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In Vitro Anti Bacterial Potential of Different Extracts of *Tagetes Erecta* and *Tagetes Patula*

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Abstract

The antimicrobial activity of cold aqueous, hot aqueous and methanol extracts of *Tagetes erecta* and *Tagetes patula* flowers was evaluated by agar well diffusion method against ten different pathogenic species of Gram-negative bacteria viz., *Escherichia coli, Proteus vulgaris, Proteus mirabilis, Aeromonas sobria, Aeromonas hydrophila, Plesiomonas shigelloides, Salmonella enterica* serotype Typhi, *Salmonella enterica* serotype Typhimurium, *Salmonella enterica* serotype Aboni and *Salmonella enterica* serotype Enteritidis, four different pathogenic species of Gram-positive bacteria such as *Bacillus subtilis*, *Bacillus cereus*, *Bacillus circulans* and *Staphylococcus aureus*. Methanol extract of *Tagetes erecta* at 40 mg/ml concentration was found to have better inhibitory activity when compared to cold and hot aqueous extracts, which were evident through the increased zones of inhibition against Gram-negative and Gram-positive bacteria. Methanol extract of *Tagetes erecta* showed highest inhibition zone of 26 mm against *Aeromonas sorbia, Aeromonas hydrophila, Staphylococcus aureus* (MTCC7405) and *Staphylococcus aureus* (clinical isolate), while lowest inhibition zone of 12 mm with *Bacillus subtilis*. Similarly, hot aqueous extracts of *Tagetes patula* had better activity as compare to cold aqueous extract and methanol extract at 40mg/ml concentration. It had highest and lowest zone of inhibition with *Proteus vulgaris* OX19 (30 mm) and *Staphylococcus aureus* (clinical iolate) (13 mm) respectively. Minimal inhibitory concentrations (MICs) were between concentrations of 20 - 160 mg/ml with aqueous or methanol extracts of *Tagetes erecta* and *Tagetes patula* flowers for most of the tested bacteria. Results of antimicrobial activity of extracts indicate that they possess potential broad spectrum antibacterial activity.

Keywords: *Tagetes* flower extracts, antibacterial activity

1. Introduction

Medicinal plants represent a rich source from which antimicrobial agents may be obtained. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava *et al*., 1996). Medicinal plants are used by 80% of the world population as the only available medicines especially in developing countries (Hashim *et al*., 2010). Clinical microbiologists have great interest in screening of medicinal plants for antimicrobial activities and phytochemicals as potential new therapeutics. Medicinal plants, *Tagetes erecta* and *Tagetes patula* (Family, Asteraceae) were widely used in olden days for the treatment of wounds. They are common aromatic annual herbs and are popular garden plants. They yield strongly aromatic essential oil (tagetes oil), which is mainly used for the compounding of high-grade perfumes. Different parts of these plants including flower are used in folk medicine to cure various diseases viz., colic's, diarrhea, vomiting, fever, skin diseases and hepatic disorders (Farjana *et al*., 2009; Ivancheva and Zdravkova, 1993). Flowers are especially used in fevers, epileptic fits (Ayurveda), astringent, carminative, stomachic, scabies and liver complaints and are also employed in diseases of the eyes. They are said to purify blood and flower juice is given as a remedy for bleeding piles. They are also used in rheumatism, colds and bronchitis (Ghani, 1998; Kirtikar and Basu, 1987)

Phytochemical studies carried out with different species of *Tagetes* have revealed the presence of flavonoids and terpenes displaying pharmacological and insecticidal properties (Tereschuk *et al.,* 1997; Perich *et al.,* 1995). The essential oil of *Tagetes terniflora* (Tereschuk *et al.,* 2003) and *Tagetes lucida* have reported antibacterial activity against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes* (Caceres *et al.,* 1991), *Escherichia coli*, *Salmonella enteritidis*, *Salmonella typhi*, *Shigella dysenteriae* and *Shigella flexneri* (Caceres *et al.,* 1990). The antibacterial and antifungal activity of the essential oil of *T. minuta* and *T. tilifolia* has also been reported (Zygadlo *et al.,* 1994). Essential oil of *T. patella* is also reported to show activity against *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa* (Rondón *et al*., 2006). The alcoholic extracts of *Tagetes erecta* L. leaves showed promising

antimicrobial activity against *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Salmonella typhi*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Escherichia coli* (Reddy, 2010).

