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Antibacterial effect survey of total extract and different fractions of aerial part of *Teucrium orientale var glabrescens* on several pathogenic bacteria

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ABSTRACT

According to the vast widespread drug resistance among pathogenic microorganisms and the side effects of many chemical drugs, research on novel antimicrobial compounds is urgently needed. Some herbs have been shown to have antimicrobial properties and can be used to produce antimicrobial drugs. In this study, different extracts effects of *Teucrium orientale* was practiced on several pathogenic bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, *Staphylococcus expidermidis*). The plant specimens were dried and powdered after transferring to the laboratory and final approval. Extraction was performed using methanol and water solvents and then ethyl acetate, diethyl ether and chloroform fractions using standard methods. The extracts were kept in standard conditions after determination of net weight until the continuation of monitoring. Serial concentrations of each extract and fraction were prepared in appropriate solvent for microbial tests and then their antibacterial activity was performed according to standard agar diffusion method and mean diameter of growth zone was recorded as antimicrobial activity. Pseudomonas aeruginosa showed the highest resistance to different concentrations of the extracts according to obtained results. The highest sensitivity was observed in *B. subtilis* at concentration of 50 mg / ml ethyl acetate extract with 25.9 mm diameter of growth zone. The results of this study have shown that the extract of *T. orientale* has the ability to treat microbial infections in humans. Further studies and identification of its constituents are essential for the use of this plant as a drug.

1. Introduction

Medicinal plants have been used for centuries to treat diseases. Although many drugs are chemical, at least one-third of the drugs are of plant origin or have been deformed after being extracted from the plant (Esmaili, 2009). At present, the use of medicinal plants for the treatment of various diseases has been growing scientifically and traditionally. According to studies in the United States, sales of herbal ingredients as medicines have grown 33 percent. Iran is one of the best regions in the world in terms of climate and geographical location and has been a source of production and consumption of medicinal plants in the past (Iranmanesh et al., 2010).

In recent years, due to the widespread complications of the use of chemical drugs and the inefficiency of these drugs in the treatment of some diseases, attention has been paid to finding effective drugs of natural origin, and extensive research is ongoing around the world (Ayoubi et al., 2016). Iran is one of the rich countries in the field of medicinal plants that has not been scientifically studied on many of its

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species. The use of medicinal plants and natural compounds has been suggested by the World Health Organization in the treatment of parasitic diseases (Edwards, 1993).

T. orientale var glabrescens is one of the traditional herbs that has attracted many researchers today. There are four species of *Teucrium* in our country such as: *T. orientale*, *T. polium*, *T. melissoides* and *T. stocksianum*, among which *T. polium* and *T. orientale* are the most frequent (Emad et al., 2012). Studies on *T. orientale* have revealed that it contains tannin, terpenoids, saponins, sterols, flavonoids and leukoanthocyanins and also has antibacterial effects but has no obvious antifungal effects (Emad et al., 2012).

Regarding the pathogenic bacteria confrontation also due to the observed increase of antibiotic resistance of the pathogenic bacteria to the synthetic antibiotics and the high cost of production of these antibiotics ، there is a special tendency towards the use of medicinal plants (Pedramnia et al.,2018). One of the herbs that has been considered in traditional medicine even by Hippocrates and Galen is the *Teucrium orientale*. *T. orientale* is a herbaceous plant that has been reported in recent years for its anti-diabetic, antispasmodic, analgesic, anti-inflammatory and antioxidant effects, but very little research has been done to investigate its antimicrobial effect (Emad et al., 2012).

In 2012, the antibacterial effect of *T. orientale* was also confirmed by Golshani et al (Golshani et al., 2012). They performed their disc diffusion assay on two gram-positive bacteria, *Clostridium perfringens* and *S. aureus*, and two gram-negative bacteria *Salmonella* and *E. coli*. The results of this study showed that the plant showed good effects on gram-positive bacteria *C. perfringens* and *S. aureus*, but in contrast the growth inhibition zone in disk diffusion method was not observed in fighting against gram-negative bacteria *Salmonella* and *E. coli* (Golshani et al., 2012).

In 2010, Darabpour et al. identified about 29 compounds of *T. orientale*. After analysis, data on identified chemical compounds and studied ecotypes of *Teucrium* in Lorestan province were identified (Darabpour et al., 2010). If priority is given to the production of chemicals such as: betapinin, beta-cariophyllene, francis-B, carvacrol, bicyclogermacrene, alpha-pinin, germacrene-d, the Khorramabad ecotype is introduced and recommended (Darabpour et al., 2010).

