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Antibacterial and antifungal activities of *teucrium royleanum* (Labiatea)

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Abstract
The crude methanolic extract and subsequent fractions of *Teucrium royleanum* (Labiatea) were screened for antibacterial and antifungal activities. Against tested pathogens, crude extract and subsequent fractions demonstrated moderate to excellent antibacterial activities. Highest antibacterial activity was displayed by the ethyl acetate fraction against *S. typhi* (100%), against *E.coli* (76.7%) and against *P. aerugenosa* (70.8%) followed by the chloroform fraction against *S. typhi* (85.7%). Similarly, the crude extract and its subsequent fractions showed mild to excellent activities in the antifungal bioassay with maximum antifungal activity against *M. canis* (87%) by the chloroform fraction followed by the ethyl acetate (71%) and *n*-butanol (70%) fractions.

Keywords: Teucrium royleanum, *antibacterial and antifungal activity*

Introduction

The recognition of verocytotoxin (VT)-producing *Escherichia coli* (VTEC) as etiological agents of diarrhoea represents one of the most important and exciting recent advances in the field of enteric infections. Not only do VTEC rival non-typhoidal *Salmonellae* and *Ampylobacter* species as the most frequent causes of diarrhoea in some geographical settings [1,2], but a significant risk of two life-threatening complications, hemorrhagic colitis and the hemolytic uremic syndrome (HUS) [3], makes VTEC infection a public health problem of serious concern.

Pseudomonas aeruginosa is an opportunistic pathogen. It rarely causes disease in healthy persons. In most cases of infection, the integrity of a physical barrier to infection (e.g., skin, mucous membrane) is lost or an underlying immune deficiency (e.g., neutropenia, immunosuppression) is present. Adding to its pathogenicity, this bacterium has minimal nutritional requirements and can tolerate a wide variety of physical conditions. The pathogenesis of pseudomonal infections is multifactorial and complex. *Pseudomonas* is both invasive and toxigenic and causes respiratory tract, CNS, otitis media, eye infections, bone and joints infections, GIT, UTI, endocarditis and skin infections.

Salmonella typhi is a flagellated, gram-negative bacillus belonging to the family Enterobacteriaceae responsible for typhoid fever, which is a prolonged bacteraemic, systemic illness with minimal, at least initially, diarrhoea [7]. Bacteria of the genus of *Salmonella* can produce a wide range of infections in humans, including gastroenteritis, typhoid fever, bacteraemia and localized infections, other than an asymptomatic carrier state. [8,9]. Multidrug-resistant (MDR) *S. typhi* (resistant to chloramphenicol, ampicillin, and trimethoprim-sulphamethoxazole) and isolates with reduced susceptibility to fluoroquinolones (indicated by resistance to nalidixic acid) are known. Alternative synthetic drugs are reasonably effective but quite expensive and have many side effects [10,11].

Plant background

Species of the *Teucrium* genus are bitter, astringent, antirheumatic herbs that reduce inflammation, stimu-

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late the digestion and have been used as herbal medicines for coughs and asthma since ancient times. Several studies concerning bacteriostatic, spasmolytic, antioxidant, antinflammatory and antifungal activity of *Teucrium* species have been reported in the literature [12–15]. As a folk medicine, *Teucrium royleanum* is used as an antispasmodic, astringent, antipyretic, and for skin rashes [16]. The constituents of *Teucrium montanum* are montanins A, B, C and D, teucrins A and E from *Teucrium chamaedrys*, 6-ketoteuscordin and teuscordinin from *Teucrium scordium* [17] and from *Teucrium fruticans*, neo-clerodanes, namely 11-hydroxyfruticolone, deacetylfruticolone and 6- acetyl-10- hydroxyteucjaponin B [18].

Interest in the phytochemical exploration of *Teucrium royleanum* began in 1992 when Mashooda Hassan isolated triterpenoids, aliphatic, steroidal and acidic compounds, a mixture of triaocontane and hentriacontane, lupeol and β-amyrin, hexacosianic acid and stigmastatrien-3-ol [19]. The plant extract displayed outstanding inhibition on acetylcholinesterase and butyrylcholinesterase [21]. Keeping in mind its uses as folk medicine, though deserved, this plant has not been extensively subjected to phytochemical and pharmacological investigation. The present study is the continuation of our previous work [22,23].

Methods and materials

Plant material

Teucrium royleanum (Labiatea) as the whole plant was collected from Dir district, N.W.F.P (Pakistan) in June-August 2004 and identified by Professor Dr. Jehandar Shah, Plant Taxonomist, Vice Chancellor University of Malakand, Chakdara Dir. A voucher specimen (CA-013) has been deposited in the herbarium of the University of Malakand.

