurrence of geographic variations of the yield and composition of volatiles. The different essential oil compositions of a species found for different origins reflect the different environmental conditions of each particular location and culture conditions (different altitudes, different solar exposition, different soil types, etc.) [1]. The literature reveals that the intraproveance variation for content and chemical composition has been analyzed in Eucalyptus oils among different districts of Punjab, India [2]. Similar studies have been reported on chemical variation of essential oils with respect to different geographical locations [3-5]. Clove oil has been used in human medicine as topical anesthetic, particularly in dentistry [6], as an agent for the treatment of inflammatory diseases and as radical scavengers due to its antioxidant properties [7]. Clove oil has been used in aromatherapy as mouth sterilizer or painkiller [8]. Lemongrass oil has various medicinal,

herbal and therapeutic properties. It is expected to have analgesic, antibacterial, carminative, deodorizing, febrifuge, fungicidal, antiseptic, antidepressant, astringent, diuretic, galactagogue, antipyretic, antimicrobial and sedative properties. Its fungicidal properties have been already well reported [9]. Lemongrass oil is a good insect repellent [10]. Biosynthesis of secondary metabolites, in particular essential oil, even though determined genetically, is strongly affected by ontogenetic and environmental factors [11-13]. Thus considering these facts the possibility of geographical condition for the compositional variation of essential oils the present study was conducted by studying the physical properties and chemical composition of clove oil and lemongrass oil by GC-MS analysis. The objective of the study is to identify and report the chemical variation of essential oils like clove oil and lemongrass oil with respect to change in geographical locations.

## Materials and Methods

### Extraction of essential oils

The Clove bud phytosamples were collected from three different sources of India: Ootacamund hills of Tamil Nadu, Nainital Hills of Uttar Pradesh and Munnar of Kerala. The Lemongrass leaf samples were collected from Bhopal of Madhya Pradesh, Ootacamund hills of Tamil Nadu and Azhapuzha of Kerala. The voucher specimen of all the samples were deposited at Raninat herbarium, Centre for molecular systematics, St. Joseph's college (campus), Tiruchirappalli, Essential oils were extracted by initial crushing followed by hydrodistillation.

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## Analysis of physical and chemical properties

The specific gravity of various samples was yielded out using a pycnometer, while optical rotation and refractive index were determined using a polarimeter and Abbe's refractometer.

## GC-MS equipment

The analysis was performed in GC-MS Instrument named SHIMADZU-QP 2010 PLUS. The column used was RXi-5MS, L: 30 m, T: 0.25  $\mu$ m, d: 0.25 mm. The method used for the Clove oil analysis is as follows. The column temperature was maintained at 100°C. The flow rate was retained at 5.6 ml/min. The temperature suite is devised as initial 100°C for 2 min, rate: 6°C/min, 200°C for 1 min, rate: 20°C/min and 250°C for 2 min. The sample preparation was done by dissolving 0.2  $\mu$ l Clove oil in 50 ml Acetonitrile (solvent). The system of analysis of the Lemongrass oil samples were devised as follows. The column temperature was maintained at 50°C. The stream rate was sustained at 11 ml/min. The temperature suite was formulated as: initial 50°C for 1 min, 100°C for 1 min, rate: 10°C/min and 150°C for 1 min, rate: 10°C/min. The sample preparation was done by dissolving 0.2  $\mu$ l Clove oil in 50 ml methanol (solvent).

