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Comparison of Essential Oil Content and Composition of Different Parts of *Cymbopogon citratus* (DC.) Stapf (Poaceae) Grown in Sri Lanka

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Abstract The Lemongrass, *Cymbopogon citratus* (DC.) Stapf, is an important species of Poaceae family commonly used in the cosmetic, food and in folk medicines in many countries. The aim of this study was to compare the variation of essential oil content and composition of different parts of the plant with the maturity of lemongrass. The Essential oil content and composition of different parts of lemongrass were determined using Clevenger type apparatus and GC-MS respectively. Essential oil content was significantly higher in leaves (0.91%) followed by sheath (0.7%) and roots (0.2%) respectively. Leaf essential oil content was increased with the maturity of the plant. The number of constituent' presence in leaf, sheath and root of *C. citratus* were 9, 14 and 17 respectively. Moreover, out of 20 constituents identified from all 3 parts of lemongrass, only 6 constituents were common for all three parts, while the composition of root essential oil was significantly different from leaf and sheath essential oil. The major compounds found in leaf and sheath essential oil of *C. citratus* were geranial, neral, β- myrcene and geraniol while root essential oil contained mainly Selina-6- en-4-ol (28.08%) followed by geraniol (10.70%), t-muurolol (9.61%), neral and longifolene respectively. Based on results, it could be concluded that in addition to the lemongrass leaf, sheath and root also equally important for use in multipurpose industries.

Keywords: *Cymbopogon citratus, Essential oil, Lemongrass, Poaceae, Geranial, Neral*

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1. Introduction

Cymbopogon citratus (DC.) Stapf (Poaceae), Lemongrass in English, is economically important, essential oil bearing perennial plant which is widely distributed in Indonesia, Madagascar, India, Sri Lanka and countries in Africa and South America [1,3,5]. This plant is widely cultivated for its high quality essential oil comprising a mixture of an array of bioactive chemical constituents which are widely used in several industries for the production of an array of products all over the world. The essential oil of lemongrass has characteristic strong lemony odour due to the presence high content of aldehyde citral which has two geometric isomers, geranial (citral a) and neral (citral b) [18]. In addition to citral, it consists of geraniol, myrcene, geranyl acetate, caryophyllene and monoterpene olefins, such as limonene [18,20,21]. Due to the presence of large number of compounds and bioactive molecules essential oils as well as aqueous extracts have been widely used in traditional and western medicines for the treatment of nervous, gastrointestinal disturbances, pneumonia, coughs, fevers, reducing cholesterol, uric acid, stimulating of digestion and cleansing of the liver, pancreas, kidney, bladder and the digestive tract [13,17]. Moreover, essential oil of lemon grass plays a pivotal role in food and cosmetic industries [12].In addition to that, constituent presence in lemongrass essential oil is highly valued as a natural weedicide and recent research revealed that lemon grass essential oil is highly effective for the control herbicide resistant biotypes of *Bidens pilosa* and *Bidens subalternans* and inhibited germination and seedling growth of *Echinochloa crus-galli* [7,16]. Furthermore, Essential oil of lemongrass can be used as an insecticide. It has showed good results against aphids, thrips, red flour beetle and larval stages of housefly [9,15]. Meanwhile it was revealed that essential oil content and composition obtained from different parts of lemongrass is significantly varied [6]. Even though, lemongrass is commercially used in an array of industries, compositional analysis of essential oil presence in different parts well as changes of oil content with the plant maturation are scattered or lacking. Present study demonstrates the essential oil content and composition of different parts of *C. citratus* and variation of essential oil yield with different harvesting stages.

2. Materials and Methods

2.1. Harvesting and Sample Preparation

Plants which previously authenticated and cultivated under the same soil and climatic conditions were uprooted carefully without destroying the roots. Then the roots, leaves and sheaths of those plants were separated. Those separated parts were cut into 2-3 cm pieces with secateurs and prepared three fresh samples (350g each) from each part (roots, leaves, sheaths) of *C. citratus*.

