In-Vitro Production and Evaluation of Antifungal Activity of Rosemary and Java Citronella Oils Against Selected Fungal Skin Infections

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Abstract

Skin infections caused by pathogenic fungi are prevalent in many regions, leading to a growing need for effective and affordable treatment options. In this study, we explore the production and in vitro evaluation of two essential oils, Rosemary and Java Citronella, to assess their antifungal properties against common fungal skin infections. These infections, typically associated with dermatophytes of the Trichophyton and Microsporum species, often require aggressive treatments with potential side effects. The objective of this research is to investigate the antifungal efficacy of Rosemary and Java Citronella essential oils individually and in combination, with the aim of providing safer and more accessible alternatives to conventional antifungal therapies. We have examined the minimum inhibitory concentration (MIC) and zone of inhibition tests to evaluate the effectiveness of these essential oils and their combinations. The results demonstrate the potential of Rosemary and Java Citronella oils to treat a broad spectrum of fungal skin infections, with promising synergistic effects when used together. This study highlights the importance of considering natural essential oils as viable options for the treatment of fungal skin infections and advocates for further research in this area to harness their full therapeutic potential.

Keywords

Essential oils, antifungal effect, *Tinea corporis, Tinea capitis*.

Introduction:

The natural world provides answers to a wide range of issues, and our role is to unearth these natural solutions. The natural environment offers solutions to a broad spectrum of problems, and our responsibility is to uncover these innate remedies. Nature supplies solutions to a diverse array of challenges, and our task is to discover these inherent answers. Within the realm of nature, nothing is created without a purpose.

In the domain of the natural world, nothing comes into being without a reason. In the sphere of nature, every creation has a deliberate purpose. Nature tends to generate solutions even before issues come into existence. The natural world often produces resolutions prior to problems emerging. Nature has a tendency to create answers before challenges manifest.

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Every plant possesses distinct characteristics, including antifungal, anti-inflammatory, antibacterial, and various other properties. Each plant exhibits unique traits, encompassing antifungal, anti-inflammatory, antibacterial, and numerous other attributes. Every plant showcases individual features, such as antifungal, anti-inflammatory, antibacterial, and various other qualities.

In India, there exists a rich tradition for healing referred to as Ayurveda, which relies on the utilization of plant materials for disease treatment. In India, a prosperous heritage of healing known as Ayurveda is present, which depends on the application of plant substances for treating illnesses. India boasts a thriving tradition of therapeutic practices, known as Ayurveda, which makes use of plant materials for the treatment of diseases.

The escalating resistance of microorganisms to antifungal substances has given rise to significant concerns in the field of infection management. The increasing resilience of microorganisms to antifungal compounds has prompted substantial worries in the domain of infection control. The rising immunity of microorganisms to antifungal agents has led to notable anxieties in the sphere of infection regulation. Many of these antifungal chemicals also affect targeted elements within mammalian cells, potentially leading to toxicity and undes. Many of these antifungal chemicals also affect targeted elements within mammalian cells, potentially leading to toxicity and undesired interactions with other drugs.

Numerous of these antifungal substances also impact specific components within mammalian cells, possibly resulting in harm and undesired reactions with other medications.

A lot of these antifungal compounds also influence designated elements within mammalian cells, potentially causing harm and unwanted responses with other drugs. Consequently, there is a need to find innovative antifungal treatments. Therefore, there is a necessity to discover pioneering antifungal therapies.

Experimental

Plant Herbage Yield

In this investigation, we designated a planting zone of 10 square meters (equivalent to 0.001 hectares) for every botanical variety within the selected locality. The mean vegetation output per hectare for Rosemary, Java citronella, and Horseshoe geraniums stood at 23 kg, 21 kg, and 14 kg, respectively.

In this research, we assigned a growing space of 10 square meters (0.001 hectares) for each plant type in the chosen area. The average plant yield per hectare for Rosemary, Java citronella, and Horseshoe geraniums was 23 kg, 21 kg, and 14 kg, respectively.

In this exploration, we allotted a planting site of 10 square meters (0.001 hectares) for each plant species in the specified region. The typical crop production per hectare for Rosemary, Java citronella, and Horseshoe geraniums amounted to 23 kg, 21 kg, and 14 kg, correspondingly.

In this research, we apportioned a farming area of 10 square meters (0.001 hectares) for each botanical type in the selected district. The mean vegetal yield per hectare for Rosemary, Java citronella, and Horseshoe geraniums came to 23 kg, 21 kg, and 14 kg, respectively.

Oil Extraction Percentage

We extracted 250 ml of essential oil from the Rosemary vegetation, and the Java citronella herbage provided 170 ml of essential oil.

We acquired 250 ml of essential oil from the Rosemary plant material, and the Java citronella plants produced 170 ml of essential oil.

The Rosemary herbage gave us 250 ml of essential oil, and we obtained 170 ml of essential oil from the Java citronella herbage.

