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The Antimicrobial Activity of Elderberry (*Sambucus nigra* L.) Extract Against Gram Positive Bacteria, Gram Negative Bacteria and Yeast

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Abstract: The methanolic extract of *Sambucus nigra* L. berries was studied for its *in vitro* antimicrobial activities through the Agar Dilution Method. The extract of the elder berry inhibited the growth of *Bacillus subtilis*, *Staphylococcus aureus* (ATCC15156), *Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli*. Also, growth of *Candida albicans* was inhibited by crude extract of *Sambacus nigra* berries. The Minimum Inhibitory Concentration (MIC) of the extract ranged from 0.625-15 μg mL^{-1}. The phytochemical analysis of the crude extract of this medicinal plant revealed the presence of anthocyanin, vitamins A and C a well as a good source of calcium, iron and vitamin B6. It also contains sterols, tannins and essential oils and can readily be considered as a healthy food. However, more evidence is needed to really sustain any claim related to their medicinal values. The results of the present study suggest that *Sambucus nigra* L. can be used in treating diseases caused by the test organisms. It can also be used as a strong food preservative.

Key words: Antimicrobial activity, elder berry, pathogenic microorganisms, inhibitory activity, Iran

INTRODUCTION

Nature is a valuable source of medicinal plants. In Iran, almost all plants have therapeutic properties and the application of medicinal plants, especially in traditional medicine is currently well acknowledged and is established as a viable profession (Amin, 1991). Preparation of extracts which contain bioactive compounds from medicinal plants permits the demonstration of their physiological activity. It also facilitates pharmacological studies which lead to the production of more potent drugs with reduced toxicity (Ebana *et al*., 2001; Pamplona-Roger, 1999; Manna and Abalaka, 2000). However, these complementary components give the plant as a whole a safety and efficiency much superior to those of its isolated and pure active components (Shariff and Shariff, 2001). There is therefore the need to search much more for plants of medicinal value and their medicinal applications. The World Health Organization (WHO) along with other national authorities now recognizes the antimicrobial resistance in both medicine and agriculture as a major emerging problem of public health importance.

In traditional medicine, elderberries were used for their diaphoretic, laxative and diuretic properties (Manganelli *et al*., 2005; Merica *et al*., 2006). In fact, they were utilized in order to treat various illnesses such as sinus congestion, stomachache, sore throat, constipation, diarrhea, common cold and rheumatism (Manganelli *et al*., 2005; Novelli, 2003). The plants flowers are said to have diaphoretic, anti-catarrhal, expectorant, circulatory stimulant, diuretic and topical anti-inflammatory actions (Merica *et al*., 2006). Some of these properties result from the elderberry fruits, containing viburnic and acid tannins both known to have a positive effect on nasal congestion, diarrhea and improvement of respiration (Novelli, 2003). Leaves and inner bark have also been used for their purgative, emetic, diuretic, laxative, topical emollient, expectorant and diaphoretic actions (Merica *et al*., 2006).

Elderberry medicinal potential comes from its antioxidant components. Elderberry is believed to have major health benefits due to its anthocyanins which is absorbed and significantly increase the plasma antioxidant capacity (Cao and Prior, 1999; Netzel *et al*., 2002).

Probably, the most interesting properties of elderberry extracts were reported by Zakay-Rones *et al*. (1995). Following the earlier researches done by Konlee (1998), a mixture containing elderberry extract had an

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inhibitory effect on haemagglutinin found in mycovirus. More studies conducted by Barak *et al.* (2001, 2002) have shown that such a mixture could inhibit the replication of 11 strains of the influenza virus and increase the cytokines production. The aim of this study is to determine the antimicrobial activity of *Sambacus nigra* berry extract against some gram positive bacteria, gram negative bacteria and yeast.

MATERIALS AND METHODS

Collection of plant materials: Elder berry was collected at the plains and jungles of Malayer, Hamadan, Iran. The plant was duly authenticated at the College of Agriculture of Shiraz University, Shiraz, Iran.

Source of microorganisms: The organisms used in this study were *Bacillus subtilis*, *Escherichia coli* (RITCC 2310), *Pseudomonas aeruginosa*, *Staphylococcus aureus* (ATCC 15156), *Candida albicans* (clinical: isolated from oral lesions caused by candidiasis) and *Salmonella typhi* (CT18). The organisms were obtained from the Department of Pathobiology, College of Veterinary Medicine, Shiraz University, Shiraz, Iran.

Standardization of microorganisms: Exactly 0.2 mL of overnight pure cultures of each organism was dispensed into 20 mL of sterile nutrient broth and was incubated for 3-5 h at 37°C to standardize the cultures to 10^6 cfu mL^{-1}. A loop full of the standard cultures was used for the antimicrobial assay.

Extract preparation: The method of Okogun (2000) was used to obtain the berry extract. The 50 g of freeze-dried berry were extracted with 200 mL of the solvent (in the ratio of 9:1 mL distilled methanol:water, respectively). Extraction was allowed to proceed for 48 h. The extract was decanted and the solvent was then removed by evaporation at the room temperature (28±2°C) in order to obtain the extract. The air dried extract was stored for 48 h in sterile universal bottles at the room temperature. The sterility of the extract was tested before use.

