

Evaluation of the Phytochemical Properties of Eucalyptus Citriodora Exudates and its Potency as Coagulant in Water Purification

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Abstract
This research work was carried out with the aim to investigate the phytochemical properties and the effectiveness of Eucalyptus citriodora exudates as coagulant for water purification and to compare the effectiveness of this plant extract as coagulant to alum (standard conventional coagulant) and this was done using Completely Randomized Design with loading doses from 0.02g - 0.10g of the extract and alum as standard coagulant. The phytochemical screening was carried out on the plant extract of Eucalyptus citriodora exudates according to the methods as described by Sofowora for testing phytochemical compounds. The following parameters namely; pH, TDS, turbidity, and electrical conductivity of the pond water used were determined before and after coagulation in this study. From the results obtained, the phytochemical properties analysed in this research work indicated that carbohydrate, glycoside, saponins, tannins, flavonoids, resins, proteins and terpenoids were found to be present in high concentration in this plant extract. Alkaloids and steroids were observed to be present in medium concentration, oil was seen in low concentration while reducing sugar and acidic compound were absent in this plant extract. The values obtained in this study indicated that Eucalyptus citriodora exudates showed 86.39% reduction in TDS at optimal dosage of 0.10g but showed increase in turbidity as dosage increases. The pH values (6.6-7.4), electrical conductivity (567-621 µS/cm) and TDS (100-455 mg/L) obtained in this study for the water purification were in the recommended range of permissible limits by WHO and USEPA except for turbidity values (45-85 NTU) which were above the set standard for drinking water quality.
Keywords: Phytochemical properties, Eucalyptus citriodora exudates, Alum, Coagulation and Water purification
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1.0 Introduction

Water purification process using aluminium sulphate (alum) and calcium hypochlorite which is a general method has been a major challenge on the nation's economy, since they are imported products thereby making treated water very expensive in most developing countries and the poor ones cannot afford it. Since water is an essential component that is needed by all the living organisms for all purposes in life and it depends on physical activity, age, health issues, and environmental conditions. It is also the key parameter that is influencing survival and growth of micro-organisms in foods and other microbial environments in the water which turns to affects the water quality which results in water borne diseases (Ana & Lidija, 2018; Amodio *et al*. 2014; Edema *et al*. 2011). Water borne diseases are one of the main problems in most developing countries which are caused by rapid industrialization and urbanization that have drastically affected water quality as industries spew out large volume of pollutants into the water bodies. This has led to reduced volume of potable water available for use and about 1.6 million people are compelled to use contaminated water and more than a million people die from diarrhea yearly (Crapper *et al*. 1973). Due to these constraints, this plant called *Eucalyptus citriodora* was evaluated for water treatment. *Eucalyptus citriodora* is an evergreen tree in the family myrtaceae with lemon scented leaves which medicinal values which works as powerful antiseptic, releasing cough and cold are known to be associated with this plant from a long time and within the family, the Eucalyptus genus has been planted and exploited on a large scale for many decades. *Eucalyptus citriodora* is a natural plant of Australia and there are varieties of species but only *E. microtecha* and *E. citriodora* have been found to form exudate in the tropics (Ukoha *et al*. 2005). Preliminary analysis of the exudates of *E. citriodora* gave tannin contents of 33.3% and 22.7% non-tans. Further analysis has revealed that extract from water has about 60% of tannin and extract from ethanol about 70% of tannin was obtained. These showed that, this plant matter has high quantity of tannins. This makes the exudate a viable source of tannin for the leather industry. *E. microtecha* is readily soluble in water at room temperature whereas *E. citriodora* exudates is only slightly soluble in water at 25 °C but dissolves readily in warm water at 40 °C and polar organic solvents (Ukoha *et al*. 2005). Earlier findings have shown that *Eucalyptus citriodora*, *Eucalyptus teretcorni* and *Eucalyptus globulus* have antimicrobial, analgesic and anti-inflammatory properties and have been reported by researchers from different parts of the globe and are non-toxic, hence; their use as flocculants is recommended especially in developing countries (Ali *et al*. 2012; Gideon & Clinton, 2010). The use of this plant coagulant has added advantages over the chemical ones because it is biological and some of these plants have been reported as edible plants (Chemistry world, 2013). Other plants which have been shown to be very effective as primary coagulants and can be compared to alum (conventional chemical coagulant) are powder from cactus plant, Hyacinth bean and *prosopis julifora* (Ghebremichael, 2004). Among all the plants that have been tested over the years, powder processed from the seeds of *Moringa Oleifera* plant has been shown to be the most effective (Gideon & Clinton, 2010). Qualitative phytochemical screening tests are used to find out the phyto-constituents present in individual fraction and it is important and necessary in this study. Many researchers have carried out several works on phytochemical characteristics of plant extracts by qualitative phytochemical tests. It is imperative that, this study be carried out in order to find out an eco-friendly water purification process using plant extract from *Eucalyptus citriodora exudates* which is available in most of the urban and rural areas in Africa for the benefits of human beings.

