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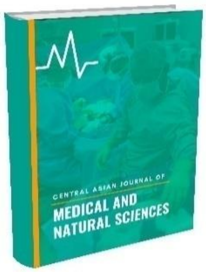

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THE EFFECT OF AQUEOUS AND ALCOHOLIC EXTRACTS OF PEGANUM HARMALA SEEDS ON SOME BACTERIAL SPECIES PRESENT IN QAYYARAH GENERAL HOSPITAL WASTEWATER

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Abstract: The effect of the aqueous and alcoholic extract of the seeds of the plant (Peganum harmala) was studied and the concentrations of four (25%, 50%, 75%, 100%) g/ml were tested on the bacterial species that were diagnosed in the study sites of Qayyarah General Hospital, namely Staphylococcus aureus and Escherichia. coli, salmonella typhi, Raoultella planticola, Klebsilla pneumonaei, and Enterobacter aerogenes. The two extracts, at all their concentrations, produced an inhibition in the number of bacterial cells after treatment with it. The results showed that the effect of the alcoholic extract was higher than the effect of the aqueous extract, as the results showed that the positive bacteria (S.aureus) were less The diameter of inhibition among the types at all concentrations, the highest diameter of inhibition for (E.coli) bacteria at a concentration of 100%, and the lowest effect at a concentration of 25%, as the effect of the two extracts increases with increasing concentration against all bacterial species. The detection of active chemical compounds in Peganum harmala seeds was studied, and the results showed that they contain a high percentage of active compounds (phenolic, alkaloid, and tannins, and the absence of flavonoids and glycosides) through qualitative (inferential) detection.

Key words: aqueous extract, alcoholic extract, peganum harmala seeds, bacterial contamination, wastewater.

1. Introduction

Hospitals play an essential role in human well-being through the health services they provide, and at the same time are responsible for generating large amounts of wastewater (Parida and his group, 2022). In developed countries, hospitals generate approximately 400-1200 liters of wastewater per bed per day, while in developing countries, hospitals generate 200-400 liters/capita/day compared to 100-400 liters/capita/day of domestic wastewater (Kumari and his group, 2020). Hospital wastewater differs in nature from wastewater and from other sources (Elmorabet and his group, 2020). This type of wastewater has become a global problem due to its stability and persistence in the environment (Rodriguez-Moza 2015; Verlicchi et al.,2015). The Peganum harmala plant is widely used in herbal medicine in many countries of the world to treat many diseases. It is considered a medicinal plant because it contains alkaloids of the B-Carbolines type, which are the medicinal active substances in the plant, where the percentage of active compounds ranges between (2-7)%. In dried seeds, the biological and pharmacological activity of the plant is due to its alkaloids (Ayoub and his group, 1989). Peganum harmala belongs to the family Zygophyllaceae, this family includes about 25 genera. There are more than 250 species, and because of its bitter taste and strong odor that repels most grazing animals, this plant is not used for grazing, as all grazing animals are sensitive to its toxicity (Mohmoudian and his group, 2002.)

