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PJBS

ISSN 1028-8880

# Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 11 (21): 2454-2463, 2008
ISSN 1028-8880
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# Antimicrobial Activity of Some Macrophytes from Lake Manzalah (Egypt)

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**Abstract:** The antimicrobial activities of aqueous and organic solvents (chloroform, ethanol and methanol) extracts of four plants *Ceratophyllum demersum* L., *Eichhornia crassipes*, *Potamogeton crispus* and *Potamogeton pectinatus* were tested *in vitro* against seventeen different microorganisms including Gram-positive and Gram-negative bacteria and fungi. Nine of these identified organisms were obtained from different sources, *Bacillus subtilis* 1020, *Bacillus cereus* 1080, *Staphylococcus aureus*, *Erwinia carotovora* NCPPB 312, *Candida albicans*, *Candida tropicalis*, *Aspergillus niger*, *Fusarium oxysporum* and *Penicillium italicum*. The other eight organisms were isolated from Manzalah lake water and identified using API 20E strip system (BioMereux). One hundred pathogenic bacterial isolates representing eight genera were identified to species level. These organisms are *Escherichia coli* (20%), *Pseudomonas aeruginosa* (16%), *Klebsiella pneumoniae* (14%), *Salmonella colerasuis* (13%), *Shigella* sp. (11%), *Serratia liquefaciens* (10%), *Proteus vulgaris* (9%) and *Brenneria nigrifluens* (7%). The extracts of all tested plants demonstrated antimicrobial activity against the used organisms. The efficiency of the extracts varied with, solvent used in the extraction as well as plant species and the part of plant used. The aqueous extract appeared to be the highly effective extract against all tested orgamisms especially *Fusarium oxysporum* causing inhibition zone 48±0.01 mm, *Pseudomonas aeruginosa* 59±0.02 mm and *Salmonella cholerasuis* 55±0.01 mm when using *P. crispus*, *P. pectinatus* and *C. demersum*, respectively. Ethanol extracts of *C. demersum*, *P. crispus* and *E. crassipes* root showed antimicrobial activities against all tested organisms except *Aspergillus niger*. At the same time the extract of *P. pectinatus* had no effect also on *Fusarium oxysporum* and the extract of *E. crassipes* leaves have no effect on *Penicillium italicum*. On using chloroform extracts *Escherichia coli*, *Aspergillus niger* and *Penicillium italicum* showed resistance. Comparing the effect of different plants extracts *C. demersum* appeared to be the most effective followed by *P. pectinatus*. Furthermore, the extracts of *E. crassipes* leaves being more effective than that, of its roots. Elemental analysis were also takes place in water and plant samples and the results revealed the presence of Mn and Pb in higher concentration in *P. pectinatus* (Mn 603±4.243 ppm and Pb 44±2.828 ppm), at the same time the highest values of Fe 1680±2.2 ppm, Zn 31.5±2.1 ppm and Cu 26.5±2.1 ppm were recorded for *C. demersum*. Comparing the two parts of *E. crassipes* (leaves and roots), the roots have the highest values of all studied metals.

**Key words:** Antimicrobial activity, plant extracts, microorganisms

## INTRODUCTION

One of the most important factors of water pollution is the microbial contamination; especially with pathogenic microorganisms. Enteric pathogens are typically responsible for waterborne sickness (Karaboze *et al*., 2003). Pathogens are a serious concern for managers of water resources, because excessive amounts of faecal bacteria in sewage and urban run-off have been known to indicate risk of pathogen-induced illnesses in humans (Fleisher *et al*., 1998). The need for new antimicrobial agents and strategies for their use in the treatment of serious Gram-positive infections is evident (Menichetti, 2005). Furthermore, several species of Gram-negative bacteria present in municipal wastewater are pathogenic. Thus, identification of these pathogenic agents in water resources is beneficial for controlling and prevention planning of the infectious diseases (Rabeh, 2007).

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Bacterial diseases results in major economic losses to fish and animals production, moreover, it represents a potential hazard to human health. Since the advert of antibiotics in the 1950s, bacteria and fungi have been relied upon for sources of antimicrobial agents (Cowan, 1999). For treatment of bacterial disease antibiotics are sometimes used. The misuse of antibacterial agents increase the incidence of resistant strains. One of the solutions to solve antibiotic resistant incident problem among pathogenic bacteria is to develop new drug from natural sources such as plant. Moreover, most antimicrobial agents have many side effects. However, herbal therapy if effective serves both as synergistic and corrective factors (Diab, 2002). Traditionally, plants are used as source of treatment of diseases in different parts of the world (Cowan, 1999; Eisenberge *et al*., 1993; Hostettmann *et al*., 2000; Stock Well, 1988).

The plant is widely used in Angola against diarrhea and dysentery, especially amoebic dysentery. In Nigeria, extracts or exudates of the plant are used as ear drops and in the treatment of boils, sore and promoting wound healing (Igoli *et al*., 2005).