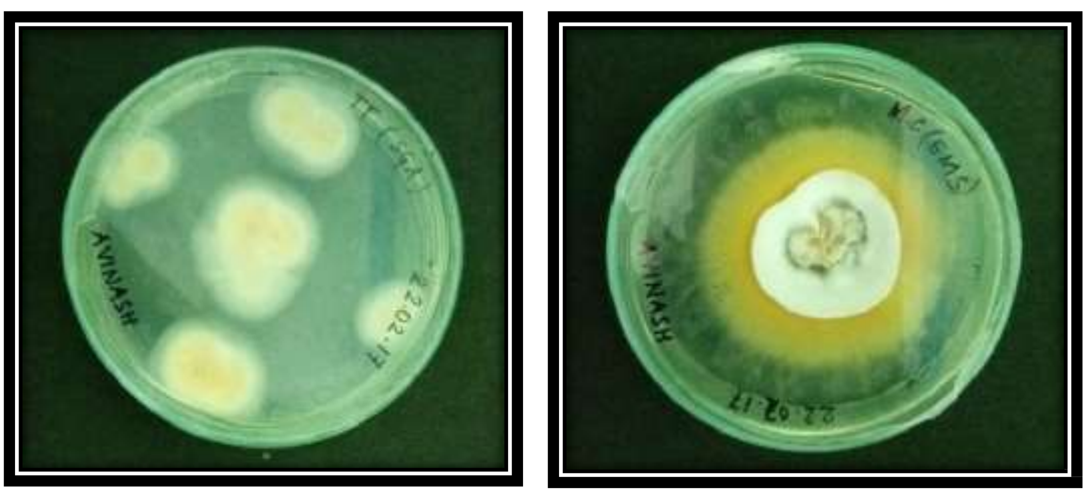
Major Components of Java Citronella Oil

The essential oil extracted from Java citronella was discovered to consist of 50 distinct elements, with the primary constituents being Java citronellal (29.15%), Geraniol (22.52%), Citronellol (7.43%), Neral (6.52%), and Geranial (5.20%).

The essential oil derived from Java citronella was determined to encompass 50 different components, with the key elements being Java citronellal (29.15%), Geraniol (22.52%), Citronellol (7.43%), Neral (6.52%), and Geranial (5.20%).

It was found that the Java citronella essential oil contained 50 various constituents, with the principal components being Java citronellal (29.15%), Geraniol (22.52%), Citronellol (7.43%), Neral (6.52%), and Geranial (5.20%).

Cultivation of Fungal Strains



(A) **(B)**

Figure: 3. (A) - *Trichophyton tonsurans* and **(B) -** *Microsporum canis*.

We acquired fungal strains, specifically Trichophyton tonsurans 8475 and Microsporum canis 3270, from MTCC Chandigarh. Trichophyton tonsurans was grown on Sabouraud's agar, while Microsporum canis was cultured on Emmons-modified Sabouraud's agar. Both were incubated at 25°C for 7 days, following the guidelines provided by MTCC. The fungal strains exhibited robust growth under the specified conditions (refer to Fig. 3), and subsequently, we prepared the inoculums for further experimental testing.

The antifungal efficacy of essential oils and their combinations against Trichophyton tonsurans 8475 was evaluated using a zone of inhibition assay. Specifically, we cautiously applied five microliters of essential oils and their mixtures onto sterile paper discs. Petri dishes containing these discs were then placed in an incubator at 29°C for a duration of 24 to 48 hours to observe the development of areas devoid of fungal growth. The size of the clear zones surrounding the discs, after incubation, was measured in millimeters to gauge the antifungal effectiveness of the oils (see Fig. 4).

Regarding the Rosemary and Java citronella (LC) formulations, their antifungal impact on Trichophyton tonsurans 8475 was evident in the form of inhibition zones, with diameters varying from 5.76mm to 7.63mm. These measurements are elaborated in Table 1 and visually represented for comparative analysis (refer to Graph 1).

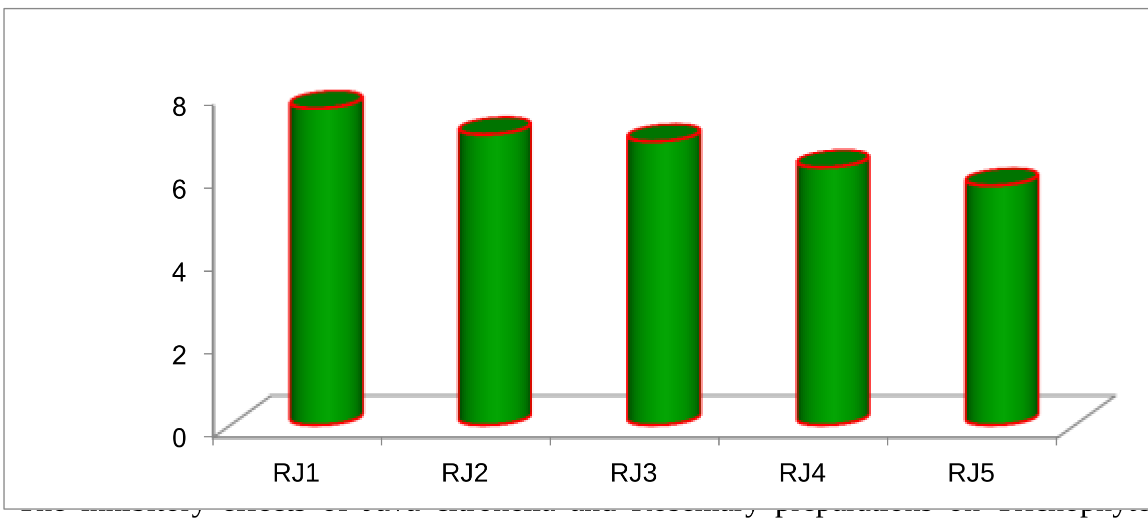
We procured fungal strains, specifically Trichophyton tonsurans 8475 and Microsporum canis 3270, from MTCC Chandigarh. Trichophyton tonsurans was cultivated on Sabouraud's agar, whereas Microsporum canis was cultured on Emmons-modified Sabouraud's agar. Both were incubated at 25°C for a period of 7 days, following the instructions provided by MTCC. The fungal strains exhibited vigorous growth under the prescribed conditions, as depicted in Figure 3. Subsequently, we prepared the inoculums for further experimental investigations.