In 2009, Esmaili and Amiri identified 44 compounds in essential oil of *T. orientale* collected from north of Borujerd in Lorestan province, alpha-pinin, beta-cariophyllene, germacrene-d, Beta-pinene and limonene and it was also found that the essential oil of this plant against the most gram-positive and negative bacteria tested has significant antimicrobial effects and is greater than gentamicin antibiotics (Esmaili et al., 2009).

The effects of *T. orientale* on blood sugar control in type 2 diabetic patients have been studied and compared with oral glibenclamide. Research has shown that *T. orientale* herbal medicine has an average blood sugar level over a period of time (Emad et al., 2012). It controls its use and its effects are similar to glibenclamide tablets. Also its effect on the amount of food consumed and appetite of patients as well as body mass index and blood lipids are significant and it is recommended that this plant be considered as a suitable drug to control blood sugar in diabetic patients (Emad et al., 2012).

In this study, due to the effective constituents of *T. orientale* that can have significant antimicrobial effects, and according to the fact that there are no studies on antimicrobial effects of this plant on *S. aureus*, *S. epidermidis*, *E. coli*, *B. subtilis*, *Pseudomonas aeruginosa* and *S. typhimurium*, it was decided to conduct this study.

2. Materials and Methods

2.1. Preparation of extracts

Aerial parts of *T. orientale* were collected from Qotb Abad area of Hormozgan province in June 2018 and identified in the herbarium of pharmacy faculty of Islamic Azad University and registered under AUF-112 code. The aerial part of this plant was extracted after drying in open air and crushed by a mill. Extraction was carried out by maceration method (Iranmanesh et al., 2010). The antimicrobial effect of the extract of the plant against the mentioned bacteria was evaluated by both qualitative and quantitative methods.

2.2. Antimicrobial screening

In order to evaluate the antimicrobial effects of extracts extracted from *T. orientale*, disk and well plate diffusion methods were used (Edwards 1993). These methods are performed in mueller Hinton Agar medium. This culture medium was originally formulated for the isolation of pathogenic Neisseria spp. Most commonly used in high-powered antibiotic discs today to determine antibiotic susceptibility patterns.

2.2.1 Disk Plate Method

For this method, Muller Hinton Agar (MHA) culture medium was prepared for 5 bacterial strains. The materials required for preparation of Meuller Hinton agar medium include: Beef Extract 2g, Acid Hydrolysate of Casein 17.5g, Starch 1.5g and Agar 17g. This medium is used for antibiogram testing. It also provides the growth conditions for the *Neisseria* family, which in the latter case should be incubated in the environment after cultivating the suspect sample at 5-10% CO_2 pressure. First, the Meuller-Hinton media were prepared according to the US Pharmacopoeia (USP) formula. The required amount of Mueller Hinton powder was poured into an erlene containing distilled water and brought to the desired volume. Then, with the help of heat, the powder was dissolved in distilled water until the environment became completely transparent. At this stage, the pH of the medium was adjusted 7.2 - 7.4 by 0.5M NaOH and then autoclaved for sterilization at 121°C at 15 PSI for 15 minutes. After the ambient temperature reached 40-50°C, the media was divided into 6 cm disposable sterile plates under sterile conditions.

The standard 0.5 McFarland solution was used to prepare the microbial suspension. This is because the number of bacteria used for testing should be compared with the standard, as negative or false results may be obtained if the number of bacteria used is not appropriate.

Bacterial suspension was removed by sterile swab and cultured in Mueller Hinton Agar. The medium was kept in the laboratory for a period of time and after drying the surface, the discs containing the plant extracts and the positive and negative control groups were placed in a suitable distance from each other and from the wall. Plates were then placed in the refrigerator for 15 minutes at 5°C to completely infiltrate the extract and the agar medium. After this stage, the media were cultured for 24 hours at 35°C and then measured using callipers to read the results of growth inhibition zone diameter.

2.2.2 Well Plate method

In this method, like the disk plate method, the bacterial suspension was first removed by sterile swab and cultured in Muller Hinton agar medium. The required materials for the microplate method include: plant extract 4mg, Half McFarland Bacterial Suspension, DMSO 10% (2ml), Gentamycin 25g/ml and vancomycin 25mg/ml. After bacterial culture, wells were pipetted to 6 mm in diameter by pipette paste. Each well had different concentrations of suspension, gentamicin, and vancomycin as positive control and blank solvent as negative control was added to 100 µLit. Plates were then incubated in a 37°C incubator for 24 hours and, like the disk plate method, the growth inhibition zone diameter was measured using a callipers and compared with control groups. It is worth noting that all steps were performed both under disk and well plate method under the hood and next to the flame, and each test was repeated three times to reduce test error.

