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Short Communication

Antibacterial activity of Licorice (*Glycyrrhiza glabra* L.) root extract

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Abstract

Consumers have recently become significantly more interested in natural products made from plants, largely due to the perception that natural ingredients are safe. Licorice root represents a good source of phytochemicals that could be used as functional ingredients. The principal medical bioactive component of licorice root is glycyrrhizin. Due to the presence of glycyrrhizin, licorice extracts are frequently employed in the pharmaceutical and confectionery sectors. The aim of the current study is to determine the antimicrobial activities of licorice extract in ethanol and water on bacteria by using agar well diffusion method. The ethanolic licorice extract showed significantly higher antibacterial activities against two gram-positive (*Bacillus cereus* and *Staphylococcus aureus*) and two gram-negative (*Escherichia coli* and *Klebsiella pneumoniae*) bacteria.

Keywords: Antimicrobial activity, Glycyrrhizin, Phytochemicals, Yashtimadhu

Highlights

- Antibacterial activity of licorice extract was evaluated against selected microorganisms.
- Ethanolic extract showed high antimicrobial activity ($p<0.05$) against selected bacteria.

In recent years, there has been a lot of interest in the research of medicinal plants due to their antioxidant and antimicrobial properties, which could be used as natural medicine. Plants have been utilized for thousands of years as drugs and remedies for a wide range of illnesses. An important area of research is the regulation of microbial action and redox status in the human body by the characteristics of diet and food components (Dimitrios, 2006). The use of commercial antimicrobial drugs to treat several infectious diseases increased the resistance power against various microorganisms. Oxidative stress is a result of an imbalance between the production of reactive oxygen species (ROS) and the ability of antioxidant defenses (Hazra *et al.*, 2008). Due to these circumstances, scientists have been searching for novel anti-microbial and therapeutic antioxidant compounds from a variety of sources including medicinal plants (Karaman *et al.*, 2003). The licorice (*Glycyrrhiza glabra* L.) belongs to the Fabaceae family, and it is frequently used in the food and tobacco industries as a sweetener and flavoring ingredient (Peng *et al.*, 2021). Licorice is commonly known as 'Yashtimadhu', which flourishes in subtropical regions of Western Asia, the Middle East and Europe. Licorice extracts and its main compound glycyrrhizin are widely used in herbal medicine, food, tobacco products and snuff. Licorice is a perennial herb possessing sweet taste which has awide range of pharmacological effects on humans (Tian *et al.*, 2008). Licorice is frequently used to treat upper respiratory conditions like bronchitis, hoarseness, and sore throats (Fujisawa *et al.*, 2000). The principal medical bioactive component i.e., glycyrrhizin (triterpenoid saponin glycoside) has attracted a lot of interest. Its bioactive components have been demonstrated to have anti-inflammatory, gastroprotective, antitumoral, antiviral, antihepatotoxic, antifungal, antibacterial and anticancer actions as well as to be effective in treating ulcers, rheumatoid arthritis and menopausal heat flashes while lowering low density lipoprotein (Wang *et al.*, 2015; Mostafa, 2017). Natural antioxidants found in licorice, includes phenols, saponins, flavanones, isoflavonoids, isoglycyrrhizin, 18-glycyrrhetinic acid, liquiritigenin, licochalcone A, licochalcone E and glabridinhelpto, prevent oxidative damage. It may also provide protection from cancer, pathogens and oxidative stress-induced physiological malfunctions (Asif, 2015; Mircea *et al.*, 2015; Kwon *et al.*, 2020).

Rancid tastes and odours are caused by the oxidative degradation of fats and oils in food which also lowers the nutritional quality and safety of the product. To maintain flavour, colour and prevent vitamin destruction, antioxidants like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate are

