ADDIS ABABA UNIVERSITY

INSTITUTE OF BIOTECHNOLOGY

Evaluation of Coagulation Efficiency of Protein Extracts from *Lupinus albus* L., *Moringa stenopetala* (Baker f.) Cufod., *Trigonella foenum-graecum* L. and *Vicia faba* L. for Water Purification

By

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A Thesis Submitted to the Institute of Biotechnology, Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biotechnology

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Declaration

I, the undersigned, declare that this Thesis is my own work and has not previously been submitted to another university. The materials obtained from other sources have been duly acknowledged in the Thesis.

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This is to certify that the Thesis prepared by **Mr. Neway Adele** entitled **"Evaluation of Coagulation Efficiency of Protein Extracts from *Lupinus albus* L., *Moringa stenopetala* (Baker f.) Cufod., *Trigonella foenum-graecum* L. and *Vicia faba* L. for Water Purification**" submitted in partial fulfillment of the requirement for the Degree of Master of Science in Biotechnology complies the regulation of the university and meets the accepted standards with respect to originality and quality.

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List of abbreviations/acronyms

BSA	Bovine serum albumin
CAWST	Centre for Affordable Water and Sanitation Technology
CD	Charge density
EPA	Environmental Protection Agency
LAPE	*Lupinus albus* protein extract
MOH	Ministry of Health
MSPE	*Moringa stenopetala* protein extract
NTU	Nephelometric turbidity unit
PE	Polyelectrolyte
TFPE	*Trigonella foenum-graecum* protein extract
TLC	Technical Learning College
VFPE	*Vicia faba* protein extract

Abstract

Access to clean drinking water is a basic human right. However, an estimated 1.2 billion people across the world consume unclean water daily. An interest has been growing towards natural coagulants as the health and environmental concerns on conventional chemical coagulants are rising. Natural coagulants have the potential to serve as alternative water treatment agents. In this study, Lupinus albus, Moringa stenopetala, Trigonella foenum-graecum and Vicia faba protein extracts were evaluated as natural coagulants for water treatment. The protein extracts were purified from crude extracts using a protein purifier, and protein concentrations were determined by spectrophotometric method. Small volume coagulation efficiency tests were conducted on raw water taken from Legedadi water treatment plant. These were done using completely randomized design (CRD) experiment with settling times of 0 min (initial time), 90 min, 180 min and 270 min and, protein extract doses of 5 mg/L, 10 mg/L, 15 mg/L and 20 mg/L. Raw water as negative control and polyelectrolyte as positive control were also included. The optical density (OD) values were measured for all the samples. At 270 min and 20 mg/L, the coagulation efficiency percentages for Lupinus albus, Moringa stenopetala, Trigonella foenum-graecum and Vicia faba protein extracts were 71%, 89%, 12% and 67% in the water sample collected in April, 2019 respectively. Similarly, Lupinus albus, Moringa stenopetala and Vicia faba achieved 17%, 92% and 12% at 270 min settling times and 5 mg/L, 20 mg/L and 10 mg/L concentration in the water sample collected from August, 2019 respectively. Negative control (raw water) and polyelectrolyte (positive control) were also 6 − 10% and 89 − 94% at 270 min settling time in April and August, 2019 respectively. Among the four protein extracts, Moringa stenopetala showed the highest coagulation efficiency similar to polyelectrolyte. This study concluded that Moringa stenopetala protein extract can be used as natural coagulant for water purification in both sampling times.

Keywords/phrases: Coagulation efficiency, Extraction, Natural coagulant, Protein extract, Purification

1. INTRODUCTION

Water for human consumption must be palatable and safe (OpenWASH, 2016). However, humans have inadequate access to drinking water and use water sources contaminated with pathogens or polluted with suspended and dissolved solids in large parts of the world. Drinking such water or using it for food preparation leads to wide-spread waterborne diseases like cholera. Over 40 countries in the world suffer from a safe drinking water deficit, with an estimated 1.2 billion people drinking unclean water on a daily basis and five million people, mostly children, dying every year from water-related diseases. The United Nations estimates that by 2025, 2.7 billion people will not have access to safe drinking water (Zaman *et al.*, 2014). In Ethiopia, only 52% of the population has access to safe water; as a result, 60-80% of the population suffers from waterborne and water related diseases (Birhanu Hailu, 2017).

