Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading

Antimicrobial properties of *Teucrium polium* against some clinical pathogens

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ARTICLE INFO

Article history:
Received 7 December 2009
Received in revised form 30 December 2009
Accepted 10 January 2010
Available online 20 February 2010

Keywords:
Teucrium polium
Antibiotic
Herbal medicine

ABSTRACT

Objective: To explore the antibacterial effect of the alcoholic extracts of aerial parts of *Teucrium polium*, native in Iran on some pathogenic bacteria. **Methods**: Antibacterial activity of ethanolic extract (50 to 400 mg/mL) and methanolic extract (400 and 600 mg/mL) was evaluated by disc diffusion method. **Results**: The ethanolic extract results showed that *Bacillus anthracis* was the most sensitive species, while *Escherichia coli* and *Proteus mirabilis* were more resistant than others. In the case of the methanolic extract, *Bordetella bronchiseptica* was the most sensitive and *Proteus mirabilis* and *Arcanobacterium pyogenes* were the most resistant species. The hydroalcoholic extract of *Teucrium polium* had a relatively satisfactory effect on *Salmonella typhi*. The minimal inhibitory concentration (MIC) of *Staphylococcus aureus* and *Salmonella typhi* was 40 mg/mL and *Bordetella bronchiseptica* and *Bacillus anthracis* was 10 mg/mL. The minimal bactericidal concentration (MBC) against *Bacillus anthracis* was 10 mg/mL while against other species were not found (>200 mg/mL). The methanolic extract had also synergistic effect with methicillin, vancomycin against *Staphylococcus aureus* and with novobiocin against *Salmonella typhi*. **Conclusions**: These results suggest that this plant contains relatively good antibacterial activity and it can be used as a source of antiseptic compounds for medicinal uses.

1. Introduction

In recent years, due to alarming increase in the rate of infections with antibiotic resistant microorganisms and side effects of some synthetic antibiotics, there is increasing interest in medicinal plants as a natural alternate to synthetic drugs. Plants are known to produce a variety of compounds to protect themselves against a variety of their own pathogens and therefore can serve as antimicrobial substances[1,2]. *Teucrium polium* (*T. polium*) locally named "*Kalpooreh*" has been known as an important traditional medicinal plant in Khuzestan, South West of Iran. *T. polium* is a member of the *Lamiaceae* family, a grass plant, durable, with 10–30 cm in height and callous white exterior that ordinarily have dispersal in rocky and sandy area of Europe zones, North of Africa and South West of Asia like Iran[3]. Medical reputation of this plant was noticed in traditional medicine by Socrates and Jalinous[3]. Researchers showed that this plant have anti-diabetic, anti-spasmodic, anti-inflammatory, analgesic and anti-oxidant effects[4-10]. The antimicrobial effects of *T. polium* have been rarely studied by research. The aim of this study was to explore antibacterial properties of ethanolic and methanolic extracts of *T. polium* against some of clinical pathogens.

2. Materials and methods

2.1. Plant collection and identification

The plants used in this study were collected in April, 2008 from hills around of Behbahan (south east of Khuzestan, Iran). The taxonomic identification of this plant was done by Herbarium in Department of Agricultural College, Shahid Chamran University.

2.2. Preparation of plant extract

The aerial parts of *T. polium* were dried at room temperature for ten days and then ground to a fine powder. One gram of powder was extracted by using 10 mL of alcohol (ethanol or methanol)-distilled water solution (8∶2 v/v), centrifugation (3 000 rpm) for 15 mins and collecting of the supernatants. This process was repeated for three times. Solvents were then removed by evaporation[11,12].

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2.3.Bacterial strains

Twelve bacterial species were used in this study. The gram-positive species were *Bacillus pumilus* (*B. pumilus*), *Bacillus anthracis* (*B. anthracis*), *Bacillus licheniformis* (*B. licheniformis*), *Bacillus cereus* (*B.cereus*), *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*) and Gram negative species bacteria were *Yersinia enterocolitica* (*Y. enterocolitica*), *Escherichia coli* (*E. coli*), *Salmonella typhi* (*S. typhi*), *Bordetella bronchiseptica* (*B. bronchiseptica*), *Proteus mirabilis* (*P. mirabilis*), *Arcanobacterium pyogenes* (*A. pyogenes*). These species were originally isolated from patients and identified by standard biochemical reactions.

2.4. Antibacterial susceptibility testing

The isolates were grown in trypone soya broth (TSB) medium at 37 ℃ for 22 hrs. Final inoculums bacterial numbers were adjusted to 10^8 cfu mL^{-1} with reference to the Mc Farland turbidometry[13,14]. One milliliter of the test tubes suspension was added to each plate containing Muller Hinton Agar (MHA, Merck) by sterile cotton swab and allowed to remain in contact for 1 min. Four concentrations of ethanolic extract (50, 100, 200 and 400 mg/mL) and two concentrations of methanolic extract (400, 600 mg/mL) were prepared. The sterile filter paper discs (6 mm diameter) were saturated by addition of 50 μL portions of different concentrations of both extracts[15,16]. Then discs were placed on cultured plates. The plates were incubated at 37 ℃ for 24 hrs and the inhibition zone around each disc was measured. As positive controls, discs containing different concentrations of eight antibiotics including nafcillin 1 mcg, colistin 1mcg, tetracycline 30 mcg, novobiocin 30 mcg, penicillin 10 mcg, vancomycin 30 mcg, methicillin 5 mcg and oxacillin 1mcg were used. All these synthetic antibiotics were produced by Difco.

