

Pharmacognostic, Antimicrobial and Toxicological Studies of a Seasonal Medicinal Plant; *Tagetes Patula L*

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Authors' Contributions

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ABSTRACT

Purpose of this study: This study has been carried out to explore the medicinal significance of *Tagetes patula* by evaluating its in vitro antibacterial, antifungal and toxicological effects. Pharmacognostic standardization is done to maintain the purity and quality of the drug.

Methodology: Pharmacognostic studies (macro morphology and microscopy i.e. Histology and powder microscopy), solubility and color reaction of *T.patula* were carried out followed by in vitro antimicrobial studies of ethanol extract of the flowers. In this regard antibacterial, antifungal, and toxicological studies were performed by well diffusion, Agar dilution, and larvicidal activity respectively.

Results: Flower extract of *T. patula* showed good antibacterial effects against gram +ve and gram -ve bacteria except *Shigella flexanri*. Moreover it showed powerful antifungal effects against various human (*Aspergillus flavus*, *Candida glabrata*, *Trichophytonlongifusus*), plant (*Fusariumsolani*)and animal pathogens (*Microsporumcanis*). Moreover remarkable larvicidal activity was observed against brine shrimp at 100 and 1000 µg/ml.

Conclusion: This research study makes the valuable medicinal plant a good candidate for skin infections and wound management for topical use. Such studies will help in formulating topical herbal preparations to combat inflammatory and infectious skin disease.

Keywords: *Tagetespatula*, Pharmacognostic standardization, antimicrobial, *Shigella flexanri*, *Trichophyton longifusus*, *Fusarium solani*, brine shrimp.

INTRODUCTION

Plant derived products serves as potential source of modern drugs. In developed countries 13% of medicine consumed are based on herbs However, only fewer plants i.e. 15% were undergone proper investigation. According to report generated by World Health Organizations herbal medicines are easier to access, effective, nontoxic, relatively cheap and have lesser side effects[1]. Natural plant products offered

great treasure of secondary metabolites which have been displayed enormous biological and pharmacological activities [2]

The ornamental plant *Tagetespatula* (Asteraceae) is well known for its reddish yellow, orange colored beautiful flowers [3]. Name of plant is common as French marigold and Genda[4]. *Tagetespatula* is rich in commercially important carotene compounds, helenien, xanthophyll, essential oil ,thiophene, steroids and terpenoids [5, 6].The plant can be used

in the form of extracts, powder, decoction, juices, infusions and oils for the treatment of various diseases. i.e. juice of flowers are used in this remedy given to a patient produces strengthening of connective tissues, enhance healing process and decreases inflammation and act as analgesic. Crushed flowers are used as remedy to purify blood in 15 days. Traditionally flowers heads are used to kill internal worms and proved as anthelmintic. Commercially flower oil can be used as insect repellent and modifier in hair lotions also the oil has been used as food additive and food colorants(4). oil also acts as antiseptic as well as antifungal in candidiasis and other infections whereas roots and seeds are employed as purgative. Aqueous extract of the flower showed antibacterial while methanolic extract possessed anti inflammatory activity [5].

MATERIALS AND METHODS

Plant Material

The fresh flowers of *T.patula* (2 kg) were purchased from local nursery of Karachi, in November 2016 and identified by Prof. Dr. Surraya Khatoon, Department of Botany, Faculty of Science, University of Karachi voucher specimen number (0043) were deposited in herbal museum of Department of Pharmacognosy, University of Karachi.

Preparation of Extracts

Fresh flowers of *Tagetes patula* were first clean and after that ray and disc florets were removed and percolated in ethanol at room temperature for 10- 15 days. After that percolate was filtered by whatman filter paper no 1 and dried in rotary evaporator at 39-40 °C under reduced pressure. This procedure was repeated thrice. Obtained semisolid ethanolic extract was lyophilized on freeze drying apparatus under reduce pressure, to get powder brown extract (24gm).

Histological Evaluation

The transverse section of the florets of *T.patula* were examined through cellular sequences by making permanent slides. Method was adopted by staining and glutening. After that complete histology was studied by means of electronic microscope. [7,8].

Microscopic Examination

Flower were first clean to remove dirt and unwanted debris and then dried under shade thereafter dried powder material evenly ground and analyzed for

powder microscopy. The powdered material treated one by one with glycerin (50%), iodine solution (5%) and chloralhydrate solution (10%) [7,9].

