Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 3
Open Access

Antibacterial effect of two plants used in remediation of Al-Sadr clinical city wastewater

Ruaa M. Abdul – Jabbar*, Batool M. Al-Adily , Nuha F. Kadhim

Department of Biology, Collage of Science, University of Babylon, Iraq
*Corresponding Author**:** ruaa11789@gmail.com

Cite this paper as: Ruaa M. Abdul – Jabbar, Batool M. Al-Adily,Nuha F. Kadhim (2024) Antibacterial effect of two plants used in remediation of Al-Sadr clinical city wastewater. *Frontiers in Health Informatics, 13* (3),4135-4143

Abstract

Current study shows the efficiency of two types of aquatic plant, as extracts after used them in the phytoremediation of wastewater that taking from treatment plant Al-Sadr clinical city. These extracts included ceratophyllum demersum, leman monir with two concentrations (50000,150000mg/l) as antimicrobials to inhibitions five species of pathogenic antimicrobial-resistant bacteria, are Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, and proteus mirabilis, that isolated from same wastewater treatment plant of Al-Sadr clinical city. The results showed that the high effectiveness of these extracts against these five species of bacteria, where the C. demersum extract recorded the highest inhibition diameter for bacteria S. aureus, E. faecalis and P. aeruginosa, P. mirabilis was (14,11,9,8mm) respectively, at concentration (150000mg/l), The plant that used in remediation of wastewater were more effective than those plants which grow in tub water (control) on bacteria growth. Results explain these extracts contain on the phytochemical compounds were had turn in increase inhibition diameters antimicrobials resistance bacteria including alkaloids, tannins, phenolics, saponins and flavonoids that acts as antioxidants were, in the C demersum treated recorded 21.52,14.07,44.60,12.58,19.31 respectively,

1. Introduction

One of the reasons to use of plant extracts as antimicrobials was due to the prescription of antibiotics carelessly and irrationally in a variety of illnesses resulted in the creation of antibiotic-resistant microorganisms. This unprecedented usage of antibiotics that caused resistance has an impact on human health globally (AlSheikh *et al*., 2020; Dhingra *et al.,* 2020). Because excessive faecal bacteria in sewage and urban runoff have been shown to imply a risk of pathogen-induced illnesses in humans, pathogens are an important problem for managers of water resources (Sabae *et al.,* 2007). It is clear that novel antimicrobial drugs and approaches are required for the treatment of severe Gram-positive infections (Corona *et al.,* 2023). Research indicates that naturally occurring antimicrobial agents derived from plant extracts contain components that have an inhibitory impact and can be applied therapeutically for a more successful course of treatment. (Dong *et al.,* 2019; Nekratova *et al.,* 2023) be less dangerous than synthetic ones (Fareed *et al.,* 2008).

Antimicrobial phytochemicals such as flavonoids, tannins, terpenoids, sterols, glycosides, saponins, carotenoids, alkaloids, terpenes, and phenols are abundant in hydrophytes (Devi *et al.,* 2005; Metwally *et al.,* 2020). They showed antibacterial activities (Fareed *et al*., 2008; Sridevi *et al.,* 2010; Ziada *et al*., 2008), antifungal activities (Metwally *et al.,* 2020) and antiviral activities (Shin *et al.,* 2010; Sohail *et al.,* 2011). It is well known that hydrophytes manufacture flavonoids in response to microbial infection, and that these compounds exhibit antimicrobial action against a broad range of bacteria in vitro (Bhowmik *et al.,* 2013).

Because they may combine to form complexes with soluble and extracellular proteins, flavonoids have biological actions that include anti-inflammatory, antiallergic, antioxidant, and antibacterial properties. Also, tannins included in hydrophytes are recognized as antimicrobial agents, they impede the growth and development of germs by rendering the protein essential for microbe nourishment unavailable (Yoshino *et al.,* 2004). Research has been done on saponin in a variety of aquatic plant-herbivore relationships (Metwally *et al.,* 2020; Smolders *et al.,* 2000). According to research by Kurashov et al. (2016), the essential oil of C. demersum possesses antifungal, anti-inflammatory, antibacterial, and antineoplastic properties. According to Gobrial et al. (2015), the acetone extract of C. demersum shown antibacterial activity against negative gram bacteria. Rich supplies of biologically active chemicals against Aspergillus, Penicillium, Acremonium, Rhizopus, Cladosporium, Torula, and Alternaria can be found in Polygonum senegalensis and P. crispus (Omar et al., 2018).

