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Biological Activity of *Saussurea amara* (L.) DC.

Shagjjav Oyungerel
National University of Mongolia, sh_oyungerel@yahoo.com

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Sh. Oyungerel

Abstract

The medicinal application of specific plants over the long period in traditional medicine, suggests the presence of biologically active substances in those plant species. *Saussurea amara* (L.) DC. is a wild plant with a high feeding value because of its high protein content, vitamins, and minerals. *S. amara* is used in the traditional Mongolian medicine for the treatment of hepato-biliary disorders. The goal of this study was to evaluate the antioxidant and antimicrobial activities of *S. amara*. The antioxidant capacity of a methanol extract of plants was evaluated by analysing its DPPH free-radical scavenging activity and reducing power and determination of total phenolic compounds. In the experiment of DPPH, the IC_{50} for the commercial standard BHA was 4.4±0.41 µg/ml. *S.amara* has a weak antioxidant activity by DPPH, FRAP and TPC respectively that, the IC_{50} value was 346 ± 6.03 µg/ml, 12.90 ± 0.42 µg equivalent to 1 µg BHT and 49.03 ± 0.81µg equivalent to 1 µg pyrocatechol. Antimicrobial assays of the test extract were performed on three clinical microorganisms, including Gram-positive (*Staphylococcus aureus* KCTC 3881), Gram-negative (*Escherichia coli* KCTC 1039) bacteria and one fungus (*Candida albicans* KCTC 7965). *S. amara* has two antibacterial (*S. aureus, E. coli*) activities. *S. amara* has two antibacterial (*Staphylococcus aureus, E. coli*) activities by the DCM fraction with an inhibition zone of *S. aureus* of 38 ± 0.2 mm, and that of *E. coli* was 14 ± 0.1 mm. The water fraction of *S. amara* did not show not any antibacterial effect. The results implicate, that *S. amara* has a relatively high antibacterial activity and that continuative studies on its natural compounds would be promising.

Keywords: *Saussurea amara*, medicinal plant, antioxidant activity, antimicrobial activity

1. Introduction

Over the years, the medicinal plants have been investigated, recently in the scientific food field. Recently, there has been an upsurge of an interest in the therapeutic potential of plants, as antioxidants in reducing free radical-induced tissue injury. The medicinal application of specific plants used for long time in traditional medicines, suggests the presence of biologically active substances in plant species (KLETTER et al. 2008, VINAY et al. 2010). Several pathogenic microbes, especially Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*, cause several human infectious diseases. Despite reports on the development of several new antibiotics each year, microbes with antibiotic resistance capacities are evolving daily by creating serious challenges in health care settings all over the world. The most successful antibiotics that have been applied to combat diseases are a small molecule and secondary metabolites including penicillin molecule that were originally isolated from the fungi. Therefore, the novel antibiotics against the specific pathogenic bacteria need to be urgently explosed in order to meet the rate of evolution of a super pathogen (BABITA et al. 2008). Plants are an important source of potentially useful structures for the development of new chemotherapeutic agents (PALOMBO et al. 2001). Many reports are a variable on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants (SAMY et al. 2000, STEPAMOVIC et al. 2003, TONA et al. 1998). Therefore, in the present study, a considerable attention has been directed towards the identification of antioxidant and antimicrobial activities.

2. Material and method

S. amara was collected from Mongolia (N 48°11′319′′/E 106°44′262′′) in August 2011 (fig. 1) and taken to Korea Polar Research Institute in Incheon of Korea for the study.

***Morphology*:** Herbs 9-70 cm tall and perennial. Stem solitary, 3-7 mm in diam., erect, branched, and usually wingless. Corolla pale purple, rarely white, 1.3-1.8 cm, gland-dotted, tube 8-10 mm, limb 5-7 mm, lobes 3-5 mm. Achene brown, cylindrical, 3-3.5 mm, 4-ribbed. Pappus dirty white; outer bristles 1-5 mm; inner bristles 1.2-1.7 cm. Flowers and fruits: Jul-Oct. 2n = 26. ***Growth conditions*:** Wastelands, by trails, forest steppes, steppes, saline and alkaline soils, dikes of streams, dunes, river and lakeshores, sandy soils; 500-3200 m.

***Distribution*:** Gansu, Hebei, Heilongjiang, Henan, Jilin, Liaoning, Mongol, Ningxia, Qinghai, Shaanxi, Shanxi, Xinjiang (Kazakhstan, Kyrgyzstan, Mongolia, Russia, Tajikistan, Uzbekistan; E Europe);
(http://www.efloras.org/flora-taxon.aspx? flora_id=2&taxon_id).

