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The Possibility of Treating the Surface Water of Ayin Kiam with Aleppo Pine Extracts

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A B S T R A C T

The present work aimed to treatment the surface water of Ayin Kaim using the aqueous extract of *Aleppo pine* leaves as a coagulant. Several analyses were performed on water before and after treatment included pH, total dissolved solids, conductivity, turbidity, iron ion concentration, and total coliform count, also GC-MS analysis was performed on *Aleppo pine* leaf powder. The coagulation properties of *Aleppo pine* seeds and cones were found to be promising, but several limitations prompted the search for more effective alternatives. Notably, the utilization of aqueous leaf extract from *Aleppo pine* in water purification has not been previously reported. Our findings unequivocally demonstrated that the aqueous extract of *Aleppo pine* leaves significantly enhanced water quality parameters, including pH (8.63-8.44), total dissolved solids (6360-4140) mg/L, conductivity (9.67-6.59) mS/cm, turbidity (123-59.6) NTU, iron ion concentration (0.08-0.0) mg/L, and total coliform count (398-247) cfu. The improvement in water quality is attributed to the presence of various chemical compounds in *Aleppo pine* leaves, including carbohydrates, proteins, and carboxylic acids, which were confirmed through GC-MS analysis. Studies have shown that these compounds possess the ability to adsorb compounds on their surfaces, thereby supporting the efficacy of aqueous extracts of *Aleppo pine* leaves as an alternative in water purification. Given the severe health implications associated with chemical coagulants, the use of *Aleppo pine* leaves as natural coagulants in water purification presents a cost-effective solution due to their year-round availability and independence from complex climatic conditions.

إمكانية معالجة المياه السطحية لعين كعام باستخدام مستخلصات نباتية مختلفة

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الكلمات المفتاحية:
معالجة الماء
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جهاز مطيافية الكتلة
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.

الملخص

هدفت الدراسة الحالية إلى معالجة المياه السطحية في عين كعام باستخدام المستخلص المائي لأوراق الصنوبر الحلبي كمواد متخثرة. وقد أجريت عدة تحاليل على المياه قبل وبعد المعالجة شملت الرقم الهيدروجيني، والمواد الصلبة الذائبة الكلية، والتوصيل، والعكارة، وتركيز أيونات الحديد، وعدد القولونيات الكلية، كما أجريت تحليلات كروماتوغرافيا الغاز-مطياف الكتلة على مسحوق أوراق الصنوبر الحلبي. وقد تبين من دراسات سابقة أن خصائص التخثر لبذور وأقماع الصنوبر الحلبي واعدة، ولكن العديد من القيود دفعت إلى البحث عن بدائل أكثر فعالية. والجدير بالذكر أن استخدام المستخلص المائي لأوراق الصنوبر الحلبي في تنقية المياه لم يتم الإبلاغ عنه من قبل. أظهرت نتائجنا بشكل لا لبس فيه أن المستخلص المائي لأوراق الصنوبر الحلبي عزز بشكل كبير معايير جودة المياه، بما في ذلك الرقم الهيدروجيني (8.44-8.63)، وإجمالي المواد الصلبة الذائبة (4140-6360) ملجم / لتر، والتوصيل (6.59-9.67) ملي سيمنز / سم، والعكارة (59.6-123) وحدة نترات الأمونيوم، وتركيز أيونات الحديد (0.0-0.08) ملجم / لتر، والعدد الكلي للبكتيريا القولونية (247-398) وحدة تشكيل مستعمرة. ويمكن أن يعزى التحسن في جودة المياه إلى وجود مركبات كيميائية مختلفة في أوراق الصنوبر الحلبي، بما في ذلك الكربوهيدرات والبروتينات والأحماض الكربوكسيلية، والتي تم تأكيدها من خلال تحليل كروماتوغرافيا الغاز-مطياف الكتلة. وقد أظهرت الدراسات أن هذه المركبات تمتلك القدرة على امتصاص المركبات على أسطحها، وبالتالي دعم فعالية المستخلصات المائية لأوراق الصنوبر الحلبي كبديل في تنقية المياه. ونظراً للعواقب الصحية

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الشديدة المرتبطة بالمواد الكيميائية المسببة للتخثر، فإن استخدام أوراق الصنوبر الحلبي كمواد طبيعية مسببة للتخثر في تنقية المياه يمثل حلاً فعالاً من حيث التكلفة بسبب توفرها على مدار العام واستقلالها عن الظروف المناخية المعقدة.

1. Introduction

Water is a necessary resource for life and is needed by every living organism. It plays a vital role in all the processes that occur within living organisms, starting with the primary organisms, passing through plants, and ending with humans. However, this resource has become very limited in its pure state, as there was a dangerous belief that rivers, lakes and oceans are the most suitable place for dumping urban, industrial waste and any other waste to be disposed of. In addition, the aquatic environments have sufficient capacity to mitigate this pollution and thus restore their usual balance. This belief may be somewhat true in the event that the pollutants are few, but this natural weapon is weak and becomes weaker with the increase in industrial growth. Moreover, the increase of pollutants that are ejected in high concentrations, causing a serious imbalance in the ecological balance of these water bodies [1-2].