2. Materials and Methods

1. Plants collection, adaptation and water treatment

Both C. demersum and L. minro were collected from rivers in AL Najaf governorate then put for 7 days in a tab water then used divided in two groups first one put in the wastewater of Al-Sadr clinical city that effluent from treatment plant in clinical city, while second put in a tab water (control) after 25 of treatment plants were collected for prepare extracts.

2. plant extracts

Plants samples collected from wastewater after used 25 days of phytoremediation and were Transfer it to the laboratory under appropriate conditions. The samples were allowed to dry in a shade and breathing place, dried *C. demersum* and *L. minro* samples were cut and pulverized. 60 g of the of the plants were extracted with soxhlet extractor with (250 ml) of ethanol (EtOH) at 50 ° C for about 6 hours. The extracts obtained were concentrated by rotary evaporator (Harborne, 1998).

3. phytochemical composition of plant extracts

Total alkaloids.

HCl 2N used to dissolved amount of plant extract, and then added chloroform, then BCG solution and finally 5 ml of phosphate buffer were added to this solution, then the absorbed the optical density at 417 nm.

Total tannin content (TTC).

TTC were estimated according to method of Price and Butler(1977) .

Total flavonoid content (TFC).

By using 5% NaCl then 10% $AlCl_3$ and finally 1N NaOH respectively then measured at 510 nm.

Total Phenolic contents.

1 ml of plant extract, then 0.5 ml 2N of the Folin-Ciocalteu reagent and 1.5 ml 20% of Na_2CO_3 solution, finally, 2 hours after which the absorbance was taken at 765 nm (Hagerman, *et al*., 2000).

Total saponins

Total saponins was determined according to the method that described by Makkar *et al*., 2007).

4. Antibacterial effect

Five species of purified bacteria *S. aureus, E. faecalis*, *E. coli, P. aeruginosa and p. mirabilis* were used to the effect of plant extracts according to (APHA 1999; Holt et al. 1993; CLSI 2012).

3.Results

Phytochemical compounds in used plants in treatment

In the current study it found that Phytochemical compounds (alkaloids, tannins, phenolics, saponins and flavonoids) concentrations that studied in *C. demersum* and *L. minor* that used in the treatment of Al-Sader clinical city wastewater were differ according to plants species and type of water where they put table (1).Wastewater caused a significant increase in Alkaloids content in *C. demersum* but caused significant decrease in *L. minor* p-value 0039 and 0.001 respectively, while it induced increasing of Tanins content in both plant but in *C. demersum* the increasing was non-significant (p-value 0.610). *L. minor* recorded significant increases in both Phenoles and Flavonoids (p-value 0.013 and 0.001 respectively), while *C. demersum* recorded non-significant increases in them after used these plants in the treatment experiment. Wastewater made non-significant in Saponins concentrations in both plants.

Table (1): concentrations of some phytochemical compound's mg/l in *C. demersum* and L. *minor* that used in the phytoremediation of Al-Sader clinical city wastewater.

Phytochemicals	*Ceratophyllum demersum*		p-value	*Lemna minor*		p-value
	Control	**Treated**		**Control**	**Treated**	
Alkaloids	18.82±0.7	21.52±1.3	**0.039***	22.19±0.7	12.42±1.5	**0.001****
Tannins	12.01±1.9	14.07±1.6	**0.610**	17.27±1.8	26.41±2.4	**0.004****
Phenolics	40.17±0.6	44.60±3.5	**0.103**	49.62±3.3	33.50±1.5	**0.013***
Saponins	11.05±1.9	12.58±1.2	**0.305**	13.94±1.4	13.19±1.1	**0.513**
Flavonoids	16.33±1.3	19.31±2.2	**0.132**	14.63±3.1	23.34±0.8	**0.001****