In present study, aqueous and methanol extracts of the flowers of *T. erecta* and *T. patella* were prepared to assess their antibacterial activity against 14 human pathogens.

2. Materials and Method

2.1 Plant material

Fresh flowers of *T. erecta* and *T. patulla* (Figure 1a and 1b) were collected from local village area (Sirol) of Gwalior, during the month of December to January and taxonomically identified by Dr. K. N. Nagaich, Professor of Horticulture, Rajmata Vijaraje Scindia Krishi Vishwa Vidyalaya, Gwalior.

2.2 Bacterial cultures

Standard bacterial cultures of *Escherichia coli* (MTCC1698), *Aeromonas sobria* (MTCC1608) , *Aeromonas hydrophila* (MTCC1739), *Plesiomonas shigelloides* (MTCC1737), *Salmonella enterica* serotype Typhi (MTCC733), *Salmonella enterica* serotype Typhimurium (MTCC 3231), *Salmonella enterica* serotype Enteritidis (MTCC3219), *Salmonella enterica* serotype Aboni, *Bacillus subtilis* (MTCC2756), *Bacillus cereus* (MTCC4079), *Bacillus circulans* (MTCC9720) and *Staphylococcus aureus* (MTCC7405) were provided by Dr. H. V. Batra, Defence Food Research Laboratory, Mysore. Cultures of *Proteus vulgaris* OX19, *Proteus vulgaris* OX2 and *Proteus mirabilis* OXK were obtained from Central Research Institute, Kasuali. and clinical isolates of *Bacillus cereus* and *Staphylococcus aureus* were obtained from Microbiology Division, DRDE, Gwalior. All the cultures were maintained on Nutrient Agar for further use.

2.3 Preparation of extracts

Fresh flowers collected were surface sterilized with 0.1% $HgCl_2$ and washed repeatedly with sterile phosphate buffer saline (pH 7.2) followed by distilled water. Flowers were than dried at 50^{o}C using electric drier and crushed with the aid of a mechanical grinder to powdered form. These powdered flowers were used to prepare different extracts as described below.

2.3.1 Cold aqueous extract

Fifty grams (50g) of the flower powder was weighed out and soaked in 200 ml of distilled water in 500ml conical flask, stoppered with a rubber cork and left for 24 hours. This soaked material was filtered using a sterile Whattman no. 1 filter paper into a sterile conical flask ((Akueshi *et al*., 2002). Filterate was then subjected to water-bath evaporation at 100°C to dryness. The standard extracts obtained were stored in a refrigerator at 4°C until required for use (Okigbo and Omodamiro, 2006).

2.3.2 Hot aqueous extract

Fifty grams (50g) of the dried flower powder was soaked in 200 ml of water and boiled for thirty minutes in a conical flask. Flask was left undisturbed for 24hrs and then contents were filtered using sterile filter paper and evaporated to dryness at 100^{o}C. The standard extracts obtained were stored in a refrigerator at 4°C until required for use (Okigbo and Mmeka, 2008).

2.3.3 Methanol extract

Fifty grams (50g) of dried flower powder was soaked in 400 ml methanol in 1000 ml air tight Schott Duran bottle at room temperature for 24 h with shaking in orbital shaker at 50 rpm. The extract obtained was protected from sunlight by wrapping bottle with black paper. The extract was filtered with Whatman No.1 filter paper. The filtrate was allowed to dry at room temperature until dry methanol extract was obtained (Pavithra *et al*., 2009). Extracts obtained were stored in a refrigerator at 4°C until required for use.

