Research Article

Natural Coagulants Extracted from Leaf, Shell, and Kernel of *Jatropha curcas* for Turbidity Treatment

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Abstract
This research investigates the extraction of coagulants from the leaves, kernel shells, and kernels of *Jatropha curcas,* analyzing the effects of pH, temperature, and wastewater turbidity on coagulant activity. The coagulant extracts were obtained by mixing water with dried leaves, shells, and kernel samples in a proportion of 1:50. Turbidity removal was used as an indicator of coagulant activity. Coagulant activity was evaluated by applying different concentrations of the extracts (0.5, 1, 2, 3, 4, 5, and 6% v/v) to synthetic wastewater prepared at 770 NTU. The results showed maximum turbidity removal values of 81.2%, 76.6%, and 66.7%, identifying optimum dosages of 1, 0.5, and 4% v/v for the leaf, shell, and kernel extracts, respectively. These optimum dosages were subsequently applied to pharmaceutical wastewater, tap water, Dead Sea water, and groundwater samples. The turbidity removal results indicated that the extracts from all three fractions performed most effectively at pH 3, followed by notable turbidity reduction at pH 9. Increasing temperature further enhanced the coagulating activity of all extracts. Among the three, the Jatropha leaf extract exhibited the highest coagulant efficiency, except in the case of Dead Sea water, where its performance was limited due to high salinity. Overall, turbidity removal ranged between 80% and 90% in all water samples, except for the Dead Sea sample, where removal was 63%. This study concludes that the leaf fraction of *Jatropha curcas* possesses the highest coagulating power.

Keywords: Colloid, Coagulating activity, Dead sea, Electrical double layer, Wastewater, Zeta potential

1 Introduction

Jatropha, falling under the Euphorbiaceous family and consists of approximately 170 known species. Its name derives from the Greek words: jatr'os (doctor) and troph'e (food), indicating medicinal uses. Research on *J. curcas* has resulted in the isolation and characterization of many biologically active compounds, highlighting its potential applications in traditional medicine [1]. In addition, *J. curcas* is recognized as a significant source of seed oil for biodiesel production [2] and green bio lubricants [3], [4]. Recent findings indicate that the seeds, shell, or press cake residues or these fractions, which are typically discarded after oil extraction, contain protein that can serve as an effective coagulant in wastewater treatment [5]. Because of their cost-effectiveness, ecological and safety, these natural coagulants are considered preferable to synthetic chemical alternatives. Given the background that chemical coagulants represent a risk to human health due to the residual amount of chemical products present in treated water [2]. Some studies have examined the use of *J. curcas* seed kernels and shells, in powdered form or as dried press cake, to treat turbid water. A natural coagulant made from these materials was tested on a kaolin solution, simulating wastewater with turbidity levels between 100 and 8000 NTU. The highest turbidity removal rate, over 98%, occurred at pH 3 with a dosage of 120 mg/L [5]. Another study on pharmaceutical wastewater using *J. curcas* seeds found that the optimal performance of *J. curcas* occurred at pH 3 with a coagulant dose of 200 ppm, achieving 51% turbidity removal and 32% reduction in chemical oxygen demand (COD) [6].

To the best of our knowledge, the leaves of Jatropha have not yet been investigated to reduce turbidity, and the use of Jatropha fractions as coagulating extracts has not been completely investigated either. Coagulation is a critical primary treatment step for removing suspended solids from water in water treatment processes, where various types of coagulants are utilized [7].

The coagulants are classified into synthetic or chemical types and natural ones. The most common chemical coagulants are aluminium chloride, iron sulphate, alum, and polymers, which were used to enhance the efficiency of coagulation processes and decrease the necessary coagulant amount [7]–[9]. Natural coagulants have recently garnered attention due to their advantages. Its ability to clean water effectively, along with being environmentally friendly and cost-effective, makes it a sustainable choice for water treatment in regions where traditional chemical coagulants may not be readily available [7]. Natural coagulants help reduce waste and minimize environmental impacts because they produce biodegradable sludge, which can be repurposed for agricultural use [7]. These coagulants are derived from renewable natural sources, improving safety by lowering the potential health risks associated with residual chemicals, such as aluminium [7]. On the other hand, certain natural coagulants, like *Moringa oleifera*, offer additional advantages, including antimicrobial properties [10]. Examples of coagulants derived from natural sources, like leaves and seeds of the moringa plant [10], [11], or chitosan from crustacean shells [8]. Moreover, these coagulants are an economical choice due to their low operational costs, which result from reduced pH adjustment and sludge management needs, along with lower dosages required [12].

