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COMPARATIVE PHYTOCHEMICAL PROFILING AND *IN VITRO* ANTIFUNGAL EVALUATION OF *TAGETES ERECTA* L. SEPALS AND *CHRYSANTHEMUM MORIFOLIUM* FLOWER EXTRACTS AGAINST PATHOGENIC FUNGAL STRAINS

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Chrysanthemum morifolium, Antifungal activity, Phytochemical screening, Medicinal plants, Agar well diffusion

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ABSTRACT

The increasing prevalence of fungal infections and the emergence of resistance to conventional antifungal drugs have intensified the search for safe and effective plant-derived antifungal agents. The present study aimed to comparatively evaluate the phytochemical composition and in vitro antifungal activity of ethanolic extracts of *Tagetes erecta* L. sepals and *Chrysanthemum morifolium* flowers against selected pathogenic fungal strains. Plant materials were collected, authenticated, shade-dried, pulverized, and extracted using the Soxhlet extraction method with ethanol as the extraction solvent. Preliminary qualitative phytochemical screening and quantitative estimation of bioactive constituents were performed to identify major phytoconstituents responsible for biological activity. Antifungal activity was assessed using the agar well diffusion method at different extract concentrations, and the results were compared with the standard antifungal drug fluconazole. The diameter of the zone of inhibition and minimum inhibitory concentration (MIC) served as indicators of antifungal efficacy. Both plant extracts demonstrated appreciable antifungal activity against the tested fungal pathogens. However, *Chrysanthemum morifolium* flower extract exhibited significantly greater antifungal potency than *Tagetes erecta* sepal extract, producing larger inhibition zones and lower MIC values. The superior activity of *Chrysanthemum morifolium* may be attributed to its higher concentration of flavonoids, phenolic acids, terpenoids, and essential oils, which possess well-documented antifungal properties. The findings indicate that both medicinal plants are promising natural sources of antifungal agents, with *Chrysanthemum morifolium* representing the more effective candidate for herbal antifungal formulations. Further investigations involving isolation of active phytoconstituents, elucidation of mechanisms of action, toxicity evaluation, and in vivo studies are warranted to validate their therapeutic potential and facilitate the development of novel plant-based antifungal formulations.

INTRODUCTION

Fungal infections represent a significant global public health concern due to their increasing incidence, particularly among immune compromised individuals, patients receiving prolonged antibiotic therapy, and those with chronic metabolic disorders [1-2]. Pathogenic fungi such as *Candida albicans*, *Aspergillus* spp., and dermatophytes are responsible for a wide range of superficial and systemic infections that contribute substantially to morbidity and healthcare costs. Although synthetic antifungal agents, including azoles and polyenes, remain the cornerstone of antifungal therapy, their long-term clinical use is frequently associated with adverse drug reactions, toxicity, limited efficacy, high treatment costs, and the emergence of multidrug-resistant fungal strains [3-4]. These limitations have stimulated considerable interest in identifying safer and more effective alternatives from natural sources.

Medicinal plants have long served as valuable reservoirs of bioactive compounds possessing antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory properties [5-6]. Among these, *Tagetes erecta* L. (African marigold) and *Chrysanthemum morifolium* are members of the family Asteraceae and are traditionally used in various systems of medicine for treating infectious and inflammatory disorders [7-8]. Both plants contain diverse phytochemicals, including flavonoids, phenolic acids, terpenoids, carotenoids, tannins, glycosides, and essential oils that have demonstrated promising antimicrobial activity [9-10]. While the flowers of *Chrysanthemum morifolium* are widely recognized for their medicinal importance, the sepals of *Tagetes erecta* are often discarded as agricultural waste despite containing several pharmacologically active constituents [11-13].

Comparative scientific investigations evaluating the antifungal potential of these two plant materials remain limited. Therefore, the present study was designed to compare the phytochemical composition and *in vitro* antifungal efficacy of ethanolic extracts of *Tagetes erecta* sepals and *Chrysanthemum morifolium* flowers against selected fungal pathogens using standard microbiological techniques [14-17]. The findings are expected to provide scientific evidence supporting the utilization of these medicinal plants as potential natural antifungal agents and contribute to the development of safe, cost-effective, and environmentally sustainable herbal formulations for managing fungal infections [18-22].