Interest in plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics (Shiota *et al*., 2004; Abu-Shanab *et al*., 2004). The plant compounds were used to treat infections in age-old practice in a large part of the world, especially in developing countries, where there is dependence on traditional medicine for a verity of diseases (Gangoue-Pieboji *et al*., 2006; Shiba *et al*., 2005). Plants remain a common source of antimicrobial agents which is reported to have minimal side effects (Biobitha *et al*., 2002; Maghrami *et al*., 2005).

The aesthetic, medical and antimicrobial properties of aqueous plants extracts have been known since ancient times. The extracts of several wild and medicinal plants have been tested against some bacterial and fungal growth and for antimicrobial properties (Bilgrami *et al*., 1980; Arora and Ohlan, 1997; Shatter, 2001; Diab, 2002; El Anezy *et al*., 2006; Erdeny, 2006; Khalil, 2006; Qadir and Hoshyar, 2006). Few studies were reported on the effect of macrophytes extracts on growth of some bacterial and fungal orgamisms (Abd-Alla *et al*., 2001; Ballesteros *et al*., 1992; Bushmann and Stephen, 2006; Haroon, 2006).

Lake Manzalah is the largest Egyptian, costal deltaic lake in Egypt. It is situated at the Northeast quadrant of the Nile Delta, along Southeastern Mediterranean coast between Damietta branch of the River Nile and the Suez Canal. It serves five provinces namely; Damietta, port-said, Ismailia, Sharkiya and Dakahliy (Zahran and Willis, 1992). This lake is often inhabited by mixed stands of aquatic plants, some of these plants were selected for this study. *Ceratophyllum demersum* L. (family Ceratophyllaceae), *Eichhornia crassipes* (family Potederiaceae), *Potamogeton crispus* and *Potamogeton pectinatus* (family Potamogetonacea). Morphologically, these plants were briefly described by Tackholm (1974), Migahid (1978) and Pandey (1982).

The present investigation aimed to study the effects of some extracts obtained from these four macrophytes using different solvent, as well as its dry powdered samples on growth of some strains of pathogenic bacteria and fungi.

## MATERIALS AND METHODS

**Source of plant materials:** The macrophytes used in this investigation were collected during June 2005 from two stations situated at the southern coast of lake Manzalah (Fig. 1) namely:

**El-Raswa:** From this place *Ceratophyllum demersum* L., *Potamogeton pectinatus* L. and *Potamogeton crispus* L. were collected.

**El-Shiboh:** From this place samples of *Eichhornia crassips* were collected.

The collected samples washed with tap water, left to dry in shade to constant weight. Samples of *Eichhornia crassips* were separated into leaves and roots where the analysis takes place in each part separately. For the rest of plants the analysis takes place in the whole plant because these plants have no true roots and the absorption of elements takes place through the whole plant. The dry samples were ground to fine powder and preserved in well stopper sample vessels.

**Water analysis:** Water samples at a depth of 5-20 cm was taken from each stand dominated by the studied hydrophytes. Water pH was determined using a combined pH meter digital (Model 5986). Total alkalinity was determined according to Welch (1952). Determination of $Na^+$, $K^+$ and $Ca^{++}$ in water samples was carried out using a Corning 410 flame photometer. Microelements were determined by Flame Atomic Absorption Spectrophotometry (Pertkin Elmer 2100 Flame Atomic Absorption Spectrophotometer with an Autosampler, Allen, 1989).

**Plant analysis:** For determination of metals, dried plant tissue was digested at 80°C for 2 h, using nitric acid (Allen, 1989). Metals were determined by Flam Atomic

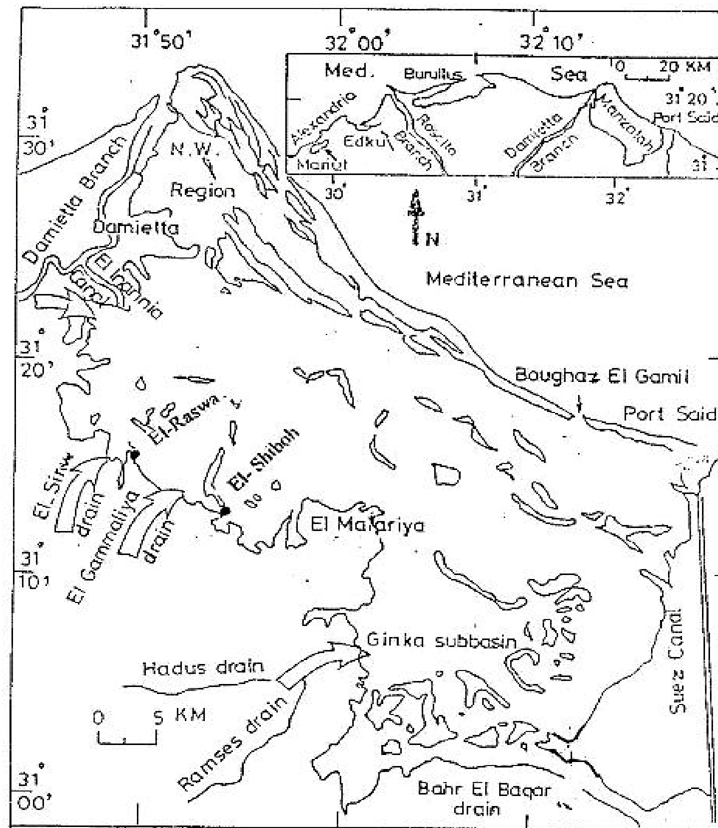


Fig. 1: Map of Manzalah Lake with points of samples collection, El-Shiboh, El-Raswa

Absorption Spectrophotometry (Pertkin Elmer 2100 Flam Atomic Absorption Spectrophotometer with an Autosampler).

