



The comparison of antibacterial effect of *Schrophularia striata* Boiss. and *Stachys schtschegleevii* Sosn. extracts on pathogens isolated from urinary tract infections

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ABSTRACT

Background & Aim: The present study was aimed to evaluate and compare the antibacterial activity of *Schrophularia striata* Boiss. and *Stachys schtschegleevii* Sosn. Extracts against seven clinical isolates. The test isolates were *Streptococcus agalactiae*, *Enterococcus faecalis*, *Staphylococcus saprophyticus*, *E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Enterobacter dissolvens*. *E. coli* (ATCC 25922) and *S.aureus* (ATCC 29213) were used as quality control strains.

Experimental: Clinical isolates were identified using standard microbiological methods. The methanol extracts from the aerial parts of these plants were assessed using agar well diffusion and broth microdilution methods. Considering the wide application of ciprofloxacin in treatment of Urinary Tract Infections (UTIs), susceptibility test was performed towards antibiotic.

Results & Discussion: The methanol extracts of *Schrophularia striata* and *Stachys schtschegleevii* exhibit both high bacteriostatic and bactericidal activities. The Minimum Inhibitory and Bactericidal Concentration (MIC and MBC) values of *Schrophularia striata* extract ranged between 0.39-12.5 mg/ml and 0.78-25 mg/ml, respectively. The extract of *Stachys schtschegleevii* was effective in MIC and MBC ranges of 1.56-12.5 mg/ml and 3.12-50 mg/ml, respectively. On comparing the efficiency of the two extracts, *Stachys schtschegleevii* extract exhibited the highest activity against gram-negative bacteria, whereas, the extract of *Schrophularia striata* inhibited the growth of *Streptococcus agalactiae* and *Staphylococcus* spp.

Industrial and practical recommendations: On considering antibacterial activity of both the extracts, these herbal extracts may treat urinary tract infections caused by some of the test isolates.

1. Introduction

The genus *Scrophularia* (Scrophulariaceae) comprises about 200 species of flowering plants, commonly known as 'figwort' (GRIN, 2011) which are widely distributed in the world, especially in the Mediterranean area (Amiri et al., 2011). In traditional medicines, some species of the genus have been found to possess antibacterial, antifungal (Fernandez et al., 1996; Ahmad et al., 2012a; Tasdemir et al., 2008), antitumor (Tasdemir et al., 2008; Garcia et al., 1998), anti-inflammatory (Diaz et al., 2004), diuretic and cardiac stimulant activities (Ahmad et al., 2012b), and have been used in the treatment of gastrointestinal disorders (Loi et al., 2005). *Scrophularia* genus contains biologically active compounds, e.g., irridoids, irridoids glycosides (Miyase and Mimatsu, 1999; Boros and Stermitz, 1990; Stevenson et al., 2002), phenylpropanoids and terpenoids (Monsef-Esfahani et al., 2010).

Another medicinal plant considered in this study, *Stachys schtschegleevii* Sosn. (Lamiaceae), is one of the 34 Iranian *Stachys* species (Rechinger, 1982; Mozaffarian, 1996). This plant (traditionally named poulk), has been used in infectious, rheumatic, inflammatory and respiratory diseases (Rezazadeh et al., 2005). The antibacterial property of the essential oil and methanol extract obtained from the leaves of *Stachys schtschegleevii* has been assessed (Sonboli et al., 2005; Abichandani et al., 2010). Phytochemical studies on this plant revealed the presence of phenolic compounds (Nazemiyeh et al., 2006), and terpenoids (Sonboli et al., 2005; Norouzi-Arasi et al., 2004).

In respect of high resistance of nosocomial isolates responsible for urinary tract infections (UTIs) to antibiotics, introducing of the new antimicrobial agents is critical in treatment of such infections. UTI is one of the most common infectious diseases worldwide. Moreover, UTIs account for 35- 45% of the nosocomial infections (Kamat et al., 2009). *Escherichia coli* is the most frequent pathogen in acute infections (Beyene and Tsegaye, 2011; Amin et al., 2011). In this study we evaluated and compared the antibacterial activity of methanol extracts of *Scrophularia striata* and *Stachys schtschegleevii* against some of bacterial uropathogens.

2. Materials and Methods

2.1. Plant materials

Scrophularia striata Boiss. and *Stachys schtschegleevii* Sosn. were collected from Shahvar mountain and the Abr Forest (Shahrood, Semnan province, Iran) in April 2014. Voucher specimens (No. 582 and 673) were deposited at the Herbarium of the Research Center for Agriculture and Natural Resources (Shahrood).

2.2. Preparation of the extracts

The aerial parts of these plants washed, dried and powdered (40g). Methanol extracts (200 ml) were concentrated in rotary evaporator (Heidolph, Germany) at 45 °C for 30 min. The dried extracts were stored at refrigerator. Extracts were resuspended in dimethyl sulphoxide (DMSO), (Merck, Germany) to achieve the stock concentration 250 mg/ml. The stock solutions were sterilized using 0.22 µm filters.