Water treatment is any unit process that changes or alters the chemical, physical, or bacteriological quality of water with the purpose of making it safe for human consumption or appealing to the customer. It is made up of various stages or unit processes combined to form one treatment system (Spellman, 2014). Coagulation is one of the processes of water treatment that involves addition of coagulants that promote the clumping of fine particles into larger floc for ease of separation (Jiang, 2015).

Coagulants are either chemical or natural substances. Chemical coagulants are usually salts of aluminum or iron, and they are dosed to the raw water under controlled conditions. Aluminum sulphate (alum) and other synthetic chemicals are commonly used in the treatment of drinking water. They are expensive for areas where the chemicals have to be imported, and may have negative effects on consumers' health. Moreover, sludge volume and disposal to the environment are of concern (Ghebremichael *et al.,* 2006). The presence of excessive amounts of aluminum sulphate has been associated with Alzheimer's disease (Crapper *et al.*, 1973).

A part of possible solution of these problems might be development of natural coagulants which are safe for human health as well as environment (Muthuraman *et al.*, 2013). Natural coagulants are extracted from natural sources such as microorganisms, animals and plants. At present, a number of effective coagulants have been identified of plant origin. *Moringa oleifera* is the best natural coagulant discovered so far that can replace aluminum sulphate (alum), which is used

widely for water treatment in many parts of the world (Ali *et al*., 2010). In addition, indigenous knowledge indicates that there are several plant species that can be used as a coagulant and disinfectant (Moa Megersa *et al.,* 2014).

Natural coagulants have been used in water treatment since ancient times. But lack of knowledge on the exact nature and mechanism by which they work has impeded their wide spread application and they have been unable to compete with the commonly used chemicals. In recent years, there has been a resurgence of interest to use natural materials due to cost and associated health and environmental concerns of synthetic organic polymers and inorganic chemicals (Ghebremichael, 2004).

Hence, this study was initiated to evaluate the coagulation performance of selected plant species: *Lupinus albus*, *Moringa stenopetala, Trigonella foenum-graecum* and *Vicia faba* as coagulants for drinking water treatment.

1.1. Research question

How efficient are *Lupinus albus, Moringa stenopetala, Trigonella foenum-graecum* and *Vicia faba* protein extracts for reducing the optical density of raw water?

1.2. Objectives of the study

1.2.1. General objective

To determine the concentration and evaluate the coagulation efficiency of *Lupinus albus, Moringa stenopetala, Trigonella foenum-graecum* and *Vicia faba* protein extracts.

1.2.2. Specific objectives

- To determine the concentration of *Lupinus albus, Moringa stenopetala, Trigonella foenum-graecum* and *Vicia faba* protein extracts.
- To evaluate the coagulation efficiency of *Lupinus albus, Moringa stenopetala, Trigonella foenum-graecum* and *Vicia faba* protein extracts at different settling times, doses and raw water sampling times.

1.3. Research hypothesis

Lupinus albus, Moringa stenopetala, Trigonella foenum-graecum and *Vicia faba* protein extracts reduce the optical density of raw water.

2. LITERATURE REVIEW

2.1. Water sources

We obtain the water we use from three basic sources. These are: ground water, surface water and rainwater. Ground water is water that fills the spaces between rocks and soil making an aquifer. The depth and quality of ground water varies from place to place. About half of the world's drinking water comes from the ground. Surface water is water that is taken directly from a stream, river, lake, pond, spring or similar source. The quality and quantity of surface water varies from one place to another and over time, due to factors such as geology, climate and surrounding land use. Surface water quality is generally unsafe to drink without treatment. Rainwater is water that replenishes both groundwater and surface water, and can also be collected directly (CAWST training manual, 2009 and OpenWASH, 2016).

2.2. Water pollution

Water pollution is any undesirable change in the state of water, contaminated with harmful substances (Dwarapureddi and Saritha, 2016). It occurs when surface water or groundwater is adversely affected by the addition of pollutants. For surface water, the quality of the water will be determined by the geology, by precipitation and by what happens in the catchment. Rainwater that lands in a catchment flows into the river. River water can be contaminated from pollution sources in the catchment even though they may be some distance away. For groundwater, the situation is similar but the boundaries are less distinct and pollutants can seep into aquifers that extend below more than one catchment. Water quality can be affected by pollution from point sources and non-point sources. Point sources are identifiable locations that discharge directly into a body of surface water. Non-point sources are those where pollution arises over a wide area and it is often difficult to locate the exact place of origin (OpenWASH, 2016). Surface water sources, polluted by man and nature, are likely to contain suspended and dissolved organic (plant or animal origin) and inorganic (mineral) material, and biological forms such as bacteria, spores, cysts and plankton (EPA, 2002).