**Preparation of plant extracts:** The powdered plants samples were subjected to extraction using different organic solvents (methanol, ethanol and chloroform) in addition to water according to the method described by Saber (1976) and Alghalibi (2004). Twenty grams of each plant materials were soaked in 200 mL solvent (80%), shaken for about 90 min and left in the laboratory in a sealed container for 24 h. The above steps was repeated daily throughout five successive days, thereafter the extract was filtered through Whatman No. 1 filter paper and concentrated under vacuum at 4°C in a Rota-vapor apparatus to dryness. The residues were dried to constant weights and kept in a vacuum desiccator for further study.

**Microorganisms**

**Antagonistic organisms:** Seventeen different microorganisms including Gram-positive and Gram-negative bacteria and fungi were used in this study as indicator organisms. Out of these organisms 9 identified isolates were obtained from different sources and the other isolated from Manzlah water samples.

**Identified bacteria:** *Bacillus subtilis* 1020, *Bacillus cereus* 1080 and *Staphylococcus aureus* obtained from the culture collection of the Microbiological Resource Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Resource, Cairo, Egypt), *Erwinia carotovora* NCPPB 312 was kindly provided by Professor Essam Azab. *Candida albicans*, *Candida tropicalis*, *Aspergillus niger* and *Fusarium oxysporum* were kindly provided by Professor Yhya Abd El-Gleel Mahmood. *Penicillium italicum* was isolated from disease of *Cirtus sinensis* fruits and identified by Azab *et al.* (2006).

**Unidentified bacteria:** The unidentified bacteria were isolated from Manzlah water samples.

**Isolation and purification of Gram-negative bacteria:** Isolation of Gram-negative bacteria in Lake Manzala water

samples were performed using MacConkey agar supplemented with 0.001 g $L^{-1}$ crystal violet (cited in Rabeh and Azab, 2006). One hundred isolates were purified, screened and the suspected similar ones were grouped for the purpose of selection and identification processes.

**Identification of some Gram-negative pathogens:** One hundred isolates from the examined water samples were subjected to identification by biochemical characteristics using API 20E strip system (BioMereux). Each API 20E strip consists of twenty wells containing dehydrated media. The isolate to be tested was suspended in sterile saline and added to each well. The inoculated strip was incubated for 16-24 h and the color reactions were noted either positive or negative.

**Inocula preparation:** Overnight cultures of the bacterial indicators were prepared in culture broth and diluted to $1\times10^6$ cfu $mL^{-1}$ while the fungi were grown in PDA and there spore suspension of $5\times10^5$ spore $mL^{-1}$ were prepared and served as inocula.

**Antimicrobial assay:** The antibacterial activities of the macrophytes aqueous and organic solvent extracts were determined by paper disc method described by Dulger (2005). The dried plant extracts were dissolved in there solvents and sterilized paper discs having a diameter 6 mm (Whatman No. 1) were impregnated with 40 μL of each extract (130 mg $mL^{-1}$) and placed on the surface of previously inoculated petri dishes. Nutrient agar plates were prepared and 100 μL of $1\times10^6$ cfu $mL^{-1}$ was spread on the surface. The inoculated plates were kept at 4°C for 2 h and incubated at the suitable temperature (*Bacillus subtilis* 1020 and *Bacillus cereus* 1080 at 30°C and the other bacteria at 37°C) for 24 h. At the end of incubation period, the appearance of inhibition zones were considered positive results. The antifungal activities of the studied plant extracts were measured using the well technique described by Holmalahti *et al.* (1994). Wells (6 mm diameter) were punched in the previously inoculated Potato Dextrose Agar (PDA) plates with 50 μL of $5\times10^5$ spores $mL^{-1}$ of each fungus, using a sterilized cork-borer. One hundred microliter aliquots (300 mg $mL^{-1}$) of each sterilized extract inserted into the wells and the plates were incubated at 30°C for 3 days and the antifungal activities were evaluated by measuring inhibition zone diameters. This experiment was performed in triplicate.

