African Journal of Microbiology Research Vol. 5(19), pp. 2909-2911, 23 September, 2011
Available online http://www.academicjournals.org/ajmr
DOI: 10.5897/AJMR11.276
ISSN 1996-0808 ©2011 Academic Journals

Full Length Research Paper

Antibacterial activity of plant extracts against oral and skin pathogens

Majid Alipour* and Omid Khanmohammadi

Department of microbiology, Islamic Azad University (IAU) - Babol Branch, Babol, Iran.

Accepted 23 June, 2011

The aim of this study is to examine the antibacterial activity of plant extracts. Both broth dilution and disc diffusion methods were used to assess the antibacterial activity of these extracts against skin and oral pathogens. *Eryngium caucaseum* Trautv, *Eryngium bungei* Boiss and *Adiantum capillus-veneris* have shown antibacterial activity against *Streptococcus pyogenes*, *Streptococcus mutans*, *Staphylococcus aureus* and *Streptococcus sanguis*. *E. caucaseum* Trautv showed the highest inhibition zone (> 30 mm) against *S. pyogenes.* The growth of *S. pyogenes* was remarkably inhibited by the ethanolic extract of the three plant extracts.

Key words: *Adiantum capilis–veneris*, *Eryngium bungei* Boiss, plant extracts.

INTRODUCTION

Plants and plant extracts have been used for the treatment of skin disorders for centuries. Because of increasing resistance to antibiotics of many bacteria, plant extracts are of new interest as antiseptics and antimicrobial agents. *Eryngium caucaseum* Tratuv and *Eryngium bungei* Boiss are found in northern Iran, especially in the Mazandaran Province. The leaves of these two plants are used for flavouring cooked vegetable in different local foods; moreover, leaves of these plants are used in soup or mixed with Yoghurt (Khoshbakht et al., 2007). The *Adiantum capillus*-veneris is found in the Mazandaran province. The local people prepare the leaves of this plant as an infusion and use to treat colds, cough, bronchitis, flu, pneumonia and urinary tract infections. The aerial part of this plant has showed antibacterial activity (Mahmoud et al., 1989). There is increasing interest in using natural plants on resident oral bacteria and as antibiotic for eradication pathogenic bacteria. Dental caries is associated with microorganisms present on the tooth surface in dental plaque but only the presence of *Streptococcus mutans* has been linked with the causation of dental caries (Aliviano et al., 2008; More et al., 2008). Staphylococcal skin infections are common because they are nearly always present on the skin. Most bacterial infections of the skin are caused by two bacteria, *Staphylococcus aureus* and *Staphylococcus pyogenes.* The *S. pyogenes* can be carried asymptomatically, and can cause common illness such as pharyngitis, impetigo and scarlet fever.

S. pyogenes is also associated with the post-infection sequelae of rheumatic fever and acute glomerulonephritis (Mitchell, 2003; Cogen et al., 2008). The objective of this study is to find plants that are used both as food material and as antibacterial agent.

MATERIALS AND METHODS

The leaves of *E. caucaseum*, *E. bungei* and *Adiantum capillus* - veneris were collected from the Mazandaran Province, northern Iran. The leaves were washed with distilled water, shade dried, powdered and stored in an air-tight container separately for further use.

Preparation of ethanol extracts

100 g of each powdered plant material was soaked separately into 200 ml of ethanol in a conical flask with rubber corks and left for 24 h undisturbed. The resulting liquid was filtered (Whatman Grade No. 3 filter paper). The extraction was repeated four times. The

*Corresponding author. E-mail: alipourrna@yahoo.com. Tel: 98-0111-3265231.

Table 1. Antibacterial activity of various plant extracts by disk diffusion method.

Botanical name	Diameter of zone inhibition (mm)			
	S. *pyogenes*	***S. mutans***	***S. aureus***	***S. sanguis***
Eryngium caucaseum	30	12	8	-
Eryngium bungei Boiss	20	10	-	14
Adiantum capillus-veneris	25	8	-	-

Note: No inhibition zone; ND: not done.

Table 2. Minimum inhibitory concentration (MIC in mg/ml) of plant extracts on different bacteria.

Botanical name	MIC (mg/ml)			
	S. *pyogenes*	***S. mutans***	***S. aureus***	***S. sanguis***
Eryngium caucaseum	12.5	50	ND	ND
Eryngium bungei Boiss	50	ND	ND	50
Adiantum capillus-veneris	12.5	ND	ND	ND

Note: ND: not done.

filtrate was collected and concentrated by the rotary evaporator at 45°C for ethanol elimination. The aqueous residue was then placed in an oven 45°C for about 48 h to remove the water. The final product was dissolved in 1% DMSO (dimethyl sulfoxide) to yielding a concentration of 400 mg/ml for subsequent experimentation (Vaijayanthimala et al., 2000). The solvent was used as the negative control (1% aqueous DMSO).

Bacteria and culture condition

The strains *S. mutans* PTCC1601, *s. aureus* ATCC22923, *S. pyogenes* PTCC1447 and *Streptococcus sanguis* PTCC1449 were used for antibacterial activity. All strains were cultured on blood agar, brain heart infusion agar (BHI agar) or broth.