2. Materials and methods

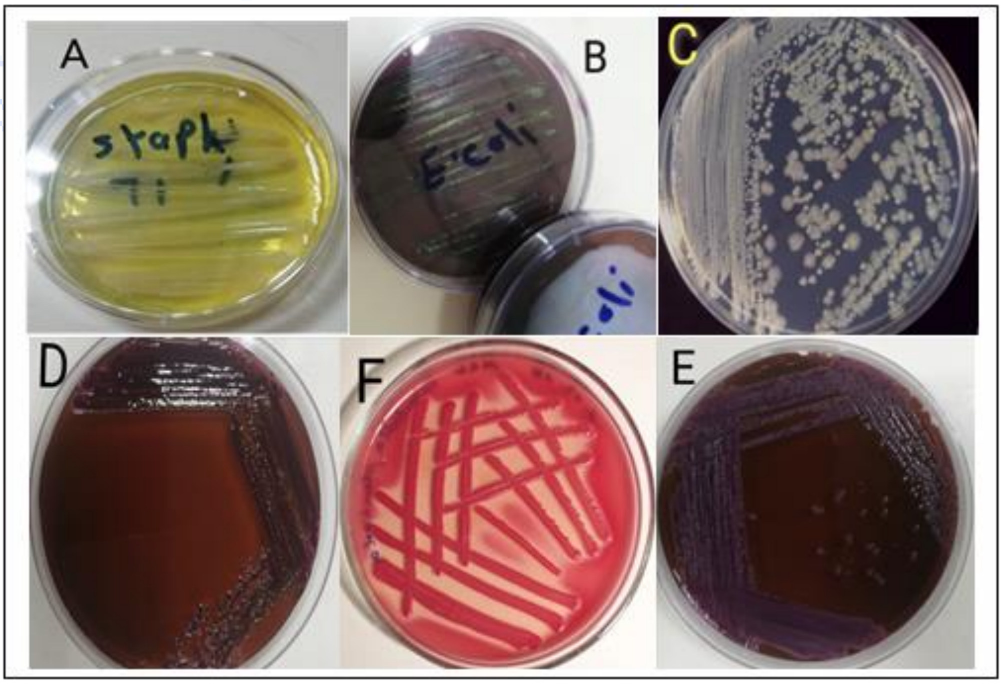
2.1. Isolation and diagnosis of bacteria

2.1.1. Preparing the cultivation media

Culture media (Nutrient agar, MacCkonkey agar, mannitol agar) were prepared as indicated by the manufacturer by dissolving the culture media in 1 liter of distilled water and then sterilizing it using an autoclave at 121°C under a pressure of 15 lb/ang2. For 15 minutes.

2.1.2. Isolation and diagnosis of bacteria

Bacterial isolates taken from the hospital's study stations were diagnosed using the Vitek 2 compact system, which is used to diagnose most types of bacteria through a special diagnostic kit for the device. The diagnostic kit contains 47 holes that are inoculated with a 24-hour-old bacterial suspension and incubated for 24 hours. The device records the color changes that occur. As a result of bacterial growth, the results of diagnosing bacterial species were as follows: (Raoultella planticola, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Staphylococcus aureus)



fige (1): Bacterial species after diagnosis A- Staph. aureus B-E.coli C- Entero.aerogenes D-Salmonella.typhi E-K.pneumoniae F- R.planticola

2.2. Fundamental study

2.2.1. Plant samples

The plant sample, L. Peganum harmala seeds, was obtained from local markets. Then the plant samples were washed with distilled water to get rid of dust and microorganisms, and they were placed in a special room at a temperature of 22-26°C, taking into account continuous stirring to ensure good drying as well as to prevent them from rotting. After ensuring that they were dry, they were crushed with a It was milled, weighed, and stored in opaque glass boxes. It was labeled with a piece of paper indicating the name of the plant and plant part. Then it was stored in the laboratory until the extraction process was carried out.

2.2.2. Preparation of Plant Extracts

Two types of plant extracts were prepared, namely aqueous and alcoholic extracts, and the extraction methods were as follows- :