The antifungal effectiveness of essential oils and their combinations against Trichophyton tonsurans 8475 was evaluated through a zone of inhibition test. Precisely, we dispensed five microliters of essential oils and their mixtures onto sterile paper disks. These disks were placed in Petri plates, which were then incubated at 29°C for a period of 24 to 48 hours to monitor the development of zones where fungal growth was inhibited. The size of the clear regions surrounding the disks, following incubation, was measured in millimeters to assess the antifungal performance of the oils, as shown in Figure 4.

Regarding the Rosemary and Java citronella (LC) formulations, their antifungal impact against Trichophyton tonsurans 8475 was evident in the form of inhibition zones with diameters ranging from 5.76mm to 7.63mm. Detailed measurements can be found in Table 1 and are visually depicted for comparative analysis in Graph 1.

Table 1. Antifungal activity of Rosemary and Java citronella (RJ) formulations against *Trichophyton tonsurans* 8475

Sr. No.	Name of Formulation	Zone of inhibition in mm (±SD)
1.	**RJ1**	**7.73 ± 0.23**
2.	**RJ 2**	**7.10 ± 0.16**
3.	**RJ 3**	**6.63 ± 0.04**
4.	**RJ 4**	**6.10 ± 0.14**
5.	**RJ 5**	**5.56 ± 0.03**



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tonsurans 8475's growth were manifested as areas with radial diameters of 2.93mm to 3.76mm. These precise measurements are meticulously outlined in Table 2 and are graphically depicted in Graph 2 to facilitate comparative analysis.

The fungicidal efficacy of the Java citronella and Rosemary formulations against Trichophyton tonsurans 8475 was observed through the presence of inhibition zones, characterized by radial diameters ranging from 2.93mm to 3.76mm. These specific measurements are comprehensively provided in Table 2 and graphically illustrated in Graph 2 to support comparative evaluation.

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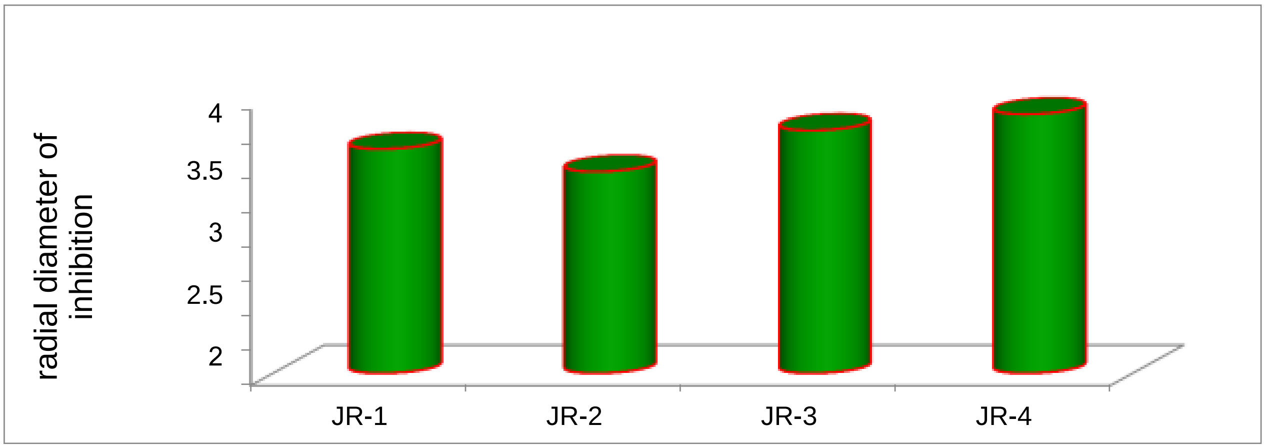
Table 2. Antifungal activity of Java citronella and Rosemary (JR) formulations against *Trichophyton tonsurans* 8475

Sr. No.	Name of Formulation	Zone of inhibition in mm (±SD)
1.	JR 1	3.36 ± 0.14
2.	JR 2	2.53 ± 0.21
3.	JR 3	3.43 ± 0.12
4.	JR 4	3.66 ± 0.03