Plate 1: Pictures showing of *Eucalyptus citriodora* plants and its exudates

2.0 Materials and Methods

Appropriate quality assurance procedures and precautions were carried out to ensure the reliability of the results. To eliminate possible contamination from detergents or other sources, all glass wares and polyethylene material used were soaked with a 1M nitric acid for 48 h and then rinsed several times with distilled water before use.

Distilled water used in this study was produced from the Department of Pure Chemistry, Akwa Ibom State University, Ikot Akpaden, Akwa Ibom State, Nigeria and the physical chemistry laboratory of the Department of Pure and Industrial Chemistry, University of Nigeria, Nsukka which was used for all washings and dilutions in this research work.

2.1 Apparatus

Beakers, conical flasks and test tubes (pyrex), Water bath (German Ramp), Spatula, Glass funnels (Pyrex), Cotton Wool, Electronic balance (B. Bran Scientific and instrument Company England), Hot air oven (Gallen Kamp) and Filter paper (Whatman filter paper No.4), A pH meter (Crimson pH meter Basic C $_{20}$), Mortar and pestle, A digital turbidimeter (Hach Model no.: 2100 P), Magnetic Stirrer (B. Bran Scientific and instrument Company England), 0.8 mm mesh size sieve.

2.2 Collection of the Sample

The exudates of *Eucalyptus citriodora* plant extract used in this research work were collected from a plantation farm located in Layin in Zorno, Zaria local government area, Kaduna State, Nigeria and was later transported to the South Eastern, Nigeria. The plant extracts was identified by a staff of the Department of Plant Science and Biotechnology of the University of Nigeria, Nsukka and deposited at the herbarium to be used for the investigation/analysis.

2.3 Sample Preparation

The samples were air-dried, grounded with a laboratory mortar and sieved with 0.8mm mesh size sieve to obtain a fine powder extracts and then stored for the practical analysis. The fine powdered extracts which was obtained and stored was used as the natural coagulant in this research work. Five (5) litres of water sample was fetched from Enyirije pond in Amuda Village, Ezza-North local government area, Ebonyi State, Nigeria. The water sample was later transferred into conical flasks. A Completely Random Design was used for this experiment. Five different concentrations of the coagulants for the loading dose were prepared by weighing 0.02g, 0.04g, 0.06g, 0.08g and 0.10g of alum and *Eucalyptus citriodora exudates* respectively. The plant exudates (fine powdered extracts) used as a natural coagulant is insoluble in cold water, their stock solutions was not prepared. The fine powdered extracts (natural coagulant) were added directly to the raw water for coagulation test as described below. The treatments given were the varying concentrations of the plant extracts from *Eucalyptus citriodora exudates* and that of the standard coagulant (alum) which was used. Each treatment effect on the response to turbidity, pH, electrical conductivity (EC) and total dissolved solids (TDS) was repeated for four (4) times in this research work.