2.2. Oil Distillation

350g of freshly cut pieces (2-3cm long) of leaves, sheaths and roots of *Cymbopogon citratus* were introduce to a 2.5L round bottom flask at the rate of 1/2.5 (1g of plant material for 2.5mL of water) and hydro-distilled in a Clevenger-type apparatus for 5hrs. The extracted volatile oils were dried over anhydrous sodium sulphate and stored in sealed vials at 4˚C until analysis. The oil content in the *C. citratus* was calculated as percentage yield, which is determined as the percent of the ratio of weight of oil to weight of lemon grass leaves used to extract it.

$$Yield\ of essential oil = \frac{\text{Amount of essential oil}(g)\text{obtained}}{\text{Mass of raw materials}(g)\text{used}} \times 100.$$

Results are presented as means of triplicate and standard deviation.

2.3. Observation for Organoleptic Properties of Oil

The colour, aroma and the oil content was observed of each essential oil derived from different parts (Leaf, Sheath, Roots) of *C. citratus.*

2.4. GC-MS Analysis

Essential oils derived from different parts (Leaf, Sheath, Roots) of *C. citratus* was subjected to GC-MS analysis. The oil analysis was carried out using GC/MS. The GC apparatus was Agilent technology 6890 series, capillary column of HP-5MS (30 m×0.25 mm, film thickness 0.25 μm). The oven temperature program was initiated at 50°C, held for 5 min then raised up to 280°C at a rate of 5°C /min held for 10 min. Helium was used as the carrier gas at a flow rate 0.9 ml/min. The injector temperature was 250°C. GC/ MS analysis was conducted on a HP 6890 GC system coupled with 5973 network mass selective detector with a capillary column the same as above, carrier gas helium with flow rate 0.9 ml/min with a split less injection mode, injector and oven temperature programmed was identical to GC. The compounds of the oil were identified by comparison of their retention indices (RI), mass spectra fragmentation with those on the stored Wiley W9N08 database and NIST (National Institute of Standards and Technology) database.

2.5. Variation of Oil Content with Maturity

This experiment was designed to determine the variation of oil yield of leaves of *C. citratus* with the maturity of plants. Three months old uniformly grown lemongrass plants were selected for this experiment. After collecting the first harvest the leaves were allowed to grow. Samples were collected after 14, 28, 42, 56 and 70 days after first cut. The samples were cut into pieces and distilled using Clevenger type apparatus for five hours and essential oil content was recorded.

2.6. Statistical Analysis

Data were statistically analyzed using Analysis of variance (ANOVA) and means were compared using Tukey test. Statistical analysis was performed with Minitab 17 software.

3. Results and Discussion

3.1. Essential Oil Content and Organoleptic Properties

Plant materials for determination of essential oil content and composition of different parts of *C. citratus* were obtained from previously authenticated healthy 3 months old cultivation maintained at institute's research field. Therefore, results presented in this study may exhibit true phytochemical variation presence in different parts of the plant. As shown in Table 1, essential oil content of leaves, sheath and roots of *C. citratus* were 0.91%, 0.7% and 0.2% (on fresh weight basis) respectively. The order of increasing of essential oil content of different parts of lemongrass was varied as leaf>sheath>root. Our results are in agreement with previous studies which reported that oil content of lemon grass leaves varied from 0.66-0.90% [5,8,20]. As shown in Table 1, organoleptic properties such as oil colour and aroma of essential oil obtained from different parts of *C. citratus* varied depending on the parts used. This clearly indicates the presence of different constituent in different parts of the plant [18]. Moreover, our results of the organoleptic properties of *C. citratus* essential oil are in agreement with reference [6] who investigated bioactive compounds of *C. citratus* essential oil from different parts of the plant.

Table 1. Essential oil content and organoleptic properties of essential oil extracted from different parts of *Cymbopogon citratus* (Mean of 3 treatments, followed by the same letter do not differ by Tukey test at $P < 0.05$)

Plant Part	Oil Yield (Fresh Weight Basis)	Oil Colour	Aroma
Leaves	0.91 ± 0.29^a	Yellow	Strong Lemony Aroma
Sheath	0.7 ± 0.05^b	Yellowish green	Strong Lemony Aroma
Roots	0.2 ± 0.13^c	Pale Yellow	Strong Aroma

3.2. Essential Oil Composition

Composition of essential oils is an important character when analyzing the commercial potential and the ability of usage in perfumery.

