Original Research Article

Antimicrobial Activities of *Teucrium creticum* Against Reference Microbial Strains and Multi-Drug Resistant Bacteria Isolated at an Oncology Ward

Brief title: Antimicrobial Activities of Teucrium creticum

ABSTRACT

Aim: To determine antimicrobial activity of *Teucrium creticum* (*T. creticum*) leaves extract against bacterial and fungal reference strains and multidrug resistant bacteria isolated at an oncology ward.
Study design: Cross-sectional study.
Place and Duration of Study: The study was carried out in department of biology and biotechnology in An-Najah National University in cooperation with the laboratory of the hospital of the university. An-Najah National University is in West Bank in Palestine. The research was performed from 8th of February to the 15th of April 2017.
Methodology*: Teucrium creticum* plant leaves were collected in Palestine, from which aqueous and methanolic extracts were prepared. Antimicrobial activities of *T. creticum* extracts were determined against reference bacterial and fungal strains as well as against 8 multidrug resistant bacteria isolated at an oncology ward. Antibacterial and anti-yeast activities were determined by Micro broth dilution method, while anti-mold activities were determined by agar dilution method. *Teucrium creticum* methanolic extract strongly inhibited the growth of the studied reference bacterial strains, which were *Staphylococcus aureus* (MIC= 1.56 mg/ml) and *Shigella sonnie* (MIC=3.125 mg/ml). In addition, most of the 8 multi-drug resistant bacterial strains isolated from patients with cancer (*Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia Coli* and *Enterobacter cloacae*) were also highly susceptible to methanolic extract (MIC=3.125 mg/ml). Both the *Staphylococcus aureus* and *Shigella sonnie* reference strains were inhibited at lower level by the aqueous extract (MIC=12.5 mg/ml). All the bacterial strains isolated from patients with cancer were susceptible to aqueous extract at different levels (3.125 – 25 mg/ml). *Epidermophyton floccosum* mold and *Candida albicans* yeast were strongly inhibited by aqueous extract, where the MIC values were 1.56 and 3.125 mg/ml, respectively.
Conclusion: *T. creticum* plant extracts showed promising antimicrobial activities against multidrug resistant bacterial isolates as well as against reference bacterial and fungal strains.

Keywords: Teucrium creticum, antimicrobial activities, multidrug resistance, oncology

1. INTRODUCTION

A major public health problem worldwide is the spread of antimicrobial resistance. This may be attributed mostly to the widespread use of antimicrobials. Infections due to multi-drug resistant (MDR) pathogens have become a therapeutic challenge and a cause of significant morbidity and mortality [1, 2, 3].
Complementary and alternative medicine (CAM) are widely used and still increasing in the Western world [4, 5]. Genus of *Teucrium* L. is a large and polymorphic one. This genus is common in mild parts of Europe, North Africa and Asia. The species of *Teucrium* genus in Turkey were applied for years in treatment of digestive system as well as diabetes mellitus. Furthermore, Turkish traditional drugs included *Teucrium* genus use as expectorant (choler and urine) and uses in treatment of inflammation, breath opening, flavoring, appetizing, antiseptic and worm removal [6, 7]. In Cyprus, the genus *Teucrium* was