Microplate method was used to quantitatively investigate the antimicrobial effects of *T. orientale* extract.

2.2.3. Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC)

In this study, 4 mg of the extract was diluted in 2 ml of 10% DMSO and the initial concentration was 2000 µg / ml and all microplate wells were added with 100 µL DMSO 10%. For dilution, 100 µL of the essential oil was added to the first well and the concentration in the first well was 1000 µg / ml. To continue after mixing the contents of the first well, 100 µl of it was removed and added to the second well and continued until the eleventh well and the concentration was diluted.

Subsequently, 100µl of bacterial suspension was added to each well and this was done separately for each strain. Gentamicin and vancomycin were used as positive control and 10% DMSO as negative control. At the end of

the work, each well contained 200 microliters. The microplates were incubated at 37°C for 24 h under aerobic conditions and the results were read after 24 h. Concentrations at which turbidity was generated were recorded and the concentration of the last transparent well was reported as MIC. By definition, MIC is a concentration of antibody that stops bacterial growth. Bacterial suspension of half the size of McFarland prepared containing 150 million bacteria per millimeter and the concentration of well that does not grow is considered MBC.

3. Results

As the concentrations of the extracts increased, the diameter of the growth zone increased and the Pvalue for methanol, chloroform, diethyl ether, ethyl acetate and aqueous extracts by well were 0.002, 0.001, 0.000, 000.0 and 0.001, respectively. Comparison of different concentrations of extracts with gentamicin and vancomycin at concentrations of 25 mg / ml were all significant in terms of control, indicating ineffectiveness of different concentrations of extracts compared with control groups.

3.1 Antibacterial activity

In the Table 1, the antibacterial activity (mm) of different concentrations of extracts and fractions of *T. orientale* against the bacteria in the well plate method and in the Table 2, the antibacterial activity (mm) of concentrations various extracts and fractions of *T. orientale* against specific bacteria have been demonstrated by disk plate method.

3.2 MIC and MBC results using microplate method

In graphs 1 to 6, comparison of MBC and MIC fractions of chloroform, diethyl ether, ethyl acetate and aqueous, methanol extract of *T. orientale* on the bacteria in this study is shown.

4. Discussion

T. orientale belongs to *Teucrium* genus and its medicinal use goes back to the time of Hippocrates and Galen. Its medicinal part, which has flowering shoots, has a potent and anticonvulsant effect and is considered useful for the treatment of genitourinary disorders and delayed or irregular menstruation, as well as being antioxidant, antifever and antimicrobial, analgesic, anti-ulcer and anti-spasmodic (Esmaili 2004).

In the present study, three concentrations of 10, 25 and 50 mg / ml were used. The results of this study showed that 50 mg / ml was the most effective concentration. In this study two methanol and aqueous extracts and three fractions of diethyl ether, ethyl acetate and chloroform were used. The effects of these 5 concentrations against *E. coli*, *S. aureus*, *S. epidermidis*, *B. subtilis*, *S. typhimurium* and *P. aeruginosa* have been evaluated. In this study, the antibacterial effects of disk and well plate methods were investigated. In this extract, the growth inhibition zone diameter was higher in most extracts, but this difference was not statistically significant.