2.3. Bacterial isolates

Seven bacterial uropathogens including *Streptococcus agalactiae*, *Enterococcus faecalis*, *Staphylococcus saprophyticus*, *E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter dissolvens* and standard strains *E. coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 29213) were used to assess the antibacterial activity of both the plant extracts. Pathogens were isolated from midstream urine samples of hospitalized patients in Imam Hossein hospital of Shahrood. Urine samples were cultured on 5% blood agar, MacConkey, and Eosin-Methylene blue (EMB) agar (Merck, Germany) using calibrated loops for semi-quantitative method (Beyene and Tsegaye, 2011) and incubated for 24 h at 37°C. Samples with colony count $\geq 10^5$ CFU/ml were considered positive. The isolates were identified using standard microbiological methods (Cheesbrough, 2002).

2.4. Antibacterial assay

2.4.1. Well diffusion method

The well diffusion method was applied to screen preliminary antibacterial activity of methanol extracts (Perez et al., 1990). Using a sterile cotton swab bacterial suspensions (1.5×10^8 CFU/ml adjusted to 0.5 McFarland turbidity standard) were inoculated onto the Mueller-Hinton agar (Merck, Germany) plates. Four wells of 8 mm diameter were punched into the agar plates using a sterile well cutter. Three wells were filled with 50µl each of the extracts dissolved in DMSO (50mg/ml). The fourth well was filled with

50µl Ciprofloxacin (10µg/ml), which served as the standard drug. *E. coli* (ATCC 25922) and *S. aureus* (ATCC29213) were used as quality control strains. The plates were allowed on the bench for 50min for prediffusion of the extracts and incubated overnight at 37C. The experiment was carried out in triplicates and the mean zones of inhibition were regarded as antibacterial activity. All results are expressed as mean \pm standard deviation.

2.4.2. Broth microdilution method

Minimum Inhibitory and Bactericidal Concentrations (MIC and MBC) were evaluated according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Different concentrations of the extracts were prepared as follows: 100, 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 mg/ml. The initial concentration of ciprofloxacin in the first well was 500 µg/ml. The experiment was carried out in triplicate. The isolates containing MBC values 4 µg/ml or more were regarded to be resistant to ciprofloxacin (Chaudhry et al., 1999).

3. Results and discussion

In well diffusion assay, the extract of *Stachys schtschegleevii* was most active against *S. saprophyticus* (19.36 \pm 0.32 mm) while for other isolates the inhibitory zones of extract ranged from 10.6-18 mm, approximately. *S. striata* extract exhibited less inhibitory zones on gram-negative isolates (about 8.8-10.23 mm) when compared to another extract (Table 1).

The methanol extracts of *Schrophularia striata* and *Stachys schtschegleevii* exhibited both high bacteriostatic and bactericidal activities (Table 1). In comparison to *Schrophularia striata* extract, *Stachys schtschegleevii* extract showed the greatest antibacterial effect on *E. coli* and *Enterobacter dissolvens* among gram-negative uropathogens (MIC range 1.56-6.25 mg/ml). Bacteriostatic property of *Stachys schtschegleevii* extract against gram-negative bacteria was significant. The extract of *Schrophularia striata* inhibited the growth of *S. agalactiae* and *Staphylococcus* spp. rather than other isolates (MIC values 0.39-3.12 mg/ml). According to results, *S. saprophyticus* was the most sensitive isolate towards both extracts. The higher MIC values of *Schrophularia striata* extract (6.25-12.5 mg/ml) were found to inhibit

the growth of *E. faecalis* and gram-negative isolates. The highest MIC value for ciprofloxacin was 256 µg/ml against *E. coli*. All the clinical isolates were resistant to ciprofloxacin (MBC \geq 4 µg/ml).

Our results are partially in agreement with previous ones which have proved the antibacterial effects of *Schrophularia striata* and *Stachys schtschegleevii* (Abichandani et al., 2010; Mikailiet al., 2011). The antibacterial property of *Stachys schtschegleevii* extract obtained from the leaves and stems has been known. The methanol extract was the most potent among the extracts and the most active against ampicillin-resistant *E. coli* (MIC =1.56 mg/ml), (Abichandani et al., 2010). In our study *Stachys schtschegleevii* extract showed the greatest antibacterial effect on *E.coli* and *Enterobacter dissolvens* (MIC range 1.56-6.25 mg/ml), so this plant extract might be used to treat UTIs.

In another study, the bioactivity of the oil of *Stachys schtschegleevii* considered by Sonboli et al. (2005) indicated moderate effect on gram-positive bacteria. *S. aureus* was the most sensitive. Whereas gram-negative bacteria, except for *E. coli* were resistant at the concentrations of the oil used (15ml/disc). Similarly, in another study on the antibacterial activity of four *Stachys* species from Iran, methanol extracts were found to be more active against gram-positive bacteria (Saeedi et al., 2008). Antimicrobial activity of *Stachys* species can be attributed to presence of monoterpenes such α -pinene as destroys the cellular integrity of gram-positive bacteria, but gram-negative ones are more resistant (Andrew et al., 1980).