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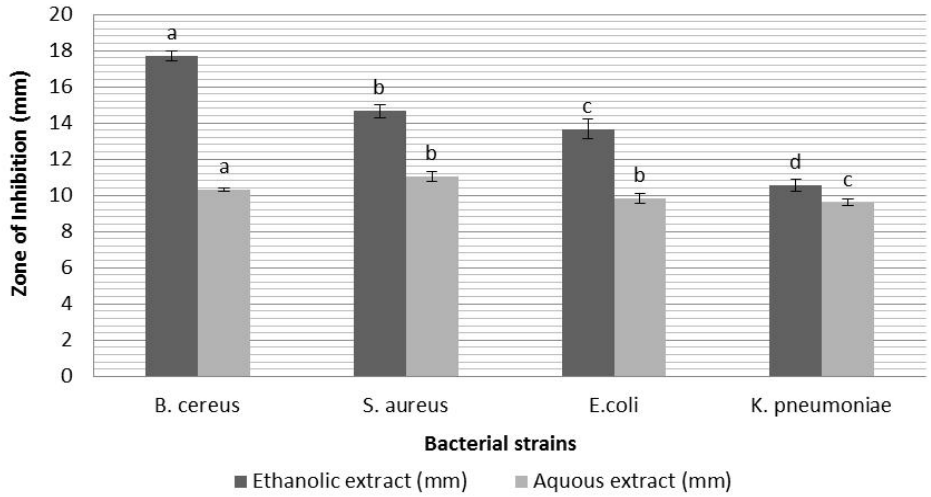
added for prolonging the shelf life of fish and fishery products. Nevertheless, toxicological effects and consumer preference for natural products have resulted in increased interest in the use of and research on natural antioxidants. Consequently, the significance of natural antioxidants has grown considerably during the past few years, particularly those of plant origin (Kwon *et al.*, 2020). Microbial activity is the main reason for the deterioration of foods, loss of quality and safety against food-borne illnesses. Plant extracts possess a distinct flavor and exhibit antioxidant as well as antimicrobial activity (Negi and Jayaprakasha, 2003). The purpose of this investigation was to evaluate the antibacterial activity of licorice root extract against a few gram-positive and gram-negative bacteria for extending the shelf life of value-added fish and fishery products.

Roots of licorice species of *Glycyrrhiza glabra* L. were purchased from Garia market of Kolkata, West Bengal. The roots were dried in a hot air oven at 40°C for 4-5 hours, grounded in a mixer grinder, packed in a polybag and stored in an air-tight container for further use. Pulverized licorice root powder was added to 70% ethanol (v/v) and water for extract preparation. The ratio of licorice root powder and solvent was 1:10 (w/v). The flasks were kept for 24 hours at room temperature (25±2°C) with occasional stirring. At the end of the extraction, the mixtures obtained from two different samples were centrifuged at 5000 rpm for 10 minutes individually. The supernatants from all the samples were filtered through Whatman no. 1 filter paper. Then, the filtrates were evaporated to dryness in a rotary evaporator at 45°C. The stocks were stored in glass bottles and kept in a refrigerator at 4±1°C for further use.

The antimicrobial activity of different solvent extracts of licorice was evaluated by the agar well diffusion method (Hamad *et al.*, 2020). The agar cultures of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Klebsiella pneumoniae* were prepared to assess the licorice extracts inhibitory effects. The bacteria were grown by inoculating a loopful of the strain in the nutrient broth (5 mL) and incubated at 37°C for 8 hours. From this, 50 µL was evenly spread over sterile Muller Hinton Agar plates using sterile cotton buds and allowed to dry. For the agar well diffusion, a well was made in the seeded plates with the help of a cup borer (6 mm). 100 µL of each extract was loaded slowly onto the well without overflowing and allowed to dry. Ampicillin (10 mcg) was used as a positive control. The plates were incubated for 24h at 37°C, and the zone of inhibition around the well was checked for the presence of antimicrobial activity and measured in mm. Results were analyzed statistically using the Statistical Package for Social Science Software (SPSS VERSION 16.0, Chicago IL, USA). One-way ANOVA (analysis of variance) was carried out, and the significant differences among means of the treatments were determined by Duncan's Multiple Range Test (DMRT). The level of significance was set up at $p \leq 0.05$.

The antimicrobial effect of ethanolic licorice extract (ELE) and aqueous licorice extract (WLE) was tested against two gram-positive bacteria – *Staphylococcus aureus* (MTCC7443), *Bacillus cereus* (MTCC 1306) and two gram-negative bacteria – *Escherichia coli* (MTCC443), *Klebsiella pneumoniae* (MTCC3384) by agar well diffusion method. The diameter of the inhibition zone (mm) of different extracts of licorice root are shown in Fig. 1.



