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## Antibacterial activity of two water plants *Nymphaea alba* and *Salvinia natans* leaves against pathogenic bacteria

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### Abstract

The present study was carried out to evaluate the antibacterial activity of two water plants *Nymphaea alba* and *Salvinia natans* leaves against three pathogenic bacteria *Escherichia coli*, *Vibrio sp.* and *Staphylococcus aureus* collected from branches connected to Shatt Al-Arab river in Al-Qurna city.

The whole plant extracts from ethanol and Acetone were tested for their activity against pathogenic bacteria (*E. coli*, *Vibrio sp.* and *S. aureus*). The zone of inhibition produced by different extracts against the test bacteria was measured and compared with standard antibiotic disc. The extracts obtained from ethanol, Acetone showed higher inhibition zones of 26.2 mm and 18.8 mm, respectively against *Vibrio sp.*

Minimum inhibition concentration values of Acetone extracts were demonstrated at low concentration ranging from 20.0 mg/mL up to 160.0 mg/mL compared to ethanol extracts which were observed ranging from 10.0 mg/mL up to 80.0 mg/mL.

**Keywords:** pathogenic bacteria, water plant, susceptibility, phytochemical.

### 1. Introduction

Plants have been an important source of pharmaceuticals for maintaining human health, According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. About 65-80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants<sup>[1]</sup>.

The use of crude extracts of plants parts and phytochemicals, of known antimicrobial properties, can be of great significance in the therapeutic treatments. In recent years, a number of studies have been conducted in various countries to prove such efficiency. Many plants have been used because of their antimicrobial traits. The screening of plant products for antimicrobial activity has shown that the higher plants represent a potential source of novel antibiotic prototypes<sup>[2]</sup>. There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This has forced scientist to search for new antimicrobial substances from various sources like the medicinal plants<sup>[3]</sup>.

For this reason, aquatic plants have attracted the interest of researchers and have proven to be promising sources of antimicrobial agents<sup>[4]</sup>.

*Nymphaea alba* is plant belongs to Nymphaeaceae family. Most of these species in this family are aquatic plant, whose leaves float or submerge in water (Abu-Zaida *et al.*, 2008)<sup>[5]</sup>. This plant prefers clear, warm, still and slightly acidic water and is localized to Central and Southern Europe, Asia, the Middle East, North Africa. Many bioactive and pharmacologically important compounds have been obtained from *Nymphaea spp*<sup>[6, 7]</sup>.

*Salvinia natans* (Salviniaceae family), is a native aquatic plant from South America and East Asia is commonly found in freshwater lakes<sup>[8]</sup>. Currently few studies show the potential of *S. natans* in the remediation of water sources contaminated with heavy metals<sup>[9]</sup>, and no studies in the literature report on the antimicrobial agents of their chemical constituents and active extracts. In a preliminary study performed with this plant, The subsequent results illustrated a strong potential of this plant to combat *Staphylococcus aureus*, the main etiologically causative agent of bovine mastitis<sup>[10]</sup>.

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This disease, an inflammatory response found in cows' udders, is the leading infectious disease affecting dairy cattle today [11, 12].

Many authors have reported the antibacterial activity of water plants extracts collected worldwide, information about Iraqi water plants extracts are still little. In Iraq, most of these studies have been attempted to evaluate the antibacterial activity of some wild plant extracts [13- 6].

The aim of this study is to investigation the antibacterial activity of the crude ethanol Acetone for two water plants *Nymphaea alba* and *Salvinia natans* leaves samples from branches connected to Shatt Al-Arab river near Al-Qurna city.

## Materials and methods

### Plant materials

The leaves of water plant *Nymphaea alba* and *Salvinia natans* were collected from the branches of Shatt Al-Arab river near Al-Qurna city during the February month, 2016. The plants were cleaned of extraneous matter, and necrotic parts were removed and washed with fresh water. The plant was transported to the laboratory in polythene bags. In the laboratory, the leaves were washed thoroughly three times with running water and once with distilled water.

### Plant Identification

The water plant *Nymphaea alba* and *Salvinia natans* were identified and confirmed by Dr. Saleh Abud Kader Al-Essa of Department of Fisheries and Aquatic Resources, Agriculture collage University of Basrah, Basrah, Iraq.

### Microbial cultures

Fresh clinical isolates of *E. coli*, *V. sp.* and *S. aureus* isolated from some infectious sources of human and shrimp and fish, respectively. for period from October 2016 to March 2017 were obtained from Laboratory biology Technologies in the marine biology Department, marine science center, University of Basrah. Stock cultures were maintained on a nutrient agar slant at 4 °C until needed.

### Preparation of plant extracts

Preparation of extracts fresh leaves were cleaned with deionized water and dried in shade for two to four weeks. After drying, the leaves were pulverized into fine powder by a grinding machine and stored in airtight container. This powder as used to prepare ethanol and acetone extracts.

A sample (50 g) of the shade-dried powdered of *S. natans* and *N. alba* leaves were soaked in 95% ethanol and Acetone (100 ml) for 72 h. At the end of the extraction, the extract was filtered using Whatman No.1 filter paper. The filtrate was concentrated in vacuum at 30°C. After complete evaporation, the extract was weighed and stored in the deep freezer at -18 °C till further use.

