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Research Article

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Biological activities of extract prepared from *Sorbus sibirica* fruit

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ABSTRACT

In this paper, we investigated the biological activities of extract prepared from Sorbus. sibirica fruits using various in vitro assays. It possessed antibacterial and antifungal activities as determined by growth inhibition assay. Among the eight different microorganisms tested, Staphylococcus aureus was most sensitive while Aspergillus niger was least sensitive to inhibition. In addition to antimicrobial activity, the extract also inhibited the growth of cancer cells, such as HeLa and BGC823 cells. At the molecular level, S. sibirica fruit extract substantially protected both protein and DNA against oxidation-induced damage and loss caused by Fenton's reagent, in which the principle oxidative agent is hydrogen peroxide. In conclusion, this preliminary study showed that S. sibirica could be considered as a potential preservative for application in food production.

Key words: antimicrobial, anticancer, *Sorbus sibirica*, DNA damages, protein damages

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INTRODUCTION

Sorbus sibirica is a small arbor originated from New Siberia, Russia [1-2]. It is distributed in the upper stream of the Boolean river in China. The fruits of *S. sibirica* contain abundant edible vitamins, including ascorbic acid (200mg /100g), Carotene (18mg/100g), VP, sorbitol, organic acid, VC. The content of total flavonoids is about 0.25% to 0.35%[3-4]. *S. sibirica* fruits are popularly consumed as tea, and are believed to relieve coughing. Therefore, *S. sibirica* is considered as a valuable natural resource with the potential for greater application, especially in the food sector.

To our knowledge, the antimicrobial, anticancer and antioxidant activities of *S. sibirica* have not been investigated. Our preliminary work has focused on the antioxidant properties of the ethanol extract from *S. sibirica* fruits, which included anti-lipid peroxidation capability, and superoxide anion radical-scavenging activity, hydroxyl radical-scavenging activity, and reducing power. The extract exhibited strong scavenging capability with respect to hydroxyl and superoxide anion free radicals, and effectively inhibited the lipid peroxidation of polyunsaturated fatty acids. Hence, the present study examined the total antioxidant activity of *S. sibricia* fruit extract and its ability to protect DNA and protein against free radical-induced damages, as well as its antimicrobial and anticancer activities[5-7]. The results suggested that *S. sibirica* fruits could offer a promising source of antisepsis agent that may have the potential to be used as a food additive.

EXPERIMENTAL SECTION

Sorbus sibirica was cultivated in Xinbin, Liaoning province in China. The fruits were harvested in October 2011 and dried in a 50^{o}C oven for 24 h[8].

Whole dry *S. sibirica* fruits (100 g) were crusted into smaller pieces using a mortar and pestle. Ten grams of the crusted fruit was soaked in 100 ml of 60% ethanol for 24 h and then sonicated three times, each for 1 h at 60-70□.

The mixture was filtered through Whatman No 1 paper (Whatman, Maidstone, UK) and the ethanol in the filtrate was removed with a rotary evaporator (CCA-1110; Eyela, Tokyo,Japan). The residual extract was freeze dried, and the powder which was designated as SsFE (*S. sibrica* fruit extract) was used in subsequent experiments. It was stored at 4^{o}C until used[8-9].

Microorganisms[10]: *Escherichia coli* LNU102, *Bacillus thuringiensis* LNU103, *Staphylococcus aureus*LNU104 , *Bacillus gasoformans*LNU107, *Bacillus subtilis*LNU BZ, *Saccharomyces cerevisiae*LNU201, *Penicillium chrysogenum*LNU307 *and Aspergillus niger* LNU308were obtained from the strain preservation center in Liaoning University . These strains were used to test the antimicrobial activity of the extract prepared from the fruits of *S. sibirica*.

Hela cells and BGC823 cells (from Life Science College of Liaoning University) were cultured in DMEM medium supplemented with 10% FBS and 100 U/ml penicillin and 100 mg/ml streptomycin[11-14].

The effect of *S. sibirica* extract on protein oxidation and DNA damage were carried out according to the method of Hu et al[15-17], performed with the image analysis software Quantity one (Bio-Rad; Hercules, CA).

RESULTS AND DISCUSSION

Antibacterial activities of the extracts

The extract prepared from *S. sibirica* fruits was able to inhibit the growth of a variety of bacteria by plate culture analysis, with the strongest inhibition against *Staphylococcus aureus* (Fig. 1). It also inhibited the growth of the fungus *Aspergillus niger*, although the inhibition was the weakest among the eight strains of microorganisms tested. Thus it appeared that *S. sibirica* fruit extract possessed both anti-bacterial and anti-fungal activities, demonstrating a broad spectrum of anti-microbial activity.

