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Application of Thymus Species as Natural Coagulant for Elimination of Turbidity of Water and Its Comparison with the Conventional Coagulant

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ABSTRACT

Introduction: The most important pollutant in surface water sources is turbidity. The aim of the study is to determine the most effective thyme plant extract as a natural coagulant to remove water turbidity and compare it with alum as a chemical coagulant..

Materials and Methods: In this study, plant extracts obtained from Thymus vulgaris (Tv), Thymus kotschyanus (Tk), and Thymus eriocalyx (Te) were used as natural coagulants for elimination of water turbidity. Then, the best of plant extract was confirmed by ninhydrin and FTIR analyses and the effect of simultaneous use of plant extract with chemical coagulant of aluminum sulfate (alum) was investigated in the hybrid ratio of 1:1, 2:1, and 1:2. Then, the effect of parameters include of pH, coagulant dose, turbidity removal efficiency, and the most suitable solvent for coagulant extraction were studied.

Results: The results of this study showed that among the natural coagulants, Tv had a higher efficiency in elimination of water turbidity, and the highest amounts of turbidity elimination for Tv, alum, and Tv/alum hybrid coagulants was 94, 90, and 96, respectively. According to the results of this study, the most effective solvent for preparing plant extracts was 1M KCl solvent.

Conclusion: The natural coagulant removes more turbidity from water than the chemical coagulant, and the simultaneous use of natural and chemical coagulants with a 1:1 ratio was more effective.

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Introduction

Providing safe drinking water is one of the indicators of health development, which today has faced serious challenges due to the entry of various pollutants into surface and underground water sources. Therefore, continuous monitoring of water quality is one of the main priorities of health experts and water researchers. One of the most important parameters determining water quality is turbidity, the cause of which is attendance of colloidal and suspended substances in water. One of the conventional methods for elimination of water turbidity is coagulation and flocculation processes[1]. In

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coagulation and flocculation processes, combinations for example aluminum chloride, ferric sulfate and aluminum sulfate are used as coagulants. Among these common coagulants, aluminum sulfate (alum) is the most widely used. Today, in addition to the relatively high cost of providing such coagulants, the remaining of such substances in drinking water can have harmful effects on water quality and human health. For instance, remaining aluminum in treated water can reason Alzheimer's disease[2], spasms, and digestive diseases among consumers[3]. In addition, the disposal of sludge resulting from such chemical coagulants can be one of the operation problems in water treatment plants[4-6]. Considering the disadvantages of chemical coagulants, researchers have turned to using natural coagulants. The advantages of natural coagulants include removing organic and inorganic turbidity, reducing appearance color, removing pathogens, algae, and plankton, and eliminating odor-causing factors[7-9].These coagulants are extracted from plants and are usually not toxic and corrosive like chemical substances[10-12]. The removal of turbidity by a natural coagulant is significantly influenced by the properties and characteristics of the coagulant[13, 14]. Therefore, by comparing the advantages and disadvantages of chemical and natural coagulants, the best way to remove turbidity from water is to use natural coagulants, including plant extracts. Thymus plant belongs to the Lamiaceae family. This plant contains phenolic and antioxidant compounds, Eugenol, carvacrol, thymol, etc. The most important effective compounds in thymus to remove turbidity from water are the presence of protein and amines in the extract[15-17]. So far, there was no study related to process of coagulation or removal of turbidity from water with the extract of Thymus species, for this reason, the effectiveness of the extract of different species of Thymus in removing turbidity and its comparison with aluminum sulfate has been investigated, and the authors have evaluated the simultaneous use of plant extract and alum based on ninhydrin and FTIR tests. In the present study parameters include of pH the most effective kind of solvent for extract extraction, coagulant dose, and turbidity removal efficiency has investigated.

Material and Methods

Preparation of natural coagulants

For prepare plant extract was used of Thymus vulgaris (Tv), Thymus kotschyanus (Tk), and Thymus eriocalyx (Te) that obtained of eastern areas of Iran. Then, the aerial portions of the plants were separated, dried and milled. Two gram of each milled plant was well mixed with combinations include of NaCl, KCl, $NaNO_3$ and KNO_3 salts (1M) were used for extraction of plant extract. The resulting mixture was stirred for 30 minutes by magnetic stirrer and filtered with whatman paper No. 42. The obtained solutions was used as natural coagulants to remove turbidity and compared their removal efficiency. Then prepared a 2% alum stock solution ($Al_2(SO_4)_3.18H_2O$) by addition of 2 gr of powder of alum to 100 ml of distilled water. Finally, the effect of hybrid of plant extract and alum was studied using Tv/alum in ratios of 1:2, 2:1, and 1:1 by mixing appropriate amounts of plant extract and alum[18]

Preparation of turbid water

In this study, a synthetic turbid solution was used to remove confounding factors during the study. To prepare the synthetic turbid solution, 5 grams of sieved clay was added to one liter of distilled water. Then, to obtain a uniform dispersal of clay particles in distilled water, the suspension of clay was blended by magnetic stirrer for 2 h. In order to complete the hydration process, particles of clay and their suspension remained at room temperature for 24 hours.