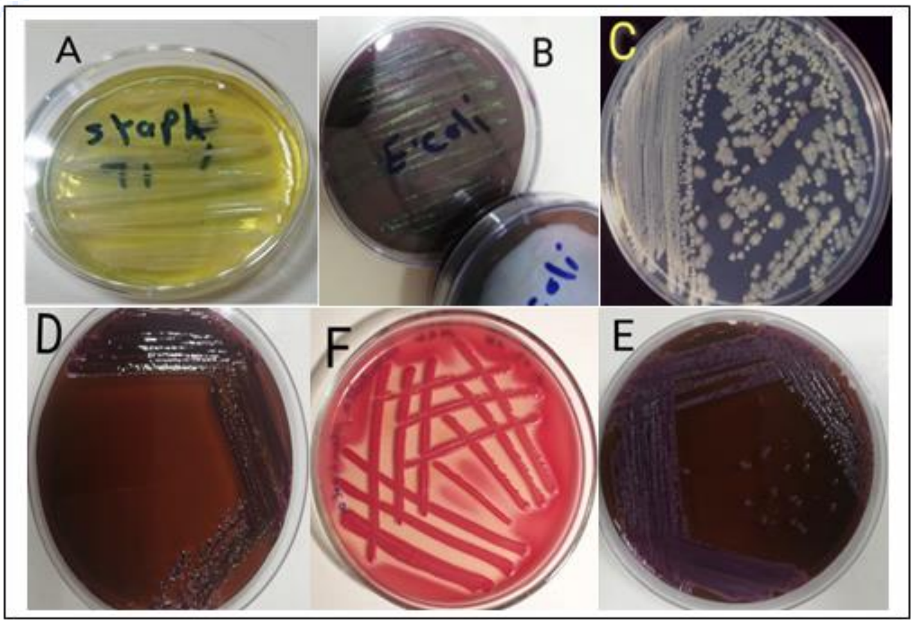
2.2.2.1. Hot Aqueous Extraction

The hot water extraction method was used according to what was stated in the study (AlRifai, 2005), with some modifications when needed, as follows:

The hot water extraction process was carried out by mixing 100 g of plant powder with 500 ml of distilled water (i.e. a ratio of 5:1 weight: volume), which was heated to a boiling temperature of 100 °C. Then it was removed from the heat source and the plant powder was added to it, then it was placed in a vibrating incubator. At a temperature of 37°C for 24 hours, it was then filtered through several layers of medical gauze and placed in glass dishes containing a piece of thermal nylon to preserve the quantity of the extract. Then it was placed in an electric oven at a temperature of 38°C for three days, and after ensuring that the extract was dried, it was placed in bottles. Dark, with a tight lid and stored in the refrigerator at 4°C until used.

2.2.2.2. Alcoholic Extract

To obtain the alcoholic extract, I followed the steps for preparing the aqueous extract, but using 80% ethyl alcohol instead of water, fige (2).



fige (2): Extraction stages of p. harmala

3.2.2. Chemical study of plants

Chemical detection of the types of active compounds in the seeds of the Peganum harmala plant was carried out as follows- :

3.2.2.1. Preliminary Qualitative Screening:

A group of laboratory-prepared chemical reagents was used to conduct the initial inferential detection of active chemical compounds.

3.2.2.2. Alkaloids Reagents

– Wagners Reagent: According to the method mentioned in (Fahmy, 1933), the brown color appears.

3.2.2.3. Phenols Reagent

I use the potassium dichromate reagent according to the method mentioned in (Harborne, 1973) to reveal the yellow color.

3.2.2.4. Tannins Reagant

Lead acetate reagent: The method mentioned in (Shihata, 1951) was proven. A gelatinous color forms with the presence of a precipitate.

3.2.2.5. Flavonoids Reagent

- Concentrated sulfuric acid detector, $H2SO4$. The method mentioned in (Newall and his group, 1996) was used. An orange ring was formed.

3.2.2.6. Glycosides Reagent

This was used as a sodium hydroxide reagent, and as in the method (Harborne, 1973), a yellow color appeared.

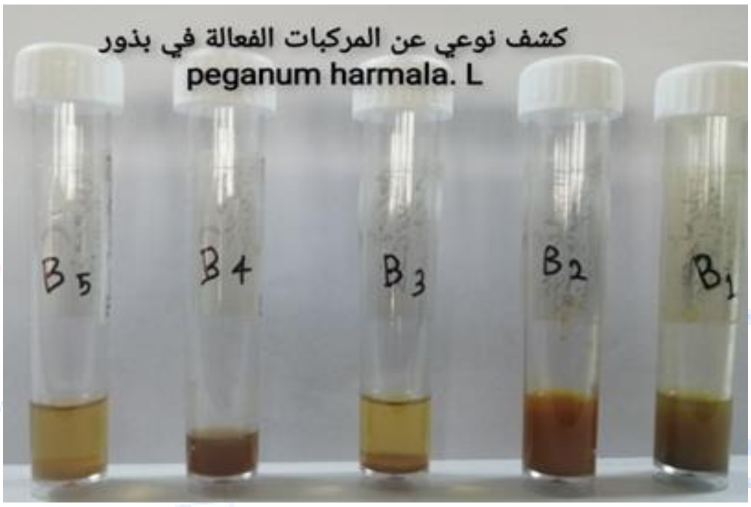
3.2.2.7. Study of the biological antibacterial effectiveness:

For the purpose of using the extracts in the inhibition experiment, the method (Mitschr and his group, 1972) was adopted in preparing the stock solution and sterilizing it. 0.5 g of dry plant extract powder was taken and dissolved in 10 ml of sterile distilled water, so we have Stock solution with a concentration of 50 mg/ml. Sterilize the solution by filtration to get rid of the bacterial contaminants present in it and obtain a sterile storage solution. Use this solution as the main source to prepare the percentage concentrations (100, 75, 50, 25)% mg/ml. The culture medium (Muller Hinton agar) was prepared according to the manufacturer’s instructions and according to the method (Atlas, 1995). The medium was sterilized in an incubator at a temperature of 121 °C and a pressure of 15 pounds per inch for 15 minutes. The medium was distributed in Petri dishes (diameter 9 cm), and the dishes were left under Laboratory temperature until the medium solidifies.

The agar diffusion method was used by well drilling according to (Egorove, 1985) to observe the sensitivity of microorganisms to the extracts of the studied plants at concentrations (100, 75, 50, 25) mg/ml, and the bacterial isolates were grown in dishes. Petri container on Muller Hinton agar medium, by spreading the bacterial isolates for each bacterial species on the dishes using Swap, and then making holes with a sterile cork drill with a diameter of 10 mm, 4 holes for each dish. The agar tablets were removed and discarded, then the concentration of the extract was marked over each hole and arranged in the direction of the hand. Hours (25, 50, 75, 100). The name of the plant, the type of extract, and the type of bacteria were on each plate. A concentration of 250 microliters/hole of plant extracts was taken from each and placed in the holes prepared for it. Then the plates were incubated at a temperature of 37°C for 24 hours, and then measurements were taken. The diameter of the inhibition zone, if any, using a ruler in millimeters, then prepare two control parameters for positive and negative bacteria in the previous manner, except for making one hole instead of 4 holes, by placing sterile distilled water in the hole.

4. Results and discussion

The results of detecting the active compounds of Peganum harmala seeds showed that there was a large amount of active compounds in the seeds. The efficiency of the Wakner reagent was demonstrated in terms of the formation of a brown precipitate in detecting alkaloids, the potassium dichromate reagent showed a yellow precipitate indicative of detecting phenols, and the lead acetate reagent showed Gelatinous consistency in detecting tannins, and the concentrated sulfuric acid reagent did not show an orange ring to detect flavonoids, and the sodium hydroxide reagent did not give a yellow color to detect glycosides, as the results showed the presence of alkaloids, phenols, tannins, and flavonoids, and this may be due to their absence or lack of presence, or due to The quality and efficiency of the detector in inferring it. fige (4).



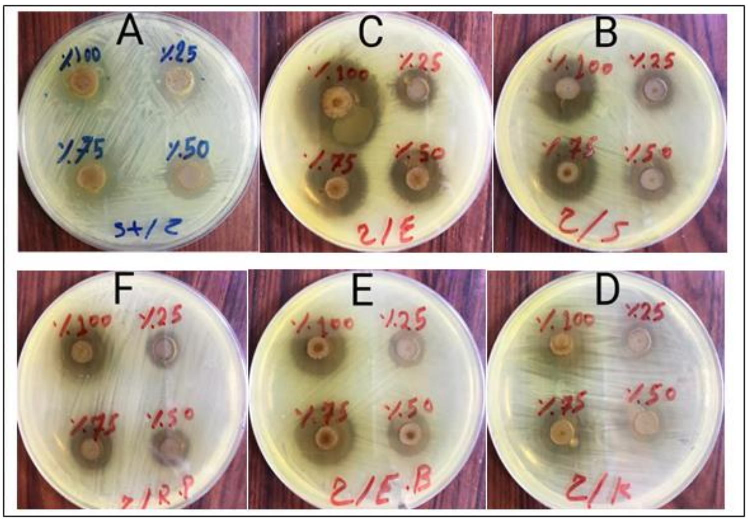
fige (3): Specific chemical detection of the active compounds of P. harmala seeds