Water pollution means any change in the physical, chemical and biological properties of water. In general, water pollution is classified into: natural pollution where the natural properties of water change and become unpalatable for human use. Chemical pollution, water becomes toxic, due to the presence of toxic and unwanted chemicals. Biological pollution, it means the presence of microorganisms in the water or the presence of plant organisms that cause a change in the nature and quality of water and affect the safety of its use. Water pollutants are classified into Oxygen-depleting substances, which are used by aerobic microorganisms in the presence of oxygen. Water-soluble inorganic substances which include heavy metals and their compounds, inorganic nutrients for plants and organic materials such as petroleum, products are also pollute the water. Pathogenic microorganisms are important pollutants that directly affect human health. Water-borne diseases (such as diarrhea and gastrointestinal diseases) are caused by various types of bacteria, viruses, protozoa, algae and fungi [3].

In Libya, the lakes, valleys (Wadi), and springs (Ayin) are the sources of the survival of life in coastal areas. They are importance of water availability for many agricultural and industrial products. For example, water of Ayin Kiam (in the west of Zliten city) is used for agriculture project and planting of vegetables and fruit trees. However, this Ayin water currently suffers from high contamination indicators due to the discharge of solid waste from residential areas, fertilizers, pesticides in the agriculture areas and man-made activities, which lead to effect on quality of water [4]. The pervious study of Ayin Kiam water showed that water is chemically polluted due to the present of high concentration of metal ions, and thus the quality of this water deteriorated [5]. There is also a clear effect of various pollutants in Ayin Kiam water and it is considered not in conformity with the standard specifications for drinking water [6]. In addition, according to studies conducted on water in the Kiam area, salmonella is more common in Surface water in Ayin Kiam, in other words, the Ayin is polluted by microorganisms [7].

More than 5000 years ago, interest in water quality began, but because of the limited knowledge in those times, this interest was limited to the color, taste and smell of water, during different historical periods some treatment processes such as boiling, filtering, sedimentation and adding some salts were done. In addition, scientific and industrial development, the treatment processes have become mainly dependent on coagulation and flocculation processes [8].

Coagulants are classified into: Synthetic coagulants (organic polymers such as polyethyleneimine, and Inorganic coagulants such as aluminum sulfate), and natural coagulants such as chitosan and microbial coagulants [10]. Inorganic coagulants and synthetic organic polymers, despite their good performance and efficiency, the residual content in the water after treatment and the financial cost are the main problems of these products, especially in developing countries. On the other hand, natural coagulants are biodegradable and considered safe for human health. Many studies and research have been conducted on natural coagulants and various natural coagulants have been obtained from microorganisms, animals or plants [10].

The *Pine* tree (*Pinus*) is one of the most widespread medicinal plants in the northern hemisphere, comprising nearly 100 species. Aleppo pine is a tall, evergreen, monoecious tree. It up to 3-80 m in length, the majority of species reaches 15-45 m in height and has wide deep crowns; open with long erect branches, very scaly bark, small, smooth branches, olive green. The leaves are needle-like, in bundles of 3, 6-10 cm. *Pine* seeds are 17-23 mm long and 5-7 mm wide, and have a thin shell and a rudimentary wing. Pine trees grow well on acidic soils, most need well-drained soil, and prefer sandy soil. It is also a popular ornamental tree that is widely planted in parks and gardens in hot and dry areas [11-12]. *Aleppo pine* trees are an important component of the flora of the Mediterranean basin, which is characterized by extraordinary geographical and topographical diversity. In addition to their health benefits [13-14]. Therefore, the present study is aim to investigate the use of *Aleppo pine* leaves extract as natural coagulant to treat the surface water of Ayin Kiam.

2. Materials and Methods

2.1. materials

Fresh *Aleppo pine* leaves were collected from trees planted around Ayin Kiam in the city of Al-Khums, northern Libya, between the geographical coordinates of latitude 32.29° to 32.34° North and longitude 14.21° to 14.28° East during the winter of 2022. The leaves were initially washed with tap water, followed by distilled water to ensure thorough cleaning. The leaves were then dried in the shade to prevent any contamination or spoilage. Finally, they were ground into a fine powder and stored properly for future use.

Water samples were collected using plastic bottles that were thoroughly cleaned with distilled water before use. The bottles were submerged under the surface of the water, removed, and then properly closed. Each bottle was labeled and stored until needed for analysis. [15].

Chemicals: All solvents and chemicals were analytical grade and purchased from BDH Chemicals, United Kingdom.

2.2. *Methods*

2.2.1. Extraction *and preparation of crude extract*

The solvent used in this study is distilled water for several reasons, the most important of which is its cheapness and its suitability to the environment and the subject of the study. The extraction was carried out using distilled water according to [17] with some modifications where 30 g of leaf powder and 450 ml of boiled distilled water was mixed by magnetic stir for 48 hours, then the extract was cooled to room temperature and centrifuged by 3000 rpm for ten minutes, then filtered. Using a rotary evaporator at 50 ºC with low pressure, the filter was dried and then the yield was calculated and then stored until needed.

