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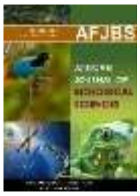

GC-FID, LC-QToF-MS, NMR, quantum chemical analysis, and toxicological evaluation of lemongrass (*Cymbopogon flexuosus*) essential oil yields in Mayurbhanj district of Odisha state

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Abstract

The main objective of this research is to characterize the volatile chemical components found in essential oil of lemongrass leaves. Lemongrass cultivation is done in very few districts of Odisha and leaf sample collected from Mayurbhanj district. Essential oil from leaves was extracted by steam distillation process and oil was analyzed by gas chromatography (GC), liquid chromatography mass spectroscopy (LCMS) and nuclear magnetic resonance (NMR). We have reported the phytochemicals, material safety and toxicological effects observed in lemongrass leaves cultivated at Mayurbhanj district of Odisha state. Lemongrass oil chemistry profile reveals 1.4805 refractive index and 0.888 specific gravity at 27˚C, exceeding norms. GC analysis reveals phytochemicals such as Camphene (0.834%; Rt. Time: 6.872 min), 6-Methyle-5-heptene-2-One (0.452%; Rt. Time: 7.251 min), 4-Nonanone (1.082%; Rt. Time: 9.755 min), and Citronellol (0.104%, Rt. Time: 15.341 min). Using LCMS analysis, 40 phenolic compounds were discovered. Proton-induced chemical shift on carbon displays signals at δ1.484, 2.000, 7.187, and 9.747 ppm, corresponding to methyl, methylene, aromatic, and aldehyde groups. Carbonyl group is present in the δ1.484-1.967 range. Quantum chemical structure analysis of citral isomers revealed highest and lowest electron charge occupancy. Not previously reported, lemongrass leaves from Mayurbhanj had the highest citral content. We also stressed hazard identification, and toxicological studies showed LD 50 was 4400 mg/Kg in oral rat treatment. Not previously reported, lemongrass oil was stable at room temperature and had a flash point of 71˚C. This research also expects new farming methods to boost essential oil, citral yields, and toxic levels.

Keywords: *Cymbopogon flexuosus*; gas-liquid chromatography; nuclear magnetic resonance; citra; essential oil.

Article History

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1 Introduction

Lemongrass is the common name for the plant *Cymbopogon flexuosus*, which is a member of the Gramineae family [1]. Citral, a cyclic monoterpene, is responsible for the prefix lemon's characteristic lemon-like aroma. Numerous essential oils, tannins, flavonoids, and alkaloids are among the phytoconstituents found in lemongrass [2]. Numerous components' secondary active metabolites have also been linked to the diverse pharmacological actions of this plant. From the Greek word "kymbe-pogon" which translates as "boat-beard," gets the name *Cymbopogon* [3]. The plant's essential oil is utilized in culinary herbs, medicinal tea, aromatherapy, and treatments for skin conditions. It is recognized as a source of traditional remedies [4]. In several regions of the world, *C. flexuosus* is used to treat rheumatism, fevers, menstrual abnormalities, and other joint difficulties in addition to digestive problems [5]. This species is a member of the Gramineae family, which consists around 8,000 plant species and about 500 genera [6]. Lemon grass is a tufted perennial grass that may reach a height of 1 meter and has several stiff, green stems that emerge from roots that are short and rhizomatous. Practitioners of folk medicine have continued to treat illnesses and crippling diseases mostly with whole herbs and extracts [7]. It is used in the food and perfume sectors as a good source of citral [8]. It serves as the raw material to produce ionones, which create vitamin A. Due to its extensive usage in the cosmetics, food, pharmaceutical, agricultural, and taste sectors, it grows throughout subtropical and tropical parts of the world [9]. The subtropics and tropics are where *Cymbopogon* grasses are widely cultivated. *Cymbopogon flexuosus* smells like lemons because it contains more aldehydes than other plants [10]. It has two geometric isomers: neral (Citral-β) and geranial (Citral-α). One of the elements that affects the creation of fragrant lemongrass is the usage of exceptional seeds. Farmers' revenues will rise as production levels of fragrant lemongrass change [11]. According to research, growers of lemongrass who used premium varietals made more money than those who used local seeds. In addition to using excellent seeds, the best and most efficient labor will yield the greatest advantage. Through farmer organizations or community leaders, it is necessary to promote the use of better seeds by supplying seed sources, encouraging community involvement, and offering pertinent technical assistance.

Agro-climatic zones of Odisha

In India, there are fifteen main agroclimatic zones. Odisha, a state on the eastern coast of India, belongs to the Easter Plateau and Hilly Region agroclimatic zone VII. However, twelve agroclimatic zones can be further subdivided into the state at the regional level based on climate, temperature, relief, and rainfall [12]. The details of agro-climatic zones of Odisha given below:

Figure 1: Physiography of different agro-climatic zones in Odisha.

Table 1: Details of agro-climatic zones with climate, soil profile and rain fall [13].

