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Evaluation of the combined antifungal effects of *Azadirachta indica* (Neem) and *Salvia coccinea*

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Abstract

Fungal infections are increasing rapidly across the world and have become a major public health concern. The growing resistance to conventional antifungal drugs and their associated side effects have encouraged researchers to explore safer and more effective alternatives from natural sources. Medicinal plants are widely used because of their rich phytochemical constituents and lower toxicity. The present study was carried out to evaluate the combined antifungal effects of *Azadirachta indica* and *Salvia coccinea* in the formulation of a herbal antifungal cream. Neem is known for its strong antimicrobial, anti-inflammatory, and antifungal properties due to compounds such as azadirachtin, nimbidin, and flavonoids. *Salvia coccinea* also contains phenolic compounds, terpenoids, and essential oils that contribute to antimicrobial activity.

The antifungal cream was prepared using neem extract, salvia extract, stearic acid, cetyl alcohol, glycerin, liquid paraffin, preservatives, and distilled water. The formulation was developed by emulsification technique under controlled temperature conditions. The prepared cream was evaluated for antifungal activity using Sabouraud Dextrose Agar (SDA) medium through standard microbiological methods. The results indicated significant antifungal activity of the formulation, with neem showing comparatively higher activity than salvia. However, the combination of both extracts demonstrated enhanced antifungal potential due to possible synergistic interactions between their bioactive compounds.

The study concludes that the combined herbal formulation of neem and salvia may serve as a promising natural alternative for the treatment of fungal infections with fewer side effects and reduced risk of resistance development.

Keywords: Terpenoids, Antifungal, Emulsification technique, Extracts

1. Introduction

Fungal diseases are escalating as a threat to public health worldwide from superficial skin infections to disseminated systemic ones. (Ospina Salazar *et al.* 2015a) ^[10] Moreover, the increasing challenge of antifungal resistance as well as the adverse effects of classical antifungal drugs have raised the necessity to develop new, more efficient and safer therapeutic options. (Raghavendra and Balsaraf 2014a) ^[12] Due to their broad phytochemical profile and minimum toxic nature, medicinal plants have been widely investigated for novel antimicrobial agents. *Azadirachta indica* (Neem) is one of the widely explored herbs in traditional system of medicine especially in south asia. (Carvalho, Cavaco, and Brodelius 2011a) ^[3] The pharmacological actions of neem are such as: Anti-fungal, Anti-bacterial, Anti-viral and Anti-Inflammatory. (Carvalho, Cavaco, and Brodelius 2011b) ^[4] These activities have been attributed to bioactive compounds like azadirachtin, nimbin, nimbidin and several flavonoids that have proven efficacy against a range of fungal infections. *Salvia coccinea* (Scarlet sage) belongs to the family of Lamiaceae is an ornamental and medicinal plant used for treating various diseases. Salvia is rich in essential oil, phenolic compounds and terpenoids, of which several proved to be antibacterial and antioxidant. (Sedghi *et al.* 2019) ^[16] While relatively little research has been performed on *Salvia coccinea* contrasted with neem, the initial results suggest that it possesses considerable antifungal properties and therein would be an excellent candidate for future research. (Ospina Salazar *et al.* 2015b) ^[11] Current scientific investigation has focused on the use of plant extracts in combination to enhance antibacterial activity through synergistic interactions.

(Raghavendra and Balsaraf 2014b) [13] These combinations may result in potentiated antifungal activity, lower dosage, and diminished risk of resistance development. However, there is little information on the antifungal effect of the mixtures of *Azadirachta indica* and *Salvia coccinea*. (Saima Tauseef *et al.* 2024)

2. Materials and methods

2.1 Plant Material

The fresh neem leaves (*Azadirachta indica*) and *Salvia coccinea* leaves were collected from FRI which is located in Dehradun at the state of Uttarakhand. The leaves were cleaned by washing them with distilled water to remove all dust and contaminants. A qualified botanist from the Department of Botany authenticated the plants. The process of authentication confirmed the correct botanical identity of the plant samples while maintaining their original purity.

2.2 Preparation of the etanolic extract

About 100 grams of powdered plant material were measured with precise accuracy. The powder was packed into a thimble and placed inside the Maceration apparatus. Ethanol served as the solvent for the extraction process. The process of continuous extraction lasted between 1 and 2 hours. The extract obtained was filtered. Evaporate the solvent using water bath to obtain a semi-solid extract. Store extract in air tight container. Keep in a cool and dry place (Osman Mohamed Ali *et al.* 2017) [9].

