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Comparative Evaluation of Mycostatic Effect of *Tagetes* spp

KEYWORDS: Tagetes erecta, Tagetes patula, Antifungal activity, leaf and flower extracts

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ABSTRACT *Current study has made comparative analysis of the phytochemical profile and mycostatic activity of leaf and flower extracts of Tagetes erecta Linn and Tagetes patula Linn in ethanol. Phytochemical screening has indicated the presence of alkaloids, flavonoids, steroids, tannins and phenolic compounds as the major secondary metabolites in extracts of both species. Bioassay for mycostatic activity of the extracts was carried out by disc diffusion method. The assay has revealed the inhibitory effect of all the extracts on growth of Candida albicans, Aspergillus niger, Saccharomyces cerevisiae and Aspergillus flavus. T. erecta leaf extract showed highest anti-fungal activity among all the four extracts tested.*

INTRODUCTION

Ayurveda or herbal medicine has been in practice since long time as one of the basic treatments for cure of various diseases in India (Valsaraj, Pushpangadan, Smitt, Adersen and Nyman, 1997). Many indigenous plants have been evaluated and used as a source of effective and potent drugs against various diseases (Srivastava, Lambert and Vietmeyer, 1996). Microbial infections represent a major domain of challenges in health care sector throughout the world. Researchers have great enthusiasm in exploring medicinal plants for biochemical constituents and antimicrobial activities. The potential new therapeutics used as drugs obtained from plants are mostly secondary metabolites. Major groups of secondary metabolites include phenols, tannins, alkaloids, flavonoids, steroids and gums (Ghani, 1990) (Dobelis, 1993).

The bacteriostatic efficacy of crude extracts derived from different parts of *Tagetes patula* has been analyzed and found to be effective against a number of enteropathogenic bacteria (Ramya, Mehna, S., Bhanumathi, Samanta P. and Bhat, S. K, 2012). New compounds inhibiting growth of microorganisms such as benzoin and emetine, isolated from plants are being commercially produced (Cox, 1994). Infectious diseases have become the world leading cause for death of more than 50, 000 people per annum. The cause for this is the resistance being developed by microbial pathogens against the drugs (Mahipal, Mashkoor, Yadavab and Solus, 1992). Therefore, development of new drugs becomes crucial in curing such diseases and hence the search for new drugs remains to be an active domain of biological research.

Tagetes patula L. and *Tagetes erecta L.* (marigold) belong to family Asteraceae. It is very popular as a garden plant and yields a strongly aromatic essential oil (tagetes oil), which is mainly used in perfumes. Occurrence of 18 active compounds, most of them terpenoids has been reported from these plants. These compounds are known to exhibit antioxidant, antimycotic, and analgesic activities (Vasudevan, Kashyap and Sharma, 1997).

Current study is aimed to compare the phytochemical profile and antifungal activity of partially purified ethanolic leaf & flower extracts of *T. patula* and *T. erecta*.

MATERIALS AND METHODS

Preparation of Extract: The leaves and flowers of *T. patula* and *T. erecta* were collected in fresh polythene bags from Bangalore, Karnataka. The leaf and flower samples were initially washed in tap water, then with distilled water to remove soil and other contaminants. They were dried on paper towel at 37^{0}C for 72 hours and powdered separately and kept for future use.

50g of dried flower was extracted with 200 ml of 100% ethanol using forced evaporation method. The solidified extracts were stored in refrigerator for further use.

Biochemical Analysis for Detection of Organic compounds
Test for carbohydrates, proteins, lipids, saponins, glycosides, tannins, alkaloids, organic acids, phenolic compounds and flavonoids of all the extracts were conducted by using standard biochemical protocols [Table 1].

Separation of bioactive components
10µl of leaf and flower extracts of *T patula* and *T erecta* were separated through TLC using Methanol- Chloroform mixture (1:9) as mobile phase._

Test Micro-organisms: The following micro fungi, *Candida albicans, Saccharomyces cerevisiae, Aspergillus niger* and *Aspergillus flavus* were obtained from Department of Microbiology, M. S. Ramaiah Medical College, Bangalore.

