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Invitro Study of Antimicrobialand Antibacterial Activity of Tagetes Erecta

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ABSTRACT:
Tagetes erecta is a widely used decorative herbaceous plant that has been used traditionally for therapeutic purposes in many different nations. The Asteraceae family includes Tagetes erectus. This little shrub can reach a height of 1-2 meters, and our Traditional System of Medicine makes extensive use of it to treat a wide range of illnesses.The purpose of the current study was to look into the antibacterial and antimicrobial properties of this common plant that is found nearby. Using the Agar well diffusion method, the anti-microbial and antibacterial activity of Tagetes erecta leaves extract and aqueous extract was assessed against gram positive and gram negative bacterial strains. The standard antibiotics streptomycin and amikacin were utilized for their antimicrobial and antibacterial properties. The purpose of this study was to determine the minimum inhibitory concentration (MIC) of T. erecta plant by utilizing the broth microdilution method and to assess the antimicrobial and antibacterial activities of an extract from plant sections against four bacteria using the Agar Well Diffusion Method. The outcome suggests that this plant part's bloom exhibited a wide range of antibacterial and antimicrobial properties.

I. INTRODUCTION:

The Tagetes erecta plant, also known as Genduphul, is a member of the Asteraceae family. Another name for these is African Marigold. The primary chemical components of Tagetes erecta include caratenoids, tannin, flavanoids, and lutein, an oxycarotenoid. Additionally, the presence of essential oil, which is extracted from plant leaves and flowers[(5)].High pigment marigold Tagetes erecta has been the subject of multiple studies assessing metabolite production, phytoremediation capacity, and stress tolerance strength. The active ingredient used to cure conditions including rheumatism, bronchitis, ulcers, and eye disorders is found in it 118. Lutein, found in flower extract, has dual uses as a culinary coloring and nutritional supplement[(1)].In tests using Salmonella typhi, Proteus vulgaris, Bacillus subtilis ATCC6633, Enterococcus faecalis ATCC 29212, Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 700603, Pseudomonas aeruginosa ATCC 27853, Candida albicans ATCC 10231, and Aspergillus niger ATCC 16404, the leaves of Tagetes erecta L. demonstrated encouraging antimicrobial activity[(1,3)].

II. MATERIALS AND METHODS:

Tagetes erecta fresh plant leaves were gathered from several localities. Every flower petals was divided and cleaned using running tap water, isopropyl alcohol (5%), and distilled water. After 15 days (about 2 weeks) of shade drying, all petals were homogenized to create a coarse powder. For later extraction, this powder was kept in an airtight container[(2)].

EXTRACTION METHODS:

METHANOL EXTRACTION:

First, 5 g of dried powder have been extracted by maceration in 75 ml of a methanol: water combination at a 4:1 ratio for 24 hours at room temperature and in the dark conditions. In a span of 72 hours, this phase was carried out three times. A rotary evaporator was used to gather, filter, and concentrate the extracted substances following each stage, resulting in an ending weight in dry form of 300 mg at 40 °C. Prior to being tested for microbes, the extracts were resuspended with 1 milliliter of 80% methanol and kept at -86 °C[(1)].

COLD AQUEOUS METHOD:

A 500 ml conical flask was filled with 200 ml of distilled water, 50 grams (50g) of floral powdered substance, sealed with a rubber cork, and let to stand for 24 hours. This soaking mixture was placed in a sterile conical flask and filtrated through a sterile Whattman no. 1 filter paper. The filtrate was then dried by evaporating it in a water

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bath at 100°C. The obtained standard extracts were kept at 4°C in a refrigerator until they were needed[3].

HOT AQUEOUS METHOD:

A conical flask containing 50g of dried flower substance was filled with 200 ml of water and brought to a boil for a period of thirty minutes. After a full day of inactivity, the contents of the flask were filtered through sterile filter paper and dried at 100 degrees Celsius. Until they were needed, the standard extracts were kept in a refrigerator at 4°C[3].

INVITRO METHODS:

Agar well difussion and Broth microdilution methods are used. For this theculture media was prepared by using nutrient agar[1,2]

CULTURE MEDIA:

For antimicrobial and antibacterial activity, nutrient agar medium was used and it was made with distilled water[2].

The media's demographics were as follows:

1. Agar - 20gm
2. Beef Extract - 3gm
3. Peptone - 10gm
4. Glucose - 25gm
5. Distilled water 1000ml

The prepared medium was autoclaved for 20 minutes at a pressure of 15(lbs) pounds per square inch[2].

PREPARATION OF CULTURE MEDIA:

In a conical flask, 1000 ml of distilled water was used to suspend the nutrient agar medium, which had been precisely weighed. In order to totally dissolve the medium, it was warmed up on a water bath. Since direct heating could cause the medium components to burn and become unusable,it was avoided.A non-absorbent cotton plug was used to plug the conical flask housing the nutrient agar medium. Aluminum foil was appropriately placed above the cotton plug and the conical flask's mouth. After that, the medium was autoclaved for 20 minutes at a pressure of 15 pounds per square inch to sterilize it[2].

AGAR WELL DIFUSSION METHOD:

The extracts were evaluated by Agar well diffusion technique against indicator strains. The Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI M100-S24, CLSI M44-A2, CLSI M51-A) were followed in interpreting the data. As standards, amikacin for antimicrobial activity[1] and streptomycin for antibacterial activity[2] were employed, respectively. Mueller Hinton Broth (MHB) was used to cultivate indicator strains until their OD600 value reached 1.0. Next, 100 ml of melted (at 45 °C) MHA was carefully combined with 3.3 ml of the cultures. Mixtures of media microorganisms were added to Petri dishes and let to solidify. Using a cup borer (6 mm), 50 µl of each extract was put to the wells created in the solid media. Each Petri dish was maintained for two hours at +4 °C. Next, colonies of bacteria were incubated about 12 to 15 hours at 37°C. The diameter (mm) of the inhibition zones that surrounded the wells was measured. Two technical replicates were employed, and the experiment was carried out twice. The trials also included a methanol control[1].