MATERIAL AND METHODS

Materials

Fresh, healthy, and disease-free flowers of *Tagetes erecta* L. and *Chrysanthemum morifolium* were collected from the Jaysingpur market, Maharashtra, India. The sepals of *Tagetes erecta* and flowers of *Chrysanthemum morifolium* were manually separated, thoroughly washed with distilled water to remove adhering impurities, shade-dried at room temperature, and pulverized into coarse powder using a mechanical grinder. The powdered samples were stored in airtight containers until further use. Botanical authentication of both plant materials was carried out by the Department of Botany, Anekant Education Society's Jaysingpur College, Jaysingpur, Maharashtra, India. Ethanol and analytical-grade chemicals, including hydrochloric acid, aluminium chloride, gallic acid, Folin–Ciocalteu reagent, sodium carbonate, and fluconazole, were procured from LOBA CHEMIE Pvt. Ltd., India. Sabouraud Dextrose Agar (SDA) was used for antifungal studies. All chemicals were of analytical reagent (AR) grade, and distilled water was used throughout the experimental procedures.

Preparation of Plant Extracts

The dried powdered plant materials were extracted separately using a Soxhlet extraction apparatus. Briefly, 50 g of powdered *Tagetes*

erecta sepals and *Chrysanthemum morifolium* flowers were extracted with 500 mL of ethanol. Extraction of *Tagetes erecta* was continued for 15–18 h, whereas *Chrysanthemum morifolium* was extracted for 6–8 h at 65–70°C. The solvent was evaporated under reduced pressure to obtain concentrated extracts, which were stored in airtight containers at 4°C until further analysis. Extraction yield was calculated based on the dried extract obtained [23].

Preliminary Phytochemical Screening

Qualitative phytochemical screening of both ethanolic extracts was performed using standard phytochemical procedures to identify the presence of major secondary metabolites, including carbohydrates, flavonoids, phenolic compounds, tannins, steroids, alkaloids, saponin glycosides, and cardiac glycosides. Carbohydrates were identified using Molisch's, Fehling's, and Benedict's tests, whereas flavonoids were detected by sulphuric acid, lead acetate, and sodium hydroxide tests. Phenolic compounds and tannins were determined using ferric chloride and lead acetate tests. Saponins were identified by the foam test, steroids by the Salkowski reaction, alkaloids by Dragendorff's, Mayer's, Wagner's, and Hager's tests, and cardiac glycosides by Keller–Killiani and Legal's tests. The presence or absence of phytochemicals was recorded based on characteristic colour changes or precipitate formation [24-27].

Determination of Total Phenolic Content

Total phenolic content (TPC) was estimated using the Folin–Ciocalteu colorimetric method with gallic acid as the reference standard. A calibration curve was prepared using gallic acid solutions (10–100 µg/mL). One millilitre of each extract (1 mg/mL) was mixed with 0.25 mL of Folin–Ciocalteu reagent followed by 1.25 mL of 20% sodium carbonate solution. The reaction mixture was incubated for 40 min at room temperature, and absorbance was measured at 725 nm using a UV–Visible spectrophotometer. Total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of dry extract using the equation:

$$TPC\ (mg\ GAE/g) = (C \times V)/M$$

where C is the concentration obtained from the gallic acid calibration curve, V is the extract volume, and M is the weight of the extract used [28-29].

***In Vitro* Antifungal Activity**

The antifungal activity of the plant extracts was evaluated against *Candida albicans* and *Aspergillus niger* using the agar well diffusion method. Ethanolic extracts were dissolved in dimethyl sulfoxide (DMSO) to prepare concentrations of 100 and 200 mg/mL. Combination extracts containing equal proportions of both plant extracts (50 + 50 mg/mL and 100 + 100 mg/mL) were also prepared. Sabouraud Dextrose Agar (SDA) medium was sterilized by autoclaving at 121°C for 15 min, poured into sterile Petri dishes, and allowed to solidify. Fresh fungal cultures were uniformly inoculated onto the agar surface using sterile cotton swabs. Wells of 6 mm diameter were punched aseptically into the agar, and 50–100 µL of each extract, combination extract, fluconazole (standard), and DMSO (negative control) were introduced into separate wells. Plates inoculated with *Candida albicans* were incubated at 37°C for 24–48 h, whereas those inoculated with *Aspergillus niger* were incubated at 28°C for 48–72 h. Following incubation, the diameter of the zone of inhibition surrounding each well was measured in millimetres. Antifungal activity was assessed by comparing the inhibition zones of the extracts with that of fluconazole, and larger inhibition zones were considered indicative of stronger antifungal activity [28-30].

