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Antibacterial activities of *Stachys lavandulifolia* Vahl. extract against eight bacteria

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ABSTRACT

Background & Aim: Interest in alternative medicine and plant-derived medications that affect the "mind" is growing. *Stachys lavandulifolia Vahl* subsp. *lavandulifolia* (Lamiaceae) is widely used in south Anatolia as herbal tea. It is used for the treatment of gastrointestinal and respiratory disorders. The aim of the present study was to investigate the effects of the hydro-alcoholic extract of *Stachys lavanduifolia* Vahl against eight bacteria.

Experimental: The present study was done to determine the antimicrobial activity and minimum inhibitory concentration (MIC) of extract *Stachys lavandulifolia* against human pathogenic bacteria. The antimicrobial effect of ethanolic extracts of *Stachys lavandulifolia* was determined using a deep-well broth micro dilution method on commercially available bacterial strains.

Results: The result indicated that the MICs were observed ranges from 25 to 100 ppm for antibacterial activity of the extract from *S. lavandulifolia* against eight bacteria. The least MIC value was observed against *P. mirabilis* and *E. faecalis.*

Recommended applications/industries: It suggests that the extract from *Stachys lavanduifolia* might be a promising approach for developing new anti-bacterial drugs.

1. Introduction

The genus *Stachys* (Lamiaceae) includes about 200 to 300 species in the world In Iran, 34 species of this genus are present among which, 13 are endemic (Mozaffarian, 1996). *Stachys* species are traditionally used in different conditions: headache, neuralgia, nervous conditions, as tonic at dyspepsia and for treating wounds and skin inflammation as astringent and antidiarrheal (Grieve, 1971). In pharmacological studies, *Stachys* species showed variety of effects, including: anti-inflammatory (Khanavi *et al.*, 2005), antibacterial (Skaltsa *et al.*, 1999; Skaltsa *et al.*, 2003) anti-nephritic (Hayashi *et al.*, 1994), and anxiolitic (Rabbani *et al.*, 2003).

Investigators reported that the extracts or constituents of plants belonging to the genus *Stachys* exert significant anti-inflammatory, antitoxic (Zinchenko *et al.*, 1981), antihepatitis (Savchenko, *et al.*, 1978), antibacterial (Skaltsa *et al.*, 1999), anti-anoxia (Yamahara *et al.*, 1990) and anti-nephritic (Hayashi *et al.*, 1994) effects. As active principals

phenylethanoid glycosides (Nishimura *et al.*, 1991; Miyase *et al.*, 1996) triterpenoids, steroids (Ross *et al.*, 1975; Yamahara *et al.*, 1990), and flavonoids (Zinchenko and Farm, 1970; EL-Ansari *et al.*, 1991) were identified in the genus *Stachys*. The present study was done to determine the inhibitory concentrations of *Stachys lavandulifolia* Vahl extracted against pathogenic bacteria.

2. Materials and methods

2.1. Preparation extracts

Plant material was dried at 35 °C for three days. After drying, the material was ground within industrial grinder. Sample was submerged in 100 ml of 96% ethanol and left to macerate for seven days, after maceration the plant material was filtered and 100 ml 96% ethanol was added to the water extracts to allow faster solvent removal. The solvent was evaporated to complete dryness using a standard Buchi rotary-evaporator. The resulting dry extracts were re-suspended in 5 ml distilled water. In order to determine the real concentration of each extract, 1 ml of previous homogenization of the respective extracts was removed and again completely oven-dried and then weighed to determine amount of extract per ml of final solution. The remaining extract was used for MIC assays.

2.2. Agar disk diffusion assay

The susceptibility of all antibiotics was done using disc diffusion method on Muller-Hinton agar as recommended by CLSI (2012). The procedure followed is briefly described here. *Streptococcus pyogenes* ATCC® 19615™, *Streptococcus pneumoniae* ATCC 49619, *S. saprophyticus* ATCC®1535, *Hafnia alvei* ATCC 51873, *Acinetobacter. baumannii* ATCC 19606, *Enterococcus faecalis* ATCC 29212, *Proteus mirabilis* ATCC 35659, and *Serratia marcescens* ATCC 274 plates were grown overnight on blood agar. 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. ceftazidim (30 µg), erythromycin (15 µg), ceftazidime (30 µg), and tetracyclin (30 µg).

2.3. Antimicrobial assays

Streptococcus *pyogenes* ATCC® 19615™, *Streptococcus pneumoniae* ATCC 49619, *Staphylococcus saprophyticus* ATCC®15305, *Hafnia alvei* ATCC 51873, *Acinetobacte baumannii* ATCC 19606, *Enterococcus faecalis* ATCC 29212, *Proteus mirabilis* ATCC 35659, and *Serratia marcescens* ATCC 274 were used and was sub-cultured on Nutrient agar (Oxoid) and stored at 4 ^{o}C. Following the initial incubation, the organisms were suspended in 10 ml of physiological saline solution and optical density readings were compared to a 0.5 McFarland standard. For the MIC determination, bacterial solutions of 5 × 10^5 colony-forming units (cfu/ml) were employed.