And after 24 hours, turbid water with a turbidity of 700 NTU was prepared using the supernatant before the coagulation process, in order to survey of the influence of pH on the removal of turbidity, calibrated the initial pH of turbid water with 1 M NaOH NaOH or HCl solution. The neutral pH range was considered

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for the turbid water used in turbidity removal experiments with natural coagulants[19].

Coagulation tests

The coagulation, flocculation, and sedimentation procedures used in this report were conducted using a standard Jar Test device and a set of six one-liter beakers. (VELPScientifica, model: JLT6, Italy). After filling each flask with turbid water containing various levels of turbidity, different extracts, alum, and Tv/alum hybrid were added discretely to each flask. For coagulation, the obtained mixture was blended at 120 rpm for 2 minutes fastly, and for the flocculation, the speed of mixing was lowered to 40 rpm which continued for twenty minutes. After these 2 processes (coagulation and flocculation), to settle the formed flocs, the beakers were put to rest. After thirty minutes, the samples of filtered were gathered from the on the beakers. In addition, turbidimeter was used to measure their remaining turbidity (Hach Turbidimeter Model 2100N), and the effect of turbidity elimination was computed by utilizing the following Equation (1) [18.]

$$\eta = \frac{(C0 - C)}{C0} \times 100 \quad (1)$$

C_0:The turbidity concentration before coagulation

C: Turbidity concentration after coagulation and sedimentation

Ninhydrin test

The amount of primary amines in natural coagulants of Thymus vulgaris (Tv), Thymus kotschyanus (Tk), and Thymus eriocalyx (Te) was determined using the ninhydrin test[18].

FTIR test

After determining the maximum amount of amine in the plant extracts that were studied, the significant functional groups in the extract structure were identified using a Fourier transform infrared spectrometer (FTIR). Within the wavelength range of 4000-500, this analysis was carried out. In this step, the KBr tablet preparation method was used for preparation. The resulting spectrum was obtained by FTIR and the peaks were analyzed (Figure. 1) [18].

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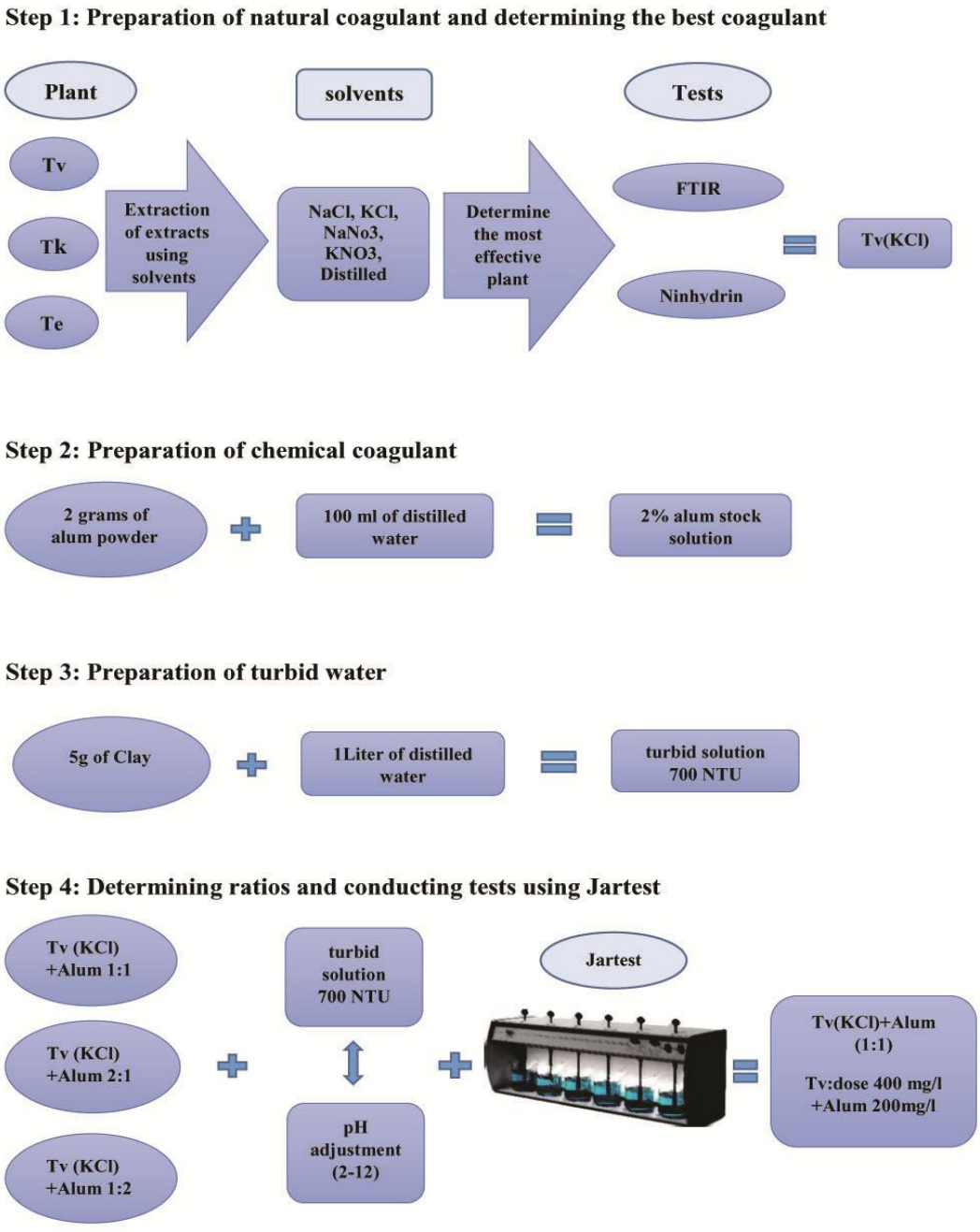


