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Inhibitory Effects of Sage Extract on the Growth of Enteric Bacteria

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Abstract: Antibacterial activity of Sage extract at concentrations of 0.1, 0.05, 0.025, 0.0125, 0.00625, 0.003125, 0.00156, 0.0005 and 0.00025 g dL^{-1} against *Salmonella typhi*, *Shigella sonnei*, *S. flexneri*, *Proteus vulgaris*, *Staphylococcus aureus*, ETEC *Escherichia coli* and *Pseudomonas aeruginosa* was evaluated. Susceptibility testing of bacterial strains against 18 antibiotics was also performed for comparison. The results showed that *P. aeruginosa* and ETEC *E. coli* were completely resistant to Sage extract even at concentration of 0.1 g dL^{-1}. Its antibacterial activity (0.1 g dL^{-1}) against *P. vulgaris*, *S. flexneri* and *S. sonnei* was the same as nitrofurantoin and ampicilline respectively. Sage extract (0.1 and 0.05 g dL^{-1}) exhibited the same effects as ampicilline and streptomycin against *S. typhi*. Its antibacterial activity (0.1, 0.05 and 0.25 g dL^{-1}) against *S. aureus* was the same as ceftazidim, chloramphenicol, gentamycin, neomycin and nitrofurantoin and was more significant compared to streptomycin and vancomycin. The results suggest Sage can be considered as an alternative herbal in the treatment of infections caused by the above-mentioned bacteria.

Key words: Sage, antibacterial activity, enteric bacteria

INTRODUCTION

Historical records indicate that, as long as 2,600 years ago, the Chinese already developed drugs using a number of forms of phytotherapeutic agent elaboration. This was also the case with the Egyptians, Greeks and Romans (Pereira *et al*., 2004). Salvia is an important genus consisting of about 900 species in the family Lamiaceae. Some species of Salvia have been cultivated worldwide for use in folk medicine and for culinary purposes (Imanshahidi and Hosseinzadeh, 2006). Sage (*S. officinalis*), *S. leriifolia*, *S. haematodes*, *S. triloba* and *S. divinorum* are some species with important pharmacological effects (Imanshahidi and Hosseinzadeh, 2006). The essential oil extracted from *S. officinalis* has antibacterial activity due to the presence of 1, 8-cineol and an antifungal substance (Pereira *et al*., 2004).

The leaves of Sage are well known for their anti-oxidative properties (Hohmann *et al*., 1999; Baricevic and Bartol, 2000), applicable to the area of human health (Pearson *et al*., 1997). The plant is reported to have a wide range of biological activities, such as anti-bacterial, fungistatic and virustatic effects (Miladinović, 2000; Horiuchi *et al*., 2007; Glamoclija *et al*., 2006; Lawless, 2002; Sivropoulou *et al*., 1997).

The anti-microbial properties as well as the tannins' based astringent activities of *S. officinalis* (active ingredient of dental-care herbal medicinal preparations) benefit the reduction in plaque growth, the inhibition of gingival inflammation and have positive effects on caries prophylaxis (Willershausen *et al*., 1991).

Furthermore, due to the anti-viral activity of its water and alcohol extracts, *S. officinalis* is included as an active ingredient also in combined plant preparations for the treatment of acute and chronic bronchitis, officially approved for clinical use in Bulgaria (Manolova *et al*., 1995). The antimutagenic potential of Sage extracts was demonstrated on *Escherichia coli* repair proficient strains (Baricevic and Bartol, 2000; Filipic and Baricevic, 1998). Some constituents of the plant, such as the triterpenes oleanolic and ursolic acids or the diterpene carnosol, were shown to present anti-inflammatory properties or related biological activities (Baricevic *et al*., 2001).

Nevertheless, the anti-inflammatory activity of *S. officinalis* and the role of these components in the anti-phlogistic action of the plant are not yet clearly defined.

In light of the recent emergence of bacteria which are resistant to multiple antimicrobial drugs, posing a g dL^{-1}challenge for the treatment of infections, the need to discover new antimicrobial substances for use in combating such microorganisms becomes patent (Pereira *et al*., 2004). Recently, antimicrobial activity of *Salvia officinalis* (Sage) leaves was showed against vancomycin-resistant enterococci (Horiuchi *et al*., 2007).

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Horiuchi *et al.* (2007) isolated the effective compound from the extract and identified it as carnosol, one of diterpenoids. Carnosol showed a weak antimicrobial activity and greatly reduced the MICs of various aminoglycosides (potentiated the antimicrobial activity of aminoglycosides) and some other types of antimicrobial agents in VRE.

In the present study, *in vitro* antibacterial activity of *S. officinalis* extract on growth of *S. typhi*, *S. flexneri*, *S. sonnei*, *P. vulgaris*, *S. aureus*, ETEC *E. coli* and *P. aeruginosa* was evaluated.

