RESEARCH ARTICLE

Formulation and Evaluation of *In-vitro* Antifungal Activity of Lemongrass and Citronella Oil against Selected Fungal Skin Infections

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ABSTRACT

The essential oil collected from *Cymbopogon flexuosus* (Lemongrass) and *Cymbopogon winterianus* (Citronella) exhibited strong inhibition against all the selected fungi, evaluated in this study. Formulation of lemongrass and basil exhibited especially strong synergistic inhibition against *Trichophyton tonsurons* and *Microsporum canis.* In conclusion, we suggest the formulation of lemongrass and basil (BL-1) essential oils for the treatment of *Trichophyton* and *Microsporum* species, especially *T. tonsurons* and *M. canis.* This spreaded to many regions and parts of the world in recent decades and may reduce the efficacious dose of formulations of essential oils and thus minimizes the side-effects of synthetic antifungal agents. The therapeutic use of essential oils may also provide a solution for the rapid development of fungal resistance that is problematic with the currently available common antifungal therapeutics.

Keywords: Antifungal effect, Essential oils, *Tinea corporis, Tinea capitis*.

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INTRODUCTION

Nature has the cure of all the problems exists; we need to find out the solution from the nature to solve the problems. In nature nothing is waste. If nature has problem than before creating the problem it create solution. All the plants carry some unique property like antifungal, anti-inflammatory, antibacterial and many more properties. In India a separate strike exists to cure the disease known as Ayurveda. In Ayurveda, plant materials are used for the cure the disease.

The emerging resistance of microbes to antifungal agents has also placed serious implications in the management of infections. These antifungal chemical substances also act on aimed object presented in mammalian cells which may result in toxicity or adverse drug exchanges. Therefore, the discovery of novel antifungal has emerged. The plant phytochemistry sagest phytochemicals can be a better medicine as compared to synthetic drugs. The use of plant extract as drug started form early man civilization 29. These traditional medicines based on medicinal plants have been used for centuries. In India the process of treatment by plant extract is known as Ayurveda.

EXPERIMENTAL

Herbage Production of Selected Plants

In the present study the cultivation area of 10-meter square (0.001 Hectare) for each plant was taken at selected region. The average production of herbage of lemongrass, citronella and basil was 23, 21, and 14 kg per hectare, respectively.

Percentage of Oil Yield

The herbage of lemongrass was produced 250 mL essential oil. 170 mL essential oil was obtained from citronella herbage.

Assessment of Bioactive Compounds in Essential Oils

The bioactive compounds in essential oil were assessed by GC-MS technique.

Major Bioactive Compounds in Lemongrass Oil

There were 64 different components identified in the lemongrass essential oil. Geranial 43.67%, Neral 30.94%, Nerol

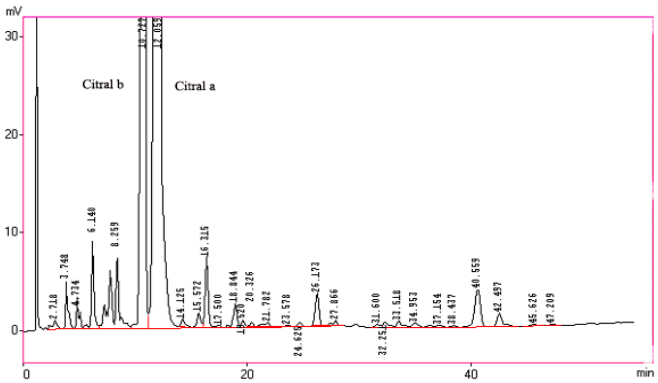

Figure 1: GC-MS graph of lemongrass oil

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3.92% and β-Myrcene 2.33% were the major components of lemongrass oil.

Major Bioactive Compounds in Citronella Oil

There were 50 different components recorded in the citronella essential oil. Citronellal 29.15%, Geraniol 22.52%, Citronellol 7.43% Neral 6.52% and Geranial 5.20% were the major components of citronella oil.