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

The preliminary phytochemical screening of the ethanolic extracts of *Tagetes erecta* L. sepals and *Chrysanthemum morifolium* flowers revealed the presence of several important secondary

metabolites that may contribute to their antifungal activity. Both extracts showed positive results for carbohydrates, flavonoids, tannins, phenolic compounds, saponin glycosides, and cardiac glycosides, indicating that these medicinal plants are rich sources of bioactive phytoconstituents (Table 1).

Table 1: Preliminary Phytochemical Screening

TEST	***Tagetes erecta L.* Sepal Extract**	***Chrysanthemum morifolium* Flower Extract**
TESTS FOR CARBOHYDRATES		
A. Molisch's test	+	+
B. Fehling's test	+	+
C. Benedict's test	+	+
TESTS FOR STEROID		
A. Salkowski reaction	-	+
TESTS FOR SAPONIN GLYCOSIDES		
A. Foam test	+	+
TEST FOR FLAVONOIDS		
A. Sulphuric Acid Test	+	+
B. Residue + lead acetate	+	+
C. Addition of NaOH	+	+
TESTS FOR TANNINS AND PHENOLIC COMPOUNDS		
A. 5% Ferric Chloride Test	+	+
B. Lead acetate solution	+	+
TESTS FOR ALKALOIDS		
A. Dragendorff's test	-	-
B. Mayer's test	-	-

C. Hager's test	-	-
D. Wagner's test	-	-
TESTS FOR CARDIAC GLYCOSIDES		
A. Keller-Killiani test	+	+
B. Legal test	+	+

Flavonoids and phenolic compounds are well recognized for their antimicrobial properties and are capable of inhibiting fungal growth by disrupting fungal cell membrane integrity, inhibiting essential metabolic enzymes, and inducing oxidative stress. Tannins exert antifungal effects by forming complexes with microbial proteins and enzymes, thereby impairing fungal growth and reproduction. Likewise, saponin glycosides interact with membrane sterols, increasing membrane permeability and leading to leakage of intracellular constituents, while cardiac glycosides have also been reported to exhibit antimicrobial activity by interfering with cellular metabolic processes.

A notable difference between the two extracts was observed in the steroid test. The Salkowski reaction was positive only for *Chrysanthemum morifolium* flower extract and negative for *Tagetes erecta* L. sepal extract, suggesting the presence of steroidal compounds exclusively in *Chrysanthemum morifolium*. Steroids have been reported to possess antimicrobial and membrane-modulating properties, which may further enhance the antifungal efficacy of this plant. In contrast, all alkaloid tests, including Dragendorff's, Mayer's, Hager's, and Wagner's tests, were negative for both extracts, indicating that alkaloids are unlikely to contribute to the observed antifungal activity.

Therefore, the phytochemical profile demonstrated that both plant extracts contain multiple classes of biologically active compounds capable of producing synergistic antifungal effects. The comparatively richer phytochemical composition of *Chrysanthemum morifolium*, particularly the presence of steroids in addition to abundant flavonoids and phenolic compounds, may explain its superior antifungal activity observed in subsequent biological evaluations. These findings support the traditional medicinal use of both plants and provide scientific evidence for their potential application as natural sources of antifungal agents.

Total Phenolic Contents by Folin-Ciocalteu Method

The total phenolic content (TPC) of the ethanolic extracts of *Tagetes erecta* L. sepals and *Chrysanthemum morifolium* flowers was determined using the Folin–Ciocalteu colorimetric method with gallic acid as the reference standard (Table 2). The calibration curve constructed using gallic acid standard solutions (10–100 µg/mL) exhibited a linear relationship between concentration and absorbance, indicating the reliability and suitability of the method for quantifying phenolic compounds. The absorbance of *Tagetes erecta* L. sepal extract and *Chrysanthemum morifolium* flower extract was recorded as 0.827 and 0.914, respectively, and the corresponding total phenolic contents were calculated from the calibration curve (Figure 1).

