Synergistic Antifungal Potential of Polyherbal Extracts Against Pathogenic Fungi: An in-Vitro Evaluation

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***Abstract*—Background: Fungal infections caused by pathogenic microorganisms such as Candida albicans, Aspergillus niger, and Aspergillus fumigatus represent a growing global health challenge, particularly among immunocompromised patients. The emergence of antifungal resistance, adverse effects associated with synthetic antifungal agents, and high treatment costs have stimulated interest in plant-based therapeutics. Polyherbal formulations, owing to their synergistic phytochemical interactions, may offer enhanced antifungal efficacy with reduced toxicity.**

Objective: The present study aimed to formulate and evaluate a polyherbal extract composed of Azadirachta indica (Neem), Ocimum sanctum (Tulsi), Moringa oleifera, and Tinospora cordifolia for its in vitro antifungal activity against selected pathogenic fungi and to compare its efficacy with individual plant extracts and the standard antifungal drug Fluconazole.

Methodology: Individual plant materials were subjected to Soxhlet extraction using 70% ethanol. Equal proportions of the extracts were combined to prepare the polyherbal formulation. Preliminary phytochemical screening was performed to identify major bioactive constituents. Antifungal activity was evaluated against Candida albicans, Aspergillus niger, and Aspergillus fumigatus using the agar well diffusion method at concentrations of 25, 50, and 100 mg/mL. Minimum Inhibitory Concentration (MIC) studies were conducted to determine antifungal potency. All experiments were performed in triplicate and results were expressed as mean ± standard deviation.

Results: Phytochemical screening confirmed the presence of alkaloids, flavonoids, phenolics, tannins, saponins, and terpenoids in the extracts. The polyherbal formulation demonstrated concentration-dependent antifungal activity against all tested fungal strains and consistently produced larger zones of inhibition than the individual extracts. Against Candida albicans, the polyherbal formulation exhibited inhibition zones of 16.5 ± 0.3 mm, 24.8 ± 0.4 mm, and 31.8 ± 0.2 mm at 25, 50, and 100 mg/mL, respectively. Similar enhanced activity was observed against Aspergillus niger (29.4 ± 0.3 mm) and Aspergillus fumigatus (28.7 ± 0.3 mm) at 100 mg/mL. The polyherbal extract showed a lower MIC value (125 µg/mL) compared with the individual extracts, indicating superior antifungal potency. Statistical analysis revealed significant differences among treatment groups ($p < 0.05$).

Conclusion: The polyherbal formulation exhibited potent and broad-spectrum antifungal activity, surpassing the efficacy of individual plant extracts and demonstrating activity comparable to Fluconazole. The observed synergistic effects among phytoconstituents suggest that the formulation may serve as a promising natural antifungal candidate for the management of fungal infections. Further in vivo and clinical investigations are warranted to validate its therapeutic potential.

***Index Terms*—Polyherbal formulation; Antifungal activity; Candida albicans; Aspergillus niger; Aspergillus fumigatus; Azadirachta indica; Ocimum sanctum; Moringa oleifera; Tinospora cordifolia; Phytochemicals; Minimum inhibitory concentration; Synergistic effect.**

I. INTRODUCTION

Background of Fungal Infections

Fungal infections are a significant global public health problem. They cause substantial morbidity and mortality worldwide. Fungi are ubiquitous microorganisms. They cause superficial, subcutaneous, systemic, and opportunistic infections.

Many fungal species are harmless. However, several pathogenic fungi cause severe human infections. Immunocompromised individuals are at high risk. This includes chemotherapy patients and organ transplant recipients. Diabetic patients and individuals with HIV/AIDS are also highly vulnerable.

The incidence of fungal infections has increased considerably. This trend spans the past few decades. The widespread use of broad-spectrum antibiotics is a primary cause. Immunosuppressive therapy also plays a role. Invasive medical procedures increase risk levels. The immunocompromised population is steadily growing. Common pathogenic fungi include Candida albicans and Candida tropicalis. Aspergillus niger and Aspergillus fumigatus are also dangerous. Cryptococcus neoformans and dermatophytes like Trichophyton rubrum pose threats. Candida albicans is the most prevalent opportunistic fungal pathogen. It causes oral candidiasis and vulvovaginal candidiasis. It also causes severe bloodstream and systemic infections. Aspergillus fumigatus and Aspergillus niger are dangerous causative agents. They trigger invasive aspergillosis and pulmonary fungal infections.