found to includes four species*; T. cyprium ssp., T. micmpodioides, T. divaricatum ssp. canescens* and *T. kotschyanum* [8]. In a previous study in Palestine, 3 species of *Teucriium* were detected, which included *Teucrium capitatum*, *Teucrium creticum* and *Teucrium divaricatum* [9].
In Palestine, *Teucrium creticum* L. (Lamiaceae) (*T. creticum*) is locally known under the common name Ja'adh. The plant is used traditionally to treat diabetes [10]. Due to its limited distribution in Palestine [11], few phytochemical and antimicrobial studies were conducted on this plant.
Teucrium creticum plant's aerial parts have been reported for its astringent, vulnerary, antipyretic and depurative properties. Furthermore, aerial parts of *T. creticum* is known for it's attractiveness to bees [12]. Among the natural sources of neoclerodane as well as 19-nor-neo-clerodane diterpenoids, *Teucrium* genus was found to be one of the most abundant sources. These compounds were reported to possess useful antifeedant activity [13]. In *Cyprus*, *T. creticum* is also known to contain clerodane diterpenoids [14]. In a study carried by Omar et al [15], *Teucrium creticum* was not shown to significantly affect human coagulation cascade. It was demonstrated in a previous study that *T. creticum* is an efficient scavenger of free radical [16]. On the other hand, ethanolic *T. creticum* extract possessed no or very low antimicrobial activities against *Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumonia, Escherichia coli*, *Salmonella typhi*, *Candida albicans, Microsporum canis*, and *Trichophyton rubrum* [17].
This study aimed to evaluate antimicrobial activity of *T. creticum* leaves extract against bacterial and fungal reference strains and multidrug resistant bacteria isolated at oncology ward of An-Najah National university.

2. MATERIALS AND METHODS

2.1 Bacterial isolates

Reference microbial strains were obtained from American Type Culture Collection. Bacterial strains were *Staphylococcus aureus* ATCC 25923 and *Shigella sonnie* ATCC 25931, while fungal isolates were *Candida albicans* ATCC 90028 and *Epidermophyton floccosum* ATCC 52066. In addition, 38 isolates recovered from different wards at An-Najah national hospital were provided with their identification. These isolates were collected from 8th of February to the 15th of April 2017.These strains were isolated from various clinical samples (blood, wound swabs, sputum, urine, etc.). The bacterial isolates were *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Enterobacter cloacae*.

2.2 Antibiotic susceptibility test

In order to select representative clinical multidrug-resistant isolates recovered at oncology ward and to examine their susceptibility to *T. creticum*, antibiotic susceptibility test was performed. Disc diffusion technique according to CLSI [18], was used for antibiotic susceptibility testing. The used antibiotic discs were Cephalothin (30 µg), Cefoxitin (30 µg), Nalidixic acid (30 µg), Amoxicillin (20 µg), Ciprofloxacin (5 µg), Imipenem (10 µg), Amikacin (30 µg), Aztreonam (30 µg), Ceftazidime (30 µg) and Amoxycillin-Clavulanic Acid (20/10 µg). In addition, *S. aurues* isolates were examined against Vancomycin (30µg). All antibiotics were obtained from OXOID (UK).

2.3 Determination of antimicrobial activities of *T. creticum*

2.3.1 Plant collection

The plant was classified and kindly provided by Dr. Ghadeer Omar. The plant was collected from Palestine and included leaves, which was ground into powder.

3.3.2 Plant extracts preparation

Plant powder (20g) was separated into two equal parts then 100 ml of 100% methanol and 100 ml of deionized water were added separately. The powder was soaked with the solvents with continues

shaking for 7 days. Then the extracts were centrifuged for 5 minutes at 1000 g and the supernatant was aspirated. The organic extract was dried using rotary evaporator, and the aqueous extract was dried using lyophilizer. Aqueous extract was dissolved in water and organic extract was dissolved in 100% DMSO (Dimethyl sulfoxide). The concentration of both extracts was 50 mg/ml. The extracts were sterilized by syringe filtration method.

2.3.3 Antimicrobial activity determination by the method of micro- broth dilution

Into each well of a micro titration plate (Greiner bio-one, China), a volume of 100 µl of Mueller Hinton broth (Becton dickinson and company, France) was placed. Plant extract (100 µl) was pipetted in the first well and mixed. Then 100 µl was transferred to next well. This was repeated to well number 11, from which 100 µl were discharged after mixing. Well number 12 (positive control of microbial growth) was free from plant extract. Bacterial suspension was prepared equivalently to 0.5 McFarland standards, and 2 ml from suspended bacteria was diluted with 4 ml Mueller Hinton broth, this suspension was inoculated in each well (1µl) except well number 11 (negative control of microbial growth). For the examined bacterial strains, the plates were placed in an incubator adjusted at 35℃ and the results were read after 24 hours [19, 20]. The yeast species (*Candida albicans)* suspension was prepared equivalently to 0.5 McFarland standards, and diluted 1:20 then 1:50 with Mueller Hinton broth, this suspension was inoculated in each well (100 µl) except well number 11 (negative control of microbial growth). Although the Plate was incubated at a temperature (35℃) similar to that of bacteria, the incubation period was extended to 48 hours. The lowest concentration, at which no visible microbial growth could be observed was considered the MIC value[19, 20, 21].