Screening the extracts for antibacterial activity

The disc-diffusion assay was used to determine the growth inhibition of bacteria by the plant extracts (Nostro et al., 2000). The extracts were checked for their antibacterial activity by using disc diffusion method. The bacteria were cultured at 37°C and prepared to turbidity equivalent to 0.5 McFarland standard (McFarland, 1907). Then 100 µl of the suspension was spread on the BHI agar or blood agar. The sterile discs were impregnated with 20 µl of the plant extract and placed on the surface of the test plate. Penicillin G (10 units) was used as positive control, and Disc saturated with DMSO was used as negative control. The diameters of the inhibition zones were measured in millimeters (mm). All extracts and controls were tested in duplicate and the experiment was repeated twice (Rani and Khullar, 2004).

Determination of minimum inhibitory concentration (MIC)

The ethanolic extracts that showed antibacterial activity were later tested to determine the minimum inhibitory concentration for each bacterial sample. The MIC of the extracts against the individual test organisms were determined by the macrodilution broth antibacterial assay. Two-fold serial dilutions of the extracts (400 mg/ml) were made in brain heart infusion broth (BHI) to achieve a concentration range of approximately 12.5 to 200 mg/ml (w/v). The inoculums for each tested strain was adjusted to the turbidity of 0.5 MacFarland (~ 1.5×10^8 cfu/ml). Afterward, 100 µl were added to the test tubes (2 ml). The tubes were incubated at 37°C for 24 h in a 5% CO_2 atmosphere and observed for the presence or absence of growth. The lowest concentrations which did not show any growth (turbidity) of tested organisms after cultural evaluation were determined as the MIC expressed in milligram per milliliter (mg/ml). The control experiments were performed only with sterile DMSO (Lodwin et al., 1998; Okoli et al., 2002).

RESULTS

The various plants have been examined but *E. caucaseum* Trautv, *E. bungei* and *A. capillus-veneris* have shown antibacterial activity against *S. pyogenes*, *S. mutans*, *S. aureus* and *S. sanguis* using a disc diffusion method on agar (Tables 1 and 2). *E. caucaseum* showed the highest inhibition zone (> 30 mm) against *S. pyogenes*. The inhibitory effect of *E. caucaseum* was also found against *S. mutans* and *S. aureus*. The growth of *S. pyogenes* and *S. aureus* were inhibited by *E. bungei* Boiss. In addition, ethanolic extract of *E. bungei* Boiss showed antibacterial activity against *S. sanguis* (> 14 mm). Ethanolic extract of *A. capillus-veneris* showed antibacterial activity against *S. pyogenes* and *S. aureus*.

DISCUSSION

Previous studies have reported that *Eryngium billarderi* possesses a significant anti-inflammatory activity. The

aerial parts of this plant extract are not only eaten but also applied to wound (Yesilada et al., 1989). *E. billarderi* roots have been used for maturation abscess (Ozcelik et al., 2004). Other studies have also indicated that ethanol extract *Eryngium* species showed anti-inflammatory activity (Kupeli et al., 2006). *E. bungei* Boiss is locally called Anarigeh. This is the first report on antibacterial activity of *E. bungei* Boiss against oral and skin pathogens. The ethanolic extracts show better antibacterial activity than aqueous (Arias et al., 2004). In this study, the growth of *S. pyogenes* and *S. aureus* was remarkably inhibited by the ethanolic extract of the three plants. These results are significant because a clinically identical infection (pyoderm) can be caused by *S. aureus* and *S. pyogenes*. In some cases, *S. aureus* can cause skin infections alone, and in some other both *S. pyogenes* and *S. aureus* contribute in skin infection. The *A. capillus*-veneris is traditionally used to treat infectious diseases (Singh et al., 2008). Compounds derived from *Adiantum lunulatum* have been shown antibacterial activity against gram positive and negative bacteria (Reddy et al., 2001). In one study, it was observed that *A. capillus-veneris* is active against *S. aureus* (Banerjee and sen, 1980). The results of current investigation are consistent with previous reports. The treatment of skin infection with these plant extracts is suitable because antibiotic theraphy has side effect on patients. The extracts of the three plants has shown antibacterial activity against *S. pyogenes*; therefore, instead of using penicicllin can be used *A. capillus- veneris* as infused and extracts of *Eryngium* spp can be consumed with yogurt or soup.

Rheumatic fever is an inflammatory disease that occurs following an *S. pyogenes* (Group A streptococcus) infection. Prevention of recurrence is achieved by eradicating the acute infection and prophylaxis with antibiotics. Therefore, these plant extracts can be used as alternative drug and possibly for eradication of *S. pyogenes* in carriers. The extracts of *Eryngium* spp have showed antibacterial activity against *S. mutans* and *S. sanguis*. Therefore, these extracts can be used for decreasing these bacteria from the surface of the teeth. These results show that extracts of the three plants possess antibacterial activity that could be the basis for their medicinal use.

ACKNOWLEDGEMENT

We thank Reza Mohammadizadeh Astalaki for excellent technical assistance.

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