## Results and Discussion

The physical properties like refractive index, optical rotation, boiling range and weight per ml were calculated from the corresponding methods of determination for the various samples extracted from various sources. The variations in physical properties of Clove oil and Lemongrass oil are well elucidated in Tables 1 and 2. Analysis of chemical composition of the samples was performed using GC-MS. In the analysis of Clove oil, the major peaks were identified as Eugenol at the retention time 9.4 and Caryophyllene at 10.9. Thus it is evident that the Eugenol and the Caryophyllene are the major constituents of the Clove bud essential oil which concurs with the dissertations of various researchers [14-16] (Table 3). Among the three locations, Eugenol was present in higher concentration (in percentage) in the essential oil isolated from buds collected from Munnar, Kerala and Caryophyllene was present in higher concentration (in percentage) in the oil isolated from the buds collected from Ootacamund hills, Tamilnadu when compared with other locations. The other components were  $\alpha$ -Humulene (2.26%) in CLV01 and 3-carene (1.24%) in CLV03, where the latter was found to be new chemotype in clove oil (Figure 1). In GC-MS analysis of Lemongrass oil, it was found that the major peaks were at the retention times 10.29 and 10.775 representing Neral and Geranial respectively. Thus Neral and Geranial being the isomers of Citral, comprises the role as the major constituent of the Lemongrass oil confirming to the literature [17-19]. The other major components present were Limonene (2.7%), isocamphene (5.1%), endo isocamphene (5.3%), p-cymene (2.4%), citronellol (1.73%) and linalyl acetate (1.35%). Isocamphene and endo isocamphene were found to be new chemotypes in LGR02 (Table 4). (+) fenchone was present in the range 5.6-7.76% in lemongrass oil collected from two different locations (LGR 01 and LGR 02). Isopiperitone (6.23%) and geranyl butyrate (3.37%) were found to be new chemotypes in the essential oil obtained from Bhopal, Madhya Pradesh (LGR 03) (Figure 2). There is strong variation in the composition of essential oils obtained from lemongrass leaves collected from three different locations. The concentration of citral (in percentage) was high in the lemongrass leaves collected from Kerala when compared to other locations, whereas the oil obtained from Ootacamund hills have shown additional number of components

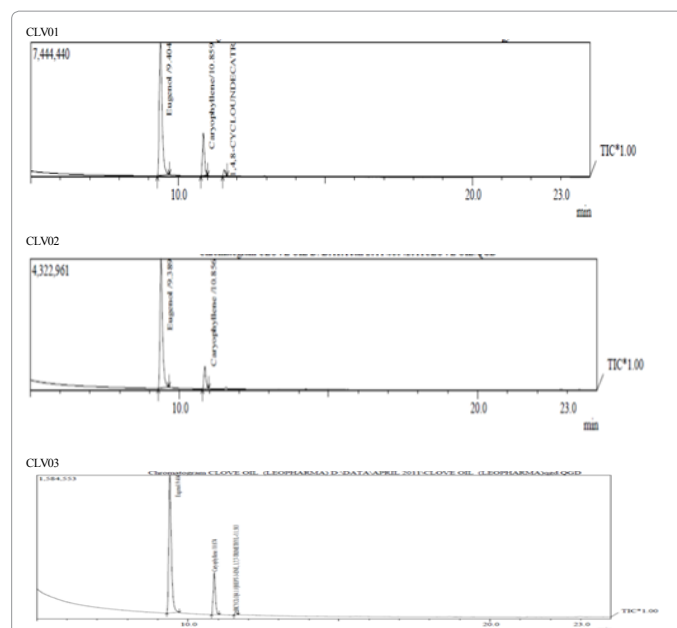


Figure 1: GC chromatograms of clove oil obtained from three different locations of India.

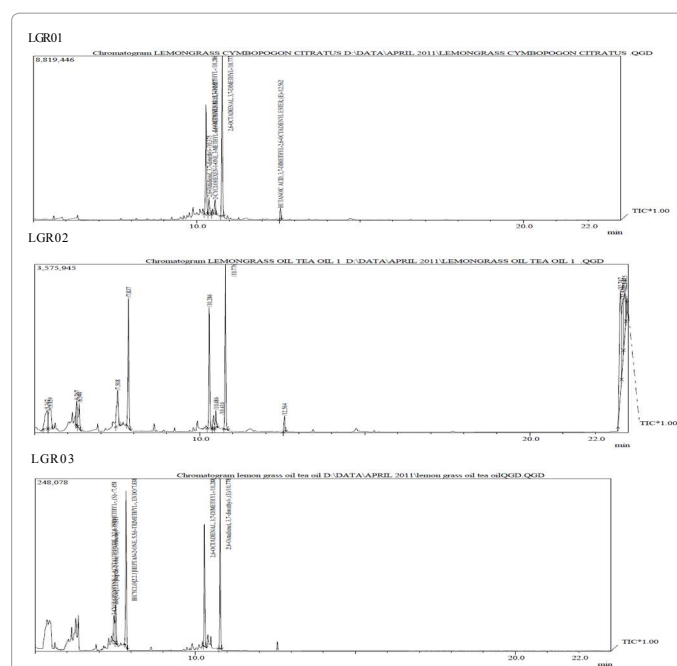


Figure 2: GC chromatograms of lemongrass oil obtained from three different locations of India.

