

## Antibacterial Activity of Methanolic Extract of *Tagetes Erecta* (Marigold)

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**Abstract** – Infectious diseases have always been one of the important concerns of human and have continuously attracted the attention of a large number of various medical and laboratory professionals. On the other hand, treatment with antibiotics has other problems such as drug resistance and side effects, so the use of new herbal medicines with fewer side effects can be a great help in treating these types of infections. The objective of this study was to investigate the antibacterial activity of marigold (*Tagetes erecta*) methanolic extract on five reference strains including *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus cereus* and *Streptococcus pyogenes*. Then, their antimicrobial effects were investigated using agar well diffusion and tubular dilution methods. The extract from the flowers of *T. erecta* showed a great potential antibacterial activity against both Gram negative and Gram positive bacteria.

**Keywords:** *T. erecta*, Pathogens, Agar Well diffusion assay.

### Introduction

In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects<sup>1</sup>. India possesses almost 8% of the estimated biodiversity of the world with around 0.126% million species<sup>2</sup>. The World Health Organization (WHO) estimate edthat approximately 80% of

world population relies mainly on traditional medicines, mostly plant drugs in their health care. Today, Ayurveda coexists with modern system of medicine, and is still widely used and practiced. About 30% of the currently used therapeutics is of natural origin<sup>3</sup>. In the indigenous health care delivery system, numerous plant species and natural products derived from plants are to treat diseases of infectious origin<sup>4</sup>.

Plants are used medicinally in different countries and are a source of many potent and powerful drugs<sup>5</sup>. Traditionally, plants have been used to treat many diseases, especially infectious diseases, including diarrhea, fever and cold, as well as birth control and oral hygiene through the world<sup>6</sup>.

On the other hand, the emergence of resistant strains among Gram-negative bacilli and Gram-positive cocci such as *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Staphylococcus* and *Enterococcus* has resulted in some problems in the treatment of infections caused by these bacteria<sup>7</sup>. Plant-derived antimicrobials eliminate bacteria with different mechanisms from antibiotics which this is clinically important in the treatment of infections caused by resistant microbial strains<sup>8</sup>.

Many studies have been conducted on extracts prepared from plants that have been collected randomly or in one of the above methods. These studies have further focused on the evaluation of antimicrobial activity, anti-worm activity, anti-viral

activity, cytotoxic and mutagenic activities as well as general pharmacological activities<sup>9-14</sup>.

Clinical microbiologists have great interest in screening of medicinal plants for antimicrobial activities and climatic phytochemicals as potential new therapeutics. The active principles of many drugs found in plants are secondary metabolites<sup>15</sup>. Nowadays, scientific studies have returned to "Natural" products<sup>16</sup>. Researches have focused intensively on finding pharmaceutical equivalents of traditional uses of medicinal plants, as well as research on the discovery of new antimicrobial compounds from a wide variety of plants<sup>17-18</sup>.

*Tagetes* species, contains about 56 species belonging to the Asteraceae family, popularly known as marigold, are grown as ornamental plants and thrive in varied agroclimates. Bioactive extracts of different *Tagetes* parts exhibit nematocidal, bactericidal, fungicidal and insecticidal activity. Nematocidal activity of roots is attributed to thienyls while the biocidal components of the essential oil from flowers and leaves are terpenoids. Also carotenoid pigments from *Tagetes* are useful in food coloring.



**Fig. 1:** *T. erecta* (Marigold) grown in garden

Extracts of *Tagetes* sp. has vast amount of orange-yellow carotenoids<sup>18</sup>.

*Tagetes erecta* L., known as marigold, is a single or perennial plant and can spread from tropical to temperate climate under wide conditions. *T. erecta* extracts are ingredient of medicinal drugs used to treat common cold, inflammation, bowel and stomach illnesses, skin infections, cough, cold and, wound<sup>19</sup>. Phytochemical studies of its different parts have resulted in the isolation of various chemical constituents such as thiophenes, flavonoids, carotenoids and triterpenoids. The plant *T. erecta* has been shown to contain quercetagenin, a glucoside of quercetagenin, phenolics, syringic acid, methyl-3, 5-dihydroxy-4-methoxybenzoate, quercetin, thienyl and ethyl gallate<sup>19-26</sup>. The flavonoid -Patulitrin is one of the potential elements for its antibacterial activity<sup>27</sup>.

The study aimed at to isolate the antibacterial substance from the fresh flowers of *T. erecta* by using extraction method using methanol as the solvent and to assess its antibacterial activity against some human pathogens.

### **Material and Methods**

**Collection of *T. erecta* flowers:** The fresh flowers from *T. erecta* was collected from the garden, were dried in an oven at 35 °C for 48 h.

**Extraction in solvent- Methanol:** An extraction procedure was used to isolate the antibacterial substance from the flowers. Firstly, 5 g of the dried materials were extracted by maceration within 75 ml of a 4:1 ratio of methanol: water mixture under the dark conditions and at room temperature for 24 h. This step was repeated three times during 72 h. The extracts from each step were collected, filtered, and concentrated at 40 °C using a rotary evaporator to give a final dry weight of 300 mg. The extracts were stored at -86 °C and resuspended with 1 ml of 80% methanol before antimicrobial testing.