Figure 1: Schematic process of experiments in 4 steps

Result

The effect of different natural coagulants on turbidity removal

As shown in Figure 2, among natural coagulants, Tv was able to show the highest removal efficiency among coagulants with a dose of 400 mg/L, removing 91% of water turbidity. But Tk and Te coagulants with the same dose removed 84 and 76% of water turbidity, respectively. In the continuation of the research, the results showed that the efficiency of removing turbidity from water using a dose of 200 mg/l of chemical coagulant (alum) is 90%.

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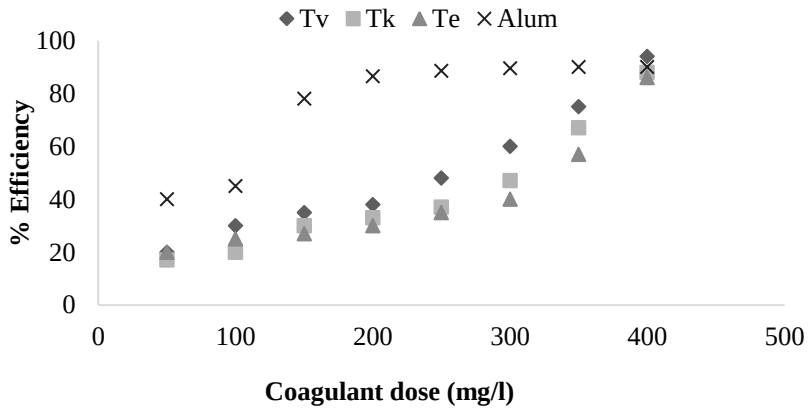


Figure 2: Influence of dose of natural coagulants on turbidity removal (turbidity: 700 NTU, pH = 7). **Tv:**Thymus vulgaris - **Tk**:Thymus kotschyanus - **Te:**Thymus eriocalyx

Ninhydrin test

Figure 3 shows the results of the ninehydrin test. The findings of this test showed that among natural coagulants, the highest amount of amine group is present in Tv plant extract. It is worth mentioning that performing this test is very important to determine the most effective plant extract for use in FTIR and hybrid coagulant tests.

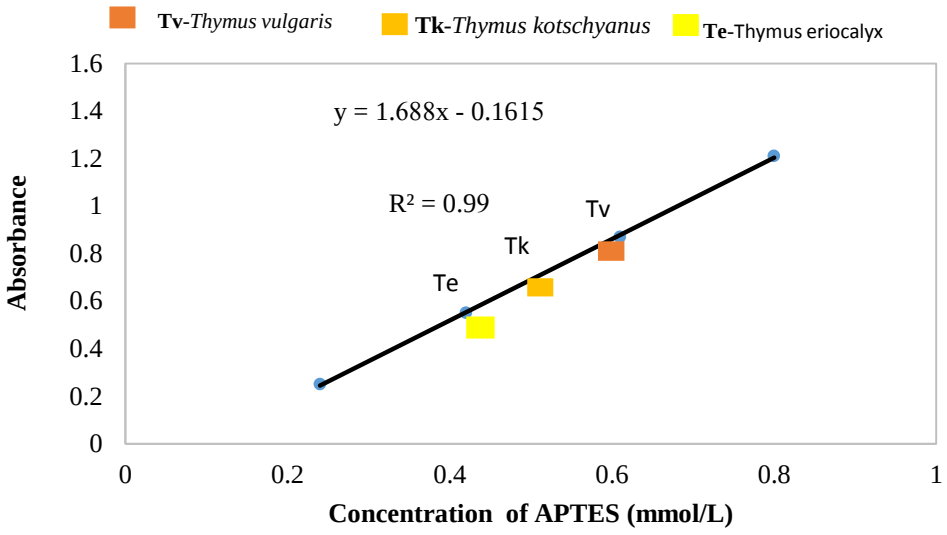


Figure 3: APTES concentration calibration curve. **Tv:**Thymus vulgaris - **Tk**:Thymus kotschyanus – **Te:**Thymus eriocalyx

