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Short Communication

Antimicrobial Activities of *Teucrium Polium* Against *Salmonella Typhimurium*

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ABSTRACT

Objective: *Teucrium polium* L. (family Lamiaceae) is a wild-growing flowering plant, found abundantly in South-Western Asia, Europe and North Africa. Traditionally, *T. polium* has been used for different pathological conditions such as gastrointestinal disorders, inflammations, diabetes and rheumatism. The purpose of this study was to evaluate the antimicrobial effect of ethanol extract of *Teucrium polium* against *Salmonella typhimurium* isolates from poultry is resistant to penicillin. **Methods:** *Teucrium polium* extract using vacuum from the center (Rotary) were 12 strains of *Salmonella typhimurium* was isolated from poultry in the city of Zabol. Minimum inhibitory concentrations of MBC *Teucrium polium* extract in different concentrations by dilution in the wells was determined on bacteria. Susceptibility to several antibiotics by Kirby-Bauer disk diffusion standard were evaluated. **Results:** The result of herbal extraction showed the most MIC (the minimum inhibitory concentration) was 10mg/ml concentration that 2 strains of them were inhibited by this concentration *Teucrium polium*. The lowest MIC was 2/5 ppm concentration that two strain of *Salmonella* were inhibited. Although the clinical relevance of extracts and essential oils because of fewer side effects than other current treatments for common antibiotics, it seems valuable, but more research to clinical application of the mechanism of action of ethanol extract and purified effective composition of this plant is on microbial agents.

1.INTRODUCTION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects (Belmekki et al., 2013; Farnsworth, 1989). Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found *in vitro* to have antimicrobial properties (Bajpai et al., 2005). The genus *Teucrium*, which belongs to the family Labiatae, includes 300 species widespread all around the world (Bonnier, 1990; Coste, 1909). A large number of known medicinal species belonging to the genus *Teucrium* are used in folk medicine and pharmacy

(Chang et al., 2006). The species of the genus *Teucrium* are very rich in phenolic compounds with very strong biological activity (Acar and Goldstein, 1996). Among the species, *T. polium* (L.) is widely used in folk medicine for many treatment interventions (Ali Shtayeh et al., 2000). This plant is well known for its antinociceptive (Abdollahi et al., 2003), antioxidant (Couladis et al., 2003), hypolipidemic (Rasekh et al., 2001), antiinflammatory, anti-rheumatoid, and hypoglycemic (Gharaibeh et al., 1988) properties. The purpose of this study was to evaluate the antimicrobial effect of ethanol extract of *Teucrium polium* against *Salmonella*

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typhimurium isolates from poultry is resistant to penicillin.

2. MATERIAL AND METHOD

2.1. Plant materials

The leaf *T. polium* was collected in the region of Iran dried at room temperature. Samples were crashed and transferred into glass container and preserved until extraction procedure was performed in the laboratory.

2.2. Preparation of extracts

Plant was properly dried and pulverized into a coarse powder. Each of 20 g grinded powders was soaked in 60 ml ethanol 95 %, separately for one day (shaking occasionally with a shaker). After one day of dissolving process, materials were filtered (Whatman No. 1 filter paper). Then the filtrates were evaporated using rotary evaporator.

2.3. Bacterial strains

All strains were isolated at different times during 2013-2014 from contaminated bird. Samples were diluted and/or homogenized in TSB medium, and isolates obtained by *Salmonella* selective enrichment in Rappaport-Vassiliadis (RV) medium after 24 h incubation at 43°C.

2.4. Agar disk diffusion assay

The susceptibility of all antibiotics was carried out using disc diffusion method on Muller-Hinton agar as recommended by CLSI (CLSI, 2002). The procedure followed is briefly described here. *Salmonella* isolated plates were grown overnight on Nutrient agar and colony suspension was prepared using the sterile saline water equivalent to a 0.5 McFarland standard. Suspension (100 µl) was spread over the media plate and antibiotic disc was transferred aseptically on the surface of inoculated media plate. Isolated plates were tested with different antibiotics and their concentration shown in parenthesis viz. ampicillin (10 µg) and penicillin (10 µg). Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of plant extracts:

The broth microdilution method was used to determine MIC and MBC. All tests were performed in Mueller Hinton broth supplemented with Tween 80 at a final concentration of 0.5% (v/v). Briefly, serial doubling dilutions of the extract were prepared in a 96-well microtiter plate ranged from 0.3 mg/ml to 10.00 mg/mL. To each well, 10 µl of indicator solution and 10 µl of Mueller Hinton Broth were added. Finally, 10 µl of bacterial suspension (10⁶ CFU/mL) was added to each well to achieve the concentration of 10⁴ CFU/mL. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and then they were placed in an incubator at 37°C for 18- 24 h. The color change was then assessed visually. The lowest concentration at which the color change occurred was taken as the MIC

value. The average of 3 values was calculated providing the MIC and MBC values for the tested extracts. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate the visible growth. The microorganism growth was indicated by turbidity. The MBC was defined as the lowest concentration of the extract at which the incubated microorganism was completely killed.