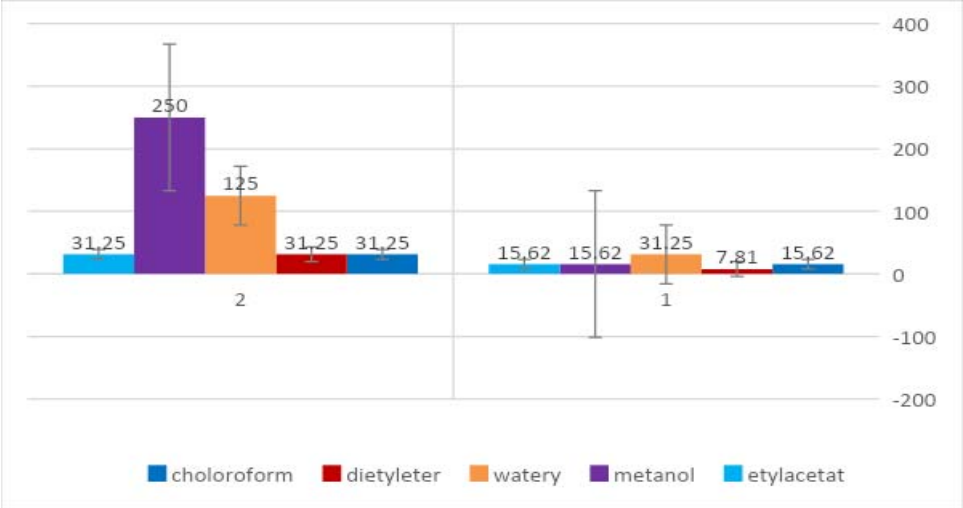
According to the results of this study, the effects (growth inhibition zone diameter) of fractions of chloroform, ethyl acetate, diethyl ether and methanol and aqueous extracts on *S. typhi* at concentrations of 50 mg / ml were 19.4, 19.3, 21.3, 21.7 and 18.8 mm respectively that were significant for the control group. The effects of *T. orientale S. typhi* were more dramatic than other plants studied and it can be hoped that the drug can be used to treat *S. typhi* infections. In the study of Saeedi et al, in the 2011, fennel was used at concentrations of 2.5 to 12.8 mg/ml which resulted in a concentration of 12.8 mg/ml of growth inhibition zone diameter of 14.5 mm. Which was less effective than gentamicin with a significant difference. In the Saeedi study, gentamicin produced a 31-mm growth in diameter that was lower than our study and had weaker effects as expected (Saeedi et al., 2011). In the study of Alamhulu in 2014, 100, 200 and 400 mg / ml concentrations of methanol and ethanol extracts of *Dendrostellera* root were used. Methanol and ethanolic extracts of this plant at a concentration of 400 mg / ml had a growth inhibition zone diameter of 16.66 and 21.33 mm, respectively (Alamhulu 2014). In the present study, although the maximum concentration used was 50 mg / ml, under the same conditions, it had better effects than *Dendrostellera*, which was statistically significant ($P < 0.005$) and indicates more effective compounds in *T. orientale*.

Table 1. Antibacterial activity (mm) of different concentrations of extracts and fractions of *T. orientale* against *bacteria* using well plate method

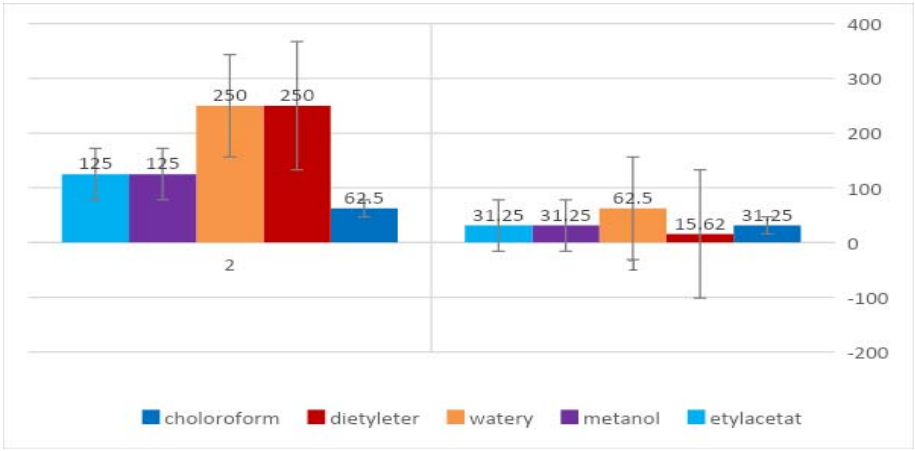
T.orientale against	Controls		Aqueous extract	Ethyl acetate fraction	Diethyl ether fraction	Chloroform fraction	Methanol extract	Extract tape / Density (mg/ml)
	Gentamycin 25(mg/ml)	Vancomycin 25(mg/ml)						
S.aureus	32.1	31.5	8.8	9.7	5.2	10.5	9.9	10
	32.1	31.5	11.9	15.8	9.7	17.4	12.7	25
	32.1	31.5	19.0	23.0	15.4	25.5	13.9	50
S.epidermidi	30.9	30.8	8.6	9.2	9.8	7.1	7.8	10
	30.0	30.8	11.6	13.3	13.1	11.8	14.4	25
	30.9	30.8	17.0	18.8	22.9	20.0	15.3	50
S.typhimuriu	34.9	34.8	13.4	14.0	5.5	10.3	16.7	10
	34.9	34.8	16.3	17.5	8.6	15.5	18.2	25
	34.9	34.8	23.5	25.1	19.6	20.5	21.1	50
B.subtilis	28.0	26.0	9.3	17.5	6.6	12.4	13.7	10
	28.0	26.0	9.8	21.8	9.1	18.1	17.7	25
	28.0	26.0	17.8	25.9	19.2	25.5	19.8	50
E.coli	34.6	31.8	10.6	10.5	5.9	8.9	12.9	10
	34.6	31.8	16.2	17.1	6.9	14.9	15.8	25
	34.6	31.8	24.6	25.5	10.12	18.7	18.9	50
P.aeroginosa	32.3	31.5	9.3	9.3	5.8	7.4	12.3	10
	32.3	31.5	15.5	13.8	9.4	9.7	13.3	25
	32.3	31.5	19.7	21.9	14.7	15.2	14.7	50