2.4 Coagulation Experiment

A given volume of wastewater was collected and the turbidity, pH, conductivity, and total dissolved solids (TDS) were measured. Raw water sample (200 ml) was measured into 250 ml beaker and *Eucalyptus citriodora exudates* plant powder (0.02 g) was added to the mixture and was stirred with magnetic stirrer vigorously for two (2) mins. and then slowly for ten (10) mins. The mixture was then poured into a glass cylinder and allowed to settle for thirty (30) mins. The procedure was repeated for the 0.04 g, 0.06 g, 0.08 g and 0.10 g respectively in this research work.

The same procedure as described above for the plant extracts was also applied for the five (5) different concentrations of alum. After the settling period of thirty (30) mins., the supernatants of the three (3) coagulants at different concentrations were collected and their turbidity, pH, electrical conductivity (EC) and total dissolved solids (TDS) levels were measured and recorded respectively in this study.

2.5 Phytochemical Screening of *Eucalyptus citriodora* exudates Extract

The phytochemical properties screening was carried out on the plant extract of *Eucalyptus citriodora* exudates according to the methods as described by Sofowora; Trease and Evans for testing phytochemical compounds (Sofowora, 1993; Trease and Evans, 2002).

2.5.1 Test for Carbohydrate

0.1g of powder of the sample was boiled with 2 ml of distilled water and filtered respectively. To the filterates, few drops of Molisch’s reagent were added. Concentrated sulphuric acid was then poured gently down the side of the test tube to form a lower layer and observed for purple colour.

2.5.2 Test for Reducing Sugar

0.1g of powder of the sample was shaken vigorously with distilled water (5 ml) and filtered. The filterates was added equal volumes of Fehling’s solution 1 and 2 then boiled on water baths for few minutes and was observed for brick red precipitate.

2.5.3 Test for Alkaloids
0.5g of the sample was boiled with 15 ml of 5% H_2SO_4 in ethanol. The mixtures was cooled and filtered. 5 ml of the filterates was added to 2 drops of Dragendoff's reagent and was observed for brick precipitate.

2.5.4 Test for Glycosides
Dilute Sulphuric acid (5 ml) was added to about 0.1g of the powder sample in a test tube and boiled for 15 minutes in a water bath then cooled and neutralized with 20% potassium hydroxide solution. 10 ml of a mixture was observed for a more dense brick red precipitate in the sample.

2.5.5 Test for Saponins
0.5g of the sample was measured and extracted with 5 ml of distilled water by boiling in a water bath for 2 minutes and filtered. The solution was shaken vigorously and observed for any persistent froth in the sample.

2.5.6 Test for Tannins
0.2g of the sample was boiled in 10 ml of distilled water in a test tube and then filtered. A few drops of 0.1% Ferric Chloride was added to 3 ml of the filterate of the sample and was observed for a greenish black precipitate.

2.5.7 Test for Flavonoids
A few pieces of magnesium turning were added to 5 ml of the sample followed by 5 ml of concentrated hydrochloric acid. A pink or magneta-red colouration was observed.

2.5.8 Test for Resins
0.2g of the sample was extracted with 15 ml of 96% of ethanol. The alcoholic extract was then poured into 20 ml of distilled water in a beaker observed for precipitate formation.

2.5.9 Test for Proteins
To a little portion of the filterate in a test tube; few drops of millions reagent was added and observed for white precipitate.

2.5.10 Test for Oils
0.1g of powdered sample was passed between filter paper and the paper observed for translucency.

2.5.11 Test for Steroids and Terpenoids
9 ml of ethanol was added to 1g of the sample and return for a few minutes and filtered. The filterate was concentrated to 2.5 ml on boiling water bath and 5 ml of hot water was added. The mixture was allowed to stand for 1h and the waxy matter filtered off. The filterate was extracted with 2.5 ml of chloroform. 0.5 ml of the chloroform extract in the test tubes and I ml of concentrated sulphuric acid was carefully added then there was an observation for a reddish brown interface.