FTIR test

The results of the FTIR test showed that Tv plant extract has a peak of 3478.82 cm^{-1} corresponding to the amine stretching group, a peak of 1638.97 cm^{-1} corresponding to the amine bending group and a peak of 609.43 cm^{-1} corresponding to Amide III. By performing this test, the presence of amines as the most effective substance in Tv extract was proven to increase the efficiency of turbidity removal (Figure 4).

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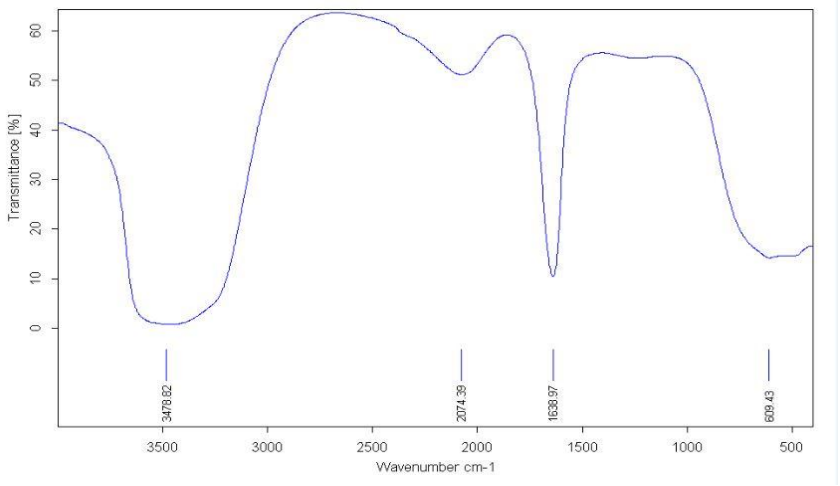


Figure 4: FTIR spectra of Thymus vulgaris extract

The effect of using hybrid TV and alum on removing turbidity

As shown in Figure 5 and Figure 5-1 the use of Tv/Alum hybrid coagulant with a proportion of 1:1 was able to remove 96% of the turbidity of the cloudy aqueous solution. However, the use of hybrid coagulants with ratios of 2:1 and 1:2 showed 91 and 92% of the removal rate, respectively.

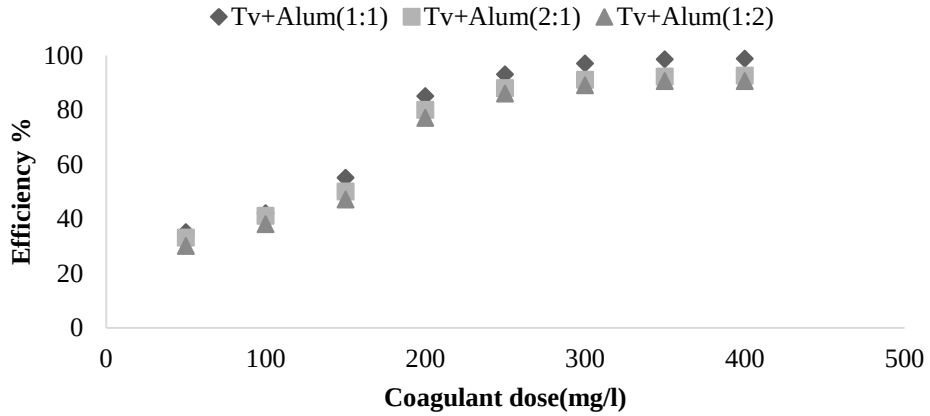


Figure 5: The impact of hybrid coagulant dose on removing turbidity (turbidity: 700NTU, pH = 7).
Tv: Thymus vulgaris - **Tk**:Thymus kotschyanus - **Te:**Thymus eriocalyx.-Alum=Aluminium Sulfate- Al2(SO4)3)

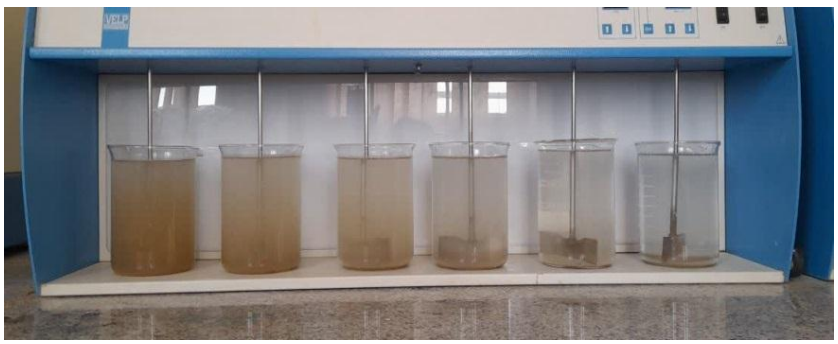


Figure 5-1. The impact of hybrid coagulant dose on removing turbidity (turbidity: 700 NTU, pH = 7).