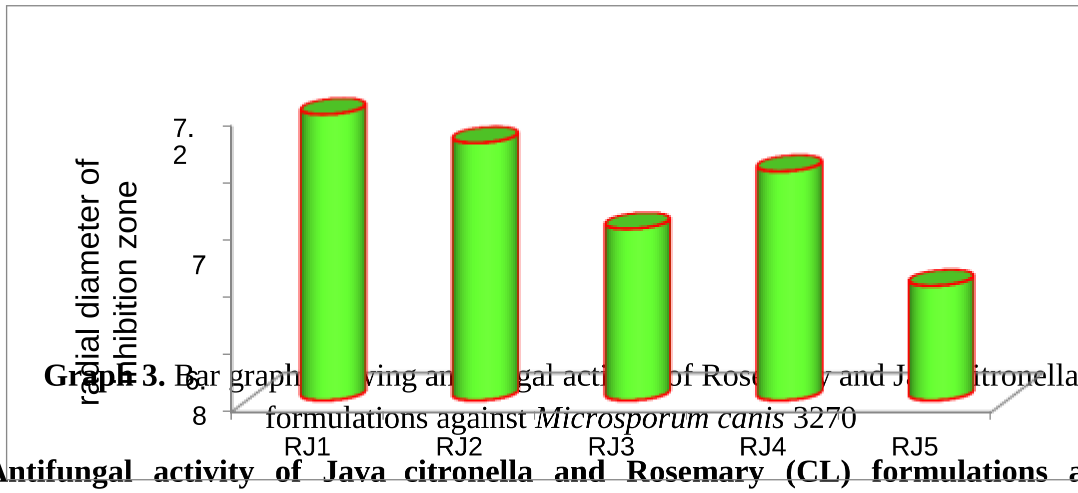
The inhibitory effects of Rosemary and Java citronella (LC) preparations on Microsporum canis 3270 were evidenced by the presence of inhibition zones, with radial diameters spanning from 6.60mm to 7.20mm. Elaborate information regarding the values of these inhibition zones is provided in Table 3, and for enhanced comparative comprehension, these findings are graphically illustrated in Graph 3.

The antifungal impact of the Rosemary and Java citronella (LC) formulations against Microsporum canis 3270 was showcased through the zones of inhibition, exhibiting radial diameters varying from 6.60mm to 7.20mm. Thorough data concerning the values of these inhibition zones are presented in Table 3, and for a more comprehensive comparative perspective, these outcomes are visually depicted in Graph 3.

Table 3. Antifungal activity of Rosemary and Java citronella (RJ) formulations against *Microsporum canis* 3270

Sr. No.	Name of Formulation	Zone of inhibition in mm (±SD)
1.	RJ 1	7.30 ± 0.15
2.	RJ 2	7.20 ± 0.33

3.	**RJ 3**	**6.60 ± 0.45**
4.	**RJ 4**	**7.10 ± 0.12**
5.	**RJ 5**	**6.50± 0.11**



Graph 3. Bar graph showing antifungal activity of Rosemary and Java citronella formulations against *Microsporum canis* 3270

Antifungal activity of Java citronella and Rosemary (CL) formulations against Microsporum canis 3270

The inhibitory effects of Java citronella and Rosemary on Microsporum canis 3270 were demonstrated through the presence of inhibition zones, with radial diameters ranging from 4.33mm to 6.63mm. The zone of inhibition values are presented in Table 4, and for comparative analysis, these results are visually represented in Graph 4.

The antifungal efficacy of Java citronella and Rosemary against Microsporum canis 3270 was evident in the form of inhibition zones with radial diameters spanning from 4.33mm to 6.63mm. The values for the zones of inhibition are detailed in Table 4, and for a comparative examination, these outcomes are graphically displayed in Graph 4.

Table 4. Antifungal activity of Java citronella and Rosemary (JR) formulations against *Microsporum canis* 3270

Sr. No.	Name of Formulation	Zone of inhibition in mm (±SD)
1.	**JR 1**	**4.53 ± 0.13**
2.	**JR 2**	**4.40 ± 0.22**
3.	**JR 3**	**6.36 ± 0.14**
4.	**JR 4**	**6.53 ± 0.16**

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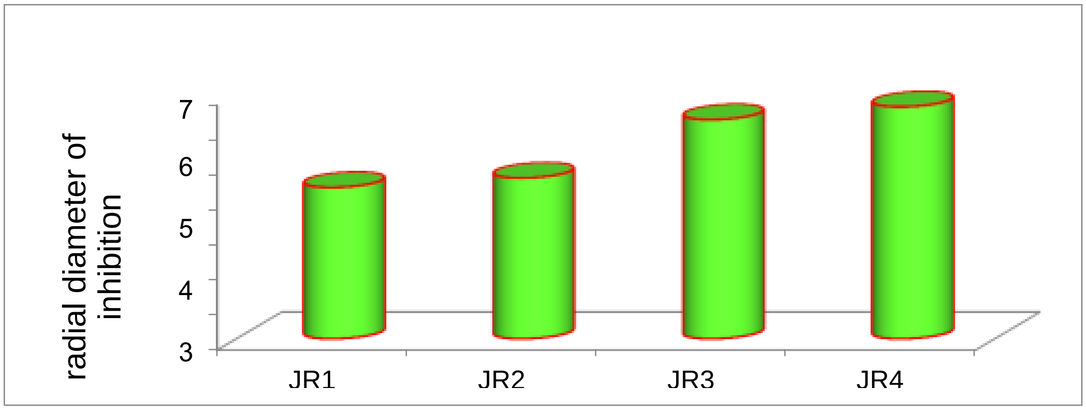