The results of the current study showed the effectiveness of the aqueous extract of the seeds of the P. harmala plant and its clear inhibitory effect against the bacterial species studied, but it is less effective as an inhibitor compared to the alcoholic extract at a concentration of 80%. The aqueous extract showed its least effect against the S. aureus type at concentrations of 25, 50, 75, and 100. mg/ml with inhibition diameters (13, 17, 19, 21 mm), respectively. While its highest effect was against E.coli at concentrations of 25, 50, 75, and 100 mg/ml with diameters of inhibition (19, 22, 24, 26 mm), respectively, and its effect was against S.typhi with diameters of inhibition (15, 17, 22, 24 mm), respectively, while its effect against the type Entero. aerogenes with diameters of inhibition reached (14, 16, 21, 23 mm), respectively, and its effect against the type R. planticola with diameters of inhibition reached (14, 17, 20, 23 mm). As for the K.pneumoniae type, its inhibition diameters reached (16, 19, 21, 24 mm), respectively. The lowest diameter of inhibition for S.aureus at 25% concentration was 13 mm, while the highest diameter of inhibition for E.coli was 19 mm, for K.pneumoniae it was 16 mm, while for S.typhi it was 15 mm. As for the two types R.planticola and Entero.aeerogenes, their inhibition diameter reached 14 mm at 25% concentration. At the concentration of 50%, the lowest diameter of inhibition reached (16 mm) for the type Entero.aeerogenes, while the highest diameter of inhibition for the type E.coli reached 22 mm, while the type K.pneumoniae reached 19 mm, as for the types S.aureus and S.typhi and R.planticola, the diameter of inhibition reached 17 mm at 50% concentration. At the concentration of 75%, the highest diameter of inhibition for E. coli reached 24 mm, while the lowest diameter of inhibition for S. aureus reached 19 mm, and the inhibition diameter for S. typhi reached 22 mm, as for Entero. aerogenes and K. pneumoniae. Their inhibition diameters reached 21 mm, and the inhibition diameter of R. planticola reached 20 mm. At 100% concentration, the lowest diameter of inhibition for S. aureus was 21 mm, while the highest diameter of inhibition for E. coli was 26 mm, and the inhibition diameter for S. typhi and K. pneumoniae was 24 mm, while for Entero. aerogenes and R. planticola 23 mm. As for the effect of the alcoholic extract against bacterial species at its concentrations (25%, 50%, 75%, and 100%), at a concentration of 25%, the lowest diameter of inhibition for the Entero. aerogenes type reached 14 mm, and the highest diameter of inhibition for the E. coli type reached 20 mm, and the diameter of The inhibition diameter for S.aureus, R.planticola, and K.pneumoniae species was 16 mm, while for S.aureus species, its inhibition diameter reached 15 mm. At the concentration of 50%, the highest diameter of inhibition for E.coli species reached 22 mm, the lowest diameter of inhibition reached 18 mm for S.aureus species, the inhibition diameter for Entero.aeerogenes and K.pneumoniae species reached 20mm, while for S.typhi and R. planticola 19 mm, and at a concentration of 75%, the highest diameter of inhibition for E. coli was 25 mm, the lowest diameter of inhibition for R. planticola was 20 mm, the inhibition diameter was 23 mm for K. pneumoniae, and the inhibition diameter for S. typhi was 22 mm. As for the two species, Entero.aeerogenes and S.aureus, their diameter of inhibition was 21 mm. At 100% concentration, the highest diameter of inhibition for E. coli was 27 mm, while the lowest diameter of inhibition for S. aureus, Entero. aerogenes, and R. planticola was 23 mm. The inhibition diameter for K. pneumoniae was 26 mm, and for S. typhi. Its damping diameter was 25 mm, as shown in Table 1 and fige 5 and 6.

