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Cultivate And Assess The Antifungal Potential Of Java Citronella And Horseshoe Geraniums Oils Against A Variety Of Fungal Infections.

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Abstract

Mixtures of different essential oils often provide an alternative approach to promoting human health. Numerous researchers have extensively examined the mechanisms of action of essential oils, their individual constituents, and various combinations. There are approximately 90 different types of essential oils, with over a thousand potential combinations recommended for dermatological applications. This study delves into the antifungal properties of essential oils, specifically citronella and Horseshoe geraniums oil, as natural remedies against pathogens responsible for dermatological infections like Tinea corporis and Tinea capitis. The main aim of this research is to elucidate the characteristics of essential oils, particularly their antifungal attributes, and their effectiveness when used in blends or combinations.
The essential oil extracted from Cymbopogon flexuosus (Lemongrass), Ocimum Horseshoe geraniumsicum (Horseshoe geraniums), and Cymbopogon winterianus (Java citronella) demonstrated significant inhibitory effects against all the fungi tested in this study. The therapeutic application of essential oils may also provide a solution to tackle the issue of the rapid development of fungal resistance associated with commonly used antifungal treatments available today.

Keywords: Essential oils, antifungal effect, Trichophyton tonsurans Microsporum canis.

Cultivation Techniques for Java Citronella

I. Propagation:

Java citronella plants were multiplied using slips. Directly sowing seeds in the field or using a nursery was considered impractical. Slips were obtained from mature parent plants and subsequently transplanted into the field. The use of seeds was avoided to prevent genetic variations and disparities in oil yield.

II. Soil Conditions:
Java citronella can thrive in a variety of soil types, with loamy to sandy loam soils containing abundant organic matter being the most suitable. The optimal soil pH for germination and growth of these plants falls within the range of 6.0 to 7.5. Clayey, waterlogged soils are not suitable for cultivating Java citronella.

III. Climate:
Java citronella crops germinate effectively during the rainy season when a warm and humid climate prevails. The selected region for cultivating Java citronella, Kannauj, had a warm and humid climate with humidity levels and temperatures ranging from 20-30°C, which was ideal for plant growth and optimal oil production.

IV. Planting Time:
Java citronella crops can be successfully cultivated from July to August in North Indian regions. Consequently, the Java citronella plants were planted during the first week of July.

V. Slips Preparation:
Slips were prepared from mature Java citronella parent plants, with each slip consisting of a 2–3-inch stem length along with roots. These prepared slips were fresh, robust, green, and succulent.

VI. Land Preparation and Transplanting:
The field was plowed three times before the Java citronella transplantation process. Additionally, 15 tons per hectare of farmyard manure were applied to the soil. NPK fertilizers were added in a ratio of 180:80:50. A 0.02% Bavistin solution was applied to the slips just before transplanting to prevent fungal infections. Subsequently, the Java citronella slips were transplanted into the well-prepared field with a spacing of 60×60 cm between plants, which is considered ideal for Java citronella cultivation. Post-transplantation, irrigation was applied to ensure plant health.

VII. Crop Nutrition:
Nitrogen treatment was administered to the crop field at the time of transplanting, in addition to farmyard manure and other biofertilizers. Biopesticides were also applied to the Java citronella crop for disease prevention and higher oil yields.

VIII. Weed Control:
Weeding was conducted twice during the growth period of the crop. Once the plants reached maturity, the presence of the Java citronella inhibited the growth of various weed types, making further weeding unnecessary.

IX. Irrigation:
Java citronella crops require irrigation once or twice a month, particularly when rainfall is insufficient. Typically, 6 to 8 irrigation cycles are necessary throughout the entire growth period.

X. Harvesting:
To ensure the quality and quantity of oil production and to prevent contamination, harvesting Java citronella requires careful handling. A clean surface area is recommended for placing harvested plants. The timing of harvesting significantly affects oil quality and yield. Harvesting is typically conducted on bright, sunny days.

The Java citronella crop was harvested 95 days after transplantation. The herbage was left in a shaded area for 10-12 hours to reduce moisture.

XI. Processing and Isolation of Essential Oil:
Hydro-distillation was employed to extract Java citronella essential oil, resulting in a yield of 160 kg per hectare. The essential oil was stored in aluminum containers in a cool, dry location.

XII. Yield:
An average yield of fresh herbage ranged from 20 to 22 kg, and the entire herbage produced approximately 160-170 ml of essential oil..

Cultivation Techniques for Sweet Horseshoe Geraniums

I. Propagation:
Java citronella plants were propagated using slips. Sowing seeds directly in the field or utilizing a nursery was considered impractical. Slips were obtained from mature parent plants and subsequently transplanted into the field. The use of seeds was avoided to prevent genetic variations and differences in oil yield.

II. Soil Conditions:
Java citronella can flourish in a variety of soil types, with loamy to sandy loam soils containing ample organic matter being the most suitable. The optimal soil pH for germination and growth of these plants falls within the range of 6.0 to 7.5. Clayey, waterlogged soils are unsuitable for cultivating Java citronella.

III. Climate:
Java citronella crops germinate effectively during the rainy season when a warm and humid climate prevails. The selected region for cultivating Java citronella, Kannauj, featured a warm and humid climate with humidity levels and temperatures ranging from 20-30°C, which was ideal for plant growth and optimal oil production.

IV. Planting Time:
Java citronella crops can be successfully cultivated from July to August in North Indian regions. Consequently, the Java citronella plants were planted during the first week of July.

V. Slips Preparation:
Slips were prepared from mature Java citronella parent plants, with each slip consisting of a 2–3-inch stem length along with roots. These prepared slips were fresh, robust, green, and succulent.