Outgrowth of Prepared of Inoculums

Fungal strain of *Trichophyton tonsurans* 8475 and *Microsporum canis* 3270 was collected from MTCC Chandigarh. *T. tonsurans* was cultured in Sabourauds agar and *M. canis* was cultured in Emmons modification of Sabourauds agar incubated at 25°C for 7 days (Media and incubation period was prescribed by the MTCC). The outgrowth of both stains was good (Figure 3) in relative media. The inoculums were ready to use for further experimental test.

Antifungal Activity of Essential Oils by Zone of Inhibition Test

Determination of antifungal activity of the selected essential oils and formulations against *T. tonsurans* 8475 was by the zone of inhibition test. Five microliters of essential oils and formulations of essential oils were pipetted onto sterile paper disks. The petri plates were incubated at 29°C for 24 to 48 hours to check zone of inhibition. Areas of clearing around the disks after incubation were measured and indicate that the oils have some antifungal activity. Diameters of zones of inhibition were measured in millimeters and recorded (Figure 4).

Antifungal Activity of Lemongrass and Citronella (LC) Formulations against T. tonsurans 8475

The antifungal activity of lemongrass and citronella formulations against *T. tonsurans* 8475 was observed in the zone of inhibition from the diameter range of 5.76 mm to 7.63 mm. The values of zone of inhibition displayed in the Table 1 and showed graphical form (Graph 1) for comparative study.

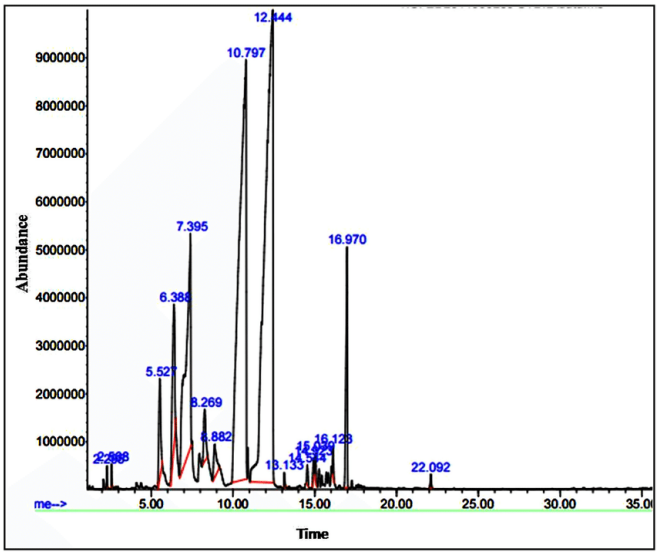


Figure 2: GC-MS graph of citronella oil

Antifungal Activity of Citronella and Lemongrass (CL) Formulations against T. tonsurans 8475

The antifungal activity of citronella and lemongrass formulations against *T. tonsurans* 8475 was identified in the zone of inhibition from the radial diameter range of 2.93 to 3.76 mm. The values of zone of inhibition showed in the Table 2 and showed graphical form in the Graph 2.

Table 1. Antifungal activity of lemongrass and citronella (LC) formulations against *T. tonsurans* 8475

Sr. No.	*Name of formulation*	*Zone of inhibition in mm (±SD)*
1.	LC1	7.63 ± .25
2.	LC2	7.00 ± .17
3.	LC3	6.83 ± .05
4.	LC4	6.20 ± .17
5.	LC5	5.76 ± .05