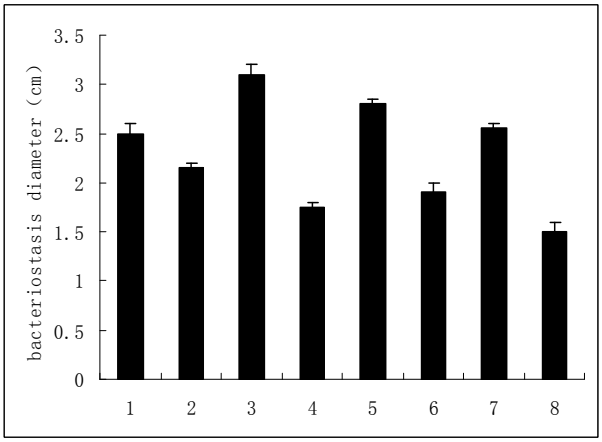


Figure 1. Antimicrobial activity of *S. sibirica* fruit extract

The numbers (1-8) represent the different microorganisms: 1, Escherichia coli ; 2, Bacillus thuringiensis ; 3, Staphylococcus aureus; 4, Bacillus gasoformans; 5, Bacillus subtilis; 6,Saccharomyces cerevisiae; 7, Penicillium chrysogenum; 8, Aspergillus niger

Effect of S. Sibirica fruit extract on BGC 823 and HeLa cells

S. sibirica fruit extract also inhibited the growths of two cancer cell lines, BGC 823 and HeLa cells with similar intensity (Figs. 2&3). In the case of BGC 823 cells, around 15% of the cells was inhibited by 250 μM SsFE (the highest concentration used) after 24 h of treatment time, compared to around 40% and 55% after 48 h and 72 h of treatment time (Fig. 2). There was a sharp increase in the percentage of inhibition at 100 μg/mL SsFE after 72-h treatment time, whereas the increase in inhibition was more gradual after 48-h treatment time. A sharp increase in inhibition at 100 μg/mL SsFE was also observed for the inhibition of HeLa cells in both 48 h- and 72 h-treatment times. Maximum inhibition of HeLa cell growth achieved with the highest concentration of SsFE was around 50% for both 48 h- and 72 h- treatment times (Fig. 3). For 24 h-treatment time, there was essentially no increase in inhibition beyond an SsEF concentration of 100 μg/mL. Both results showed that 72 h and 250 μg/mL would be the most effective exposure time and SsFE concentration, respectively, to achieve 50% inhibition of the growths of these two cancer cell lines.

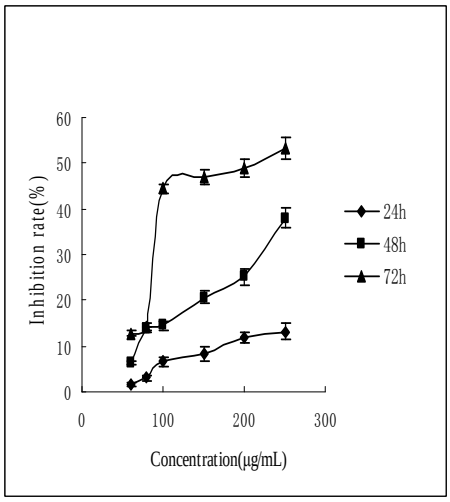


Figure 2. Effects of time and concentration of *S. sibirica* fruit extract on the growth of BGC 823 cells

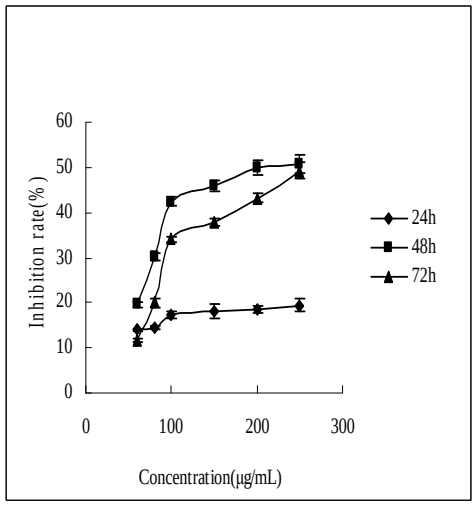
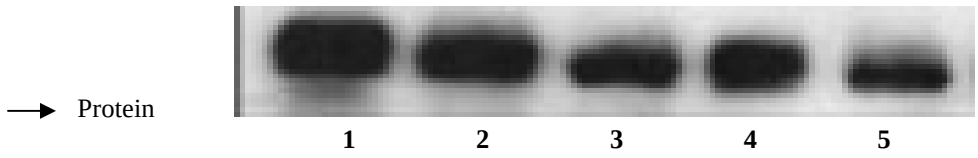


Figure 3. Effect of time and concentration of *S. sibirica* fruit extract on the growth of HeLa cells

Protection against protein damage

The protective effect of *S. sibirica* fruit extract against protein damage was investigated by determining the extent of protein loss caused by the Fe^{3+}/H_2O_2/ascorbic acid system (Fenton reagent) in the presence of SsFE. The loss of BSA was most pronounced (80%) when it was incubated with Fenton reagent only (land 5, Fig. 4). SsFE and BHA were both able to reduce the loss of protein caused by Fenton reagent. More protection was achieved by SsFE at the higher concentration (5 mg/mL) used, resulting in just 20% protein loss, compared to 50% loss when a lower concentration of 1 mg/mL was used. SsFE appeared to be less effective than BHA under the same concentration, but the result clearly demonstrated the protective effect of SsFE against protein damage caused by oxidation.