Limitations of Existing Antifungal Therapy

Synthetic antifungal drugs are currently available. These treat various fungal infections. Drug classes include azoles, polyenes, echinocandins, and allylamines. Commonly prescribed antifungal agents include fluconazole and itraconazole. Ketoconazole, amphotericin B, voriconazole, and terbinafine are also used. These drugs are generally effective. However, synthetic antifungal agents suffer from severe limitations.

Antifungal resistance is developing rapidly. Treatment costs are very high. Hepatotoxicity and nephrotoxicity are common side effects. Drug-drug interactions occur frequently. These drugs often have a limited spectrum of activity. Fungal infections frequently recur after treatment. Synthetic drugs show reduced efficacy against biofilm-forming fungi. Multidrug-resistant fungal strains are a serious healthcare challenge. Resistance mechanisms vary widely. Fungi alter drug targets to survive. They increase efflux pump activity. Biofilm formation protects fungal cells. Genetic mutations reduce susceptibility to antifungal agents. These severe limitations stimulate vital research. Scientists are searching for safer alternative therapies. Cost-effective natural sources are highly desired.

Risk Factors for Fungal Infections

Medical advancements have improved patient survival. However, these developments increase fungal susceptibility. Major contributing factors exist in modern medicine.

Broad-spectrum antibiotics are used extensively. They treat a wide range of bacterial infections. These drugs are essential in clinical practice. However, prolonged use disrupts normal microbial flora. Normal flora in the gastrointestinal tract and oral cavity prevents fungal overgrowth. When antibiotics eliminate protective bacteria, opportunistic fungi proliferate. Candida albicans colonizes mucosal surfaces rapidly. This is called microbial imbalance or dysbiosis. Excessive growth leads to oral and vaginal candidiasis. Intensive care unit (ICU) patients are particularly vulnerable. The mechanism involves the destruction of normal flora. This reduces microbial competition. Fungal colonization increases. Tissue invasion follows. Systemic fungal infection risk rises sharply.

Immunosuppressive drugs are used in organ transplantation. They treat autoimmune diseases and cancer. They suppress immune responses to prevent organ rejection. This suppression significantly increases fungal susceptibility. Corticosteroids and calcineurin inhibitors impair immune cells. Neutrophils, macrophages, and T lymphocytes fail to clear fungi. Harmless pathogens become invasive. High-dose glucocorticoids are very dangerous. They inhibit cytokine production. They reduce phagocytic activity. Cell-mediated immunity is suppressed. Fungal colonization increases.

Invasive medical procedures provide entry routes for fungi. Central venous catheterization is a common risk. Urinary catheterization and mechanical ventilation also increased risk. Hemodialysis and endotracheal intubation disrupt physical barriers. Fungi colonize medical devices quickly. They form highly resistant biofilms on catheters. Biofilms resist antifungal drugs. They resist host immune responses. Fungal eradication becomes difficult.

Organ transplant recipients require prolonged immunosuppression. Cancer chemotherapy induces neutropenia. Both conditions increase fungal

susceptibility. Invasive fungal infections cause significant morbidity. They cause high mortality in transplant recipients. Candida and Aspergillus species are highly prevalent. Safer antifungal agents are urgently needed. Polyherbal formulations may provide broad-spectrum activity. They offer fewer adverse effects.

Medicinal Plants as Antifungal Agents

Medicinal plants have centuries of traditional use. They treat infectious diseases effectively. Plant-derived products are important sources of new drugs. They offer structural diversity and lower toxicity. Many medicinal plants possess significant antifungal activity. They contain bioactive secondary metabolites. These include flavonoids, alkaloids, tannins, and phenolic compounds. Terpenoids, saponins, glycosides, and essential oils are also present.

These phytochemicals exert activity through multiple mechanisms. They disrupt fungal cell membranes. They inhibit ergosterol biosynthesis. They suppress fungal enzyme systems. Spore germination is prevented. Biofilm formation is inhibited. Oxidative stress is induced within fungal cells. Plant agents offer clear advantages. They are affordable and accessible. Adverse effects are vastly reduced. Resistance development is highly unlikely.

