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# ANTIBACTERIAL POTENTIAL OF METHANOLIC EXTRACTS AND SUB-FRACTIONS OF TEUCRIUM STOCKSIANUM BIOSS COLLECTED FROM MALAKAND DIVISION, PAKISTAN

Shah, S.M.M.[1*]; Sadiq A.[1], Gul F.[2]

[1]University of Malakand, Chakdara 18000, Dir (L), KPK, Pakistan
[2]Pakistan Council of Scientific and Industrial Research, KPK, Pakistan

*shahpharmacist@yahoo.com

## Abstract

In the current study the methanolic extract of the whole plant of *Teucrium stocksianum* (MEWTS) exhibited marked antibacterial activity against most of the tested pathogens. The MEWTS displayed profound antibacterial potential against *Salmonella typhi* (68.6%), good against *Escherichia coli* (46%) and *Bacillus cereus* (43.3%), while moderate to poor activity was observed against rest of the tested pathogens. The methanolic extract of the roots of *Teucrium stocksianum* (MERTS) was found comparatively more effective against *S. typhi* (55.6%). The extract displayed good activity against *E. coli* (40.3*%), Streptococcus aureus* (35.6%) and *Enterococci faecalis* (32.3%). Methanolic extract of the aerial parts of *T. stocksianum* (MEATS) was found less effective against most of the bacterial strains and demonstrated maximum percent inhibition against *S. typhi* (48.6%). Among the sub-fractions of MEWTS, ethyl acetate fraction (EAF), exhibited remarkable inhibition (83.6%) against *S. typhi*, excellent against *E. coli* (53.3%) and moderate against *B. cereus* (30.6%) while poor activity was recorded against the rest of the strains. The aqueous fraction has shown no antibacterial activity against the test organisms. Based on our findings it is concluded that the methanolic extract of whole plant and the ethyl acetate fraction are potential target for the bioassay guided isolation of novel antimicrobial compounds.

**Key words:** *Teucrium stocksianum,* Antibacterial, Lamiaceae.

## Introduction

Human beings from the very beginning are dependent upon plants for the fulfillment of their basic needs like food, health maintenance and shelter. According to WHO, 70-80% population of developing countries acquire their primary pharmaceutical care from medicinal plants [1]. Since time immemorial, numerous medicinal plants have been focused by human being for the treatment of different diseases. The history of plants revealed that plants have been used as medicine, preservatives, beverages and spices for flavoring the food [2-4]. Medicinal plants posseses a varity of pharmacological activities due to occurence of various classis of phytochemicals [5]. *Teucrium stocksianum* belongs to the family Lamiaceae. Most of the genera of this family have been reported to possess a variety of biologically active compounds [6]. Most of the members of the genus *Teucrium* are herbaceous plants. There are about 7000 species of this genus mostly distributed in the temperate areas. Some of the species are reported from Pakistan including *Teucrium stocksianum, Teucrium scordium, Teucrium royleanum* and *Teucrium quadrifarium* [7-10]. Various species of this genera have been traditionally used for the treatment of various ailments, *Teucrium polium* is used ethno-medicinally for the treatment of pregnancy pains, flatulence, analgesia, liver disorders, jaundice, coughing and miscarriage [11-17].

It has been reported that *Teucrium* species possesses antiseizure [18], antioxidant [19], analgesic and hepatoprotective [20]. Ahmad et al. have shown that the crude methanolic extract and the subsequent fractions of *T. royleanum* possess strong antibacterial and antifungal activities [21]. The extracts have also shown significant inhibition of acetylcholine and butarylcholine esterase in enzyme inhibition activities [21,22].

*Teucrium stocksianum* is used in folk medicines for the treatment of fever, sore throat, as expectorant, diabetes, foot burning sensation, body coolant and blood purifier [23,24]. The methanolic extract and essential oils of *T. stocksianum* is reported to possess potent analgesic activity [25,26]. In our previous work the crud saponins, methanolic extract and sub-fraction have shown profound cytotoxic, phytotoxic and insecticidal activity [27]. It has been documented that the ethyl acetate fraction of the crude methanolic extract of this plant has shown significant antidiabetic effect in Alloxan induced diabetic rabbits, which endorse the folkloric use of the plant [28]. Islam et al explored the antiulcerogenic and cytoprotective effects of the alcoholic extract of *T. stocksianum* [29]. Thus looking to the pharmacological potentials and abundant availability, we designed the current study with the aim to provide scientific evidences for the folkloric use of *T. stocksianum* in the management of gastrointestinal infections and typhoid fever.