## RESULTS

Table 1 showed the biochemical characteristics of pathogenic Gram-negative bacteria isolated from Lake Manzalah water. The isolates of genus 1 were differentiated and confirmed by API 20E as *E. coli*. It is

Table 1: API 20E biochemical characteristics of pathogenic Gram-negative bacteria isolated from Lake Manzalah water

| Biochemical characteristics | Genus 1 | Genus 2 | Genus 3 | Genus 4 | Genus 5 | Genus 6 | Genus 7 | Genus 8 |
|---|---|---|---|---|---|---|---|---|
| ONPG | + | - | + | - | - | + | - | - |
| ADH | - | + | - | - | - | - | - | - |
| LDC | - | - | - | + | + | - | - | - |
| ODC | - | - | - | + | + | - | - | - |
| CIT | - | + | + | - | - | + | - | - |
| $H_2S$ | - | - | - | - | - | - | + | - |
| URE | - | - | + | - | - | - | + | - |
| TDA | - | - | - | - | - | - | + | - |
| IND | + | - | - | - | - | - | + | - |
| VP | - | - | + | - | - | + | - | + |
| GEL | - | - | - | - | - | - | - | - |
| GLU | + | + | + | + | + | + | + | + |
| MAN | + | - | + | + | - | + | - | + |
| INO | - | - | + | - | - | + | - | - |
| SOR | + | - | + | + | - | + | + | + |
| RHA | + | - | + | + | - | - | - | + |
| SAC | - | - | + | - | - | + | + | + |
| MEL | - | - | + | - | - | + | - | + |
| AMY | - | - | + | - | - | + | - | + |
| ARA | + | - | + | - | - | - | - | + |
| OX | - | + | - | - | - | - | - | - |
| $NO_2$ | + | - | + | + | + | + | + | + |
| $N_2$ | - | - | - | - | - | - | - | - |
| MOB | + | + | - | + | - | + | + | + |
| McC | + | + | + | + | + | + | + | + |
| OF-O | + | + | + | + | + | + | + | + |
| OF-F | + | - | + | + | + | + | + | - |

According to the biochemical reactions of API 20E eight G-negative bacteria were identified as follows: *E. coli* (1) *Pseudomonas aeruginosa* (2), *Klebsiella pneumoniae* (3), *Salmonella cloerasuis* (4), *Shigella* sp. (5), *Serratia liquefaciens* (6), *Proteus vulgaris* (7) and *Brenneria nitrifluens* (8).+: Positive, -: Negative

Table 2: Mean values ±SD of water characteristics expressed as ppm of the two stations from which plants were collected

| Water characteristics (ppm) | Station | |
|---|---|---|
| | El-Raswa | El-Shiboh |
| pH | 9.00±0 | 9.400±0.14 |
| Temp. (°C) | 30.00 | 29.000 |
| TSS | 1752.00±1.83 | 1714.000±1.83 |
| $CO_3$ | 0.00 | 0.000 |
| $HCO_3$ | 127.00±2.05 | 117.800±2.4 |
| Cl | 761.10±1.6 | 752.300±3.5 |
| $SO_4$ | 179.00±2.1 | 167.000±3.1 |
| Ca | 62.60±0 | 72.300±1.8 |
| Mg | 148.10±2.1 | 146.000±2.2 |
| Na | 378.60±3.3 | 371.100±3.7 |
| Fe | 0.01±0 | 0.020±01 |
| Zn | 0.00 | 0.000 |
| Mn | 0.04±0.01 | 0.025±0.01 |
| Cu | 0.01±0.0 | 0.010±0 |

Table 3: Mean values ±SD of macro and microelements expressed as % of dry wt. and ppm in the studied plants

| Plant species | Macro and microelements as % of dry wt. in ppm | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Ca | Na | Mg | Fe | Zn | Mn | Cu | Pb |
| *C. demersum* | 1.63±0.04 | 2.17±0.0 | 6.00±0.00 | 1680.0±2.2 | 31.5±2.1 | 390.0±4.14 | 26.5±2.10 | 23.00±1.40 |
| *E. crassipes* roots | 1.22±0.13 | 2.34±0.0 | 7.60±0.01 | 1552.0±0.8 | 234.0±2.8 | 340.0±1.40 | 85.0±1.40 | 12.02±0.01 |
| *E. crassipes* leaves | 0.92±0.00 | 2.17±0.0 | 6.40±0.00 | 691.5±0.1 | 64.5±0.3 | 191.5±0.70 | 36.5±2.10 | 10.50±0.70 |
| *P. crispus* | 1.98±0.00 | 2.01±0.0 | 2.10±0.00 | 1470.0±1.1 | 29.0±1.4 | 405.0±0.07 | 10.5±0.70 | 31.50±2.12 |
| *P. pectinatus* | 2.48±0.00 | 2.42±0.0 | 2.13±0.00 | 1503.0±0.5 | 21.0±1.4 | 803.0±4.20 | 11.0±1.40 | 44.00±2.80 |

the main indicator of faecal pollution, constitute 20% of the identified Gram-negative bacteria in the examined water. This also indicated that the water of Lake Manzala is subjected to sewage pollution. Based on morphological and API 20E biochemical reactions, the members of genus 2 are identified as *Pseudomonas aeruginosa* which is an opportunistic pathogen of humans. In contrast to most enterobacteria, this pathogenic bacterium is the most significant example of bacteria capable of multiplying in water. Thus, *P. aeruginosa* is common (16%) in the tested water of Lake Manzala.

The isolates of genus 3 were identified as *Klebsiella pneumoniae* according to morphological and biochemical characteristics. *Klebsiella pneumoniae* represented 14% of the identified gram-negative bacteria isolated from Lake Manzala. This pathogenic bacterium has been previously isolated from surface water (Podschun *et al*., 2001). The species of Genus 4 was classified as *Salmonella cholerasuis* because of its biochemical characteristics, such pathogenic bacterium constituted 13% of Gram-negative bacteria from the examined water.