Essential oil composition of different parts (Leaf, Sheath and Root) of *C. citratus* was demonstrated in Table 2. As shown in Table 2, major compounds present in leaf, sheath and root were identified representing 90.59%, 95.45% and 88.68% of total essential oil profile respectively. Moreover, it is interesting to note that number of constituent presence in leaf, sheath and root of *C. citratus* were 9, 14 and 17 respectively. The similar observations were showed by [14] where found that number of compounds present in different parts were varied as leaf<sheath<roots. Meanwhile five major constituents were identified from leaf essential oil majoring geranial (41.21%) followed by neral (31.13%), β-myrcene, geraniol and geranyl acetate respectively. Results on essential oil composition of leaf essential oil are in agreement with [4] and [19], who investigated oil content and composition of leaves of *C. citratus* and they observed that 15.69% myrcene, 34.98% neral and 40.72% of geraniol in fresh leaves of *C. citratus*.

Richness of the important compounds like geraniol, neral, etc. determines the commercial potential of large scale cultivation. Geraniol is highly valued as a perfume and a starting material for a large number of synthetic aromatic chemicals. Citral is used as a starting material for synthesis of perfumery grade ionones and vitamin A. In the present study, among 20 constituent identified in all 3 parts, only β- myrcene, neral, geranial, geraniol, and selina-6-en-4-ol were observed as major constituents present in different parts of *C. citratus* (Figure 1).

Table 2: Essential oil composition of Lemongrass

Order no	R.T.	Compound	Compound percentage*		
			Leaf	Sheath	Root
1	14.93	β- Myrcene	11.17	3.03	1.49
2	16.457	β-Ocimene	0.37	1.783	ND
3	18.471	β-Linalool	0.76	0.81	1.32
4	23.03	Neral	31.13	30.20	7.18
5	23.694	Geraniol	3.47	2.75	10.70
6	23.978	Geranial	41.21	45.45	ND
7	26.276	Geranic acid	0.82	0.39	1.49
8	26.667	Geranyl acetate	1.48	0.74	ND
9	27.709	Caryophyllene	0.18	0.32	2.09
10	29.876	Cuparene	ND	ND	2.73
11	30.066	α-Amorphene	ND	ND	1.30
12	30.267	δ- Cadinene	ND	0.51	2.01
13	32.268	α- Eudesmol	ND	ND	2.07
14	32.695	Selina-6- en-4-ol	ND	5.86	28.08
15	32.955	Calarene	ND	ND	2.38
16	33.121	α- Cadinol	ND	1.74	5.38
17	33.204	α- Cubebene	ND	ND	1.10
18	33.453	t-Muurolol	ND	ND	9.61
19	33.571	Longifolene	ND	1.09	6.32
20	34.353	Juniper camphor	ND	0.78	3.37
Total oil profile			**90.59%**	**95.45%**	**88.68%**

ND- Not Detected (< 0.05)
*values are the average of triplicates.

Out of 5 major compounds, only 3 compounds β-myrcene, neral and geranial were common for all three parts tested. Our results are agreed with reference [14], who compared essential oil composition of leaf, sheath and roots and found that β- myrcene, neral and geranial were common compounds presence in *C. citratus* grown in Nigeria. Moreover, geranial was observed as major compound presence in leaf and sheath, however, these were not observed in root essential oil.

Findings of this study are partially agreed with reference [6] who investigated essential oil composition of leaf and sheath essential oil of *C. citratus*. Moreover, previous study [5] showed that geranial (40.72%), neral (34.98%) and β-myrcene (15.69%) as major compounds available in oils extracted from fresh leaves of lemongrass. Furthermore, a recent study [10] and [5] showed that they have found that geranial (34.8%), neral (30.72%), β-myrcene (11.28%), geraniol (5.54%), 1,3,4-trimethyl 3-cyclohexene-1-Carboxaldehyde (2.20%), citonellol (1.34%). Essential oil composition of roots of *C. citratus* was quite different from leaf and sheath essential oil composition. It was demonstrated that root essential oil is a mixture of 19 individual compounds majoring. selina-6- en-4-ol (28.8%), followed by geraniol (10.70%), t-muurolol (9.61%), neral (7.81%), longifolene (6.32), α- cadinol (5,38%) and juniper camphor (3.37%) respectively. Even though, geranial was the major constituent of leaf and sheath essential oil, it was not observed in root essential oil. Moreover, the content of neral and β- myrcene are significantly low in root essential oil (Figure 1).