Table 2. Antibacterial activity (mm) of different concentrations of extracts and fractions of *T. orientale* against *bacteria* by disk plate method

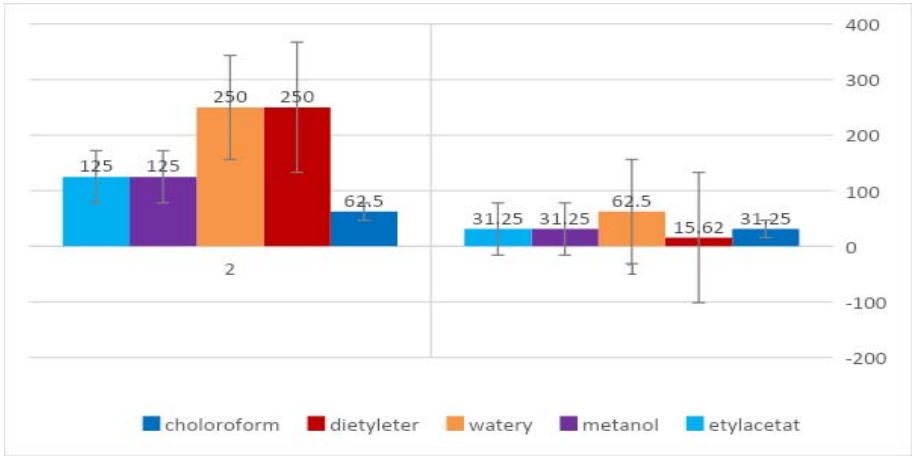
T.orientale against	Controls		Aqueous extract	Ethyl acetate fraction	Diethyl ether fraction	Chloroform fraction	Methanol extract	Extract tape / Density (mg/ml)
	Gentamycin 25(mg/ml)	Vancomycin 25(mg/ml)						
S.aureus	30.6	30.3	2.4	8.7	5.0	9.1	8.1	10
	30.6	30.3	3.2	14.8	11.0	14.9	10.0	25
	30.6	30.3	14.3	18.4	16.0	22.8	15.1	50
S.epidermidi	29.0	29.4	5.1	6.9	5.9	5.9	6.6	10
	29.0	29.4	9.8	11.2	9.6	10.5	11.5	25
	29.0	29.4	21.3	15.8	15.2	14.9	14.8	50
S.typhimuriu	32.6	33.2	4.8	11.0	12.7	8.5	13.6	10
	32.6	33.2	7.3	15.5	15.0	13.5	16.2	25
	32.6	33.2	18.8	21.3	19.3	19.4	21.7	50
B.subtilis	20.6	27.2	4.2	14.3	6.6	11.4	11.0	10
	20.6	27.2	7.7	20.0	8.8	15.5	16.1	25
	20.6	27.2	19.5	24.8	14.2	23.9	19.1	50
E.coli	32.5	30.5	3.0	8.6	9.1	6.3	9.9	10
	32.5	30.5	5.1	16.1	14.4	11.6	13.2	25
	32.5	30.5	8.3	24.3	20.3	14.8	17.5	50
P.aeroginosa	31.3	30.4	3.6	6.5	7.5	5.4	7.8	10
	31.3	30.4	6.6	11.3	12.1	7.6	10.5	25
	31.3	30.4	14.1	18.3	17.5	11.5	14.8	50



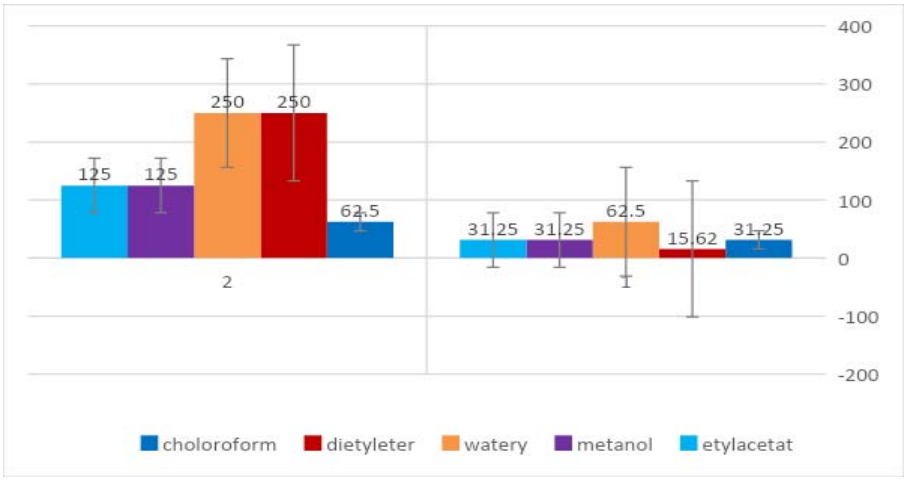
Graphs (1): Comparison of MBC and MIC fractions of chloroform, diethyl ether, ethyl acetate and aqueous, methanol extracts of *T. orientale* on *B. subtilis*