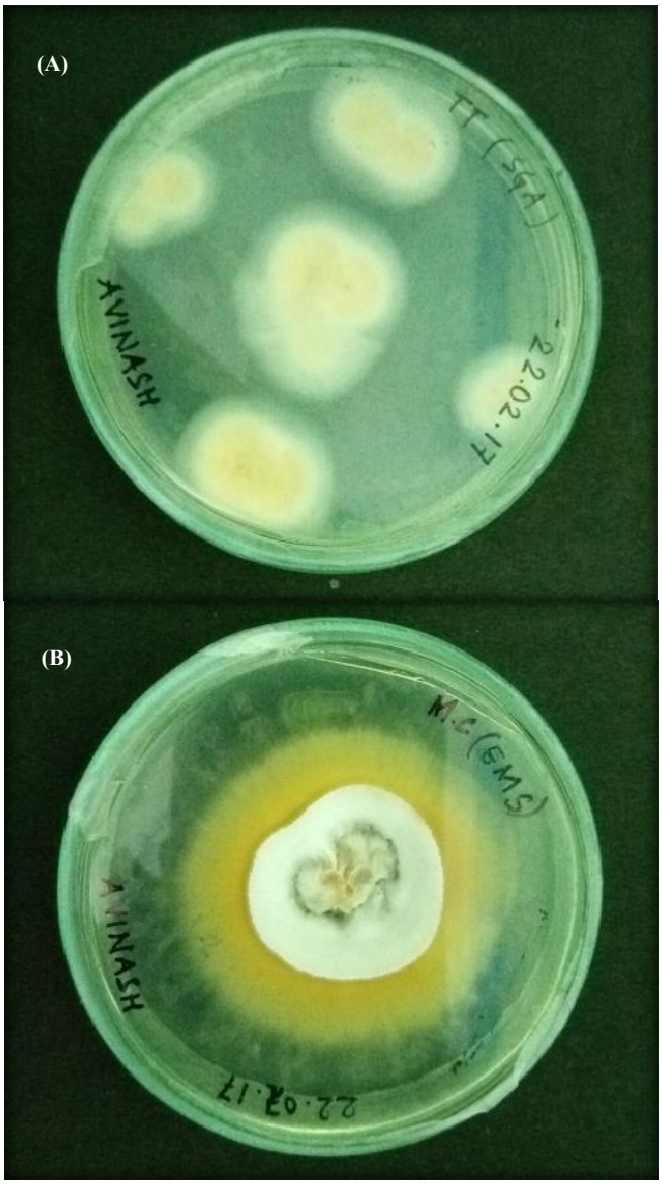


Figure 3: (A) – *T. tonsurans* and **(B) –** *M. canis*.

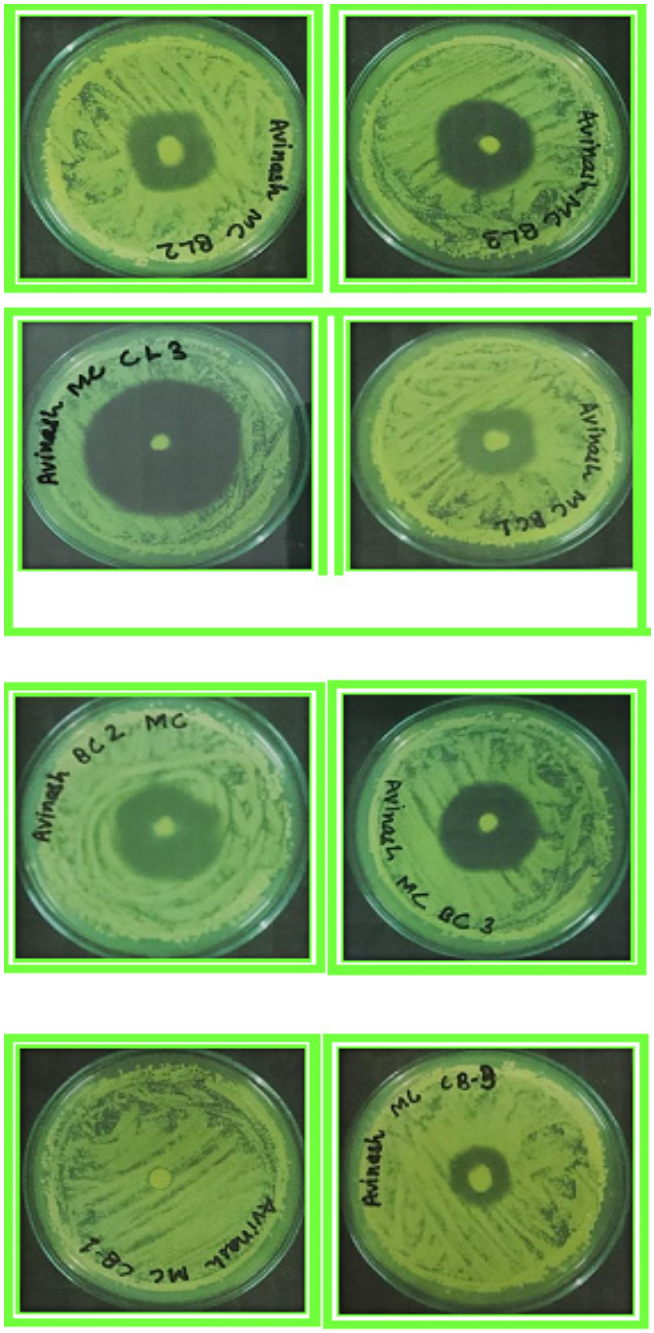
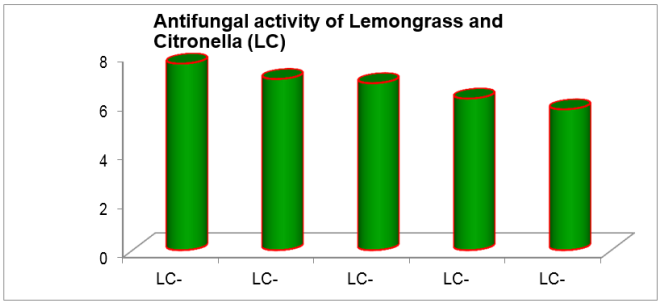