Solubility and Reaction of Powder Drug with Various Reagents

Tagetes patula showed different solubility patterns and changes of colour when mix up in various chemical agents [10].

Antimicrobial Activity

Microorganisms were obtained in this research work from HEJ, research Institute of Chemistry, University of Karachi. a-Gram-Positive Bacteria: *Staphylococcus aureus*, *Bacillus subtilis*. b-Gram-Negative Bacteria: *Escherichia coli*, *Salmonella typhi*, *Shigella flexenari*, *Pseudomonas aeruginosa*. Three types of fungal organism were used in the study i) Plant Pathogens: *Fusarium solani*, ii) Animal Pathogens: *Microsporium canis*, and iii) Human Pathogens: *Aspergillus flavus*, *Candida albicans*, *Trichophyton longifusus*, *Candida glabrata*.

Antibacterial Activity

The antibacterial activity was evaluated by Well diffusion method. Amoxicillin was used as standard drug and as medium, nutrient agar was employed. Inoculation of nutrient plates were done within 24 hrs. Old bacterial cultures around 10^8 - 10^6 CFU/ml were taken. Wells with size of 8 mm were made in the nutrient media. Reference drug along with samples were incorporated into the media with concentration of 100mg/ml. DMSO was used as negative control. The inhibitory zone of Gram+ve and Gram-ve organisms were noted and then compared with standard.[11].

Antifungal Activity

The method used to screen antifungal activity of crude extract of *Tagetes patula* was Agar dilution. Miconazole, and Amphotericin B were used as reference drug. Sabouraud dextrose agar (SDA) subjected to maintain fungal organism growth. The broth was kept in incubator for 24 hrs. at 37 °C. A dilution of 1:100 in distilled water was used in the test. The petri plates prepared with SDA. In the media wells (6mm) were dug for test and standard samples each having concentration (100mg/ml) respectively. While for negative control DMSO was used. The plates were remains in incubator for 24 hrs. at 37°C. [12] Results were noted by measuring zone of inhibition in mm and then compared with standard.

The obtained mean values were calculated as follows:

100-Linear growth in test (mm)/Linear growth in control (mm) x100.

Larvicidal Activity

The toxic manifestations of bioactive compounds depicted some information about high or low level of toxicities by Brine shrimp bioassay method which is a rapid and preliminary method. The brine shrimps eggs were conveniently hatched in the artificial sea water at 25 C in 24 hrs. This was prepared with 3.8 g sea salt in 1000ml of distilled water and thereafter filtered. By this method large number of larvae have been produced. Ethanolic extract in concentration of 10,100 and 1000µg/ml with 5 ml of sea water and ten shrimps in each vial were kept in incubator for 24 hrs. at 27 °C. The numbers of shrimps survived was counted after 24 hrs. In this protocol, the negative control was served as solvent. While the standard drug Ectoposide with LD50=7.465µg/ml was used as positive control. [13].

RESULTS

Cellular profile of crude drug was examined on the basis of microscopic techniques shown in [Figure.1]. The diagnostic characters of *T. patula* flower in various detecting reagent are summarized in [Figure. 2]. The overall solubility pattern of crude disc and ray florets in various polar and non polar solvents are observed in Table 1.

Results of antibacterial and antifungal activity against various pathogens are shown in (Table 2 and 3) respectively. Flower extract of *T. patula* showed good antibacterial effects against gram +ve and gram -ve bacteria except *Shigella flexanri*. Moreover it showed powerful antifungal effects against various human (*Aspergillus flavus*, *Candida glabrata*, *Trichophyton longifusus*), plant (*Fusariumsolani*) and animal pathogens (*Microsporumcanis*). At various concentrations the extract behaves different larvicidal activity which is depicted in Table 4.