2.2.2. Phytocomponent identification by GC-MS

The chemical composition of the samples was analyzed using a Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) equipped with a direct capillary column TG-5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially set at 35 °C and then increased at a rate of 3 °C per minute until reaching 200 °C, where it was held for 3 minutes. Subsequently, the temperature was further increased to the final temperature of 280 °C at a rate of 3 °C per minute and maintained for 10 minutes. To maintain optimal conditions, the injector and MS transfer line temperatures were maintained at 250 °C and 260 °C, respectively. Helium gas was used as the carrier gas, flowing at a constant rate of 1 ml/min. A solvent delay of 3 minutes was employed, and diluted samples of 1 µl were automatically injected using the Autosampler AS1300 in the split mode. Electron ionization (EI) mass spectra were collected at an ionization voltage of 70 eV over the range of m/z 40-1000 in full scan mode. The source temperature was set at 200 °C. Identification of the components was achieved by comparing their retention times and mass spectra with those in the WILEY 09 and NIST 11 mass spectral databases [18-19]

2.2.3. Coagulation Procedure

A magnetic stirrer apparatus was utilized to evaluate the effectiveness of *Aleppo pine* leaf extracts in coagulating water for purification purposes. Following the methodology outlined by [21-22] with some modifications, different concentrations (0 mg/L, 25 mg/L, 50 mg/L, and 100 mg/L) of natural coagulants extract and ferric chloride were added to separate beakers. The contents were then agitated at varying speeds: 200 rpm for 5 minutes to facilitate rapid mixing, followed by a slower stirring at 80 rpm for 20 minutes. The resulting supernatant underwent sedimentation, filtration, and subsequent analysis of various physicochemical and biological properties. It should be noted that all experimental procedures were conducted under normal room temperature conditions.

2.2.4. Analysis of the physicochemical and biological properties

To validate the efficacy of the plant extracts in purifying the water samples, both untreated and treated samples were analyzed using standard methods. The analysis focused on several key physicochemical parameters, including turbidity, pH, total dissolved solids (TDS), and conductivity.

2.2.4.1. Turbidity measurement

Turbidity was assessed using the nephelometric method with a turbidimeter (HACH 2100Q) to measure the turbidity levels of water samples, following the established methodology by [23]. The water samples were analyzed before and after treatment with varying doses of plant extracts and ferric chloride. To conduct the turbidity measurement, a test tube was filled with the water sample up to the 10 ml mark. The turbidity meter reading was then taken against a blank tube. The resulting turbidity value was directly read from the display of the turbidity meter and reported in nephelometric turbidity units (NTU). This experimental procedure allowed for the quantitative assessment of turbidity levels in the water samples, enabling the evaluation of the effectiveness of different doses of plant extracts and ferric chloride in reducing turbidity. The use of a standardized and widely accepted nephelometric method ensures the reliability and comparability of the turbidity measurements obtained in this study.

2.2.4.2.PH measurement

The pH meter (HACH HQ40D) was utilized to measure the hydrogen ion concentration of the water samples before and after treatment with varying doses of plant extracts and ferric chloride. The pH meter was calibrated before measurement.

2.2.4.3.TDS and conductivity measurement

In accordance with the methodology outlined by [22], the concentration of conductivity and total dissolved salts in water samples was measured using a (HACH HQ40D) conductivity meter. This measurement was conducted both before and after treatment with various doses of plant extracts and ferric chloride. To ensure precise readings, the conductivity meter was calibrated using a buffer solution. The procedure involved immersing the electrode carefully in the water sample to measure its conductivity and total dissolved salts. The values for conductivity and total dissolved salts were then directly read from the meter screen. By adhering to this rigorous scientific approach, the study effectively assessed the changes in conductivity and total dissolved salts resulting from the treatment process. The utilization of an electrical conductivity meter, along with the calibration using a buffer solution, guarantees the reliability and accuracy of the conductivity and total dissolved salts measurements. Consequently, this enables a comprehensive evaluation of the impact of different doses of plant extracts and ferric chloride on the conductivity and total dissolved salts levels in water.

2.2.4.4. Iron ions measurement

The determination of iron ions in water samples was accomplished through the utilization of atomic absorption spectrophotometry. This analytical method relies on the sample's capacity to absorb radiant energy from the light spectrum and generate a distinct signal, enabling the quantification of iron ion concentration within the sample. In line with the methodology outlined by [22], a spectrophotometer (HACH DR2800) was employed to measure the iron ion concentration in water samples before and after treatment with varying dosages of plant extracts and ferric chloride. To ensure precise measurements, the spectrophotometer was calibrated using a standard solution.

2.2.4.5. Biological analysis

The Compact Dry method is a valuable culture media technique that enables the direct enumeration of microorganisms. In this study, the methodology employed was in accordance with the practices established by [24]. To execute the experiment, 100 ml of both treated and untreated water samples was inoculated onto a Compact Dry Total Count plate. This particular plate contains a selective medium specifically designed to support the growth of bacteria. Subsequently, the plates were incubated for 48H. After the completion of the incubation period, the growing colonies on the plates were carefully counted. The number of colonies in each sample was determined using the website provided by the manufacturer of the Compact Dry Total Count plates (https://bactlab.colony-app.com). By utilizing the Compact Dry method, researchers were able to obtain a direct and accurate measurement of the colonies count in both treated and untreated water samples. This technique offers a reliable means to assess the effectiveness of the treatment process in reducing or eliminating colonies.