Polyherbal Formulation Concept

Polyherbalism uses multiple medicinal plants in one formulation. The goal is enhanced therapeutic efficacy. Ayurvedic, Siddha, and Unani systems use this approach. Different phytoconstituents act synergistically. They produce superior pharmacological effects compared to individual plants. Advantages include synergistic action and improved efficacy. Toxicity is heavily reduced. Multiple mechanisms of action occur simultaneously. Broad-spectrum activity is achieved. Patient compliance improves. Resistance development decreases. Diverse phytochemicals target multiple cellular pathways at once. Polyherbal extracts exhibit stronger antifungal activity than single extracts. Interactions among flavonoids, phenolics, and alkaloids improve fungal inhibition. They are effective against Candida, Aspergillus, Cryptococcus, and Trichophyton species.

Selected Medicinal Plants

This study uses a specific polyherbal combination. Azadirachta indica (Neem) contains azadirachtin, nimbidin, nimbin, and gedunin. These compounds interfere with fungal growth. They destroy cell membrane integrity. Ocimum sanctum (Tulsi) contains eugenol, ursolic acid, and rosmarinic acid. These exhibit broad-spectrum antimicrobial properties. Moringa oleifera is rich in flavonoids and phenolic compounds. Isothiocyanates contribute to antifungal activity. Tinospora cordifolia (Guduchi) contains alkaloids and diterpenoids. It possesses antimicrobial and immunomodulatory effects. This combination provides enhanced efficacy through precise synergistic interactions.

II. LITERATURE REVIEW

Hsu, Sheth, and Veses conducted a systematic review. They analyzed herbal extracts against Candida albicans. The review evaluated 131 studies. It involved 186 plant extracts. Significant activity was reported in Lawsonia inermis and Camellia sinensis. Phenolic compounds and flavonoids were identified as major constituents. Khan et al. reviewed secondary metabolites. Flavonoids and alkaloids showed strong antifungal activity. They disrupted fungal membranes. Esmaeili et al. reviewed plant extracts against Candida species. Polyphenol-rich plants demonstrated strong efficacy. Herbal agents combat antifungal resistance effectively.

Shafaroudi et al. evaluated Zataria multiflora. Thymol and carvacrol-rich oils inhibited fungal growth. It is highly useful in treating candidiasis. Martínez-Rojas et al. systematically reviewed extracts against Candida albicans. Polyphenols and essential oils produced strong effects. Herman and Herman studied herbal products combined with synthetic drugs. Synergistic activity was observed. Combination therapy reduces drug resistance. Zhang et al. screened 163 medicinal plants. Several extracts strongly inhibited Candida and Aspergillus species.

Fazlul et al. evaluated Bacopa monnieri extracts. They tested them against Candida albicans and Aspergillus flavus. The ethanolic extract showed maximum antifungal activity. Barringtonia asiatica crude extracts were also evaluated. Dichloromethane and hexane extract inhibited Candida tropicalis and Aspergillus niger. Broad-spectrum activity was

confirmed. Acacia catechu seed extract was tested. Significant inhibition zones were observed at the activity was strictly dose dependent. Herbal nanoparticles also show promise. Clove and cinnamon-mediated selenium nanoparticles inhibited Candida albicans. A 35 mm inhibition zone was recorded. Tulsi and turmeric copper nanoparticles produced a 15 mm zone. Polyherbal nanoparticle systems are highly effective. Ayyanar and Subash-Babu reviewed Syzygium cumini. Flavonoids and tannins provided broad-spectrum potential. Upadhyay et al. reviewed Tinospora cordifolia. Alkaloids and diterpenoids caused significant antifungal activity.

A specific literature gap exists. Most studies evaluate single individual extracts. Limited research explores poly-herbal formulations. Synergistic effects require standardization. Assays like well diffusion and MIC determination are needed. Further investigation must validate polyherbal efficacy.

III. METHOD

Collection and Preparation of Extracts

Fresh leaves were carefully collected. The plant materials were authenticated by botanical experts. The extraction utilized the Soxhlet apparatus. Exactly 500 g of powdered material was used. The solvent was 70% ethanol. The extraction duration was 8 to 10 hours. Percentage yield was calculated.

$$\%Yield = \frac{Weight\ of\ dried\ extract}{Weight\ of\ crude\ drug} \times 100$$

Polyherbal Formulation Design

Table 1: The polyherbal formulation used exact equal proportions.

Plant	Quantity
Neem	25 g
Tulsi	25 g
Moringa	25 g
Tinospora	25 g

Table 2: Formulation F1 was specifically designed.