Sl. No.	**Agro-climatic Zone**	**Agricultural Districts**	**Broad Soil groups**	**Different parameters of climate (Normal)**			**Climate**
				Mean annual rain fall (mm)	Mean maximum summer temp(°C)	Mean minimum winter temp(°C)	
1.	North Western Plateau	Sundargarh, parts of Deogarh, Sambalpur & Jharsuguda	Red, Brown Forest, Red & Yellow,	1600	38.0	15.0	Hot & moist sub-humid

			Mixed Red & Black				
2.	North Central Plateau	Mayurbhanj, Majorparts of Keonjhar, (except Anandapur & Ghasipura block)	Lateritic, Red & Yellow, Mixed Red & Black	1534	36.6	11.1	Hot & moist sub-humid
3.	North Eastern Coastal Plain	Balasore, Bhadrak, parts of Jajpur & Hatdihi block of Keonjhar	Red, Lateritic, Deltalc alluvial, Coastal alluvial &Saline	1568	36.0	14.8	Moist sub-humid
4.	East & South Eastern Coastal Plain	Kendrapara, Khurda, Jagatsinghpur, part of Cuttack, Puri, Nayagarh & part of Ganjam	Saline, Lateritic, Alluvial, Red &Mixed red& Black	1577	39.0	11.5	Hot & Humid
5.	North Eastern Ghat	Phulbani, Rayagada, Gajapati, part of Ganjam & small patches of Koraput	Brown forest, Lateritic Alluvial, Red, Mixed Red & Black	1597	37.0	10.4	Hot & moist, sub-humid
6.	Eastern Ghat High Land	Major parts of Koraput, Nabarangpur	Red, Mixed Red & Black, Mixed Red & Yellow	1522	34.1	7.5	Warm & humid

7.	South Eastern Ghat	Malkangiri & part of Keonjhar	Red, Lateritic, Black	1710	34.1	13.2	Warm & humid
8.	Western Undulating Zone	Kalahandi & Nuapada	Red, Mixed Red & Black and Black	1352	37.8	11.9	Hot & moist sub-humid
9.	Western Central Table Land	Bargarh, Bolangir, Boudh, Sonepur, parts of Sambalpur & Jharsuguda	Red & Yellow, Red & Black, Black, Brown forest, Lateritic	1614	40.0	12.4	Hot & moist sub-humid
10.	Mid central table land	Angul, Dhenkanal, parts of Cuttack & Jajpur	Alluvial, red, lateritic, mixed red& black	1421	38.7	14.0	Hot & moist sub-humid

2 Materials and method

Plant material collection and preparation

Lemongrass sample (Sample ID: LG24CUTM2024) has been collected from the cultivating field in Baripada block (Co-ordinates: Latitude- 21.998709^0; Longitude- 87.011419^0) of Mayurbhanj district of Odisha state. The slips of leaves are cut into 70-80 cm and dried in sunlight for 24 hours.

Oil extraction by steam distillation

The steam distillation unit is equipped with two vessel/drum with 500 Kg ×2 nos. which is equal to 1 ton capacity. Vessel is manufactured with 304 stainless steel (SS) with 3mm thickness. Vapour sealing is done with water. A boiler unit (Water tube) is attached to each vessel for warm water supply [14]. Boiler (Water tube) outer shell and fire box is made with mild steel (MS), top cover and chimney is made up of MS or Galvanized iron (GI). Boiler quality tubes are used and glass tube water gauge with auto release pressure gauge is attached with a drain valve 1” ball valve. SS 304 quality separator is used to separate oil from water. MS shell base attached with SS ball valve 80 mm x 2 nos. A connecting pipe with SS 304 73 mm outer diameter connects boiler to shell. 1” mm GI pipe used to supply water from condenser to boiler. Two hot water outlet valve with 1”mm brass ball valve is attached to shell grid which is made with SS 304 (1550 dia × 2 nos.). On the top of each vessels are attached to a cone shaped steam collector which is directly connected to a condenser and further the condenser is connected to an oil collecting tube. Lemongrass essential oil is collected through steam distillation method, each drum is filled with 500 Kg dried glass and oil is collected through separator [15].

Figure 2: Diagrams showed the steam distillation unit from different views.

Physical and chemical properties

Physical and chemical properties are measured with appearance and smell, Specific Gravity was measure by Bottle with Stopper - 25 ml and refractive index was measured by Digital refractometer model DR602.

