

Comparative Analysis of Antidermatophytic Potential of *Cymbopogon citratus* Essential Oil Stored in Different Temperature Condition for Two Years

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ABSTRACT

Background: *Cymbopogon citratus* commonly known as lemon grass is a valuable medicinal plant. In present investigation variation in the antidermatophytic potential of *C. citratus* essential oil was estimated in different temperature and storage periods. **Materials and Methods:** *C. citratus* essential oil was extracted through hydrodistillation methods and kept in amber bottles in three different temperature conditions like room temperature (variable from 8-45°C), refrigerator temperature (2-6°C) and 30°C for 2 years. Minimum inhibitory concentration was determined after every fourth month of storage against selected dermatophytes. **Results:** Antidermatophytic activity of *C. citratus* oil was found prominent due to high concentration of citral derivatives. MIC was found to be stable against *Trichophyton rubrum* (MTCC 296) (0.1 µL/mL) and *T. mentagrophytes* (MTCC 7687) (0.1 µL/mL) at the end of experiment in all three temperature conditions. 30°C temperature condition was also found quite stable. A very slight increase of MIC was found against *Candida albicans* (MTCC 3018) and *Microsporium fulvum* (MTCC 2837) at 24 months (Sixth testing) of storage. Oil stored at room temperature showed variation in MIC as compared to initial one. MIC was found to double against *M. fulvum* (MTCC 2837) at sixth testing (0.4 µL/mL) of storage as compared to first testing (0.2 µL/mL). **Conclusion:** Present work concluded that essential oil stored in low temperature condition can maintain their primary composition and properties for long time. Therefore, storing of essential oil products in low temperature (below 8°C) condition is best practice for long time application of medicinal products.

Keywords: Longevity, Essential oil, Dermatophytes, MIC, Storage, *C. citratus*.

INTRODUCTION

Plant derived bioactive substances are very important source of medicines that play a vital role in human health and also applied against several microbial infections.^[1-4] Essential oils obtained from various aromatic plants are known to show a broad spectrum of antimicrobial activity against both human and plant pathogenic microorganisms.^[5-9] Quality and quantity of essential oils components varies among species, it is affected by several factors including the topographical condition, climatic factors, the stage of maturity, extraction methods and collection time. These chemical differences are directly interrelated to differences in biological activities.^[10]

Difference in the chemical constituents of essential oils not only inherently controlled, but also influenced by several other factors like the origin source and ecological conditions, reaping and post reaping stages, extraction and drying methods, storage conditions, and distillation time and type.^[11-13] Essential oil composition may be changed during processing and storage conditions which affect the quality and

biological activities of oil. Light, temperature and oxygen presence are important key factors that play vital role on the chemical modification mechanism. Several works on the quality and quantity changes of essential oil have been reported on the storage condition.^[14-15] During storage reduction in the quantity of oil and degradation of color was observed.^[16-18] Due to unstable biological behaviors of essential oil, it is very necessary to check quality and stability of essential oil before any pharmaceutical preparation.

In present investigation we selected *Cymbopogon citratus* which is used in various pharmaceutical and perfume industries due to presence of high concentration of citral compound.^[9] *C. citratus* commonly known as lemongrass, widely cultivated in lake city and the surrounding rural area due to their broad-spectrum utilities. In previous work we studied biochemical constituents and antifungal potential of *C. citratus* essential oil and their five fractions collected from different

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temperature conditions.^[19] *C. citratus* essential oil showed outstanding antidermatophytic activity against human pathogen fungi. No data regarding longevity or stability of *C. citratus* essential oil has been reported till date. Therefore, the purpose of the present investigation was to explore the changes in the biological activities of essential oil stored in different temperatures condition for two years.

MATERIALS AND METHODS

Collection of plant material and extraction of essential oil: One hundred fifty kg leaves of *C. citratus* were collected early in the morning from Jhadol Tehsil (24.3533°N 73.53°E) Udaipur district. Prof. S. Misra, Herbarium (Voucher number RUBL21182), Department of Botany, University of Rajasthan, Jaipur, India identified the plant species. Leaves were dehydrated in shade and cut into small pieces. For the extraction of essential oil dried 500g leaf semi-crushed leaves were macerated with a small amount of distilled water and the slurry was hydrodistilled with hydrodistillation unit (Clevenger's apparatus) for 4 hr. Essential oil collected in tubes was dried with anhydrous sodium sulphate. Moisture free oils were stored in amber coloured bottles in three different temperature conditions, room temperature, 30°C temperature and refrigerator temperature (2-8°C) for two year for experimental analysis. Experiments were conducted once for each condition.

