

Isolation and Characterization of Essential Oil from *Cymbopogon citrates* Grown in Nepal

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Abstract

In this work essential oil was obtained by hydro-distillation from air dried leaves of *Cymbopogon citrates* using Clevenger apparatus and analyzed by Gas chromatography–mass spectrometry (GC-MS) technique. Twenty three chemical components were identified in the essential oil of *Cymbopogon citrates* leaves. Among them geranial (citral- α) 32.51%, neral (citral- β) 28.38% and myrcene 14.23% were found as major components. The preliminary phytochemical analysis of the methanolic extract of *Cymbopogon citrates* leaves showed that it has alkaloids, tannins, carbohydrates and flavonoids whereas negative results for phenols.

Keywords: *Cymbopogon citrates*, Essential oil, Citral- α , Citral- β , GC-MS, Myrcene

Introduction

Plants having essential oils are significantly important in medical practice. The chemical composition of essential oil is complex and may contain hundreds of components. It is a mixture of fragrant volatile substances such as monoterpenes, sesquiterpenes, aromatic compounds, and their derivatives. Essential oils have been used as antimicrobial, anti-inflammatory, sedative, expectorants, and diaphoretics etc. There are numerous medicinal plants possess essential oils in their tissues such as sage (*Salvia officinalis* L.), eucalyptus species, peppermint (*Mentha piperita* L.), Scotch pine, elecampane (*Inula helenium* L.), linden (*Tilia cordata* Mill), garden heliotrope (*Valeriana officinalis* L.), etc¹.

Lemongrass, *Cymbopogon citratus*, is a perennial medicinal plant belonging to family Gramineae, and it is distributed worldwide in tropical and subtropical areas of Asia, Africa, and America²⁻⁴. Some species (particularly *Cymbopogon citratus*) are commonly cultivated as culinary and medicinal herbs because of their scent resembling that of lemons (citrus lemon). Common names includes lemon grass, sarbed wire grass, silky heads, citronella grass, fever grass, tangled hierba or gavati chahapati among many others².

Lemongrass is widely used as culinary herb in Asian cuisines and also as medicinal herb in India. It has a subtle citrus flavour and can be dried and powdered or used fresh. It is commonly used in teas, soups, and curries. It is also suitable for the use with poultry fish, beef and sea food. It is often used as a tea in African countries such as Togo, South Eastern Ghana Volta region and the democratic republic of

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Congo and Latin American countries such as Mexico⁵. Lemongrass oil is used as pesticides and preservative. Research shows the lemongrass oil has antifungal properties. Despite its ability to repel some insects such as mosquitoes, its oil is commonly used as 'lure' to attract honey bees⁶.

Lemon grass *Cymbopogon nardus* and *Cymbopogon winterianus* grow to about 2 m (6.6 ft) and have magenta coloured base stems. These species are used in the production of soaps, insect repellent (especially mosquitoes) in insect sprays and candles and in aromatherapy. The principal chemical constituents i.e. geraniol and citronellol of citronella, are the antiseptics⁷. It is used as a pesticide and preservative, is put on the ancient plant leaf manuscripts found in Nepal and India preservative⁸. It is also used as an addition to tea, and in preparations such as *kadha*, which is a traditional herbal brew used against coughs, colds, etc. It has medicinal properties too and is used widely in ayurvedic medicine. It is supposed to help with relieving cough and nasal congestion. It shows antioxidant, anti-cancer properties and anti-inflammatory property⁹.

Herbal drugs have been used worldwide during the last few decades as evidenced by rapidly growing global and national markets of herbal drugs¹⁰. Several studies indicated that approximately 33% of drugs produced in the developed countries are derived from plants¹⁰. The present research was carried out to evaluate phytoconstituents of methanolic extract of *Cymbopogon citratus* leaves and investigate the composition of its extracted essential oil via Gas chromatography-mass spectrometry (GC-MS) technique.

Experimental Methods

Materials and Sample Preparation

The fresh leaves of *Cymbopogon citratus* were collected from the Nepal Agricultural Research Council Lalitpur, Nepal. Fresh and dried leaves were subjected to hydro-distillation for 8 h, using Clevenger type apparatus, according to method recommended by Guenther⁵. The extracted essential oils were dried with anhydrous sodium sulphate and stored in sealed cubet at low temperature before analysis. The extracted oil was analyzed by GC-MS using Varian 240 GC-MS system equipped with VF-5 fused capillary column (30m x 0.25 mm, i.e. film thickness 0.25 μm); oven temperature was 50-180 °C at a rate of 5 °C/min, transfer line temperature 250 °C, carrier gas was helium with a flow rate of 1 mL/min, split ratio 1:20, ionization energy 70 eV, and mass range 35-390 amu. The constituents of the oil were identified by comparison of their mass spectra with those of a computer library or with authentic compounds.

Phytochemical screening

For phytochemical screening, the plant samples were crushed and blended into smaller pieces. 200 g of powdered *Cymbopogon citratus* samples (leaf) were taken into 2000 mL capacity conical flask. 1000 mL methanol was poured to the sample. The conical flask containing the mixtures was placed on a shaker for 24 hours and filtered the content through muslin cloth. The filtrate was then filtered again using suction pressure with the aid of a vacuum pump. The filtered extract was concentrated using the rotary evaporator equipment then dried on an evaporating dish at a temperature of 50-60 °C to a semi-solid form. A sticky semi-solid greenish substance obtained which was subjected for phytochemical

screening using standard procedures¹¹⁻¹⁴. The following qualitative tests for both the metabolites were done as follows:

Test for alkaloids

About 10 mg of extract was taken and few drops of Wagner's reagent were added and the formation of a reddish brown precipitate indicates the presence of alkaloids.