Gas chromatography (GC)

Modifications were made to the approach described by Uraku (2015) and Thenmozhi and Rajan (2015) for the GC analysis [16, 17]. Environmental Monitoring Services, Auroville, conducted the analysis. Using a flame ionizing detector (FID), the GC-FID (Model: Thermo Scientific Trace 1110 Series) was used to perform the GC analysis. The apparatus is equipped with a dual micro channel plate ion detector, low acceleration ion-transfer system, reflection type TOF analyser, EI ion source, and rotary pump ventilation facilities. The apparatus has a capillary column (SGE Analytical Science - Model: BP-1) measuring 25 meters in length, 0.32 millimeters in diameter, and 0.5 micrometers in thickness. The carrier gas was helium, which was used at a split ratio of 1:45 with an injection volume of 1 ml and a flow rate of 1 ml/min. Oil sample was made as 25 ml sample in 0.5 ml hexane. The programming temperature for the injector line was set at 180°C and 250°C, respectively. The oven was set to start at 90 degrees Celsius (isothermal for two minutes), increase by 8 degrees Celsius per minute to 250 degrees Celsius, grow by 8 degrees Celsius per minute (isothermal for three minutes) to reach 250 degrees Celsius, then increase by 5°C per minute to 280 degrees Celsius, culminating in five minutes of isothermal operation at 280°C. The GC-FID ran for 37 minutes in total [18].

Liquid chromatography mass spectroscopy (LCMS)

Electrospray ionization mass spectrometry (ESI-MS) was used to identify the chemical components of the essential oil of lemongrass. High-resolution mass spectroscopy (HRMS) (Waters; Xebo-G2-XS-QTof) was used for the study. An ESI-equipped Q-TOF mass spectrometer was interfaced with the HPLC. During MS scanning, the full-scan mode was configured with a 200°C source temperature and a mass range of m/z 50 to 2000. A maximum flow velocity of 5 µl/min was employed to provide the methanol solvent. The positively charged ion mode was used to get the MS spectra. The nebulizing pressure (N_2) was 25 psi, the boiling point of the drying gas (N_2) was 350°C, and the gas flow rate was 6 ml/min. Before analysis, about 0.5 g of extracts from the samples were mixed with methanol and screened through a 0.22 µm nylon filter. The mass fragmentations were determined using the organic chemical spectrum-database found in MASS Lynx 4.1 (Waters, USA) [19].

Nuclear magnetic resonance (NMR) spectroscopy

NMR spectroscopy was performed to obtain 1H-NMR spectra of *C. flexuosus* extract, which was recorded using NMR (Bruker Avance III running under TOPSPIN 2.4, Wilmad 535-PP-7 tube) spectrophotometer with operating frequency of 700 MHz at 289 K temperature, the spectra obtained of 10 ppm width. The evaluation of the authenticity and determination of the major constituents of commercial citronella oils were performed by the 1H NMR method developed herein. The analyses were carried out to acquire high-quality spectra; sample run for single scans and chemical shifts were reported in parts per millions (ppm). Stock solution of OMCTS in $CDCl_3$ (5.59 mg/ml) were prepared to determine repeatability and accuracy/precision of the NMR method. The ^{1}H spectra were with a single scan in just 15 seconds per sample. To remove the carbon satellites from the ^{1}H spectra, the signal was acquired in the presence of carbon decoupling [20-23].

Quantum chemical structure analysis of citral

The quantum chemical analysis of citral isomers, α and β-citral, was conducted to gain insights into their electronic structure and potential reactivity. This analysis involved computational methods to simulate molecular structures and calculate various electronic properties, providing valuable information on their chemical behavior and interaction. DMol3 in the Material Studio programme (Biovia, France) employing the B3LYP functional and a 6-31G (d,p) basis set was used to accurately calculate the molecular and electronic structure of the oil.

Other specifications and measurements

With above tests, hazard identification, first aid measures, fire fighting measures, toxicological tests, and regulatory measures are done.

3 Results and discussions

Lemongrass sample identification and compound characterization are shown in Table 2 and 3

Table 2: Composition Identifications lemongrass essential oil.

Composition Identifications

Identity	:	Lemongrass oil (Krishna Variety)
Components	:	Essential oil 100%
CAS Number	:	8007-02-1
EC (EINECS) Number	:	295-161-9
FEMA Number	:	2624

Table 3: Physical and chemical parameters with tabulated data.

Analytical test	Specification range	Result
Appearance	Mobile oily liquid bright yellow	Conforms
Odour	Characteristic citral, fresh and intense	Conforms
Relative density at 27°C (g/ml)	0.886– 0.896	0.888
Refractive index at 27°C	1.4830–1.4890	1.4805

Optical rotation	-3˚ to +1˚	
Solubility in 70% ethyl alcohol 1in 2-3 parts	Soluble/ not soluble	Soluble

Gas chromatography analysis

As shown by the chromatogram, a total of twelve components with various retention durations were eluted from the GC column and subjected to further analysis using an electron impact MS voyager detector. The retention period and mass spectroscopy library search were used to identify the ingredients. These are the mass spectrographs of the components that have been identified. GC peak regions were used to compute the relative amounts of each component. Gas chromatography analysis detected the presence of certain phytochemicals in the sample. These include Camphene 0.834% with retention time: 6.872 min, 6-Methyle-5-heptene-2-One 0.452%; retention time: 7.251 min, 4-Nonanone 1.082%; retention time: 9.755 min, and Citronellol 0.104%; retention time: 15.341 min (Figure 3, Table 4).