The results showed that the positive bacteria (S.aureus) had the lowest rate of inhibition diameter among the species. The reason for this is that the positive bacteria possess a capsule, which is a layer covering the cell wall of the bacterial cell. Scientists and researchers differed in defining this capsule, some of them such as (Gupta, 1989) defined it as regular clusters of gelatinous substances on the cell wall of bacteria, while others described it as extracellular polysaccharides covering the bacterial cell wall and attributed the resistance of these bacteria to antibiotics to the presence of this capsule (Al-Muslimaoui, 1999), as shown by The results showed that inhibition of the growth of bacteria of both sexes increased with increasing concentration of the extract. The concentration of 100% was the highest concentration and was the most effective in inhibiting the growth of all bacterial species (Zgurskaya and his group, 2015). The results of the current study are consistent with the findings of (Benbot et al., 2012), as His results showed that the alcoholic extract of Haramel seeds has a higher antibacterial effect on Gram-negative and Gram-positive bacteria than the aqueous extract.

Table (1) Diameter of the measured inhibitory activity (in mm) of the aqueous extract of Peganum harmala seeds against the bacterial species studied.

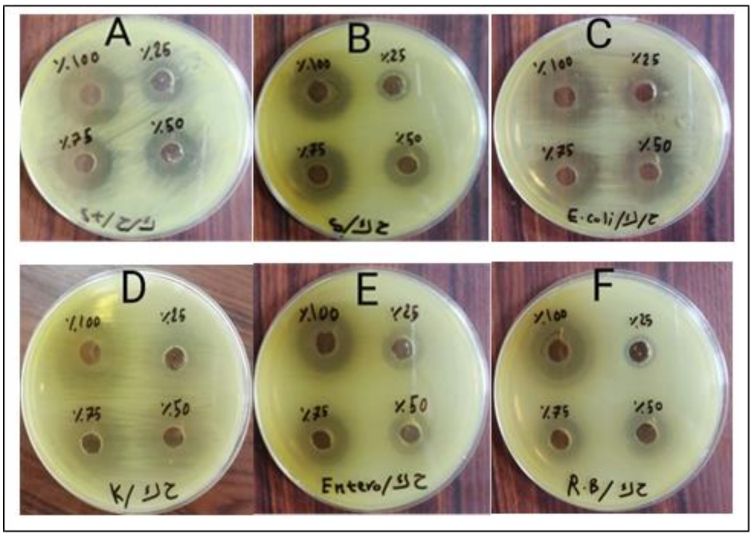
%25	%50	%75	%100	Extract concentration / Type of bacteria
13	17	19	21	*S.aureus*
19	22	24	26	*E.coli*
15	17	22	24	*S.Typhi*
14	16	21	23	*Entero.aerogenes*
14	17	20	23	*R.planticola*
16	19	21	24	*K.pneumoniae*



fige (4): The effectiveness of the aqueous extract of P.harmala seeds against bacterial species.
***Salmonella. typhimurium* - B *Staphylococcus aureus* –A**
***Enterobacter.aerogenes* - *Klebsiella pneumoniae* E - *E.coli* D -C**
***Raoultella planticola* - F**

Table (2) Diameter of the measured inhibitory activity (in mm) of the alcoholic extract of P. harmala seeds against the bacterial species studied.

%25	%50	%75	%100	Extract concentration / Type of bacteria
15	18	21	23	*S.aureus*
20	22	25	27	*E.coli*
16	19	22	25	*S.Typhi*
14	20	21	23	*Entero.aerogenes*
16	19	20	23	*R.planticola*
16	20	23	26	*K.pneumoniae*



fige (5): The effectiveness of the alcoholic extract of P.harmala seeds against bacterial species
***Salmonella. typhimurium* - B *Staphylococcus aureus* –A**
***Enterobacter.aerogenes* - *Klebsiella pneumoniae* E - *E.coli* D -C**
***Raoultella planticola* - F**