***Significance*:** It is a wild plant with a high feeding value for its high protein content, vitamins, and minerals. This plant plays an important role in keeping the ecological balance and molding landscape characteristic of saline and alkaline lands (SONG et al. 2002). These plants are exposed to several environmental stresses such as drought and salinity, which could adversely affect the plant growth and production (KANG WANG et al. 2011). *S. amara* is used in the traditional Mongolian medicine for the treatment of hepato-biliary disorders. To determine the plant's effect on the bile-salt independent bile flow (hydrocholeresis) as a measure of liver exocrine functions, different extracts were investigated in the isolated rat liver perfusion system (GLASL et al. 2006).

Fig. 1: *Saussurea amara* (L.) DC., (photo: CHAN KIM, 2010).

A. Extraction and purification of active compounds

A freeze-dried sample (20 g) was extracted in a methanol-water mixture (70:30) at room temperature. The solvent was evaporated under vacuum at 45° C and finally freeze-dried. The test sample was stored at -20°C until the further use.

B. Screening for biological activity

Antioxidant assays: following antioxidant assays are performed to analyse the antioxidant activity of natural compounds.

***1. DPPH free-radical scavenging assay*:** the free-radical scavenging activity of the M5 extract was estimated by using a previously described method (BLOIS 1958). One ml of DPPH solution (0.1 mmol/l of DPPH in methanol) was mixed with 3 mL of various concentrations of the test sample. The mixture was incubated at room temperature for 30 minutes and the quantity of reduced DPPH, which formed a yellow colour was measured in terms of absorbance at 517 nm in a UV-Visible spectrophotometer (SCINCO). A reaction mixture without the test sample was used as a negative control and with BHA as a positive control. The experiment was conducted in triplicate.

***2. Reducing power determination*:** the reducing power was determined by a previously described method (OYAIZU 1986). Various concentrations of the test sample (1 ml in methanol) were mixed with phosphate buffer (2.5 ml, 0.2 mol, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] solution (2.5 ml, 1 %) in distilled water. The reaction mixture was incubated at 50°C for 20 minutes. A trichloroacetic acid solution (2.5 ml, 10 %) in distilled water was added and centrifuged (3,000 rpm) at room temperature for 10 minutes. The supernatant (0.5 ml) was mixed with 1 ml of distilled water and 0.5 ml of ferric chloride solution (0.1 % in distilled water). The absorbance was measured at 700 nm in a UV-Visible spectrophotometer to estimate the quantity of Fe^{++}. A reaction mixture without test sample was used as a negative control and with BHT as a positive control. The experiment was conducted in triplicate.

***3. Determination of total phenolic compounds*:** Total soluble phenolic compounds were determined as described previously (SLINKARD and SINGLETON, 1997) using pyrocatechol as a standard. A test sample (1ml) of various concentrations was added to 1 ml of Folin-Ciocaltue reagent and mixed thoroughly. After five minutes of incubation at room temperature, 1 ml of Na_2CO_3 (2 %) was added and allowed to stand for 2 hours with intermittent shaking. The absorbance at 760 nm was measured. The concentration of total phenolic compounds was determined as microgram of pyrocatechol equivalent by comparing with the pyrocatechol standard curve.

C. Antimicrobial assay:

***1. Target microorganisms and cultivation conditions*:** antimicrobial assays of the test extract were performed on 3 clinical microorganisms, including Gram (+) positive (*Staphylococcus aureus* KCTC 3881), Gram-negative (*Escherichia coli* KCTC 1039) bacteria and 1 fungus (*Candida albicans* KCTC 7965). All strains were purchased from Korean Collection Type Culture (KCTC), Deajeon, South Korea. Bacterial strains were grown on a nutrient agar (NA) at 30-37 °C and *C.albicans* was grown on a yeast mannitol (YM) agar at 25°C.

***2. Antimicrobial test*:** the qualitative antimicrobial test was performed by the paper disk diffusion assay as described previously (BHATTARAI et al. 2006) to measure the zone of inhibition of the target microorganism. Sterile paper disk (Adventic, Japan) of 8 mm size were loaded with the sample extract at a concentration of 1 mg/disk in triplicate and allowed to dry at room temperature under sterile conditions. The disks were kept on the surface of NA or YM agar, which had been freshly swabbed with the overnight grown broth culture of the microbial target strain. Plates were incubated at the optimum growth temperature for each strain for 24 hours. Zones of inhibition around the plant extract loaded paper disks were reflective of the antimicrobial effectiveness of the extract.