2.3 Primary phytochemical screening

2.3.1 Test for flavonoids

The flavonoids content will be measured by using the aluminium chloride method. 1ml of test solution (1mg/ml), 1.5ml of ethanol, 0.1ml of 10% aluminium chloride hexahydrate ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$), and 0.1 ml of 1M sodium acetate (CH_3COONa), and 2.3ml of distil water will be added. (Álvarez-Caballero & Coy-Barrera, 2019) [1] After incubating the mixture at room temperature for 40 minutes, the absorbance will be recorded at 435nm against a blank prepared identically but without aluminium chloride. Rutin served as the reference standard, and results will be expressed as milligrams of rutin equivalents (RE) per gram of extract. All measurement will be conducted in triplicate for accuracy.

2.3.2 Test for Alkaloids

To determine the alkaloid content take five gram of sample in 250ml of beaker and add 200ml of 10% acetic acid in ethanol to the sample. Cover the beaker with foil paper and allow the beaker to stand for 4 hours. After that filter the solution and take out the extract. Now by using the water bath make the solution concentrated to $\frac{1}{4}$ of its initial volume. Furthermore, add the conc. NH_4OH slowly to obtain the extract until the precipitation is completed. After the precipitation formed (after allowing the solution to rest for settle) the sample is washed with Dil. NH_4OH and filter the sample again. Later weigh the sample and record the content of sample (Álvarez-Caballero & Coy-Barrera, 2019; Singh *et al.*, 1980) [1, 18].

2.3.3 Test for tannins

The tannins content will be observed by using the Ferric chloride method. Mix 0.5 g of the powdered plant extract with 10 ml of distilled water or ethanol then filter it. Take 2

ml of the filtrate and add a few drop of 1%-10% ferric chloride solution. Observe colour dark blue indicates tannins presence (Gupta *et al.*, 2011) [6].

2.3.4 Test for saponins

Mix 0.5 grams of dried plant extract or crude sample powder with 5 to 10 mL of distilled water in a test tube. Stopper the test tube and shake it vigorously by hand for 30 seconds to 5 minutes. Allow the tube to stand undisturbed for 15 to 30 minutes. The formation of a persistent, stable foam that remains over 1-2 cm high after standing confirms the presence of saponins (Li *et al.*, 2023) [7].

2.3.5 Test for terpenoids

Take 5 mL of the plant extract (or mix 0.5 g extract with water/suitable solvent). Add 2 mL of chloroform (CHCl_3) to the extract. Carefully add 3 mL of concentrated sulfuric acid (H_2SO_4) along the side of the test tube to form a layer. The formation of a reddish-brown color at the interface between the two liquids confirms the presence of terpenoids.

2.4 Fungal Culture

The preparation of Sabouraud Dextrose Agar (SDA) medium was carried out according to established microbiological methods. The steps of the procedure are as follows. The required amount of SDA powder was dissolved in distilled water. The solution was heated until complete dissolution. (Deja-Sikora *et al.*, 2019) [5] The pH value was set to 5.6. The medium was disinfected through autoclave sterilization at 121°C for 15 minutes. Sterile media were poured into Petri plates under aseptic conditions (Nagarkoti *et al.*, 2023) [8].

2.5 Test for Anti-fungal Activity

2.5.1 Agar Well Diffusion Method Procedure

Sterile SDA plates received fungal suspension to establish their inoculation. The sterile cork borer produced wells through its use. The different plant extract concentrations were applied into the wells and plates were maintained at 28°C temperature for a period of 48 to 72 hours. The zone of inhibition was measured. The measurement used millimetres as the unit of distance (Sedghi *et al.*, 2019b) [17].

2.5.2 Disc Diffusion Method

The evaluation of antifungal effectiveness was done through measurement of inhibition zones after they placed sterile discs which contained fungal extracts on the treated fungal plates. The team evaluated antifungal performance by observing the size of the inhibition zones which developed during their experiments (Sumalan *et al.*, 2020) [20].

2.5.3 Determination of Minimum Inhibitory Concentration (MIC)

The broth dilution method served as the method for MIC testing. In this serial dilutions of the extracts were conducted. Here the fungal inoculum introduced into each tube (Sosnowski *et al.*, 2020; Turbant *et al.*, 2021) [19, 21] The tubes maintained at controlled temperatures for a period of 48 hours. Later the minimum inhibitory concentration was identified which represented the lowest concentration that prevented any visible growth. It include synergistic Activity Evaluation and Checkerboard Dilution Assay (Boudghene Stambouli *et al.*, 2017) [12].