## Methods

### *Plant material*

The whole plant of *Teucrium stocksianum* Bioss was collected from the hilly areas in the proximity of University of Malakand, Chakdara (Lower dir) Khyber Pakhtunkhwa. The plant specimen was identified by plant taxonomist Professor Dr. Nasrullah, Department of Botany University of Malakand, Chakdara Dir, Khyber Pakhtunkhwa, Pakistan. The plant specimen was deposited with voucher number (H.UOM.BG.199) in the herbarium of the same University. Plant material (2 kg) was cleaned physically from foreign material and darts. The whole plant was properly washed with running tap water in ordered to remove the mud especially from roots. A small portion of the whole plant was separated while the rest of the plant, the aerial parts were cut from roots. The plant material was cut, shad dried and pulverized to fine powder.

### *Extraction*

The powdered plant materials were soaked in 70% methanol initialy for two weeks in an extraction tank. The extract was filtered through double folded nylon cloth in a stainless steel bucket. The plant material was again socked in 70% methanol for one week. Finally the marc remained was discarded and the filtrate having dark greenish color was concentrated under reduced pressure using rotary evaporator at 40 to 45˚C, yielded about 475 gm (whole plant), 150 gm (aerial parts) and 84 gm (roots) extracts. The methanolic extract of aerial parts was fractionated with different organic solvents.

### *Fractionation*

Fractionation was carried out using consecutive solvent-solvent extraction method, from low polar solvent to high polar solvent. The extract was suspended in sufficient volume of distilled water in a 04 liters separating funnel and with vigorously shaking. Then an equal volume of previously distilled *n*-hexane was added to the aqueous suspension and was shacked two to three times with specific intervals. The two immiscible solvents were allowed to stand for sufficient time till formation of two distinct layers in ordered to separate the *n*-hexane

soluble/miscible compounds from the aqueous suspension. The *n*-hexane layer was collected and the same process was repeated twice for maximum recovery of *n*-hexane soluble compounds. The collected portions of *n*-hexane were combined and concentrated under reduced pressure using rotary evaporator at 40-45oC. The remaining aqueous portion was mixed and shacked with chloroform and ethyl acetate in ordered to get the chloroform and ethyl acetate fractions respectively. At the end the aqueous fraction was recovered and was concentrated in a temperature controlled water bath [30]. Fractionation resulted in 26 g, 38 g, 47 g and 23 g of *n*-hexane, chloroform, ethyl acetate and aqueous fraction respectively.

***Antimicrobial activity***

Antimicrobial activity of the methanolic extract and subsequent fraction of *T. stocksianum* were performed using standard procedures [21].

***Preparation of growth media for antibacterial activity***

Nutrient agar media is most commonly used for the determination of the antibacterial activity of phytomedicines. Nutrient agar media was prepared by dissolving 40 g of nutrient agar in one liter of hot distilled water with constant stirring. The media and the required glassware were sterilized in autoclaving at 15 psi at 121 for 15 min.

***Antibacterial assay***

The antibacterial potential of the crude methanolic extract of *T. stocksianum* and sub-fractions were screened against various common strains of human pathogens. Both Gram-positive and Gram-negative strains like *Staphylococcus aureus, Proteus merablus* and *Bacillus cereus,* (Gram-positive bacteria), *Salmonella typhi, Pseudomonas aeruginosa, Enterococci faecalis, K. pneumonia* and *Escherichia coli* (Gram-negative bacteria) were included in this study. Antibacterial assay was performed using agar well diffusion method [21]. The stock cultures of the bacterial strains were refreshed by incubating it in nutrient broth at 37oC for 24 h. The broth culture (0.6 ml) of the test organisms were mixed with 60 ml of molten agar, cooled to 45oC. The mixtures were poured in the previously sterilized Petri dishes and were allowed to solidify in the sterile environment of laminar flow hood. Then the required numbers of wells were dug in each plate. Triplicate plats for each strain were prepared. Stock solutions of various extract in The concentration 1 mg/ml in dimethyl sulfaoixid (DMSO) were prepared and 100 µl and 200 µl from each dilution were added in the respective wells. Imipinem was used as reference standard and the control well received 100 µl and 200 µl DMSO. For proper diffusion of the test solutions the plates were left at room temperature for 2 h.

All the plates were incubated at 37oC for 24 h, then the zone of inhibition developed by the different concentrations of test samples were measured in nearest mile meter and percent inhibition were determined with the following formula

## Results and Discussion

In the current era one of the core issue in the field of microbiology which is more focused than before is the multiple drug resistance developed by microorganisms. This is due to irrational use of commercial antibiotics for the treatment of various infections of both human and plants [31]. Antimicrobial agents from plants origin provide a good natural alternatives for the treatment of different infections and as will as food preservatives [32]. The essential oils of most of the *Teucrium* species revealed marked antimicrobial activities [33].