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The effect of pH on the efficiency of removing turbidity from aqueous solutions

Figure 6 shows the influence of pH on turbidity removal from turbid aqueous solutions. As shown in the figure, the highest turbidity removal efficiency was obtained at pH=7, and the removal efficiency did not change with increasing pH to 12.

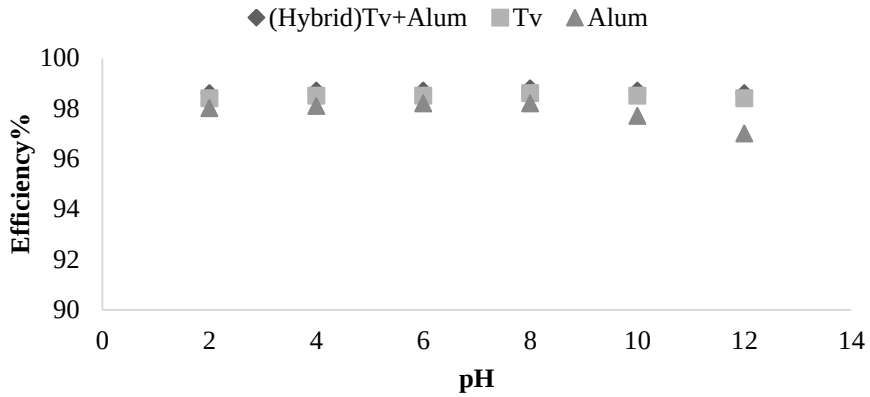


Figure 6. The impact of pH on the removal of turbidity (Tv:Thymus vulgaris, turbidity: 700 NTU, coagulant dose: 400 mg/L)

Features and images of flocs resulting from natural and chemical coagulants

As shown in Figure 7, Flocs obtained from Tv/Alum hybrid coagulants (Figure 7, C, E, F, G, H) are larger than the natural coagulant (Tv) (Figure 7, A) and chemical coagulant (Alum) (Figure 7, B).

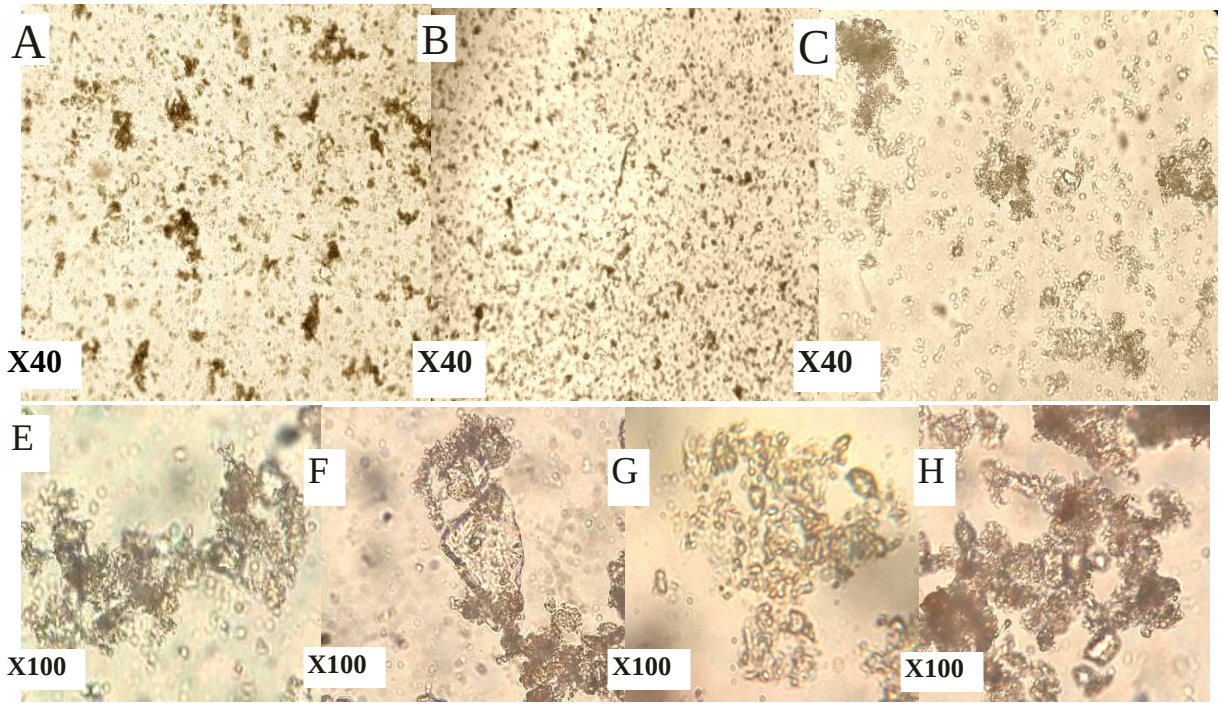


Figure 7: Examining the effects of natural and chemical coagulants on flocs , initial turbidity: 700 NTU(A:Thymus vulgaris (Tv), B:Alum, C,E,F,G,H:Thymus vulgaris (Tv) + Alum)