Typically biodegradable, natural coagulants have lower environmental impacts compared to industrial counterparts. Furthermore, natural coagulants are often preferred in regions where chemical usage is a concern due to their organic and eco-friendly characteristics [7]. Numerous studies have explored the potential of replacing synthetic chemicals with eco-friendly coagulants. For instance, pumpkin seeds were tested for treating the same turbid water samples, but showed weaker performance (79%) compared to other seeds and alum [13]. Additionally, papaya seeds and *Moringa oleifera* seeds have been identified as excellent alternative coagulants, capable of reducing turbidity by 92–94% at a dosage of 1 g/L. In comparison, alum achieved a removal rate of around 92% at a higher dosage of 40 mg/L [13]. The neem was applied at a concentration of 0.3 mg/L in aquaculture wastewater, reducing turbidity, total suspended solids, and colour by 83%, 81%, and 66%, respectively [11]. In another study, *Plantago ovata* extract, recognized as an effective bio-coagulant, was used in coagulation experiments on turbid water samples ranging from 50 to 300 NTU. The research eliminated 96% of the initial turbidity [14]. The leaves of various plants were examined as coagulants, using dried powder extracts of thirteen different plants at concentrations ranging from 500 to 10,000 mg/L. The highest removal rate achieved was 76%, which was obtained using buttonwood [15]. The objective of this study is to assess the coagulant activity of the leaves, kernel shells, and kernel coagulant extract of *J. curcas*, and analyse the effect of temperature, pH, and turbidity of wastewater on the coagulant activity.

2 Materials and Methods

2.1 *Materials*

The seeds and leaves of *Jatropha curcas* were acquired from a supplier in Saudi Arabia and from the southern region of Jordan, respectively. Sulfuric acid (95%–97%) and sodium hydroxide (99.9%) were purchased from Sigma Aldrich and were used as received for pH adjustment. Water samples were collected from the Dead Sea, Ain Lahda, in southern Jordan, and from pharmaceutical wastewater at the Jordan River Company for Pharmaceutical Industries located in Jordan.

2.2 *Preparation of the coagulant*

The leaves and seeds were dried overnight at 80 °C. Posteriorly, the seeds were peeled and separated into kernels and external shells. The three parts or fractions were ground and sifted through a 300 µm sieve, ASTM No. 50. The powder originating from each fraction was stored in well-sealed containers until used. The extract solutions were prepared at room temperature using distilled water at a ratio of 1:50 (g/ml) and stirred for 30 min. The resulting solution was filtered, and the filtrate was used as a coagulant extract [5], [6], [15]. The experimental design is summarized in Figure 1.