2.4 Agar well diffusion for antibacterial susceptibility testing

The technique used for *in vitro* antibacterial susceptibility test was the agar well diffusion method by Perez *et al*., (1990). From fresh broth culture, 0.2 ml of each of the bacterial isolates were spread uniformly on nutrient agar plate. Afterwards, six wells at adjacent distance of about 30 mm between wells, and towards the periphery of the plates and one at centre were punched with a sterile cork borer of diameter 6mm on the agar plate. Six peripheral wells were loaded aseptically with 0.3 ml of the flower extracts (cold aqueous, hot aqueous and methanol) of *T. erecta* and *T. patula* at concentration 40 mg/ml. Distilled water (0.3ml) was introduced into the 7th central well to serve as control. The plates were allowed to stand on the laboratory bench for about 40 minutes at room temperature in order to allow for pre-diffusion of the extracts in the agar wells (Esimone *et al*., 1998). Four replicates were made. The plates were afterwards incubated at 37^{0}C for 24 hours. After this period, the plates were observed for zones of inhibition around the well. The diameters of observed zones of inhibition were

measured and were recorded in millimeters (mm). The degree of antibacterial activity was evaluated taking mean of the values obtained for the zone of inhibition on each of the replicate agar plates.

2.5 Determination of minimum inhibitory concentrations (MIC)

The determination of the MIC of the crude extracts was carried out using the test tube dilution method (Cruickshank *et al.*, 1975). Each of the extracts was constituted by dissolving 0.4 g of the concentrates in 10 ml of nutrient broth, making the concentration to be 80 mg/ml. Five tubes of 5 ml of nutrient broth were set up, and 5 ml of the 80 mg/ml of the extracts were taken and used for two-fold dilution into the five tubes of the nutrient broth, making the concentration to be 80 mg/ml, 40 mg/ml, 20 mg/ml, 10 mg/ml and 5 mg/ml. Normal saline was used to prepare a turbid suspension of all test bacteria. The dilution of the test bacteria was done continuously in the normal saline until the turbidity marched that of 0.5 Mc-Farlands standard by visual comparison. At that point, microorganism has concentration of about 1.5 x 10^8 cfu/ml. 0.1 ml of this suspension was transferred into the test tubes containing broth at different concentrations of extracts. The tubes were incubated at 37^{o}C for 24 h. The minimum inhibitory concentration was regarded as the lowest concentration that inhibited visible growth.

3. Results and Discussion

3.1 Antimicrobial susceptibility testing

Both the methanol and aqueous extracts of *T. erecta* and *T. patula* flowers showed varying degree of antibacterial activity against the test organisms (Table 1 and 2).

The methanol extract of *T. erecta* was found to be more effective than cold aqueous and hot aqueous extracts against all the test organisms. In methanol extract, all the test organisms gave good susceptibility with zone of inhibition ranging from 18 mm to 26 mm; accept for *Bacillus subtilis* (12 mm). For the cold aqueous extract, *Staphylococcus aureus* (MTCC7405) and *Staphylococcus aureus* (clinical isolate) were most susceptible to the extract (26 mm) and *Bacillus subtilis* showed no visible zone of inhibition. However, hot aqueous extract gave maximum inhibition with *Bacillus cereus* (24 mm) and minimum inhibition with *Plesiomonas shigelloides* and *Bacillus subtilis* (10mm). Phytochemical studies of different parts of *T. erecta* have resulted in the isolation of various chemical constituents such as thiophenes, flavonoids, carotenoids and triterpeniods. The plant *T. erecta* has been shown to contain quercetagetin, a glucoside of quercetagetin, phenolics, syringic acid, methyl-3, 5-dihydroxy-4- methoxy benzoate, quercetin, thienyl and ethyl gallate (Nikkon *et al*., 2009; Ghani, 1998). These classes of compounds are known to have curative activity against several pathogens and therefore could suggest the use of traditionally for the treatment of various illnesses (Usman and Osuji, 2007; Hassan *et al*., 2004). Based on earlier reports, flowers of *T. erecta* are shown to contain significant amount of free flavonoids and flavonoid glycosides (Leigh Hadden *et al*., 1999; Gayle *et al*., 1989). Antibacterial activity observed with *T. erecta* in the present study may also be associated with these compounds. The organic extract provided more powerful antimicrobial activity as compared to aqueous extracts and indicates existence of non-polar residues in the extracts which have higher bactericidal activity. Cowan (1999) mentioned that most of the antibiotic compounds already identified in plants are reportedly aromatic or saturated organic molecules which can easily solubilized in organic solvents.