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The effects of various solvents on coagulant extraction

According to Figure 8 and 9, the results showed that among the solvents used in this research, KCl solvent with 1M concentration causes the highest removal efficiency.

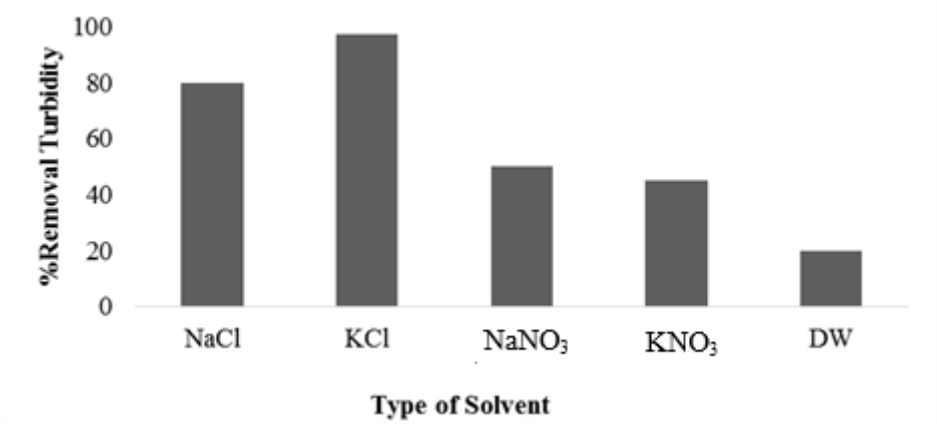


Figure 8: Influence of type of salt utilized for coagulant extraction on turbidity elimination (turbidity: 700 NTU, pH 7, salt concentration: 1 M, Tv dose: 400 mg/L).

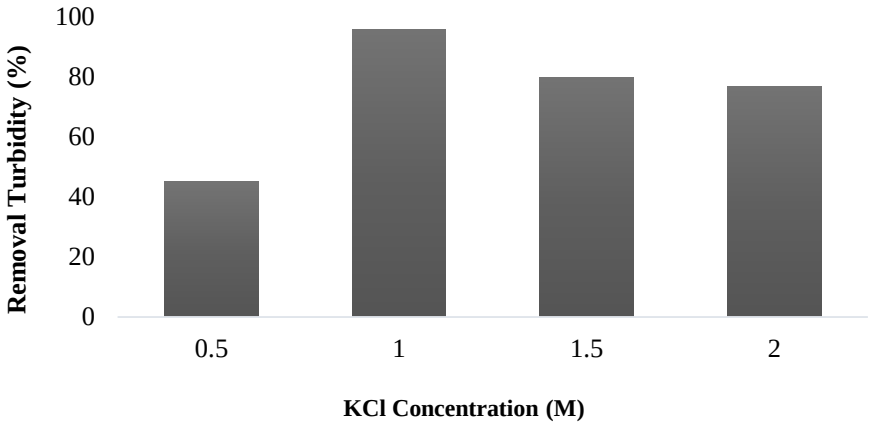


Figure 9: Influence of KCl concentration on turbidity elimination (turbidity: 700NTU, pH 7, Tv dose: 400 mg/l).

Discussion

In this report, different natural coagulants with doses of 50, 100, 150, 200, 250, 300, 350, and 400 mg/liter were used. The amount of synthetic turbidity of water in all experiments was 700 NTU. As can be seen in Figure 2, the highest amount of turbidity removal using Tv, Tk, and Te (400 mg/liter) was achieved by 91, 84, and 76% respectively. Therefore, among the used coagulants, Tv had the highest turbidity removal efficacy. The high turbidity removal efficiency can be attributed to the high amount of amine in Tv plant extract, which was proved using ninhydrin and FTIR tests.