2.5.12 Test for Terpenoids
0.5 ml of the chloroform extracts from steroid test was evaporated to dryness on a water bath and heated with 3 ml of concentrated acid for 10 minutes on a water bath and a grey colour observed.

2.5.13 Test for Acidic Compounds
0.1g of the sample was placed in a clean and clear dry test tubes and sufficient water was added. These was warmed on a hot water bath and then cooled. A piece of moist litmus paper was dipped into the filterate and observed for any colour change.

3.0 Results and Discussion
Results obtained from the plant extraction and phytochemical screening analysis is shown in Tables 1-5 below. The obtained results of the phytochemical characteristics analysed in this study indicated that carbohydrate, glycoside, saponins, tannins, flavonoids, resins, proteins and terpenoids were found to be present in high concentrations in this plant extract. Alkaloids and steroids were observed to be present in medium concentrations, oil was seen in low concentration while reducing sugar and acidic compound were absent in this plant extract are shown in Table 1 below. Several research works have been carried out by various researchers on the phytochemical property of this plant extracts using qualitative method (Okore *et al*. 2007; Ayandele & Abebiyi, 2007) and the obtained results for the analysis of the phytochemical properties of *Eucalyptus citriodora* exudates plant extracts were in supports of their earlier findings.

Table 1: Result of Phytochemical Analysis

Sample	**A**
Carbohydrate	+++
Reducing sugar	-
Alkaloids	++
Glycoside	+++
Saponins	+++
Tannins	+++
Flavonoids	+++
Resins	+++
Proteins	+++
Oil	+
Steroids	++
Terpenoids	+++
Acidic compound	-

Key: - Absent
\+ Low in concentration
++ Medium in concentration
+++ High concentration
A = *Eucalyptus citriodora exudates* extract

Table 2: Reduction in turbidity in pond water

	Turbidity values (NTU)	
Concentration (g/200ml)	**Alum**	***Eucalyptus citriodora* exudates**
0.02	40	55
0.04	20	50
0.06	10	45
0.08	7	75
0.10	5	85