Hot aqueous extract of *T. patula* was found to be more effective than cold aqueous and methanol extracts against all the test organisms. In hot aqueous extract, *Proteus vulgaris* OX19 (30 mm) was more susceptible and *Staphylococcus aureus* (13 mm) showed the least susceptibility. Also, for the cold aqueous extract *Proteus vulgaris* OX19 gave better zone of inhibition (24 mm) as compare to other organisms. Three organisms viz. *Bacillus subtilis, Bacillus cereus* MTCC4079*, Bacillus cereus* (clinical isolate) gave no visible zone of inhibition with cold aqueous extract. However, methanol extract gave maximum inhibition with *Bacillus circulans* (24 mm) and minimum inhibition with *Salmonella enterica* serotype Enteritidis and *Salmonella enterica* serotype Typhimurium (10mm). In one of the chemical study, conducted on the different parts of *T. patula* (roots, leaves and flowers) were found to contain thiophenes, steroidal and terpenoidal type of constituents (Bano *et al*., 2002). The essential oil extracted by steam distillation from Indian *Tagetes patula*, thirty compounds were identified, representing 89.1% of the total detected. The main components were piperitone, piperitenone, terpinolene, dihydro tagetone, cis-tagetone, limonene, and allo-ocimene. Results of study conducted by Moleyar and Narasimham (1987) suggest that the cell membrane is a very important target of the essential oil components for antibacterial activity. The interaction of the lipophilic compounds with the cytoplasmic membrane also depends on the presence of water-soluble compounds (Knobloch *et al*., 1988). Thus, there may be possibility that presence of aliphatic alcohol in *T. patula* hot extracts are higher as compare to other extracts, such as linalool, and a ketone, such as piperitone, which increases its antibacterial activity. Such observations are demonstrated by Romagnoli *et al*., (2005) while studying antifungal activity of essential oils of *T .patula.* Moreover, hot

extracts contain more essential oil contents as compare to cold extracts. However, it is required to do further investigations with hot extract used in the study to identify to components involve in antibacterial activity.

3.2 Minimum inhibitory concentrations (MIC)

The minimum inhibitory concentration (MIC) of both the methanol and aqueous extracts of *T. erecta* and *T. patula* against the different test organism ranged from 20 mg/ml to 160 mg/ml (Table 3 and 4).

With *T. erecta* extracts, best MIC result was recorded with methanol extract at 20 mg/ml against *E.coli*, *Aeromonas sorbia, Aeromonas hydrophila, Staphylococcus aureus* (MTCC 7405) and *Staphylococcus aureus* (clinical isolate). However, for *T. patulla* better MIC activity was observed with hot aqueous extract as compare to cold aqueous and methanol extracts at 20 mg/ml. At this concentration growth of *Proteus vulgaris OX19, Plesiomonas shigelloides, Bacillus cereus* and *Bacillus circulans* was inhibited.

The minimum inhibitory concentration (MIC) results of both the methanol and aqueous extracts of *T. erecta* and *T. patula* were in accordance with the observations recorded during antimicrobial susceptibility testing.

4. Conclusion

The flower extracts of *T. erecta and T. patula* have demonstrated significant biological activity against the test pathogenic organisms. This has introduced flowers of these plants as a potential candidate for drug development for the treatment of diseases caused by various pathogens. However, it is required to identify various pharmacologically active components present in methanol extract of *T. erecta* and hot aqueous extract of *T. patula*, seen to give promising results.