VI. Land Preparation and Transplanting:
The field was plowed three times before the Java citronella transplantation process. Additionally, 15 tons per hectare of farmyard manure were applied to the soil. NPK fertilizers were added in a ratio of 180:80:50. A 0.02% Bavistin solution was applied to the slips just before transplanting to prevent fungal infections. Subsequently, the Java citronella slips were transplanted into the well-prepared field with a spacing of 60×60 cm between plants, which is considered ideal for Java citronella cultivation. Post-transplantation, irrigation was applied to ensure plant health.

VII. Crop Nutrition:
Nitrogen treatment was administered to the crop field at the time of transplanting, in addition to farmyard manure and other biofertilizers. Biopesticides were also applied to the Java citronella crop for disease prevention and higher oil yields.

VIII. Weed Control:
Weeding was carried out twice during the crop's growth period. Once the plants reached maturity, the presence of the Java citronella inhibited the growth of various weed types, rendering further weeding unnecessary.

IX. Irrigation:
Java citronella crops require irrigation once or twice a month, particularly when rainfall is insufficient. Typically, 6 to 8 irrigation cycles are necessary throughout the entire growth period.

X. Harvesting:
To ensure the quality and quantity of oil production and to prevent contamination, harvesting Java citronella requires careful handling. A clean surface area is recommended for placing harvested plants. The timing of harvesting significantly affects oil quality and yield. Harvesting is typically conducted on bright, sunny days.

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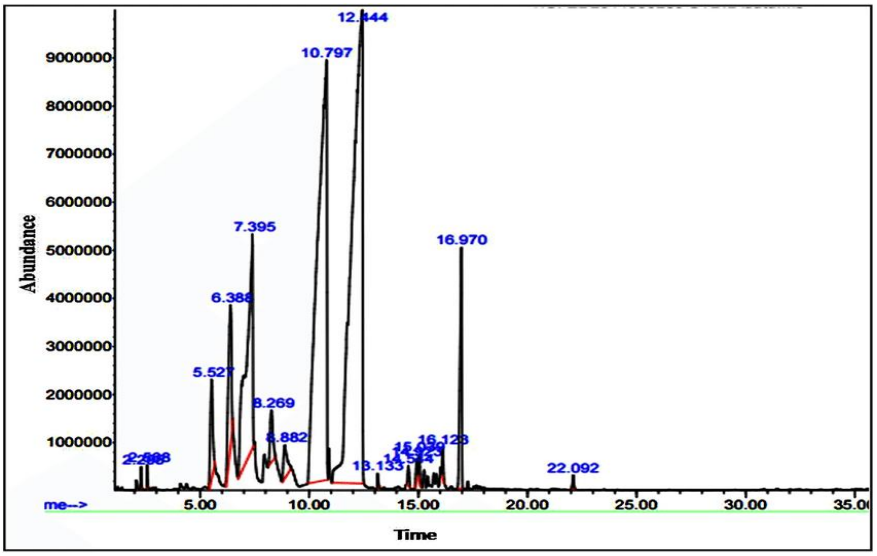
XI. Processing and Isolation of Essential Oil:
Hydro-distillation was employed to extract Java citronella essential oil, resulting in a yield of 160 kg per hectare. The essential oil was stored in aluminum containers in a cool, dry location.

XII. Yield:
An average yield of fresh herbage ranged from 20 to 22 kg, and the entire herbage produced approximately 160-170 ml of essential oil.

Assessment of selected oils components

The various constituents of lemongrass, Java citronella, and Horseshoe geraniums essential oils were determined using gas chromatography mass spectrometry (GC-MS). A total of 50 chemical components were identified in Java citronella essential oil, while Horseshoe geraniums essential oil contained 21 chemical components.