Comparing the results of turbidity removal efficiency in this study with the efficiency obtained in studies conducted by Pricilla et al. on Chrysopogon zizanioides plant seed extract[20] and Saravanan et al. on Azadirachta indica plant extract showed that Tv had more efficiency to remove turbidity from aqueous solutions[21]. Such high turbidity removal efficiency had also been reported by the studies performed by Alenazi et al. on the coagulant of Strychnos potatorum [22]and Gaikward and Munavalli on the coagulant of Moringa oleifera and Strychnos potatorum[23]. Furthermore, Pritchard et al. foundthat the addition of Moringa oleifera, Jatropha curcas, and Guar gum plant extracts can

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significantly improve water quality[24]. Yarahmadi et al. also showed that Descurainia Sophia seed extract had an acceptable potential to improvecoagulation and flocculation process in surface water treatment process[25]. By conducting a review, Shukla disclosed that plant species can advance the goals of using water treatment technologies and concluded that the use of plant extract could be an economical and environmentally friendly approach[26].

The results of this study showed that the maximum efficiency of turbidity removal by alum chemical coagulant (a dose of 200 mg/l) was 90%, which did not change with rising the dose of alum up to 400 mg/liter (Figure. 2). This was while the study by E. M. Elsayed et al. obtained the highest turbidity removal efficiency of 85% by alum[27].

In this study, the use of the Ninhydrin test was considered for determining the amount of amine in plant extracts. This test was performed using a spectrophotometer, and An evaluation was made on the impact of amine groups in the extract using a ninhydrin dye reagent. In this study, the amount of absorption for Tv, Tk, and Te extracts was obtained as 0.82, 0.74, and 0.6 respectively. By utilizing the equation derived from the standard curve(Figure. 3), all the amines in the examined extracts were identified. The results showed that the concentration of amine in Tv, Tk, and Te extracts was 0.58, 0.53, and 0.45 mmol/liter, respectively, and it was determined that the highest amount of amine groups were present in the Tv plant extract. Therefore, the efficiency of the Tv plant extract was proved by the ninhydrin test. Dalvand et al. also utilized ninhydrin test to verify the number of amine groups and the number of proteins in Moringa plant extract [19]. Surleva and Drochioiu also used ninhydrin test to investigate the cyanogen content in apple and flax seeds[28], and Forlani and Funck used this test to quickly and easily measure the concentration of L-proline in Arabidopsis thaliana and Oryza sativa extracts[29].

The results of FTIR analysis related to Tv extract are shown in Figure 4. In this Figure, the peak at 3478.82 cm^{-1} was attributed to the amine stretching group; the peak at 1638.97 cm^{-1} was related to the amine bending group, and the peak at 609.43 $cm^{-1}$was ascribed to Amide III, and the amines were confirmed as the most effective substance in removing turbidity from aqueous solutions. Ridwan Fahmi revealed that there was a significant correlation between protein content in plant extract as a natural coagulant and the efficiency of removing turbidity from water, and finally, the results of the study conducted by this researcher showed that the protein in Okra plant could potentially be an active agent in the coagulation process[30].

The results of Kristianto with emphasis on FTIR analysis showed that the presence of active agents such as proteins in plant extracts could be an important reason for using such substances as natural coagulants[31]. However, studies by Okuda et al. and Madrona et al. indicated that other factors could also play a role in coagulation. They also showed that lectin in M. oleifera extract was an active factor in natural coagulation process[32, 33].

Figure 5 shows the simultaneous application of natural coagulant of Tv and alum in different ratios. As Figure 5, 5-1 shows, the highest turbidity removal efficiency of 96% was obtained using a dose of 400 mg/l and a ratio of Tv/alum equal to 1:1, while in the proportion of 2:1 and 1:2, the turbidity elimination efficiency was 91% and 92%, respectively. The high turbidity removal efficiency can be attributed to the high amount of amine in the Tv plant extract, which was provedusing ninhydrin and FTIR tests. Moreover, the effectiveness of 1:1 ratio in Tv/alum hybrid could be related to the optimal concentration of amine in coagulation process. When plant extract and alum are used at the same time, the concentration of needed coagulant decreases. At water treatment plants, decreasing the amount of coagulant use can have an important effect on decreasing the amount of sludge produced in the sedimentation units of the water treatment plant and greatly decline the costs of sludge disposal. On the other hand, reducing the amount of alum for water treatment reduces the risks caused by chemical coagulants in terms of diseases such as Alzheimer's and problems such as sludge production; therefore, a ratio of 1:1 brought the

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most useful result in laboratory and theoretical aspects[18]. It was found that coagulation and flocculation of particles are often based on the mechanism of bridging between particles and neutralization. In this regard, Dalvand et al. used combined coagulants to prevent excessive sludge production by alum[19]. One of the reasons for the concurrent use of natural coagulant with alum (Tv/alum) was the production of less sludge and, as a result, the reduction of water treatment costs. In this study, the TOC (Total Organic Carbon) level of the treated water was 1 mg/l.