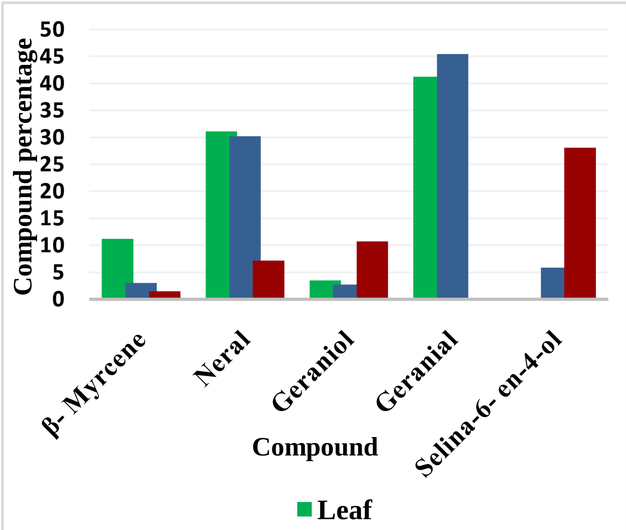

Figure 1. Percentage major compounds available in all three parts (leaf, sheath and root) of *Cymbopogon citratus*

3.3. Variation of Oil Content with Maturity

As demonstrated in Figure 2, essential oil content of lemongrass leaves after 14 days from 1st cut is 0.75% and it was not significantly different even after 28 days from the last cut (0.70%). However, the oil content of 3rd, 4th and 5th harvests were significantly increased. (Increased up to 1.06% at 5th harvest after 70 days).This results clearly indicate the leaf essential oil content significantly increased with the maturity of the plant. Previous studies

[21] and [11] indicated that harvesting of lemongrass can be commenced at 90 days after planting and continue up to 5-6 years at 50-60 days' interval which resulted only 0.35 to 0.6% of the dried biomass. Our results revealed that lemongrass leaves can be harvested after 56 days from the last cut for higher oil content.

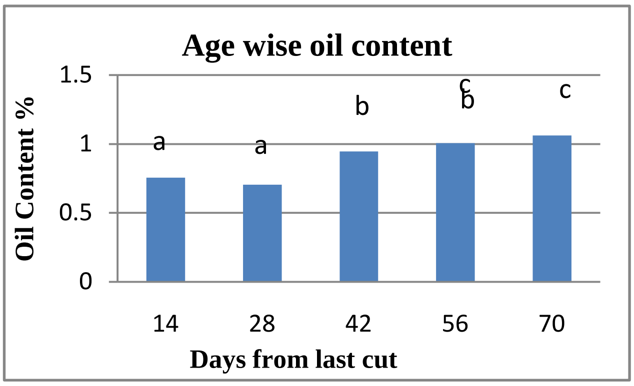

Figure 2. Variation of oil content of *Cymbopogon citratus* leaves with maturity (Mean of 3 treatments, followed by the same letter do not differ by Tukey test ($P < 0.05$)

This result is also compatible with the oil yield data showed in [2]. According to [2], oil yield of lemongrass has been increased gradually up to 62 days from the last cut.

4. Conclusion

The results of the present study clearly indicated that essential oil content and composition of *C. citratus* are mainly depending on part of the plant used for essential oil extraction. Availability of the highest oil content of leaf clearly validate the traditional claim of using lemongrass leaf for oil extraction in commercial level. Moreover, results of the current study are vital important for understanding of the pharmacological activities of different parts of the plant. Further, lemongrass can be harvested at 14 days' interval for increased oil content. Since plant materials for all these studies are taken from the plants maintained under same soil and climatic conditions, observed results reflected the true chemical variation. Based on all these it could be concluded that in addition to the lemongrass leaf, sheath and root also could be incorporated for oil extraction.

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