2.3.4 Anti-mold activity determination by agar dilution method

The prepared SDA media (Becton dickinson and company, France) were kept in melted state (1ml in each test tube) in water bath at 40 ℃. Six test tubes for each plant extract (water and methanolic) were prepared. A volume of 1 ml of plant extract was added into tube number one and mixed. Then 1ml was transferred from tube number 1 to next tube. The process was repeated up to tube number 6. Then tubes were put on slant position. Tube number six was used as negative control of fungal growth. One tube was free from plant extract (positive control of fungal growth) [20, 22]. In addition, serial dilution of 100% DMSO was examined.

A suspension of mold (*Epidermophyton floccosum)* was prepared to be equivalent to a McFarland standard with 90% transmission. A total of 20µ1 of fungal suspension was placed on surface of each slant in the tubes with the exception of negative control of fungal growth. The tubes were incubated at 25 ℃ for 14 days [20, 22].

Each of plant extract and DMSO were examined by micro-broth and agar dilution methods two times.

3. RESULTS

3.1 Antimicrobial activities of *T. creticum* extracts

3.1.1. Inhibitory activity against reference strains

Methanolic extract of *T. creticum* was more effective than aqueous one against *S. aureus* and *S. sonnie*. In more detail, growth of *S. aureus* and *S. sonnie* was inhibited by methanolic extract concentration of 1.56 and 3.125 mg/ml, respectively (Table 1, Figure1). On the other hand, higher concentration of aqueous plant extract (MIC= 12.5 mg/ml) was required to stop the growth of both bacterial types. Both fungal types were more susceptible than bacteria to aqueous extract of *T. creticum*, where MIC for *E. floccosum* and *C.albicans* were 1.56 and 3.125 mg/ml, respectively. The limited solubility of methanolic *T. creticum* extract caused impediment for the determination of MIC due to the antimicrobial activity of solvent DMSO. Where the container (well or tube) showing no growth of fungi and containing plant extract was also containing an inhibitory concentration of DMSO (determined by MIC value of DMSO alone).

3.2.2 Inhibitory activity against bacteria isolated from patients with cancer

In general ,the frequencies of resistance to different antibiotics were higher among isolates collected at oncology ward in comparison to other sources. All the 8 isolates of bacterial strains isolated at the oncology wards were multidrug resistant (resistant to 4 or more antibiotics). Table 1 and Figure 2 show the antimicrobial activities of *T. creticum* extract against bacteria isolated from patients suffering from cancer. Among the extract for which MIC was determined, both methanolic and aqueous *T. creticum* extract showed inhibitory activity against all bacteria isolated from cancer patients. The 4 *P. aeruginosa* isolates were strongly inhibited by methanolic extract (MIC=3.125 mg/ml) and inhibited mostly at lower level (3.125-12.5 mg/ml) by aqueous extract. Out of the 2 *E. coli* isolates, one was similar to *P. aeruginosa* isolates, where the MIC values were 3.125 and 12.5 mg/ml for methanolic and aqueous extracts, respectively. On other hand, the second *E. coli* isolate was more resistant to activity of *T. creticum* extract, where the MIC of aqueous extract was 25 mg/ml and MIC of methalonic extract was not determined. *S. aureus* and *E. cloacae* isolated at oncology wards expressed more resistance to aqueous extract (MIC= 25mg/ml) and were more susceptible to organic extract, where MIC was 3.125 mg/ml for both.