Antibacterial effect of extracts of used plants

In the current study, two types of plants (*C. demersum, L. minor*) were used in treating Al-sadr clinical city wastewater, after the end of the experiment period, the used plants were collected and extracts were prepared, these extracts were used to inhibit five species of pathogenic bacteria, *S. aureus, E. faecalis* (gram positive bacteria) and *E.coli, P. aeruginosa, P. mirabilis* (gram negative bacteria), that were isolated from the same wastewater of Al-Sadr clinical city, were high effectiveness of these extracts against these five species of bacteria, where the *C. demersum* extract treated recorded the highest inhibition diameter for bacteria *Staphylococcus aureus, Enterococcus faecalis and Pseudomonas aeruginosa, proteus mirabilis* was (14,11,9,8mm) respectively, with a concentration of (150000mg/l), compared with a concentration (50000mg/l), ile the inhibition diameter for bacteria *Escherichia coli* by the *C. demersum* extract treated was (10mm) equal for the two concentrations (50000, 150000mg/l). For *C. demersum* extract control, it recorded high inhibition rate for all species of bacteria at concentration (150000mg/l) compared to concentration (50000mg/l) as in the table (2). While, *L. minor* extract treated recorded high inhibition diameters for all species used bacteria, with a concentration of (150000mg/l), compared with a concentration (50000mg/l). Also, the same results for *L. minor* extract control with concentrations (150000mg/l) and (50000mg/l). The results showed in the table (2) explain significant difference between extracts plants and its concentration during period study.

Plant extract	**Extract plant concen.**	**Inhibition diameters in mm for bacterial isolates**				
		Staphylococcus aureus	***Enterococcus faecalis***	***Escherichia coli***	***Pseudomonas aeruginosa***	***Proteus mirabilis***
		Mean±S.D				
Control	**0**	0	0	0	0	0
***C. demersum* extract treated**	**0.1**	12±1.7	10±1.4	10±1.4	8±2.3	0
	0.3	14±2.0	11±2.0	10±1.4	9±1.7	8±2.3
***C. demersum* extract control**	**0.1**	0	0	8±2.3	8±1.1	0
	0.3	10±2.2	7±0.8	10±1.4	12±1.7	8±2.3
***L. minor* extract treated**	**0.1**	8±2.3	8±1.1	10±1.4	8±2.3	0
	0.3	11±1.5	10±1.4	13±1.9	12±1.7	8±2.1
***L. minor* extract control**	**0.1**	16±3.1	18±4.4	14±2.0	10±1.4	11±1.5
	0.3	20±2.9	22±3.8	16±3.1	12±1.7	15±3.3
LSD(0.05)		**1.552**	**1.224**	**1.512**	**1.087**	**2.028**

Table (2): Antibacterial effect of plants extracts that used in the phytoremediation of Al-Sader clinical city wastewater and inhibition diameters in mm for bacterial isolates.

Plant extract	Extract plant concen. Mg/l	Inhibition diameters in mm for bacterial isolates				
		Staphylococcus aureus	***Enterococcus faecalis***	***Escherichia coli***	***Pseudomonas aeruginosa***	***Proteus mirabilis***
		Mean±S.D				
Control	**0**	0	0	0	0	0
***C. demersum* extract treated**	**50000**	12±1.7	10±1.4	10±1.4	8±2.3	0
	150000	14±2.0	11±2.0	10±1.4	9±1.7	8±2.3
***C. demersum* extract control**	**50000**	0	0	8±2.3	8±1.1	0
	150000	10±2.2	7±0.8	10±1.4	12±1.7	8±2.3
***L. minor* extract treated**	**50000**	8±2.3	8±1.1	10±1.4	8±2.3	0
	150000	11±1.5	10±1.4	13±1.9	12±1.7	8±2.1
***L. minor* extract control**	**50000**	16±3.1	18±4.4	14±2.0	10±1.4	11±1.5
	150000	20±2.9	22±3.8	16±3.1	12±1.7	15±3.3
LSD(0.05)		**1.552**	**1.224**	**1.512**	**1.087**	**2.028**