On the other hand, isolates of genus 5 were identified as *Shigella* sp. and represented 11% of the identified Gram-negative bacteria. Isolates of genus 6 were identified as *Serratia liquefaciens* and represented 10% of Gram-negative bacteria isolated from the tested water samples. This bacterium is considered a pathogen of fish (McIntosh and Austin, 1990). Genus 7 (9%) and genus 8 (7%) of Gram-negative bacteria which were identified as *Proteu vulgaris* and *Brenneria nigrifluens*.

Different values of water characteristics were recorded for the stations of samples collection, with the highest values except $Ca^{++}$ content and pH value for samples from El-Raswa. The pH values lies in the alkaline side and ranged from 9.0 at El-Raswa to 9.4±0.141 at El-Shiboh. The values of TSS fluctuated between 1752±1.83 and 1714±1.83 ppm and the water temperature ranged from 29 to 30°C for the two stations, respectively. Low values of micro-elements were recorded for the two stations (Table 2).

As shown in Table 3 different concentrations of all studied metals ( Ca, Na, Mg, Fe, Zn, Mn, Cu and Pb) were found in the different plants as well as in different parts of the same plant. The submerged macrophyte *P. pectinatus* has the highest values of Mn 603±4.243 ppm and Pb 44±2.828 ppm followed by *P. crispus* (Mn 405±7.07 ppm and Pb 31.5±2.121 ppm), while the highest values of Zn 234±2.828 ppm and Cu 85±1.414 ppm were recorded for *E. crassipes* roots.

The free floating plant *E. crassipes* shows different concentrations of various elements within its tissues and the highest values were recorded for its roots.

The antimicrobial activities of *C. demersum*, *P. pectinatus*, *P. crispus* and *E. crassipes* (leaves and roots) against previously isolated strains of bacteria and fungi were investigated. The results are shown in Table 4-7. As presented in this study, the extracts of all selected plants displayed some kind of activity on the tested organisms. The aqueous extract appeared to be the most effective extract but, being more effective on fungal

Table 4: The antimicrobial activities of aqueous, chloroform, ethanol and methanol extracts of *Ceratophyllum demersum*

| | Inhibition zones (mm) | | | |
|---|---|---|---|---|
| Micro organisms | Aqueous extract | Chloroform extract | Ethanol extract | Methanol extract |
| *Escherichia coli* | 17.0±0.00 | 0.0 | 15±0.02 | 0.0 |
| *Pseudomonas aeruginosa* | 24.0±0.03 | 13.0±0.00 | 15±0.00 | 14.0±0.00 |
| *Proteus vulgaris* | 13.0±0.01 | 15.0±0.00 | 26±0.00 | 18.0±0.00 |
| *Salmonella cholerasuis* | 55.0±0.01 | 15.0±0.00 | 16±0.00 | 13.0±0.00 |
| *Shigella* sp. | 21.0±0.02 | 10.0±0.00 | 16±0.00 | 15.0±0.00 |
| *Klebsiella pneumoniae* | 33.0±0.06 | 17.0±0.01 | 20±0.00 | 13.0±0.00 |
| *Serratia liquefacieus* | 26.0±0.02 | 18.0±0.01 | 18±0.00 | 20.0±0.00 |
| *Brenneria nigriflneus* | 16.0±0.01 | 12.0±0.00 | 16±0.00 | 13.0±0.00 |
| *Bacillus subtilis* 1020 | 12.0±0.00 | 27.0±0.00 | 33±0.00 | 13.0±0.00 |
| *Bacillus cereus* 1080 | 28.0±0.01 | 37.0±0.01 | 33±0.00 | 23.0±0.01 |
| *Erwinia carotovora* NCPPB 312 | 8.0±0.00 | 16.0±0.01 | 23±0.00 | 14.0±0.00 |
| *Staphylococcus aureus* | 18.0±0.01 | 17.0±0.01 | 17±0.00 | 18.0±0.01 |
| *Candida albicans* | 20.0±0.01 | 22.0±0.01 | 11±0.00 | 11.0±0.00 |
| *Candida tropicalis* | 22.0±0.01 | 24.0±0.00 | 11±0.00 | 12.0±0.01 |
| *Aspergillus niger* | 53.0±0.02 | 0.0 | 0.00 | 0.0±0.00 |
| *Fusarium oxysporum* | 45.0±0.02 | 23.0±0.00 | 24±0.00 | 11.0±0.00 |
| *Penicillium italicum* | 12.0±0.02 | 0.0±0.00 | 16±0.00 | 0.0 |

