

ANTIBACTERIAL POTENTIAL OF ETHANOL EXTRACT
OF MARIGOLD (*TAGETES ERECTA* L.)

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Abstract

Antimicrobial resistance is a pandemic which affects the therapeutic options of many people in need by rendering most known antibacterial agents ineffective. The answer to the problem may be found in the old books written by ancient philosophers who used various plants to treat the sick. Our objective was to study the antibacterial properties of the commonly found plant, *Tagetes erecta* L., and to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the crude ethanol extract. A maceration protocol was used to obtain crude ethanol extract of dried aerial parts of *T. erecta* and broth microdilution method to test the antibacterial activity of the extract against selected Gram-positive and Gram-negative bacteria. The inhibitory effect of the *T. erecta* extract was documented with MICs for *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* being 368 mg/ml and 182 mg/ml for *Enterococcus faecalis*. The MBCs for all bacterial species were 368 mg/ml. The current study demonstrates the antibacterial properties of *T. erecta* and its potential use as a source of antibacterial compounds.

Key words: antibacterial effect, *Tagetes erecta*, marigold

Introduction. Infectious diseases are disorders, caused by microorganisms (bacteria, viruses, fungi, and parasites) that invade the human body. They often

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lead to severe complications and even death. Despite the availability of many treatment options, one of the most problematic conditions leading to high mortality are bacterial infections. The most common cause of unfavourable outcome is considered to be the resistance to the routinely used antibacterial agents [1].

A year ago, the World Health Organization (WHO) announced that the production and introduction of new antibiotics is inadequate and insufficient to tackle the hidden pandemic of antimicrobial resistance, as only twelve drugs have been approved in the last seven years, ten of which belong to already known families of antibacterial agents. WHO believes that urgent and major measures are needed to address the situation and prevent the imminent catastrophe due to antibiotic resistance [2].

For many years, various plants have been used in folk medicine to deal with various infectious and non-infectious diseases [3]. Nowadays, more and more scientists are considering these plants as the key to opposing the emerging antibiotic resistance [4].

The genus *Tagetes* L. belongs to the family *Asteraceae* and consists of more than 50 different species. *Tagetes* spp. originated in South America, but are also distributed in other continents such as Africa, Asia and Europe. Among the most famous representatives of the genus are *T. erecta*, *T. tenuifolia*, *T. lunulata* and *T. patula* [5,6]. It is known that the plants of this family have different medicinal qualities – anti-inflammatory, antibacterial, antimycotic, wound healing, and etc. [7]. These properties, usually, are due to the production of phenylpropanoids, carotenoids, flavonoids, thiophenes [5]. One of the representatives of *Tagetes* spp., *T. erecta*, also known as marigold, grows rapidly, is undemanding and is believed to possess all the positive qualities of the family *Asteraceae* [7].

The aim of this study was to evaluate the antibacterial properties of one of the commonly cultivated plants in Bulgaria, *Tagetes erecta* L., and to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the crude ethanol extract.

Material and methods. Scheme of the experimental design is shown in Fig. 1.

Extract preparation. The fresh flowers of *T. erecta* were collected in the city of Varna, Bulgaria, in June 2023. Voucher specimens were deposited under the number TUV1343 in the herbarium of Technical University of Varna.

Ten grams of cleaned and air-dried at room temperature *T. erecta* flowers (*Tageti flos*) were mixed with 100 ml 96% ethanol (1:10) and stirred constantly at room temperature for 24 h. The mixture was subjected to evaporation with rotary evaporator at 40 °C for 8 h. From 10 g of dried plant 1.84 g of final extract was obtained. The extract was resuspended in 5 ml 70% ethanol with a final concentration of 0.368 g/ml (368 mg/ml) and filtered. The mixture was used immediately to determine the antibacterial properties of the plant.

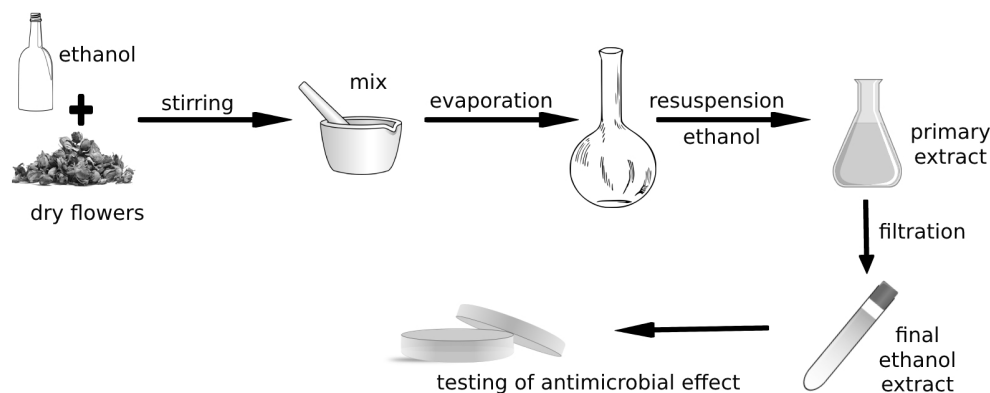


Fig. 1. Scheme of the experimental design

Preparation of bacterial suspension. For the purpose of the study, the following Gram-positive and Gram-negative bacteria were selected from the American Type Culture Collection (ATCC): *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 (Ridacom, Bulgaria). The lyophilized bacteria were revived according to the manufacturer's instructions. Blood agar (Oxoid, UK) was used for cultivation of the bacteria. One or two identical colonies were suspended in a sterile saline solution until 0.5 McFarland turbidity was reached.