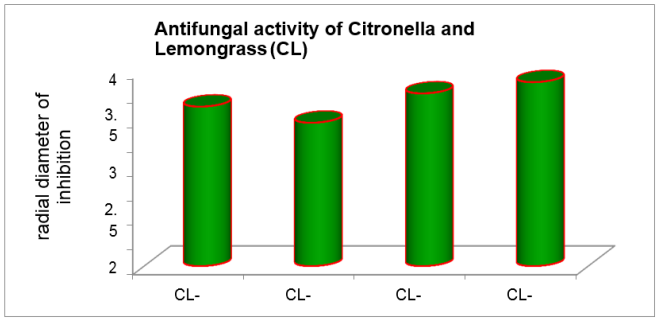
Figure 4: Clear area around the disk (zone of inhibition) showing antifungal activity of some different formulations of lemongrass, and basil oils against *T. tonsurans* 8475 and *M. canis* 3270.



Graph 1: Bar graph showing antifungal activity of lemongrass and citronella formulations against *Trichophyton tonsurans* 8475.

Table 2: Antifungal activity of Citronella and lemongrass (LC) formulations against *T. tonsurans* 8475

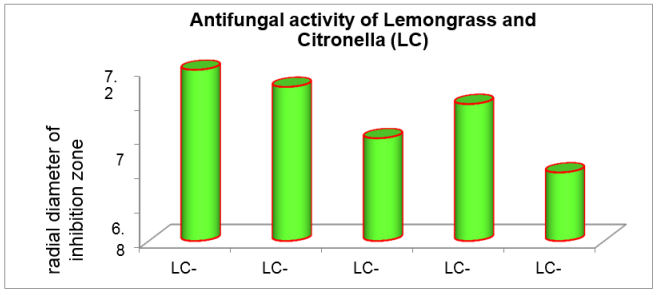
S. No.	*Name of formulation*	*Zone of inhibition in mm (±SD)*
1.	CL1	3.26 ± 0.15
2.	CL2	2.93 ± 0.20
3.	CL3	3.53 ± 0.11
4.	CL4	3.76 ± 0.05



Graph 2: Bar graph showing antifungal activity of citronella and lemongrass formulations against *T. tonsurans* 8475

Table 3: Antifungal activity of lemongrass and citronella (LC) formulations against *M. canis* 3270

Sr. no.	*Name of formulation*	*Zone of inhibition in mm (±SD)*
1.	LC1	7.20 ± 0.17
2.	LC2	7.10 ± 0.34
3.	LC3	6.80 ± 0.43
4.	LC4	7.00 ± 0.17
5.	LC5	6.60± 0.10



Graph 3: Bar graph showing antifungal activity of lemongrass and citronella formulations against *M. canis* 3270