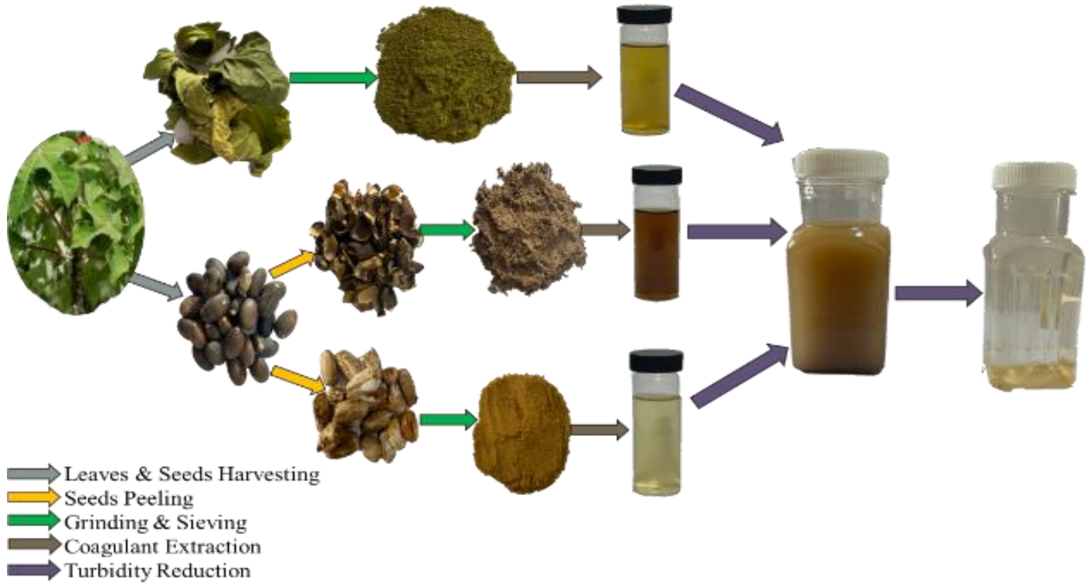

Figure 1: Sample scheme of the process.

2.3 *Coagulation activity experiments*

2.3.1 Determination of optimum dosages

To study coagulation activity and determine optimum dosages, a synthetic wastewater sample was prepared using granular soil, which passes a sieve mesh No. 50 ASTM (300 microns of particle size). The synthetic turbid water was prepared by mixing soil with tap water in a ratio of 1:100 (g/ml) and stirring to ensure homogeneity, achieving a turbidity of 770 NTU. Afterward, 200 mL of turbid water was used for each exploratory run. Initial and final turbidity values were measured using a turbidimeter model Milwaukee Mi 415. The batch coagulation process begins with the addition of coagulant extract and rapid mixing for 2 min, followed by slow mixing for 10 min, and concludes with a settling period of 30 min. Rapid stirring effectively distributes the coagulant, while slow stirring allows impurities to clump together, forming flocs that can be easily removed. After sedimentation, the coagulated samples are collected to measure the final turbidity [5], [6], [16]. The experiments were conducted using coagulant extracts from three fractions at room temperature and neutral conditions, with varying doses of 0.5, 1, 2, 3, 4, 5, and 6 v/v%. The optimum dosages were identified at maximum turbidity removal.

2.3.2 Effect of pH, temperature, and turbid water

Several samples were collected to analyse the effect of pH, temperature, and turbid water. The coagulation activity performance at varied pH (3, 5, 7, 9, and 11) is tested. All experiments were conducted using turbid tap water samples with the optimal dose of each Jatropha coagulant at room temperature. Hydrogen potential values (pH) of turbid water were adjusted using solutions of 4M H_2SO_4 and 5M NaOH. Thereafter, the turbidity removal was measured at various temperatures of turbid tap water samples (15, 25, 35, and 45 ℃) using an optimal dose of each Jatropha coagulant under neutral conditions. The coagulation efficiency of Jatropha fractions was tested on synthetic turbid water samples from the Dead Sea, groundwater, and pharmaceutical wastewater, comparing the results to those from tap water. These types of water samples are analysed based on electrical conductivity EC, salinity, and total dissolved solids TDS using Wiher, a water quality tester pen meter. All experiments were conducted at room temperature using the optimal dose of each Jatropha coagulant, without any modifications to the pH.

2.3.3 Phytochemical analysis

Phenolic compounds were determined according to the methods developed by [17]. While esters and amino acids were quantified using liquid chromatography coupled with mass spectrometry (HPLC/MS) analysis, the methodology followed by [18]–[20].

2.4 *Statistical analysis*

Three replicates were taken from each sample, and their results were used for statistical analysis. The values for the presented variables are reported as the mean ± standard deviation (SD) represented by error bars. A statistical t-test was conducted to determine whether there were significant differences, with a significance level set at p-value ≤ 0.05.

3 Results and Discussion

3.1 *Assessment of coagulating activity*

The coagulation activity is demonstrated by the quantity of turbidity removal. The percentage values of turbidity removal to different doses of extract solutions for leaf, kernel shell, and kernel fractions are presented in Figure 2. The results reveal maximum percentages of turbidity removal of 81.2%, 76.6%, and 66.7%, achieving minimum values of 54.8%, 47.5%, and 41.36 % for leaf, kernel shell, and kernel fractions, respectively. These maximum values of turbidity removal were obtained at doses 1, 0.5, and 4 v/v% of extract for each solution fraction.