4. Discussion

Numerous antimicrobial-resistant bacteria (ARB) are discharged from hospitals through wastewater systems, making hospitals major suppliers of ARBs and key players in their emergence and dissemination (Hocquet et al., 2016) In this study five species of pathogenic antimicrobial-resistant, are *Staphylococcus aureus, Enterococcus faecalis* (gram positive bacteria) and *Escherichia coli, Pseudomonas aeruginosa, proteus mirabilis* (gram negative bacteria), were isolated from wastewater treatment plant of Al-Sadr clinical city. Similar study isolated these five species of pathogenic antimicrobial-resistant from a tertiary care teaching hospital (Kalaiselvi *et al.*, 2016). Another study that show *E.coli, P. aeruginosa, E. faecalis and S. aureus*, have the greatest resistance to various antibiotics(Lykov *et al.*, 2021). Because of wastewater treatment plants could promotes the ARB, and described as hotspots for the transfer of antibiotic resistance genes between bacterial species, furthermore, metals such as mercury and antibacterial cleaning agents may encourage the persistence of antimicrobial-resistant bacteria (ARB) in wastewater (Hocquet *et al*., 2016).

In other study, the total amount of antibiotics removed by traditional physical and biological treatment methods was found to be 79% in the warmer months and 36% in the winter. This indicates that the antibiotic removal effectiveness at the WWTP is insufficient., so, should use advanced treatment processes (Aydin *et al.*, 2019). In the current study, where using extracts of the *C. demersum*, and *L. minor* to treatment antimicrobial-resistant bacteria, because these extracts contain on phytochemical compounds that acts as antioxidants (Hoang *et al.,* 2022).

During the current study it found that phytochemical compounds (alkaloids, tannins, phenolics, saponins and flavonoids) concentrations that studied in *C. demersum* and L. minor that used in the treatment of Al-Sadir clinical city wastewater were differ according to plants species and type of water where they put, where, all these phytochemical compounds recorded high values in the *C. demersum* extract treated more than in the *C. demersum* extract control, were the highest value recorded by phenolics (44.6), while in *L. minor* extract treated only flavonoids and tannins recorded high values (23.34, 26.41) respectively compared with *L. minor* extract control . Where Syed *et al.*, (2018) proved the extract of *C. demersum* contains on these phytochemical compounds. So, there was significant different between values of these compounds of extract treated and extract control for study plants.

These compounds that derived from plants could provide mothed approaches against pathogenic bacteria (Vaou *et al.*, 2021). Where the results of this study, show high effectiveness of these extracts against these five species of bacteria, where the *C. demersum* extract treated recorded the highest inhibition diameter for bacteria *S. aureus, P. mirabilis, E. faecalis and P. aeruginosa* was (14,11,9,8mm) respectively, with a concentration of (150000mg/l), compared with a concentration (50000mg/l), and inhibition diameter (10mm) for *Escherichia coli*, equal for the two concentrations (50000,150000mg/l), the results showed that the *C. demersum* extract treated was more efficient in inhibiting bacteria than the *C. demersum* extract control, this results agree with Fareed *et al.,* (2008) that proved the extract of *C. demersum* was the most effective as antimicrobial activities against all tested bacteria.

While, *L. minor* extract treated recorded high inhibition diameters for all types of used bacteria with a concentration of (150000mg/l), compared with a concentration (50000mg/l), where the highest inhibition diameter was for *Escherichia coli* (13mm). While the *L. minor* extract control recorded the highest inhibition diameters against, *Staphylococcus aureus, Enterococcus faecalis and Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis* was (20,22,16,12,15mm) respectively, compared with the *L. minor* extract treated.

Where match with GÜLÇİN *et al.,* (2010) had an antibacterial effect against *Staphylococcus* because its source of natural antioxidants and contains phenolics and flavonoids.

Also, Miras-Moreno *et al..,* (2022) explained that the secondary metabolism of the plant leads to the raising of the content of non-enzymatic antioxidants such as flavonoids.