Antifungal Activity of Lemongrass and Citronella (LC) Formulations against M. canis 3270

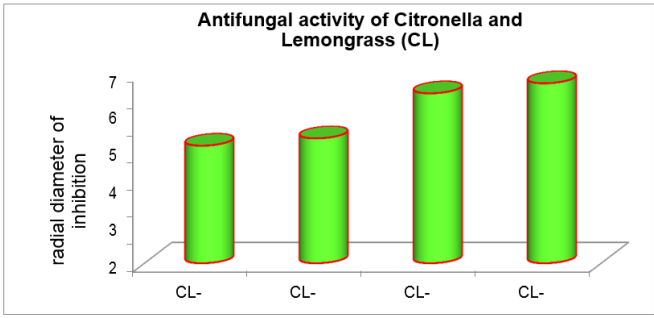
The antifungal activity of lemongrass and citronella against *M. canis* 3270 was showed in the zone of inhibition from radial diameter range of 6.60 to 7.20 mm. The values of zone of inhibition displayed in the Table 3 and showed graphical form (Graph 3) for comparative study.

Antifungal Activity of Citronella and Lemongrass (CL) Formulations against M. canis 3270

The antifungal activity of citronella and lemongrass against *M. canis* 3270 was showed in the zone of inhibition from radial diameter range of 4.33 to 6.63 mm. The values of zone of inhibition displayed in the Table 4 and showed graphical form (Graph 4) for comparative study.

Table 4: Antifungal activity of citronella and lemongrass (CL) formulations against *M. canis* 3270

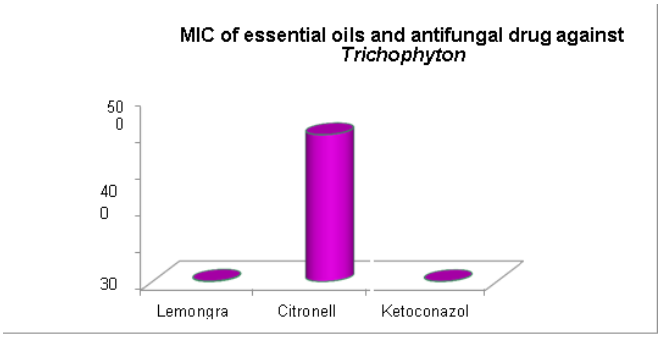
S. No.	*Name of formulation*	*Zone of inhibition in mm (±SD)*
1.	CL1	4.33 ± 0.11
2.	CL2	4.60 ± 0.20
3.	CL3	6.26 ± 0.15
4.	CL4	6.63 ± 0.11



Graph 4: Bar graph showing antifungal activity of citronella and lemongrass formulations against *M. canis* 3270

Table 5: MIC of lemongrass, citronella, basil oils and ketoconazole against *T. tonsurans* 8475.

S. No.	*Antifungal agents*	*MIC (µL/mL)*
1.	Lemongrass	1
2.	Citronella	400
4.	Ketoconazole	0.10



Graph 5: Bar graph showing MIC of lemongrass, citronella and ketoconazole against *Trichophyton tonsurans* 8475

Determination of Minimum Inhibitory Concentration (MIC)

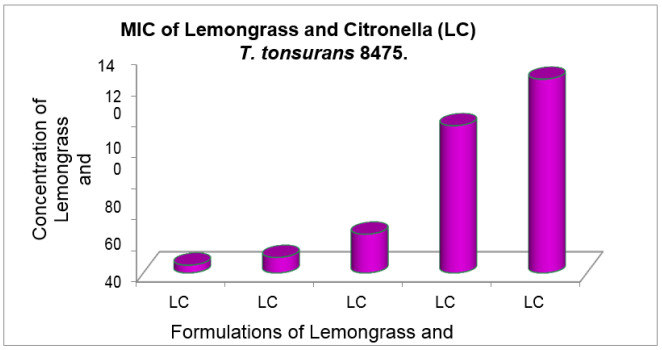
Minimum inhibitory concentration (MIC) of essential oils and formulations as antifungal agent was determined after 24 hours of incubation period. The process of inhibition of fungal growth was started around the disks as an initial clear line that sowed the MIC of respective oil and formulation. MIC of different formulations against *T. tonsurans* 8475 and *M. canis* 3270.