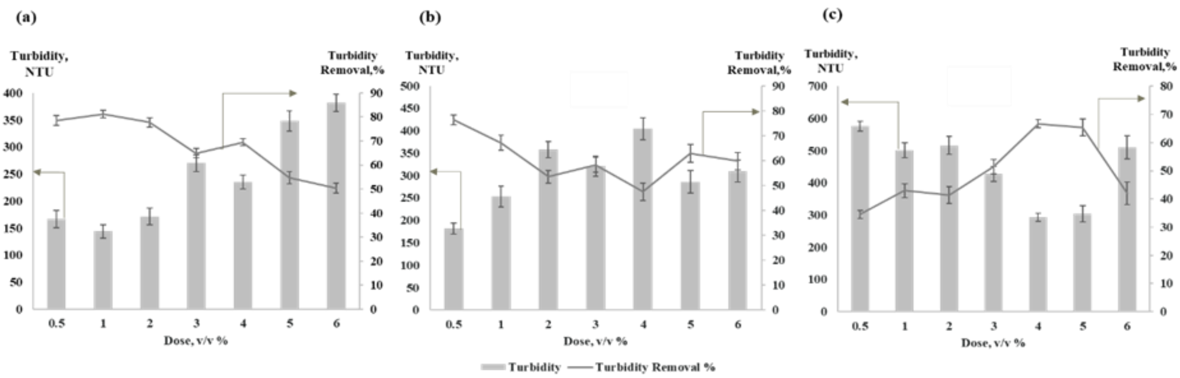

Figure 2: Percentage of turbidity removal to different dosages of coagulant extracts for leaf (a), kernel's shell (b), and kernel (c) at room temperature and neutral conditions.

Table 1: Phytochemical composition of *J. curcas*.

	Leaf	Kernel Shell	Kernel
Crude Protein (%)	5.870	3.71	61.80
Total Phenolic (mg GAE/gDM)	27.70	9.16	3.60
Free Amino Acids (mg /gDM)	15.09	11.13	9.07
Total Flavonoid (%)	4.40	0.60	0.04

Notes: mg GAE/gDM: mg of Gallic Acid Equivalent/ gram of Dry Matter.

Finding the highest coagulation activity in the leaf, followed by the kernel shell, and kernel fractions. High removal of turbidity occurred, which can be explained by studying the amino acids present in the leaf of *Jatropha curcas*. These are associated with the phenomena of Van der Waals forces combined with the physical concept of the electrical double layer (EDL) and zeta potential [21]. Table 1 reports the phytochemical composition of each fraction. Notable values of total phenolic (27.78 mg GAE/ g DM), free amino acids (15.09 mg/gDM), and total flavonoid (4.4%) were observed for leaves. Followed by the kernel shell and kernel fraction. Results comparable to those obtained by [22]–[25]. These values revealed that the fraction of Jatropha leaf presents the highest levels of free amino acids, flavonoids, and phenols. It leads us to think that a major percentage of these oxygenated and nitrogenous functional groups enhances the coagulating capability of each fraction of *J. curcas* [26]. This is because certain asymmetric molecules of oxygenated or nitrogenous functional groups present in the solution of jatropha, such as amines (R-NH2), phenols (C6H5-OH), and flavonoids, tend to form dipole-dipole bonds [27], [28]. This tendency is due to their high values of non-null permanent dipole moment between 1.3 to 1.5, characteristic of these functional groups H-O and H-N [29], [30]. Inducing the formation of dipoles that are easily joined by attractive intermolecular Van Der Waals forces. The double electrical layer (EDL) around the negatively charged colloidal solid, which generates the repulsive electrostatic force, is annulled by the presence of dipoles. Destabilizing the colloid, favouring coagulation and quantifying values of Electrokinetic or Zeta potential (ζ) close to zero (0) at the slipping plane [21]. This double layer (EDL) is constituted of cations of the rigid Stern layer, plus the ions of the diffuse zone, adsorbed on the surface of the colloidal solid (Figure 3). Being the diffuse zone rich in H^+ and H_3O^+ cations, coming from of electrolytic Jatropha solution. Additionally, the high content of aspartic and glutamic amino acids present in the leaves fraction, 119 and 186 g/kg protein, respectively [22], accentuates its particular cationic-acid character. Other research carried out by [12] also demonstrated high coagulating capability for extracts of *J. curca*'s seeds treated with NaOH and NaCl, in accordance with the chemical-electrical mechanism focused on this investigation. Studies about coagulation-flocculation using chemical coagulants presented by [27] support the EDL analysis discussed in this work.

