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Antimicrobial Activity of *Tagetus Erectus* against MDR Pathogens

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Abstract: ***The antimicrobial activity of Tagetus erectus leaves against pathogenic bacteria was studied. Plant extract was prepared using Methanol and was suspended in DMSO solution. Comparative study of leaf extract was evaluated by Agar well diffusion method against Staphylococcus aureus, Bacillus cereus, Salmonella typhi and Escherichia coli. The study showed maximum Zone of Inhibition (ZOI) against Gram -positive bacteria rather than Gram - negative bacteria which is due to the difference in the cell wall composition of the bacteria. Thus, the Antimicrobial Activity found in the Methanol extract of the leaves showed better inhibitory effect on the Gram - positive bacteria i. e. S. aureus and B. cereus.***

Keywords: Tagetus erectus, Leaf Extract, Antimicrobial Activity, Agar well diffusion method, MDR bacteria

1. Introduction

Medicinal plants and their constituents are widely in use around the globe. They are becoming increasingly popular due to their natural effectiveness. In the past few decades, there has been an exponential growth in the field of Ayurveda due to their natural therapeutic properties, their origin and minimized synthetic use of chemicals with lesser side effects. More than over a population of 1.1 billion Indians , about 70 % are still in use of non-allopathic system of medicines **(Gopi*et al.*,2012).** Microbial infections represent a set of infirmities combating human health all over the world **(Bhat *et al., 2013).*** There has been a keen approach of the researchers in the screening of medicinal plants for their biochemical constituents and their antimicrobial activities **Padalia and Chanda (2015)**. The therapeutics serve as a major source for secondary metabolites which include phytochemicals like alkaloids, flavonoids, steroids, gums, tannins, etc. **(Bhat *et al.*, 2012) .**

Clinical microbiologist have two major interests in antimicrobial study, which are phytochemical study in the use of making a potential drug and in biopreservation for increasing the shelf life of food products **Cowan (1999).** *Tagetus erectus* or Marigold commonly known as Genda (in Hindi) is an ornamental plant. This herb is found all over the world. The whole plant contains essential oil lunionene, ocimene, linalool, linalyl acetate, tagetone and n-nonyl aldehyde components. It's petals have tagetin and hydroxyfalvones**Gupta and Vasudeva (2012).** The whole herb is used as an epileptic, bronchodilator, stomachic, astringent and carminative. Flowers and leaves are used as a vermifuge and diuretics and helps in treating indigestion, colic, cough and rheumatism.**Verma&Verma (2012).**

The aim of the present study is to extract active constituents of leaves of *Tagetus erectus* to evaluate the antimicrobial activity against bacterial pathogens viz. *S. aureus, B. cereus, E.coli and S. typhi.* The different parts of *Tagetus erectus* are also helpful for their Wound healing activity **(Chatterjee *et al.*, 2011),** Antioxidant activity **(Chivde*et al.*, 2011) [Karwani and Sisodia (2015)]**. Thus, it was thought worthwhile to explore this plant for its antimicrobial activity of plant extract in methanol against four bacterial strains by agar well diffusion method.

2. Materials and Methods

Place of work
The present work entitled "Antimicrobial Activity of *Tagetus erectus* against bacterial pathogens '' was conducted in Centre for Microbiology, Department of Botany, Ewing Christian College, Prayagraj.

Study sample
The study was conducted to evaluate the Antimicrobial activities of Methanolic extract of the leaves of *Tagetuserectus* by Agar well diffusion method using DMSO.

Collection of plant material
Fresh leaves were collected from Botanical Garden of Ewing Christian College and from outside vendors.

Preparation of plant extract
Fresh plant materials were washed under tap water to remove dust and surface impurities. And was kept for drying in hot air oven at the temperature of 72 degree Celsius for 3 days and then dried leaves were homogenised into a fine powder with the help of pestle and mortar. The 50 gm powder of leaf was then prepared into an extract using 400 ml of methanol in a conical flask. The extract was then filtered into a beaker using Whatman paper No. 1. The extract obtained was protected from sunlight by wrapping the flask with a black paper. The filtrate was allowed to dry at room temperature until dry extract was obtained. **(Jain *et***

***al.* 2012).** Extracts obtained were stored in the refrigerator at 4°C until further use.

Microorganisms used

Four MDR bacterial pathogens were used , out of which two were gram-positive bacteria *i.e. Staphylococcus aureus* and *Bacillus cereus* and two were gram- negative bacteria *i.e. Escherichia coli* and *Salmonella typhi*. All bacterial strains used in the practical were maintained on Nutrient Agar medium in Microbiology lab of Ewing Christian College.