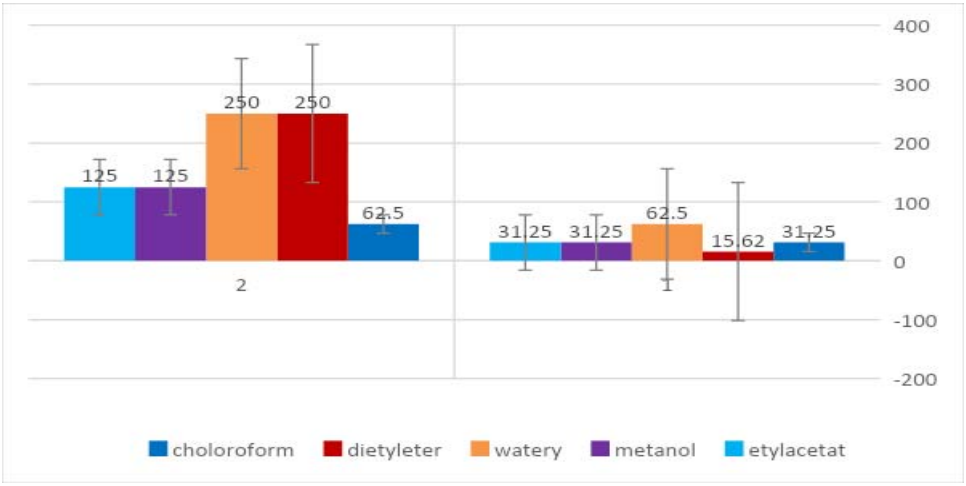
Graphs (2): Comparison of MBC and MIC fractions of chloroform, diethyl ether, ethyl acetate and aqueous, methanol extracts of *T. orientale* on *P. aeruginosa*



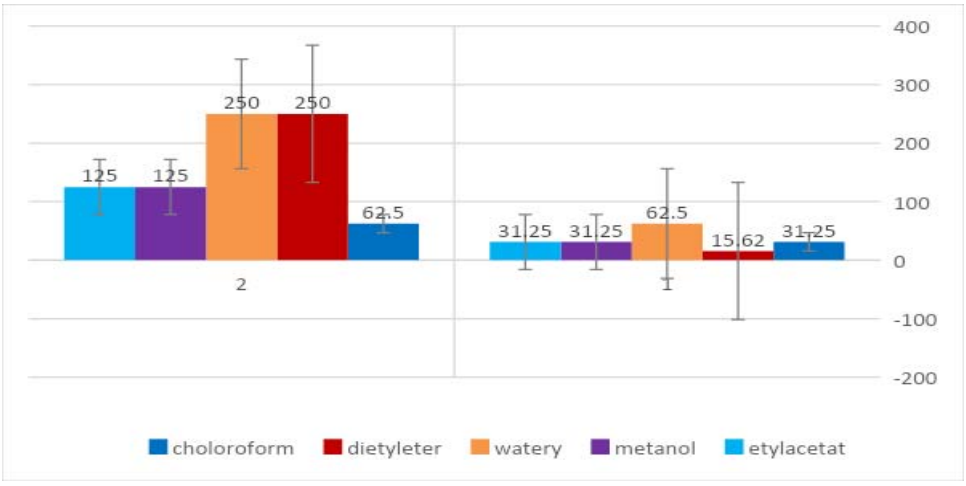
Graph (3): Comparison of MBC and MIC fractions of chloroform, diethyl ether, ethyl acetate and aqueous, methanol extracts of *T. orientale* on *S. aureus*



Graph (4): Comparison of MBC and MIC fractions of chloroform, diethyl ether, ethyl acetate and aqueous, methanol extracts of *T. orientale* on *S. epidermidis*



Graph (5): Comparison of MBC and MIC fractions of chloroform, diethyl ether, ethyl acetate and aqueous, methanol extracts of *T. orientale* on *E. coli*