Initial turbidity = 90NTU

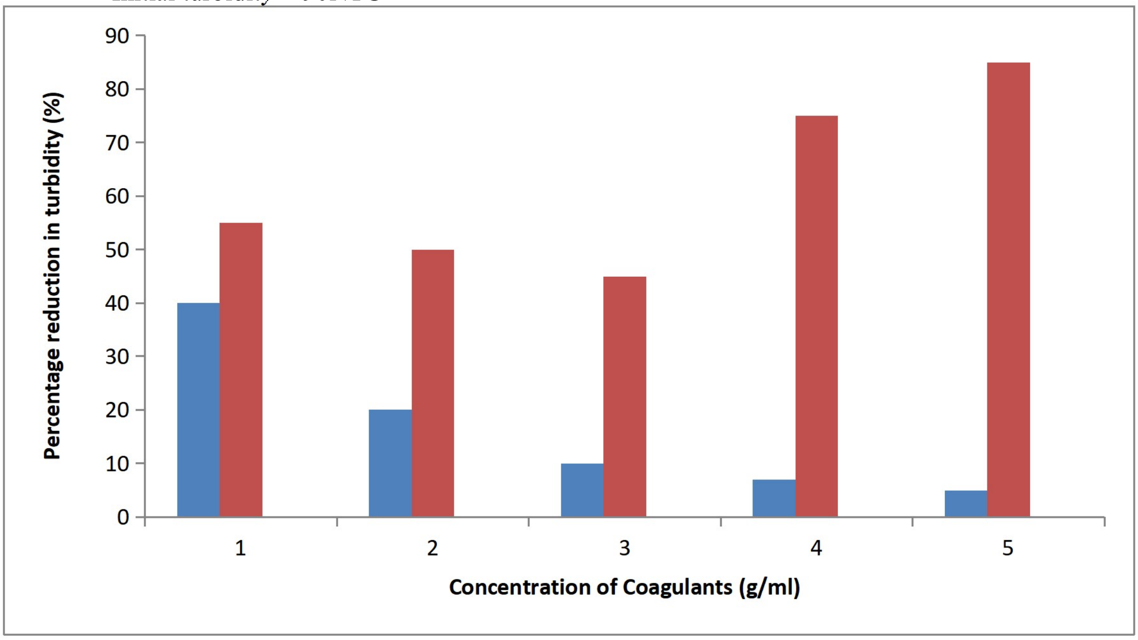

Figure1: Percentage reduction of turbidity at different concentration of coagulants

Table 3: pH values of treated water at various concentrations of coagulants

Concentration (g/200ml)	Alum	*Eucalyptus citriodora* exudates
0.02	7.00	6.60
0.04	6.80	6.80
0.06	6.60	7.00
0.08	6.40	7.20
0.10	6.20	7.40

Initial pH of raw water = 7.2

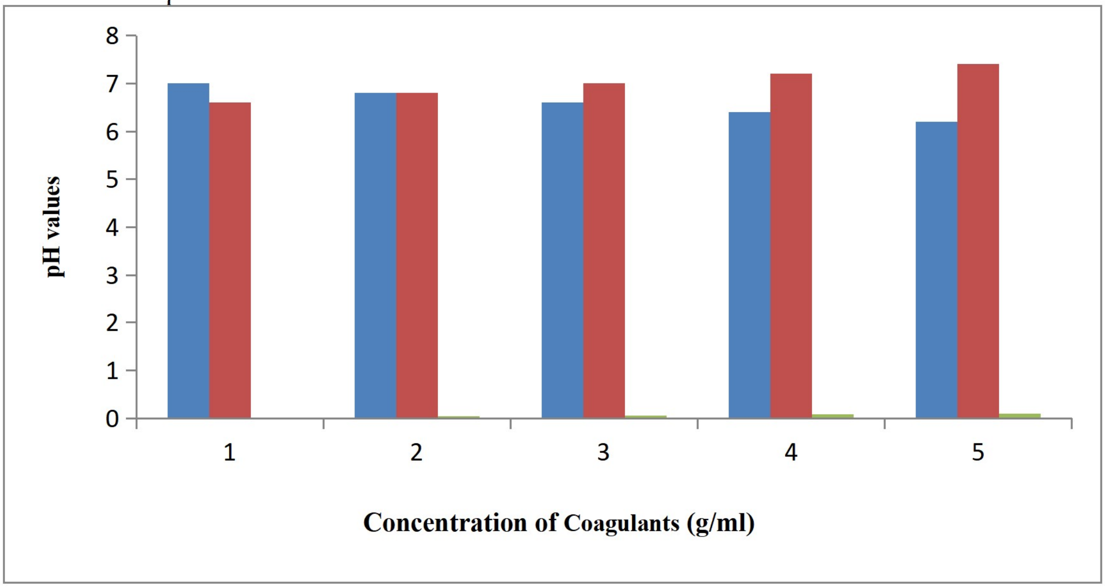

Figure 2: Change in pH value of water at different concentrations of coagulants

Table 4: Conductivity values of treated water at various concentration of coagulants

Concentration (g/200ml)	Alum	*Eucalyptus citriodora* exudates
0.02	690	621
0.04	520	600
0.06	362	587
0.08	175	575
0.10	140	567

