

Antibacterial Activity of the Flavonoid, patulitrin isolated from the flowers of *Tagetes erecta* L.

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Abstract: Fresh flowers of *Tagetes erecta* L., the flavonoid was isolated by extraction and fractionation with different solvents. It was then identified as patulitrin (10mg/100ml) by various tests. It was then subjected to disc diffusion assay to assess its antibacterial potentials against *Alcaligenes faecalis*, *Bacillus cereus*, *Campylobacter coli*, *Escherchia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Streptococcus mutans* and *Streptococcus pyogenes*. The flavonoid possesses antibacterial activity against all the tested strains and shows maximum zone of inhibition for *Klebsiella pneumoniae* (29.50 mm). *T.erecta* flavonoid patulitrin is one of the potential elements for therapeutic use.

Keywords : *Tagetes erecta* L., Flowers, Flavonoid patulitrin, Antibacterial activity.

Introduction

Plants have been one of the important sources of medicine, since the beginning of human civilization. In spite of the tremendous development in the field of synthetic drugs and antibiotics during the twentieth century, plants still constitute one of the major sources of drugs in modern as well as traditional medicine throughout the world. Moreover, as antibiotics mostly have clear effects, the chance of finding antimicrobial active traditional medicine is considered high (1).Flavonoids are a large group of compounds naturally occurring in lower and higher plants. Flavonoids have been shown to be able to affect various biological functions: capillary permeability, cellular secretary processes involved in the inflammatory response and inhibition of enzymes, receptors and carriers (2).The inhibitory activities of flavonoids against bacteria and yeast have been

investigated by a number of researchers, especially in Latin America. More than four hundred flavonoids have been identified in plants. Flavonoids are beneficial nutrients for health and a diet for optimal immunity. These nutrients enhance the activity of white blood cells and boost the body's defenses against a broad range of bacterial and viral infections, from urinary tract infections to HIV(3). Studies have found that the flavonoids in these foods protect against heart disease and cancer (4).

Here the flowers of marigold were taken for the present study to isolate the flavonoid and to assess its antibacterial activity against some human pathogens.

Tamil name of marigold is Tulukka samandi, and is very popular as a garden plant and for its flowers, owing to its bright- yellow flowers and

beautifully dissected foliage. The flower heads were employed at religious ceremonies for decorative purposes (5).

Materials and Methods

Plant material:

During the month of February, fresh flowers of *Tagetes erecta* L., were collected from various gardens in Thiruvavur district, Tamilnadu, India. The plant was identified and carefully examined with the help of regional floras(6,7). Specimens were further confirmed with reference to herbarium sheets available in the Rapinat Herbarium, St. Joseph's college, Tiruchirapalli. Voucher specimens were prepared and deposited in the herbarium section of the STET Women's College, Mannargudi, Thiruvavur district, Tamil Nadu, India.

Isolation and identification of flavonoid:

Eight hundred gram of the fresh flowers was extracted with methanol under reflux. The alcoholic extract was concentrated *in vacuo* and the aqueous concentrate was successively fractionated with petroleum ether, peroxide-free diethyl ether and ethyl acetate. No crystalline solid could be recovered from petroleum ether as well as diethyl ether fractions. The ethyl acetate fraction was concentrated *in vacuo* and left in an ice-chest for a day until a yellow solid is separated, which was filtered and studied.

When crystallized from methanol, it came out as yellow crystals. It was freely soluble in aqueous sodium hydroxide, hot water, ethanol and ethyl acetate but insoluble in diethyl ether and chloroform. It gave a greenish-brown colour with alkaline FeCl₃, an intense yellow colour with NaOH, red colour with Mg-HCl

and yellow precipitate with aqueous lead acetate. It appeared as a dark purple spot in UV light which turned yellow on fuming with NH₃. It answered Wilson's boric acid, Gibb's and Molisch's tests but did not respond to Horhammer-Hansel test. It was identified as patulitrin based on R_f values in comparison with an authentic sample isolated from *Tagetes patula* (Table 1).