Test for Flavonoids

Shinoda Test: 10 mg of extract was added to pinch of magnesium turnings and 1-2 drops of concentrated hydrochloric acid was added. Formation of pink color indicates the presence of Flavanoids.

Lead acetate test: 10 mg of extract was taken and few drops of 10% lead acetate solution was added. Appearance of yellow colour precipitate indicates the presence of flavonoids.

Test for Phenols and Tannins

Lead acetate test: 10 mg of extract was taken and 0.5 mL of 1% lead acetate solution was added and the formation of precipitate indicates the presence of tannins and phenolic compounds.

Ferric chloride test: Five mg of extract was taken and 0.5 mL of 5% ferric chloride was added. The development of dark bluish black color indicates the presence of tannins.

Sodium hydroxide test: Five mg of extract was dissolved in 0.5 ml of 20% sulphuric acid solution. Followed by addition of few drops of aqueous sodium hydroxide solution, it turns blue which indicates the presence of phenols.

Test for Carbohydrates

Fehling's test: Five ml of Fehling's solution was added to 0.5 mg of extract and boiled in a water bath. The formation of yellow or red precipitate indicates the presence of reducing power.

Benedict's test: Five ml of Benedict's solution was added to 0.5 mg of extract and boiled in water bath. The appearance of red or yellow or green precipitate indicates the presence of reducing sugars.

Results and Discussion

A pale yellow essential oil with yields of 0.67% (on fresh weight basis) was obtained from fresh lemongrass plant. This result agrees with some works who reported that oil content of a normal cut should average 0.25-0.5%, but with good management and selected strains could be yielded up to 0.66-0.90%^{15,16}.

Twenty three components were identified in the essential oil of fresh air dried *C. citratus* leaves by different drying methods, which represented 100 % of the oil components. The chemical constituents of oils are presented in Table 1. The components are listed in order of their retention time on the VF-5 column.

Table 1: Essential oil components of *Cymbopogon citratus*

Peak #	R. Time	Area	Area%	Name
1	14.271	2179810	1.95	Hept-5-en-2-one<6-methyl->
2	14.439	15880294	14.23	Myrcene
3	16.693	862645	0.77	Ocimene <(Z)-,beta->
4	17.192	375209	0.34	Ocimene <(E)-,beta->
5	19.404	941462	0.84	Linalool
6	19.594	1107383	0.99	RT: 19.592
7	19.711	2816978	2.52	Sabinene hydrate <trans->
8	20.485	202256	0.18	Octa -2, 4-dienol <trans->
9	21.647	218224	0.20	Thujone <beta->
10	21.908	378737	0.34	Nona-(2E,6Z)-dienal
11	22.165	400660	0.36	Chrysanthemol <trans->
12	22.859	1721067	1.54	Verbenol <trans->
13	23.392	2029535	1.82	Rose furan oxide
14	23.722	2310227	2.07	Isogeranial
15	23.891	1013333	0.91	Ethanol <(2Z)-(3,3-diementhylcyclohexylidene)->
16	26.126	240743	0.22	Oct-7-enol<3,7-dimethyl>
17	26.548	31667250	28.38	Neral
18	27.114	6499793	5.83	Geraniol
19	27.934	38505521	34.52	Geranial
20	30.916	257042	0.23	Deca-(2E,4E)-dien-1-ol
21	32.499	282930	0.25	Undec-(8Z)-enal
22	32.809	1456983	1.31	Linalool isobutyrate
23	42.475	224109	0.20	Eudesmol <epi-gamma->
		111572191	100.00	

The major components of the essential oils were geranial (34.52%), neral (28.38%) and myrcene (14.23%) in oils extracted from air dried lemongrass leaves. The quality of lemongrass is generally determined by its citral content. Citral is a combination of bioactive isomers geranial and neral. According to Ref.¹⁷ the lemongrass essential oil is usually made up of citral at an average of 65 to 80%.

This result showed 23 major components of the essential oil of lemongrass. Among 23 compounds, only 10 compounds (6-Methylhept-5-en-2-one, Myrcene, trans-Sabinene hydrate, trans-Verbenol, Rose furan oxide, Isogeranial, Neral, Geraniol, Geranial and Linalool isobutyrate) had a concentration of greater than 1%. This is in agreement with several research work; reported the variations of 20 to 50% of

geranial and 30 to 40% of neral in lemongrass chemical composition^{15, 18-20}. According to Schaneberg and Khan, the essential oil of lemongrass contains mainly geranial and neral²¹. Other isolated components, such as β -myrcene, ocimene, β -cimene, linalool, citronellal, citronellol, caryophyllene and β -pinene, were present as minor components reported in Ref²².

Table 2 shows the phytochemical constituents of methanolic extract of lemongrass leaf. The result shows the presence of alkaloids, tannin, flavonoids and carbohydrate whereas phenol is absent. This agrees with the findings of Umar *et al.*¹⁰ and Aina *et al.*²³.

Table 2: *Phytochemical constituents of Cymbopogon citrates leaf*

Phytochemical Constituents	Leaf extract (methanol)
alkaloids	+
Tannin	+
Flavonoids	+
Phenol	-
Carbohydrate	+

Conclusions

The essential oil was successfully extracted from the plant *Cymbopogon citratus* by means of hydrodistillation method *via* clevenger type apparatus. The volatile constituents which is present in this plant was analyzed by the Gas Chromatography-Mass Spectroscopy. Twenty three chemical components were identified in the essential oil of *Cymbopogon citratus* leaves and dried citrus leaves obtained by GC-MS technique including geranial (citral- α) 32.51%, neral (citral- β) 28.38% and myrcene 14.23% as a major component. The result of phytochemical screening of methanolic extract of *Cymbopogon citratus* leaves showed the presence of tannins, flavonoids and carbohydrates whereas phenol is absent.

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