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EVALUATION OF ANTIBACTERIAL ACTIVITY OF *NELUMBO NUCIFERA* (GAERTN.) FLOWER EXTRACTS AGAINST GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

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ABSTRACT

The antibacterial activity of *Nelumbo nucifera* (Gaertn.) flower extracts in different solvents (methanolic, ethanolic and aqueous) was studied against gram-positive (*Micrococcus luteus* and *Bacillus subtilis*) as well as gram-negative bacteria (*Escherichia coli*). The extracts were tested for the presence of antibacterial activity by agar well diffusion assay (AWDA) method. The patterns of inhibition varied with the solvent used for extraction and the organisms tested. The results showed that *M. luteus* and *B. subtilis* were highly susceptible to the extracts of *N. nucifera* as compared with the *E. coli*. The methanolic extract of *N. nucifera* was the most effective as the widest inhibitory zone was observed as compared to the ethanolic as well as aqueous extract used. The remarkable antibacterial activity of the extracts against these gram-positive as well as gram-negative bacteria suggests that there is a scientific basis for its utilization as antibacterial agents in designing and developing new drugs.

Key Words: Antibacterial; *Nelumbo nucifera*; Solvents; Zone of Inhibition.

INTRODUCTION

In recent years, the emergence of multiple antibiotic resistance pathogenic bacterial strains is a major medical problem worldwide and poses a big threat to human society [1, 2]. Moreover, almost all of the antibiotics have side effects, including hypersensitivity, immune-suppression and allergic reactions, and they are expensive too [3]. These circumstances make it essential to search new and more potent antimicrobial compounds to combat these pathogens. Interest in plant-derived drugs have been increasing, mainly due to the current

widespread belief that “green medicine” is safer and more dependable than costly synthetic drugs, many of which have adverse side effects [4].

According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary health care needs [5]. The rediscovery of the connection between plants and health is responsible for launching of a new generation of multi-component botanical drugs, dietary supplements and plant-produced recombinant proteins [6]. In the developing nations, maximum people depend on these plants for medicine because of their easy availability and low cost of treatment.

The use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used in traditional medicine to contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance [7]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds [8]. Screening of the plants for antimicrobial activities is important for the finding of potential new compounds for therapeutic uses [9]. 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 mankind [10]. Various aquatic plants have the ability to produce bioactive materials that showed antibacterial activities [11, 12].

Nelumbo nucifera (Gaertn.) belongs to Nymphaeaceae family, is a perennial plant that grows up to 45 cm in height. It is a herbaceous aquatic plant, whose leaves float or submerged in water [13]. This plant prefers clear, warm, still and slightly acidic water. Many bioactive and pharmacologically important compounds have been obtained from *Nymphaea* spp. and used in medicine and pharmacy [14]. 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 [15].

Hence, the present study was initiated to evaluate the antibacterial activity of methanolic, ethanolic and aqueous crude extracts of *N. nucifera* flower against gram-positive as well as gram-negative bacterial strains.

MATERIALS AND METHODS

Plant collection and extract preparation

Flowers of *N. nucifera* (Gaertn.) were collected from the water reservoir used for drinking after the treatment at M. D. University, Rohtak, Haryana, India. 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 6 hrs 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. The supernatant was taken and used to determine the antibacterial activity by agar well diffusion assay (AWDA) method.

Source of Microorganisms

Micococcus luteus (MTCC106) (Dr. S. K. Tiwari, Department of Genetics), *Bacillus subtilis* (Dr. Bijender Singh, Department of Microbiology), and *Escherichia coli* strain DH5α (Dr. K. K., Department of Microbiology) M. D. University, Rohtak, Haryana, India. The 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.

Bioassay for Antibacterial Activity

The antibacterial activity of crude solvent extracts (methanolic, ethanolic and aqueous) of *Nelumbo nucifera* (Gaertn.) flower against gram-positive as well as gram-negative bacterial strains were evaluated by agar well diffusion assay (AWDA) method [16]. 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 (~10^6 cfu/ml). Aliquots of the test compound were introduced into the well and the plates were incubated for overnight at 37 ºC. The diameters of the inhibition zones were measured in millimeters. For each bacterial strain, controls were maintained in which pure solvents were used instead of the extract.

Statistical analysis

The experiment was carried out in three independent sets, each consisting of 3 replicates. Values shown here represent mean ± standard error of the mean (SEM).