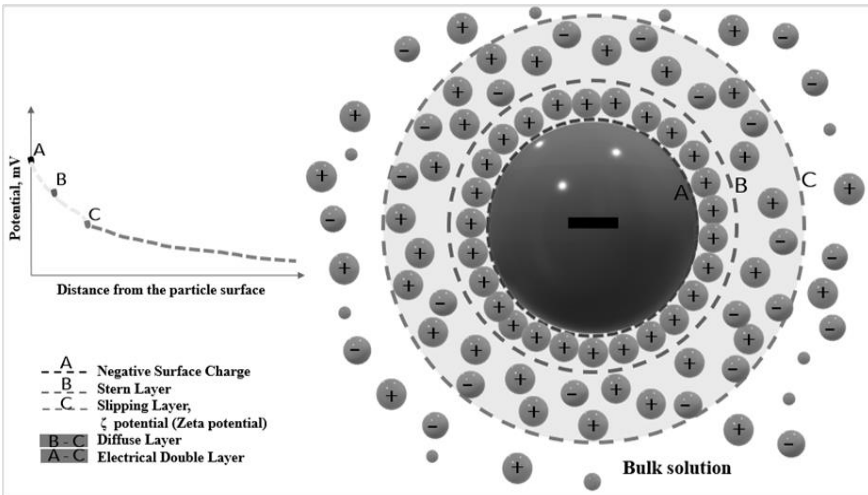

Figure 3: Electric double layer (EDL)around the colloidal particle with Zeta potential (ζ) at the slipping plane.

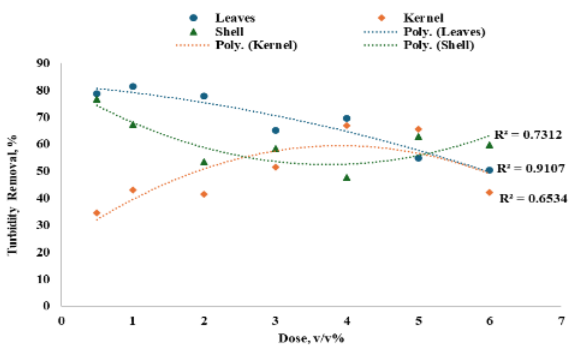

Figure 4: Evolution of coagulating activity.

Figure 4 presents the evolution of turbidity removal with different dosages of coagulant. This monitoring allowed identifying the optimal dosage, which appears at the maximum peaks of the curves. This analysis is comparable with other works presented for *J. curcas* seed [6], [28]. Results shown in Figure 4 discovered that once the maximum remotion dose is reached at the optimum dosage, the coagulating effect of the Jatropha solution tends to decrease. This phenomenon occurred for the coagulant extracts of three fractions.

The reduction of coagulant activity is explained according to Derjaguin-Landau-Verwey-Overbeek's theory (DLVO theory) for lyophobic colloids. DLVO enounces that the repulsive electrostatic forces can be considerably altered by changing concentration, pH, valence, and nature of the electrolytes. For this reason, an excessive dose of Jatropha solution would alter the concentration of the electrolyte H^+ derived from amines and proteins, increasing the cations present and consequently reestablishing the repulsive electrostatic forces of the double layer due to excess protons. Stabilizing again the colloid and consequently, the coagulating effect of the Jatropha solution would decrease [6], [12]. However, the shell presented a peculiarity: once it reaches the minimum coagulant activity, it increases again. This could be justified by the highest phorbol esters content present in the shell fraction. Table 2 presents the results of phorbol esters, and the percentage of turbidity removal assessed in the coagulant extracts. Maximum results of 0.38% of esters and 86% of Turbidity Removal in Dead Sea water, suggesting a potential link between ester concentration and coagulant efficiency in ionic-cationic environments.

An attribute that could enhance the shell extract's effectiveness as a coagulant in highly saline environments. This phenomenon may be produced by the notable reactivity of the hydrogen ion in the carboxyl group toward metal cations. This promotes the formation of carboxylate salts or ester-derived salts. Favouring the formation of salts in ionic environments, restoring polarity, and reactivating the coagulating capacity [31].