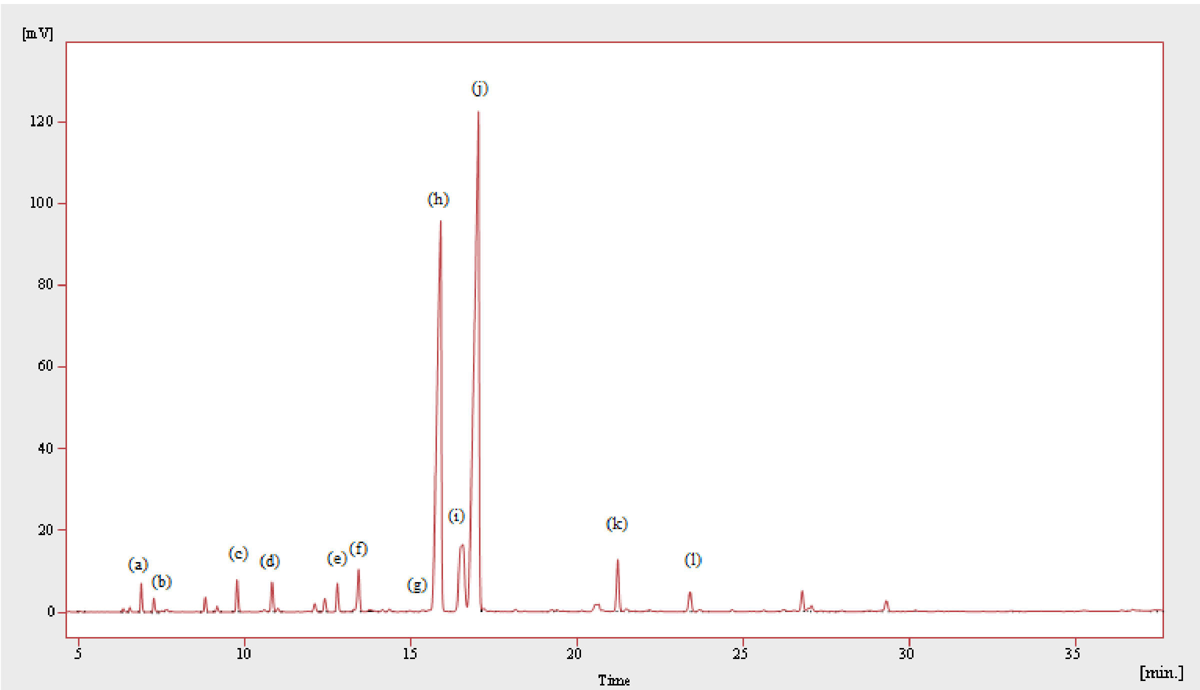

Figure 3: Chromatogram of lemongrass oil.

Table 4: Shows retention time in minute, area in mV/s height in mV, height in % and area in % along with name of compounds found in lemongrass essential oil.

Sl. No.	Reten. Time [min]	Area [mV/s]	Height [mV]	Height [%]	Area [%]	Compound Name
1.	5.019	0.883	0.246	0.1	0.031	
2.	6.331	2.873	0.864	0.3	0.100	
3.	6.528	4.096	1.231	0.4	0.143	
4.	6.872	23.931	6.996	2.1	0.834	Camphene (a)
5.	7.251	12.957	3.467	1.0	0.452	6-Methyl-5-heptene-2-0ne (b)
6.	7.488	1.289	0.362	0.1	0.045	
7.	7.651	4.667	0.655	0.2	0.163	
8.	8.797	14.024	3.695	1.1	0.489	
9.	9.149	5.269	1.402	0.4	0.184	
10.	9.755	31.041	7.868	2.4	1.082	4-Nonanone (c)
11.	10.568	2.449	0.524	0.2	0.085	
12.	10.808	30.876	7.251	2.2	1.076	Linalool (d)
13.	10.979	4.967	0.971	0.3	0.173	
14.	12.096	10.024	1.976	0.6	0.349	
15.	12.395	16.597	3.349	1.0	0.578	
16.	12.773	30.306	6.976	2.1	1.056	Isoneral (e)
17.	13.259	2.026	0.488	0.1	0.071	
18.	13.411	52.605	10.374	3.1	1.833	Isogeranial (f)
19.	13.733	3.154	0.475	0.1	0.110	
20.	14.131	2.763	0.591	0.2	0.096	
21.	14.344	3.845	0.718	0.2	0.134	
22.	14.960	1.434	0.288	0.1	0.050	
23.	15.341	2.988	0.393	0.1	0.104	Citronellol (g)
24.	15.885	893.591	95.784	28.8	31.139	Citral β (h)
25.	16.547	193.087	16.408	4.9	6.729	Geraniol (i)
26.	17.027	1276.310	122.405	36.8	44.476	Citral α (j)
27.	17.181	5.275	0.805	0.2	0.184	
28.	18.136	2.746	0.569	0.2	0.096	
29.	19.221	3.526	0.522	0.2	0.123	

