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A COMPARATIVE INVITRO STUDIES OF PHYTOCHEMICAL ANALYSISAND ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACTS OF TAGETES ERECTA AND TAGETES PATULA.

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ABSTRACT

The focus of the current work is on analysing Tagetes erecta and Tagetes patula for their antimicrobial and phytochemical properties. Humans have employed natural plant products for a variety of uses throughout history. Ayurveda, an Indian system of medicine, primarily employs plant-based treatments or formulations to treat a variety of ailments, including cancer. The market for natural medicines has significant room for growth. Dried flower petals of T.erecta and T. patula were extracted with methanol, macerated for 24 hours at room temperature. The filtered methanolic extracts were evaporated to form thick residue used for Phytochemical screening and evaluating antimicrobial activity seperately .The presence of T.erecta contains Alkaloids, Phenols and Aminoacids. T.patula contains Alkaloids, Phenols and Flavonoids. MHA media was prepared and inoculated with bacterial strains Gram positive bacteria [Bacillus subtilis and Staphylococcus aureus] and Gram negative bacteria [Escherichia coli and Aeromonas sorbia]. For invitro antimicrobial activity by disc diffusion method various concentrations (10 µg/ml, 20 µg/ml, 30 µg/ml, and 40 µg/ml) of methanolic extractions of T erecta and T. patula were prepared in DMSO. The comparative study revealed that of T.erecta exhibited highest zone of inhibition 26 mm for Staphylococcus aureus (gram +ve) and Aeromonas sorbia (gram -ve). flower extracts of T.patula showed less zone of inhibition towards same strains.Results of antimicrobial activities of extracts indicate that T. erecta shows broad spectrum of activity.

INTRODUCTION

Tagetes erecta (African Marigold)

It is an annual or perennial herbaceous plant that grows to a height of 30 to 110 cm. The root is cylindrical, rotating, and has a superficial, fibrous branching structure. The stem is cylindrical, elliptical, and striated, occasionally ridged, smooth or somewhat with villi, herbaceous to slightly woody, with resin channels in the bark that are fragrant when squeezed. Up to 20 cm long, pinnate, with 11 to 17 leaflets, lanceolate to linear-lanceolate, up to 5 cm long and 1.5 cm wide, acute to acuminate, serrated to sub-holders, the lower ones of each leaf frequently setiform (in the form of threads), the superiors are occasionally completely setiform; and with numerous round glands. Opposing leaves at the bottom alternate at the top.[1]

Tagetes patula (French Marigold)

Annual Tagetes patula can grow up to 0.5 metres (1.6 feet) tall and 0.3 metres (1.0 feet) in width. It blooms from July to October in various areas. Blooms are producedin the plant's natural habitat, the highlands of central Mexico, from September until the first deadly frost. Within two weeks following the beginning of bloom, achenes ripen and are shed. The heads are predominantly pollinated by beetles in the wild, as well as by tachinid flies and other insects, and contain mostly hermaphrodite (containing both male and female organs) florets. All marigold species have oil glandson their leaves. The oils smell strongly.[2] Both sandy and clay soils, as long as they have sufficient drainage, are suitable for its growth. It needs direct sunlight to some shade. endures cold upto -1 °C, beyond which it is vulnerable to frost and unable to grow in the shade.[3]

MATERIAL AND METHODS

Methodology

Plant material

The flowers of Tagetes erecta and Tagetes patula were purchased from the local flower market. The flowers were completely cleaned in tap water, with the petals separated and shade dried for 10 days. The Tagetes erecta and Tagetes patula are grind into a fine powder by using the electrical grinder and stored in airtight containers separately, for future use.

Extraction by maceration

1. Transfer 50g of T. erecta and T. patula powder, which have been finelyground, and add 50ml of methanol separately into 2 iodine flasks.

2. macerated for 24 hours at room temperature with occasional shaking.
3. After 24 hours, the above solvents were filtered using Whatman filter paperseparately.
4. Transfer the filtrates to two beakers, and the remaining solvents to two iodine flasks, each with 50 ml of methanol.
5. Repeat the same above procedure and collect the filtrate.
6. Mix both the filtrates and keep them on a water bath at 100 ^{o}C until you get a thick solvent.

Disc Diffusion Method

1. A previously solidified medium appropriate to the assay is inoculated with the required quantity of suspended microorganisms.
2. The suspension is added to the medium at a temperature of 40–45^{o} C, and the inoculated medium is poured immediately into the petridish.
3. Solutions of known concentrations of the standard Doxycycline 10 µg/ml and the test samples of T. Erecta and T.Patula (10 µg/ml, 20 µg/ml, 30 µg/ml, and 40 µg/ml) were prepared in DMSO seperately.
4. Keep the blank discs in plates after cutting them to 6 mm in diameter.
5. Discs are handled with foreceps and dipped into separate extracts of T.erecta and T. patula before in MHA placed on MHA media.
6. Allow it to sit for 10 minutes at room temperature before incubating it for 48 hours at 40^{o}C and measure the diameters of the circular inhibition zones.

RESULTS AND DISCUSSION

Phytochemical Analysis

The study of medicinal plants' chemical constituents and active principles has grown in importance around the world. The current study includes phytochemical screening of the plants. The plants were gathered and examined. They were then shade dried and powdered before going through phytochemical testing[4]. Methanol was used to extract the dried powdered leaves separately. Qualitative chemical tests were performed on the methanolic extracts.