MIC of Lemongrass, Citronella, Basil oils and Ketoconazole against T. tonsurans 8475

The MIC of lemongrass, citronella, basil oils and ketoconazole against *T. tonsurans* 8475 was showed from the range of 0.10 to 500 µL/mL. The values of MIC displayed in the Table 5 and showed graphical form (Graph 5.) for comparative study (Figure 5).

Table 6. Minimum inhibitory concentration of lemongrass and citronella (LC) formulations against *T. tonsurans* 8475

S. No.	*Name of formulation*	*MIC (µL/mL)*
1.	LC1	5
2.	LC2	10
3.	LC3	25
4.	LC4	95
5.	LC5	125



Graph 6: Bar graph showing MIC of lemongrass and citronella (LC) formulations against *T. tonsurans* 8475

MIC of Lemongrass and Citronella (LC) Formulations against T. tonsurans 8475.

The MIC of these formulations against *T. tonsurans* 8475 was recognized from the range of 5 to 125 µL/mL. The values of MIC displayed in the Table 6 and showed graphical form (Graph 6).

MIC of Citronella and Lemongrass (CL) Formulations against T. tonsurans 8475

The MIC of CL formulations against *T. tonsurans* 8475 was calculated from the range of 165 to 380 µL/mL. The values of MIC showed in the Table 7 and graphical form showed in the Graph 7.

MIC of Basil and Lemongrass (BL) Formulations against T. tonsurans 8475

The MIC of BL formulations against *T. tonsurans* 8475 was recognized from the range of 140 to 305 µL/mL. The values of MIC showed in the Table 8 and graphical form showed in the Graph 8.

MIC of Lemongrass and Basil (LB) Formulations against T. tonsurans 8475

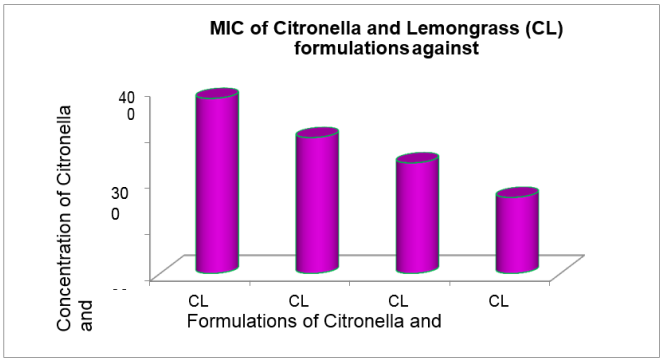
The MIC of LB formulations against *T. tonsurans* 8475 was recorded from the range of 5µl/ml to 65µl/ml. The values of MIC displayed in the Table 9 and graphical form showed in the Graph 9.

MIC of Lemongrass, Basil and Citronella (LBC) Formulations against T. tonsurans 8475.

The MIC of LBC formulations against *T. tonsurans* 8475 was recognized and the MIC value of all these formulations was equal which displayed in the following table 10 and graphical form showed in the Graph 10.

Table 7: MIC of Citronella and Lemongrass (CL) formulations against *T. tonsurans* 8475

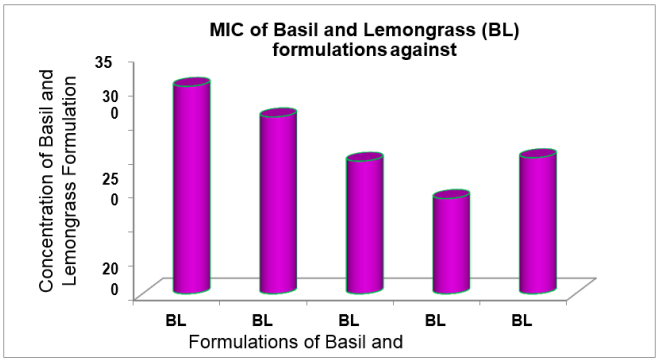
Sr. No.	*Name of formulation*	*MIC (µL/mL)*
1.	CL1	380
2.	CL2	295
3.	CL3	240
4.	CL4	165