2.5. MIC and MBC Determination

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of ethanolic and methanolic extracts of *T. polium* were determined against four species including, *S. aureus* for ethanolic extract and *B. bronchiseptica*, *B. anthracis* and *S. typhi* for methanolic extract (somewhat important and more sensitive bacteria). MIC was determined by macro broth dilution assay method[17,18]. In the tube dilution assay, standard bacterial suspension was added to tubes containing 1 mL Muller Hinton Broth (MHB, Merck) and different concentrations of extracts (5, 10, 20, 40, 80, 160, 200 mg/mL). The tubes were incubated at 37 ℃ for 24 hrs. The first tube in the above series with no sign of visible growth was reported as the MIC. MBC was determined by culture done by one loop of the culture medium from those tubes (in MIC assay) showing no apparent growth on MHA and subsequent incubation at 37 ℃ for 24 hrs. The least concentration that showed no colony formation on agar was assumed as MBC for the extracts.

2.6. Study of the synergistic effect

To determine the synergistic effect of the *T. polium* with synthetic antibiotics against *S. aureus*, *S. Typhi* and *B. anthracis*, methanolic extract at 400 mg/mL was added to the discs containing antibiotics including novobiocin 30 mcg, methicillin 5 mcg and vancomycin 30 mcg[19] .

3. Results

Table 1 showed the results of antibacterial assays of ethanolic and methanolic extracts of *T. polium* against various bacterial species. The results showed that antibacterial effects of both extracts of *T. polium* against *B. Pumilus*, *B. anthracis*, *B. licheniformis*, *B. cereus*, *S. aureus*, *S. epidermidis*, *Y. enterocolitica*, *E. coli*, *S. typhi*, *B. bronchiseptica* at lower concentrations were decreased. However, antibacterial effect of *T. polium* ethanolic extract agaist *A. pyogenes* was increased with decreased concentrations. Also, antibacterial effect of ethanolic extracts against *P. mirabilis* was observed only at 10 mg/mL. These results suggest that ethanolic extract was effective against all of bacterial species even at one concentration. *A. pyogenes* and *P. mirabilis* were resistant to methanolic extract even at highest concentration. However, *B. anthracis* and *B. bronchiseptica* were most susceptible microorganism to ethanolic and methanolic extracts, respectively. *E. coli* and *P. mirabilis* were the species most resistant to both extracts. Furthermore, results showed anti-staphylococcal effect of ethanolic extract of *T. polium* was better than methanolic extracts. MIC and MBC results showed that MIC of ethanolic extract against *S. aureus* was 40 mg/mL and for methanolic extract against *B. bronchiseptica* was 10 mg/mL, but MBC against both bacteria were not found. Also MIC value for methanolic extract against *S. typhi* and *B. anthracis* was 40 mg/mL and 10 mg/mL, respectively. MBC value against *S. typhi* was not found (>200 mg/mL) while against *B. anthracis* was 10 mg/mL. The study of synergistic effect of *T. polium* with antibiotic discs showed that the methanolic extract of this plant in combination with novobiocin against *S. typhi* and in combination with methicillin and vancomycin against *S. aureus* have synergism while the combination of this extract with methicillin against *B. anthracis* didn't have change (Table 2).

4. Discussion

Nowadays, increased resistance of pathogenic microorganisms to synthetic antibiotics has caused a concern in universal health and pharmaceutical industries which are looking for new way for production of novel antibacterial products. Medicinal plants are good choices, because these natural resources have fewer side effects, with low-cost and satisfactory effect on broad spectrum of antibiotic resistant microorganisms. In many parts of the world, the extracts of medicinal plants are used for their antibacterial,

Table 1
Antibacterial activity of ethanolic and methanolic extracts from the aerial part of *T. polium* against some pathogenic bacteria.

Bacterial species	Different concentrations of extract (mg/mL)						Antibiotic							
	Ethanolic				Methanolic									
	50	100	200	400	400	600	VA	TE	P	NF	NB	CL	MT	OX
B. anthracis	10	11	14	18	20	22	21	25	R	R	–	–	23	R
B. pumilus	8	9	10	10	12	14	18	14	R	R	–	–	16	R
B. licheniformis	R	R	7	8	10	13	16	29	R	R	–	–	16	R
B. cereus	R	R	9	10	11	15	–	–	–	–	–	–	R	R
S. aureus	8	9	11	12	8	9	22	R	R	R	–	–	R	R
S. epidermidis	7	7	10	13	9	10	–	–	–	–	–	–	R	R
A. Pyogenes	11	11	9	R	R	R	–	–	–	–	–	–	–	–
E. coli	R	R	R	9	R	9	–	–		R	26	10	R	R
S. typhi	9	10	10	13	14	16	–	–		R	12	R	R	R
P. mirabilis	R	11	R	R	R	R	–	–		R	30	12	R	R
B.bronchiseptica	9	9	10	11	22	26	–	–		R	24	R	R	R
Y. enterocolitica	7	7	9	9	10	11	–	–		–	–	–	–	-

R: Resistant, –: Not used;
VA: Vancomycin 30 mcg; TE: Tetracycline 30 mcg; P: Penicillin 10 mcg; NF: Nafcillin 1 mcg; NB: Novobiocin 30 mcg; CL: Colistin 10 mcg; ME: Methicillin 5 mcg; OX: Oxacillin 1 mcg.