MATERIALS AND METHODS

Sage was obtained in the form of dry plant. The extract was made using Perculation method and was diluted in methanol at concentrations of 0.1, 0.05, 0.025, 0.0125, 0.00625, 0.003125, 0.00156, 0.0005 and 0.00025 g dL^{-1}. In order to prepare sage discs, blank discs were dipped into different sage dilutions and kept refrigerated for 24 h. Bacterial suspensions were prepared by inoculating 2-5 bacterial colonies into test tubes filled with physiologic saline and incubating at 37°C for 3-5 min. After incubation, their opacities were compared with that of 0.5 Mac Farland's to ensure that the amount of tested bacteria was proper.

In well diffusion method, after inoculating of the bacterial strains on culture media, holes were filled with antibiotic or sage solutions in separate culture media. Following on from that, they were incubated at 37°C for 18-24 h.

In disc diffusion method, culture media were loaded with antibiotic or sage containing discs. Annular zone diameters were measured following 18-24 h of incubation.

RESULTS AND DISCUSSION

Comparison of results showed that sage extract did not have antibacterial activity against *P. aeroginosa* (Table 1). Further more, sage extract had no effect on bacterial growth in the case of ETEC *E. coli*. Its antimicrobial activity (0.1 g dL^{-1}) against *P. vulgaris* was the same as nitrofurantoin. Antibacterial activity of sage extract (0.1 g dL^{-1}) against *S. flexneri* and *S. sonnei* was the same as ampicillin. Sage extract (0.1 and 0.05 g dL^{-1}) exhibited the same effects as ampicillin and streptomycin against *S. typhi*. Its antimicrobial activity (0.1, 0.05 and 0.25 g dL^{-1}) against *S. aureus* was the same as ceftazidim, chloramphenicol, gentamicin, neomycin and nitrofurantoin and was more significant compared to streptomycin and vancomycin. All the procedures mentioned above were done two more times and the same results were achieved.

Herbal traditions have been passed down and refined with scientific understanding, providing information to assist in health maintenance. Approximately 25% of all prescription drugs are derived from trees, shrubs, or herbs. Their increasing use in recent years is an evidence of a public interest in having alternatives to traditional medicine (Shirazi *et al.*, 2007).

In the present study, sage extract exhibited no activity against *P. aeroginosa* that is similar to the results of a study carried out by Pereira *et al.* (2004). Unlike to that study in which 96.2 % of *E. coli* samples were susceptible to essential oil extracted from sage, here no antibacterial activity against ETEC *E. coli* was observed.

In another study, Hammer *et al.* (1999) investigated 52 plant oils and extracts including sage oil for antibacterial activity against some gram positive and

Table 1: Average inhibition zone diameters of sage in well diffusion and disc diffusion methods

		Average zone diameter (mm)						
Methods	Concentration (g dL^{-1})	*S. typhi*	*S. sonnei*	*S. flexneri*	*P. vulgaris*	*S. aureus*	ETEC *E. coli*	*P. aeroginosa*
Disc diffusion method	0.10	12	12	11	12	20	7	5
	0.05	10	10	9	8	19	0	0
	0.025	8	8	7	0	16	0	0
	0.0125	6	0	0	0	14	0	0
	0.00625	0	0	0	0	12	0	0
	0.003125	0	0	0	0	9	0	0
	0.00156	0	0	0	0	8	0	0
	0.0005	0	0	0	0	7	0	0
	0.00025	0	0	0	0	6	0	0
Well diffusion method	0.10	17	16	14	16	30	11	8
	0.05	12	12	10	8	27	6	6
	0.025	9	9	8	0	20	0	0
	0.0125	6	5	5	0	18	0	0
	0.00625	5	0	0	0	14	0	0
	0.003125	0	0	0	0	12	0	0
	0.00156	0	0	0	0	10	0	0
	0.0005	0	0	0	0	8	0	0
	0.00025	0	0	0	0	7	0	0

gram negative bacteria including *E. coli*, *P. aeruginosa*, *S. typhi*, *S. aureus* and other bacteria. They found that sage did not inhibit any organisms at the highest concentration.

Furthermore, Koga *et al.* (1999) examined sage essential oil for bactericidal activity against a range of gram-positive and gram-negative bacteria by viable count determinations. Generally, gram-positive bacteria showed higher resistance to sage essential oil than gram-negative bacteria. However in our study the antibacterial activity of sage extract against *S. typhi* and *S. aureus* was comparable with antibiotics. In other studies the antibacterial activity of sage oil against the strain INRA L2104 of Bacillus cereus (Valero and Salmeron, 2003) and *Helicobacter pylori* (O'Mahony *et al.*, 2005) were also demonstrated.

Finally, we concluded that antibacterial and anti inflammatory activities of sage provide hope that it can form the basis for an alternative therapeutic agent in the management of infections.

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