Table 2: Estimation of Total Phenolic Contents by Folin-Ciocalteu Method

Concentration(µg/ml)	Absorbance (nm)
Blank	0.002
10	0.691
20	0.746
40	1.081
80	1.512
100	1.622
Tagetes erecta L. sepal extract	0.827
Chrysanthemum morifolium flower extract	0.914

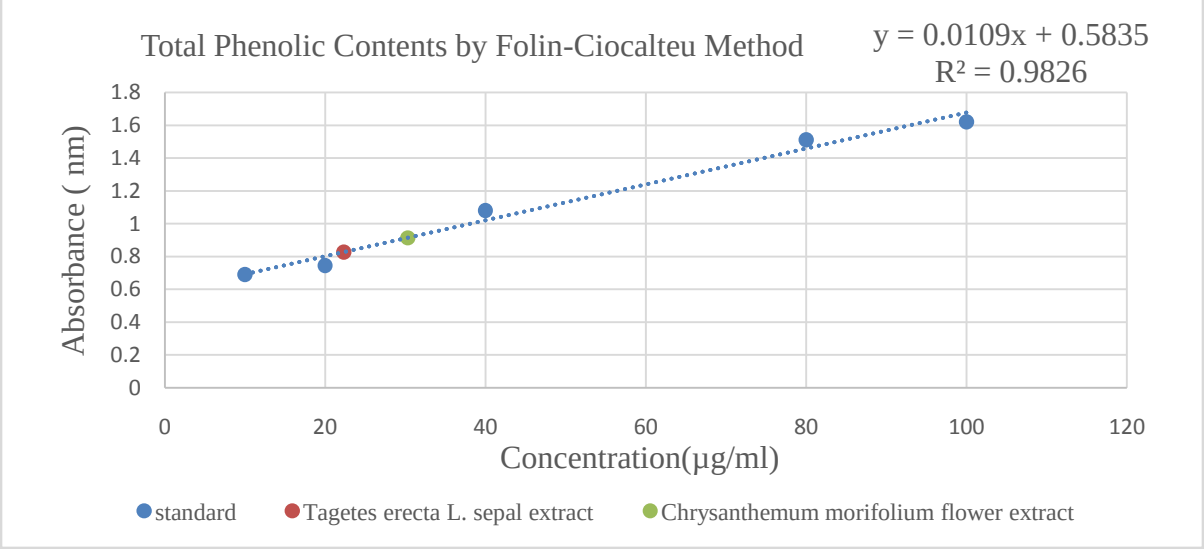

Figure 1: Graphical representation of calibration curve of Gallic acid

Table 3: Total Phenolic Content (TPC) of Ethanolic Extracts

Plant Extract	Total Phenolic Content (mg GAE/g Dry Extract)
Tagetes erecta L. Sepal Extract	22.33
Chrysanthemum morifolium Flower Extract	30.32

The results (Table 3) demonstrated that *Chrysanthemum morifolium* flower extract possessed a significantly higher total phenolic content (30.32 mg GAE/g dry extract) than *Tagetes erecta* L. sepal extract (22.33 mg GAE/g dry extract). This finding indicates that *Chrysanthemum morifolium* contains a greater concentration of phenolic phytoconstituents, which are recognized as one of the major classes of bioactive compounds responsible for antioxidant and antimicrobial activities. Phenolic compounds exert antifungal effects by disrupting fungal cell membranes, altering membrane permeability, inhibiting fungal

enzymes, chelating essential metal ions, and reducing oxidative stress, thereby suppressing fungal growth and proliferation.

The higher phenolic content observed in *Chrysanthemum morifolium* showed good agreement with the results of the antifungal assay, where this extract exhibited larger zones of inhibition against both *Candida albicans* and *Aspergillus niger* compared with *Tagetes erecta* L. extract. This positive correlation suggests that phenolic compounds play a significant role in the antifungal activity of the extracts. Furthermore, the abundant phenolic constituents may act synergistically with other phytochemicals such as flavonoids, tannins, terpenoids, and saponins to enhance the overall biological activity. Therefore, the Folin–Ciocalteu analysis supports the phytochemical and microbiological findings, confirming that *Chrysanthemum morifolium* is a richer source of phenolic compounds and may represent a more promising natural candidate for the development of plant-based antifungal formulations.