The fungi were sub cultured in Potato Dextrose Agar with pH 5.6±0.2 and incubated at 28°C in an aerobic atmosphere.The tubes were observed for the growth of the organisms and stored at 4^{0}C in refrigerator.

Bioassay for Mycostatic Activity of Extracts
Mycostatic activity of the extracts was evaluated by disc diffusion method. The PDA plates were inoculated with each fungal culture (10 days old) by point inoculation. The partially purified ethanolic extracts of the leaf & flower of *T. patula* and *T. erecta* were impregnated on filter paper discs (5mm in diameter) with 100, 50, 25, 12.5 µg ml^{-1} concentrations. Ethanol was used to dissolve the extract and was completely evaporated before application on test-organism-seeded plates. Nystatin (100µg/mL) was used as positive control and sterile distilled water was used as negative control. The fungal plates were incubated at room temperature. The activity was determined after 72h of incubation at 28°C. The diameters of the inhibition zone were measured in mm and tabulated.

RESULTS

Biochemical Analysis for Detection of Organic Compounds
A total of 11 tests were carried out for detection of the phytochemical components present in the plant extracts. Results

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of the experiments showed the presence of alkaloids, carbohydrates, tannins, phenols and flavonoids as the major constituents in the extracts (Table1).

Separation of bioactive components Based on the Standard TLC staining technique, the leaf and flower extracts of *T.erecta* showed the presence of two different phenolic compounds. The leaf extract of *T.erecta* showed the presence of three different ketone compounds while the flower extract of *T.erecta* showed the presence of six different ketone compounds.

The leaf and flower extract of *T. patula* showed the presence of four different phenolic component and three components containing ketone groups.

Bioassay on Antifungal Activity of Extracts
Comparison of all the four extracts at different concentrations against *C albicans, A. niger, A. flavus* and *S. cerevisiae* are illustrated in Figure 2.1, 2.2, 2.3 and 2.4 respectively. The zone of inhibition varied significantly among different combinations of the extracts and type of microorganisms. Maximum inhibition of growth was observed in *C. albicans* followed by *A. flavus, S. cerevisiae* and *A. niger* on exposure to the leaf extract of *T. erecta*. The zone of inhibition is more for *T. erecta* leaf extract followed by *T. patula* flower as compared to other two samples against all the fungi tested.

DISCUSSION:
Screening of the phytochemical profiles of *T. patula* and *T. erecta* leaf and flower extract has indicated the presence of alkaloids, flavonoids, steroids, tannins and phenolic compounds as the major secondary metabolites.

The phytochemical constituents such as alkaloids, flavonoids, steroids, tannins and phenolic compounds have been reported to act as bacterial growth inhibitors. Studies have indicated that phenolic compounds and tannins show significant level of antimicrobial activity (Barnabas and Nagarajan, 1988). Our previous study has confirmed significant antibacterial properties associated with crude ethanol extracts of leaf and flower of *T.erecta* and *T.patula* with *T.erecta* flower extract being more effective at concentration of 150µg/mL (Ramya et al, 2012).

The activities of hydro alcohol extracts of *Cassia fistula* has been reported to exhibit significant antifungal activity against *C albicans, A niger, S aureus* and *A clavatus* at 250µg/mL (Nayan, Bhalodia, Pankaj and Shukla, 2011). Presence of tannins, flavonoids and sterols has been correlated with antifungal activity of some plants (Harish, Ravikumar and Ramkrishana, 2007).

Results of the current study have confirmed significant mycostatic activity of the crude ethanol extracts of leaf and flower of *T. erecta* and *T .patula* at 100µg/mL, which is lesser than that reported for *C. fistula* by earlier research (Nayan *et al*, 2011). Therefore it can be concluded that antimycotic potential of *Tagetes* species is more than *C. fistula*. *T. erecta* leaf extract showed maximum antifungal activity against all the test fungal species. Differential levels of activities exhibited by the extracts may be attributed to the relative concentration of the active phytochemical component(s) in the extracts. More focused investigation for identification, isolation and evaluation of the activity of the active component(s) is required to be done for confirming the lead molecule of future drug.