Wang et al. showed that the simultaneous use of polyacrylamide chemical coagulants and iron aluminum chloride polymer could remove water turbidity up to 98% [34]. Moreover, Mir et al. expressed that the combined extracts of moringa leaves and seeds had better efficiency than single leaf or seed extracts for water treatment[35]. The influence of the initial pH of the water on turbidity elimination process by simultaneous application of alum, Tv, and Tv/Alum coagulants is shown in Figure 6. The coagulation efficiency by Tv/alum hybrid with a proportion of 1:1 at pH=7 was obtained as 96%, and by enhancing the pH to 12, the efficacy did not change significantly. Nevertheless, when alum was used alone, the maximum removal efficiency was obtained at neutral pH, so by increasing the pH from 4 to 7, the efficiency increased with a very gentle slope; however, efficiency decreased again with its increase up to 12. Mousavi et al. confirmed that alum and Ci/alum could reduce pH because of the hydrolysis of alum and the creation of Al^{3+} and SO_4^{2-} ions in water, where Al^{3+}reacted with OH^- or alkalinity; this caused the consumption of alkalinity agents and creation of the acidic agents in water[18]. Examining the variation in pH values after the coagulation and flocculation displayed that when plant extract was used alone, the resulted pH value of treated water did not change much and was almost constant. Therefore, the use of such herbal coagulants in waters with low alkalinity did not cause any problems, while the use of alum or Tv/alum decreased the pH of the water, which in many water treatment plants, led to a need for adding alkaline materials such as $CaCO_3$, $Ca(OH)_2$, and NaOH to the water treatment process to prevent the decrease in pH. These additives would eventually lead to an increase in sludge produced in sedimentation units.pH control is very important in coagulation, so if the pH range is not suitable, the formed clots will be small, light, and fragile[35, 36].

Yarahmadi et al. reported that the final pH of water was almost constant when Descurainia Sophia plant extract was employed as a coagulant, but with the use of ferric chloride as a chemical coagulant, the final pH of water decreased[25]. On the other hand, the study conducted by Mir Waqas Alam et al. indicated that the simultaneous use of moringa leaf and seed extracts had a greater effect in reducing the final pH of water compared to the single use of their extracts[37].

After the process of coagulation and flocculation and the formation of flocs on the bottom of Jar Test containers, a light microscope was used to observe the flocs as shown in Figure.7. As seen in Figure. 7, larger flocs were formed when Tv/alum hybrid coagulant was used (Figure. 7, C, E, F, G, H). In addition, the comparison of the size of the flocs showed that the flocs obtained from Tv extract were larger than the flocs obtained from alum coagulant and were settled at a greater distance from each other, but such flocs were relatively smaller than the flocs obtained from Tv/alum hybrid (Figure 7, A). In addition, the flocs obtained from Alum were finer than other flocs, had more aggregate characteristics, and were deposited at a short distance from each other (Figure 7, B). The comparison of the flocs of this study as well as the results in the previous article confirmed the influence of the concurrent application of plant extract and alum on the quality of floc formation[18].For the formation of flocs, usually the higher the water turbidity was, the easier was the coagulation of particles and colloids. But, in water with low turbidity, coagulation could not be done easily.

In this study, distilled water and salt solvents such as KCl, NaCl, $NaNO_3$, and KNO_3 with different concentrations were used to obtain plant

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extracts. The results showed that the efficiency of the extract obtained by KCl solvent with a concentration of 1 M had the highest removal efficiency (Figure. 8, 9). In similar studies, different salts had been used to extract plant extracts, and it had been proved that different salt solutions had different efficiencies for extracting different types of plant extracts. So, Bouchareb et al. and Nkurunziza et al. used NaCl salt, and Ridwan Fahmi et al. also employed distilled water and NaCl as a solvent[30,38-40] The previous study also disclosed that 1 M NaCl solution had the highest efficiency for extracting Cichorium intybus extract[18]

Conclusion

Water turbidity is considered as one of the influencing factors on health and aesthetic aspects of water, and solving this turbidity is one of the problems facing societies. This research was done to find useful and effective solutions for water purification, so the results of this researchshowed that theconcurrent use of natural and chemical coagulants is more effective in removing turbidity from aqueous solutions compared to the single use of chemical or natural coagulants. Among natural coagulants, Tv extract showed better results than other coagulants. One of the most important reasons for the high efficiency of Tv compared to other natural coagulants is the high amount of amines in the plant extract, which was proven by FTIR and ninhydrin tests. In investigating the influence of pH, it was found that pH changes do not have much effect on the efficiency of natural coagulants, and this is one of the advantages of such coagulants. Also, the results related to obtaining extracts from plants showed that KCl salt with a concentration of 1M has a better effectivenessfor extracting amines from the extract.

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Conflict of interest

The authors declared no conflict of interests.

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Authors' contribution

H.K. devised the project, and the main conceptual ideas and proof outline. H.K. and S.M.M. carried out the experiment. S.D.A and A.A and L.M. processed the experimental data, performed the analysis, and designed the figures. All authors discussed the results and contributed to the final manuscript.

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