Table 2: Content of esters and turbidity removal.

Coagulant Extracts	Phorbol Esters, wt.%	Turbidity Removal, %		
		Tap Water	Dead Sea	Groundwater
Leaves	0.18	81.30	66.73	91.08
Shell	0.38	76.36	86.60	89.43

3.2 *Parameters that affect coagulating activity*

3.2.1 Effect of pH

Hydrogen Potential (pH) affects the coagulant's solubility and charge. Influencing the particles' stability in the water and impacting the turbidity reductions [27], [28]. Figure 5 shows the effect of pH on the turbidity removed from the water treated. This study found that the best performance of removal rate was for leaf extract (96.55 %), closely followed by shell's result (96.28 %), and finally kernel extract (82.14 %). These results demonstrated that the highest removal rate was under acidic conditions, at pH 3. This suggested that at pH 3, the isoelectric potential (IP) was achieved. Being IP, the pH, which is obtained at the maximum coagulation point, once the zeta potential has reached the zero value $\zeta \sim 0$. Hence, this result demonstrated that due to the presence of glutamic (IP 3.2) and aspartic amino acids (IP 2.77), compounds abundantly found in the leaf, kernel's shell, and kernel [32]; the coagulation was favoured between its IP range of 3.2 to 2.77 (at pH 3). This analysis was also discussed by [33] for the Quality assessment profile of *Jatropha curcas* seed oil from Nigeria.

The results were also compared with studies conducted by [26] on turbidity removal using synthetic coagulants in prepared kaolin–tap water samples. The synthetic coagulants achieved turbidity removal rates of around 97.7% at pH 6.5 for aluminium, 90.8% at pH 7 for copper sulphate, and 87% and 72% for ferric and ferrous sulphate at pH 4. In this study for coagulants of *J. curcas*, a high efficiency of the extracted coagulants was found for all fractions at pH 3. Reporting values of turbidity removal between 96.55% to 82.14%. Highlighting the advantages of using natural coagulants from Jatropha due to their high efficiency and simple chemical-free extraction method. This promotes sustainability and significantly reduces environmental damage. Results illustrated in Figure 5 also discovered that the coagulants' performance is significantly notable under basic conditions (pH 9) in comparison to neutral conditions (pH 7).

The leaves, shells, and kernel extract reduced the turbidity by 93,2%, 91.59%, and 73.68%, respectively, at pH 9. These results revealed that in all environments, acid and basic, the leaves-based coagulant presented the best coagulating activity. That may be related to the reduction in the coagulant's solubility and the colloids' destabilization compared with acidic conditions [5], [15]. The analysis of this study is consistent with investigations presented by [5], where the high removal rate exceeded 96% by Jatropha-based coagulants in both environments, between pH 1-3 and pH 11–12, for acid and basic medium, respectively.

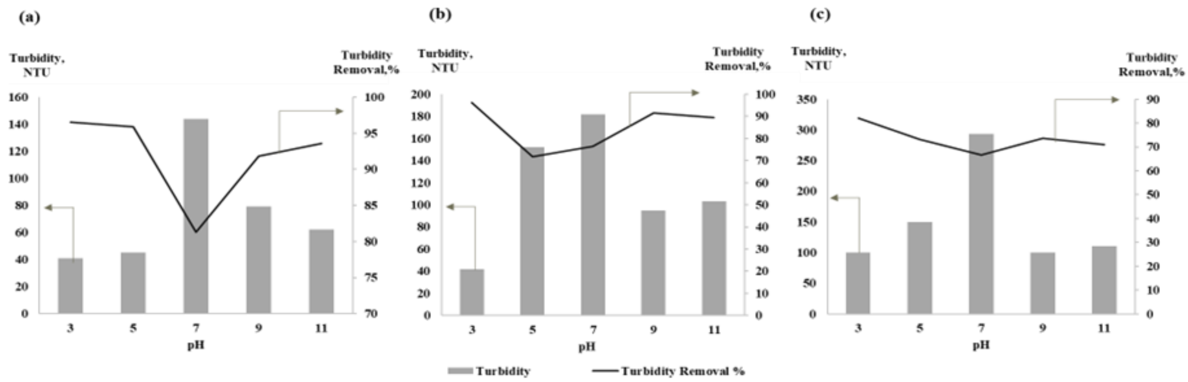

Figure 5: Percentage of Turbidity Removal vs pH for leaf (a), kernel's shell (b), and kernel (c) at room temperature using optimal dosages (1, 0.5, and 4 v/v%, respectively).