And González-Renteria *et al.,* (2020) demonstrated effectiveness of *L. minor* extract as an antimicrobial. So, the high values of phytochemical compounds in the current study that resulted from degradation or absorption of the antibiotics by *C. demersum* extract treated were had turn in increase inhibition diameters antimicrobials resistance bacteria, this results similar with Silva *et al.,* (2010) that explain the biological characteristics of some plant species that are employed in the antibacterial activity of various plants are often caused by the active chemicals generated during secondary vegetal metabolism. In the current study, may be high concentration of flavonoids in the extracts of the *C. demersum* and *L. minor* led to an increase in the diameter of inhibition of bacteria, this result similar with Xie *et al.,* (2015) where refer to the structure of flavonoids as antibacterial agents, and explained, the antibacterial mechanism of flavonoids, where hydroxyls at special sites on the aromatic rings of flavonoids improve the antibacterial activity, these flavonoids mechanisms involving, inhibition of energy metabolism, inhibition of the porin on the cell membrane, and attenuation of the pathogenicity.

The findings also demonstrated significant variation in the sensitivity of bacteria to the extract from plants., this might result from variations in the kind and quantity of their active ingredients (Al-Janae'e et al., 2017). linked to how well they absorb and migrate inside the cell (Pourmorad et al., 2006). The type, composition, thickness, and fat and protein content of cellular membranes, as well as the mechanism of transition operation of effective materials, all influence the differences in bacterial sensitivity to these substances. As a result, certain bacteria exhibit a higher sensitivity against a particular compound (Lee et al., 1999). This variation could also be explained by the presence of protective substances within the bacteria's cells, which may have been brought about by exposure to outside forces or by efficient environmental transport. These substances can increase a cell's level of protection based on the kind of microorganism, the type of external effect, and other abnormal factors. The most significant protective materials are glutamate, carbohydrates and mannitol (Salih & Hussein, 2014).

4. Conclusions

The phytochemical composition of plants was very effected by types of water were plant grow. So, the *C. demersum* was more effective in the inhibition of bacteria, thus it can used both *C. demersum* and *L. minor* as a source to prepare antibacterial to control the resistance bacteria

AlSheikh, H. M. A., Sultan, I., Kumar, V., Rather, I. A., Al-Sheikh, H., Tasleem Jan, A., & Haq, Q. M. R. (2020). Plant-based phytochemicals as possible alternative to antibiotics in combating bacterial drug resistance. *Antibiotics*, *9*(8), 480.

Dhingra, S., Rahman, N. A. A., Peile, E., Rahman, M., Sartelli, M., Hassali, M. A., ... & Haque, M. (2020). Microbial resistance movements: an overview of global public health threats posed by antimicrobial resistance, and how best to counter. *Frontiers in Public Health*, *8*, 535668.

Sabae, S. Z., & Rabeh, S. A. (2007). Evaluation of the microbial quality of the River Nile waters at Damietta Branch, Egypt.

Corona, A., De Santis, V., Agarossi, A., Prete, A., Cattaneo, D., Tomasini, G., ... & Latronico, N. (2023). Antibiotic therapy strategies for treating gram-negative severe infections in the critically Ill: A narrative review. *Antibiotics*, *12*(8), 1262.

Dong, Y., Chen, H., Gao, J., Liu, Y., Li, J., & Wang, J. (2019). Bioactive ingredients in Chinese herbal medicines that target non-coding RNAs: promising new choices for disease treatment. *Frontiers in Pharmacology*, *10*, 515.

Nekratova, A. N., & Zinner, N. S. (2023). Medicinal plants of the Tomsk region and possibilities of their application. *Advances in Traditional Medicine*, *24*(1), 41-66.

Fareed, M. F., Haroon, A. M., & Rabeh, S. A. (2008). Antimicrobial activity of some macrophytes from Lake Manzalah (Egypt). *Pakistan Journal of Biological Sciences: PJBS*, *11*(21), 2454-2463.

Devi, S. R., & Prasad, M. N. V. (2005). Antioxidant capacity of Brassica juncea plants exposed to elevated levels of copper. *Russian journal of plant physiology*, *52*, 205-208.

Metwally, F. E., Mohamed, A. A., Mahalel, U. A., & Sheded, M. G. (2020). Evaluation of certain cosmopolitan hydrophytes in the Nile River, Aswan district for their ecological and bioactivity potentials: A review. *Int. J. Sci. Technol. Res*, *9*(1).

Sridevi, M., Kondala Rao, B., & Sathiraju, D. (2010). Sensitivity of Bacteria Isolated from Champavathi Estuary to Some Medicinal Plants of Vizianagaram district, East coast of India. *Drug invention today*, *2*(7).