3. Results and Discussion

3.1. Phytocomponent identification by GC-MS

GC-MS is a widely recognized and established analytical technique renowned for its high accuracy in identifying active compounds. In this particular investigation, GC-MS analysis was employed to characterize the active compounds present in *Aleppo pine* leaves. The analysis led to the identification of various active compounds, which were subsequently categorized into different classes, such as: Flavonoids, Phenols, Terpenes, Terpene alcohols, Terpene derivatives, Terpenoids, amino acids, Amides, Steroids, Fatty acid derivatives, Fatty acid esters, Heterocyclic compounds, Phenanthrene derivatives, Siloxanes, Silicone compounds, Spiro compounds, Sterols, Alcohols, Aldehydes, Alkanes, Aromatic compounds, Carboxylic acids, Esters, Ethers, and Fluorinated compounds. For a comprehensive understanding of the identified active compounds, detailed information regarding their chemical structures, retention times (RT), peak areas (%), molecular formulas, and molecular weights (MW) can be found in Table 1. This table provide valuable insights into the composition and characteristics of the active compounds present in the respective plant leaves.

In the analysis of *Aleppo pine* leaves, a total of 44 compounds were identified. Among these compounds, triacontylhexane was found to be the most abundant (peak area 9.32%), 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)- (peak area 4.46%), (3β)-Stigmast-5-en-3-ol (peak area 4.39%), 4,4'-Methylenebis(2-tert-butyl-6-methylphenol) (peak area 2.71%), and (Z)-docos-13-enamide (peak area 2.15%).

Table 1. Active Compounds Identified in *Aleppo pine* leaves by GC-MS.

Peak	Compound name	RT	Area %	formula	MW
1	N,N-dimethyl-2h-pyran-2-iminium chloride	11.26	0.10	$C_7H_{10}ClNO$	159
2	1,4-cyclohexadiene, 1-methyl-4-(1-methylethyl)	12.81	0.77	$C_{10}H_{16}$	136
3	3,4-dihydrothieno[3,4-B]thiophene-5-carboxylic acid	14.35	0.10	$C7H_6O_2S_2$	186
4	(1E,4E)-20-methyl-11-[(2s,3s)-3-hydroxy-1-azabicyclo[2.2.2] octan-2-yl]methylidene]cyclohexane-3-one	14.55	0.27	$C_{22}H_{31}NO_2$	341
5	1-piperidinecarboxaldehyde, 2-phenyl	15.19	0.11	$C_{12}H_{15}NO$	189
6	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, exo	17.13	0.20	$C_{10}H_{18}O$	154
7	3-cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	17.70	4.46	$C_{10}H_{18}O$	154
8	A-terpineol	18.23	1.04	$C_{10}H_{18}O$	154
9	3-cyclohexene-1-methanol, α,α,4-trimethyl	18.29	0.29	$C_{10}H_{18}O$	154
10	-Cis-4-methoxy thujane	18.81	2.12	$C_{11}H_{20}O$	168
	7-oxo-2-oxa-7-thiatricyclo[4.4.0.0(3,8)] decan-4-ol	19.41	0.10	$C_8H_{12}O_3S$	188