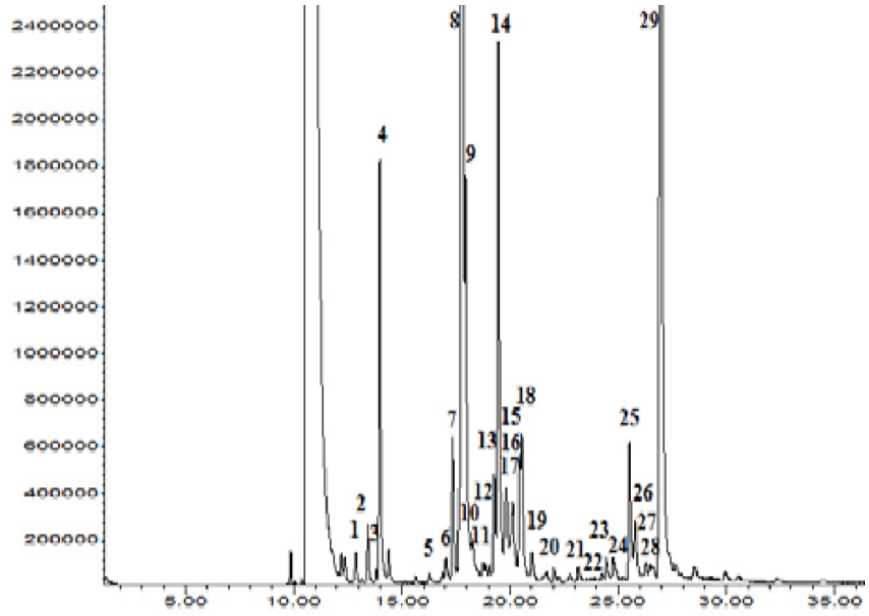


GC-MS Graph of Java citronella oil

Table 1. Name and percentage of components of Java citronella essential oil

Sr. No.	Retention Time	Name of the oil components	Percentage of components
1.	5.26	Bergamal	0.06
2.	11.70	Terpineol	1.21
3.	12.63	Linalool	1.62
4.	14.41	Ctronellol isobutanoate	0.11
5.	15.57	Lavandulyl acetate	0.50
6.	16.04	Java citronellal	29.15
7.	16.21	Mentha-2,8-dien-1-ol	0.30
8.	16.43	Limonene	0.45
9.	16.78	Decenal	0.16
10.	17.41	Decenal-1-ol	0.06
11.	17.79	Citronellol	7.43
12.	18.19	Neral	6.52
13.	19.20	Geraniol	22.52
14.	19.52	Geranial	5.20
15.	19.78	Neryl acetate	1.86
16.	20.12	Elemene	1.26
17.	20.42	Dodecanal	0.10
18.	20.55	Caryophyllene	0.12
19.	24.00	Germacrene	1.02
20.	24.22	geranyl acetate	2.63
21.	24.51	Bergamotene	0.11
22.	24.72	Isoeugenol	0.03
23.	25.04	Germacrene	1.09
24.	25.32	Elemol	1.92

25.	25.51	Elemol acetate	1.32
26.	26.02	Limonene	1.27
27.	26.88	Cadinene-γ	0.04
28.	28.04	Methyl linolate	0.02
29.	28.23	Damascene-α	0.52
30.	28.54	Eudesmol -γ	0.30
31.	28.92	Eremoligenol	0.46
32.	29.26	Bisabolene-α	0.01
33.	29.58	Damascene-α	0.02
34.	29.89	Farnesol	0.60
35.	30.09	Methyl isoeugenol	1.30
36.	30.31	Isophorene	1.40
37.	30.64	Myrtanol	0.60
38.	30.89	Linalyl acetate	0.54
39.	31.09	α - pinene	0.16
40.	31.26	Camphene	0.20
41.	31.64	β- pinene	0.21
42.	32.08	Sabinene	0.04
43.	32.32	β- caryophyllene	0.57
44.	32.53	4- Terpineol	1.05
45.	33.20	Cis- ocimene	0.07
46.	33.58	Trans- ocimene	0.70
47.	33.87	p- cymene	0.55
48.	34.56	Terpinolene	1.24
49.	34.95	1- hexanol	0.09
50.	35.09	1- borneol	0.14



GC-MS graph of Horseshoe geraniums oil

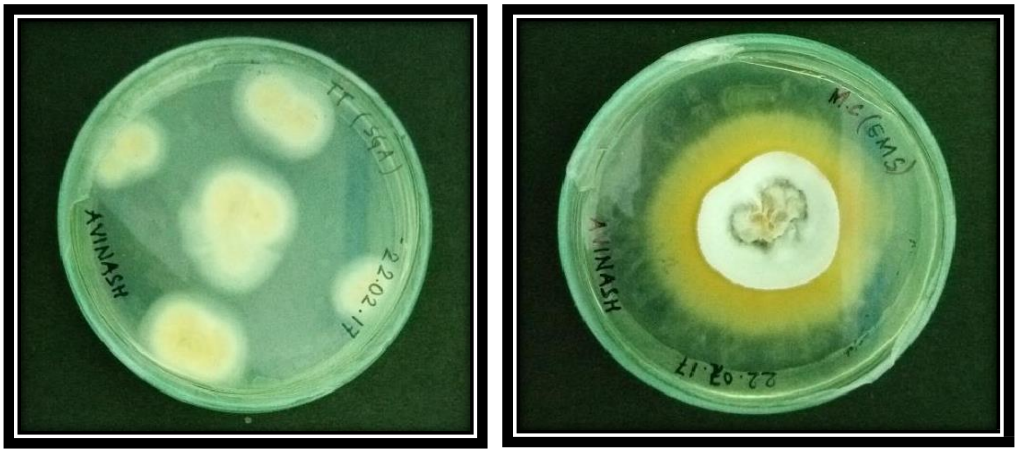
Table 2. Name and percentage of components of Horseshoe geraniums essential oil

Sr. No.	Retention Time	Name of the oil components	Percentage of components
1.	2.41	1-octen-3-ol	0.07
2.	7.97	6-methyl-5-hepten-2-one	0.19
3.	9.28	1, 8- cineole	0.06
4.	17.92	Linalool	17.61
5.	17.95	Fenchone	0.10
6.	18.31	Sapthulenol	0.61

7.	18.44	terpinen-4-ol	0.47
8.	20.11	eudesmol	0.12
9.	20.56	1, 10-di-epi-cubenol	0.17
10.	21.03	Methyl chavicol	76.36
11.	21.2	trance caryophyllene	0.46
12.	21.49	trans-α- bergamotene	0.62
13.	21.61	α – humulene	0.10
14.	22.1	germacrene-D	0.15
15.	22.26	Bicyclogermacrene	0.06
16.	22.50	germacrene-A	0.61
17.	23.40	γ cadinene	1.89
18.	25.54	trans-α-bisabolene	0.07
19.	30.05	epi-α-cadinol	0.06
20.	32.71	caryophyllene oxide	0.05
21.	36.40	humulene epoxide II	0.06

Outgrowth of prepared of inoculums

The fungal strains, specifically Trichophyton tonsurans 8475 and Microsporum canis 3270, were acquired from MTCC Chandigarh. Trichophyton tonsurans was cultured on Sabouraud's agar, while Microsporum canis was cultivated on Emmons-modified Sabouraud's agar. Both strains were incubated at 25°C for a period of 7 days, following the guidelines provided by MTCC. It was observed that both fungal strains displayed vigorous growth under these specified conditions (Fig. 5.2), and subsequently, the inoculums were prepared and made ready for further experimental testing.