Ziada, M. A., Mashaly, I. A., El-Monem, M. A., & Torky, M. (2008). Economic potentialities of some aquatic plants growing in north east Nile delta, Egypt. *J. Appl. Sci*, *8*(8), 1395-1405.

Shin, W. J., Lee, K. H., Park, M. H., & Seong, B. L. (2010). Broad-spectrum antiviral effect of Agrimonia pilosa extract on influenza viruses. *Microbiology and immunology*, *54*(1), 11-19.

Sohail, M. N., Fiaz Rasul, F. R., Asia Karim, A. K., Uzma Kanwal, U. K., & Attitalla, I. H. (2011). Plant as a source of natural antiviral agents.

Bhowmik, S., Datta, B. K., & Saha, A. K. (2013). Ethno medicinal and phytochemical screening of some hydrophytes and marsh plants of Tripura, India. *World Applied Sciences Journal*, *22*(10), 1453-1459.

Yoshino, K., Ogawa, K., Miyase, T., & Sano, M. (2004). Inhibitory effects of the C-2 epimeric isomers of tea catechins on mouse type IV allergy. *Journal of agricultural and food chemistry*, *52*(15), 4660-4663.

Smolders, A. J., Vergeer, L. H., Van Der Velde, G., & Roelofs, J. G. (2000). Phenolic contents of submerged, emergent and floating leaves of aquatic and semi-aquatic macrophyte species: why do they differ?. *Oikos*, *91*(2), 307-310.

Kurashov, E. A., Fedorova, E. V., Krylova, J. V., & Mitrukova, G. G. (2016). Assessment of the potential biological activity of low molecular weight metabolites of freshwater macrophytes with QSAR. *Scientifica*, *2016*(1), 1205680.

Ghobrial, M. G., Nassr, H. S., & Kamil, A. W. (2015). Bioactivity effect of two macrophyte extracts on growth performance of two bloom-forming cyanophytes. *Egyptian Journal of Aquatic Research*, *41*(1), 69-81.

Omar, A. M., Taha, A. S., & Mohamed, A. A. A. (2018). Microbial deterioration of some archaeological artifacts: manipulation and treatment. *European Journal of Experimental Biologya*, *8*(3), 21.

APHA 1999 Standard Methods for the Examination of Water and Wastewater. 20th edn. American Public Health Association, American Water Works Association and Water Environment Federation, Washington, DC, USA.

Mulvey, M. R., & Simor, A. E. J. C. (2009). Antimicrobial resistance in hospitals: how concerned should we be? *,* *180*(4), 408-415.

USEPA 1974 Industrial effluent guidelines. Available from: https://www.epa.gov/eg/industrial-effluent-guidelines.

Pradhan, B., & Mishra, S. K. (2010). Multiple drug resistance in bacterial isolates from liquid wastes generated in central hospitals of Nepal. *Kathmandu University Medical Journal*, *8*(1), 40-44.

MackieT. J.MacCartneyJ. E. 1996 Practical Medical Microbiology. 14th edn. Churchill Livingstone Publications, London, UK.

HoltJ. G.KriegN. R.SneathP. H.SafetyJ. T.WilliamsS. T. 1993 Chapter II, The nature of bacterial identification schemes. In: Bergey's Manual of Determinative Bacteriology (WilliamsK.WilkinsO., eds). Lippincott, Williams and Wilkins, Baltimore, USA.

CLSI (Clinical and Laboratory Standards Institute) 2012 Performance Standards for Antimicrobial Susceptibility Testing, 22nd Informational Supplement, MO2-A12.

Makkar HP, Siddhuraju P, Becker K. (2007). *Methods in molecular biology: plant secondary metabolites,* Totowa: Human Press; p. 93-100

Harborne, J.B. (1998). "Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis." 3rd edition. Chapman and Hall, London.

Harborne, J.B (1973): Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman A &Hall.London. Pp 279.

Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002;10(3):178-82.

Price ML, Butler LG. Rapid visual estimation and spectrophotometric determination of tannin content of sorghum grain. J Agric Food Chem. 1977;25(6):1268-73.

Hagerman, A., Muller, I and Makkar, H. (2000) Quantification of tannins in tree foliage. A laboratory manual, Vienna: FAO/IAEA, Pp. 4-7.