In a research, the antibacterial activity of leaf extract of *Schrophularia striata* was assessed against *E. coli* using microdilution method. In contrast with our study, the MIC and MBC values towards *E. coli* ranged between 32.1 µg/ml and 39 µg/ml (Bahrami and Valadi, 2010). In our study, MIC and MBC of *Schrophularia striata* extract on *E. coli* were 6.25 and 12.5 mg/ml, respectively. The extract of *Schrophularia striata* inhibited the growth of *S. agalactiae* and *Staphylococcus* spp. rather than other isolates (MIC values 0.39-3.12 mg/ml). The extract to concentration of 0.39 mg/ml had greatest inhibition of growth against *S. saprophyticus*. The highest concentration of used extract was 12.5 mg/ml against *Klebsiella pneumoniae*, *Proteus mirabilis* and *Enterobacter dissolvens*.

Table 1. Antibacterial activity of *Schrophularia striata* and *Stachys schtschegleevii* extracts.

M.O	<i>S. schtschegleevii</i> extract			<i>S. striata</i> extract			CP
	IZ (mm)	MIC (mg/ml)	MBC	IZ (mm)	MIC (mg/ml)	MBC	MIC (µg/ml)
<i>E.c1</i>	12.7 ± 0.45	1.56	3.12	9.26 ± 0.25	6.25	12.5	256
<i>K.p</i>	10.6 ± 0.11	12.5	50	8.8 ± 0.55	12.5	25	128
<i>P.m</i>	11.26 ± 0.25	12.5	25	10.23 ± 0.25	12.5	25	8
<i>E.d</i>	14.6 ± 0.3	6.25	12.5	9.61 ± 0.36	12.5	25	64
<i>E.c2</i>	10.4 ± 0.1	3.12	6.25	10.21 ± 1.24	6.25	12.5	0.008
<i>S.sa</i>	19.36 ± 0.32	1.56	3.12	16.2 ± 0.52	0.39	0.78	4
<i>E.f</i>	15.16 ± 0.2	6.25	12.5	11.9 ± 0.52	6.25	6.25	16
<i>S.a</i>	18 ± 0.88	3.12	6.25	14.23 ± 0.15	0.78	3.12	NA
<i>S.au</i>	15.53 ± 1.47	3.12	6.25	16.34 ± 0.65	3.12	6.25	0.31

M.O: Microorganism; *E.c1*: *E. coli*; *K.p*: *Klebsiellapneumoniae*; *P.m*: *Proteus mirabilis*; *E.d*: *Enterobacterdissolvens*; *E.c2*: *E.coli* (ATCC 25922); *S.s*: *Staphylococcus saprophyticus*; *E.f*: *Enterococcus faecalis*; *S.a*: *Streptococcus agalactiae*; *S.au*: *Staphylococcus aureus* (ATCC 29213); IZ: inhibition zone (mean ± SD). The experiment was carried out in triplicate. CP: Ciprofloxacin; NA: Not applicable.

The study on antibacterial activity of the methanol extract of root and aerial part of *Schrophularia striata* was evaluated against *S. aureus*, *E. coli* and *Bacillus cereus* using disc diffusion method. The most effective one was methanolic extract of root against *B. cereus* (inhibition zone = 21 mm), (Safavi et al., 2012). Other species of the genus *Scrophularia* have also been shown to have antibacterial activity (Ahmad et al., 2012a; Tasdemir et al., 2008; Stavri et al., 2006).

Since *Schrophularia striata* and *Stachys schtschegleevii* appear to be most promising, bioassay-guided fractionation which can elucidate their active antibacterial compounds. The antimicrobial activity of *Stachys schtschegleevii* is in relation to the presence of polar compounds, e.g. flavonoids and phenylethanoid glycosides (caffeic acid derivatives), (Saeedi et al., 2008). Moreover the studies on *Scrophularia* genus have shown flavonoids and terpenoids, e.g., caryophyllene, phytol, linalool which are common and responsible for various pharmacological activities in this genus (Pasdaran et al., 2013). Other studies reveal that the components of both *Stachys* and *Scrophularia* genera are responsible for their therapeutic effects, particularly in bacterial infections and inflammation (Azadmehr et al., 2013; Valiyari et al., 2012). The studies indicate that *E. coli* is still the most common cause of UTIs in Iran (Ranjbar et al., 2009). In

addition, coagulase negative *Staphylococcus* spp. are prevalent cause of UTI among gram-positive bacteria (Huebner and Goldmann, 1999). Our study results support traditional uses of these medicinal plants in the treatment of UTIs. As UTIs being a major part of the nosocomial infections, these herbal extracts may treatment acute infection caused by some of the test isolates.

4. Conclusion

In this study, the antibacterial effect of *Stachys schtschegleevii* and *Scrophularia striata* extracts were determined on some of prevalent uropathogens. On considering antibacterial activity of both the extracts, *Stachys schtschegleevii* extract was found to exhibit more activity against gram-negative uropathogens than *Schrophularia striata* extract while the *Schrophularia striata* extract inhibited the growth of *S. agalactiae* and *Staphylococcus* spp to the large extent. These Iranian native plants can be used as an antiseptic agent to eliminate bacterial uropathogens.

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