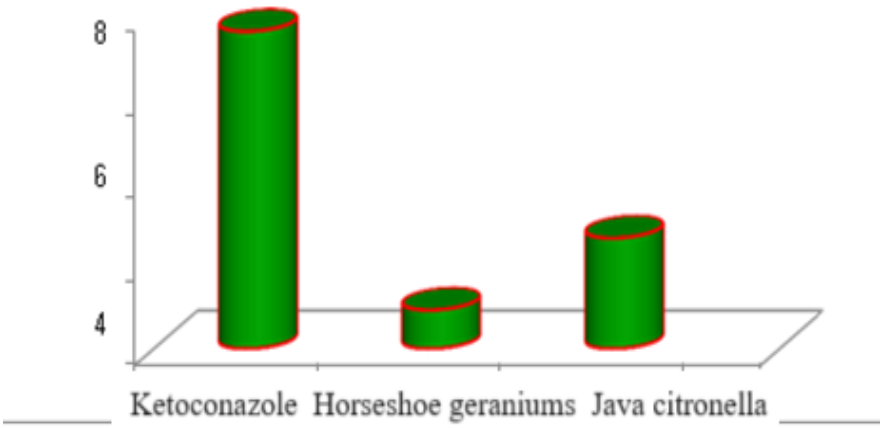
(A) (B)

Figure: 1. (A) - *Trichophyton tonsurans* and **(B) -** *Microsporum canis*.

The inhibitory effects of Java citronella, Horseshoe geraniums oils, and ketoconazole on Trichophyton tonsurans 8475 were evaluated using the zone of inhibition test, yielding a spectrum of inhibition diameters ranging from 0.93mm to 7.63mm. Specific values for the zone of inhibition can be found in Table 3, and for comparative analysis, a graphical representation is presented in Graph 1.

Table 3. Antifungal activity of essential oils and antifungal drug against *Trichophyton tonsurans* 8475

Sr. No.	Antifungal agents	Zone of inhibition in mm (±SD)
1.	Java citronella	0.94 ± 0.14
2.	Horseshoe geraniums	2.67 ± 0.21
3.	Ketoconazole	7.64 ± 0.14

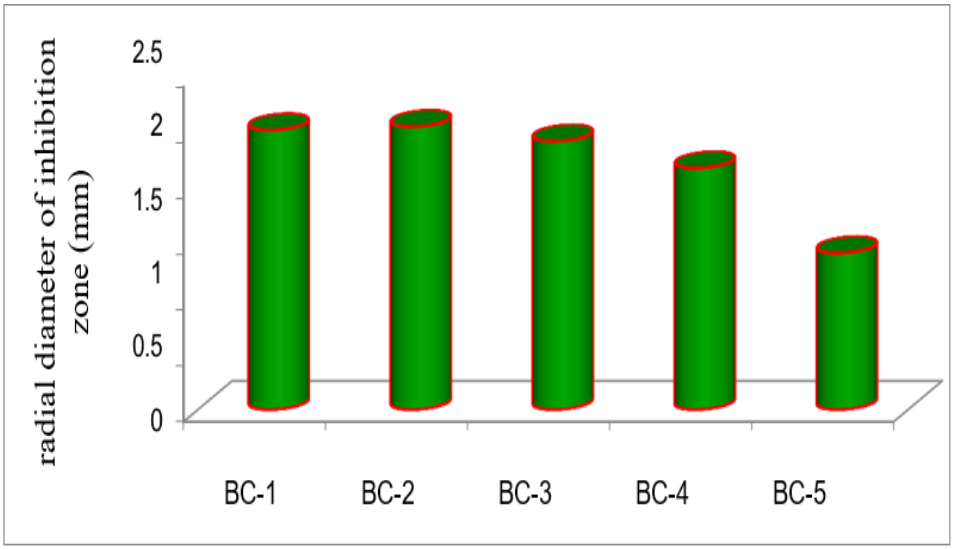


Graph 1. Bar graph showing antifungal activity of essential oils and antifungal drug against *Trichophyton tonsurans* 8475

The antifungal efficacy of Horseshoe geraniums and Java citronella (BC) formulations against Trichophyton tonsurans 8475 was ascertained via the zone of inhibition test, resulting in a spectrum of radial diameter values spanning from 1.40mm to 2.53mm. A comprehensive breakdown of these values can be located in Table 4, and for a visual comparative analysis, please consult Graph 2.

Table 4. Antifungal activity of Horseshoe geraniums and Java citronella (HJ) formulations against *Trichophyton tonsurans* 8475

Sr. No.	Name of Formulation	Zone of inhibition in mm (±SD)
1.	HJ 1	2.40 ± 0.12
2.	HJ 2	2.43 ± 0.04
3.	HJ 3	2.30 ± 0.11
4.	HJ 4	2.26 ± 0.14
5.	HJ 5	1.30 ± 0.12