Test organisms:

The flavonoid thus isolated and identified as patulitrin was tested for its antibacterial activity against *Alcaligenes faecalis* (MTCC-3104), *Bacillus cereus* (MTCC-430), *Campylobacter coli* (MTCC-1126), *Escherichia coli* (MTCC-1650), *Klebsiella pneumoniae* (MTCC-109), *Pseudomonas aeruginosa* (MTCC-667), *Proteus vulgaris* (MTCC-742), *Streptococcus mutans* (MTCC (890) and *Streptococcus pyogenes* (MTCC-1924).

Antibacterial Activity:

Nutrient agar medium was used for antibacterial assay and the inoculum was prepared by inoculating 0.2ml of overnight cultures of each organism into 20ml of sterile nutrient broth and incubated at 37°C for 3-5 h to standardize the culture to produce 10⁶ cfu/ml. Antibacterial activity of the flavonoid (10 mg/100ml) was tested by disc diffusion assay (8). The antibiotic, tetracycline (30 µg/disc) was used as a positive control for all the strains used. The plates were then tested for inhibitory zones (including the diameter of the disc).

Statistical analysis:

Random sampling was done for the entire tests. The four values were calculated for mean and standard deviation by using the formula(9).

Table 1: R_f Values to the Constituents of the flowers of *Tagetes erecta* L.

Compound	Developing solvents*							
	a	b	c	d	e	f	g	h
Flavonoid (From Ethyl acetate fraction)	18	5	15	30	65	60	50	70
Patulitrin (authentic)	18	5	15	30	65	60	50	70

(Whatmann No. 1, Ascending, 30 ± 2°C)

* Solvent Key :

- | | |
|---------------------|--|
| a- H ₂ O | e - 60% aq. HOAc |
| b- 5% Aq. HOAc | f - n-BuOH : HOAc : H ₂ O = 4:1:5 (upper phase) |
| c- 15% Aq. HOAc | g - Water Saturated Phenol |
| d- 30% HOAc | h - HOAc; Conc. HCl : H ₂ O = 30 : 31 |

Table 2: Antibacterial activity of *Tagetes erecta* L. flavonoid patulitrin

Organisms	Zone of inhibition in mm	
	Patulitrin 10 mg / 100 ml	Tetracycline 30 µg / disc
<i>Alcaligenes faecalis</i>	25.00 ± 0.81	21.80 ± 1.92
<i>Bacillus cereus</i>	21.50 ± 1.29	19.80 ± 1.48
<i>Campylobacter coli</i>	26.50 ± 1.29	19.60 ± 4.82
<i>Escherichia coli</i>	26.50 ± 1.29	21.40 ± 4.33
<i>Klebsiella pneumoniae</i>	29.50 ± 0.57	21.60 ± 4.82
<i>Pseudomonas aeruginosa</i>	21.00 ± 0.81	18.80 ± 1.78
<i>Proteus vulgaris</i>	28.75 ± 0.95	20.00 ± 5.24
<i>Streptococcus mutans</i>	26.50 ± 0.57	20.60 ± 3.78
<i>Streptococcus pyogenes</i>	26.50 ± 1.29	21.70 ± 3.96

The values of four determinations are expressed as Mean ± S.D.

Results and Discussion

The flowers of *T. erecta* were found to contain patulitrin with reference to the tests performed. Antibacterial activity of the flavonoid (10 mg/100ml) was higher for all the tested strains than that of the antibiotic tetracycline. Inhibition is maximum for *Klebsiella pneumoniae* (29.50 mm) and minimum for *Pseudomonas aeruginosa* (21.00 mm). Results obtained are presented in Table 2.

The results obtained shows that the flavonoid patulitrin is very much effective in killing the pathogens. Hence, for all human ailments, herbal medicines are available in our surrounding itself. Marigolds are naturalized to many warm climate areas

all over the world. They are also used in perfumery(10). Several recent papers reported that antibacterial activity is due to flavonoids(11). The flavonoids are also toxic to insects, which further modify the alkaloids and incorporate them into their own defense secretion(12). Plants selected for antimicrobial activity have shown appreciable results due to the presence of tannins, flavonoids and sterols(13). In conclusion, the study has showed that the flowers have properties that can inhibit the growth of bacterial pathogens and there should be need for the use of this plant and its derivatives for the purpose of antimicrobial activity.

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