Gas Chromatography

GC and GC-MS analysis were carried out as per standard protocol used in previous article.^[19] Quantitative estimation of the essential oil was carried out using a Shimadzu GC- 2010. The carrier gas nitrogen was used as at 10 psi inlet pressure with FID and Omega SPTm column (30.0 m × 0.25 mm ID, film thickness 0.25 µm). Injector and detector temperatures were 270°C and 280°C respectively. Column temperature programmed from 80°C (2 min hold), 80°C to 180°C at 4°C/min and 180°C to 230°C at 6°C/min with hold time of 6 min and 19 min, respectively. The flow rate of carrier gas was 1.21 ml/min and split ratio was 1:80. The information was managed on GC arrangements programming for oil composition.

GC/MS Analysis

GC-MS information was additionally acquired on a Shimadzu GCMS-QP-2010 or more framework utilizing same segment. Helium was used as transporter gas. Injector, mass detector, ion source temperatures, column temperature and other condition were similar as given in GC. EI source and mass range were 70 eV and 40-850 amu respectively. Compounds were identified by using Willey, NIST and Perfumery libraries. The compound identification was finally confirmed by comparison of their relative retention indices using n alkanes (C10-C40) and assessment with the similar data published in literature with literature values. These data were again proved by co-GC investigation of the available authentic compounds.

Quantitative Analysis

Calibration curve of citral, limonene, propyl amyl ketone, isogeranial, and caryophyllene oxide were generated using five concentration points. The percentage value of essential oil components was adjudicate using these calibration curves.

Fungal Culture

For present investigation four dermatophytic fungi *Trichophyton rubrum* (MTCC 296), *T. mentagrophytes* (MTCC 7687), *Microsporum canis* (MTCC2820), *M. fulvum* (MTCC 2837) and one yeast *Candida albicans* (MTCC 3018) were procured from the Imtech Chandigarh. These selected fungi are sustained on Sabouraud's dextrose agar

medium and Potato dextrose agar medium. Regular sub culturing and microscopic examination were performed for avoiding any saprophytic contamination.

Determination of MIC

Provine and Hadley^[20] suggested semisolid agar antifungal susceptibility testing method was applied for determination of MIC with slightly modification.^[21]

Preparation of Inoculum: Sterile gauze containing germ-free tween 80 was utilized to pick the sterling cluster of yeast (24 hrs old culture) and dermatophytes (3-7 days old slant at 37°C on SDA). This was then suspended in 3-4 mL of clean typical saline and vortexed. The turbidity of the homogenous suspension was kept to ~0.5 McFarland standard. The blend was vortexed and weighty particles were permitted to settle.

Immunization of oil containing tubes: The semisolid tubes of brain heart infusion agar medium containing known concentrations of test sample (oil) as well as controls (oil-free) were ready in triplet. One loopful (Himedia Flexilooop 4) of 0.5 McFarland adjusted inoculum was inserted the loop deep within the semisolid agar medium. These test tubes were incubated at 34°C for 24 hr for *Candida albicans* and 48-72 hr for dermatophytes. The inoculum suspension of tested microbes was also inoculated onto Sabouraud dextrose agar to check for purity and viability.

Ascertainment of end point: Ascertainment of end point were carried out as per protocol used in previous article.^[21] Growth was compared with oil-free control and recorded by visual examination as follows: growth equal as control: +4; minor reduction in growth: +3; momentous decline in growth (80% in yeast and 50% in dermatophytes) +2; few noticeable hyphal fragments or exceptionally slight growth: +1; no growth: 0.

RESULTS

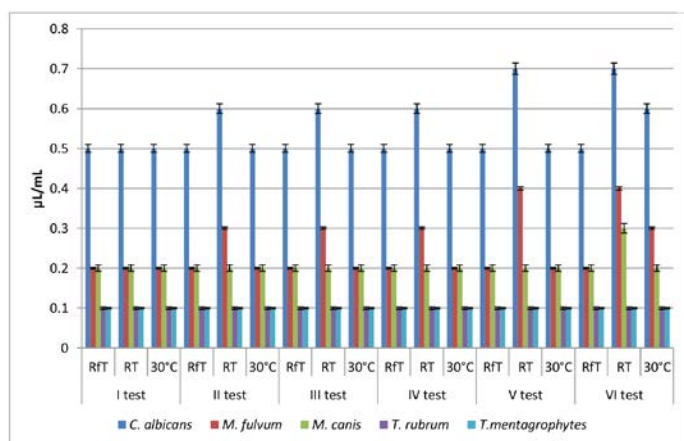
C. citratus essential oil contains varies principal and potential bioactive compounds. The key constituents of *C. citratus* essential oil were found to be citral α (48.26%) and citral β (39.85%) followed by limonene (1.70%), propyl amyl ketone (1.88%), isogeranial (1.43%) and caryophyllene oxide (1.07%).^[19] Citral, a major constituent of *C. citratus* oil is responsible for its antifungal properties (Table 1).

