**ABSTRACT**

Antibacterial activity of crude flower extracts of *Nelumbo nucifera* (Gaertn.) with different solvents (methanol, ethanol and aqueous) was evaluated for antibacterial activity against medically important human pathogenic bacteria, including two gram-positive (*Staphylococcus aureus* and *Streptococcus sp.*) as well as two gram-negative (*Pseudomonas aeruginosa* and *Salmonella typhi*). The different extracts were tested for the presence of antibacterial activity by agar well diffusion assay (AWDA) method. The flower extracts with different solvents showed significant antibacterial activity against all the tested bacterial strains. In the present study, *Streptococcus* sp. was highly susceptible to the extract as compared with other three bacterial strains. The results also showed that the methanol extract of *N. nucifera* was the most effective as the widest inhibitory zone was observed as compared to ethanol as well as aqueous extract. The use of flower extracts of the *N. nucifera*, with known antibacterial properties can be of great significance in therapeutic treatments.

**KEY WORDS:** Antibacterial activity; Agar well diffusion assay; *Nelumbo nucifera*; Human pathogens; Solvents.

**INTRODUCTION**

Bacteria are listed at first position among the microorganisms causing opportunistic diseases and innumerable antibacterial agents are currently used for treating bacterial infections.\(^1\) However, the widespread and indiscriminate use of antibacterial agents resulted in the development of drug resistance among many virulent pathogenic bacteria.\(^2\) Many of the currently used antibacterial are associated with adverse effects such as toxicity, hypersensitivity, immunosuppression, and tissue residues posing a public health hazard. Further, the newer broad spectrum antibiotics are cost prohibitive and are not within the reach of poor peoples. These disadvantages undermine the therapeutic utility of the currently available antibacterial and thus necessitating the need for finding alternative remedies for the treatment of bacterial diseases. As the global scenario is now changing towards the use of non-toxic and eco-friendly products, development of modern drugs from traditional medicinal plants should be emphasized for the control of various human as well as animal diseases.\(^3\)

Various medicinal plants have been used for many years in daily life to treat diseases in all over the world. Interest in medicinal plants reflects the recognition of the validity of many traditional claims regarding the value of natural products in healthcare.\(^4\) In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Natural products play an important role in drug development programs in the pharmaceutical.\(^5\) Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs.\(^6, 7\) A wide range of medicinal plant parts is used to extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local, used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority have not been adequately evaluated.\(^8, 9\)

Antimicrobial substances are the substance that inhibits the growth and existence of microorganisms. These microorganisms could be pathogenic or non-pathogenic hence, antimicrobial substances are used in the treatment of various ailments. Quite a large number of antimicrobial substances exist and they are gotten from diverse sources such as microbes, plants, animals and chemicals.\(^10, 11\)
Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. Hence, researchers have recently paid attention to safer phytomedicines and biologically active compounds isolated from plant species used in herbal medicines with an acceptable therapeutic index for the development of novel drugs. In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants.

It is known that the aquatic plants form, one of the most productive ecosystems of the world and essential life supporting systems, providing a wide array of benefits to humankind. Various aquatic plants have the ability to produce bioactive materials that showed antibacterial activities.

*N. nucifera* (Gaertn) is an angiosperm commonly known as Indian lotus belongs from Nymphaeaceae family. It is one of the oldest perennial aquatic herb that grows up to 45 cm in height (Fig. 1), and consumed throughout Asia. *Lotus (N. nucifera)* classified in floating leaved plants among aquatic plants. This plant prefers clear, warm, still and slightly acidic water. Pharmacological studies of the plant revealed that the whole plant possesses antidiabetic, antipyretic, anti-inflammatory, anticancerous, antimicrobial, antiviral and anti-obesity properties. Furthermore, *N. nucifera* flower has considerable reputation as a potent adjunct in the treatment of various ailments such as cancer, hypertension, diarrhea, fever, weakness, infection and body heat imbalance. The selection of crude plant extracts for screening programs has the potential of being more successful in its initial steps than the screening of pure compounds that are isolated from natural products.