The checkerboard method was applied to test how neem interacted with *Salvia coccinea* extracts and various extract combinations was prepared and evaluated to check their effectiveness against different fungal strains. (Saja-Garbarz *et al.*, 2021)

2.5.4 Fractional Inhibitory Concentration Index (FICI)

FICI values were calculated using the formula:

FICI = $\frac{\text{MIC of Drug A in Combination} + \text{MIC of Drug B in Combination}}{\text{MIC of Drug A Alone} + \text{MIC of Drug B Alone}}$

Interpretation of FICI

FICI Value	Interpretation
≤ 0.5	Synergistic
0.5-1	Additive
1-4	Indifferent
> 4	Antagonistic

3. Results and Discussion

3.1 Preliminary Phytochemical Screening

The qualitative phytochemical screening was conducted to detect major bioactive compounds which produce antifungal effects.

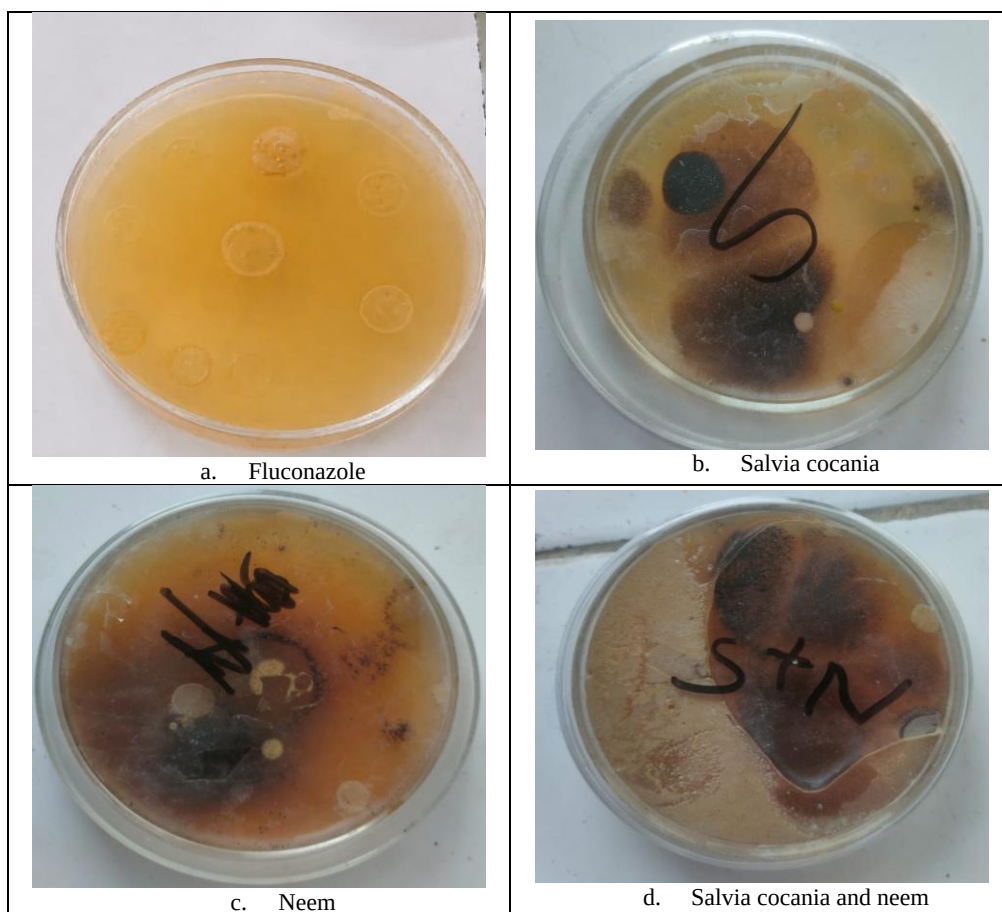
Phytochemical	Neem Extract	<i>Salvia coccinea</i> Extract
Alkaloids	Present (+)	Present (+)
Flavonoids	Present (+)	Present (+)
Tannins	Present (+)	Present (+)
Saponins	Present (+)	Present (+)
Terpenoids	Present (+)	Present (+)