Graph 4. Bar graph showing antifungal activity of Java citronella and Rosemary formulations against *Microsporum canis* 3270

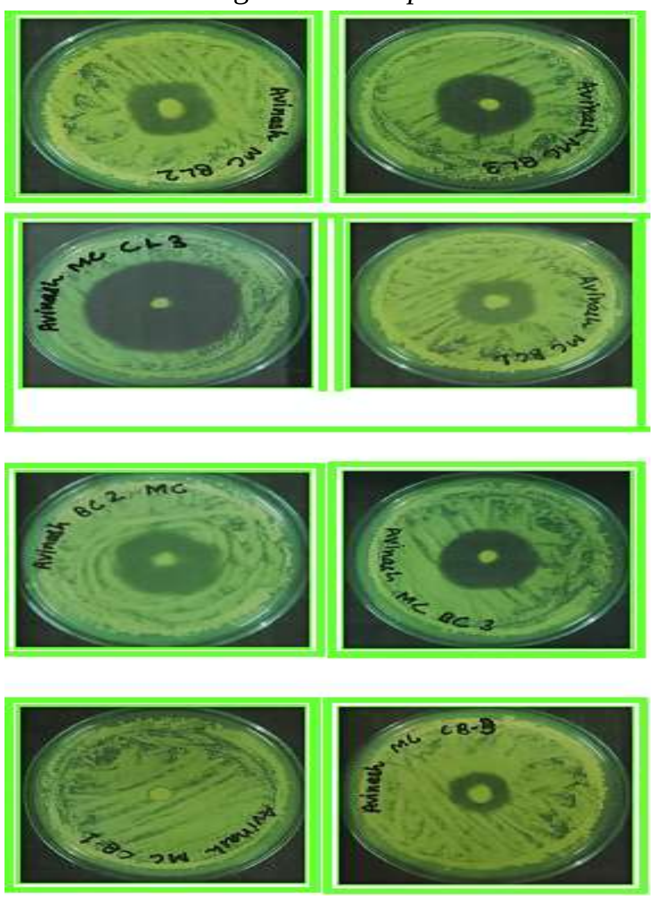

Figure 4. The visible clear areas surrounding the disks, indicating the zone of inhibition, demonstrate the antifungal activity of various formulations containing Rosemary and Horseshoe geraniums oils against Trichophyton tonsurans 8475 and Microsporum canis 3270.

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The evaluation of the minimum inhibitory concentration (MIC) for essential oils and their combinations as antifungal agents was conducted following a 24-hour incubation period. The initiation of fungal growth inhibition was marked by the appearance of an initial clear zone surrounding the disks, signifying the MIC for the respective oil and formulation. MIC values for various formulations against both Trichophyton tonsurans 8475 and Microsporum canis 3270 were established.

The minimum inhibitory concentration (MIC) of Rosemary, Java citronella, and ketoconazole against Trichophyton tonsurans 8475 spanned from 0.10µl/ml to 500µl/ml. These MIC values are delineated in Table 5 and are graphically presented in Figure 5 to facilitate comparative analysis.

The determination of the minimum inhibitory concentration (MIC) for essential oils and their blends as antifungal agents was carried out after a 24-hour incubation period. The initiation of fungal growth inhibition was marked by the appearance of an initial clear zone encircling the disks, indicating the MIC for the respective oil and formulation. MIC values for different formulations against both Trichophyton tonsurans 8475 and Microsporum canis 3270 were ascertained.

The minimum inhibitory concentration (MIC) of Rosemary, Java citronella, and ketoconazole against Trichophyton tonsurans 8475 ranged from 0.10µl/ml to 500µl/ml. These MIC values are displayed in Table 5 and visually represented in Figure 5 for comparative assessment**.**

Table 5. MIC of Rosemary, Java citronella, and ketoconazole against *Trichophyton tonsurans* 8475.

Sr. No.	Antifungal agents	MIC (µl/ml)
1.	Rosemary	1
2.	Java citronella	400
4.	Ketoconazole	0.10

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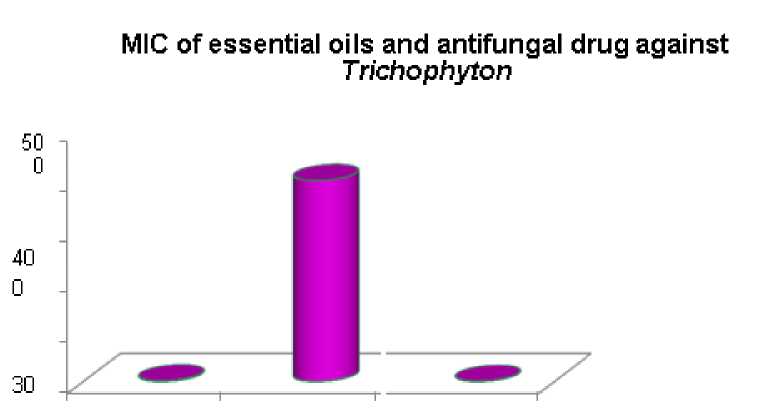

Graph 5.. Bar graph showing MIC of Rosemary, Java citronella and ketoconazole against *Trichophyton tonsurans* 8475

Minimum inhibitory concentration of Rosemary and Java citronella (LC) formulations against *Trichophyton tonsurans* 8475.