30.	19.389	4.199	0.503	0.2	0.146	
31.	20.144	1.692	0.256	0.1	0.059	
32.	20.635	22.611	1.782	0.5	0.788	
33.	21.221	66.340	12.683	3.8	2.312	Geranyl acetate (k)
34.	21.475	6.667	0.707	0.2	0.232	
35.	22.168	3.952	0.440	0.1	0.138	
36.	23.392	27.756	4.834	1.5	0.967	β-Caryophyllene (l)
37.	23.677	3.936	0.526	0.2	0.137	
38.	24.661	3.170	0.567	0.2	0.110	
39.	25.611	2.566	0.381	0.1	0.089	
40.	26.205	3.954	0.513	0.2	0.138	
41.	26.517	2.334	0.280	0.1	0.081	
42.	26.771	27.774	5.103	1.5	0.968	
43.	26.952	4.818	0.928	0.3	0.168	
44.	27.048	12.822	1.437	0.4	0.447	
45.	27.955	1.905	0.305	0.1	0.066	
46.	28.816	3.162	0.229	0.1	0.110	
47.	29.301	18.329	2.636	0.8	0.639	
48.	30.179	3.784	0.325	0.1	0.132	
49.	33.053	2.847	0.209	0.1	0.099	
50.	36.709	2.702	0.427	0.1	0.094	
51.	37.387	2.756	0.218	0.1	0.096	
	Total	2869.676	332.939	100.0	100.000	

LCMS analysis

Scientists have recently been interested in green herbaceous plants after discovering that they contain high concentrations of bioactive substances, particularly phenolic compounds, which have shown promise for health benefits in a number of studies. Phenolic compounds from lemongrass oil (*C. flexuous*) were screened and described using the most recent LC-ESI-QTOF-MS technique. With the use of sophisticated analytical techniques, we are able to identify the majority of the bioactive components found in plant samples. Agilent Mass Hunter, version B.06.00, and the Personal Compound Database Library (PCDA) were used to qualitatively describe the compounds. The data pertaining to the phenolic chemical profile of plants are listed in Table 5. Using LC/MS analysis, 40 phenolic compounds were discovered (Figure 4, Table 5).

Figure 4: LC-QToF-MS analysis of lemongrass essential oil extracted from *C. flexuosus*.

Table 5: LC-QToF-MS/ MS characterization of phytochemicals extracted from lemongrass leaves.

Formula	Mass Error (ppm)	Theoretical Isotope Distribution	Description	m/z	Retention time (min)
C27H48N6O6	-38.92298538	100 - 32.2 - 6.25 - 0.9 - 0.105	N-Acetyl-L-leucyl-L-isoleucylglycyl-L-valyl-N-methyl-L-prolinamide	288.1693	0.236667
C31H44N4O5	19.64104697	100 - 35.7 - 7.21 - 1.06 - 0.124	Pandamine	288.1693	0.236667
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	(3alpha,5beta,7alpha)-3,7,14-Trihydroxycholan-24-oic acid	373.2625	0.0506
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	ursocholic acid	373.2625	0.0506
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	beta-Phocaecholate	373.2625	0.0506
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	3Beta,7beta,12beta-trihydroxy-5beta-cholan-24-oic acid	373.2625	0.0506
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	5a-Cholic acid	373.2625	0.0506
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	3a,6a,12a-Trihydroxy-5b-cholan-24-oic acid	373.2625	0.0506
C20H36O6	10.78061116	100 - 22.3 - 3.6 - 0.433	8,8a-Deoxyoleandolide	373.2625	0.0506
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	3a,4b,7a-Trihydroxy-5b-cholanoic acid	373.2625	0.0506
C27H36O3	24.20837509	100 - 29.7 - 4.88 - 0.575	Quingestanol acetate	373.2625	0.0506
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	cholic acid	373.2625	0.0506
C24H36O3	-30.2092888	100 - 26.5 - 3.98 - 0.437	3-Ethoxyandrosta-3,5-dien-17beta-ol propanoate	373.2625	0.0506
C24H36O3	-30.2092888	100 - 26.5 - 3.98 - 0.437	ANAGESTONE ACETATE	373.2625	0.0506
C26H36N2O2	-3.298080539	100 - 29.3 - 4.56 - 0.498	2-[2-(4-Benzyl-1-piperazinyl) ethyl]-2,5,7,8-tetramethyl-6-chromanol	373.2625	0.0506
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	(3alpha,5beta,7alpha,8xi,9xi,10xi,12alpha,13xi,14xi,17xi,20xi)-3,7,12-Trihydroxycholan-24-oic acid	373.2625	0.0506
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	3alpha,7alpha,12beta-trihydroxy-5beta-cholan-24-oic acid	373.2625	0.0506
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	Vulpecholate	373.2625	0.0506