Initial conductivity of raw water = 1113 µS/cm

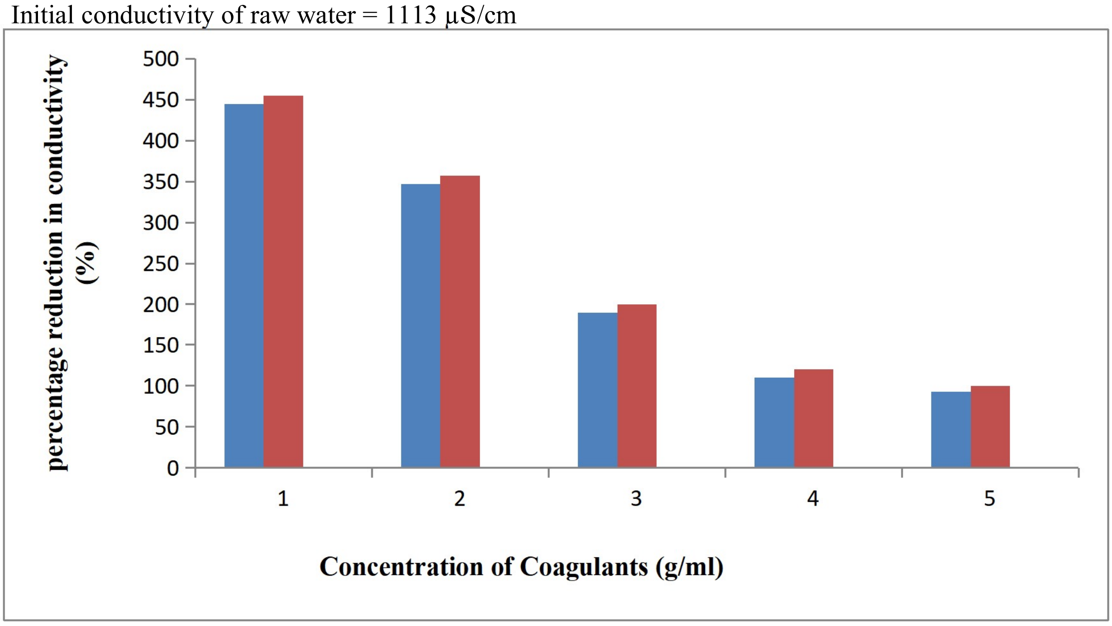

Figure 3: Percentage reduction of conductivity at different concentrations of coagulants

Table 5: TDS of treated water at various concentrations of coagulants

Concentration (g/200ml)	Alum	*Eucalyptus citriodora* exudates
0.02	445	455
0.04	347	357
0.06	190	200
0.08	110	120
0.10	93	100