12	Linalyl acetate	21.44	1.34	$C_{12}H_{20}O_2$	196
13	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1R-(1R*,4E,9S*)]	28.34	0.19	$C_{15}H_{24}$	204
14	3,4-dihydro-2h-1,5-(3''-t-butyl)benzodio xepine	31.96	0.41	$C_{13}H_{18}O_2$	206
15	1H-cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1A α,4A α,7 α,7A α,7bα)]	34.15	0.22	$C_{15}H_{24}O$	220
16	Hexadecan-2-ol	38.82	0.14	$C_{16}H_{34}O$	242
17	Hexadecamethylcyclo octasiloxane	39.68	0.12	$C_{16}H_{48}O_8Si_8$	592
18	Neophytadiene	44.32	1.06	$C_{20}H_{38}$	278
19	3,7,11,15 tetramethyl-2-hexadecen-1-ol	45.10	0.16	$C_{20}H_{40}O$	296
20	7,9-di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	45.40	0.15	$C_{17}H_{24}O_3$	276
21	Methyl hexadecanoate	46.77	0.79	$C_{17}H_{34}O_2$	270
22	Hexadecanoic acid,2,3 dihydroxypropyl ester	48.08	0.34	$C_{19}H_{38}O_4$	330
23	Cis-2-phenyl-1, 3-dioxolane-4-methyl octadec-9, 12, 15-trienoate	51.83	0.12	$C_{28}H_{40}O_4$	440
24	11-octadecenal	52.60	0.29	$C_{18}H_{34}O$	226
25	Octadecanoic acid, methyl ester	52.94	1.35	$C_{19}H_{38}O_2$	298
26	1-phenanthrenecarboxylic acid,7-ethenyl-1,2,3,4,4A, 4b,5,6,7,9,10,10A dodeca hydro-1,4A,7-trimethyl-, methyl ester, [1R-(1 α,4A α,4b α,7 α,10A α)]	55.73	0.28	$C_{21}H_{32}O_2$	316
27	9(11)-dehydrotestosterone	55.90	0.36	$C_{19}H_{26}O_2$	286
28	Effusanin E	57.69	0.11	$C_{20}H_{28}O_6$	364
29	Methyl abietate	58.08	0.24	$C_{21}H_{32}O_2$	316
30	4H-1-benzopyran-4-one,2-(3,4-dihydroxyphenyl)-6,8-di-α -d-gluco pyranosyl-5,7-dihydroxy	58.26	0.23	$C_{27}H_{30}O_{16}$	610
31	Methyl 7-isopropyl-1,4a-dimethyl-1,2,3,4,4A,10A-hexahydrophenanthrene-1-carboxylate	58.62	0.13	$C_{21}H_{28}O_2$	312
32	Methyl dehydroabietate	58.92	0.70	$C_{21}H_{30}O_2$	314
33	1-phenanthrenemethanol, 1,2,3,4,4A,9,10,10A-octa hydro-1,4A-dimethyl-7-(1-methylethyl)-,[1s-(1 α,4Aα,10Aα)]	59.22	1.49	$C_{20}H_{30}O$	286
34	4,4'-methylenebis(2-tert-butyl-6-methylphenol)	61.28	2.71	$C_{23}H_{32}O_2$	340
35	Silicone oil	65.08	0.13		
36	Spirost-8-en-11-one, 3-hydroxy-, (3α,5α,14α,20α,22α,25R)	66.30	0.14	$C_{27}H_{40}O_4$	428
37	9,12-octadecadienoic acid (Z,Z),-2,3-bis[(trimethyl silyl)oxy]propyl ester	68.42	0.10	$C_{27}H_{54}O_4Si_2$	498
38	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15 -hexadecamethyl	69.14	0.12	$C_{16}H_{50}O_7Si_8$	578
39	2,3-bis[(trimethylsilyl) oxy]propyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate	69.78	0.11	$C_{27}H_{52}O_4Si_2$	496
40	4H-1-benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy	70.82	0.28	$C_{18}H_{16}O_7$	344
41	(Z)-docos-13-enamide	71.54	2.15	$C_{22}H_{43}NO$	337
42	Heptacosane	75.55	1.73	$C_{27}H_{56}$	380
43	(3β)-stigmast-5-en-3-ol	83.10	4.39	$C_{29}H_{50}O$	414
44	Triacontylhexane	84.03	9.32	$C_{36}H_{74}$	506

Additionally, several other compounds were identified in smaller quantities. These compounds include 3,4-dihydrothieno[3,4-b] thiophene-5-carboxylic acid (peak area 0.10%), 2,3-Bis[(trimethylsilyl) oxy] propyl(9Z,12Z,15Z)-octadeca-9,12,15-trienoate (peak area 0.11%), hexadecamethyl cyclooctasiloxane (peak area 0.12%), methyl 7- isopropyl-1,4A-dimethyl-1,2,3,4,4A,10A-hexa hydrophenanthrene-1-carboxylate (peak area 0.13%), and hexadecan-2-ol (peak area 0.14%). Furthermore, several other compounds were also identified at various levels. Several chemical compounds have been identified as abundant in *Aleppo pine* leaves, each possessing distinct properties. Triacontylhexane, for instance, finds primary utility in applications where iso alkanes are unsuitable due to biological considerations, such as in detergent and protein production and the wax industry [26-25]. 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-, has been identified as a constituent in fragrances. [27] Additionally, (3β)-Stigmast-5-en-3-ol, present in pine leaves, demonstrates notable antimicrobial and lipid-lowering properties.[28-29] 4,4'-Methylenebis(2-tert-butyl-6-methylphenol), and (Z)-docos-13-enamide, exhibit anticancer properties.[30] Whereas, to the best of our knowledge, no previous research studies have been published thus far regarding the GC-MS analysis of *Aleppo pine* leaves, these findings furnish valuable insights into *Aleppo pine* leaf composition, thereby fostering further exploration and comprehension of its potential applications and benefits.

Furthermore, employing GC-MS analysis on *Aleppo pine* leaves has revealed the presence of significant constituents. These include a diverse array of compounds such as amines, amides, amino acids, proteins, carboxylic and fatty acids, along with their esters, as well as long-chain alkanes like (Z)-docos-13-enamide, triacontylhexane, Heptacosane, and (1E,4E)-20-Methyl-11-[(2S,3S)-3-hydroxy-1-azabicyclo[2.2.2]octan-2-yl]methylidene]cyclohexane-3-one, methyl hexadecanoate. These constituents positively influence water turbidity by altering particle surface charges and facilitating condensation interactions and they act as mediators, promoting the aggregation of suspended particles and the formation of clumps, thereby showing potential as natural alternatives to conventional chemical coagulants in water purification processes, where this contributes to improved environmental and economic performance in treatment procedures.[31-36] However, further comprehensive investigations and research endeavors are required to attain a thorough understanding of their underlying mechanisms of action and to optimize their efficacy in water treatment processes.