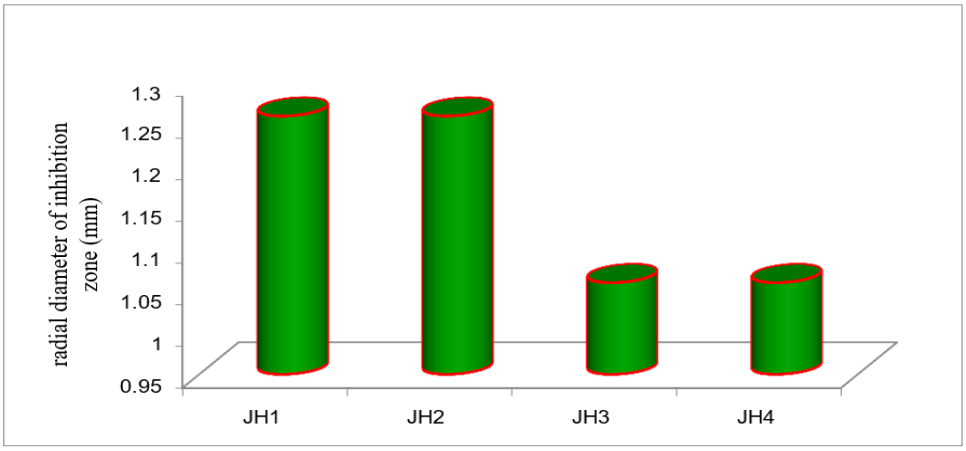


Graph 2. Bar graph showing antifungal activity of Horseshoe geraniums and Java citronella formulations against *Trichophyton tonsurans* 8475

The antifungal effectiveness of Java citronella and Horseshoe geraniums (JH) formulations against Trichophyton tonsurans 8475 was assessed using the zone of inhibition test. This yielded a spectrum of radial diameter values ranging from 1.06mm to 1.26mm. A comprehensive breakdown of these values is available in Table 5, and for visual comparison, please consult Graph 3.

Table 5. Antifungal activity of Java citronella and Horseshoe geraniums (JH) formulations against *Trichophyton tonsurans* 8475

Sr. No.	Name of Formulation	Zone of inhibition in mm (±SD)
1.	JH1	1.20 ± 0.03
2.	JH2	1.21 ± 0.21
3.	JH3	1.04 ± 0.02
4.	JH4	1.03 ± 0.12

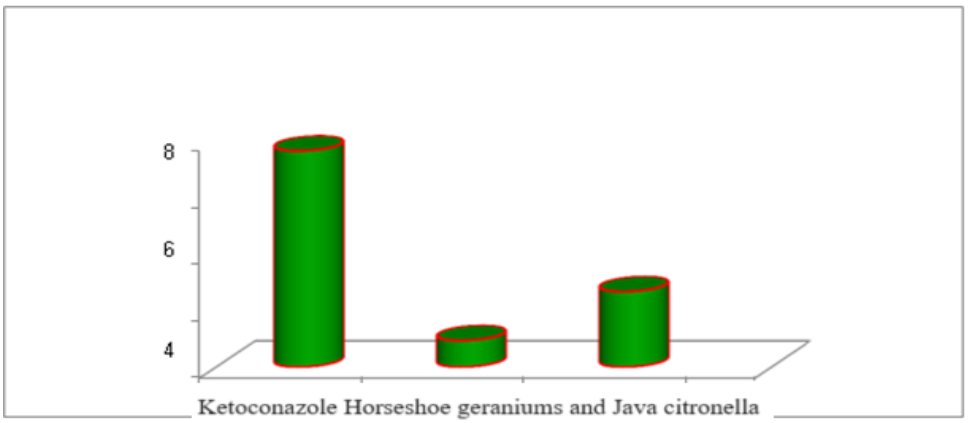


Graph 3. Bar graph showing antifungal activity of Java citronella and Horseshoe geraniums formulations against *Trichophyton tonsurans* 8475

The antifungal potential of Java citronella, Horseshoe geraniums oils, lemongrass, and ketoconazole against Microsporum canis 3270 was established through the evaluation of the zone of inhibition. The outcomes unveiled a spectrum of radial diameter values spanning from 1.40mm to 7.63mm. Elaborate data regarding these inhibitory zones can be located in Table 6, and for a visual comparative analysis, please refer to Graph 4 (Fig. 2).

Table 6. Antifungal activity of Horseshoe geraniums and Java citronella (HJ) formulations against *Microsporum canis* 3270

Sr. No.	Name of Formulation	Zone of inhibition in mm (±SD)
1.	Java citronella	1.30 ± 0.34
2.	Horseshoe geraniums	2.26 ± 0.06
3.	ketoconazole	7.43 ± 0.12

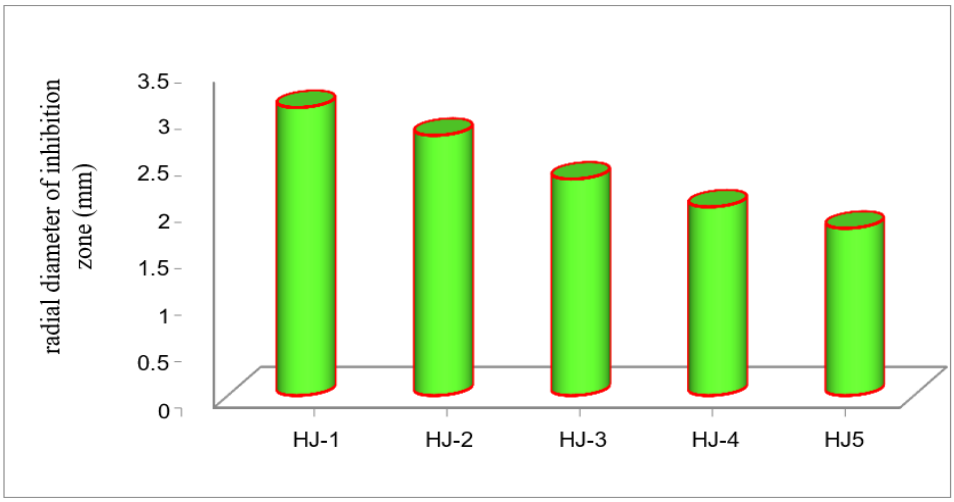


Graph 4. Bar graph showing antifungal activity of lemongrass, Horseshoe geraniums and Java citronella formulations against *Microsporum canis* 3270

The antifungal efficacy of Horseshoe geraniums and Java citronella formulations against Microsporum canis 3270 was noted, resulting in a range of radial diameter values from 1.80mm to 3.10mm. Detailed information concerning the zone of inhibition can be located in Table 7, and for a visual presentation, please refer to Graph 5.