Preparation of bacterial suspension

Colonies of different strains of bacteria were aseptically transferred to the individual test tubes containing fresh Nutrient broth and further incubated at 37°C for 24 hours. This prepared inoculum was used to spread onto Nutrient Agar (NA) using sterile cotton swab to make lawn of bacteria on the NA culture media.

Antimicrobial assay using agar well diffusion method

The Antimicrobial Activity was carried out by Agar well diffusion technique . To perform antimicrobial assay, initially a stock culture of different bacteria was revived by inoculating the broth media and incubated at 37° C for 24 hours. The culture media (NA) was prepared and poured into the petriplates and was allowed to solidify. Each plate was then swabbed with bacterial suspension using sterile cotton swab which was evenly spread on the plate under aseptic conditions. Suspension in each plate was allowed to dry for 15-20 minutes. After swabbing, agar wells were made using 5 mm borer in the center of each petriplate. Each well was then loaded with leaf extract and DMSO. All these plates were then incubated for 37°C for 24 hours. The Antimicrobial Activity of each extract was assessed by measuring the diameter of Zone of Inhibition (in mm) around each disc. **(Bhat *et al.*, 2012*)***

3. Result and Discussion

The Antimicrobial Activity of Methanolic extract of *Tagetus erectus* was evaluated against four multidrug resistant pathogenic bacteria namely *Staphylococcus aureus, Bacillus cereus, Salmonella typhi and Escherichia coli*. The study revealed comparatively more inhibition in gram-positive bacteria .*i.e. S. aureus* (24mm) and *B. cereus* (21.6 mm). However, less inhibition was observed in the case of two gram- negative bacteria *i.* e. *S. typhi* (18.33mm) and *E.coli* (17.6 mm). (Table 1 and Figure 1)

Table 1: Antimicrobial Activity of Methanolic Leaf Extract of Tagetus erectus against MDR bacterial pathogens

Leaf Extract	S.aureus	B.Cereus	S.typhi	E.coli
Zone of Inhibition (ZOI)	24 mm	21.6 mm	18.33 mm	17.6mm

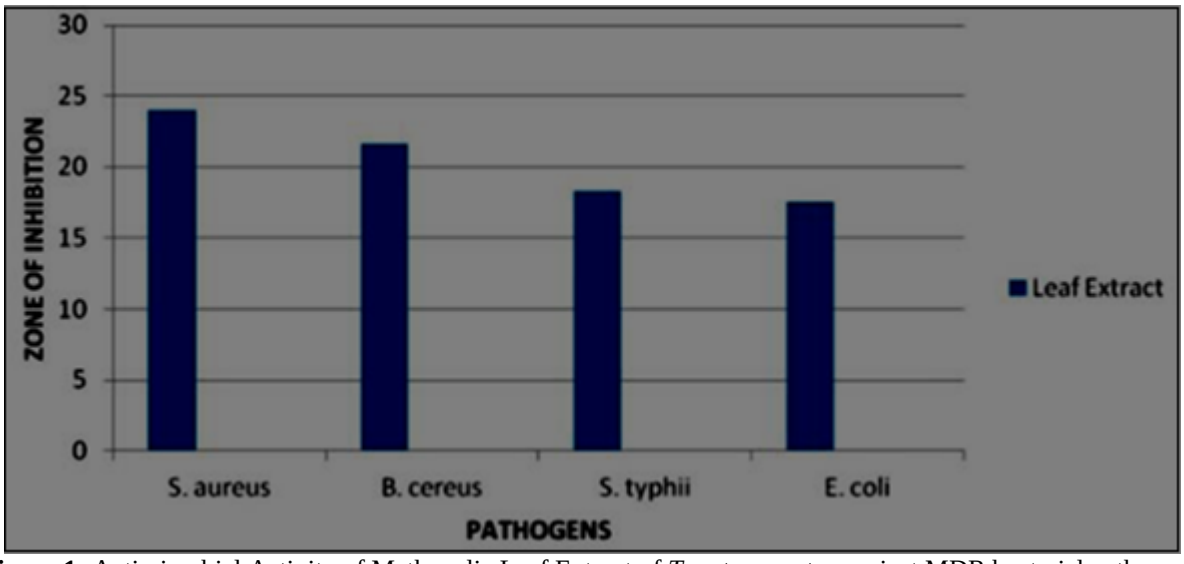

Figure 1: Antimicrobial Activity of Methanolic Leaf Extract of *Tagetus erectus* against MDR bacterial pathogens