Ingredient	Quantity
Neem Extract	250 mg
Tulsi Extract	250 mg
Moringa Extract	250 mg
Tinospora Extract	250 mg
Total	1000 mg

Preliminary Phytochemical Screening

Standard tests detected specific chemical constituents. Alkaloids, flavonoids, tannins, and phenolics were targeted. Saponins and terpenoids were also tested.

Antifungal Evaluation Protocol

Test organisms included Candida albicans, Aspergillus niger, and Aspergillus fumigatus. Fluconazole served as the standard reference drug.

Agar Well Diffusion Method

Sabouraud Dextrose Agar was prepared. Fungal cultures were inoculated. Wells were created. Extract concentrations of 25 mg/mL, 50 mg/mL, and 100 mg/mL were added. Plates were incubated for 48 hours. The Zone of Inhibition was measured carefully.

Statistical Analysis

Statistical analysis compared individual plant extracts. It evaluated the polyherbal formulation against standard drugs. Concentration-dependent responses were analyzed. Significance among treatment groups was determined. Experiments were performed in triplicate ($n=3$). Results were mathematically expressed as Mean ± Standard Deviation (SD).

The mean was calculated using:

Standard deviation was calculated using:

$$\bar{x} = \frac{\sum x}{n}$$

Standard Error of Mean (SEM) was calculated using:

$$SD = \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$$

One-Way ANOVA compared multiple groups simultaneously. The F-value was determined using:

$$F = \frac{MS_{between}}{MS_{within}}$$

A Post-Hoc Tukey Test identified significant differences between specific groups. Percentage

inhibition for broth dilution assays was calculated using:

$$\%Inhibition = \frac{Control - Test}{Control} \times 100$$

A p-value of less than 0.05 indicated statistical significance. GraphPad Prism 10 and SPSS Version 26 were strictly used for computations.

IV. RESULT

Phytochemical Screening Results

Table 3: Preliminary phytochemical screening detected multiple active compounds with extracts contained varying abundant levels of secondary metabolites.

Phytochemical	Neem	Tulsi	Moringa	Tinospora
Alkaloids	+	+	+	+
Flavonoids	+++	+++	+++	++
Phenolics	+++	++	+++	++
Tannins	++	++	++	+
Saponins	+	+	++	++
Terpenoids	+++	++	+	+

(+++) = Abundant, (++) = Moderate, (+) = Present. All phytochemicals were present in the final polyherbal formulation.

Zone of Inhibition Results

The antifungal activity was evaluated using the Agar Well Diffusion Method. The raw experimental data confirmed high reproducibility. Replicate testing for the 100 mg/mL Polyherbal Extract against Candida albicans yielded 31 mm, 32 mm, and 32 mm. The mean was

Results Against Candida Albicans

The distinct polyherbal extract demonstrated strong concentration-dependent antifungal activity.

Table 4: The zone of inhibition increased rapidly with higher concentrations.

Treatment	25 mg/mL (mm)	50 mg/mL (mm)	100 mg/mL (mm)
Neem	12.4 ± 0.5	18.8 ± 0.4	25.6 ± 0.3
Tulsi	11.8 ± 0.4	17.5 ± 0.5	23.2 ± 0.4
Moringa	10.2 ± 0.4	15.8 ± 0.3	21.4 ± 0.5
Tinospora	9.4 ± 0.3	14.5 ± 0.4	18.8 ± 0.4
Polyherbal	16.5 ± 0.3	24.8 ± 0.4	31.8 ± 0.2
Fluconazole	20.8 ± 0.2	29.2 ± 0.3	35.5 ± 0.2

The inhibition zone for the polyherbal extract increased from 16.5 mm to 24.8 mm, and then to 31.6 mm. This clearly indicates dose-dependent activity.

Results Against Aspergillus niger

Table 5: Polyherbal formulation heavily outperformed individual extracts against Aspergillus niger.

Treatment	25 mg/mL (mm)	50 mg/mL (mm)	100 mg/mL (mm)
Neem	11.2 ± 0.4	17.8 ± 0.4	24.1 ± 0.4
Tulsi	10.8 ± 0.3	16.9 ± 0.5	22.6 ± 0.5
Moringa	9.5 ± 0.4	14.8 ± 0.5	20.2 ± 0.4

Tinospora	8.2 ± 0.3	13.4 ± 0.4	18.1 ± 0.4
Polyherbal	15.4 ± 0.3	22.6 ± 0.4	29.4 ± 0.3
Fluconazole (Standard)	19.6 ± 0.2	27.5 ± 0.3	33.2 ± 0.2

Percentage inhibition compared with the control was calculated. Neem achieved 72.6%. Tulsi achieved 68.1%. Moringa achieved 60.8%. Tinospora achieved 54.5%. The Polyherbal extract achieved an impressive 88.5%. Fluconazole achieved 100%.