C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	1b,3a,7a-Trihydroxy-5b-cholanoic acid	373.2625	0.0506
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	2b,3a,7a-Trihydroxy-5b-cholanoic acid	373.2625	0.0506
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	(3beta,4beta,5beta,7alpha)-3,4,7-Trihydroxycholan-24-oic acid	373.2625	0.0506
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	1b,3a,12a-Trihydroxy-5b-cholanoic acid	373.2625	0.0506
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	omega-Muricholate	373.2625	0.0506
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	alpha-Muricholate	373.2625	0.0506
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	ÃŽÂ²-Muricholic Acid	373.2625	0.0506
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	(3beta,5beta,6alpha,7alpha)-3,6,7-Trihydroxycholan-24-oic acid	373.2625	0.0506
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C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	(3alpha,5alpha,6alpha,7beta)-3,6,7-Trihydroxycholan-24-oic acid	373.2625	0.0506
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	(3alpha,5alpha,6beta,7beta)-3,6,7-Trihydroxycholan-24-oic acid	373.2625	0.0506
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	(2beta,3alpha,5beta,12alpha)-2,3,12-Trihydroxycholan-24-oic acid	373.2625	0.0506
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	3a,4b,12a-Trihydroxy-5b-cholanoic acid	373.2625	0.0506
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	(3beta,4beta,5beta,12alpha)-3,4,12-Trihydroxycholan-24-oic acid	373.2625	0.0506
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	(3alpha,5beta,6beta,12alpha)-3,6,12-Trihydroxycholan-24-oic acid	373.2625	0.0506
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	(3beta,5beta,6alpha,12alpha)-3,6,12-Trihydroxycholan-24-oic acid	373.2625	0.0506
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	(3beta,5beta,6beta,12alpha)-3,6,12-Trihydroxycholan-24-oic acid	373.2625	0.0506
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	3b,7a,12a-Trihydroxy-5b-cholanoic acid	373.2625	0.0506
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	3beta,7alpha,12beta-Trihydroxy-5beta-cholan-24-oic Acid	373.2625	0.0506
C24H40O5	-27.51622527	100 - 26.6 - 4.43 - 0.551	3a,7b,12b-Trihydroxy-5b-cholanoic acid	373.2625	0.0506

NMR analysis

^{1}H-NMR is effective technique for metabolites study in plant extract, as it analyses all the metabolites present in extract. Figure 4 illustrated the ^{1}H-NMR spectrum of *C. flexuosus* extract. Chemical shift obtained from ^{1}H-NMR spectra can be due to proton on carbon or proton on Oxygen/ Nitrogen. Chemical shift procured from proton on carbon is shown by signal at δ1.484, 2.000, 7.187 and 9.747 ppm which correspond to methyl, methylene and aromatic and aldehyde groups, respectively. The value range between δ1.484–1.967 shows the presence of carbonyl group. Single range between δ4.023–7.416 due to the shift for proton on Oxygen/ Nitrogen which account for the presence of alcohol in the extract. Occurrence of allylic group in extract, is due to the shift acquired between δ1.619–2.046. The Peak Fabrics obtained from NMR profile illustrated that *C. flexuosus* extract comprising of mixture of terpenoids and flavonoids.