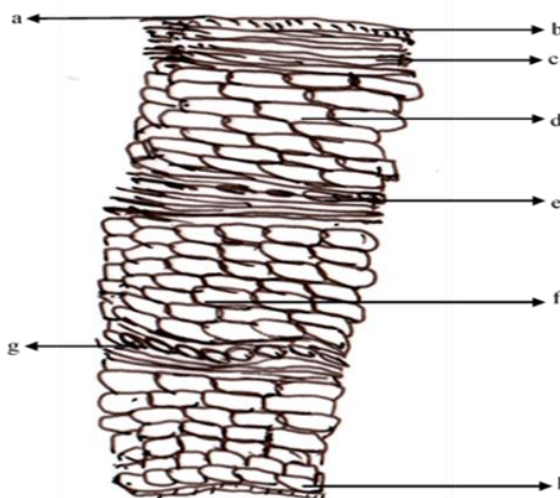


Figure 1. Transverse Section of of *Tagetes patula*.

a) Cuticle b) Upper Epidermis c) Upper zone endodermis with thin layered cells d) Hypodermal cells e) Middle zone of endodermis with lignified cells f) Mesodermal cells g) Lower zone of endodermis h) Lower epidermis.

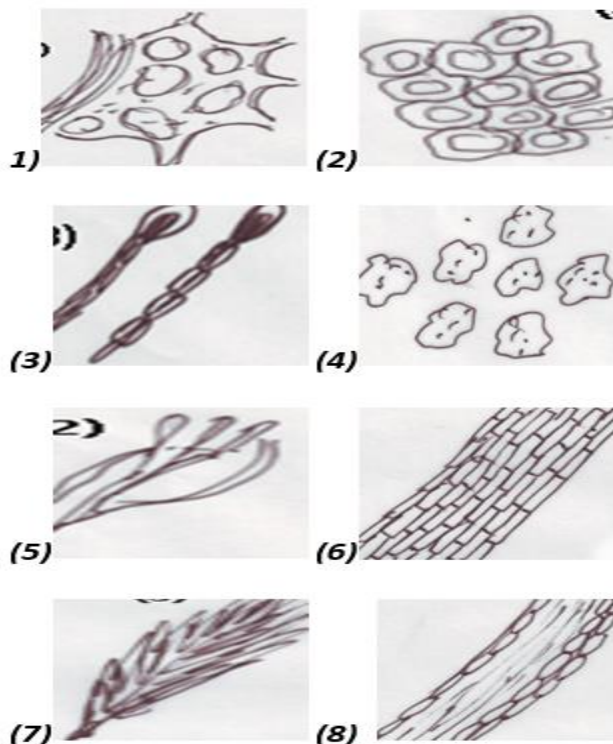


Figure 2. Microscopic examination of *T.patula* florets in chloral hydrate, glycerin and iodine solution.

1) Parenchyma of hypanthium showing an oil gland
 2) Sheet of collenchyma
 3) glandular trichome
 4) Immature pollen grains
 5) Fibers
 6) An overview of rectangular cells of epidermis
 7) More spiny trichome
 8) Segmented epidermis with fibers.

Table 1. Color Reactions and Solubility Changes of *Tagetes Patula*.

Chemicals	Solubility (Ray florets)	Actual Color of Ray florets	Color Change	Solubility (Disc florets)	Actual Color of Disc florets	Color Change
Powder+ H ₂ SO ₄	On heating soluble	Reddish Brown	Black solution	On heating soluble	Reddish Orange	Black solution
Powder+ CH ₃ COOH	On heating Slightly soluble	Reddish Brown	Yellowish Orange	On heating insoluble	Reddish Orange	Yellowish Orange
Powder+ Benzene	On heating insoluble	Reddish Brown	No change of color	On heating insoluble	Reddish Orange	No color change
Powder+ Methanol	On heating Slightly soluble	Slightly soluble	No change of color	on heating soluble	Reddish Orange	No color change
Powder+ water	On heating Slightly soluble	Reddish Brown	Light Brown	On heating Slightly soluble	Reddish Orange	Light Brown

Table 2. Antibacterial Activity of *Tagetes Patula*.

Group of Organisms	Bacterial Cultures	Zone of Inhibition of Sample (mm)	Zone of Inhibition of Amoxicillin (mm)
Gram +ve	<i>Staphylococcus aureus</i>	12	30
	<i>Bacillus subtilus</i>	20	35
Gram -ve	<i>Escheria coli</i>	17	35
	<i>Salmonella typhi</i>	16	30
	<i>Pseudomonas aeruginosa</i>	17	21
	<i>Shigella flexanri</i>	-	40

Table 3. Antifungal Profile of *T. Patula*.