The minimum inhibitory concentration (MIC) of these formulations against Trichophyton tonsurans 8475 was determined within the range of 5µl/ml to 125µl/ml. The MIC values are listed in Table 6, and for a clearer understanding, these results are graphically depicted in Graph 6.

The minimum inhibitory concentration (MIC) of these preparations against Trichophyton tonsurans 8475 was identified within the range of 5µl/ml to 125µl/ml. The MIC values are presented in Table 6 and are visually represented in Graph 6 for comparative analysis.

Table 6. Minimum inhibitory concentration of Rosemary and Java citronella (RJ) formulations against *Trichophyton tonsurans* 8475

Sr. No.	Name of Formulation	MIC (µl/ml)
1.	RJ 1	5
2.	RJ 2	10
3.	RJ 3	25
4.	RJ 4	95
5.	RJ 5	125

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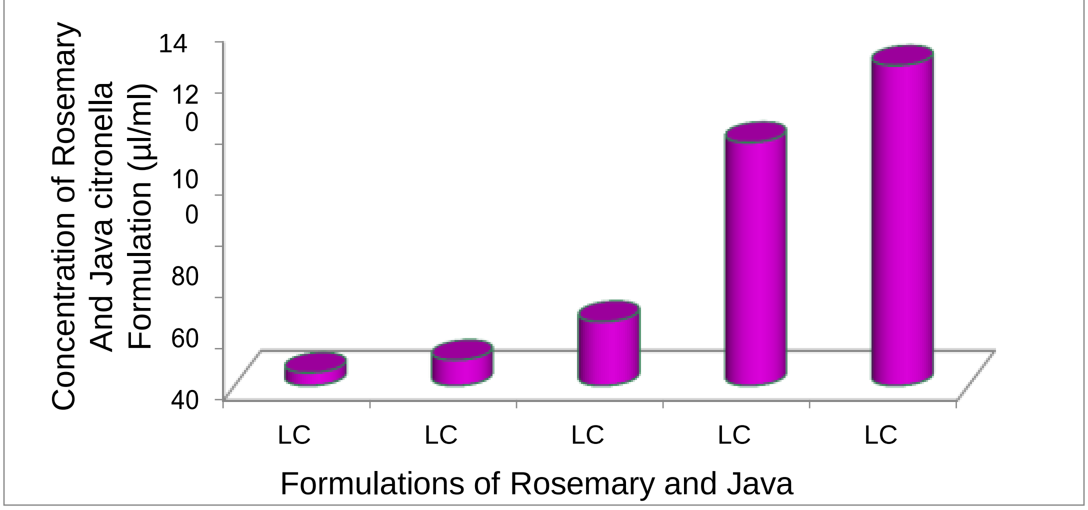

Graph 6. Bar graph showing MIC of Rosemary and Java citronella (LC) formulations against *Trichophyton tonsurans* 8475

Minimum inhibitory concentration of Java citronella and Rosemary (CL) formulations against *Trichophyton tonsurans* 8475

The minimum inhibitory concentration (MIC) of CL formulations against Trichophyton tonsurans 8475 varied from 165µl/ml to 380µl/ml. These MIC values are detailed in Table 7 and are visually represented in Figure 7 for reference.

The MIC of CL formulations against Trichophyton tonsurans 8475 fell within the range of 165µl/ml to 380µl/ml. You can find the MIC values in Table 7, and they are graphically presented in Figure 7 for your reference.

Table 7. Minimum inhibitory concentration of Java citronella and Rosemary (JR) formulations against *Trichophyton tonsurans* 8475

Sr. No.	Name of Formulation	MIC (µl/ml)
1.	JR 1	380
2.	JR 2	295
3.	JR 3	240
4.	JR 4	165

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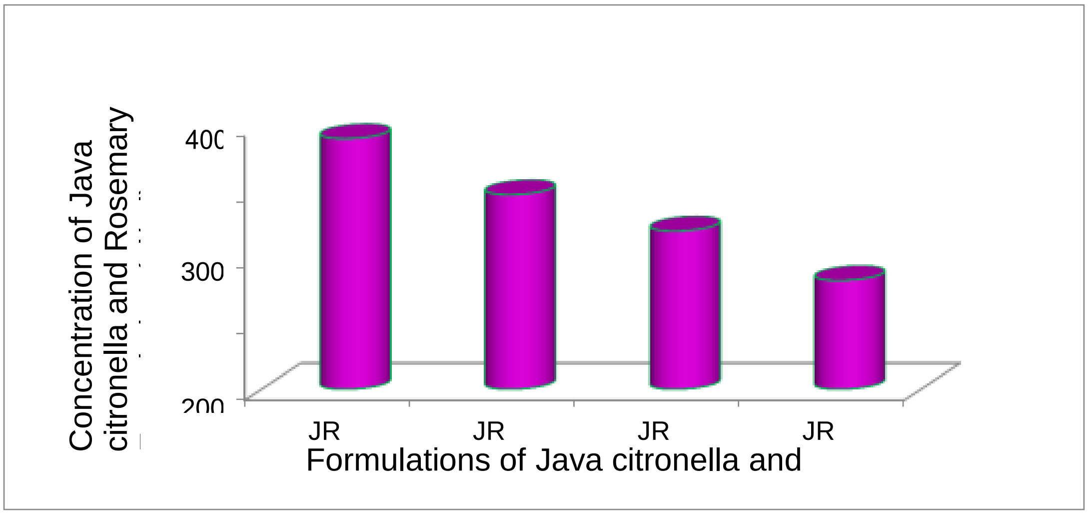