Table 7. Antifungal activity of Horseshoe geraniums and Java citronella (HJ) formulations against *Microsporum canis* 3270

Sr. No.	Name of Formulation	Zone of inhibition in mm (±SD)
1.	HJ 1	3.20 ± 0.21
2.	HJ 2	2.30± 0.12
3.	HJ 3	2.23 ± 0.14
4.	HJ 4	2.13 ± 0.03
5.	HJ 5	1.70 ± 0.11

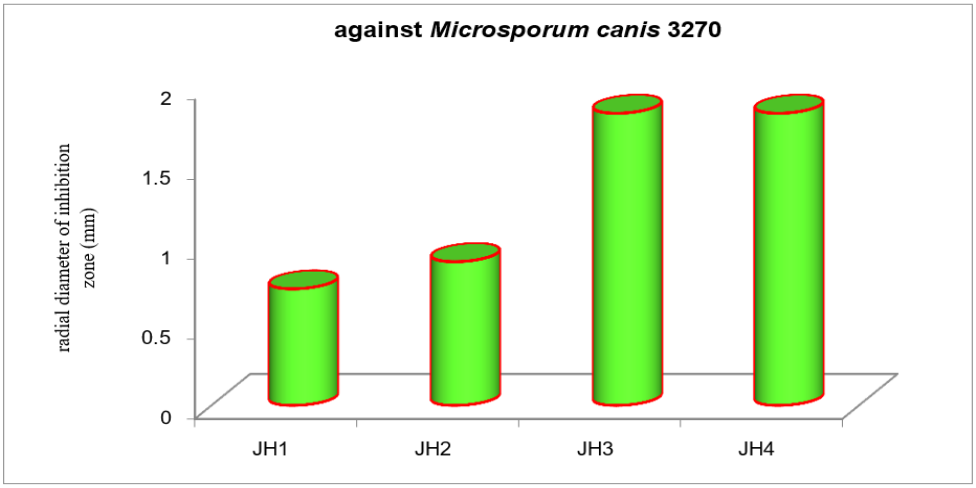


Graph 5. Bar graph showing antifungal activity of Horseshoe geraniums and Java citronella formulations against *Microsporum canis* 3270

The antifungal activity of Java citronella and Horseshoe geraniums (CB) formulations against Microsporum canis 3270 showed a zone of inhibition within the radial diameter range of 0.73mm to 2.00mm. The specific values for the zone of inhibition can be found in Table 8, and for a graphical representation, please refer to Graph 6.

Table 8. Antifungal activity of Java citronella and Horseshoe geraniums (JH) formulations against *Microsporum canis* 3270

Sr. No.	Name of Formulation	Zone of inhibition in mm (±SD)
1.	JH 1	0.74 ± 0.03
2.	JH 2	0.91 ± 0.11
3.	JH 3	1.85 ± 0.12
4.	JH 4	2.01± 0.13



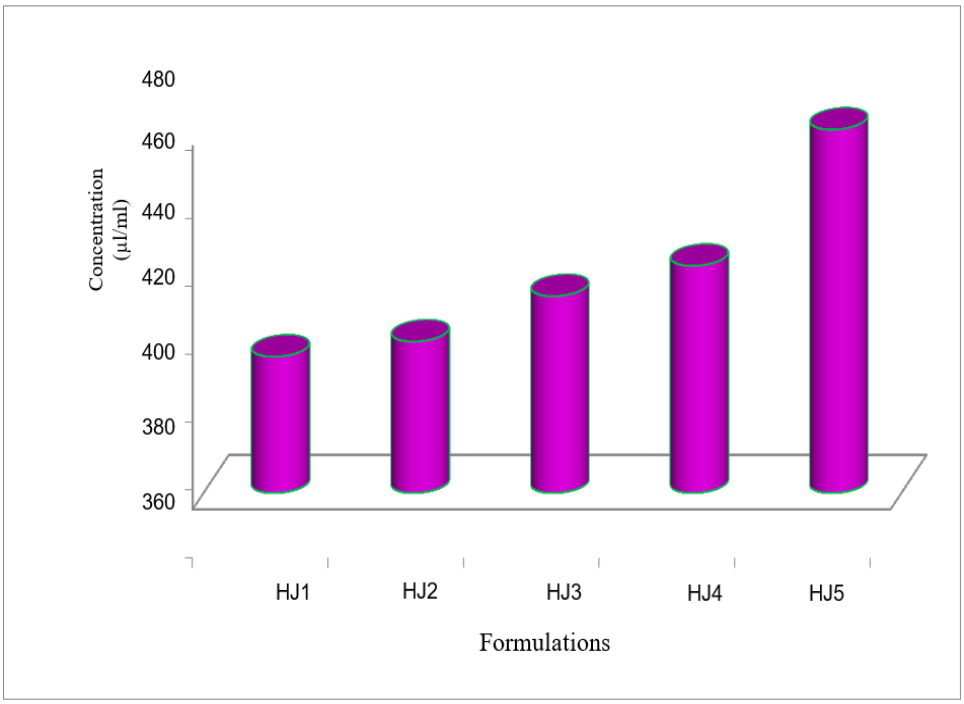
Graph 6. Bar graph showing antifungal activity of Java citronella and Horseshoe geraniums formulations against *Microsporum canis* 3270

Minimum inhibitory concentration of Horseshoe geraniums and Java citronella (BC) Formulations against *Trichophyton tonsurans* 8475

The minimum inhibitory concentration of BC formulations against *Trichophyton tonsurans* 8475 was identified from the range of 405µl/ml to 480µl/ml. The values of MIC showed in the table 9 and graphical form showed in the graph 7.