Class of Pathogen	Name of Fungus	Linear Growth		%age of Inhibition	Std Drug
		Sample	Control		
Human	<i>Trichophyton longifusus</i>	30	100	70%	Miconazole
	<i>Candida albicans</i>	70	100	30%	Miconazole
	<i>Aspergillus flavus</i>	40	100	60%	Amphotericin B
	<i>Candida glabrata</i>	40	100	60%	Miconazole
Plant	<i>Fusarium solani</i>	30	100	70%	Miconazole
Animal	<i>Microsporium canis</i>	30	100	70%	Miconazole

Table 4. Screening of Larvicidal Activities (Against Brine Shrimps) of *Tagetespatula*.

Concentration of Solution (µg / ml)	<i>Tagetespatula</i> (crude)		
	No. of survivors	No. of dead	%age of Death
Control	10	0	-
10	7	3	30%
100	1	9	90%
1000	0	10	100%

DISCUSSION

In best of our knowledge powder microscopy and histological studies of *T.patula* florets was first time conducted in this studies. Histological evaluation revealed that epidermis consist of cuticle and single layered barrel shaped cells. On lower side small cystolyth were present. Upper zone consists of thin walled endodermis. While Middle zone composed of lignified cells but various oil cells abundantly found in lower zone. Parenchyma was wavy with numerous pigments. Both upper and lower layer covered with glandular trichomes. Various oil glands seen in transverse section [Figure.1]. In powder florets regular bands of red coloring globules were observed. Glandular trichomes were multicellular, unbranched, uniseriate i.e. globular, ovoid head was present with multicellular stalk. Group of oil glands seen in parenchyma of hypanthium. Hair like fibers were abundantly seen. Spherical, spiny pollen grains were present. Rectangular segmented thin layer epidermis was noticed, air spaces were tiny or absent. [Figure. 2]. In solubility testing both ray and disc florets showed polar characteristics i.e ray florets were soluble in sulphuric acid but slightly soluble in acetic acid, methanol and water while in Benzene they were insoluble .Whereas disc florets were insoluble in acetic acid and Benzene while soluble in water methanol and sulphuric acid. (Table 1) Ethanolic extract showed good activity against gram positive bacteria i.e. *S. aureus* and *B. subtilus* with respective zone of inhibition 12 mm and 20 mm .The flower extract was also effective against 90 % gram negative bacteria (*E. coli*, *S. typhi*, *P. aeruginosa*,) with respective zone of inhibition 17 mm, 16 mm, and 17mm.when compared with standard drug Amoxicillin .It was interesting to note that in our study *T.patula* extract showed same strong inhibition against *E.coli* which is in agreement with previous research study [14] No zone of inhibition was shown in case of *S. flexanri*. Generally gram negative bacteria shown less susceptibility towards plant extracts as compare to gram positive bacteria due to the fact that their outer wall is composed of lipopolysaccharides and lipoprotein which behaves resistant for antibacterial entry (Table 2). [15]. The ethanolic extract has prominent antifungal activity against human pathogen i.e *A. flavus*, *C. glabrata* and especially *T. longifusus*, with 60% and 70% inhibition respectively. Fungal pathogen produces severe problems around world

causing a number of human, plant and animal diseases. Like pathogenic *A. flavus* which is a real cause of aflatoxins can contaminate food commodities. *T. longifusus* (dermatophyte) responsible for hair, nail and scalp. infections [16] profound activity was also shown in case of *F.solani* (70%) and *M. canis* (70%).While least antifungal activity was demonstrated in case of *C.albicans* (Table 3). Similar outcomes have been reported by other studies which further proved the antimicrobial potentials of this plant [2,17,18,19,] .The floral extract exhibited remarkable larvicidal activity, it was observed at concentration of 10 and 100 µg/ml the mortality rate was found to be 30% and 90% respectively and when the dose was increased up to 1000 µg/ml the rate of death was 100% .Therefore ,it is evident that when dose is increased the extracts works effectively. (Table 4) The strong cytotoxic activity (LC50 5.58 µg/ml) was also confirmed in methanolic leaves extract of same plant by Rahul khudus *et al* [2].

CONCLUSION

On the basis of this current study it can be concluded that *Tagetes species* showed good antibacterial and antifungal activities and might be used as a good candidate in healing wounds and other certain kind of skin infectious conditions. Moreover Pharmacognostic standardization has also performed that is necessary for quality control and authentication of the medicinal plant.

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