Table 1 *Teucrium creticum* antmicrobial activities against bacteria isolated from cancer patients and reference microorganism strains

Microbial Isolates	MIC (mg/ml)	
	Aqueous Extract	Methanolic Extract
Staphylococcus aureus ATCC 25923	12.5	1.56
Shigella sonnie ATCC 25931	12.5	3.125
Candida albicans ATCC 90028	3.125	Undetermined
Epidermophyton floccosum ATCC 52066	1.56	Undetermined
Strains isolated from patients with Cancer		
Pseudomonas aeruginosa 5	12.5	3.125
Pseudomonas aeruginosa 565	12.5	3.125
Pseudomonas aeruginosa 164	3.125	3.125
Pseudomonas aeruginosa B92	12.5	3.125
Staphylococcus aureus B209	25	3.125
Escherichia coli 131	25	Undetermined
Escherichia coli 453	12.5	3.125
Enterobacter cloacae B54	25	3.125

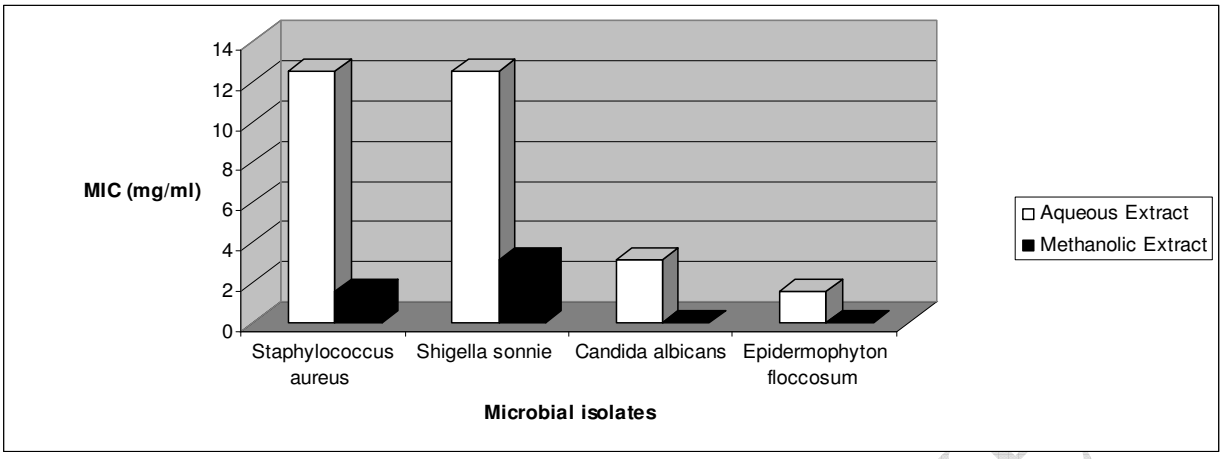

Figure 1. Antimicrobial activities of methanolic and aqueous *T. creticum* extracts against reference strains