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a. ***b.***

Figure 1: *Tegetus* plant species used to prepare various flower extracts

a. T. erecta

b. T. patula

Table 1: Antimicrobial activity of various flowers extracts of *Tagetes erecta* and *Tagetes patula* against Gram negative bacteria

Name of Organism	Zone of inhibition, diameter in mm					
	Tagetes erecta Extract			*Tagetes patula* Extract		
	Cold aqueous	Hot aqueous	Methanol	Cold aqueous	Hot aqueous	Methanol
Escherichia coli	16	12	24	18	21	12
Salmonella enterica serotype Enteritidis	22	20	22.5	20	20	10
Salmonella enterica serotype Typhi	20	12	20	20	20	12
Salmonella enterica serotype Aboni	20	20	20	20	20	14
Salmonella enterica serotype Typhimurium	18	12	18.5	19	18.5	10
Proteus vulgaris OX2	12	12	24	10	20	12
Proteus mirabilis OXK	24	14	24	14	20	16
Proteus vulgaris OX19	20	16	22	24	30	20
Aeromonas sorbia	18	12	26	14	16	14
Aeromonas hydrophila	20	14	26	19	22	14
Plesiomonas shigelloides	16	10	18	13	28	14

Table 2: Antimicrobial activity of various flowers extracts *Tagetes erecta* and *Tagetes patula* against Gram Positive bacteria

Name of Organism	Zone of inhibition, diameter in mm					
	Tagetes erecta Extract			*Tagetes patula* Extract		
	Cold aqueous	Hot aqueous	Methanol	Cold aqueous	Hot aqueous	Methanol
Bacillus subtilis	0	10	12	0	22	12
Bacillus cereus (clinical isolate)	24	12	25	0	14	14
Bacillus cereus	12	24	24	0	28	12
Bacillus circulans	12	18	18	12	24	24
Staphylococcus aureus	26	23.5	26	14	14	12
Staphylococcus aureus (clinical isolate)	26	22	26	12	13	11

Table 3: Minimum inhibitory concentration (MIC) of various flower extracts of *Tagetes erecta* and *Tagetes patula* against Gram negative bacteria

Name of Organism	MIC (mg/ml)					
	Tagetes erecta Extract			*Tagetes patula* Extract		
	Cold aqueous	Hot aqueous	Methanol	Cold aqueous	Hot aqueous	Methanol
Escherichia coli	40	80	**20**	40	40	80
Salmonella enterica serotype Enteritidis	40	40	40	40	40	160
Salmonella enterica serotype Typhi	40	80	40	40	40	80
Salmonella enterica serotype Aboni	40	40	40	40	40	40
Salmonella enterica serotype Typhimurium	40	80	40	40	40	160
Proteus vulgaris OX2	80	80	40	120	40	80
Proteus mirabilis OXK	40	80	40	40	40	40
Proteus vulgaris OX19	40	40	40	40	**20**	40
Aeromonas sorbia	40	80	**20**	80	40	40
Aeromonas hydrophila	40	40	**20**	40	40	40
Plesiomonas shigelloides	40	120	40	80	**20**	80

Table 4: Minimum inhibitory concentration (MIC) of various flower extracts of *Tagetes erecta* and *Tagetes patula* against Gram Positive bacteria

Name of Organism	MIC (mg/ml)					
	Tagetes erecta Extract			*Tagetes patula* Extract		
	Cold aqueous	Hot aqueous	Methanol	Cold aqueous	Hot aqueous	Methanol
Bacillus subtilis	160	80	80	No inhibition*	40	80
Bacillus cereus (clinical isolate)	40	80	40	No inhibition*	80	40
Bacillus cereus	80	80	40	No inhibition*	**20**	80
Bacillus circulans	40	40	40	80	**20**	40
Staphylococcus aureus	**20**	**20**	**20**	40	40	80
Staphylococcus aureus (clinical isolate)	**20**	40	**20**	80	40	160

*No inhibition in growth was seen at test concentrations of extracts