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Research Article

ANALYSIS OF PHYTOCHEMICAL COMPOSITION AND BACTERIOSTATIC ACTIVITY OF *TAGETES* SP

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ABSTRACT

Present study was carried out to investigate the antimicrobial effect of ethanolic extracts of leaves and flowers of *Tagetes erecta Linn* and *Tagetes patula Linn.* After performing preliminary phytochemical screening and thin layer chromatography, antimicrobial efficacy of the extracts was evaluated through agar well diffusion method using the bacterial species, *viz Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli,* and *Pseudomonas aeruginosa.* The study has demonstrated highest anti-bacterial activity of the flower extract of *T. erecta* among all the four extracts tested.

Keywords: *Tagetes erecta, Tagetes patula,* Antibacterial activity, leaf and flower extracts

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[1]. Many indigenous plants have been evaluated and used as a source of many effective and potent drugs against various diseases[2]. Microbial infections represent an important set of ailments challenging human health throughout the world. Researchers have great keenness in screening of 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 phenolics, tannins, alkaloids, flavonoids, steroids and gums[3, 4].

The bacteriostatic efficacy of crude extracts derived from different parts of *Argemone mexicana* has been analyzed and found to be effective against a number of enteropathogenic bacteria[5]. New compounds inhibiting growth of microorganisms such as benzoin and emetine, isolated from plants are being commercially produced [6]. 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 bacteria against the drugs[7]. 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.[8]

Occurrence of 18 active compounds, most of them terpenoids has been reported form this plants. These compounds are known to exhibit antioxidant, antimycotic, and analgesic activities [9].

The current study is aimed to compare the phytochemical profile and antibacterial 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* were collected in fresh polythene bags from the vicinity of Hooghly district, West Bengal and those of *Tagetes erecta* were collected from Bangalore, Karnataka. The samples were initially washed in tap water, then with distilled water to remove soil and other contaminants. They were dried on paper towel at 37^0C for 72 hours in the laboratory.

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].

Test Micro-organisms

Staphylococcus aureus (ATCC29737), Staphylococcus epidermidis (ATCC12228), Escherichiacoli (ATCC8739) and *Pseudomonas aeruginosa (ATCC9027)* obtained from Department of Microbiology, M. S. Ramaiah Medical College, Bangalore were used for the bioassay experiments. The bacteria were grown on Nutrient agar slants with pH 7±0.2 and incubated at 37^0C for 24 hours in an aerobic atmosphere.The tubes were observed for the growth of the organisms and stored at 4^0C in refrigerator.

Bioassay on Antibacterial Activity of the Extracts

Bioassay for antibacterial activity was carried out using agar well diffusion method. The partially purified ethanolic leaf & flower extracts of *T. patula* and *T. erecta* were dissolved in 1 ml of 0.2 M phosphate buffer. 150 µg concentrations of extract was introduced into the well (3mm depth) using a micro-pipette in Mueller Hinton swabbed agar (MHA) plates. The test organisms (0.5 Macfarland turbidity standards) were spread on the plates. Streptomycin was used as positive control. - Sterile distilled water was used as negative control. The culture plates were then incubated in bacteriological incubator for 18-24 hours at 37^0C. After incubation period, zone of inhibition was measured and tabulated.

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

Sl. No	Test	Compound screened	*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 phenolics 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

Table 2: Comparison of antibacterial effect of *Tagetes* extracts on test organisms

Bacterial strains	*T.erecta* leaf *	*T.erecta* flower *	*T.patula* leaf *	*T.patula* flower *	Positive Control Streptomycin *	Negative Control Distilled water *
E. coli	8.33±1.21	6±2.09	3.5±1.47	11.5± 1.06	11.5± 1.78	0
S. aureus	9.5±1.01	12±2.24	2.2±1.02	15.5±1.06	17±0.87	0
S. epidermidis	8.67±0.5	10±1.24	0	12.5± 1.18	19 ±1.12	0
P. aeruginosa	0	9.5±1.47	0	5.6±1.12	6.9±2.13	0

*Average diameter of zone of inhibition with SD expressed in mm at conc. of 150µg/ml

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 of the experiments showed the presence of alkaloids, carbohydrates, tannins, phenolic compounds and flavonoids as the major constituents in the extracts. (Table 1)

Bioassay for Antibacterial Activity of the Extracts

Results of the antibacterial bioassay are illustrated in Table 2. The zone of inhibition varied significantly depending upon the type of microorganisms. Maximum inhibition of growth was observed in *S. aureus* followed by *S. epidermidis and E. coli* for the flower of *T. patula.* However the extract showed mild inhibitory effect on growth of *P. aeruginosa*. The zone of inhibition is more for *T.erecta* flower extract followed by *T. patula* as compared to other two samples in all the cases against all the bacteria tested. However, the inhibitory effect of the flower extract has reached comparable level to those of standard antibiotics (i.e., Streptomycin) at the concentration of 150µg/ml.

DISCUSSION

Biochemical screening of *T.patula* and *T.erecta* leaf and flower extracts have indicated the presence of alkaloids, flavonoids, steroids, tannins and phenolic compounds as the major secondary metabolites. Many of these compounds have been reported to have various bioactive properties on living organisms including bacteristatic or bactericidal action[10-12]. Earlier investigation on antibacterial activity of *T. erecta* has identified the flavonid patulitrin isolated from the flowers of the plant as the active ingredient[13]. Current study has confirmed the highest antibacterial activity of the flower extract of *T. erecta* when compared to its leaf extract and leaf as well as flower extracts of *T. patula.* Therefore, *T. erecta* flowers can be used as the source for large scale production of the active ingredient.

The microorganisms used for bioassay in the current study are opportunistic pathogens involved in multiple types of human infections and with lot of clinical importance. Treatment against opportunistic pathogens is mainly by administration of antibiotics. Multi drug resistances by a number of pathogens have triggered an urge to find new drugs from natural sources such as medicinal plants. While considering the challenges posed by drug resistant superbugs like MRSA[14], identification and sourcing of new antibacterial compounds like patulitrin appear to be of much practical significance in the current clinical scenario. Further research and developmental work is warranted for exploiting this potential natural source for real time use in healthcare system.

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