Extraction

The shade dried plant material was chopped into small pieces and finally pulverized into a fine powder. The plant material (12 kg) was soaked in methanol with occasional shaking, at room temperature. After 15 days, the methanol-soluble materials were filtered off. The filtrate was concentrated under vacuum at low temperature (40°C) using a rotary evaporator when a blackish crude extract (350 g) was obtained.

Fractionation

The crude methanolic extract (350 g) was suspended in distilled water (500 mL) and sequentially partitioned with *n*-hexane (3 × 500 mL), chloroform (3 × 500 mL), ethyl acetate (3 × 500 mL) and *n*-butanol (3 × 500 mL) to yield *n*- hexane (41 g), chloroform (83 g), ethyl acetate (88 g), *n*-butanol (54 g) and aqueous (67 g) fractions, respectively.

Antibacterial activity

The crude extract along with fractions were screened against various human pathogens including *Escherichia coli, Bacillus subtilis, Klebsiella pneumonae, Shigella flexenari, Staphylococcus aureous, P.* aeruginosa and *S. typhi* by the agar well diffusion method [20]. In this method, 10 mL aliquots of nutrients broth (Sigma-Aldrich, Germany) were inoculated with the test organism and incubated at 37°C for 24 h. Using a sterile pipette, 0.6 mL of the broth culture of the test organism was added to 60 mL of molten agar, which had been cooled to 45°C, mixed well and poured into a sterile Petri dish (for the 9 cm Petri dish, 0.2 mL of the culture was added to 20 mL of agar). Duplicate plates of each organism were prepared. The agar was allowed to set and harden and the required number of wells were dug in the medium with the help of a sterile metallic cork borer ensuring proper distribution of the wells in the periphery and one in the center. Agar plugs were removed. Stock solutions of the test samples at a concentration of 1 mg/mL were prepared in sterile dimethyl sulfoxide (DMSO) and 100 μL and 200 μL of each dilution was added in their respective wells. The control well received only 100 μL and 200 μL of DMSO. Imipinem was used as standard drug. The plates were left at room temperature for 2hr to allow diffusion of samples then incubated face upwards at 37°C for 24 h. The diameter of the zones of inhibition was measured to the nearest mm (the well size also being noted).

Antifungal activity

Similarly the antifungal activity was evaluated by the agar tube dilution method [20]. The samples at a concentration of 24 mg/mL were dissolved in sterile (autoclaved) dimethyl sulfoxide (DMSO, Merck), which served as stock solution. Sabouraud dextrose agar (SDA, Sigma-Aldrich, Germany) was prepared by mixing 32.5 g sabouraud, 4% glucose agar and 4.0 g of agar-agar in 500 mL distilled water and the mixture was mixed thoroughly with a magnetic stirrer. Then 4 ml aliquots were dispensed into screw cap tubes, which were autoclaved at 120°C for 15 min and then cooled to 15°C. The non-solidified SDA media was mixed with stock solution (66.6 μL) giving a final concentration of 400 μg of the extract per ml of SDA. Tubes were then allowed to solidify in the slanted position at room temperature. Each tube was inoculated with a piece (4 mm diameter) of inoculums removed from a seven days old culture of fungi for non-mycelial growth; an agar surface streak was employed. Other media supplemented with dimethyl sulfoxide (DMSO) and reference anti-fungal drugs

Table I. Anti bacterial activities of crude extract & various fractions of *Teucrium Royleanum*.

Name of Bacteria	Zone of Inhibition of standard (Imipinem) 10 μg / mL	Crude Extract		$CHCl_3$ Fraction		EtOAc Fraction		BuOH Fraction	
		Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)
E. coli,	30	16.00	53%	11.00	36.6%	23.00	76.6%	5.00	16.6%
B. subitilis	33	13.00	39.3%	4.00	12.1%	14.00	42.4%	Nil	Nil
S. aurous	33	9.00	27.2%	Nil	Nil	Nil	Nil	Nil	Nil
S. flexenari	24	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
P.aerugenosa	24	15.00	60%	10.00	41.6%	17.00	70.8%	6.00	25%
S. typhi	25	15	60%	24.00	85.7%	25.00	100%	11	45.8%

Crude extract and fractions were used at a dose of 3 mg/mL.

served as negative and positive control respectively. Inhibition of fungal growth was observed visually after 7-days of incubation at 28 ± 1°C. Humidity (40-50%) was controlled by placing an open pan of water in the incubator.

Results and discusion

As shown in Table I the crude extract displayed moderate activity against *E.coli* (53%), both *P. aeruginosa* and *S. typhi* showed good activity (60%), *S. aureous* (27.2%) and *B. subtilis*, (39.3%). No activity was displayed against *S. flexenari*.