Table 2
Results of the study of synergistic effect of *T. polium* methanolic extract with antibiotic discs.

Result	Db (mm)	Da (mm)	Antibiotic Disc	Bacterial species
(Syn)	16	0	Methicillin	*S. aureus*
(Syn)	27	22	Vancomycin	*S. aureus*
(Syn)	18	12	Novobiocin	*S. typhi*
(–)	23	23	Methicillin	*B. anthracis*

Da: Antibiotic, Db: Antibiotic+Extract, Syn: Synergism, (–): Not enhanced.

antifungal and antiviral activities[20]. *T. polium* is one of the most important medicinal plants that extensively used in traditional medicine in Iran. On the basis of this study, ethanolic and methanolic extracts of *T. polium* aerial parts are effective against both gram-positive and gram-negative bacteria. Ethanolic extract at 200 and 400 mg/mL and methanolic extract at 400 and 600 mg/mL doses showed high antibacterial activity, so that these concentrations had growth inhibition on 6 out of 7 gram positive bacteria. Furthermore, ethanolic extract at 400 mg/mL and methanolic extract in 600 mg/mL extract were growth inhibitory on 4 out of 5 gram-negative bacteria. In general, considering diameter of inhibition zones showed that hydroalcoholic extract of *T. polium* was more effective against gram-positive bacteria than gram-negative bacteria. *T. polium* was effective only at 10 mg/mL concentration of ethanolic extract against *P. mirabilis*. This observed resistance could be due to cell membrane permeability or other genetic factors. The diameter of inhibition zones around the more active extracts in particular methanolic extract were comparable with standard antibiotics used as positive controls. All of the gram-positive and gram-negative bacteria were resistant to nafcillin and oxacillin. More of the bacterial species were resistant to methicillin and penicillin. Antibacterial effect of methanolic extract against *B. bronchiseptica* was more comparable to standards. DNA hybridization results suggest that *B. bronchiseptica*, *B. pertussis* and *B. parapertussis* are closely related (72%-94%), hence it is presumable that methanolic extract of *T. polium* may be effective against *B. pertussis* and *B. parapertussis* that cause pertussis[21]. The MBC for *B. anthracis* was found at 10 mg/mL and for the other species, *S. aureus*, *S. typhi* and *B. bronchiseptica*, didn't show bactericidal activity even at 200 mg/mL (MBC >200 mg/mL). This result may be due to high bacteriostatic effect of *T. polium* extracts; we can name these extracts as bacteriostatic agents as they can inhibit bacterial growth but generally do not kill them[17]. Exceptionally the MIC and MBC values for *B. anthracis* were the same. It is reported that for bactericidal antimicrobials the MIC and MBC are often near or aquiline values, so, it can be said that this extract have bactericidal activity against this species[22]. The methanolic extract had significant synergistic effect in combination with methicillin against *S. aureus* compare to methicilin alone. This could be new hope for treatment of methicillin resistant *S. aureus* (MRSA) infections and restricting resistant strains distribution. The synergism between this extract and methicilin isn't against *B. anthracis* that may be due to the same target site for these compounds in this species. The compounds found in this plant includes alkaloids, triterpenes, flavonoids, glycosides, sterols, tannin and saponin[8,23-25]. Flavonoides are one of the broadest groups of phenolic compounds[26]. Phenolic compounds with capacity to chelate transition metals, reduce reactivity of metal ion by forming an inert metal-ligand chelation

of transition metals (such as iron) and thus could reduce their bioavailability for bacteria[27]. We speculate that plant tannine have antimicrobial activity because non-live central wood of many trees has a high concentration of Tannines that assist in preventing bacterial and fungal decay[26]. On the other hand, in the essential oil of this plant, there are compounds including α-pinene, β-myrcene, cadinol, myrtenal, limonene and presumably α-pinene could play an important role in antibacterial activity of *T. polium*[28]. By studying the diameter of inhibition zones of ethanolic and methanolic extracts of *T. polium* against bacteria, in particular against *B. bronchiseptica*, we can truly realize differential ability of these alcohols in extraction of plant bioactive constituents. Hence, more researches with choice of a suitable solvent indicate antibacterial activity of *T. polium* against a specific pathogen. With additional research, we study antibacterial activity of *T. polium* with more accuracy by purification of bioactive constituents from different parts of this plant.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors wish to thank the vice chancellor for research of Shahid Chamran University for the research grant.

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