Graph (6): Comparison of MBC and MIC fractions of chloroform, diethyl ether, ethyl acetate and aqueous, methanol extracts of *T. orientale* on *S. typhimurium*

In this study, the growth inhibition zone diameter of methanol and aqueous extracts and fractions of chloroform, ethyl acetate, diethyl ether on *S. epidermidis* at concentration of 50 mg / ml were 14.9, 15.2, 15.8,14.8 and 21.3 mm, respectively. The growth inhibition zone diameter against *S. epidermidis* was lower in all extracts than aqueous extract than *S. typhi* and this showed higher resistance to *S. epidermidis*. Sadrnia used aqueous extract of *Aloe vera* against *S. epidermidis*. In this study, concentrations of 1.11 to 285.7 mg / ml were used. The highest effect was related to the concentration of 285.7 mg / ml and the growth inhibition zone diameter was 30 mm. At the concentration of 50 mg / ml, this plant had a diameter greater than 22 mm, which had better effects than the present study (Sadrnia 2014).

In this study, *S. aureus* was also used and the growth inhibition zone diameter of chloroform, diethyl ether, ethyl acetate, methanol and aqueous extracts at 50 mg / ml concentrations against this bacterium were 22.8, 16, 18.4, 15.1 and 14.3 mm, respectively. The results of this study showed that the effects of all extracts of *T. orientale* against *S. aureus* were better than *S. epidermidis*, however, this difference was not significant. Due to the importance of *S. aureus* bacteria, many studies have been done to investigate the antibacterial effects of different plants. In the study of Sadrnia the growth inhibition zone diameter at concentrations of 35.7 and 71.4 mg / ml against *S. aureus* was 20 and 25 mm, respectively, which is consistent with the present study. In a study by Saeedi et al, who used Foeniculum, the plant extract at a concentration of 12.8 mg / ml produced a 14.3 mm growth inhibition zone diameter (Saeedi et al., 2011). In the study of Zarei et al., *Alcea* plant was used at concentrations of 100, 200, 400 and 800 mg / ml which had growth inhibition zone diameterof 14, 20, 20 and 23 mm, respectively (Zareii et al., 2014). In our study, the highest concentration was 50 mg / ml, but in all extracts except chloroform extract, it performed better than 100 mg / ml *Alcea* (Zareii et al., 2014).

Pedramnia et al in 2018 to investigate the chemical composition and antimicrobial effects of methanol extract of *laurus nobilis* plant in concentrations of 100, 200, 400 and 800ppm on a number of different microbial strains including *S. aureus*, *Listeria monocytogenes*, *E.coli*, *B. cereus* and the mold *Aspergillus niger* which published the disc with diffusion results, which showed that the methanol extract of the leaves of the *Laurus nobilis* plant prevents the growth of *S. aureus*, *Listeria monocytogenes* and *E.coli* bacteria. As the antibacterial effect of *Laurus nobilis* extract increases, so does the concentration of the extract. However, it did not affect the bacterium *B. cereus* and the mold *A. niger* in the concentrations used. Finally, it can be inferred that according to the results, the extract of *Laurus nobilis* extract contains antimicrobial substances with antimicrobial effects, but it has less antifungal effect, which can be said to be consistent with our study (Pedramnia et al., 2018).

Another bacterium used in this study is *P. aeruginosa* which has growth inhibition zone diameter of chloroform, diethyl ether, ethyl acetate and methanol and aqueous extracts against this bacterium, respectively, 11.5, 17.5, 18.3, 14.8 and 14.1 mm. In study by Mohammadi Sichani et al., methanol extract and essential oil of *Achillea* was used against *Pseudomonas* bacteria and showed that these compounds were not effective on *P. aeruginosa* and no growth inhibition zone was observed (Mohammadi Sichani et al., 2011). The results of this study showed that *P. aeruginosa* was the most resistant to the other bacteria used in this study but this difference was not statistically significant. Rajabi investigated the effects of extracts of *Aloe vera* leaf and scale and Aloe vera gel on Gram positive and negative bacteria. In this study, no *Pseudomonas* sensitivity related to scale extract was observed and no growth inhibition zone was observed (Rajabi et al., 2014). Aloe vera leaf extract at a concentration of 285 mg / ml caused a growth inhibition zone of 8.4 mm in diameter. In *Aloe vera* gel, at 500 mg / ml, the growth inhibition zone was 18.9 mm in diameter, and at 333 mg / ml and lower concentrations no growth inhibition zone was observed (Rajabi et al., 2014). The results of most of the studies showed resistance of *Pseudomonas* to most of the extracts used, but the results were promising in the present study.