Moreover, the employment of GC-MS analysis on *Aleppo pine* leaves has substantiated the presence of significant bioactive compounds. These compounds manifest a spectrum of biological activities, encompassing antibacterial, anti-inflammatory, antifungal, and anticancer properties. Noteworthy examples of these compounds include Hexadecamethyl cyclooctasiloxane, (3β)-Stigmast-5-en-3-ol, (Z)-docos-13-enamide, 4,4'-Methylene bis (2-tert-butyl-6-methyl phenol), and 7,9-Di-tert-butyl-1-oxaspiro [4.5] deca-6,9-diene-2,8-dione. These compounds exert their effects through various mechanisms: they interfere with bacterial cell wall components, resulting in its weakening and subsequent rupture due to osmotic pressure; they may target ribosomes within bacterial cells, disrupting protein synthesis and inhibiting bacterial growth; they possess the potential to disrupt bacterial genetic material replication by interfering with DNA and RNA replication processes; they target essential cellular components inducing leakage, or they impede crucial metabolic pathways by interfering with the synthesis of essential metabolites. [29] [36-42]

3.2. The impact of Aleppo pine leaf extract on the surface water of *Ayin Kiam*

The study investigated the impact of plant-based coagulants, namely Moringa oleifera and *Aleppo pine* leaf extracts, alongside the industrial coagulant ferric chloride, on the surface water quality in Ain Kam. The research focused on evaluating the influence of these coagulants at concentrations of 25, 50, and 100 mg/L on some water quality parameters, including turbidity, pH, conductivity, total dissolved solids, concentrations of heavy metals (specifically iron), and coliform counts, as detailed in Table 2.

3.2.1. The turbidity

The findings of the study revealed that the turbidity of the raw water in Ayin Kiam, as measured by 123 NTU, was relatively high. It was observed that the minimum turbidity limit could be achieved by utilizing a concentration of 50 mg/L of *Aleppo pine* leaf extract. Analysis of the data presented in Table 2 and Figure 1 indicates that treating water with low or high concentrations of *Aleppo pine* leaf extract has an unfavorable impact on turbidity levels. [31][43-44] The plausible explanation for *Aleppo pine* leaf extract ability to diminish water turbidity may be in the presence of distinctive chemical compounds such as amines, amides, amino acids, proteins, carboxylic and fatty acids, along with their esters, as well as long-chain alkanes within their leaves, which could have the potential to neutralize the negative charge carried by colloidal particles and various impurities in the water, consequently reducing electrostatic repulsion and facilitating their coalescence and clumping. [22] [32-36] Additionally, these compounds whose presence has been proven by GC-MS analysis can serve as bridges connecting the colloidal particles, thereby facilitating the formation of mass aggregates with sufficient weight to settle. It is important to note that, to the best of the authors' knowledge, no previous research studies have been published to date regarding the effect of *Aleppo pine* leaf extract on water turbidity.

3.2.2. The pH levels

Based on the findings delineated in Table 2 and Figure 2, the application of plant coagulants resulted in a notable reduction in the pH levels of the treated water samples. Specifically, the pH of water treated with *Aleppo pine* leaf extract decreased to 8.44 at concentration of 100 mg/L yielded the desired pH levels. These observed pH values were ascertained to fall within the acceptable range stipulated by the World Health Organization. [45] This phenomenon may be attributed to the presence of acidic chemical compounds such as phenols inherent in the leaves of *Aleppo pine* plant.

3.2.3. Total Dissolved Solids

Total Dissolved Solids (TDS) can be defined as the cumulative amount of organic and inorganic substances existing in various forms, ranging from molecular and ionized to fine grains, within water. [46] The efficacy of total dissolved solids (TDS) removal from water was assessed employing *Aleppo pine* leaf extracts, as depicted in Table 2 and Figure 3. Treatment of untreated water with *Aleppo pine*, resulted in reductions in TDS content by up to 65.19%. Notably, the most substantial reduction was observed at a concentration of 50 mg/L when utilizing *Aleppo pine* leaf extract. The reduction in total dissolved salts content within water may be ascribed to the precipitation of these stable salts, owing to the presence of acidic and basic compounds inherent in *Aleppo pine* leaf extracts.

3.2.4. The conductivity

The electrical conductivity of water is crucial in water quality assessment, as it provides valuable insights into the overall mineral content and purity of the water. It measures the ability to conduct electric current, which is determined by several factors, including the concentration of dissolved salts. [47] In the case of treating Ayin Kiam water with *Aleppo pine* leaf extract, the conductivity values demonstrated a reduction that was contingent upon the concentration of the treatment agent. The percentage of decrease in conductivity after treatment with *Aleppo pine* leaf extracts reached to 31.85%. As illustrated in Table 2 and Figure 4, optimal treatment outcomes were observed with a concentration of 50 mg/L for *Aleppo pine* leaf extract. The observed decline in the electrical conductivity of the treated water can be ascribed to the decrease in the water content of total dissolved salts. [48]

3.2.5. The iron content

Iron is a micronutrient crucial for maintaining optimal physiological functions within the human body when consumed in moderate quantities. However, elevated levels of iron in drinking water can pose health risks. [49] When treating the surface water of Ayin Kiam, the iron ion concentration decreased following treatment with *Aleppo pine* leaf extracts reached 100%. Notably, the most significant reduction occurred at a concentration of 50 mg/L when employing pine as in Table 2 and Figure 5. The observed decline in iron ion concentration in the treated water may be attributed to the presence of compounds within *Aleppo pine* leaf extracts that form complexes with iron ions, leading to their precipitation. [50]

3.2.6. The coliform counts

The data presented in Table 2, and Figure 6,7 demonstrate a significant decrease in coliform counts within the surface water samples of Ayin Kiam following treatment with *Aleppo pine* leaf extract. *Aleppo pine* leaf extract yielded optimal results at a concentration of 100 mg per liter, reducing the coliform count to 247 colonies per ml of water.