Table 1: Major constituents and groups of *C. citrauts* essential oil.

Major constituents	%
Citral b	39.85
Citral a	48.23
Propyl amyl ketone	1.88
Limonene	1.70
Isogeranial	1.43
Caryophyllene oxide	1.07
Major Groups	
Monoterpene hydrocarbon	2.76
Oxygenated Monoterpene	0.86
Sesquiterpene	1.48
Oxygenated Sesquiterpene	1.17
Monoterpene Aldehyde	88.32
Monoterpene Alcohol	2.46
Aldehyde	0.16
Benzene derivative	0.08
Ketone	2.35
Alcohol	0.19
Fatty acid Ester	0.17

Table 2: MIC ($\mu\text{L/mL}$) of *C. citratus* essential oil store in three different temperature and storage conditions.

Fungi	Testing			I test			II test			III test			IV test			V test			VI test				
	R_f	T	RT 30°C	R_f	T	RT 30°C	R_f	T	RT 30°C	R_f	T	RT 30°C	R_f	T	RT 30°C	R_f	T	RT 30°C	R_f	T	RT 30°C		
1. <i>C. albicans</i>	0.5	0.5	0.5	0.5	0.5	0.5	0.6	0.5	0.5	0.6	0.5	0.5	0.6	0.5	0.5	0.6	0.5	0.5	0.7	0.5	0.5	0.7	0.6
2. <i>M. fulvum</i>	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.3	0.2	0.2	0.3	0.2	0.2	0.3	0.2	0.2	0.4	0.2	0.2	0.4	0.3
3. <i>M. canis</i>	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.2
4. <i>T. rubrum</i>	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
5. <i>T.mentagrophytes</i>	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

**Figure 1:** Comparative analysis of antifungal activity of *Cymbopogon citratus* essential oil stored in different temperature and storage conditions.

For the current study essential oil obtained from the leaves of *Cymbopogon citratus* was stored in three different temperature conditions like room temperature, refrigerator temperature (2–8°C) and 30°C temperature for two years. Longevity test for antifungal potential was performed after every fourth month for two years.

Data incorporated in Table 2 showed that at refrigerate temperature condition, no activity changes was recorded against all test fungi up to sixth testing. Antifungal potential of *C. citratus* oil stored at 30°C temperature remained same as initial MIC up to Vth testing. MIC was found to be increased during VIth testing only against *C. albicans* (MTCC 3018) (0.6 $\mu\text{L/mL}$) and *M. fulvum* (MTCC 2837) (0.3 $\mu\text{L/mL}$).

During screening of oil stored in room temperature changes of MIC was observed by second testing means eight months of storage. MIC of oil stored in all three temperature conditions was found to be unchanged against *T. rubrum* (MTCC 296) and *T. mentagrophytes* (MTCC 7687) at the end of the experiments. While it was increased during second testing against *C. albicans* (MTCC 3018) (0.6 $\mu\text{L/mL}$) and *M. fulvum* (MTCC 2837) (0.3 $\mu\text{L/mL}$) and remain constant up to fourth testing. MIC was further increased against *C. albicans* (MTCC 3018) (0.7 $\mu\text{L/mL}$) and *M. fulvum* (MTCC 2837) (0.4 $\mu\text{L/mL}$) and found same in VIth testing also. MIC against *M. canis* (MTCC2820) (0.3 $\mu\text{L/mL}$) was found to be increased only at last testing (Figure 1).

DISCUSSION

C. citratus an important medicinal plant collected from the Lake city (Udaipur) commonly used by local tribal and sub tribal in several diseases and preparations. *C. citratus* essential oil have a typical lemon odor. In our previous work we studied the chemical composition and antifungal potential of lemon grass oil and different fractions. *C. citratus* leave oil as well as all fractions showed potential antifungal properties

against all test dermatophytes.^[19] A lot of research regarding chemical composition and antimicrobial activity of *C. citratus* have been done by various researchers. But no data regarding changes in the activity of *C. citratus* stored in different temperature and time reported till date. Present work dealing with changes in antifungal potential oil *C. citratus* after storage in different temperature conditions and of different time interval. Most essential oils have a shelf life, but this shelf life can vary according to the type of oil. Storage conditions can affect the chemical composition of the essential oil. This situation can be due to contact and reaction with oxygen, evaporation and other undesirable variations in volatile oil components during storage period.^[22-23]