The crude methanolic extracts of *T. stocksianum* aerial parts (MEATS), roots (MERTS), whole plant (MEWTS) and sub-fractions of MEWTS were tested against eight different human pathogens [21]. Amongst the crud methanolic extracts of *T. stocksianum*, TSMEW has shown profound antibacterial potential against most of the pathogens as shown in Table 1. The MEWTS displayed marked antibacterial potential against *Salmonella typhi* (68.6%), good against *Escherichia coli* (46%) and *Bacillus cereus* (43.3%) *Staphylococcus aureus* (30.3%) while moderate activity were observed against *Pseudomonas aeruginosa* (26.3%) and *Proteus merablus* (22.3%), presented in Table 1. The MERTS was found comparatively more effective against *S. typhi* (55.6%). The extract displayed good activity against *E. coli* (40.3*%), S. aureus* (35.6%) and *Enterococci faecalis* (32.3%) and moderate against the rest of the test bacteria (Table1). MEATS has found comparatively less effective against most of the bacterial strains and demonstrated maximum percent inhibition against *S. typhi* (48.6%).

Among the sub-fractions of MEWTS, ethyl acetate fraction (EAF), exhibited remarkable inhibition (83.6%) against *S. typhi*, excellent against *E. coli* (53.3%) and moderate against B. cereus (30.6%) while poor activity was recorded against the rest of the strains. All the tested samples displayed poor activity against *K. pneumonia*. The results of ethyl acetate fraction is in conformity to the findings of

Ahmad et al showing that the ethyl acetate fraction of *T. royleanum* demonstrated 100% inhibition against *S. typhi* [21]. Both chloroform (CF) and *n*-hexane (HF) fraction displayed 37 and 40% inhibition against *Proteus merablus* respectively, low to negligible activity were observed against rest of the tested bacteria (Table 1). The aqueous fraction has shown no antibacterial activity against the test organisms.

On the basis of our results it is concluded that comparatively strong antibacterial activity is residing in the methanolic extract of whole plant and the ethyl acetate fraction. Both displayed profound percent inhibition against *S. typhi* and *E. coli*. Therefore these extract could be a potential target for the isolation of bio-active compounds for the treatment of typhoid and GIT infections.

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**Table 1.** Antibacterial activity of various crude methanolic extracts and sub-fractions of *T. Stocksianum*

| **Bacterial strains** | **Percent Inhibition of Test Samples** | | | | | | | **Std Drug** |
|---|---|---|---|---|---|---|---|---|
| | **TSMEA** | **TSMER** | **TSMEW** | **HF** | **CF** | **EAF** | **AF** | **Imipinem** |
| ***P. merablus*** | Nil | 29.3 ±1.45 | 22.3 ± 1.76 | 37.6 ± 2.90 | 40.6± 2.6 | Nil | Nil | 27±1.15 |
| ***S. aureus*** | 24.3 ± 1.45 | 35.6 ± 1.45 | 30.3 ± 2.02 | 29.6 ± 2.02 | Nil | 26.6 ± 2.02 | | 33.3 ±1.45 |
| ***E. coli*** | 33.3± 2.02 | 40.3 ± 2.02 | 46± 1.73 | Nil | 29.6± 2.6 | 53.3± 2.02 | Nil | 30 ± 1.73 |
| ***B. cereus*** | 39.6±1.45 | 31.6± 2.02 | 43.3± 2.33 | 24.6 ± 2.60 | 25 ± 1.7 | 30.6± 3.18 | Nil | 32.3±0.88 |
| ***S. typhi*** | 48.6±1.76 | 55.6±2.02 | 68.6±2.90 | 24.3±2.60 | 24.6± 2.9 | 83.6±2.02 | Nil | 25.3±0.88 |
| ***K. pneumonia*** | Nil | 21± 2.31 | Nil | 17.3± 2.33 | Nil | 25.3 ± 2.60 | Nil | 28±1.15 |
| ***P. aeruginosa*** | 16.3±2.02 | 23 ± 1.15 | 26.3 ± 2.02 | Nil | Nil | 23.6 ± 1.76 | Nil | 30.3±1.45 |
| ***E. faecalis*** | 21±1.73 | 32.3 ± 2.60 | 17.6 ± 1.45 | 20.6± 2.02 | Nil | Nil | Nil | 28.3±0.88 |