The effect of leaf extract was more pronounced in gram +ve bacteria than in gram – ve bacteria. The phytochemical studies of *T. erectus* showed the presence of different chemical compounds such as flavonoids, carotenoids, tannins, binethyl compounds. These compounds suggest curative properties against several bacterial strains. Therefore, the use of *T.erectus* justifies the use in therapeutics against severe diseases**. (Jain *et al.*, 2012).** Antibacterial activity may be therefore associated with these compounds. Maximum inhibition of growth was observed in *S. aureus* followed by *B.cereus* for leaf extract of*T.erectus*. However, the extract showed slightly milder inhibitory effect on growth of *S. Typhi* followed by *E. coli. S. aureus* and *B. cereus* are gram- positive bacteria but inhibition is observed to be comparatively more in *S. aureus* than in *B. cereus. S. aureus* is a non-spore forming bacteria and is less resistant in nature which may be one of the reasons, having maximum inhibition while *B. cereus* is spore forming bacteria, hence, is more resistant ,therefore showed less inhibition. *S. typhi* and *E. coli* are both gram- negative but *S. typhi* are more sensitive as compared to E. *coli* because *S. typhi*does not have glycine containing mucopeptide. The gram-negative bacteria showed minimum inhibition due to the complexity in its cell wall structure. They have permeability barrier made up of phospholipid membrane which shows impermeability to hydrophilic solutes and provides resistance towards the plant extracts. In case of gram- positive bacteria, their cell wall composition is made

up of only a single layer of peptidoglycan which is susceptible and is accessible towards the permeation of plant extracts. Effect on same gram- positive and gram-negative bacteria is different due to difference in strains of same group of bacteria. It was studied earlier that the different part of plant of same plant is having different Antimicrobial Activity due to presence of different phytochemical compounds and their doses. The growth of same bacterial strains have different inhibitory actions and also depends upon their growth phase *i. e*. Bacterial inoculum obtained from lag phase, stationary phase or decline phase which shows less inhibition than bacterial inoculum obtained from exponential growth phase. The culture conditions for the growth of bacteria is equally responsible in influencing the results and causing variation because some bacteria undergoes into resting phase under unfavourable conditions and does not show growth or inhibition. It can be concluded that the inhibition is observed highest in *S. aureus* followed by *B. cereus.* And lowest in *S. typhi* followed by *E. coli*. Antimicrobial Activity was found against gram- positive bacteria showing broad spectrum activity. **Padalia and Chanda (2015)**

4. Conclusion

All the four pathogenic bacterial strains showed Growth Inhibition against the plant extract. *S. aureus* and *B. cereus* were most sensitive bacteria against the leaf extract of *Tagetus erectus* in comparison to gram- negative bacteria.

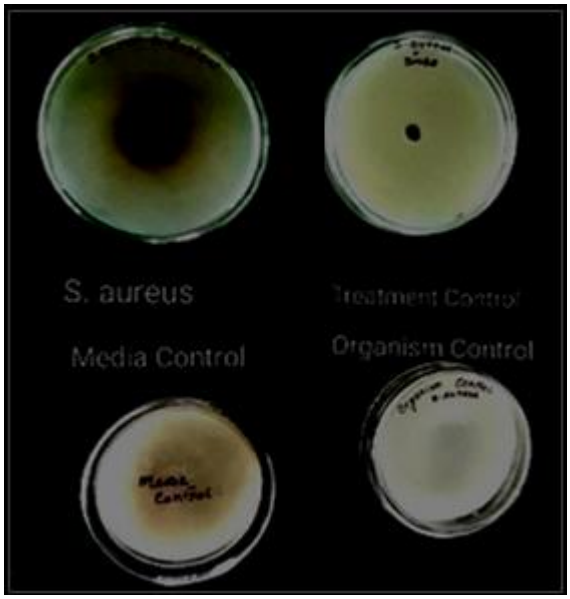

Plate 1: Antimicrobial Activity of Methanolic Leaf Extract of *Tagetus erectus* against *Staphylococcus aureus*