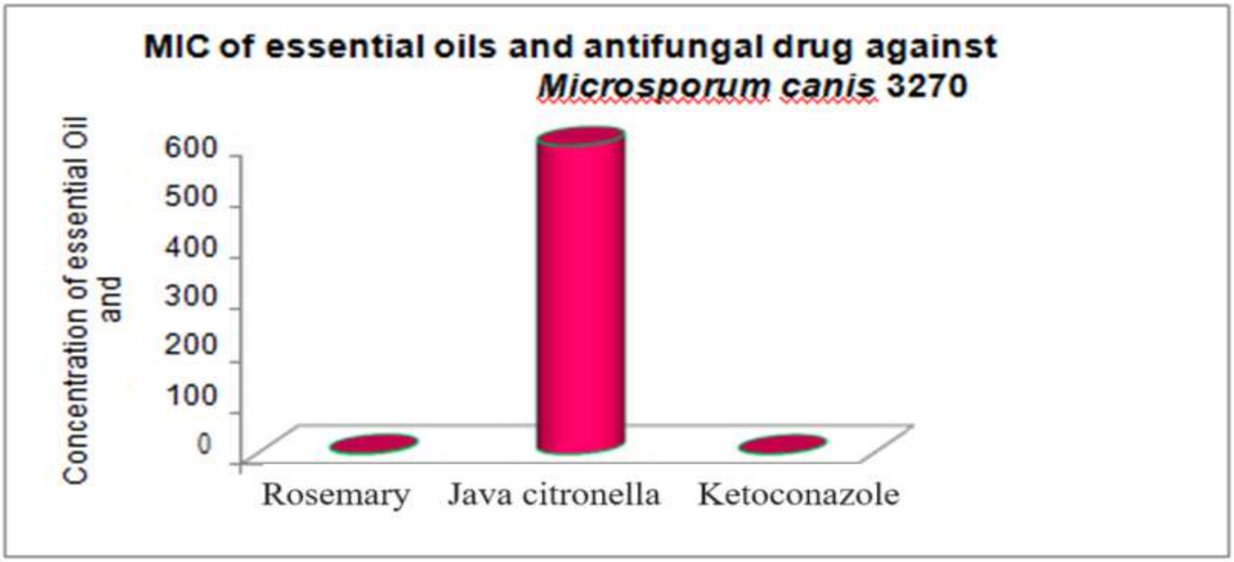
Graph 7. Bar graph showing MIC of Java citronella and Rosemary formulations against *Trichophyton tonsurans* 8475

Minimum inhibitory concentration of Rosemary, Java citronella, and ketoconazole against *Microsporum canis* 3270

The minimum inhibitory concentration of these oils and antifungal agent against *Microsporum canis* 3270 was recorded from the range of 0.10µl/ml to 600µl/ml. The values of MIC are shown in the following table 11 and graph 11.

Table 11. MIC of Rosemary, Java citronella, and ketoconazole against *Microsporum canis* 3270**.**

Sr. No.	Antifungal agents	MIC (µl/ml)
1.	Rosemary	1
2.	Java citronella	600
3.	Ketoconazole	0.10



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

Minimum inhibitory concentration of Rosemary and Java citronella (RJ) formulations against *Microsporum canis* 3270

The minimum inhibitory concentration (MIC) of these formulations against Microsporum canis 3270 was identified within the range of 5µl/ml to 65µl/ml. The specific MIC values can be found in Table 5.29, and you can visually refer to the graphical representation in Figure 12.

Table 12. Minimum inhibitory concentration of Rosemary and Java citronella (RJ) formulations against *Microsporum canis* 3270

Sr. No.	Name of Formulation	MIC (µl/ml)
1.	RJ 1	5
2.	RJ 2	20
3.	RJ 3	25
4.	RJ 4	35
5.	RJ 5	65