Graph 7: Bar graph showing MIC of citronella and lemongrass formulations against *T. tonsurans* 8475

Table 8: MIC of Basil and Lemongrass (BL) formulations against *Trichophyton tonsurans* 8475

S. no.	*Name of formulation*	*MIC (µL/mL)*
1.	BL1	305
2.	BL2	260
3.	BL3	195
4.	BL4	140
5.	BL5	200



Graph 8: Bar graph showing MIC of Basil and Lemongrass formulations against *Trichophyton tonsurans* 8475

MIC of Lemongrass, Citronella, Basil oils and Ketoconazole against M. canis 3270

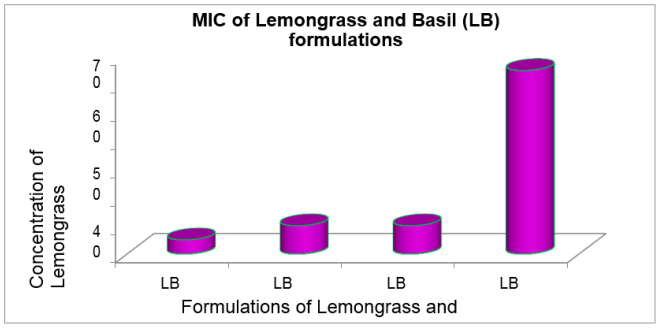
The MIC of these oils and antifungal agent against *M. canis* 3270 was recorded from the range of 0.10 to 600 µL/mL. The values of MIC showed in the following table 11 and graph 11.

MIC of Lemongrass and Citronella (LC) Formulations against M. canis 3270

The MIC of these formulations against *M. canis* 3270 was detected from the range 5µl/ml to 65µl/ml. The values of MIC displayed in the following table 5.29 and graphical form showed in the graph 5.26.

Table 9: MIC of lemongrass and basil (LB) formulations against *T. tonsurans* 8475

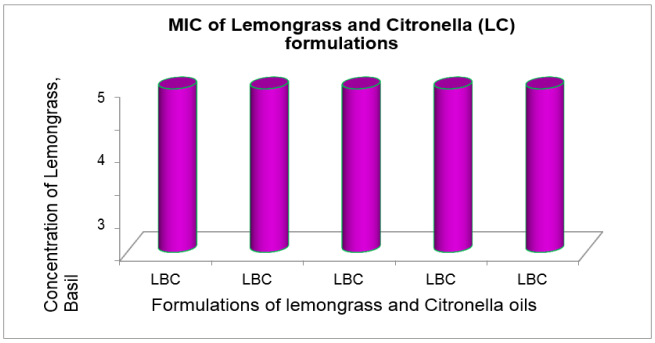
Sr. No.	*Name of Formulation*	*MIC (µL/mL)*
1.	LB1	5
2.	LB2	10
3.	LB3	10
4.	LB4	65



Graph 9: Bar graph showing MIC of lemongrass and basil formulations against *T. tonsurans* 8475

Table 10: MIC of lemongrass and citronella (LC) formulations against *T. tonsurans* 8475

S. No.	*Name of formulation*	*MIC (µL/mL)*
1.	LC1	5.0
2.	LC2	5.0
3.	LC3	5.0
4.	LC4	5.0



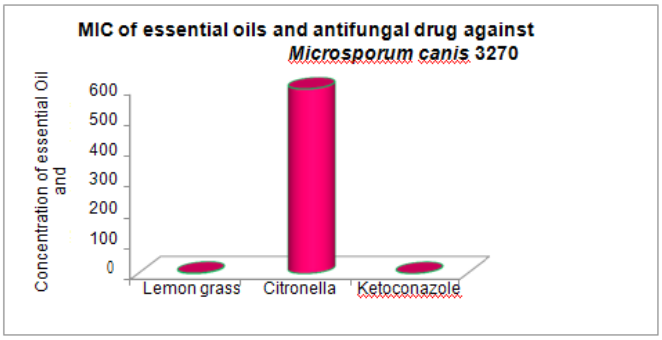
Graph 10: Bar graph showing MIC of Lemongrass and Citronella formulations against *T. tonsurans* 8475