3. Results and discussion

A. Antioxidant activity of *S. amara*

In the experiment of DPPH, the IC_{50} for the commercial standard BHA was 4.4±0.41 µg/ml. *S. amara* has a weak antioxidant activity (fig. 2) by DPPH, FRAP, and TPC respectively that, the IC_{50} value was 346±6.03 µg/ml, 12.90±0.42 µg equivalent to 1 µg BHT and 49.03±0.81µg equivalent to 1 µg pyrocatechol.

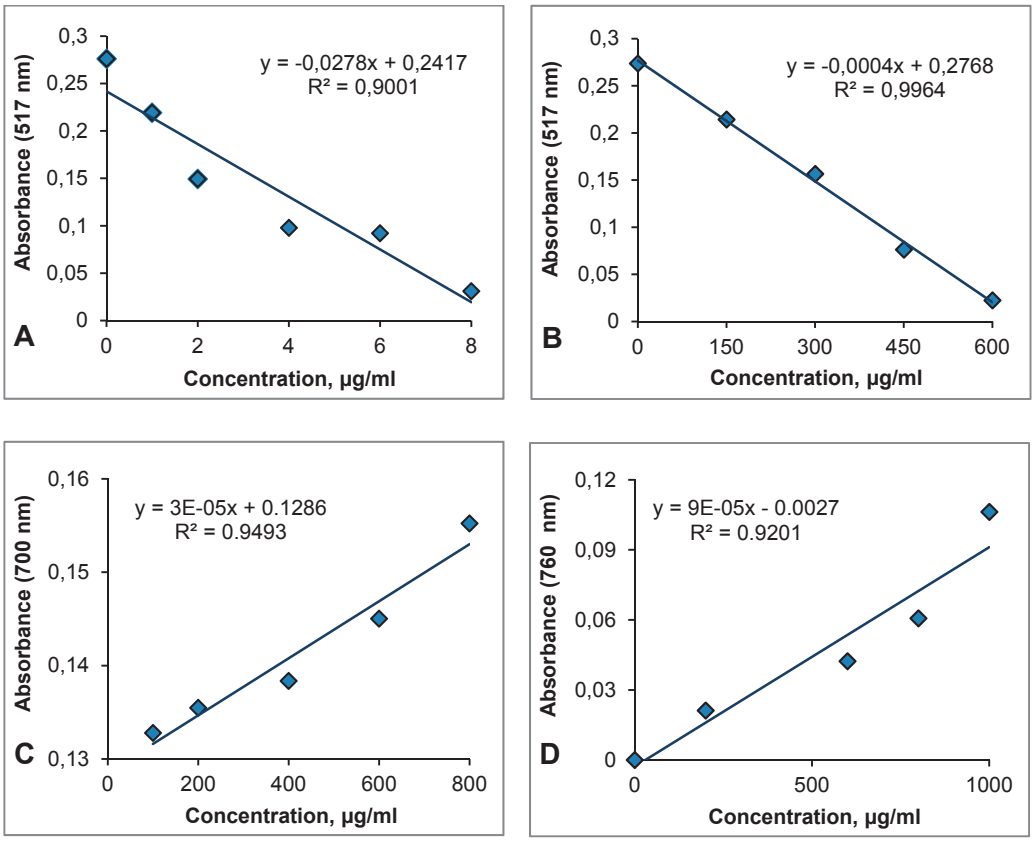

Fig. 2: **A** - DPPH free radical scavenging activity of BHA, **B** - DPPH free radical scavenging activity of *S. amara*, **C -** FRAP of *S. amara*, **D** - TPC of *S. amara.*

B. Antimicrobial activity of *S.amara*

Antimicrobial assays of the test extract were performed on tree clinical microorganisms, including Gram-positive (*Staphylococcus aureus* KCTC3881), Gram-negative (*Escherichia coli* KCTC1039) bacteria and one fungus (*Candida albicans* KCTC7965). We have tested the microbial activity of 2 mg/40 µl methanol extract of *S. amara* and it has two antibacterial (*Staphylococcus aureus, Escherichia coli*) activities. The inhibition zones of *S. aureus* and *E.coli* respectively that, 28 ± 0.2 mm and 16 ± 0.1 mm (fig. 3). This result showed that these plant with the strongest antibacterial activity and need to do a narrow study for purification of its natural compounds.