2.5. Statistical Analysis

All experiments and measurement were repeated at least three times. Statistical analyses were performed using SPSS and Excel 2010 software. All experimental results were analyzed using mean descriptive statistics and the correlation-coefficient. A value of $P < 0.05$ was regarded as statistically significant.

3. RESULTS

The result of herbal extraction showed the most MIC (the minimum inhibitory concentration) was 50 ppm concentration that 5 strains of them were inhibited by this concentration. The lowest MIC was 25 ppm concentration that 7 strain of *Salmonella* were inhibited. The highest and lowest MBC value of extract were 100mg/ml respectively (Table1).

Table1: The minimum inhibitory concentration extract against *Salmonella*

Bacterial cod	MIC/MBC for extract plant(µ/ml)	Antibiotic resistant
1	25/ 50	AM- P
2	50/ 50	AM- P
3	50/ 100	AM- P
4	50/100	AM- P
5	25/50	AM- P
6	25/ 50	AM- P
7	25/ 50	AM- P
8	25/ 50	AM- P
9	50/ 100	AM- P
10	25/ 50	AM- P
11	25/ 50	AM- P
12	50/ 100	AM- P

AM= Ampicillin

P=Penicillin

4. DISCUSSION

In recent years, drug resistance to human pathogenic bacteria has been commonly and widely reported in literature (Mulligen et al. 1993, Davis 1994, Robin et al. 1998). The result show that most MIC (the minimum inhibitory concentration) was 10mg/ml concentration that 2 strains of them were inhibited by this concentration. The lowest MIC was 2/5 ppm concentration that two strain of *Salmonella* were inhibited. The study of Belmekki, the major compounds were germacrene D (25.81%), bicyclogermacrene (13%), β -pinene (11.69%) and carvacrol (8.93%). Furthermore, the essential oil was tested against five bacteria (three Gram-positive and two Gram negative) and three fungi at different concentrations. Results showed that the oil exhibited moderate inhibitory effects on *Bacillus cereus*, *Enterococcus faecalis*, *Escherichia coli* and *Staphylococcus aureus*, with a minimum inhibitory concentrations of 3 to 5 μ l/ml (Belmekki et al., 2013). The study of minimal inhibitory concentration of the taken extracts compared to the strains resistant to *Klebsiella pneumonia* showed that all the above extracts have antimicrobial properties and they were able to prevent the growth of strains resistant to *Klebsiella pneumonia*, whereas all 8-strain klebsiella in the study were resistant to the three (aqueous, ethanolic, ethyl acetate) extracts (Shakibaiee et al., 2000). In the same study, species and sub-species of *Teucrium* had certain anti-staphylococcus effect, most of which was on the *S. epidermidis*. This bacteria was not studied in our study, but all 3 *Staphylococcus aureus* samples were resistant to *Teucrium polium* extracts (Sarac and Ugur, 2007). The study results showed that the inhibitory zone diameter Sarac and Ugur TP ethanol extract against bacteria *S.aureus* ATCC25923, *S.aureus* MU 38, *S.aureus* MU44 and *S.epidermidis* MU30 with 9, 8, 8 and 11 mm) (Sarac and Ugur, 2007).

The study of Zerroug, extracts of *T. polium* gave zones of inhibition against *Bacillus subtilis*, *Micrococcus luteus* and *Paracoccus paratrophus* of 3.7, 2.0 and 2.0 mm, respectively. *A. iva* extract only inhibited the growth of *Paracoccus paratrophus*, giving a zone of inhibition of 3.0mm (Zerroug et al., 2011). The hydroalcoholic extract of *T. polium* had a relatively satisfactory effect on *Salmonella typhi*. (Darabpour et al., 2010). The antibacterial activity of essential oil and methanolic extract of *Teucrium polium* was determined against *Pseudomonas aeruginosa*, *Pantoea agglomerans*, *Brenneria nigrifluens*, *Rhizobium radiobacter*, *Rhizobium vitis*, *Streptomyces scabies*, *Ralstonia solanacearum*, *Xanthomonas campestris* and *Pectobacterium cartovororum* by disc diffusion method. Our results indicate that both methanolic extract and essential oil did not show antibacterial activity against *P. aeruginosa*. Also the essential oil did not show antibacterial activity against *P. cartovororum*. In general, both methanolic extract and essential oil showed the same antibacterial

activity against *R. solanacearum*, *P. agglomerans*, *B. nigrifluens* and *S. scabies*(Purnavab et al., 2015).

Antibacterial activity of the crude extract, as well as with four of the isolated metabolites, was observed with *Staphylococcus aureus* anti-biofilm activity in the low μ Mol range. Diverse sesquiterpene-skeleton structure and corresponding comprehensive enzyme capacity is discussed(Elmasri et al., 2014). The extract was effective against both yeast and carrageenin pyrexia in rats. It also exhibited a marked antibacterial action against both gram positive and gram negative organisms and was found to be non toxic in acute studies(Autore et al., 1984).

CONCLUSION

The results of this work show that the extract of *T. polium* possesses antimicrobial properties, which can be used as natural antimicrobial agents for human and infectious diseases

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Authors' Contributions

All the authors had equal roles in design and writing of the manuscript.

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