Table 11: MIC of lemongrass, citronella, basil and ketoconazole against *M. canis* 3270**.**

S. no.	*Antifungal agents*	*MIC (µL/mL)*
1.	Lemongrass	1
2.	Citronella	600
3.	Ketoconazole	0.10

MIC of Citronella and Lemongrass (CL) formulations against M. canis 3270

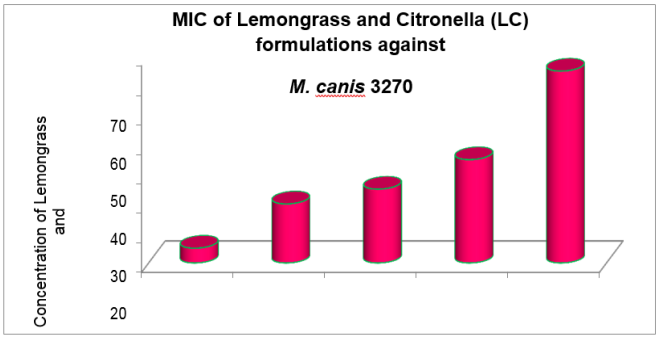
The MIC of CL formulations against *M. canis* 3270 was recognized from the range 80 to 140 µL/mL. The values of



Graph 11: Bar graph showing MIC of essential oils and ketoconazole against *M. canis* 3270

Table 12: MIC of lemongrass and citronella (LC) formulations against *M. canis* 3270

S. No.	*Name of formulation*	*MIC (µL/mL)*
1.	LC1	5
2.	LC2	20
3.	LC3	25
4.	LC4	35
5.	LC5	65



Graph 12: Bar graph showing MIC of lemongrass and citronella formulation against *M. canis* 3270

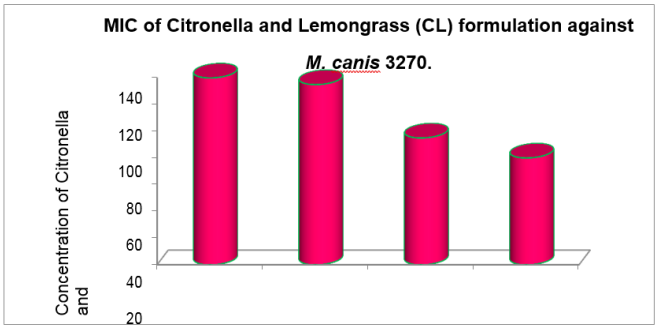
Table 13: MIC of citronella and lemongrass (CL) formulations against *M. canis* 3270

S. No.	*Name of formulation*	*MIC (µL/mL)*
1.	CL1	140
2.	CL2	135
3.	CL3	95
4.	CL4	80

MIC showed in the following table 13 and graphical form presented in the Graph 13.

SUMMARY AND CONCLUSION

As an involvement to the on-going search for alternative, available and inexpensive treatments to common skin infections in India, it is necessary to promote intense scientific research on plants as well as essential oils used for skin diseases and other cultural applications. It is clear that the antifungal effects of essential oils used for skin disorders can be either valuable or unfavorable and requires a thorough further scientific investigation of the phytochemistry, toxicity and



Graph 13: Bar graph showing MIC of Citronella and Lemongrass formulation against *M. canis* 3270

other pharmacological activities. It is also suggested that the essential oils are not only screened for antifungal properties against *T. tonsurans* and *M. canis*, but also studies on the isolated compounds be subjected to these pathogens of specific dermatological relevance. The impact on the use of traditional medicines for the treatment of fungal infections of skin can further pilot safer alternatives compared to the existing synthetic treatments which are very aggressive and have severe side. The toxicology effects of essential oils are important feature that need to be addressed, as the main aim for examining natural essential oil is to find safer, excellent quality and effective alternatives to the mainstream allopathic medications which are costly and very often require prolonged treatment schedules. The findings of current studies recommended that the use of essential oils in combinations to treat skin diseases is more effective.

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