Essential oil stored at room temperature showed variation in MIC as compared to initial one. The summer in Jaipur is exceptionally hot while winters are incredibly cold. The greatest temperatures float at 40°C to 47°C in May. The colder time of year least temperatures stay around 4–9°C. Therefore, the room temperature condition varies from 4–45°C. MIC was found to be double against *M. fulvum* (MTCC 2837) at twenty-four months of storage. No activity changes were reported against most common human pathogenic dermatophytic agents *T. rubrum* (MTCC 296) and *T. mentagrophytes* (MTCC 7687) up to last testing. MIC was found to be unchanged (0.1 $\mu\text{L/mL}$) in the both cases. These fungi were found most susceptible fungi during present studies. *C. citratus* essential oil was also found stable against *M. canis* (MTCC 2820) up to Vth testing in all the storage condition as well as in VIth testing in refrigerator and 30°C. Zoophilic strain *M. canis* is commonly isolated from dogs and other domestic animals. Jain and Sharma^[24] studied effect of temperature and storage conditions on antifungal activity of *C. martinii* essential oil. Human pathogenic fungus *T. rubrum* was reported as most resistant fungus. Variable room temperature conditions changed the quality of essential oil by influencing the antifungal potential against dermatophytes. Similar work was also carried out by Ahmed Abdulhakeem Al-Sammarraie.^[25] They suggested that all crude and fresh plant extracts gave best effectiveness against pathogens and the activity diminishes during the conservation of the plant extracts at various temperature and moisture conditions. Cool storage condition had better quality for the long stowage as compared to higher temperature condition. Higher ratio of Citral was found to be the main reason of high intensity of antifungal potential of *C. citratus* oil.

The impact of various stowage temperatures (0°C and 3°C) and periods (7, 14, 21 and 28 days) on yield and components of *Rosa damascena* Mill. oil was examined by Kazaz, *et al.*^[26] The storing temperatures on oil composition were found to not important whereas the effect of stowage time was remarkable.

During storage of essential oil monohydrocarbons constituents have a tendency to evaporate with a lower boiling temperature. Normally the capacity at low temperatures (beneath 8°C) stay away from developing or falling the centralizations of the oil parts and assist with holding the medicinal oil primary quality with the minute changes.^[27]

MIC of *C. citratus* essential oil against *C. albicans* (MTCC 3018) was found to 0.5 $\mu\text{L/mL}$ which was remain constant at VIth tasting in refrigerator storage conditions while at room temperature and at 30°C it changed

as 0.7µL/mL and 0.6µL/mL, respectively. High percentage of citral in *C. citratus* exhibited higher harm of pathogen. Meincken, *et al.*^[28] depicted that monoterpenes diffused into the cell of microorganism and caused destruction of the cell membrane.

CONCLUSION

Present study concluded that low temperature conditions prevent the degradation of quality components of essential oil. Hence at these temperature conditions essential oils can maintain its primary compositions without any changes of activity for a long time. *C. citratus* essential oil exhibited excellent antidermatophytic potential against all the test fungi up to 2 years of the study period. Stability of biological activity of *C. citratus* essential oil at low temperature condition upto 2 year is an important achievement for pharmaceutical development against dermatophytosis. This experiment ensures that if we prepared an ointment against dermatophytosis will be safe and effective for long time when stores at low temperature condition. During storage of essential oil, compounds of low boiling temperature (mainly monoterpenes hydrocarbons) get evaporate. Therefore, major activity changes were observed at room temperature conditions. Hence, we concluded that *C. citratus* essential oil containing product can be used up to two years without any alteration in activity when stored in low temperature like a refrigerator or freezer.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

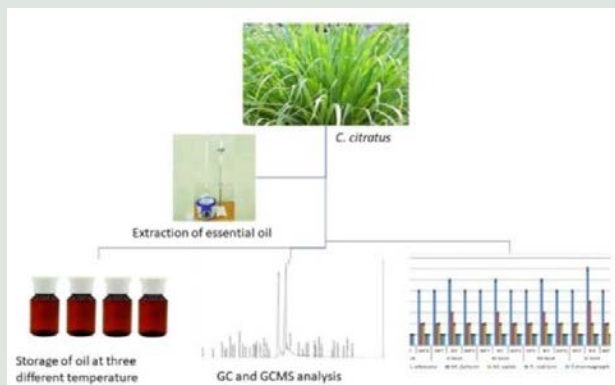
MIC: minimum inhibitory concentration; **hr:** hour; **GC:** Gas chromatography.

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GRAPHICAL ABSTRACT



SUMMARY

Present work demonstrated the longevity of *C. citratus* essential oil stored in three different temperature conditions. After every fourth month of storage antidermatophytic potential of oil was checked against human pathogenic fungi *Trichophyton rubrum*, *T. mentagrophytes*, *Microsporum canis*, *M. fulvum* and *Candida albicans*. *C. citratus* oil exhibited excellent antifungal activity against all test pathogens. Present finding revealed if essential oil store in low temperature conditions they can be used for long time without any potential changes or with negligible changes.

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