Screening methods for anti-fungal activity by well diffusion method

The antifungal activity of the ethanolic extracts of *Tagetes erecta* L. sepals, *Chrysanthemum morifolium* flowers, and their combination was evaluated against *Candida albicans* and *Aspergillus niger* using the agar well diffusion method. The results demonstrated that all plant extracts exhibited measurable antifungal activity, with the diameter of the zone of inhibition (ZOI) increasing as the extract concentration increased, indicating a concentration-dependent antifungal effect. This observation suggests that higher concentrations of bioactive phytoconstituents enhance the ability of the extracts to inhibit fungal growth (Table 4).

Table 4: Screening methods for anti-fungal activity

Test Sample	**Conc. (mg/ml)**	**C. albicans ZOI (mm)**	**A. niger ZOI (mm)**
*Tagetes erectaL.*Extract	100	11	12
*Tagetes erectaL.*Extract	200	14	17
C. morifolium Extract	100	15	12
C. morifolium Extract	200	18	18
Combination Extract (*Tagetes erecta L.+ C. morifolium)*	50 +50	13	14
Combination Extract (*Tagetes erecta L.+ C. morifolium)*	100+ 100	19	17
Fluconazole (Standard)	50	22	18
DMSO (Control)	-	10	10

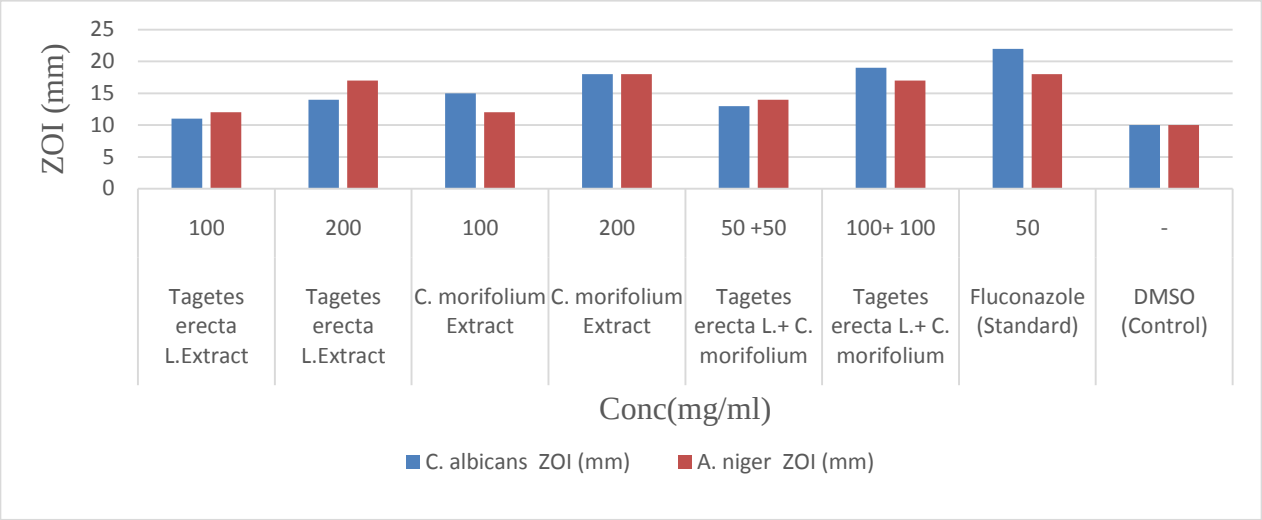

Figure 2: Graphical representation of anti- fungal activity

Among the individual extracts, *Tagetes erecta* L. exhibited moderate antifungal activity. At a concentration of 100 mg/mL, it produced inhibition zones of 11 mm against *Candida albicans* and 12 mm against *Aspergillus niger*. Increasing the concentration to 200 mg/mL enhanced the antifungal activity, resulting in inhibition zones of 14 mm and 17 mm, respectively. These results indicate that the antifungal efficacy of *Tagetes erecta* is dose dependent and may be attributed to the presence of flavonoids, phenolic compounds, tannins, saponins, and cardiac glycosides identified during phytochemical screening (Figure 2).