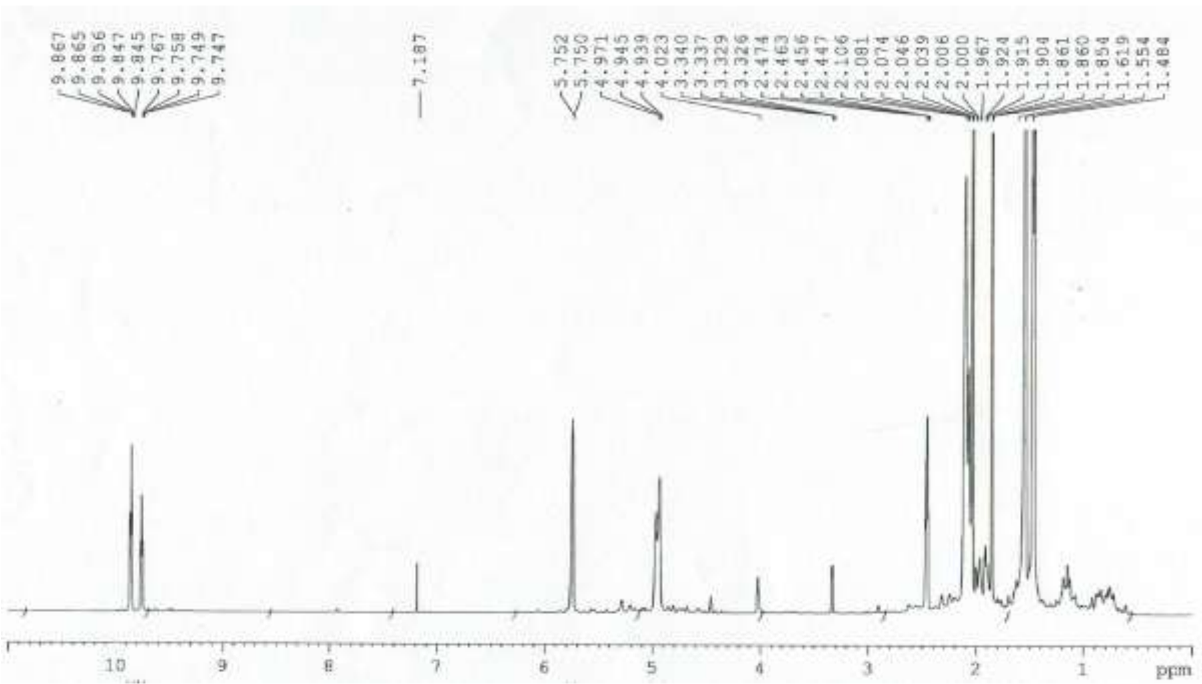

Figure 5: ^{1}H-NMR spectrum of *C. flexuosus* extract. Chemical shift obtained from ^{1}H-NMR spectra can be due to proton on carbon or proton on Oxygen/ Nitrogen.

Quantum chemical structure analysis of citral

Employing the B3LYP function with a 6-31G (d,p) basis set, quantum chemical calculations were carried out using DMol3 in the Material Studio software (Biovia, France). This analysis provided insights into the electronic structure of citral-α and β isomers derived from lemongrass oil through steam distillation, which have been shown in Figure 6. The obtained results revealed that the charges of the citral alpha and beta structures for the highest occupied molecular orbital (HOMO) are -5.289 eV and -5.331 eV, respectively. Whereas the charges of the citral alpha and beta structures for the lowest occupied molecular orbital (LUMO) are -2.457 eV and -2.511 eV, respectively. These electronic structure details are visually presented in Figure 6.

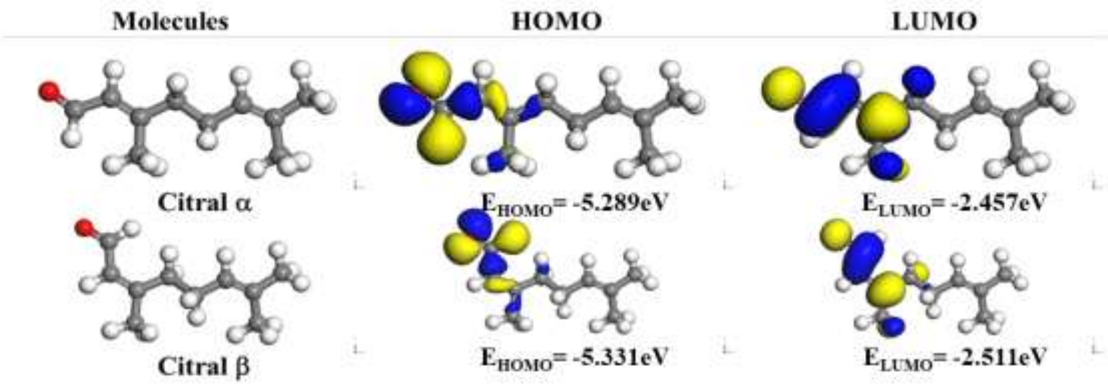

Figure 6: Highest occupied molecular orbital (HOMO) and lowest occupied molecular orbital (LUMO) chemical structure of citral-α and citral-β in lemongrass essential oil (LGEO) extracted by steam distillation (SD) method.

Hazard classification

Risk signs, risk symbols, R phrases, and/or S phrases need to be used to designate the kind of danger. Hazardous compositions and compounds are associated with two numbers and two phrases, respectively, called R and R number. S number and S phrase: they are terms and numbers corresponding to the safe management of risky preparations. There are various combinations of Risk Phrases (R) and Safety Phrases (S) as well.

GHS07

GHS05

Figure 6: Label elements GHS signal word DANGER hazard pictograms. Hazard determining components of labeling for citral (GHS07), geraniol (GHS05).

Table 6: Hazardous information for safety measures and toxic impact.

Hazards Identification
Flam. Liq.4 H227: Combustible liquid Skin Irrit.2 H315: Causes skin irritation Skin Sens.1 H317: Might result in an adverse skin response Eye Damage H318: Causes severe eye damage Aquatic Acute H402: Harmful to aquatic organisms Aquatic Chronic H411: Long-lasting toxic impacts on aquatic life

Table 7: Regulatory information for first aid and fire-fighting measures.

R phrases

R38	:	Irritating to skin
R36	:	Irritating to eyes
R43	:	May cause sensitization by skin contact.