TDS of raw water = 734.5 mg/L

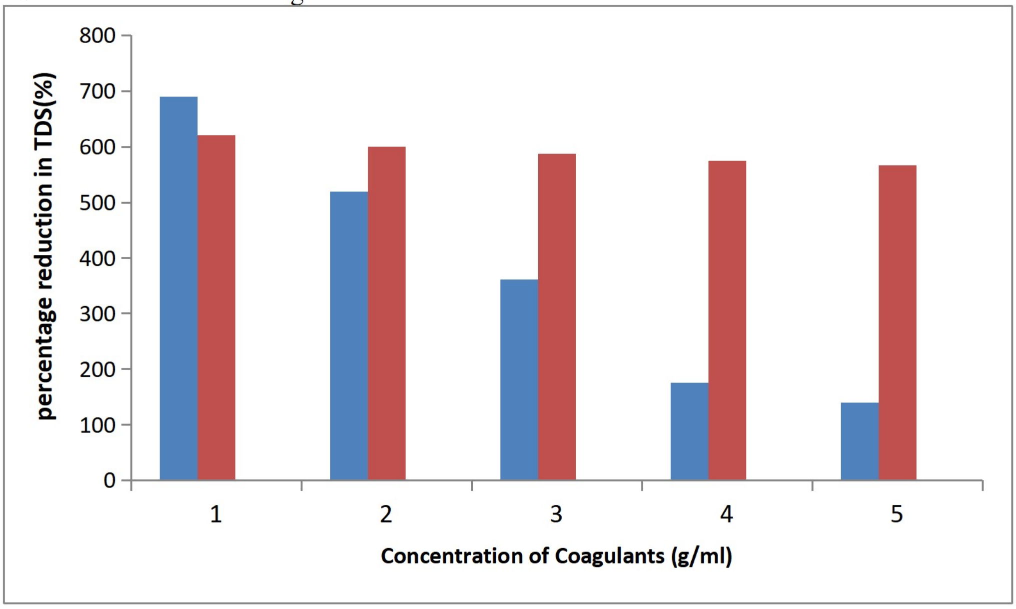

Figure 4: Percentage reduction in TDS at different concentrations of coagulants

Eucalyptus citriodora exudates showed significant reduction in turbidity at 0.02-0.06 g dosage with coagulation efficiency of 50 % at 0.06 g but show increase in turbidity value as dosage increases from 0.08-0.10 g dosages. It showed an irregular result. This could be as a result of light-purple colour water observed as the dosage increases which can block rays of light showing increase in turbidity. This light-purple coloured solution obtained could be as a result of large amount of tannins present in *Eucalyptus citriodora exudates* while alum showed a significant reduction in turbidity with 92.22 % and 94.44% efficiency at optimal dosage of 0.08 g and 0.10 g respectively as found in Table 2 and Fig.1 above. The high concentration of tannins found in this plant were in accord with the earlier research work carried out by Ukoha *et al.* (2015) which reported high concentrations of tannins in *Eucalyptus citriodora exudates* in their study.

Alum decreases the pH more rapidly as observed in the study. *Eucalyptus citriodora exudates* showed increase in pH as the dosage increases. The decrease in pH as dosage is attributed to the fact that the alum in the treated procedure produced sulphuric acid which lowered the pH levels. The increase in acidity could be due to the trivalent cation aluminium ion which serves a Lewis acid. Thus it can accept lone pair of electrons. The reason for the increase in pH with *Eucalyptus citriodora exudates* is not quite known as coagulation could be brought by resins and proteins present in the plant extract as shown in Table 3 and Figure 2 above.

There was decrease in percentage reduction in TDS and conductivity at optimal dosages of 0.08g and 0.10 g for alum. However, there is a significant reduction in TDS with *Eucalyptus citriodora exudates* showing 86.39 % reduction in TDS as 0.10 g dosage. This result shows that *Eucalyptus citriodora exudates* is an effective coagulant in the water treatment since it shows high reduction in total dissolved solids (TDS). Therefore, the increase in turbidity could be as a result of the colour change which blocks light rays showing an increase value in turbidity as shown in Tables 4-5 and Figures 3-4 above

Conclusions

The results obtained showed that, the phytochemical properties analysed indicated that carbohydrate, glycoside, saponins, tannins, flavonoids, resins, proteins and terpenoids were found to be present in high concentration in this plant extract. Alkaloids and steroids were observed to be present in medium concentration, oil was seen in low concentration while reducing sugar and acidic compound were absent in this plant extract. The plant exudates from *Eucalyptus citriodora* contain some coagulating properties which were effective in the water purification process. At optimal dosage of 0.10g/200ml, *Eucalyptus citriodora exudates* showed coagulation efficiency of 50 % at optimal dosage of 0.06 g/200ml. The coagulants are insoluble in water unlike alum; they have antimicrobial properties and are edible, so there is no risk of Alzhemier's disease. The water obtained from *Eucalyptus citriodora exudates* showed 86.39% reduction in TDS at optimal dosage of 0.10g but showed increase in turbidity as dosage increases. The pH values (6.6-7.4) obtained in this study for the water purification was in the recommended range of permissible limits by WHO and USEPA for drinking water quality.

Recommendations

It is recommended that more research works should be carried out on this interesting area in order to find out, the reason why *Eucalyptus citriodora exudates* can reduce the total dissolved solid (TDS) but increases the turbidity in the water and a combination treatment of alum in different proportions should also be investigated to establish their effectiveness in treating raw water.

Conflict of Interest

Authors did not show any conflict of Interest

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