ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACT OF TAGETES ERECTUS FLOWERS

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ABSTRACT

The methanolic extract of the flowers of *Tagetes erectus* was screened for antimicrobial activity. Antimicroboal activity was detected by observing the growth response of various microorganisms to the methanolic extract of *Tagetes erectus* which was placed in contact with them against the test organisms. Their microbiological assay is based on the comparison of inhibition of growth of microorganisms by measured concentration of plant extracts to be examined with that produced by known concentration of standard preparation of antibiotic having known activity. Positive antifungal activity was observed with methanolic extract against *Candida albicans*, no bacterial activity was observed.

Key words: Tagetes Erectus, Antibacterial activity, Antifungal activity

1. INTRODUCTION

Tagetes erectus flowers belong to the family Compositae. It is a small shrub, which grows to 1-2 m and it is used widely in our Traditional System of Medicine for curing various diseases like ulcers, laxation and in the treatment of eye diseases. The flowers are used in kidney troubles and in muscular pains and are applied on boils and carbuncles. Infusion of plant is used against rheumatism, cold and bronchitis (The Wealth of India, 2005). In Unani medicine, a confection of tender flowers and purified sugar is prescribed in anuria, retention of urine and kidney troubles. The flowers contain pigments as Quercetagetin and quercetagetrin (Khare CP, 2004). From the literature cited very few works has been carried out in this plant. Thus it was thought worthwhile to explore this plant for its therapeutic activity.

2. MATERIALS AND METHODS

Plant material

The plant was collected from the wild sources of Shirpur forest, Maharashtra, India in the month of May 2008. The plant was identified and authenticated from standard resources.

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Preparation of Extracts:

The shade dried plant material was extracted with methanol by continuous hot extraction using soxhlet apparatus for 12 hours. The extract was filtered and concentrated to remove the solvent and dried on a dessicator. The residue was used for this study (Kokate CK, 2005).

Test Organism and Inoculums:

Gram negative Bacteria- Escherchia Coli and Gram positive Bacteria- Staphylococcus aureus and microorganisms Candida albicans and Crytococcus neoformans were procured from the Department of Microbiology, NMIMS University, Mumbai.

Standard:

Anti Bacterial Amoxycillin disc of the concentration of 30ìg/disc and Antifungal Voriconazole of the concentration of 30ìg/disc were obtained from the Span Diagonistics, Mumbai.

Media:

Dehydrated nutrient agar media was used and was prepared in distilled water. The composition of the media was as given under:

Composition of nutrient agar medium

1. Agar	15.0%
2. Peptic Digest of Animal Tissu	ie 5.0%
3. Sodium Chloride	5.0%
4. Beef Extract	1.5%
5. Yeast Extract	1.5%
6. pH	7.4 ± 0.2 at 25°C
7. Distilled water	1000 ml

The medium was autoclaved at 15 lbs per square inch pressure at 121°C.

Preparation of Media:

Dehydrated nutrient agar medium (28 g) was accurately weighed and suspended in 1000 ml of distilled water in a conical flask. It was heated on a water bath to dissolve the medium completely. Direct heating was avoided as it may lead to charrig of the medium components and render it useless for the purpose (Anonymous, 1996).

Sterilization of Media:

The conical flask containing the nutrient agar medium was plugged with the help of non-absorbent cotton bung. The mouth of the conical flask and the cotton bung were properly covered with aluminum foil. The medium was then sterilized by autoclaving at 15-lbs per square inch pressure for 20 minutes.

Methods of Preparation of Test Organisms:

The test organisms were maintained on slants of medium and transferred to a fresh slant once a week. The slants were incubated at 37°C for 24 hours. Using 3 ml of saline solution, the organisms were washed from the agar slants on to a large agar surface (medium) and incubated for 24 hours at 37°C. The growth from the nutrient surface was washed using 50 ml of distilled water. A dilution factor was determined which gave 25% light transmission at 520nm. The amount of suspension to be added to each 100 ml agar or the nutrient broth was determined by use of test plates or broth. The test organisms were stored under refrigeration.

Temperature Control:

Thermostatic control is required in several stages of a microbial assay when culturing a microorganism and preparing its inoculums and during inoculation in a plate assay.

Experimental Methods:

Cup and Plate Method (Anonymous, 2002). A previously liquefied and sterilized medium was poured in to sterilized plastic Petri-plates of 100 mm size. Ten plates were prepared and were kept for solidify. Five holes were made in each plate with a stainless steel borer having 6 mm in diameter. The methanolic extract of the plant in the concentration of 10 mg/ml were made in 1% Di-methyl Sulfoxide (DMSO). Amoxycillin (Discs-

30μg/disc) and Voriconazole (30μg/disc) were used as standards. Micropipette was used to deliver the solutions in to holes. The volume of solution added to each hole was kept uniform (0.1 ml in each hole). One strip of Amoxycillin and Voriconazole (Standard) was placed aseptically to the centre hole of each plate. The plated were then left for standing for 1 h. for proper diffusion of the drug solutions. They were incubated for about 24 hours at 37°C. After 24 hours the plates were examined and the diameter of zones of inhibition was accurately measured (Anonymous, 1996).

3. RESULTS AND DISCUSSIONS

The results of antimicrobial activity (Table 1 and Table 2) indicated that the methanolic extract of *Tagetes erectus* flowers exhibited activity against *Candida albicans* and *Staphylococcus aureus*. The test concentration exhibited comparable activity with the reference control Amoxicillin and Voriconazole.

Table No.1: Anti-bacterial Activity of Tagetes erectus

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S.No.	Zone of Inhibition (mm)		
	Escherichia	Staphylococcus	
	coli	aureus	
Tagetes Erectus (20	Negative	24	
mg/ml)			
Standard Amoxycillin	26	21	
(30µg/disc)			

Table No.1: Anti-fungal Activity of Tagetes erectus

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S.No.	Zone of Inhibition (mm)		
	Candida	Cryptococcus	
	albicans	neoformans	
Tagetes Erectus (20	22	Negative	
mg/ml)			
Standard Voriconazole	20	18	
(30µg/disc)			

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