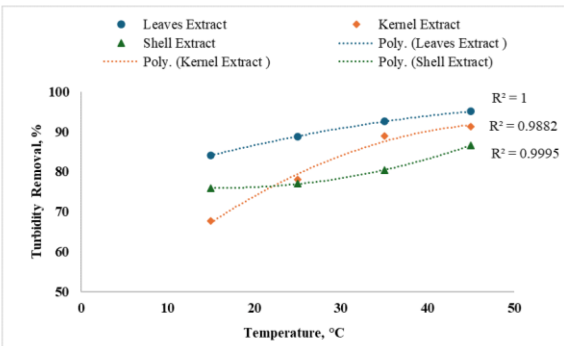

Figure 6: Effect of temperature on the turbidity removed from treated water at neutral conditions using optimal dosages (1, 0.5, and 4 %v/v, respectively).

3.2.2 Effect of Temperature

In general, the coagulants from the three fractions performed effectively with the increase in temperature. It was demonstrated by an excellent coefficient R^2 of around 1 (Figure 6). The temperature influences overall coagulation performance by its impact on molecular motion [34]. The shell-based coagulant presented the highest turbidity removal of 86.61 % at 45 °C, while its performance did not exceed 75.96 % at 15°C. The kernel-based coagulant recorded the best removal of 91.22 % at 45 °C, and the lowest reduction was around 67.74% at 15 °C. The performance of leaf-based coagulants is consistent with fractions of shell and kernel coagulants, where the highest removal reached about 95.05% at 45 °C, but the lowest removal was 84.08 % at 15 °C. The high efficiency achieved at maximum temperature may be explained because the water viscosity decreases at high temperatures. The collision and aggregation of particles enhance and form sediments, flocculating easily [35], [36], [37]. These behaviours are consistent with similar ones achieved by watermelon, where the best removal occurred at high temperatures [38].

3.2.3 Effect of Turbid Water on Coagulating Activity

The coagulants are generally affected by the turbidity of water. The turbidity is associated with other characteristics such as salinity, electrical conductivity (EC), and total dissolved solids (TDS), besides the content of organic matter [39]. Table 3 presents the results of the main features evaluated, which affect the reduction of coagulant activity. Four wastewater samples of different turbidity were used to assess the performance of Jatropha-based coagulants. The results reported in Table 3 reflect the impact of water salinity on other properties. The high salt content leads to an increase in EC due to the dissolved ions that conduct electricity. Additionally, EC is proportional to TDS as both depend on dissolved ion concentration. Whereas salinity and TDS are closely related because both measure the content of dissolved substances. In general, pH value depends on the available ions of H^+ and OH^-.

Table 3: Characterization of turbid wastewater.

	Salinity (Salts%)	Electrical Conductivity (EC) µs/cm	Total Dissolved Solids (TDS) Ppm	pH	Turbidity NTU
Tap water	0.02	512	253	8.30	770
Groundwater	0.01	371	187	8.26	785
Pharmaceutical wastewater	0.13	2697	1331	8.90	3660
Dead Sea water	3.37	63,000	30,000	5.21	520

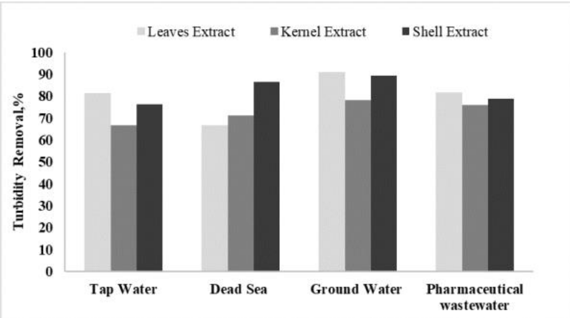

Figure 7: Turbidity removal using three coagulants on wastewater samples with varying turbidity at room temperature and optimal dosages.