Phytochemical screening of crude extracts: The phytochemical components of the medicinal plants were screened for determining the presence and amounts of anthocyanin, vitamins A and C and calcium, iron, vitamin B6, sterols, tannins, saponins, phenols, saponin glycosides and essential oils by standard methods.

Screening for antibacterial activity: The method utilized by Collins *et al.* (1995) was employed to test the antimicrobial activity of the elderberry extract. The 0.2 g of the extract was reconstituted in 5 mL sterile distilled water and was vortexed for homogeneity. Then, 1 mL of the reconstituted extract was added to Petri dishes which have sterile molten nutrient agar (Oxoid) in order to make a final concentration of 2000 μg mL^{-1}. The plates were prepared in duplicates and were set at the room temperature. A loop full of the standardized culture of test organisms was streaked on the solidified medium and was incubated for 24 h at 37°C. The control plates comprising extract without inoculum and inoculum with extract were made in parallel.

Determination of Minimum Inhibitory Concentration (MIC) of the extract: The MIC of the elder berry extract was determined on a solid medium (Nutrient agar) using the method utilized by Collins *et al.* (1995). Different concentrations of the prepared extract ranging from 0.5-5.0 μg mL^{-1} were incorporated into the nutrient agar medium followed by the application of a standardized number of cells to the surface of the agar plate. The growth was assessed after the incubation for a defined period of time (16-20 h) at the temperature of 37°C and then the MIC value was read.

RESULTS

Phytochemical screening of active fractions of the extracts: The results of the phytochemical screening of the active fractions in the extracts of *S. nigra* revealed the presence of anthocyanin, vitamins A, C, B6 and sterols. Also, calcium, iron and tannins were detected in active fractions of elderberry extract. There were no saponin, saponin glycosides and phenols were found in its extract (Table 1).

Antimicrobial activity of the crude extracts: The results revealed that the crude extract of the elderberry exhibited antimicrobial effects on all test organisms. The extract inhibited the growth of *B. subtilis* and *S. aureus*. Nevertheless, it proved to be more effective on inhibiting the growth of *Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli*. However, it mostly inhibited strongly the growth of *Candida albicans*.

Table 1: Phytochemical components of elder berry extract

Phytochemical components	Presence in elder berry extract
Saponin glycosides	-
Anthocyanin	+
Vitamin A	+
Vitamin C	+
Vitamin B6	+
Phenol	-
Sterols	+
Calcium	+
Iron	+
Tannins	+

- = Absent; + = Present

Table 2: The Minimum Inhibitory Concentration (MIC) of *S. nigra* extract for gram positive and gram negative bacteria

Bacteria/Yeast	MIC (μg mL^{-1})
Staphylococcus aureus	5.000
Bacillus subtilis	5.000
Pseudomonas aeruginosa	3.320
Escherichia coli	2.700
Salmonella typhi	1.900
Candida albicans	0.625

The Minimum Inhibitory Concentration (MIC) of the extract: The MIC of *S. nigra* extract for *S. aureus* and *B. subtilis* was 5 μg mL^{-1} whereas, it was 0.625 μg mL^{-1} for *C. albicans*. The extract inhibited the growth of *P. aeruginosa*, *E. coli* and *S. typhi* at the concentrations of 3.32, 2.7 and 1.9 μg mL^{-1}, respectively (Table 2).

DISCUSSION

The crude extract of *Sambacus nigra* L. berries studied was found to contain one or more of the following phytochemical compounds: anthocyanin, vitamins A and C, calcium, iron and vitamin B6. It also contains sterols, tannins and essential oils. Other investigators (Schwarz *et al*., 2001; Bermudez-Soto and Tomas-Barberan, 2004; Proestos *et al*., 2005) have reported the presence of these components in other members of the family such as Combretaceae and Myrtaceae to which the plant used in the present study also belong.

The inhibitory effects of this medicinal plant on the microorganisms may therefore, be due to the presence of the above phytochemical components. The results of the present study showed that the crude extracts of *S. nigra* inhibit the growth of *Candida albicans*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli* very well. This means that the extract has strong effects on these organisms.

The Minimum Inhibitory Concentration (MIC) for *Bacillus subtilis* is the same as of *Staphylococcus aureus* meaning that equal doses of antimicrobial agents will be required in infections where *B. subtilis* is the etiologic agent. The MIC value for *Candida albicans* was 0.625 μg mL^{-1} suggesting that a very small amount of the drug can inhibit the growth of the organism. Thus, *S. nigra* is highly potent against *C. albicans*.

The results of the present study also showed the presence of anthocyanin, vitamins A and C, calcium, iron and vitamin B6. Also, the occurrence of tannins in elderberry extract shows that the plant may be useful in various industries. For example, tannin is useful in food, pharmaceutical and leather industries as well as agriculture (Nguji, 1988; Dalziel, 1995). It could also be used as expectorants and decongestants.

CONCLUSION

Sambucus nigra L. has been found to be effective against some important pathogenic microorganisms involved in wounds, burns, skin infections and some microorganisms involved in enteritis, typhoid and candidiasis. Thus, the extract of *Sambacus nigra* L. berries can be used in order to treat of these ailments. The extract of the plant proved to be active against *Staphylococcus aureus* and *Bacillus subtilis* at low concentration and against C. *albicans* at very low concentrations. It is however, more effective against *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi*.

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