and new chemotypes when compared to other oils collected from other two locations. The change in composition of major constituents of Clove oil and Lemongrass oil were correlated with their geographical conditions. The percentage composition of the Eugenol and Citral in essential oils obtained from Kerala was relatively in higher percentage than other two sources which may be due to the promising climatic conditions (tropical wet climate, wet temperate vegetation, red loamy soil with annual temperature and rainfall 22.5-25°C and 400-800 cm respectively) that might have inclined the higher percentage of Eugenol in the Clove oil and Citral in Lemongrass oil.

Characteristics	Sample CLV 01	Sample CLV 02	Sample CLV 03
Location	Nainital Hills, Uttar Pradesh	Munnar, Kerala	Ootacamund Hills, Tamil Nadu
Weight/ml	1.042	1.053	1.049
Refractive Index	1.527	1.532	1.534
Boiling Range	252.2-253.5°C	253.4-254.1°C	249.3-252°C
Optical Rotation	-0.78°	-1.02°	-1.45°

**Table 1:** Analysis of physical properties of clove oil collected from three different locations of India.

Characteristics	Sample LGR 01	Sample LGR 02	Sample LGR 03
Location	Azhapuzha, Kerala	Ootacamund Hills, Tamil Nadu	Bhopal, Madhya Pradesh
Weight/ml	0.894	0.884	0.873
Refractive Index	1.492	1.487	1.489
Boiling Range	259.7-260.2°C	258.4-259.9°C	249.3-252°C
Optical Rotation	-3.2°	-4.3°	-7.05°

**Table 2:** Analysis of physical properties of Lemongrass oil collected from various locations of India.

S.No	Components	Mol wt	CLV 01	CLV 02	CLV 03
1.	Eugenol	164	79.7%	82.72%	78.52%
2.	Caryophyllene	204	18.03%	11.32%	20.23%
3.	α-Humulene	204	2.26%	-	-
4.	3-carene	136	-	-	1.24%

**Table 3:** Variation in chemical composition of clove oil obtained from three different locations of India.

S.No.	Components	Mol wt	LGR01	LGR02	LGR03
1.	Isocamphane	138	-	5.01%	-
2.	Endo-isocamphane	138	-	5.3%	-
3.	p-cymene	134	-	2.4%	-
4.	(+) Limonene	136	-	2.7%	-
5.	(+) Fenchone	152	7.66%	5.61%	-
6.	(+) Camphor	152	30.62%	12.07%	-
7.	Neral (z- citral)	152	23.17%	10.61%	34.78%
8.	(E)-geranial	152	-	1.42%	6.41%
9.	Citronellol	154	-	1.73%	-
10.	Geranial (cis-trans citral)	152	31.03%	13.69%	49.21%
11.	Linalyl acetate	196	-	1.35%	-
12.	Campholenic aldehyde	152	7.52%	-	-
13.	Isopiperitone	152	-	-	6.23%
14.	Geranyl butyrate	224	-	-	3.37%

**Table 4:** Variations in chemical composition of lemongrass oil collected from three different locations of India.

## Conclusion

The essential oil samples sourced from various geographical locations evidently prove that there are significant variations observed both in physical and chemical properties. Thus these variations remain the basis of the inconsistency in the composition of natural drug product and proper standardization of the natural sources for its chemical composition is highly required to maintain its consistency. While taking a close look at these wide variations, Kerala can be identified as a spot of well flourished geographical conditions favoring the rich constituency in the natural sources. The climatic conditions and topography are really suitable for the quantitative and qualitative production of herbal sources of drugs.

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