A



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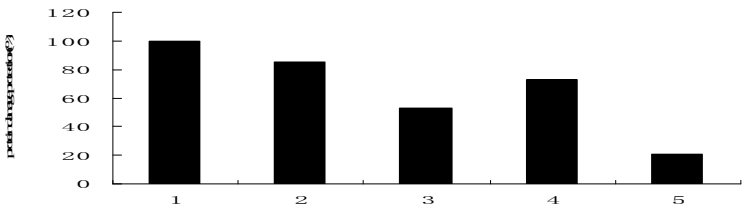

Figure 4. Effect of *S. sibirica* fruit extract on oxidation-induced protein damage. (A) SDS-PAGE showing the loss of BSA after treatment with Fenton's reagent (Fe^{3+}/ascorbate/H_2O_2 system) in the absence or presence of SsFE or BHA. (B) Histogram comparing the protection afforded by SsFE and BHA against Fenton's reagent-induced protein loss determined by densitometry. BSA incubated without Fenton's reagent (lane 1), with Fenton's reagent plus 5 mg/mL SsFE (lane 2), with Fenton's reagent plus 1 mg/mL SsFE (lane 3), with Fenton's reagent plus 1 mg/mL BHA (lane 4), or with Fenton's reagent only (lane 5)

Protective effect on hydroxyl radical-induced DNA damage

In addition to protecting protein against oxidative damage and loss, *S. sibirica* fruit extract also protected DNA against damage and loss induced by Fenton reagent (Fig 5). However, the protective effect afforded by SsFE was weaker than BHA, even at the higher concentration (10% versus 30 % loss of DNA, lane 3 versus lane 4 in Fig. 5). At the lower concentration, SsFE provided little protection against Fenton-induced DNA damage. Nevertheless, the protective effect of *S. sibirica* fruit extract against oxidation-induced DNA damage was obvious from the experimental result.

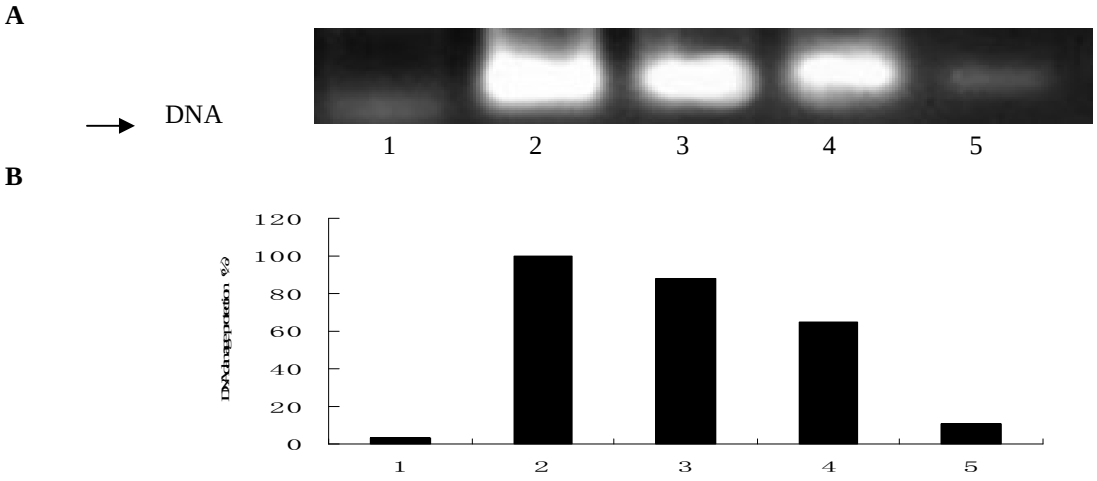

Figure 5. Effect of *S. sibirica* fruit extract on oxidation-induced DNA damage. (A) Agarose gel electrophoretic showing the loss of DNA after treatment with Fenton's reagent (Fe^{3+}/ascorbate/H_2O_2 system) in the absence or presence of SsFE or BHA. (B) Histogram comparing the protection afforded by SsFE and BHA against Fenton's reagent-induced DNA loss determined by densitometry. Genomic DNA of HEK 293 cells incubated with Fenton's reagent only (lane 1), without Fenton's reagent (lane 2), with Fenton's reagent plus 1 mg/mL BHA (lane 3), with Fenton's reagent plus 5 mg/mL SsFE (lane 4), or with Fenton's reagent plus 1 mg/mL SsFE (lane 5)

CONCLUSION

The current study which investigated the biological activities of extract prepared from *S. sibirica* fruits highlights a promising potential of this species of plant to provide renewable bioproducts with the following desirable bioactivities: antibacterial, antifungal, antitumorigenic and antioxidative activities. The ease at which the bioactive components can be extracted from the fruits of this plant using environmentally benign solvents (e.g., ethanol, water) may also offer additional benefit for large scale extraction and application.

Acknowledgements
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