Hocquet, D., Muller, A., & Bertrand, X. J. J. o. H. I. (2016). What happens in hospitals does not stay in hospitals: antibiotic-resistant bacteria in hospital wastewater systems. *93*(4), 395-402.

Kalaiselvi, K., Mangayarkarasi, V., Balakrishnan, D., Chitraleka, V. J. J. o. W., & Health. (2016). Survival of antibacterial resistance microbes in hospital-generated recycled wastewater. *14*(6), 942-949.

Lykov, I., & Volodkin, V. (2021). *Presence of antibiotic-resistant bacteria in the environment.* Paper presented at the IOP Conference Series: Earth and Environmental Science.

Aydin, S., Aydin, M. E., Ulvi, A., Kilic, H. J. E. S., & Research, P. (2019). Antibiotics in hospital effluents: occurrence, contribution to urban wastewater, removal in a wastewater treatment plant, and environmental risk assessment. *26*, 544-558.

Hoang, N. N., Nguyen, T. K., Vo, T. H., Nguyen, N. H., Nguyen, D. H., & Tran, D. L. (2022). Isolation, Characterization, and Biological Activities of Fucoidan Derived from Ceratophyllum Submersum L. *Macromolecular Research*, *30*(2), 136-145.

Syed, I., Fatima, H., Mohammed, A., & Siddiqui, M. A. (2018). Ceratophyllum demersum a free-floating aquatic plant: A Review. *Indian Journal of Pharmaceutical and Biological Research*, *6*(02), 10-17.

Vaou, N., Stavropoulou, E., Voidarou, C., Tsigalou, C., & Bezirtzoglou, E. J. M. (2021). Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. *9*(10), 2041.

GÜLÇİN, İ., KİREÇCİ, E., AKKEMİK, E., Topal, F., & Hisar, O. (2010). Antioxidant and antimicrobial activities of an aquatic plant: Duckweed (Lemna minor L.). *Turkish Journal of Biology*, *34*(2), 175-188.

Miras-Moreno, B., Senizza, B., Regni, L., Tolisano, C., Proietti, P., Trevisan, M., ... & Del Buono, D. (2022). Biochemical insights into the ability of Lemna minor L. Extract to counteract copper toxicity in maize. *Plants*, *11*(19), 2613.

González-Renteria, M., del Carmen Monroy-Dosta, M., Guzmán-García, X., & Hernández-Calderas, I. (2020). Antibacterial activity of Lemna minor extracts against Pseudomonas fluorescens and safety evaluation in a zebrafish model. *Saudi Journal of Biological Sciences*, *27*(12), 3465-3473.

Silva, N. C. C., & Fernandes Júnior, A. J. J. O. V. A. (2010). Biological properties of medicinal plants: a review of their antimicrobial activity. *Journal of venomous animals and toxins including tropical diseases*, *16*, 402-413.

Xie, Y., Yang, W., Tang, F., Chen, X., & Ren, L. (2015). Antibacterial activities of flavonoids: structure-activity relationship and mechanism. *Current medicinal chemistry*, *22*(1), 132-149.

Al-Janae'e, A. M., Ali, A. H., & Al-Edany, T. Y. (2017). Efficacy of some aromatic plant extracts on treating the eggs of the common carp (Cyprinus carpio L.) against fungal infection in comparison with traditional fungicide malachite green. *Basrah Journal of Agricultural Sciences*, *30*(2), 59-71.

Pourmorad, F., Hosseinimehr, S. J., & Shahabimajd, N. (2006). Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *African journal of biotechnology*, *5*(11).

Lee, K. K., Kim, J. H., Cho, J. J., & Choi, J. D. (1999). Inhibitory effects of 150 plant extracts on elastase activity, and their anti-inflammatory effects. *International journal of cosmetic science*, *21*(2), 71-82.

Salih, K.I. & Hussein, A.R. (2014). Application of intensive production program for common carp *Cyprinus carpio* fingerlings in fish hatcheries. A- Stage of larvae production. J. Kerbala Univ., 12(1): 75-84. (In Arabic). https://www.iasj.net/iasj?func=fulltext&aId=88021.