Table 9. Minimum inhibitory concentration of Horseshoe geraniums and Java citronella (HJ) Formulations against *Trichophyton tonsurans* 8475

Sr. No.	Name of Formulation	MIC (µl/ml)
1.	HJ1	405
2.	HJ2	410
3.	HJ3	425
4.	HJ4	435
5.	HJ5	480

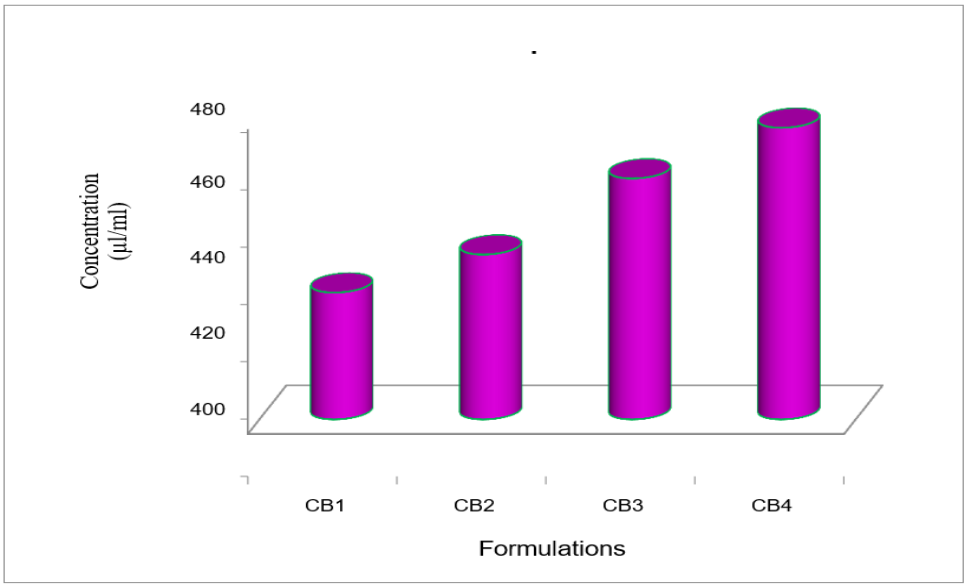


Graph 7. Bar graph showing MIC of Horseshoe geraniums and Java citronella Formulation against *Trichophyton tonsurans* 8475

The minimum inhibitory concentration of Java citronella and Horseshoe geraniums (CB) formulations against Trichophyton tonsurans 8475 was determined to be in the range of 410µl/ml to 475µl/ml. You can find the specific values in Table 10, and for a graphical representation, please refer to Graph 8.

Table 10. Minimum inhibitory concentration of Java citronella and Horseshoe geraniums (JH) formulations against *Trichophyton tonsurans* 8475

Sr. No.	Name of Formulation	MIC (µl/ml)
1.	JH 1	410
2.	JH 2	425
3.	JH 3	455
4.	JH 4	475

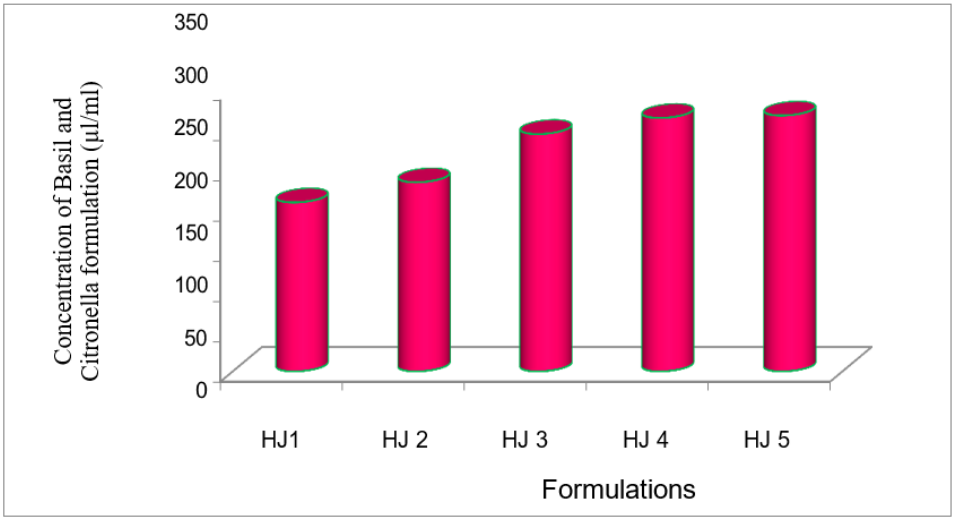


Graph 8. Bar graph showing MIC of Java citronella and Horseshoe geraniums formulations against *Trichophyton tonsurans* 8475

The minimum inhibitory concentration of Horseshoe geraniums and Java citronella (BC) formulations against Microsporum canis 3270 ranged from 210µl/ml to 318µl/ml. You can find the specific values in Table 11, and for a graphical representation, please refer to Graph 9.