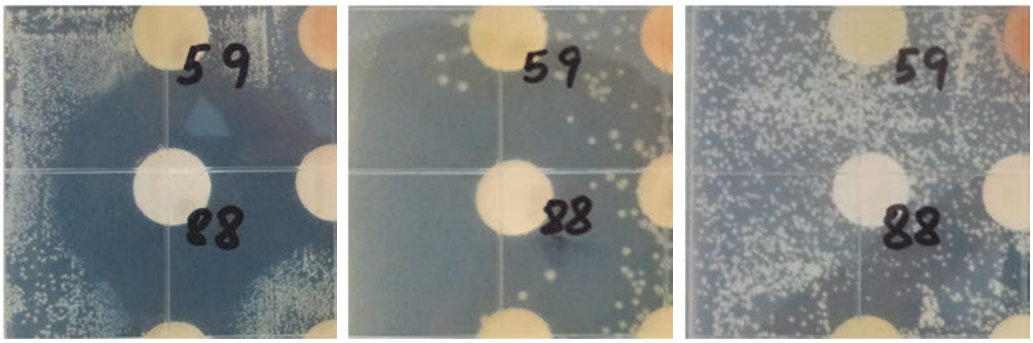

Fig. 3: Antimicrobial activity of *S. amara* (#88). **A** - Anti-*Staphylococcus aureus* activity, **B** - Anti-*Escherichia coli* activity, **C**. - *Candida albicans.*

Antibacterial activity of water and DCM fractions on Staphyloccocus amara

We have separated the water and dichloromethane (DCM) fractions of *S. amara* and have checked the antibacterial activity of 2 mg/40µl extract of both fractions. This result showed that the water fraction of *S. amara* has no antibacterial activity, but the DCM fraction has two antibacterial (*S. aureus, E. coli*) activities. The inhibition zones of *S. aureus* and *E. coli* were 38 ± 0.2 mm respectively 14 ± 0.1 mm (fig. 4).

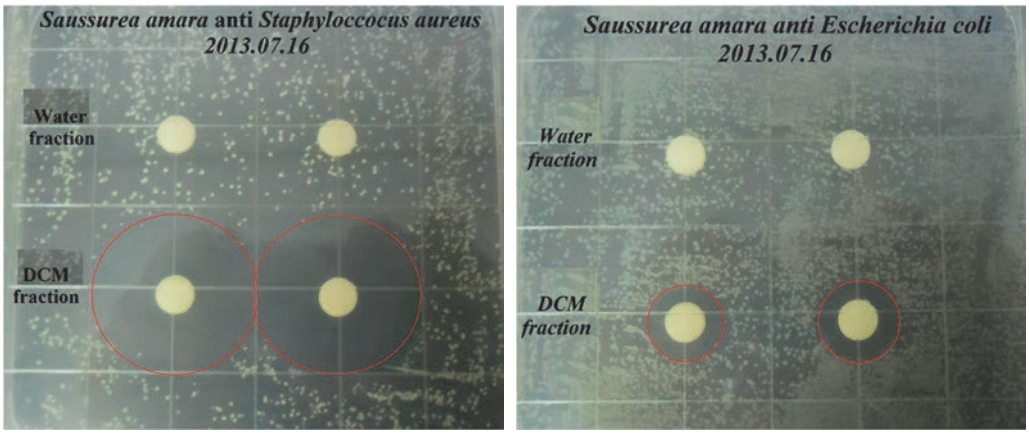

Fig. 4: Antibacterial activity of DCM fractions of *Saussurea amara*.

However, *S. amara* has a weak antioxidant activity, but this sample has two antibacterial (*S. aureus, E. coli*) activities. This result showed that there is no relationship between antioxidant and antimicrobial activities of plants. The screening of plants with high antioxidant values may not identify species with the effective antimicrobial activity because the two parameters are not highly correlated. High antioxidant levels could not predict antimicrobial activity against the tested microorganisms. The plant antioxidant and antimicrobial activities may vary based on time of harvest, storage temperatures and extraction methods. Seasonal changes, environmental factors, and the stage of plant development affect the production and distribution of bioactive constituents in the plant. *O. biennis* L. and *Glyceria grandis* S. Wats. Examples are that showed antioxidant activity above 69,000 TE but showed activity against only a single microbe. Conversely, some plant species with antioxidant levels as low as 7000 to 8000 TE, exhibited large inhibition antimicrobial inhibition zones up to 19 mm, but also against a single microorganism (JOY et al. 2008).

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Address: Oyungerel Shagjjav
Department of Biology, School of Science,
National University of Mongolia, Ulaanbaatar
E-mail: oyungerel@num.edu.mn, sh_oyungerel@yahoo.com