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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.[1] In Turkey, Teucrium species have been used for years in order to treat diabetes and diseases of the digestive system as traditional drugs for the purpose of choler expectorant, urine expectorant, antidiabetic, treatment of inflammation, antiseptic, worm removal, flavour, appetizing and breath opener [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].[4]

The genus Teucrium is considered to be one of the most abundant natural source of neoclerodane and 19-nor-neo-clerodane diterpenoids. 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 Micro broth dilution method

A 100 µl volume of Mueller Hinton broth (Becton dickinson and company, France) was pipetted in each well of a 96 well plates (Greiner bio-one, China). 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). The plates were incubated at 35°C for 24 hours for bacterial species [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). Plate was incubated at 35°C for 48 hours. MIC value was defined as the lowest concentration that inhibited any visible microbial growth [19, 20, 21].

2.3.4 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 °C. 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 °C 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

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.
It's recommended to repeat the study on larger number of patients 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.