Sources and references

1. **Al-Muslimawy, Dhikra Adnan Jawad, (1999),** Serological study of Staphylococcus aureus capsularis isolated from patients at Hilla Hospital, Master's thesis, University of Babylon.
2. **Al-Rifai, Faten Nuri Mulla Abdul Hussein (2005)**. Isolating and diagnosing polluted microbiology for cosmetics and the ability to use some plant extracts to preserve them. PhD thesis. Department of Life Sciences - College of Science - University of Mosul.
3. **Atlas, R.M.(1995)**. Laboratory Manual of Experimental Microbiology Mosbyyear book, Inc., USA.
4. **Ayoub, M.T.; Rashan, L.J.; Khazraji, A.T.; and Adday: M.H. (1989).** An Oxamide from peganum hamala seeds. phyto chemistry., 28 (7):2000-2001.
5. **Benbot. amel. yahya abdel wahab, abdel hakim (2012),** Evaluation of antibacterial activity of Cordella alkaloids extracted from peganum harmala seeds. biological sciences, university hidi M bennlarbi, algeria:(5). 568-573.
6. **Egorove, N.S (1995).** Antibiotics a scientific approach. Mir publishers. Moscow.
7. **El Morabet, R., Abad Khan, R., Mallick, J., Khan, N. A., Ahmed, S., Dhingra, A., Rahman Khan, A., Alsubih, M., Alqadhi, S., Bindajam, A. (2020).** Com- parative study of submerged membrane bioreactor and extended aeration process coupled with tubeset- tler for hospital wastewater treatment. Alexandria Engineering Journal, 59(6), 4633–4641.2020.08.021
8. **Fahmy,I.R. (1933)**."Constituents of plant crud drugs. Ist" Ed-poul Barbey-cairo. Egypt.
9. **Gupta, S. (1989)**. The short text book of medical microbiology. 3rd ed. J. PB. New Delhi.
10. **Harbone J.B. (1973).** Phtochemia methods, aguie to modem techniques po plant analysis chapman and hall Ltd. London. pp: 159-165.
11. **Kumari, A., Singh, N., Tiwari, B.(2020)**. Hospital wastewater treatment scenario around the globe. Current Developments in Biotechnology and Bio- engineering, 68(1), 1–12. -B978-0-12-819722-6.00015-8 2020 Elsevier B.V. All rights reserved
12. **Mitscher, L.A., Leu, R, Bathala, M.S., Beal, J, L. and White, R.(1972).**Antimicrobal agents from higher plants. lioydia; 35:157-166.
13. **Mohmoudian, Jalilpour, and Salehian. (2002).** Toxicity of peganum hamala. Review and a case report. Iranian J. Of pharmacology and therapeutic., 1:1-4.
14. **Newall, Anderson. and Phillipson (1996).** Herbal Medicines. A guide for health care professionals. The pharmaceuitical press.
15. **Parida, V.K., Sikarwar, D., Majumder, A., Gupta, A.K. (2022).** An assessment of hospital wastewater and biomedical waste generation, existing legis- lations, risk assessment, treatment processes, and scenario during COVID-19. Journal of Environ- mental Management, 308(January).2022.114609
16. **Rodriguez-Moza, Weinberg(2015).** Meeting report: Pharmaceuticals in water- an interdisciplin- ary approach to a public health challenge. Environ- mental Health Perspectives, 118(7), 1016–1020
17. **Shihata, I.M.(1951).**Apharmacolo-gical study of anagallis arvensis M.D.vet. thesis cairo University.
18. **Singh. (2015).** *Bio-nanoparticles: biosynthesis and sustainable biotechnological implications*. John Wiley & Sons.
19. **VerlicchiAl Aukidy, Zambello. (2015).**What have we learned from worldwide experienceson the management

and treatment of hospital efflu-ent - An overview and a discussion on perspectives. Science of the Total Environment, 514, 467–491. 2015.02.020

20. **Zgurskaya, Lopez, and Gnanakaran. (2015)**. Permeability barrier of gram-negative cell envelopes and approaches to bypass it. ACS Infectious diseases. 1(11).