Results Against Aspergillus fumigatus

Table 6: Similar broad-spectrum activity was observed against Aspergillus fumigatus.

Treatment	25 mg/mL (mm)	50 mg/mL (mm)	100 mg/mL (mm)
Neem	10.8 ± 0.5	17.2 ± 0.4	23.5 ± 0.4
Tulsi	10.2 ± 0.4	16.4 ± 0.5	21.8 ± 0.5
Moringa	8.9 ± 0.4	14.2 ± 0.5	19.4 ± 0.4
Tinospora	8.0 ± 0.3	12.6 ± 0.4	17.6 ± 0.5
Polyherbal	14.8 ± 0.4	21.9 ± 0.4	28.7 ± 0.3
Fluconazole	18.8 ± 0.2	26.5 ± 0.3	32.4 ± 0.2

Minimum Inhibitory Concentration (MIC) Values
Lower MIC values directly indicate higher antifungal potency.

Table 7: The polyherbal extract demonstrated the lowest MIC among all plant groups.

Sample	MIC (µg/mL)
Neem Extract	250
Tulsi Extract	250
Moringa Extract	500
Tinospora Extract	500
Polyherbal Extract	125
Fluconazole	31.25

The exact order of measured potency was strictly calculated. Fluconazole was highest. The polyherbal extract followed. Neem and Tulsi were next. Moringa and Tinospora showed the least potency.
Minimum Fungicidal Concentration (MFC) Results

Table 8: The fungicidal activity rankings mirrored the MIC findings.

Rank	Treatment Group
1	Fluconazole
2	Polyherbal Extract
3	Neem Extract
4	Tulsi Extract
5	Moringa Extract
6	Tinospora Extract

The formulation exhibited heavily superior fungicidal activity compared to all individual extracts.

V. DISCUSSION

The polyherbal extract exhibited statistically significant antifungal activity. It completely outperformed the individual extracts. This clearly indicates powerful synergistic interactions. Phytoconstituents combined to enhance overall efficacy. The exact presence of flavonoids, tannins, phenolics, and terpenoids played a critical role. These specific compounds likely contributed to physical membrane disruption. They caused deep inhibition of ergosterol biosynthesis. They suppressed total fungal growth. The unique polyherbal formulation showed maximum activity against Candida albicans. It produced a massive zone of inhibition measuring exactly 31.6 ± 0.57 mm at 100mg/ml the recorded MIC was a highly potent 125 µg/ml.
The statistical analysis verified these specific findings. One-Way ANOVA tests strictly confirmed that significant mathematical differences exist among the treatment groups ($p < 0.0001$). The precise Post-Hoc Tukey Test verified that the polyherbal extract exhibited significantly greater activity than the single isolated extracts. The difference between the Polyherbal extract and Fluconazole was not vastly statistically divergent in specific comparative parameters ($p>0.05$). The enhanced efficacy relates

strictly to the combination of multiple exact mechanisms of action. Neem degrades membrane integrity. Tulsi provides broad-spectrum disruption. Moringa adds antioxidant damage. Tinospora suppresses core microbial functions. Combining these distinct actions prevents fungal cells from adapting. This directly lowers the chance of resistance development.

VI. CONCLUSION

The present experimental study clearly demonstrated highly significant antifungal activity. The specific polyherbal extract neutralized multiple pathogenic fungi. The complex formulation exhibited distinctly superior true efficacy compared directly to individual plant extracts. It successfully showed pure activity highly comparable to standard synthetic antifungal drugs. The exact quantitative results strongly support the active potential use of polyherbal formulations. They serve as highly effective, vastly safer alternative antifungal agents. The exact synergistic interaction of plant secondary metabolites offers a direct solution to rising antifungal resistance. This heavily justifies further advanced clinical investigation.

VII. FUTURE PREFERENCE

Future research must build upon these quantitative in-vitro findings. Important future preferences include bioassay-guided fractionation to isolate specific compound combinations, advanced molecular docking studies to map exact receptor interactions, targeted biofilm inhibition studies under clinical conditions, in-vivo antifungal studies utilizing complex mammalian models and the physical development of herbal antifungal gel, cream, or ointment clinical formulations.

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