In a study conducted in 2018 by Sabahi et al., Entitled "Antibacterial activity of aqueous and alcoholic extracts of *Aloe vera* and *garlic* on *S. aureus*, *E. coli* and *P. aeruginosa* bacteria". They concluded that the bacterium *P. aeruginosa* was resistant to *garlic* extract and

Aloe vera. Due to the significant bacterial effect of alcoholic and aqueous extracts of *garlic* and *Aloe vera* on pathogenic bacteria, which are involved in the development of a variety of hospital samples, these extract can be considered as natural and alternative drugs that can be said that their results are consistent with the present study (Sabahi et al., 2018).

Furthermore, in this study, fractions of chloroform, diethyl ether, ethyl acetate and methanol and aqueous extracts were investigated on *E. coli* at concentrations of 50 mg / ml of *T. orientale* and they produced growth inhibition zone of 14.8, 20.3, 24.3, 17.5 and 8.3 mm respectively. This study showed that *E. coli* was the most sensitive to diethyl ether fraction and the most resistant to aqueous extract in the concentration of 50 mg / ml. In the study, Sadrnia used *Aloe vera* extract against *E. coli*. In this study no growth inhibition zone was observed up to 8.92 mg / ml. At concentrations of 17.8, 35.7, 71.4, 142.8 and 285.7 mg / ml the diameter of the growth inhabitation zone were reported to be 11, 16, 16, 28 and 28 mm, respectively (Sadrnia 2014). Comparison of this study with the present study showed that the extracts of *T. orientale* extract had higher potency even in lower concentrations in killing *E. coli*. Shahidi used methanol and aqueous extracts of *Apium graveolens* against *E. coli*, which methanol and aqueous extracts at a concentration of 80 mg / ml, produced growth inhabitation zone diameter of 0.57 and 0.55 respectively which this effect was negligible compared to our study and *T. orientale* (Shahidi et al., 2013).

In a study conducted by Ayoubi on the antibacterial properties of methanol extract of *Gundelia tournefertii* on *S. aureus* and *E. coli*. They concluded that the extract of this plant has very high antimicrobial properties that are consistent with our study (Ayoubi et al., 2016).

In a study by Ekhtelat et al. in 2018 which Entitled "Antibacterial activity of *barberry* root extracts and *Fennel* separately and in combination with Niacin and Sodium dioxide against *E. coli* " was conducted.

They concluded that the inhibitory effects of Sodium dioxide and Niacin with *barberry* root extract and *Fennel* were higher than their single effects (Ekhtelat et al., 2018).

One of the most sensitive bacteria used in this study is *B. subtilis*. Growth inhabitation zone diameter of chloroform, diethyl ether, ethyl acetate and aqueous extracts of methanol and aqueous extracts at 50 mg / ml of *T. orientale* in *B. subtilis* was 23.9, 14.2, 24.8, 19.1 and 19.3 mm, respectively. *B. subtilis* has been shown to be more sensitive than other ethyl acetate and chloroform fractions. Jalali et al, used extracts of *Pycnocycla spinosa* to kill *B.subtilis*. In this study, hydroalcoholic, hexane, chloroform and methanol extracts with concentrations of 1.78 to 480 mg / ml were used. In this study, hydroalcoholic, chloroform and methanol extracts of mentioned plant at 120 mg / ml concentration produced 12, 7 and 7.33 mm diameter growth inhabitation zone respectively and hexane extract at showed no growth inhabitation zone at this concentration (Jalali et al., 2007). A comparison of the Jalali study and the present study shows that the results of the present study were much better. According to the studies and different extracts and essential oils used, *T. orientale* extract has been shown to be a candidate for future antibiotic drug production.

In this study, in addition to the diffusion methods including disk and wells, the extracts were also calculated MIC and MBC using microplate methods. The lowest MIC in this study was 7.81 mg / ml for different bacteria and the highest MBC was obtained at 250 mg / ml. In the Sharifi et al. Study, the MIC obtained for *Escherichia coli* and *Klebsiella* was 25 mg / ml and for *Staphylococcus aureus* and *Enterococcus faecalis* 50 mg / ml, respectively, which is higher than our study, ie, to provide a minimum concentration of inhibitory dose, higher extract is needed. In Sharifi et al. study, MBC was obtained for *E. coli* 200 mg / ml and for *Klebsiella*, *S. aureus* and *Enterococcus faecalis* 100 mg / ml (Sharifi et al., 2015). The results of this study using the propagation and microplate methods were completely consistent.

The results of this study showed that *T. orientale* is one of the most effective medicinal plants against human pathogenic bacteria and can be used as a candidate for antibiotic production in the future and certainly after further tests.

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