### Antibacterial activity tests

Disc diffusion method was used to determine susceptibility of plant extracts [17]. Mueller-Hinton agar (MHA) was sterilized in flasks cooled to 45-50 °C and then poured into sterilized Petri dishes. Sterile filter paper discs of 6 mm diameter were impregnated with extract solution of concentration of extract (10 mg/mL/disc) and then placed on to agar plates which had previously been inoculated with the tested microorganisms (*E. coli*, *Vibrio sp.* and *S. aureus*). Control experiments comprising streptomycin were set up. The plates were then incubated at 37 °C for 24- 48 h. The diameters of the inhibition zones were measured in millimeters.

## Results

### Antibacterial activities

The different extracts (concentration: 10 mg/mL/disc) of two water plant leaves were screened for their antibacterial activity against three different pathogenic bacteria. Acetone extracts of two water plant leaves were showed lower inhibitory zones against *E. coli* bacteria.

The antibacterial activity of ethanol and Acetone extracts of two water plant leaves is shown in Table (1) the values of inhibition zones were showed in lower levels in a comparative way with standard antibiotic disc- Streptomycin (30 µg/disc). The present study revealed that, ethanol extract possessed the highest zone of inhibition of *N. alba* water plant extracts with 26.2 mm against *Vibrio sp.* followed by 18.8 mm against *S. aureus*, respectively. Acetone extracts of *S. natans* did not show any antibacterial activity against three pathogen bacteria. All the test extracts were somehow effective against both *Vibrio sp.* *S. aureus*.

The acetone for *N. alba* extract produced higher inhibitory zones against *Vibrio sp.* and *S. aureus* of 18.8 mm and 12.1 mm, respectively.

**Table 1:** Antibacterial activity of crude extracts (concentration: 10 mg/mL/disc) of two water plant against three different pathogens bacteria

Poathogenic bacteria	Ethanol (mm)		Acetone (mm)		Streptomycin 30ug/disc
	<i>N. alba</i>	<i>S. natans</i>	<i>N. alba</i>	<i>S. natans</i>	
<i>E. coli</i>	8.3	6.4	Nil	Nil	22.5
<i>Vibrio sp.</i>		10.3	18.8	Nil	35.4
<i>Staphylococcus aureus</i>	26.2 18.1	8.7	12.1	Nil	28.2

### Minimum inhibitory concentration (MIC)

Tables (2) showed MIC values using agar dilution method. MIC values of ethanol extracts of *N. alba* and *S. natans* were observed at concentrations ranging from 10.0 mg/mL up to 80.0 mg/mL, however, for acetone extracts of two plant, with higher concentrations it were observed between 20.0 mg/mL up to 160.0 mg/mL.

**Table 4:** Minimum inhibitory concentration (MIC) of extracts of two water plant on pathogens bacteria.

Bacteria	MIC (mg/mL) ethanol extracts		MIC (mg/mL) acetone extracts	
	<i>N. alba</i>	<i>S. natans</i>	<i>N. alba</i>	<i>S. natans</i>
<i>E. coli</i>	80	80	80	160
<i>Vibrio sp.</i>	20	40	80	80
<i>Staphylococcus aureus</i>	10	10	20	40

## Discussion

Plants are rich source of antibacterial agents, which could be effectively used in human disease management. Plants have served humans as a source of food, shelter and clothing since time immemorial [18, 19]. Before the invention of antibiotic in 19th century, man completely relied on plant sources to treat all diseases and disorders [20]. Traditional medicines or folk medicines are an important source of potentially useful new compounds for the development of chemotherapeutic agents [12]. The essential values and uses of some plants have been worked out and published, but many of them remain unexplored to date. Therefore, there is a necessity to explore their uses and conduct broad-spectrum and extensive studies

to discover their medicinal properties.

The results obtained showed that ethanol and acetone extracts of *N. alba* and *S. natans* exhibited inhibitory activities against the tested bacteria with different degrees as demonstrated by measuring the diameters of inhibition zones and these results are in conformity with the results obtained by Abu-Zaida *et al.* (2008) [5].

In the present study three different pathogenic bacteria viz. *E. coli* (human), *S. aureus* (Fish) and *Vibrio sp.* (Shrimp) were tested to know the antibacterial activity against them to ethanol and acetone crude extracts of two water plants. The extracts of ethanol have shown higher antibacterial activities (26, 18 and 10 mm) by both *N. alba* and *S. natans* against *Vibrio sp.* and *S. aureus*. The acetone extracts of *N. alba* and *S. natans* have shown higher antibacterial activity against *Vibrio sp.* and *S. aureus*.

In Iraq, many studies were showed plants leaves contain flavone as well as contain phenolic compounds which have an important role to discourage the growth of bacteria that work on the inhibition of the enzymes responsible for the metabolic basic interfere interactions in a specialist with proteins leading to the metamorphosis of protein and then the in ability of bacteria to continue [14-17, 21].

The results of this study have shown that the ethanol and acetone crude extracts of two water plants (*N. alba* and *S. natans*) leaves extracts have great potential as antimicrobial agents in the treatment of infectious organisms.

### Conclusion

On the basis of the results of this study the two plants (*N. alba* and *S. natans*) obtained from the Shatt Al-Arab river near Al-Qurna city can be regarded as a potential antibacterial agent against *E. coli*, *Vibrio sp.* and *S. aureus* isolated from some infectious sources of the patients.

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