Table 11. Minimum inhibitory concentration of Horseshoe geraniums and Java citronella (HJ) formulations against *Microsporum canis* 3270

Sr. No.	Name of Formulation	MIC (µl/ml)
1.	HJ 1	210
2.	HJ 2	235
3.	HJ 3	295
4.	HJ 4	315
5.	HJ 5	318



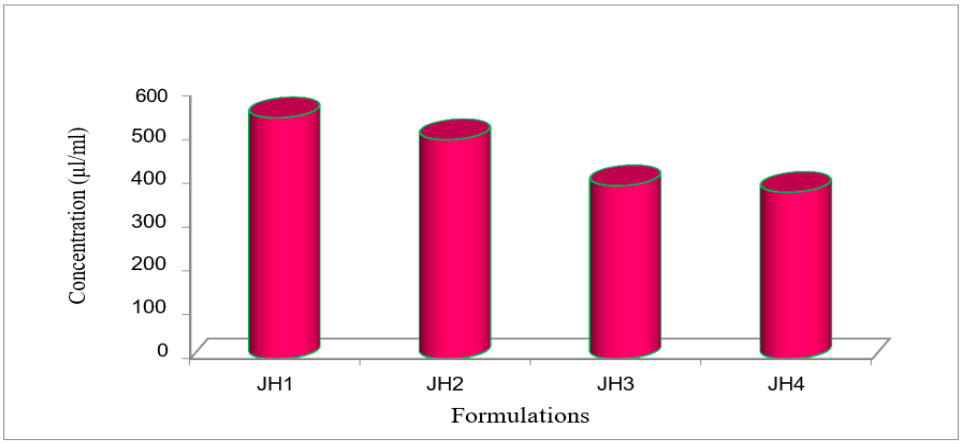
Graph 9. Bar graph showing MIC of Horseshoe geraniums and Java citronella formulations against *Microsporum canis* 3270

Minimum inhibitory concentration of Java citronella and Horseshoe geraniums (CB) formulations against *Microsporum canis* 3270

The minimum inhibitory concentration of these formulations against *Microsporum canis* 3270 was recorded from the range 385µl/ml to 550µl/ml. The values of MIC displayed in the following table 12 and graphical form presented in the graph 10.

Table 12. Minimum inhibitory concentration of Java citronella and Horseshoe geraniums (JH) formulations against *Microsporum canis* 3270

Sr. No.	Name of Formulation	MIC (µl/ml)
1.	JH1	550
2.	JH2	500
3.	JH3	395
4.	JH4	385



Graph 10. Bar graph showing MIC of Java citronella and Horseshoe geraniums formulations against *Microsporum canis* 3270

In the present research, essential oils and their formulations have demonstrated their capability to hinder the growth of Trichophyton tonsurans 8475 and Microsporum canis 3270. The formulations that exhibited substantial antifungal activity, characterized by notable zone of inhibition values, and concurrently displayed low minimum inhibitory concentrations, are regarded as the most effective. These specific formulations have exhibited remarkable efficiency against both Trichophyton tonsurans 8475 and Microsporum canis 3270. The MICs ranged from 1 µl/ml to 20 µl/ml, and the zone of inhibition spanned from 7.66 mm to 7.0 mm, which proved adequate for inhibiting the growth of the selected fungi. A comparative analysis of this data is presented in Table 13. The selection of formulations with these high and low values is imperative for establishing dosage standards against the targeted fungi.

Table 13. MIC values of all the formulations of essential oils.

Formulations	MIC (µl/ml) against *Trichophyton tonsurans* 8475	MIC (µl/ml) against *Microsporum canis* 3270
Java citronella	400	600
Horseshoe geraniums	500	200
Ketoconazole	0.1	0.1
JH-1	405	210
JH-2	410	235
JH-3	425	295
JH-4	435	315
JH-5	480	318

Table 14. Zone of inhibition values of all the formulations of essential oils.

Formulations	Zone of inhibition in (mm) against *Trichophyton tonsurans* 8475	Zone of inhibition in (mm) against *Microsporum canis* 3270
Java citronella	0.93	1.4
Horseshoe geraniums	2.66	2.36
Ketoconazole	7.63	7.56
JH-1	2.5	3.1
JH-2	2.53	2.8
JH-3	2.4	2.33
JH-4	2.16	2.03
JH-5	1.4	1.8

Summary and Conclusion

To summarize, the antifungal effects of essential oils used in the treatment of skin disorders can yield both favorable and unfavorable outcomes, emphasizing the importance of comprehensive scientific research into their phytochemistry, toxicity, and other pharmacological properties. It is advisable that research not only assesses essential oils for their antifungal properties against T. tonsurans and M. canis but also delves into studies on the isolated compounds to address their specific relevance in dermatological conditions.

The utilization of essential oils through blends or combinations, involving the careful selection and combination of two or more oils, is a skillful approach to address the holistic relief of individual symptoms. The goal of blending is to create a synergistic therapeutic effect that surpasses the efficacy of individual essential oils. This synergy can be achieved when the compounds in the respective essential oils influence different target sites.

In conclusion, this study has established that essential oil formulations have the potential to initiate a synergistic antifungal effect. However, further investigations are required to explore the synergistic effects of different oils and their compounds, as well as to determine the most effective doses and application methods in practice. This research opens promising pathways for the development of alternative and effective treatments for common skin infections.

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