Table 1: A comparative phytochemical analysis of Tagetes erecta and Tagetes patula.

Test	Tagetes erecta	Tagetes patula
Wagner's test	+	+
Mayer's test	+	-
Dragendoff's test	+	+
Liebermann's-Burchard test	-	-
Salkowski's test	-	-
Ferric chloride test	+	+
Lead acetate test	-	+
Alkaline reagent test	-	+
Xanthoproteic test	-	-
Ninhydrin test	+	-

- In the above table 1 we observed that T.erecta contains Alkaloids by (Wagner's test, Mayer's test, Dragendoff's test), Phenols by (Ferric chloride test) and Aminoacids by (Ninhydrin test).
- In the above table 6 we observed that T.patula contains Alkaloids by (Wagner's test, Mayer's test, Dragendoff's test), Phenols by (Ferric chloride test) and Flavonoids by (Alkaline reagent test).

Table 2: A Comparative Antimicrobial Activity of Methanolic Extracts of Tageteserecta and Tagetes patula.

Bacterial species	Zone of inhibition in mm							
	T.erecta				T.patula			
	10µg /ml	20µg/ ml	30µg/ ml	40µg/ ml	10µg/ ml	20µg/ ml	30µg/ ml	40µg/ ml
Gram- positive Bacillussubtilis	7	8.5	10	12	6.5	8	11	12
Staphyloco ccus aureus	19	21	23	26	7	9	10	13
Gram- negative Escherieha coli	15	19	22.5	24	9.2	10	11.5	12
Aeromonas sorbia	16	20	24	26	6.7	8	12	14

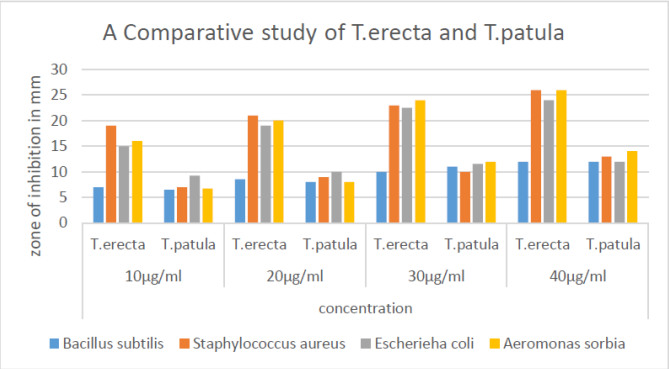

Fig. 1: A comparative study of T.erecta and T.patula.

1. The T.erecta and T. patula of methanolic extract at various concentrations [10,20,30,40g/ml] are prepared and subjected for antimicrobial activity against gram positive (Staphylococcus aureus, Bacillus subtilis) and negative bacteria (E. coli , Aeromonas sorbia) by disc diffusion method.
2. T. erecta has a higher zone of inhibition at conc 40µg/ml than T. patula when itcomes to gram-postive bacteria's(Staphylococcus aureus, Bacillus subtilis).
3. T. erecta has a higher zone of inhibition at conc 40µg/ml than T. patula when itcomes to gram-negative bacteria's (E. coli , Aeromonas sorbia).
4. The zone of inhibition expands along with the concentration.
5. Methanolic extracts of T. erecta exhibit stronger antimicrobial activity than T.patula.
6. T.patula has a lowest zone of inhibition at conc 10µg/ml than T.erecta when itcomes to gram postive bacteria's (Staphylococcus aureus, Bacillus subtilis) .
7. T.patula has a lowest zone of inhibition at conc 10µg/ml than T.erecta when itcomes to gram negative bacteria's(E. coli , Aeromonas sorbia).

CONCLUISON

This project study is mainly based on determining the antimicrobial and phytochemical analysis of *Tagetes erecta* and *Tagetes patula*. For phytochemical analysis the flower extract of both the plant species were taken. Phytochemical compounds taken for estimation were the presence alkaloids, Tannins, Carbohydrates, saponins, Proteins and Amino acids.In the phytochemical comparative analysis of both these species, the presence of alkaloids and phenolic compounds were seen indicating that these two species.*Tagetes erecta* has more antimicrobial property than the *Tagetes patula*. The T.erecta and T. patula of methanolic extract at various concentrations [10,20,30,40g/ml] are prepared and subjected for antimicrobial activity against gram positive (Staphylococcus aureus, Bacillus subtilis) and negative bacteria (E. coli , Aeromonas sorbia) by disc diffusion method. T. erecta has a higher zone of inhibition at conc 40µg/ml than T. patula when it comes to gram-postive bacteria's (Staphylococcus aureus, Bacillus subtilis) and gram-negative bacteria's (E. coli, Aeromonas sorbia).The zone of inhibition expands along with the concentration. Methanolic extracts of T. erecta exhibit stronger antimicrobial activity than T. patula. T.patula has a lowest zone of inhibition at conc 10µg/ml than T.erecta when it comes to gram postive bacteria's (Staphylococcus aureus, Bacillus subtilis) and gram negative bacteria's (E. coli, Aeromonas sorbia).

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