BROTH MICRODILUTION METHOD:

The test tube dilution method (broth microdilution method) was used to determine the MIC of the crude extracts. The concentration of each extract was 80 mg/ml, which was achieved by dissolving 0.4 g of the concentrates in 10 ml of nutritional broth. A set of five tubes containing five milliliters each of nutrient broth were prepared. Five milliliters of the extracts containing 80 mg/ml were taken and diluted twice into the five tubes of nutrient broth, resulting in concentrations of 80 mg/ml, 40 mg/ml, 20 mg/ml, 10 mg/ml, and 5 mg/ml. All test microorganisms were prepared as a turbid suspension using regular saline. The test bacteria were continually diluted in regular saline until, in visual comparison, the turbidity equaled that of 0.5 Mc-Farlands standard. The concentration of microorganisms is approximately 1.5 x 108 cfu/ml at that stage. A volume of 0.1 milliliters of this suspension was introduced into test tubes holding broth at varying extract concentrations. For 24 hours, the tubes were incubated at 37 ^{OC}. The concentration at which observable growth was inhibited was considered to be the minimal inhibitory concentration[3].

III. RESULTS AND DISCUSSION:

The antibacterial activity of T. erecta flower extracts, both in methanol and aqueous form, against the test organisms varied in degree (Tables 1).

Streptomycin drug is used as standard for Bacillus circulans and Escherichia coli, where E.coli is a Gram negative bacteria.

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Both Amikacin and Streptomycin drugs are used as standard for Bacillus subtilis and Staphylococcus aureus.

The results that are obtained are compared with the standard drugs Amikacin and Streptomycin. Where It was discovered that T. erecta's methanol extract outperformed both hot and cold aqueous extracts in its ability to kill every test organism. With the exception of Bacillus subtilis (12 mm), all test organisms shown good susceptibility to methanol extract, with a zone of inhibition spanning from 18 to 26 mm. Bacillus subtilis did not exhibit a discernible zone of inhibition in response to the cold aqueous extract, while Staphylococcus aureus (MTCC7405) and Staphylococcus aureus (clinical isolate) exhibited the highest susceptibility to the extract (26 mm). Hot aqueous extract, on the other hand, showed least inhibition with Plesiomonas shigelloides and Bacillus subtilis (10 mm) and maximum inhibition with Bacillus cereus (24 mm). Numerous chemical components, including thiophenes, flavonoids, triterpinoids and carotenoids, have been isolated as a consequence of phytochemical investigations conducted on diverse T. erecta sections. Quercetagetin, a glucoside of quercetagetin, phenolics, syringic acid, methyl-3, 5-dihydroxy-4-methoxy benzoate, quercetin, thienyl and ethyl gallate have all been found to be present in T. erecta. These chemical classes have been traditionally used to treat a variety of ailments because they have been shown to have therapeutic action against a number of infections. Previous investigations indicate that T. erecta flowers have a notable concentration of free flavonoids and flavonoid glycosides. These chemicals might also be linked to the antibacterial action that T. erecta showed in this investigation. In comparison to aqueous extracts, the organic extract exhibited more antibacterial activity, suggesting the presence of non-polar residues in the extracts that possess increased bactericidal activity.

Table 2 shows that the methanol and aqueous extracts of T. erecta had minimum inhibitory concentrations (MIC) ranging from 20 mg/ml to 160 mg/ml against the various test organisms. The T. erecta extract that showed the best MIC against E. Coli and Staphylococcus aureus was the methanol extract at a dose of 20 mg/ml. Both the methanol and aqueous extracts of T. erecta showed minimum inhibitory concentrations (MIC) that matched the findings of the antimicrobial susceptibility test.

TABLE-1 Tagetes erecta flower extract's antimicrobial efficacy against both Gram positive and Gram negative bacteria.

Name of The Organism	Zone of inhibition, diameter in mm				
	Tagetes Erecta Extract			Standard drugs	
	Cold aqueous	Hot aqueous	Methanol	Amikacin (antimicrobial activity)	Streptomycin (antibacterial activity)
Bacillus subtilis	0	10	12	29.5±2.1	24mm
Staphylococcus aureus	26	23.5	26	26.5±2.1	26mm
Bacillus circulans	12	18	18	_	27mm
Escherichia coli (Gram negative)	16	12	24	_	28mm

TABLE-2: Minimum Inhibition Concentration (MIC) for antimicrobial activity of Tagetes erecta flower against both Gram positive and Gram negative bacteria.

Name of the Organism	Zone of inhibition, diameter in mm		
	Tagetes Erecta Extract		
	Cold aqueous	Hot aqueous	Methanol
Bacillus subtilis	60	80	80
Staphylococcus aureus	20	20	20
Bacillus circulans	40	40	40
Escherichia coli (Gram negative)	40	80	20

IV. CONCLUSION:

Remarkable biological activity has been shown by T. erecta flower extracts against the test pathogenic pathogens. This has made Tagetes Erecta flower blossoms a viable option for medication research to treat illnesses brought on by different infections. To achieve the promising findings shown in the methanol extract of T. erecta, it is necessary to identify the numerous pharmacologically active components that are present in the extract.

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