Table 5: The antimicrobial activities of aqueous, ethanol and methanol extracts of *Eichhornia crassipes* (leaves and roots)

| | Zone of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | Aqueous extract | | Ethanol extract | | Methanol extract | |
| Micro organisms | Leaves | Roots | Leaves | Roots | Leaves | Roots |
| *Escherichia coli* | 11.0±0.00 | 15.0±0.00 | 0.0±0.00 | 11.0±0.00 | 12.0±0.00 | 14.0±0.00 |
| *Pseudomonas aeruginosa* | 12.0±0.00 | 32.0±0.02 | 12.0±0.00 | 11.0±0.00 | 11.0±0.00 | 10.0±0.00 |
| *Proteus vulgaris* | 16.0±0.00 | 24.0±0.01 | 10.0±0.00 | 12.0±0.00 | 11.0±0.00 | 12.0±0.00 |
| *Salmonella cholerasuis* | 20.0±0.01 | 16.0±0.00 | 15.0±0.00 | 11.0±0.00 | 16.0±0.00 | 8.0±0.00 |
| *Shigella* sp. | 25.0±0.02 | 15.0±0.00 | 13.0±0.00 | 23.0±0.01 | 23.0±0.00 | 20.0±0.00 |
| *Klebsiella pneumoniae* | 23.0±0.00 | 21.0±0.02 | 14.0±0.00 | 14.0±0.00 | 17.0±0.00 | 11.0±0.00 |
| *Serratia liquefacieus* | 24.0±0.00 | 18.0±0.00 | 1.5±0.00 | 27.0±0.02 | 24.0±0.01 | 11.0±0.00 |
| *Brenneria nitgriflneus* | 21.0±0.00 | 15.0±0.00 | 0.0±0.00 | 13.0±0.00 | 0.0±0.00 | 11.0±0.00 |
| *Bacillus subtilis* 1020 | 18.0±0.00 | 12.0±0.00 | 20.0±0.01 | 28.0±0.02 | 47.0±0.02 | 11.0±0.00 |
| *Bacillus cereus* 1080 | 18.0±0.00 | 25.0±0.01 | 15.0±0.00 | 28.0±0.01 | 47.0±0.01 | 13.0±0.00 |
| *Erwinia carotovora* NCPPB 312 | 9.0±0.00 | 17.0±0.00 | 11.0±0.00 | 21.0±0.01 | 19.0±0.00 | 11.0±0.00 |
| *Staphylococcus aureus* | 17.0±0.00 | 15.0±0.00 | 16.0±0.00 | 16.0±0.00 | 25.0±0.00 | 9.0±0.00 |
| *Candida albicans* | 32.0±0.02 | 21.0±0.00 | 11.0±0.00 | 11.0±0.00 | 21.0±0.00 | 12.0±0.00 |
| *Candida tropicalis* | 20.0±0.00 | 25.0±0.02 | 12.0±0.00 | 14.0±0.00 | 30.0±0.01 | 19.0±0.00 |
| *Aspergillus niger* | 47.0±0.01 | 16.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 12.0±0.00 |
| *Fusarium oxysporum* | 44.0±0.01 | 25.0±0.01 | 12.0±0.00 | 17.0±0.00 | 14.0±0.01 | 37.0±0.02 |
| *Penicillium italicum* | 21.0±0.00 | 15.0±0.00 | 0.0 | 17.0±0.00 | 0.0 | 16.0±0.00 |

growth especially (*Aspergillus niger* and *Fusarum oxysparum*) causing inhibition growth zone ranged from 53±0.02 mm with *C. demersum* to 48±0.01 with *P. crispus* extract.

Regarding to the efficiency of organic solvents used in extracting antimicrobial substances from the used plants, ethanol and methanol were nearly similar except in few cases, where, ethanol extracts appeared to be active against all tested organisms except *Aspergillus niger* and *Fusarum oxysparum* in case of *Potamogeton pectinatus*, *Aspergillus niger* using the extracts of *Ceratophyllum demersum*, *Potamogeton crispus* and *Eichhornia crassipes* roots, while the extract of *E. crassipes* leaves appeared to have no effect also on *Penicillium italicum*.

Chloroform extract of all tested plants seemed to have no effect against *Escherichia coli*, *Aspergillus niger* and *Penicillium italicum*.

Aqueous extract of *C. demersum* exhibited antimicrobial effects against all tested organisms and being the highly effective extract against *Salmonella cholerasuis* and *Aspergillus niger* causing inhibition growth zone (55±0.01 and 53±0.02 mm, respectively). At the same time on using methanol and ethanol extracts *Aspergillus niger* showed resistance while, the inhibition growth zone of *Salmonella cholerasuis* ranged from 13±0.0 to 16±0.0 mm, respectively (Table 4).

The results in Table 5 showed that the aqueous extract of *E. crassipes* (leaves and roots) were effective against all tested organisms and the leaves extract seem to be the highly effective especially against *Aspergillus niger* and *Fusarium oxysporum* causing inhibition zones ranged from 47±0.01 to 44±0.01 mm, respectively. At the same time methanol extract was the highly effective against *Bacillus subtilis* 1020 and