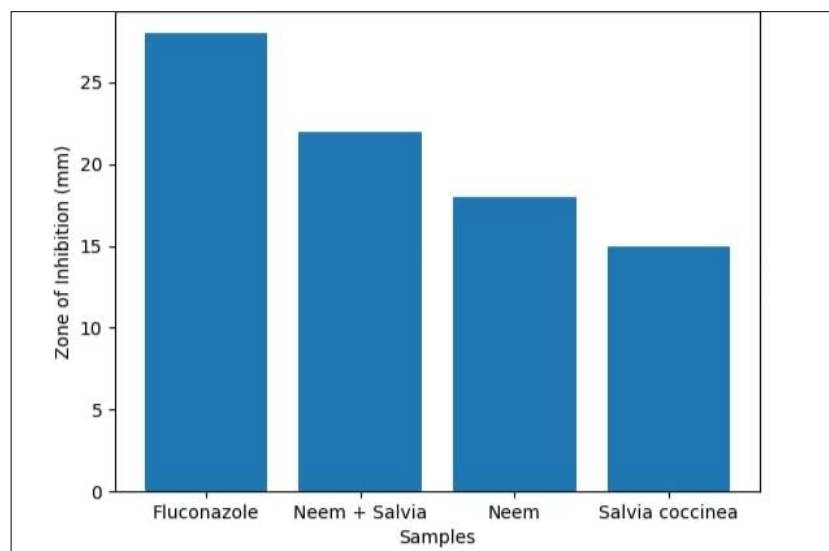
The phytochemical screening confirmed multiple bioactive compounds which exist in both plant extracts. The antimicrobial and antioxidant properties of flavonoids and phenolic compounds demonstrate their strong effectiveness against harmful microorganisms. The two compounds terpenoids and alkaloids together create membrane damage while they also stop fungal cells from growing. The neem extract contained high levels of terpenoids and tannins whereas *Salvia coccinea* showed substantial concentrations of phenolic compounds and flavonoids.

3.2 Antifungal Activity by Agar Well Diffusion Method

The agar well diffusion method shows the antifungal activities of both individual extracts and their combined extracts and standard drug of fluconazole against selected fungal strains.

Drug/ Plant Extract	Concentration (mg/ml)	Horizontal (mm)	Vertical (mm)	Zone of Inhibition
Fluconazole	50	30	26	28
<i>Salvia coccinea</i> Extract	200	16	14	15
Neem Extract	200	19	16	18
Neem + <i>Salvia coccinea</i> Extract	200	23	21	22





The antifungal activity of the extracts showed an upward trend which matched the rising extract concentration. The combined extracts exhibited significantly greater zones of inhibition than the individual extracts against all fungal strains tested. The strongest activity was observed against *Candida albicans* which showed less activity against *Aspergillus niger* and *Trichophyton rubrum*. The enhanced activity of combined extracts suggests synergistic interactions between phytochemicals present in neem and *Salvia coccinea*. Terpenoids together with flavonoids and phenolic compounds created an effect which enabled them to disrupt fungal cell membranes, inhibit spore germination, interfere with enzyme systems and impair fungal metabolism. These findings support previous reports on synergistic herbal antifungal combinations

4. Conclusion

The present study on the combined antifungal effects of *Azadirachta indica* and *Salvia coccinea* demonstrates that both plants possess significant antifungal properties against pathogenic fungal organisms. The experimental findings indicate that extracts obtained from these medicinal plants were effective in inhibiting fungal growth, with the combined treatment showing greater antifungal activity than the individual plant extracts alone. This suggests a possible synergistic interaction between the bioactive compounds present in Neem and *Salvia coccinea*.