This decrease in coliform count can be attributed to the possibility of pine extracts acting in the same way as antibiotics in combating microorganisms. The antibiotics exert their effects through various mechanisms: they interfere with bacterial cell wall components, resulting in its weakening and subsequent rupture due to osmotic pressure; they may target ribosomes within bacterial cells, disrupting protein synthesis and inhibiting bacterial growth; they possess the potential to disrupt bacterial genetic material replication by interfering with DNA and RNA replication processes; they target essential cellular components inducing leakage, or they impede crucial metabolic pathways by interfering with the synthesis of essential metabolites. [51]

To the best of the authors' knowledge, no prior research has been published to date investigating the impact of *Aleppo pine* leaf extract or any other pine genus on water properties. However, existing studies have established the efficacy of employing extracts derived from pine cones and seeds as natural, sustainable coagulants and adsorbents for the treatment of turbid water, industrial wastewater, and the removal of dyes. [31] [43-44]

Table 2. The impact of *Aleppo pine* leaf extract on the surface water of Ayin Kiam.

Water Quality Parameters	Untreated Water	Treated Water 25mg/L	50mg/L	100mg/L
Turbidity NTU#	123±0.08	61.4±0.08	59.6±0.3	62.1±0.08
Ph	8.63±0.05	8.60±0.02	8.54±0.05	8.44±0.03
TDS* mg/L	6350±0.3	4570±0.4	4140±0.2	5080±0.2
Conductivity mS/cm	9.67±0.006	7.22±0.05	6.59±0.03	8.07±0.008
Iron mg/L	0.08±0.03	0.07±0.01	0.0	0.01±0.006
Total coliform count Cfu\ml$	499±0.6	247±0.8	263±0.8	328±0.8

*TDS: Total dissolved solids; #NTU: Nephelometric turbidity unit; $cfu: colony forming unit

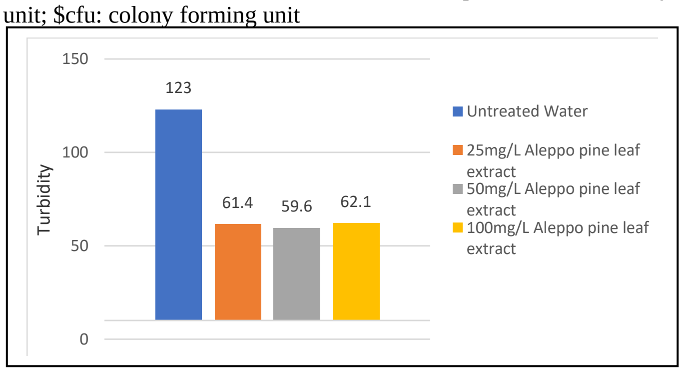

Figure 1. The impact of *Aleppo pine* leaf extract on the turbidity

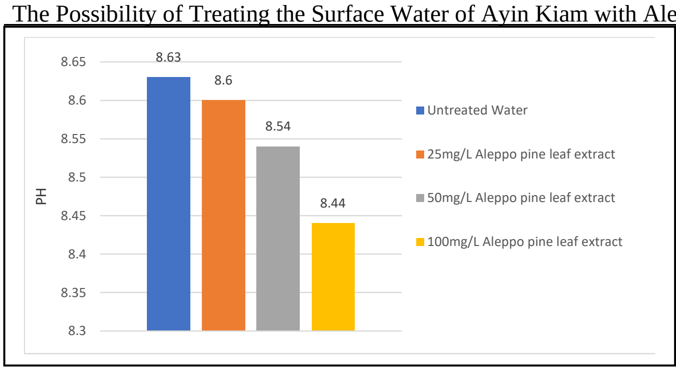

Figure 2. The impact of *Aleppo pine* leaf extract on the PH.

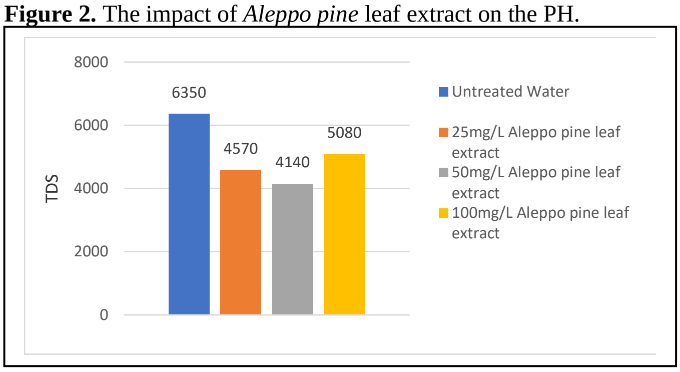

Figure 3. The impact of *Aleppo pine* leaf extract on the TDS.