Table 6: The antimicrobial activities of aqueous, chloroform, ethanol and methanol extracts of *Potamogeton crispus*

| | Inhibition zones (mm) | | | |
|---|---|---|---|---|
| Micro organisms | Aqueous extract | Chloroform extract | Ethanol extract | Methanol extract |
| *Escherichia coli* | 15.0±0.00 | 0.0±0.00 | 14.0±0.00 | 11.0±0.00 |
| *Pseudomonas aeruginosa* | 18.0±0.00 | 14.0±0.00 | 13.0±0.00 | 16.0±0.00 |
| *Proteus vulgaris* | 18.0±0.00 | 18.0±0.00 | 13.0±0.00 | 25.0±0.01 |
| *Salmonella cholerasuis* | 26.0±0.00 | 13.0±0.00 | 22.0±0.00 | 18.0±0.00 |
| *Shigella* sp. | 25.0±0.00 | 15.0±0.00 | 23.0±0.00 | 17.0±0.00 |
| *Klebsiella pneumoniae* | 15.0±0.00 | 13.0±0.00 | 17.0±0.00 | 18.0±0.00 |
| *Serratia liquefacieus* | 23.0±0.00 | 20.0±0.01 | 28.0±0.01 | 29.0±0.01 |
| *Brenneria nigriflneus* | 13.0±0.00 | 13.0±0.00 | 22.0±0.01 | 15.0±0.00 |
| *Bacillus subtilis* 1020 | 26.0±0.01 | 13.0±0.00 | 25.0±0.02 | 11.0±0.00 |
| *Bacillus cereus* 1080 | 19.0±0.00 | 23.0±0.00 | 25.0±0.02 | 11.0±0.00 |
| *Erwinia carotovora* NCPPB 312 | 12.0±0.00 | 14.0±0.00 | 12.0±0.00 | 22.0±0.01 |
| *Staphylococcus aureus* | 17.0±0.00 | 18.0±0.00 | 16.0±0.00 | 21.0±0.00 |
| *Candida albicans* | 31.0±0.02 | 11.0±0.00 | 12.0±0.00 | 12.0±0.00 |
| *Candida tropicalis* | 37.0±0.02 | 12.0±0.00 | 24.0±0.02 | 17.0±0.00 |
| *Aspergillus niger* | 30.0±0.02 | 0.0±0.00 | 0.0±0.00 | 14.0±0.00 |
| *Fusarium oxysporum* | 48.0±0.01 | 11.0±0.00 | 16.0±0.00 | 21.0±0.00 |
| *Penicillium italicum* | 30.0±0.01 | 0.0 | 38.0±0.01 | 18.0±0.00 |

Table 7: The antimicrobial activities of aqueous, chloroform, ethanol and methanol extracts of *Potamogeton pectinatus*

| | Inhibition zones (mm) | | | |
|---|---|---|---|---|
| Micro organisms | Aqueous extract | Chloroform extract | Ethanol extract | Methanol extract |
| *Escherichia coli* | 14.0±0.00 | 0.0 | 15.0±0.00 | 11.0±0.00 |
| *Pseudomonas aeruginosa* | 59.0±0.02 | 11.0±0.00 | 16.0±0.00 | 44.0±0.02 |
| *Proteus vulgaris* | 48.0±0.01 | 14.0±0.00 | 31.0±0.02 | 50.0±0.02 |
| *Salmonella cholerasuis* | 27.0±0.00 | 19.0±0.01 | 15.0±0.00 | 23.0±0.01 |
| *Shigella* sp. | 25.0±0.01 | 10.0±0.01 | 16.0±0.00 | 21.0±0.01 |
| *Klebsiella pneumoniae* | 20.0±0.00 | 16.0±0.00 | 11.0±0.00 | 12.0±0.00 |
| *Serratia liquefacieus* | 25.0±0.00 | 17.0±0.01 | 27.0±0.01 | 21.0±0.00 |
| *Brenneria nigriflneus* | 13.0±0.00 | 13.0±0.00 | 27.0±0.01 | 16.0±0.00 |
| *Bacillus subtilis* | 14.0±0.00 | 12.0±0.00 | 28.0±0.00 | 25.0±0.01 |
| *Bacillus cereus* | 15.0±0.00 | 33.0±0.02 | 28.0±0.02 | 25.0±0.01 |
| *Erwinia carotovora* NCPPB 312 | 7.0±0.00 | 7.0±0.00 | 10.0±0.00 | 16.0±0.01 |
| *Staphylococcus aureus* | 18.0±0.01 | 19.0±0.00 | 18.0±0.02 | 18.0±0.01 |
| *Candida albicans* | 25.0±0.01 | 13.0±0.00 | 11.0±0.00 | 12.0±0.00 |
| *Candida tropicalis* | 15.0±0.00 | 19.0±0.01 | 12.0±0.00 | 0.0±0.00 |
| *Aspergillus niger* | 38.0±0.01 | 0.0±0.00 | 0.0±0.00 | 18.0±0.01 |
| *Fusarium oxysporum* | 38.0±0.01 | 28.0±0.02 | 0.0 | 26.0±0.01 |
| *Penicillium italicum* | 25.0±0.02 | 0.0 | 20.0±0.01 | 19.0±0.02 |

*Bacillus cereus* 1080 causing inhibition zone 47±0.02 and 47±0.01 mm for the two species, respectively. In contrast to this the ethanol extract of roots was highly effective than that of leaves against these two bacterial species.