Figure 7 shows that for the Dead Sea water sample, which is high-saline water, the removal percentages were 86.60%, 71.20%, and 66.73% for the shell, kernel, and leaf extracts, respectively. This study discovered that the kernel's shell and kernel-based coagulants achieved higher coagulating activity with the Dead Sea sample than the tap water sample. That is due to the ionic strength in high saline water, which compacts the electrical double layers around suspended particles [40]. It contributes to the coagulant content of cationic proteins and fatty acids. Enhancing the charge of neutralization and inter-particle bridging, following the analysis presented by [25]. Additionally, the aggregation of colloids is enhanced by the hydrophobic nature of lipids, phorbol esters, and fatty acids that exist in the kernel's shell and kernel [41]. Kernel's shell and kernel-based coagulants are adapted to high ionic strength, in contrast to leaves that are rich in hydrophilic functional groups, such as hydroxyl and carboxyl, whose interactions are disrupted by high ionic strength [42]. This leads us to think that leaf extracts are more efficient in low ionic environments than kernel and shell extracts [39]. For the groundwater sample, the removal percentages were 89.43%, 78.09%, and 91.08% for the kernel shell, kernel, and leaf extracts, respectively. This can be attributed to the highest levels of amino acids in its proteins, polysaccharides, and polyphenols in the leaves fraction.

Enhancing the groundwater results, where the coagulant acts better than in a tap water sample. However, the kernel performance is limited due to its lipophilic features and the poor composition of polysaccharides and polyphenols; the composition is shown in Table 1. This analysis was also followed by [40], [41] in the study of neutralization charge and inter-particle bridging in a water sample with high salinity, EC and TDS. For pharmaceutical wastewater samples, the removal percentages were 78.96%, 75.96%, and 81.69% for the kernel shell, kernel, and leaf extracts, respectively. The highest response of leaves contributed to its amino acid content, which causes charge neutralization and provides binding sites for the aggregation of suspended solids and organic contaminants [42], [43]. In all cases, except for the Dead Sea sample, the highest turbidity removed corresponds to the leaf coagulant. For the Dead Sea sample, the most effective coagulant extract was the one derived from the shell**.**

4 Conclusions

The study of coagulation activity revealed that the coagulant extracts of *J. curcas* were able to remove the turbidity efficiently. It means that the simple and chemical-free extraction method proposed in this study is successful. Promoting sustainability, with the advantage of significantly reducing environmental damage. This would allow the development of new, more eco-friendly water treatment alternatives. To the best of our knowledge, the leaves of Jatropha have not yet been investigated to reduce turbidity, and the use of Jatropha fractions as coagulating extracts has not been completely investigated either. This study, a novel contribution in the field of natural coagulants.

Also, this work led us to conclude that it is not necessary to use exclusively dried powder of seeds, kernel, or press cake kernel chemically treated as a coagulant for water treatment. Finding that the percentage of turbidity removal varies according to the different content of the phytochemical functional groups of each fraction. Being the percentage of turbidity removal, the most important parameter to evaluate the coagulating activity and identify the optimum dosages of the coagulant extracts.

In general, three coagulants performed effectively under acidic conditions (at pH 3). Ranging the pH of maximum turbidity removal into the IP of the compounds present in the highest proportion, such as the amino acids glutamic and aspartic. Being also positively influenced by the increasing temperature.

This research successfully demonstrated that *J. curcas* fractions are good natural coagulants that can be used efficiently in pharmaceutical wastewater. It is attributed to the complex nature of the organic matter in wastewater and the synergistic interactions of the coagulant content. Finding out that, except for Dead Sea wastewater, the leaves-based coagulant was the best coagulant extract. Attributing its limited coagulating capacity to the high salinity levels.

For samples with high salinity content, as Dead Sea water, kernel's shell extract was the most effective coagulant, followed by kernel extracts. This is associated with its high content of phorbol esters, as these compounds exhibit high reactivity in ionic-cationic environments, where they readily form ester salts. Recommending for future research, a more in-depth investigation of this topic, together with industrial-scale process profitability. Finally, this study concludes that the leaf fraction has the highest coagulating power.

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Author Contributions

All authors participated in a) conceptualization, investigation, and research design, b) analysis and interpretation of the data, c) methodology, writing and original draft, d) editing and critical review, e) approval of the final version. The authors have read and approved the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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