**Test organisms:** The test organisms used in this study was taken from National Institute of Chemical Laboratory (NCIM), Pune. The organisms used in the study were *Escherichiacoli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus cereus* and *Streptococcus pyogenes*.

**Propagation and maintenance of test organisms:** The bacterial test organisms were streaked on the Nutrient Agar slants and were incubated overnight at 37°C.

**Antimicrobial activity:** The testing of the bacterial cultures for the inhibitory effect of *T. erecta* was performed by using agar well diffusion method.

**Agar Well Diffusion Assay (Zone of Inhibition Evaluation):** Antibiotic susceptibility and resistance were evaluated by agar well diffusion assay. 0.5 McFarland density of bacterial and fungal culture was adjusted using normal saline (0.85% NaCl) using densitometer to get

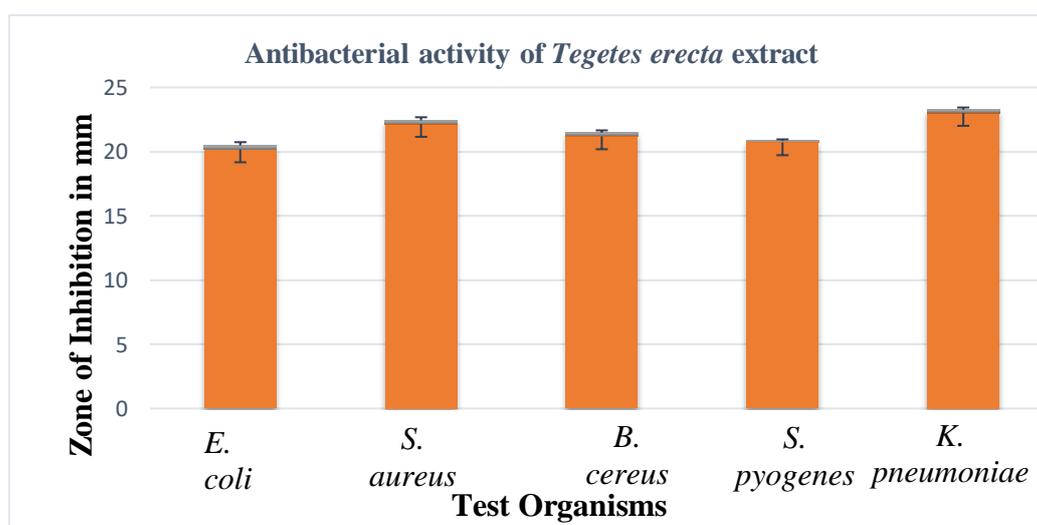
## Results and Discussion

bacterial and fungal population of  $1.0 \times 10^8$  cfu/ml. 100µl of each of the adjusted cultures were mixed into separate 100 ml of sterile, molten, cool MHA (Mueller-Hinton agar), mixed well and poured into sterile Petri plates. These were allowed to solidify and then individual plates were marked for each individual isolates. Each plate was punched to make wells of 6 mm diameter with the help of sterile cork borer at different sites of the plates. 100 µl of respective essential oil were pipette out into the well in assay plates. Bacterial plates were incubated overnight at 37°C. Following incubation, petriplates were observed for the inhibition zones, diameters of which were measured by using Vernier Calipers.

Table-1 Antibacterial activity of *Tegeteserecta* extract

S. No.	Test Organisms	Zone of Inhibition in mm
1	<i>Escherichia coli</i>	20.36 ± 0.26
2	<i>Staphylococcus aureus</i>	22.33 ± 0.24
3	<i>Bacillus cereus</i>	21.35 ± 0.23
4	<i>Streptococcus pyogenes</i>	20.80 ± 0.11
5	<i>Klebsiella pneumoniae</i>	23.15 ± 0.21

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



Graph-1 Representation of antibacterial activity of *T. erecta* methanolic extract

Antibacterial activity of the methanolic extract of *T. erecta* (10 mg/100ml) was performed. Inhibition is maximum for *Klebsiella pneumoniae* ( $23.15 \pm 0.21$ mm) and minimum for *Escherichia coli* ( $20.36 \pm 0.26$ mm) Results are presented in Table 1 and Graph 1. The results showed a variant sensitivity of *T. erecta* methanolic extract against Gram negative and Gram positive bacteria. Thus, proving *T. erecta* to be most potent plant for new drug discovery. Hence, for all human ailments, herbal medicines are available in our surrounding itself. *T. erecta* (Marigolds) are naturalized to many warm climate areas all over the world. They are also used in perfumery<sup>28</sup>. Several recent papers reported that antibacterial activity is due to flavonoids<sup>29</sup>. The flavonoids are also toxic to insects, which further modify the alkaloids and incorporate them into their own defense secretion<sup>30</sup>. Plants selected for antimicrobial activity have shown appreciable results due to the presence of tannins, flavonoids and sterols<sup>31</sup>.

### Conclusion

In conclusion, the study has showed that the flowers of *T. erecta* 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. To overcome anti biotic resistance an immense boosting should be panelized. The herbal anti microbial plants should be more high- lighted in the world of medicine.

### Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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