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TABLES:
Table 1: Preliminary phytochemical analysis results for *T.erecta* **and** *T. patula*

Sl. No	Test	Compound present	T.erecta leaf	T.erecta flower	T.patula leaf	T.patula flower
1	Fehling's test	Reducing sugars	Positive	Positive	Positive	Positive
2	Iodine test	Non reducing polysaccharide	Negative	Negative	Negative	Negative
3	Millon's test	Protein	Negative	Negative	Negative	Negative
4	Salkowski test	Steroids	Positive	Positive	Positive	Positive
5	Flavonoids test	Flavonoids	Positive	Positive	Positive	Positive
6	Mayer's test	Alkaloids	Positive	Positive	Positive	Positive
7	Tannins and phenols test	Tannins and phenolics	Positive	Positive	Positive	Positive
8	Legal test	Cardenoloids	Negative	Negative	Negative	Negative
9	Keller Killiani test	Deoxysugars	Negative	Negative	Negative	Negative
10	Saponin glycosides	Saponin glycosides	Negative	Negative	Negative	Negative
11	Calcium chloride test	Organic acids	Negative	Negative	Negative	Negative

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Fig 2.1: Comparison of Different extracts against *Candida albicans*

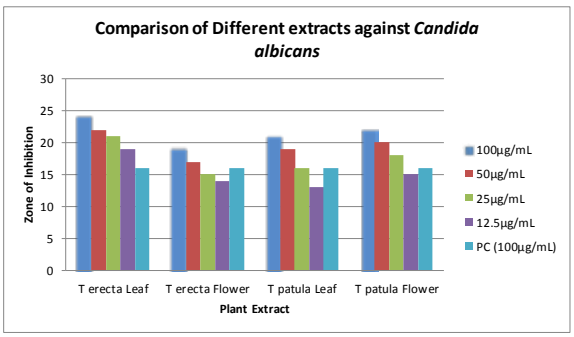


Fig 2.2: Comparison of Different extracts against *Aspergillus niger*

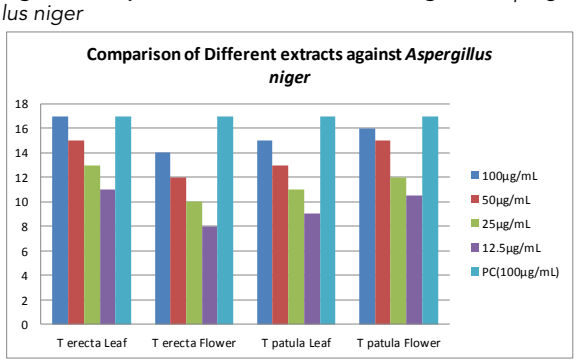


Fig 2.3: Comparison of Different extracts against *Aspergillus flavus*

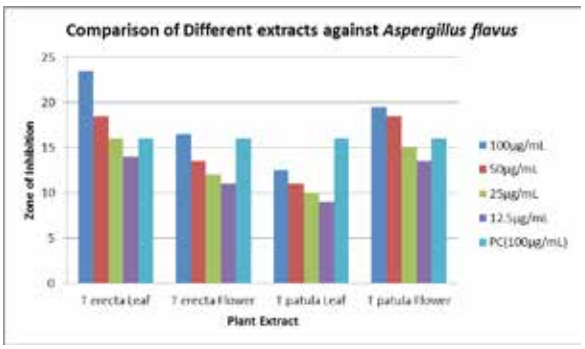
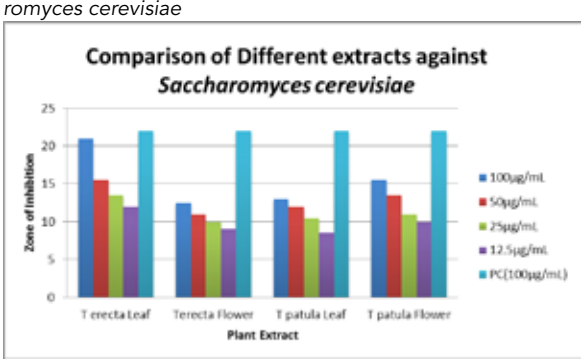


Fig 2.4: Comparison of Different extracts against *Saccharomyces cerevisiae*



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