Safety Phrases

S7	:	Keep container tightly closed.
S16	:	Keep away from source of ignition
S24/25	:	Avoid contact with skin and eyes.
FDA no	:	182.2
FEMA no	:	2831
HS Code	:	33012933

First aid and Fire-fighting measures

If oil accidentally gets in your eyes, take off your contact lenses if needed. Spend fifteen minutes flushing with a lot of water or an eye wash solution. If there has been skin contact, take off any contaminated clothes. Use plenty of water to flush and wash with soap. Apply an emollient to the affected skin. In the event that oil is accidentally consumed, dilute it with water or milk, call the poison control center, or get medical help right away. Avoid breathing in vapors in a disgusting way if you must inhale. If there was a significant inhalation, remove to fresh air. As quickly as you can, take the sufferer to a secure location. Loosen belts, waistbands, ties, and collars that are too tight. Give oxygen if breathing is difficult. Put the person via mouth-to-mouth resuscitation if they are not breathing. LGEO is purely non-flammable, its flash point>100 Deg by close cup technique. Extinguishing media can be used like dry chemical, carbon dioxide, foam. It was recommended to not use water jet. Additionally, it was mandated that firefighters use positive pressure, self-contained breathing apparatus as part of particular firefighting protocols. Water spray cools containers that are in direct flame contact. Apply extinguishing agent straight into the fire's base. There was no unexpected fire or explosive risks found.

Stability and reactivity

Under typical circumstances, LGEO is stable. It was advised to avoid using materials that were incompatible with powerful oxidizing agents, as well as heated labor and ignition sources close or on empty containers. A few other situations that were to be avoided included heat, flame, and contact with sparks, as well as potentially dangerous decomposition products such CO, CO_2, smoke, and unknown chemical compounds that could arise during combustion. It has been noted that while byproducts are produced during burning, dangerous polymerization does not take place.

Toxicological information

LGEO may be irritating to eyes, skin, and mucous membranes. Acute toxicity level in oral administration in rat was 4400 mg/Kg (LD_{50}) in primary stage and there was a secondary dosage which was greater than 2000 mg/Kg in rat. According to Plata-Rudea et al., Lemongrass topically applied was toxic against *S. granarius* adults (LD_{50} = 4.03 µg·insect^{-1}) and mortality increased in a dose-dependent manner, as also reported in other pests [19]. According to Costa et al., LGEO did not present an extensive toxic effect in rodents, since the LD_{50} in mice was around 3500 mg/Kg, which is a much higher dose than those usually taken as an infusion by humans [24]. According to Xavier et al., LGEO and citral would be safe even at the limit dose level of 2000 mg/Kg body weight after single dose oral administration to female Sprague Dawley rats and hence the LD_{50} could be proclaimed as more than 2000 mg/Kg, enabling to be labelled as category 5/unclassified in hazard category of Globally Harmonized System for classification of chemical [25]. There was no data available on chronic effects on humans. Other toxic effects on humans like LGEO can be hazardous in case of skin contact (irritant, permeator), of inhalation. Furthermore, LGRO was not listed as a carcinogen [26-31]. There are no published environmental criteria for this complicated composition. Garbage recycling methods are designed to ensure that no product leftovers remain. Discard in accordance with municipal, state, and Government requirements. Surface pollution of soil, ground, and surface water is prevented by taking preventative measures.

4 Conclusion

The whole study i.e., plant material collection, extraction and chemistry profile analysis of lemongrass essential was done at Mayurbhanj district of Odisha state which was not reported before. The chemistry profile of the lemongrass oil shows refractive index (at 27˚C) was 1.4805 (Normal range: 1.4830-1.4890) and specific gravity (at 27˚C) was 0.888 (Normal range: 0.886-0.896) which was not reported before, along with this GC analysis shows the photochemical like: Camphene (0.834%; Rt. Time: 6.872 min), 6-Methyle-5-heptene-2-One (0.452%; Rt. Time: 7.251 min), 4-Nonanone (1.082%; Rt. Time: 9.755 min); Citronellol (0.104; Rt. Time: 15.341 min), Citral-β (31.139 %; Rt. Time: 15.885), Geraniol (6.729; Rt. Time: 16.547 min), Citral-α (44.476; Rt. Time: 17.027 min), Geranyl acetate (2.312; Rt. Time: 21.221 min), β-Caryophyllene (0.967, Rt. Time: 23.392). LCMS characterization reveals 40 new phenolic compounds which were not reported before. NMR spectroscopy showed the carbon atoms experience proton-induced chemical shift, resulting in signals at δ1.484, 2.000, 7.187, and 9.747 ppm. These signals correspond to methyl, methylene, aromatic, and aldehyde groups, respectively. The δ1.484-1.967 range encompasses the presence of a carbonyl group. Quantum chemical structure analysis revealed that the charges of the citral alpha and beta structures for the highest occupied molecular orbital (HOMO) are -5.289 eV and -5.331 eV, respectively. Whereas the charges of the citral alpha and beta structures for the lowest occupied molecular orbital (LUMO) are -2.457 eV and -2.511 eV, respectively. Among all these the amount of citral is in high concentration present is lemongrass leaves cultivated in Mayurbhanj area, which was not reported before. After all, we have also emphasized on hazard identifications and toxicological findings show toxic dose or LD_{50} was 4400 mg/Kg in oral administration of rat. Lemongrass oil was stable under room temperature and had a flash point of 71˚C which was not reported before. Moreover, modern agricultural advancement and process may increase the yield of essential oil and citral also which is future perspective of this study.

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Informed consent: Each and every author involved in this study offered their informed consent.

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