The results in Table 6 and 7 showed that, the aqueous extract of *P. crispus* was highly effective extract against *Fusarium oxysporum* causing inhibition zone 48±0.01 mm while the extract of *P. pectinatus* was the highly effective extract against the pathogenic bacteria *Pseudomonas aeruginosa* (59±0.02 mm) and *Klebsiella pneumoniae* (48±0.01 mm). At the same time the methanol extract of *P. pectinatus* was also recorded as the most effective extract against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

## DISCUSSION

According to the previously mentioned results, all tested plant materials used had an variable inhibitory effect on growth of the tested microorganisms. The distribution of antimicrobial substances which varied from species to species lead to the variation of antimicrobial activity (Lustigman and Brown, 1991). The effect of aqueous extract followed by ethanol extract appeared to be the most effective extract on most tested Gram positive and Gram negative bacteria. Adomi (2006) reported that the water and ethanol extracts of the stem bark of some medicinal plants were tested on Gram positive and Gram negative bacteria, where the aqueous extract was active while the ethanol extract was not active. The efficiency of the solvent used in the extraction varied with the plant material, where *Potamogeton pectinatus* and *Potamogeton crispus* appeared to be the most effective followed by *Ceratophyllum demersum* and *E. crassipes*.

As recorded by El-Habibi *et al.* (1992) and Haroon (2006) the phytochemical screening of the tested plants (Table 8), revealed the presence of some important and active substances (tannins, flavonoids, saponins,

Table 8: A preliminary phytochemical screening of *Ceratophyllum demersum*, *Eichhornia crassipes* (roots and leaves), *Potamogeton crispus* and *Potamogeton pectinatus*

| Tests | *C. demersum* | *E. crassipes* | *P. crispus* | *P. pectinatus* | References |
|---|---|---|---|---|---|
| Alkaloids | +ve | +ve | +ve | +ve | El-Habibi *et al.* (1992) |
| Glycosides | +ve | +ve | +ve | +ve | Haroon (2006) |
| Sterols | +ve | +ve | +ve | -ve | |
| Terpenes | +ve | +ve | +ve | -ve | |
| Flavonoids | +ve | +ve | +ve | +ve | |
| Tannins | +ve | -ve | +ve | +ve | |
| Saponins | -ve | -ve | +ve | +ve | |
| Mucilage | +ve | -ve | +ve | +ve | |
| Chlorides | +ve | -ve | +ve | +ve | |
| Sulphates | +ve | -ve | +ve | +ve | |
| Resins | -ve | -ve | -ve | -ve | |

alkaloids, terpenes and glycosides) but with a few exceptions, where, *C. demersum* was characterized by the presence of all these substances while tannins were absent from *E. crassipes*, saponins were not detected in *E. crassipes* and terpenes were not found in *P. pectinatus*.

The presence of the active substances mentioned above in the extracts of the studied plants caused the inhibiting effect on growth of the tested organisms. This effect was affected by the concentrations of these active substances in the plant extract. This could be explain the greatest effect of *P. pectinatus* extracts upon the tested organisms, where, it is extracts contained the highest amount of the active substances among all tested plants.

The results also showed that, the effect of extract were not only depend on the type of solvent and plant species used, but was also depend on the part of plant used, where, the extracts of *E. crassipes* leaves being more effective than that, of its roots. Although roots have higher concentrations of heavy metals compared to the leaves it is antimicrobial effect is less and this means, that the antimicrobial activity is more affected by the presence of active substances which present in leaves in high amount compared by it in roots.

As recorded by Harding (1981), Kantrud (1990), Ali *et al.* (1999) and Serag *et al.* (1999), *P. pectinatus* was found in abundance at highly polluted sites, *C. demersum* and *E. crassipes* were characterized by its high level of heavy metals, this may be another reason describing the antimicrobial activity of these extracts. The effect of various elements on antimicrobial activity of the extracts could be observed, where, *P. pectinatus* was characterized by its high lead content and the highly effected compared with the rest of other extracts.

The powdered samples of all investigated plants gave negative results, which coming in agreement with who mentioned that, the negative results recorded by Azzouz and Bullerman (1982) for the effect of powdered Pomegranate peel on growth of *A. flavus* or *A. parasiticus* may be due to that, the powdered crude material contains ferrous ion, which bind with the active substances (tannins and flavonoids) and inhibit its effect on the cytochrome while, the extracts are characterized by the presence of these substances in free form.

## CONCLUSION

The water of Lake Manzala is subjected to fecal pollution and monitoring of microbial quality of water is a must to control the spreading of pathogens transmitted by contaminated water.

The study confirms that all plants extracts used in this investigation possess *in vitro* antibacterial and antifungal activity against the used organisms. At the same time the powdered plants samples had no antimicrobial activity.

The efficiency of the extracts varied with, solvent used in the extraction as well as, plant species and the part of plant used. The aqueous extracts appeared to be the most effective extracts against all tested organisms. So, it could be conclude to use it for food preservation or medicinal purposes after some experiments for safety and toxicity.

Comparing the effect of different plants extracts *C. demersum* appeared to be the most effective followed by *P. pectinatus*. Furthermore, the extracts of *E. crassipes* leaves being more effective than that, of its roots.

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