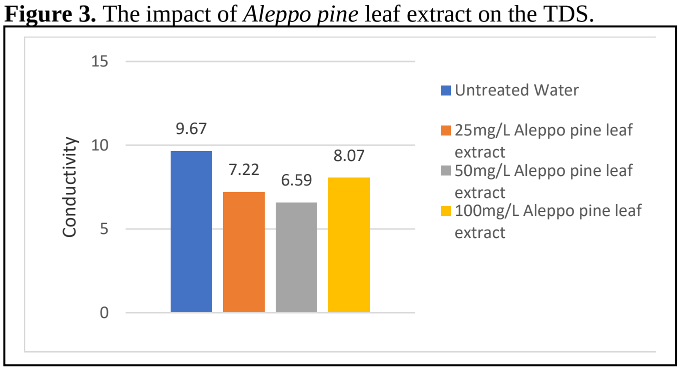

Figure 4. The impact of *Aleppo pine* leaf extract on the Conductivity.

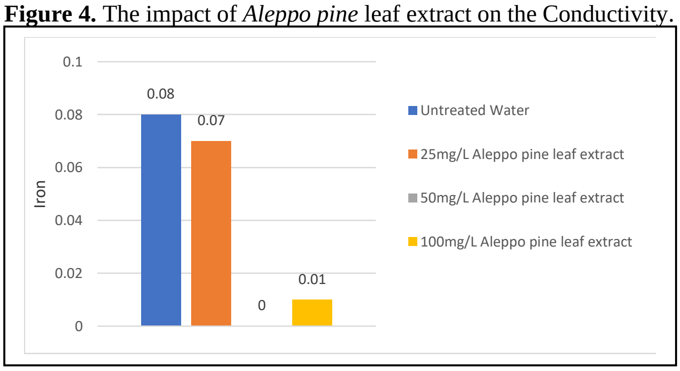

Figure 5. The impact of *Aleppo pine* leaf extract Iron.

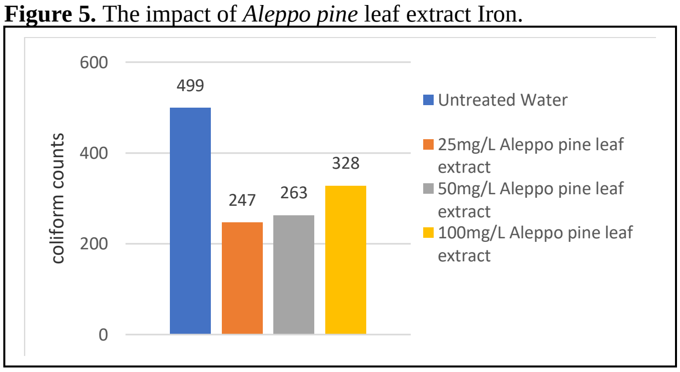

Figure 6. The impact of Aleppo pine leaf extract on the coliform counts.

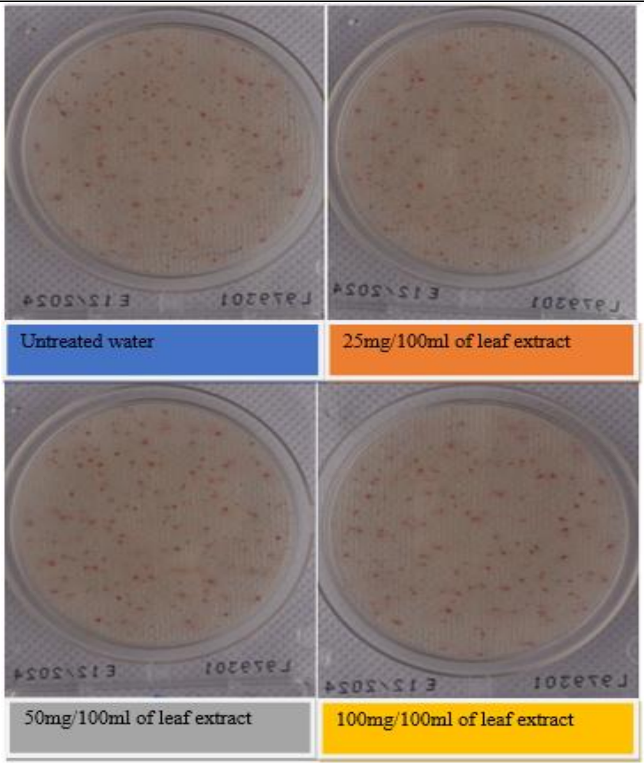

Figure 7. The microbial colonies on compact dry plates before and after water treatment.

4.Conclusion

Nature has endowed humanity with a plethora of solutions to obtain pure water and mitigate the detrimental effects of pollutants released into water bodies by human activities. Natural sources, such as plants and microorganisms, harbor substances with coagulation capabilities that have been extensively studied for their efficacy in water treatment. The current study underscores the remarkable potential of *Aleppo pine* leaf extract in the purification of water and reduction of pollution, thereby demonstrating its viability as a powerful alternative to chemical coagulants. However, further research is necessary to develop cost-effective technologies that can fully harness the potential of *Aleppo pine* leaves in water purification.

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