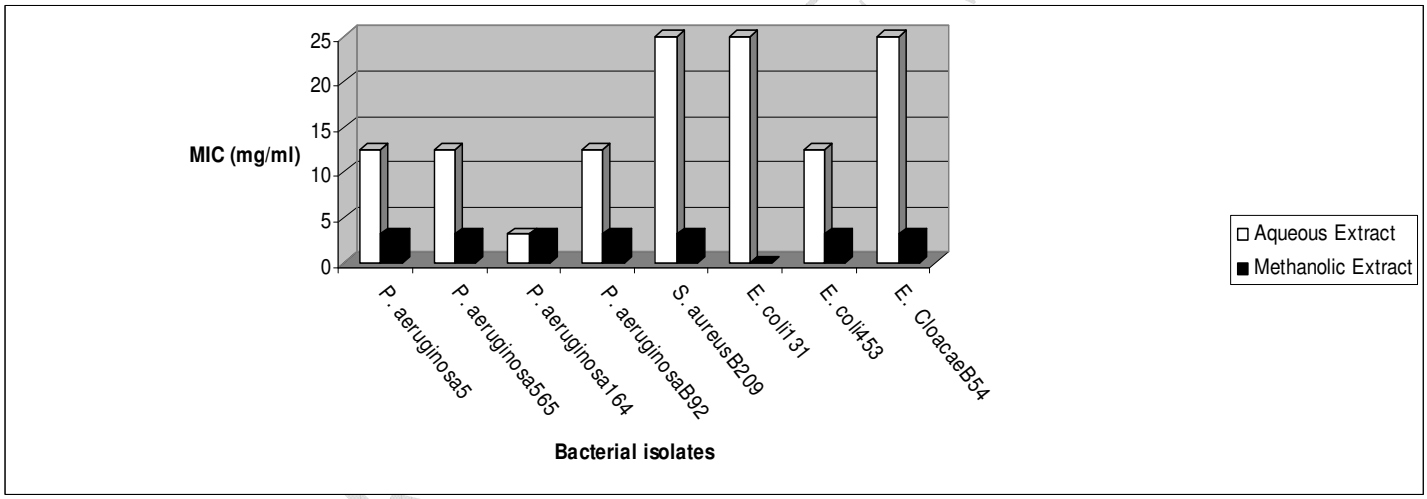

Figure 2. Antimicrobial activities of methanolic and aqueous *T. creticum* extracts against multidrug resistant strains isolated at oncology wards

4. DISCUSSION

In the present study, although the number of isolates is limited, the bacteria isolated from patients with cancer were more resistant to the examined antibiotics than that of bacteria isolated from patients without cancer. Cancer patients provide different and new environment for bacteria in compression with other patients without cancer, because cancer patients are exposed to different types of treatments (chemotherapy and radiotherapy). In addition, the immune system of cancer patients is weak, which will give bacteria more time for the development of resistant. This elevated frequency of resistance of bacteria isolated from oncology ward provoked us to include antibiotic resistant bacteria isolated from patient with cancer in the evaluation of antibacterial activities of *T. creticum*. This may provide help for treatment of multidrug resistant bacteria strains isolated from patients with cancer.

In Algeria, *Teucrium polium* L. essential oil collected at Beni Aziz region has a high antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and the yeast *Saccharomyces cerevisiae*. The

population of Boutaleb region has significant activity against *Bacillus cereus* and no action against *S. aureus* and the yeast *S. cerevisiae* [23].

In a previous research [17], ethanolic *T. creticum* extract possessed no or very low antimicrobial activities against *Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumonia, Escherichia coli*, *Salmonella typhi*, *Candida albicans, Microsporum canis*, and *Trichophyton rubrum*. In our study, aqueous plant extract of *T. creticum* showed antimicrobial activities against *Staphylococcus aureus*, *Shigella sonnie*, *Pseudomonas aeruginosa, Escherichia coli*, *Enterobacter cloacae*, *Candida albicans* and *Epidermophyton floccosum.* In addition, methanolic extract of *T. creticum* possessed stronger antimicrobial for most of isolates. The differences of the results may be due to use of different solvents in the extraction procedures. Husein *et al*. [17] in the extraction procedure used 75% ethanol on the other hand in present study, water and 100% methanol solvents were used. In addition, collection of plants from different regions and differences in extractions methods may attribute to these variations in the results. For our knowledge, we are the first to report *T. creticum* antimicrobial activities against *Shigella sonnie*, *Enterobacter cloacae*, *Epidermophyton floccosum* and multidrug resistant bacterial strains isolated from patients with cancer. Further work is needed to isolate and identify the active compounds from this plant.

Due to limited number of isolated bacteria from oncology patients no statistical analysis was done. It's recommended to repeat the study on larger number of bacterial isolates to confirm the results of the present study.

5. CONCLUSION

In conclusion, although methanolic extract of *T. creticum* possessed stronger antimicrobial activates both aqueous and methanolic extracts inhibited the growth of most examined microbial isolates including the multidrug resistant ones isolated from oncology ward, thus *T. creticum* may represent a candidate for the development of new antimicrobial agent that will be helpful for the treatment of multidrug resistant bacterial infections.

COMPETING INTERESTS

There is no competing interest to mention

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