Table1. Antimicrobial activity of the extract and Standard antibiotics

Bacterial	MIC extract plant(ppm)	Antibiotic resistance
S. pyogenes	100	-
S. pneumoniae	100	E, CE, CF
H. alvei	100	E, TE
S. saprophyticus	100	E, CF, TE
A. baumannii	100	CE, TE
E. faecalis	25	E, CE
P. mirabilis	25	E, TE
S. marcescens	100	CE

E: Erythromycin, CE: Cefixime, CF: Ceftazidime, TE: Tetracycline

The antibacterial activity of the plant extract was determined using sterile 2 ml 96-well plates (Wiegand *et al.*, 2008). The 12 wells of each row were filled with 0.5 ml sterilized Mueller Hinton agar. Sequentially, wells 2–11 received an additional 0.5 ml of a mixture of culture medium and plant extract serially diluted to create a concentration sequence from 0.512 ml to 0.008 ml. The deep-wells were incubated for 24 h at 37 °C. The resulting turbidity was observed. After 24 h, MIC was determined to be where growth was no longer visible by assessment of turbidity by optical density readings at 600 nm with a Beckman DU-70 UV-Vis Spectrophotometer. At least three repetitions were run for each assay. The levels of MIC was observed ranges from 25 to 100 ppm.

3. Results and discussion

The selection of plant material for this study was based on ethno-botanical data on the traditional use of the plants in treatment of bacterial diseases, and

conditions classified by the traditional healers as "infection" and "inflammation," the latter characterized by reddening (e.g. in wounds), or internal afflictions causing gastric discomfort. The plant species was initially tested in simple agar-bioassays, which included plants that are used or other purposes by the local population (Bussmann *et al*., 2007; Bussmann *et al*., 2008; Bussmann *et al*., 2009).

Table 1 shows the antibacterial activity of *Stachys lavandulifolia* Vahl against *P. mirabilis* and *E. faecalis*. The MIC concentrations ranged from 25 to 100 ppm. The very high values in many species indicate only a very limited antibacterial efficacy. Recent findings indicate that some natural phenolic compounds found in plants have an anti-biofouling effect on biofilm formation by Gram-negative bacteria (Jagani *et al*., 2009). There has been no large scale systematic investigation into the relationship between bacterial inhibition and total phenolic content of spices and herbs. The anti-biofouling activities of 14 selected phenol and natural phenolic compounds were tested against *Pseudomonas aeruginosa*, using a micro titer-plate (Shan *et al*., 2005). Anti-*Candida* activity of ethanolic extracts of endemic medicinal herbs against *Candida albicans* has been studied (Rohi *et al*., 2012). A study by Mahzooni-kachapi et al. (2012), the *S. lavandulifolia* Vahl oil at that concentration showed moderate activity against the two tested microorganism, but gram-negative microorganism, *E. coli*, was more sensitive to oil than other one. Whereas activity detected against the examined Gram-positive microorganism, *S. aureus*, was found to be less sensitive to the oil (Mahzooni-kachapi *et al*., 2012). Essential oils from 13 plants indicated anti-*Candida* activity; one of them is *Stachys byzantina*. The extracts of *Stachys byzantina*, *S. inflata*, *S. lavandulifolia* and *S. laxa* exhibited concentration-dependent activity against Gram-positive microorganisms (Duarte *et al*., 2005). Kursat et al. (2009) reported that the extract of *S. lavandifolia* did not show any antimicrobial activity against *B. megaterium*, and *Trichophyton* spp. while the extract showed antibacterial activity against *P. aeruginosa*, *K. pneumoniae*, *S. aureus*, *C. albicans*, *C. globrata* and *Epidermophyton* sp.

4. Conclusion

The high concentration of active constituents in *S. lavandulifolia Vahl.* revealed antimicrobial activity in this study and in the previous reports on other Stachys species.Simple agar assays alone are not reliable for determin-ing the efficacy of plants used in traditional medicine. The present study however confirms that simple laboratory methods are indeed well suited for the initial assessment of the efficacy of traditionally used medicinal plants in inhibiting bacterial growth. A comparison to the traditional uses also indicated that local knowledge can provide im-portant leads for the development of new drugs: Our hy-potheses that plants traditionally used as antibacterials do have a much higher incidence of efficacy than other me-dicinally used species, and that plants used for cleansing baths are more likely to have antibacterial properties than other species used for ritual purposes could be verified. An efficacy analysis of these mixtures, as well as assays to evaluate the toxicity of single species as well as mixtures would be an interest-ing comparative study.

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