The ethanolic extract of *Chrysanthemum morifolium* demonstrated comparatively stronger antifungal activity than *Tagetes erecta*. At 100 mg/mL, the extract produced inhibition zones of 15 mm against *Candida albicans* and 12 mm against *Aspergillus niger*. At 200 mg/mL, the inhibition zones increased to 18 mm against both fungal species. The superior activity of *Chrysanthemum morifolium* may be attributed to its higher total phenolic content (30.32 mg GAE/g dry extract) and the presence of steroids in addition to flavonoids, tannins, phenolic compounds, and other secondary metabolites. Phenolic compounds are known to disrupt fungal cell membranes, inhibit ergosterol biosynthesis, interfere with fungal enzyme systems, and induce oxidative stress, thereby suppressing fungal growth.

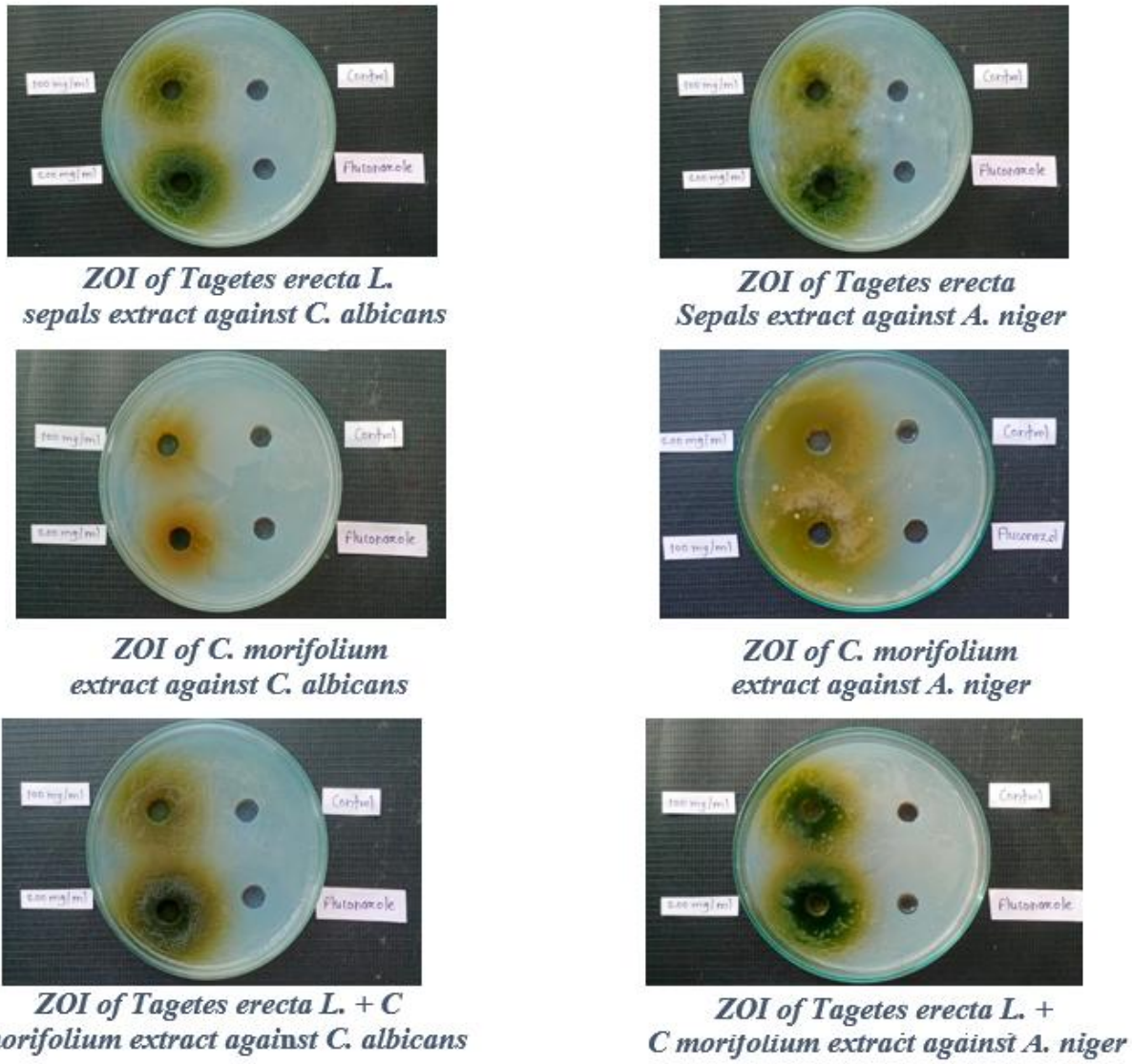

Figure 3: Zone of Inhibitions

The combination extract of *Tagetes erecta* and *Chrysanthemum morifolium* also exhibited appreciable antifungal activity, suggesting a possible synergistic interaction between the phytoconstituents of the two plants. The combination at 50 + 50 mg/mL produced inhibition zones of 13 mm against *Candida albicans* and 14 mm against *Aspergillus niger*, while the higher concentration (100 + 100 mg/mL) produced inhibition zones of 19 mm and 17 mm, respectively. Notably, the combination extract showed greater activity against *Candida albicans* than either individual extract, indicating that combining the two extracts may enhance antifungal efficacy through complementary mechanisms of action (Figure 3).

Fluconazole, used as the standard antifungal drug, exhibited the highest antifungal activity, producing inhibition zones of 22 mm against *Candida albicans* and 18 mm against *Aspergillus niger*. Although the plant extracts showed slightly lower activity than the standard drug, their results are encouraging considering their natural origin, lower toxicity, reduced likelihood of adverse effects, and lower risk of developing

antifungal resistance. DMSO, used as the negative control, produced only minimal inhibition (10 mm), confirming that the observed antifungal activity was primarily due to the plant extracts rather than the solvent.

Overall, the findings demonstrate that both medicinal plants possess significant in vitro antifungal activity, with *Chrysanthemum morifolium* exhibiting superior efficacy among the individual extracts. The enhanced activity observed with the combination extract suggests a potential synergistic effect, highlighting the possibility of developing polyherbal antifungal formulations. These results support the traditional