* Results are mean of three determinations (n=3) with s.d.; # Values of mean in the same column with different superscripts are significantly different ($p<0.05$)

Fig. 1. Antimicrobial activity of licorice root extracts against some pathogenic bacteria

Ethanolic licorice extract (ELE) at 100 µL showed the highest antimicrobial activity against *B. cereus* with an inhibition zone of 17.72±0.28 mm, followed by *S. aureus*, *E. coli and K. pneumoniae* with inhibition zones of 14.65±0.35 mm, 13.67±0.57 mm and 10.54±0.34 mm respectively. In case of aqueous licorice extract (WLE), the highest inhibitory activity was found against *S. aureus* and the least was found against *K. pneumoniae* with inhibitory zones of 11.03±0.17 mm and 9.61±0.25 mm, respectively. The ethanolic licorice extract (ELE) showed significantly higher antimicrobial activity ($p<0.05$) against both the gram-positive and gram-negative bacteria than the aqueous licorice extract (WLE).

Licorice root is a medicinal herb which is widely cultivated in China, Japan, Russia, Spain and India. The isoflavonoid components of licorice, particularly hispaglabridin and ß,4'-O-methylglabridin, glabridin, glabriol and 3-hydroxyglabrol are primarily responsible for its antimicrobial activities (Abbas *et al.*, 2015). According to Galanakis *et al.* (2013), the differentiated activity of various extracts might be due to the presence of bioactive compounds in the solvents. Alkaloids and flavonoids exert antimicrobial activity by forming complexes with soluble microbial proteins, bacterial cell walls and inactivate enzymes such as DNA gyrase etc. (Zhang *et al.*, 2008).

According to Hamad *et al.* (2020), the licorice ethanol and aqueous extract showed excellent antimicrobial activity against both the gram positive and gram-negative bacteria. In the present study, the ethanolic licorice extract (ELE) showed better antimicrobial activity against pathogenic microorganisms. These results are similar to the findings by Hamad *et al.* (2020), where the licorice ethanol extract showed good activity against *Salmonella* sp., *Staphylococcus epidermidis* and *Escherichia coli* with the highest inhibition zone of 28, 27 and 19 mm, respectively. In a study conducted by Zadeh and Kor (2013) it was reported that the extracts of licorice (*Glycyrrhiza glabra* L.) root showed antimicrobial activities against both gram-positive and gram-negative bacteria by the agar diffusion method.

Flavonoids present in licorice, particularly chalcones, play an essential role in the treatment of bacterial infections by limiting the expression of bacterial genes, obstructing bacterial development and lowering the formation of bacterial toxins (Wang *et al.*, 2015). Rosa *et al.* (2016) found that the antibacterial activity of licorice root is caused by reducing the fluidity in hydrophilic and hydrophobic regions of the cell membrane.

Therefore, it may be concluded that the ethanolic licorice extract exhibited highest antimicrobial activity against both the gram-positive and negative bacteria, which could be due to glycosides, alkaloids, terpenoids, proteins and amino acids found in licorice root extract. Smith-Palmer *et al.* (1998) stated that the reason behind higher susceptibility of gram-positive bacteria towards phenolic compounds than gram-negative bacteria is the outer structure of the cell. Gram-negative bacteria have highly hydrophobic lipopolysaccharide layer that acts as a strong permeability barrier against hydrophobic organic phenol solutes. In contrast, gram-positive bacteria only consist of a peptidoglycan layer outside the cell wall through which phenol molecules could easily penetrate, causing further cytoplasmic leakage and coagulation of proteins leading to death of the bacterial cell (Paul *et al.*, 2011). Porin protein present outside the cell wall of gram-negative bacteria act as barrier to aqueous solutes, protecting the cell (Benhammou *et al.*, 2008).

Conflict of interest: Authors have no conflict of interest in this study.

Authors' contribution: SR: First author and the researcher who conducted the work; KCD: Supervisor of the first author and planned the experimental design; SC: Corresponding author and participated in planning the experimental design and writing of the manuscript; SN: Guided the first author in conduct of the experiments done and preparation of the manuscript; PM: Assisted in the experiments and statistical analysis; AB: Assisted in statistical analysis and manuscript preparation.

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