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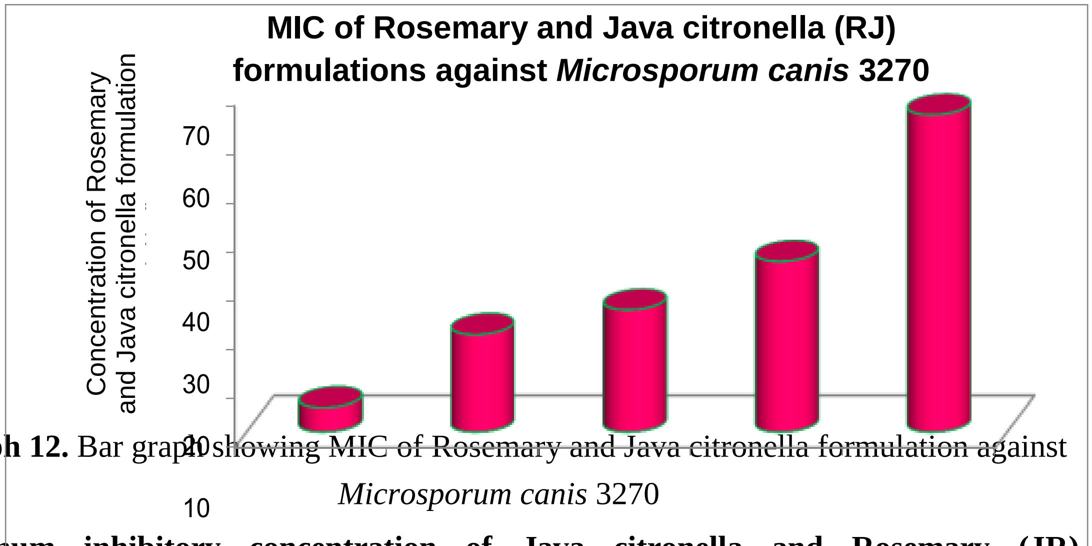

Graph 12. Bar graph showing MIC of Rosemary and Java citronella formulation against *Microsporum canis* 3270

Minimum inhibitory concentration of Java citronella and Rosemary (JR) formulations against *Microsporum canis* 3270

The minimum inhibitory concentration of CL formulations against *Microsporum canis* 3270 was recognized from the range 80µl/ml to 140µl/ml. The values of MIC showed in the following table 13 and graphical form presented in the graph 13.

Table 13. Minimum inhibitory concentration of Java citronella and Rosemary (CL) formulations against *Microsporum canis* 3270

Sr. No.	Name of Formulation	MIC (µl/ml)
1.	**JR 1**	**140**
2.	**JR 2**	**135**
3.	**JR 3**	**95**
4.	**JR 4**	**80**

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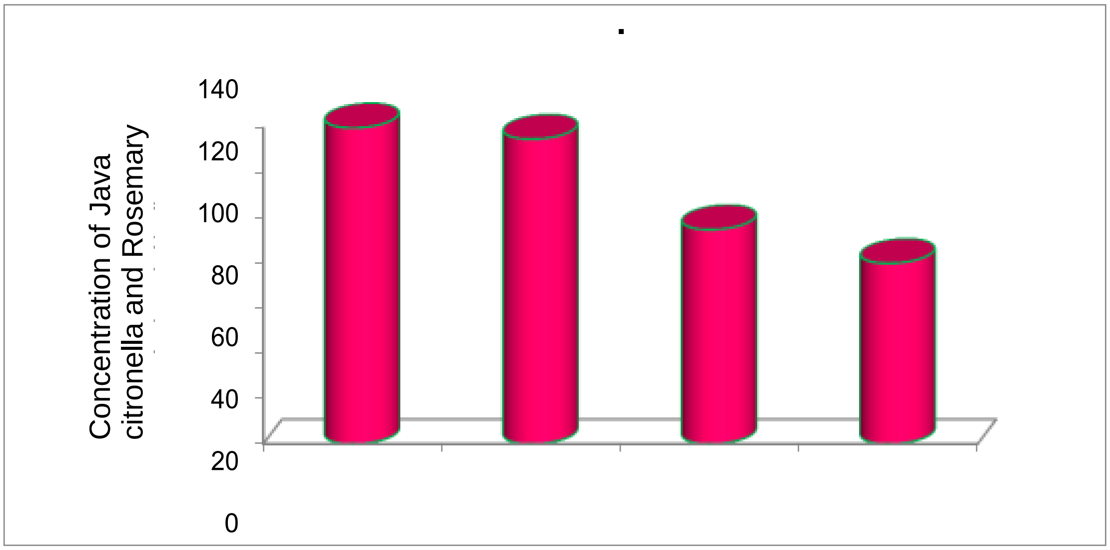

Graph 13. Bar graph showing MIC of Java citronella and Rosemary formulation against *Microsporum canis* 3270

Summary and Conclusion

In order to contribute to the ongoing quest for alternative, easily accessible, and cost-effective treatments for common skin infections in India, it is crucial to advocate for rigorous scientific research that focuses on both plants and essential oils used in the context of skin ailments and various cultural applications. The antifungal attributes of essential oils used in the management of skin disorders can produce either favorable or unfavorable results, underscoring the need for a comprehensive scientific exploration of their phytochemistry, toxicity, and other pharmacological actions.

Moreover, it is advisable that these essential oils be evaluated not only for their antifungal efficacy against T. tonsurans and M. canis but also for research that involves the isolated compounds to assess their effectiveness against pathogens that are pertinent to dermatological conditions. Embracing this approach of incorporating traditional remedies in the treatment of skin fungal infections has the potential to open doors to safer alternatives in comparison to the aggressive and frequently side-effect-prone synthetic treatments that are currently in use.

An indispensable aspect to address is the toxicological impact of essential oils. The primary objective of investigating natural essential oils is to identify safer, top-quality, and efficient substitutes to the costly and often protracted treatment regimens linked to conventional allopathic medications. The results from the present studies strongly advocate for the utilization of essential oil combinations in the management of skin diseases, given their heightened effectiveness.

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