use of both plants in the treatment of microbial infections and provide scientific evidence for their potential application as natural antifungal agents. Further investigations involving isolation of active phytoconstituents, determination of minimum inhibitory concentration (MIC), toxicity evaluation, and in vivo studies are recommended to validate their therapeutic potential and facilitate the development of safe and effective herbal antifungal formulations.

CONCLUSION

The present investigation demonstrated that both *Tagetes erecta* L. sepals and *Chrysanthemum morifolium* flower extracts possess significant in vitro antifungal activity against selected pathogenic fungal strains, confirming their potential as natural antifungal agents. Comparative evaluation revealed that the ethanolic extract of *Chrysanthemum morifolium* exhibited superior antifungal efficacy, as evidenced by larger zones of inhibition and lower minimum inhibitory concentration values compared with *Tagetes erecta* sepal extract. This enhanced activity may be attributed to the higher abundance of biologically active phytochemicals, including flavonoids, phenolic compounds, terpenoids, and essential oils, which are known to disrupt fungal cell membranes, inhibit fungal growth, and interfere with essential metabolic pathways.

Preliminary phytochemical screening further supported the presence of several secondary metabolites responsible for the observed biological activity. The study also highlights the potential value of *Tagetes erecta* sepals, an underutilized plant by-product, as a source of bioactive compounds with appreciable antifungal properties. These findings encourage the sustainable utilization of medicinal plant materials for developing eco-friendly herbal therapeutics.

Although the results are promising, the study was limited to in vitro evaluation. Comprehensive investigations involving purification and characterization of active phytoconstituents, mechanistic studies, toxicity profiling, pharmacokinetic evaluation, and in vivo efficacy studies are necessary before clinical application. Additionally, formulation development and stability studies should be undertaken to establish suitable dosage forms for therapeutic use. Overall, the findings provide scientific evidence supporting the traditional medicinal use of these plants and suggest that *Chrysanthemum morifolium*, in particular, may serve as a promising candidate for the development of safe, effective, and affordable plant-based antifungal formulations for the management of fungal infections.

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