**MATERIALS AND METHODS**

**Plant collection and extract preparation**

Fresh flowers of *N. nucifera* were collected from the water reservoir used for drinking after the treatment at M. D. University, Rohtak, Haryana. The flowers were transported to the laboratory in polythene bags where they were prepared immediately for the extraction. Twenty grams of flower material were cut into thin pieces using a sterile razor blade and combined with 5 ml solvent (methanol, ethanol and aqueous) separately. The mixture was homogenized using a sterile mortar and pestle, and the resulting solutions were incubated at room temperature for 2hrs for the extraction. At the end of the extraction, the mixture was centrifuged (1.5 ml eppendorffs) at 10,000 rpm for 10 minutes at room temperature to pellet the flower debris. Supernatant was taken and used to determine the antibacterial activity by agar well diffusion assay (AWDA) method.

**Source of bacterial strains**

In the present study four human pathogenic bacterial strains, including two gram-positive *Staphylococcus aureus* MTCC 6908 and *Streptococcus* sp. MTCC 9724 as well as two gram-negative *Pseudomonas aeruginosa* MTCC 4673 and *Salmonella typhimurium* MTCC 3224, were obtained from the microbial type culture collection (MTCC), Chandigarh, India. These bacterial cultures were grown in nutrient broth medium, pH 7.0. Stock cultures were maintained on a nutrient agar slant pH 7.0 at 4°C until needed. The media components were purchased from Hi-media, Mumbai, India.

**Antibacterial susceptibility assay**

Antibacterial susceptibility of the crude flower extracts (methanol, ethanol and aqueous) against gram-positive as well as gram-negative bacterial strains were determined by AWDA method. For this, a well (6 mm diameter) was made with the help of a borer in cooled nutrient agar plate, overlaid with soft agar (5 ml), seeded with a target strain (~10^5 cfu/ml). Aliquots (100μl) of the test compound were introduced into the well and the plates were incubated overnight at 37°C. The diameters of the inhibition zones were measured in millimeters (mm). For each bacterial strain pure solvents were used as controls.

**STATISTICAL ANALYSIS**

The experiment was carried out in three independent sets, each consisting of three replicates. Values shown here represent mean ± standard error of the mean (SEM).

**RESULTS AND DISCUSSION**

Medicinal plants are gifts of nature used to cure number of human diseases. To promote the proper use and to determine their potential as sources for new drugs, it is essential to study the medicinal plants. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with side effects and have an enormous therapeutic potential to heal many infectious diseases. The potential for developing antimicrobials from higher

**Fig. 1: Photo of Nelumbo nucifera (Gaertn.) plant**

Hence, the present study was initiated to evaluate the antibacterial activity of the methanol, ethanol and aqueous crude flower extracts of *N. nucifera* against gram-positive as well as gram-negative human pathogenic bacterial strains.
plants appears rewarding as it will lead to the development of phytomedicines to act against microbes. Therefore, researchers are increasingly turning their attention to folk medicine to develop better drugs against microbial infections. 

There are many literatures reporting the ethno-medicinal values of *N. nucifera*, but there is little scientific proof for further using this plant commercially or in a more effective form. From this viewpoint the present study was carried out to evaluate the antibacterial activity of the methanol, ethanol and aqueous crude flower extracts of *N. nucifera* against two gram-positive as well as two gram-negative human pathogenic bacterial strains by the AWDA method.

From the results obtained it was apparent that the methanol extract of *N. nucifera* was the most effective as the widest inhibitory zone was observed compared to the ethanol as well as aqueous extract used. The methanol extract is highly effective against all pathogens because more organic compounds were leached in this solvent. Although water is reported by the traditional healers and herbalists to be the most commonly used solvent for extracting the active compounds due to its easy availability. Though similar responses have been reported in many other studies against various bacteria. Many studies have revealed that plant methanol extracts inhibited the growth of testing bacteria more as compared to aqueous extracts. The present investigation also supports the above observations.

**CONCLUSION**

The crude flower extracts of *N. nucifera* showed significant antibacterial activity against tested human pathogenic bacterial strains, including both gram-positive (*S. aureus* and *Streptococcus* sp.) as well as gram-negative (*P. aeruginosa* and *S. typhimurium*). The results indicate that the methanol extract showed better antibacterial activity as compared to other two solvents (ethanol and aqueous). Therefore, they could be further subjected for screening and identification of active ingredients which are responsible for the antibacterial activity against most pathogenic microorganisms associated with various human diseases. Hence, it may be recommended that the flower extracts of this plant possess biologically active compounds with high antibacterial properties that can be used as antibacterial agents in designing and developing new drugs. Further studies should be need for isolation of bioactive compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin.

**COMPETING INTERESTS**

The author declares that he has no competing interests.

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**REFERENCES**