RESULTS AND DISCUSSION

Infectious diseases caused by bacteria, fungi, viruses and parasites are a major threat to public health in developing countries due to unavailability and high cost of medicines [17]. The plant possesses the different phytochemicals like alkaloids, tannins, phenolics, saponins, cardiac glycoside, terpenes and flavonoids. Some workers have also attributed to their observed antimicrobial effect of plant extracts to the presence of these secondary plant metabolites and it is responsible for their uses as herbs [18]. The antimicrobial activities of alkaloids and flavonoids have also been reported [19, 20]. Tannins are important in herbal medicine in treating wounds and to arrests bleedings [21]. Phyto-constituents such as saponins and phenolic compounds have also been reported to inhibit the growth of some bacteria.

There are many literatures reporting the ethno-medicinal values of *N. nouchali*, but there is little scientific proof for further using this plant commercially or in a more effective form. Similarly, in order to evaluate the antibacterial potential of crude flower extracts of *N. nucifera* against gram-positive as well as gram-negative bacterial strains by the AWDA method. In the present study, the crude flower extract showed excellent antibacterial activity against tested two gram-positive as well as one gram-negative bacterial strains as shown in Fig. 1.

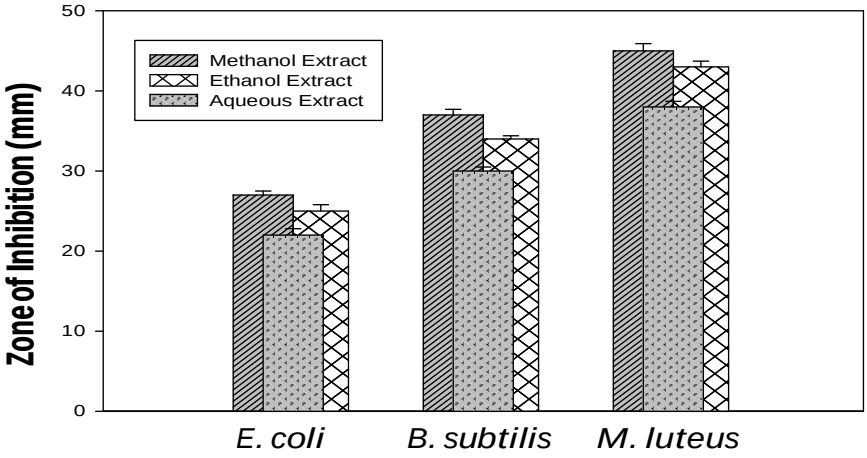

Fig. 1. Antibacterial activity of crude flower extracts of *N. nucifera*

The results shows *M. luteus* was the most sensitive organism among both the gram-positive bacteria with the inhibition zone (mm) 45 (methanol), 43 (ethanol), 38 (aqueous). However the *E. coli* exhibited less sensitivity as compared to both the tested gram-positive bacteria (*M. luteus* and *B. subtilis*) with the inhibition zone (mm) 27 (methanol), 25 (ethanol), 22

(aqueous) are given in Table 1. On the other hand pure solvent was used as control does not shows any zone of inhibition against any bacterial strain.

Table 1: Results of the antibacterial activity of crude extracts of *N. nucifera* flower

Zone of Inhibition (mm)			
Flower extracts in various solvents	***E. coli***	***B.subtilis***	***M. luteus***
Methanol	27	37	45
Ethanol	25	34	43
Aqueous	22	30	38

From the results obtained it was apparent that the methanolic extract of *N. Nucifera* was the most effective as the widest inhibitory zone was observed as compared to the ethanolic as well as aqueous extract used. Though similar response has been reported for the different extracts of *N. nouchali* by Dash et al. [22]. Many studies have revealed that methanol extracts inhibited the growth of testing bacteria more than aqueous extracts of the plant [10, 23]. The present investigation also supports the above observations.

CONCLUSION

From above study the *N. nucifera* flower extract with different solvents (methanol, ethanol and aqueous) showed remarkable antibacterial activity against the tested two gram-positive (*M. luteus* and *B. subtilis*) and one gram-negative (*E. coli*) bacterial strain. Hence, it may be recommended that the flower extracts of this plant possess compounds with high antibacterial properties that can be used as antibacterial agents in designing and developing new drugs. Further research has to be conducted on the activity of the crude extracts against a wider range of bacteria and fungi.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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