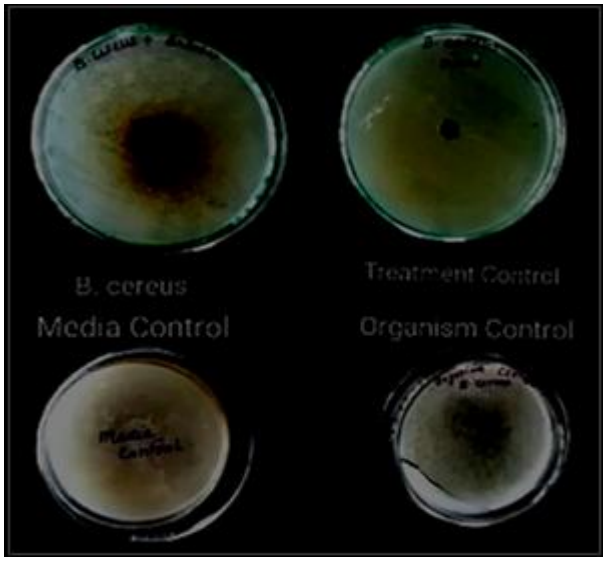

Plate 2: Antimicrobial Activity of Methanolic Leaf Extract of *Tagetus erectus* against *Bacillus cereus*

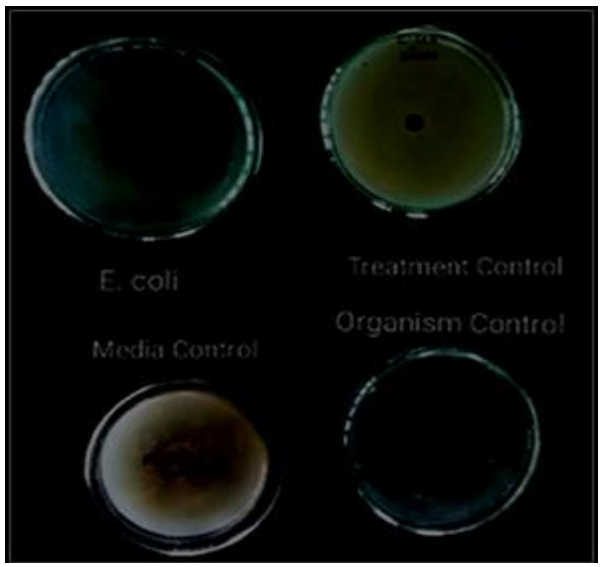

Plate 3: Antimicrobial Activity of Methanolic Leaf Extract of *Tagetuserectus* against *Escherichia coli*

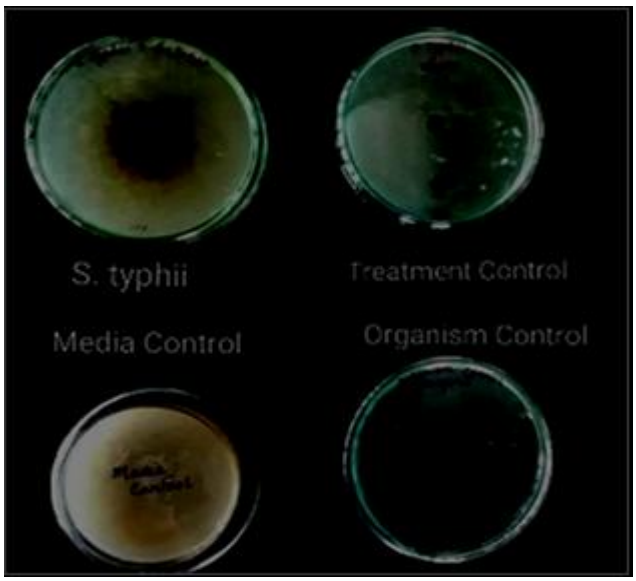

Plate 4: Antimicrobial Activity of Methanolic Leaf Extract of *Tagetus erectus* against *Salmonella typhi*

5. Future Recommendations

The crude extract can be further analysed and its bioactive compounds can be synthesised, purified and furthermore commercialised for its use in combating diseases caused by the multidrug resistant pathogens. For meeting the daunting challenge of drug resistance, the synergistic activity can be analysed which can prove to be valuable in drug development and bio preservation. This plant can also be used in Cosmetology and the presence of Essential oils makes it efficient for Aromatherapy.

6. Acknowledgement

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