References

1. Álvarez-Caballero JM, Coy-Barrera E. Chemical and antifungal variability of several accessions of *Azadirachta indica* A. Juss. from six locations across the Colombian Caribbean coast: identification of antifungal azadirone limonoids. *Plants*. 2019;8(12):555-555.
2. Boudghene Stambouli O, Amrani N, Boudghene Stambouli K, Bouali F. Dermatophytic disease with deficit in CARD9: a new case with a brain impairment. *J Mycol Med*. 2017;27(2):250-253.
3. Carvalho IS, Cavaco T, Brodelius M. Phenolic composition and antioxidant capacity of six *Artemisia* species. *Ind Crops Prod*. 2011;33(2):382-388.
4. Carvalho IS, Cavaco T, Brodelius M. Phenolic composition and antioxidant capacity of six *Artemisia* species. *Ind Crops Prod*. 2011;33(2):382-388.
5. Deja-Sikora E, Mercy L, Baum C, Hryniewicz K. The contribution of endomycorrhiza to the performance of Potato virus Y-infected solanaceous plants: disease alleviation or exacerbation? *Front Microbiol*. 2019;10:516-516.
6. Gupta K, Verma I, Khuller G, Mahajan R. Evaluation of Aro-Tal-AST complex protein as a marker for differential diagnosis of *Mycobacterium avium* infection. *J Glob Infect Dis*. 2011;3(3):259-259.
7. Li S, Yang Q, Cheng B, Liu Y, Zhou S, Ai X, et al. Neem oil against *Aeromonas hydrophila* infection by disrupting quorum sensing and biofilm formation. *Biofouling*. 2023;39(8):867-878.
8. Nagarkoti K, Prakash O, Rawat A, Kabdal T, Kumar R, Srivastava RM, et al. Chemo-profiling of methanolic and ether oleoresins of *Salvia coccinea* and *in vitro* pesticidal evaluation with *in silico* molecular docking and ADME/Tox studies. *Eur J Chem*. 2023;14(2):211-222.
9. Osman Mohamed Ali E, Shakil NA, Rana VS, Sarkar DJ, Majumder S, Kaushik P, et al. Antifungal activity of nano emulsions of neem and citronella oils against phytopathogenic fungi, *Rhizoctonia solani* and *Sclerotium rolfsii*. *Ind Crops Prod*. 2017;108:379-387.
10. Ospina Salazar DI, Hoyos Sánchez RA, Orozco Sánchez F, Arango Arteaga M, Gómez Londoño LF. Antifungal activity of neem (*Azadirachta indica*: Meliaceae) extracts against dermatophytes. *Acta Biol Colomb*. 2015;20(3):201-207.
11. Ospina Salazar DI, Hoyos Sánchez RA, Orozco Sánchez F, Arango Arteaga M, Gómez Londoño LF. Antifungal activity of neem (*Azadirachta indica*: Meliaceae) extracts against dermatophytes. *Acta Biol Colomb*. 2015;20(3):201-207.
12. Raghavendra SS, Balsaraf KD. Antifungal efficacy of *Azadirachta indica* (neem) - an *in vitro* study. *Braz J Oral Sci*. 2014;13(3):242-245.
13. Raghavendra SS, Balsaraf KD. Antifungal efficacy of *Azadirachta indica* (neem) - an *in vitro* study. *Braz J Oral Sci*. 2014;13(3):242-245.
14. Tauseef S, Tauseef F, Siddiqui K, Khan SUA. Antifungal potential of leaves and barks of *Azadirachta indica*. *Biol Sci PJSIR*. 2024;67(1):8-14.
15. Saja-Garbarz D, Ostrowska A, Kaczanowska K, Janowiak F. Accumulation of silicon and changes in

- water balance under drought stress in *Brassica napus* var. *napus* L. *Plants*. 2021;10(2):280-280.
16. Sedghi R, Heidari B, Shahmohamadi H, Zarshenas P, Varma RS. Pd nanocatalyst adorned on magnetic chitosan@N-heterocyclic carbene: eco-compatible Suzuki cross-coupling reaction. *Molecules*. 2019;24(17):3048-3048.
 17. Sedghi R, Heidari B, Shahmohamadi H, Zarshenas P, Varma RS. Pd nanocatalyst adorned on magnetic chitosan@N-heterocyclic carbene: eco-compatible Suzuki cross-coupling reaction. *Molecules*. 2019;24(17):3048-3048.
 18. Singh UP, Singh HB, Singh RB. The fungicidal effect of neem (*Azadirachta indica*) extracts on some soil-borne pathogens of gram (*Cicer arietinum*). *Mycologia*. 1980;72(6):1077-1093.
 19. Sosnowski J, Truba M, Redzik P, Toczyska E. The effect of growth regulator Tytanit dose on *Medicago × varia* T. Martin and *Trifolium pratense* L. yield and nutritional value. *Saudi J Biol Sci*. 2020;27(11):2890-2901.
 20. Sumalan RM, Kuganov R, Obistioiu D, Popescu I, Radulov I, Alexa E, *et al.* Assessment of mint, basil, and lavender essential oil vapor-phase in antifungal protection and lemon fruit quality. *Molecules*. 2020;25(8):1831-1831.
 21. Turbant F, Partouche D, El Hamoui O, Trépout S, Legoubey T, Wien F, *et al.* Apomorphine targets the pleiotropic bacterial regulator Hfq. *Antibiotics*. 2021;10(3):257-257.