The chloroform fraction showed excellent activity against *S. typhi* (85.7%) and low activity against *P. aeruginosa* (41.6%), *E. coli* (36.6%) and *B. subtilis* (12.1%). No activity was shown against *S. aureous* and *S. flexenari*. The ethyl acetate fraction showed outstanding activity against *S. typhi* (100%) and excellent activity against *P. aeruginosa* (70.8%), *E. coli* (76.6%) and low activity against *B. subtilis (*42.4%) No activity was displayed against *S. aureous* and *S. flexenari*. While the *n*-butanol fraction showed low activity against *P. aeruginosa* (25%), *E. coli* (16.6%) *and S. typhi* (48.8%), against *S. aurous, B. subtilis, S. flexenari* it exhibited no activity.

Anti-fungal activities of the crude extract and various fractions of the *Teucrium royleanum* were evaluated for activity against *Trichophyton longifusus, Candida albicans, Aspergilusflavus, Microsporum canis, Fusarium solani, and Candida glaberata,* in comparison with miconazole and amphotericin-B, The results of the antifungal activities are presented in Table II.

The crude extract displayed moderate antifungal activity against *T. longifusus* (50%) and low activity against *M. canis* (40%). While the crude extract showed no activity against, *F. solani, C. albicans, A. flavus* and *C. glaberata*.

The chloroform fraction showed an excellent result against *T. longifusus* (87%), while against *M. canis* it showed moderate activity (53%) but no activity against *C. albicans, F. solani, A. flavus, C. albicans, A. flavus and C. glaberata* (Figure 2).

The ethyl acetate fraction also showed excellent antifungal activity against *T. longifusus* (71%) and low activity against *M. canis* (40%), but no activity against *A. flavus, C. glabera, C. albicans* and *F. solani*. The *n*-butanol fraction was found inactive against *T. longifusus*, *M. canis*, *A. flavus*, *C. glabera*, *C. albicans* and *F. solani*.

As presented in Figure 1, the chloroform and ethyl acetate fractions of *Teucrium royleanum* showed an outstanding activity against *S. typhi* (100%), which is

Table II. Antifungal activities of crude extract & various fractions of *teucrium royleanum*.

Name of fungi	-Ve Control Linear Growth (mm)	Crude Extract		$CHCl_3$ Fraction		EtOAc Fraction		BuOH Fraction		Standard Drugs	
		Linear Growth (mm)	Inhibition (%)	Linear Growth (mm)	Inhibition (%)	Linear Growth (mm)	Inhibition (%)	Linear Growth (mm)	Inhibition (%)	Name	μL/mL
T. longifusus	100	50	50%	13	87%	29	71%	30	70%	Miconazole	65
C. albicans	100	100	0%	100	0%	0	0%	100	0%	Miconazole	110.8
A. flavus	100	100	0%	35	0%	100	0%	100	0%	Amphotericin - B	24
M. canis	100	60	40%	47	53%	60	40%	100	0%	Miconazole	97
F. solani	100	100	0%	100	0%	100	0%	100	0%	Miconazole	85
C. glaberata	100	100	0%	100	0%	100	0%	100	0%	Miconazole	100.8

Crude extract and fractions were used at a dose of 24 mg/mL.

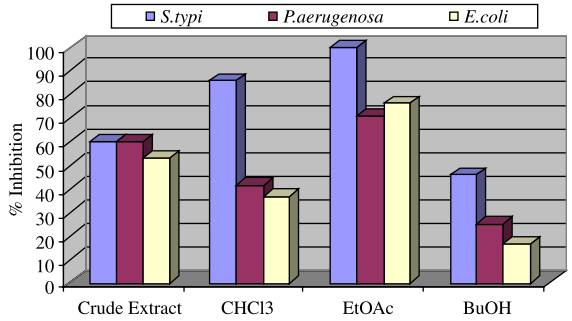

Figure 1. Antibacterial activity of the crude extract and fractions of *Teucrium royleanum* at 3 mg/mL.

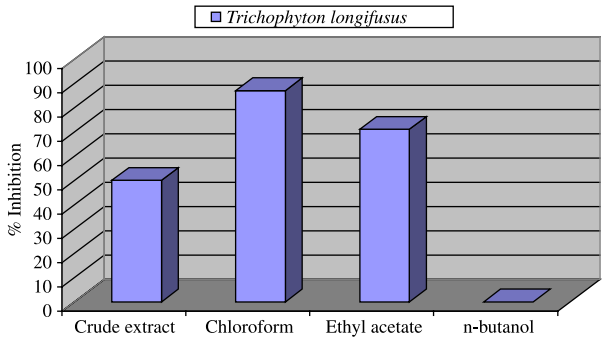

Figure 2. Antifungal activity of the crude extract and fractions of *Teucrium royleanum* at 24 mg/mL.

a facultative intracellular pathogen that causes diseases ranging from self-limiting enteritis to typhoid fever. Development of new effective and safe products for the treatment of typhoid fever urgently needed. Therefore, this plant species could be an excellent natural source for the treatment of typhoid fever and a potential target for the activity-guided isolation of active constituents in order to explore the mechanism of action and relevant uses in the indigenous system of medicine.

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