Antibacterial susceptibility testing. A microdilution method was used in 96 well plate to determine the antibacterial properties of *T. erecta* and determine the MIC and MBC. In short, 50 µl Mueller–Hinton broth (MHB) (Oxoid, UK) were added to wells 1 to 11. To well 12 100 µl MHB was added. To well 1 100 µl of the ethanol extract (368 µg/µl) was added. A double decreasing concentration of the extract was prepared by transferring and disposing of 50 µl in each subsequent well from 1 to 10. Fifty µl of pre-prepared bacterial suspension was added to all wells from 1 to 11. The plate was placed in a sterile pouch and incubated in aerobic conditions at 35 °C for 24 h. For the determination of the MBC, one loop of the mixture from each well was inoculated onto blood agar. The results were documented by two independent observers. Three control wells were also placed: a positive bacterial growth control (bacterial suspension + 100 µl MHB), a negative growth/sterility control (100 µl MHB) and a 70% ethanol control (50 µl ethanol + 50 µl bacterial suspension).

Results. Three out of the four tested bacterial species (*E. coli*, *P. aeruginosa* and *S. aureus*) demonstrated growth (cloudy medium, sedimentation or colour change of the broth) in all wells except the first one (clear medium and no sedimentation). Thus, for these three bacterial species the MIC of the crude ethanol extract of *T. erecta* was 368 µg/µl. Lower MIC for *E. faecalis* was observed – 184 µg/µl (Fig. 2).

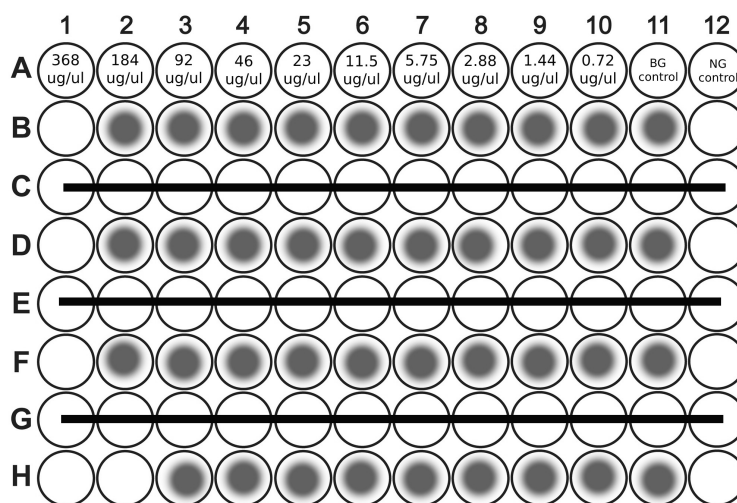


Fig. 2. Determining the MIC of *Tagetes erecta* extract. B – *Escherichia coli*; D – *Pseudomonas aeruginosa*; F – *Staphylococcus aureus*; H – *Enterococcus faecalis*; Well with black centre – bacterial growth; Well without black centre – bacterial growth inhibition; BG control – bacterial growth control; NG control – no growth control

After inoculation of samples from all wells on blood agar for the determination of MBC, 368 µg/µl is documented as the minimum bactericidal concentration for all four bacterial species. In all positive growth controls growth was documented, all negative ones were without bacterial growth, and ethanol controls did not demonstrate growth inhibition.

Discussion. The obtained extract exhibited antibacterial properties by inhibiting the growth of the tested bacteria due to the chemical substances produced by the plant.

Similar results have been reported by other scientists who have studied the antibacterial activity of *T. erecta* [8–11]. RAMYA et al. [12] also studied the bacteriostatic activity of ethanol extract of *T. erecta*. Their extracts achieved inhibitory effect against *S. aureus* and *P. aeruginosa*.

The minimum inhibitory concentration of the ethanol extract was 368 mg/ml for *E. coli*, *P. aeruginosa* and *S. aureus*, and 184 mg/ml for *E. faecalis*. AYUB et al. [13] conducted a similar study and documented antibacterial activity, with the MICs much lower than ours (0.63 mg/ml – 7.6 mg/ml).

MOTAMEDI et al. [14] also reported MICs ranging between 10 and 80 mg/ml against certain Gram-positive and Gram-negative bacteria, which are also lower than the MICs recorded in the current study. The contrast in the MICs may be due to the different amounts of dried aerial plant parts used for the extract preparation as well as the conditions under which the plants were grown, and therefore the differences in the chemical compounds found in them [5]. Two studies from Italy

and Venezuela support this theory that the major chemical components produced by the *T. erecta* depend on the environmental conditions [15,16].

Conclusion. Our study demonstrates the antibacterial activity of the crude extract of *Tagetes erecta* against bacterial species which cause infections in both hospitalized and outpatient groups. It confirms the place of *Tagetes* among the medicinal plants, as well as its potential as a source of chemical compounds for the production of new antibacterial agents to combat antimicrobial resistance.

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