2.3. Conventional surface water treatment

Water treatment is the process of removing all those substances, whether biological, chemical or physical, which are potentially harmful in water supply for human and domestic use. It produces water that is safe, palatable, clear, colorless and odorless (OpenWASH, 2016). The treatment of

water in order to make it suitable for drinking or industrial use include a combination of physical, chemical and biological methods (Gedewon Teka, 2009). Conventional drinking water treatment includes coagulation, flocculation, sedimentation, filtration and disinfection (Fig. 1). The stages of water treatment are:

1. Pretreatment: Pretreatment (preliminary treatment) is any physical, chemical or mechanical process used before main water treatment processes. Pretreatment of surface water is used to reduce and/or to stabilize variations in the microbial, natural organic matter and particulate load (MOH, 2017). It can include screening, pre-sedimentation and chemical addition (Spellman, 2014).

2. Coagulation: Coagulation is often the first unit process in main water treatment and it is very crucial for the removal of suspended and dissolved particles. It is the act of destabilizing stable colloidal particles in suspension. Destabilized particles are then flocculated for expedient removal in the sedimentation and/or filtration units (Ghebremichael, 2004). The coagulation stage occurs when a coagulant, such as alum, is added to the water to neutralize the charges on the colloidal particles in the raw water, thus bringing the particles closer together to allow a floc to begin to form (MOH, 2017). The efficiency of the coagulation process depends on raw water quality, the coagulant or coagulant aids used and operational factors, including mixing conditions, coagulation dose and pH (Amhagiyorgis Mesfin, 2007).

3. Flocculation: Flocculation is the process of gentle and continuous stirring of coagulated water for the purpose of forming flocs through the aggregation of the minute particles present in the water (Mohammed, 2009). Floc formation is controlled by the rate at which collisions occur between particles and by the effectiveness of these collisions in promoting attachment between particles. The aim of flocculation is to create a floc of a suitable size, density, and toughness for later removal in the sedimentation and filtration processes (EPA, 2002).

4. Sedimentation: Sedimentation is also called clarification. Clarification is the removal of suspended solids and floc from chemically treated water, before its application to filters (EPA, 2002).

5. Filtration: Water filtration is a physical process of separating suspended and colloidal particles from water by passing water through a granular material (Spellman, 2014).

6. Disinfection: Disinfection is used to control waterborne pathogenic organisms and prevent waterborne diseases. In water treatment, disinfection is almost always accomplished by adding chlorine or chlorine compounds after all other treatment steps (Spellman, 2014).

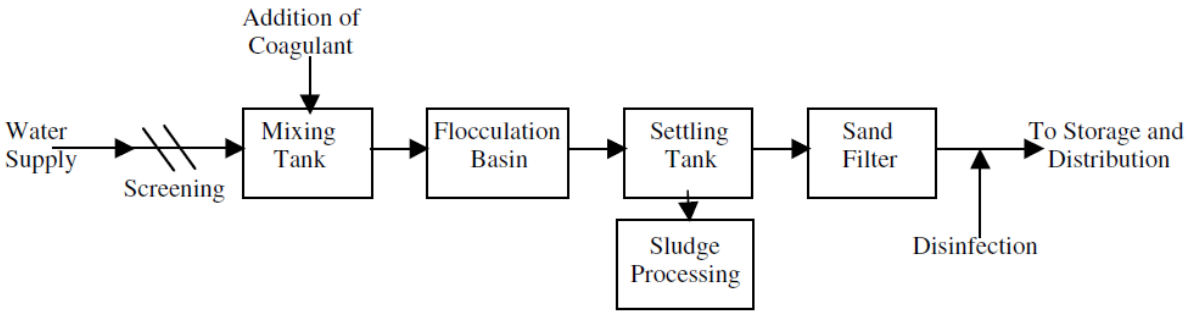

Figure 1. Conventional water treatment model (Spellman, 2001)

Coagulation, flocculation, clarification and filtration are interdependent stages of the solids separation phase of water treatment. Failure or inadequacy in any of the stages will have adverse effects on the subsequent stages and may result in the production of water with excessive turbidity and other undesirable qualities. Thus, chemical dosing which is not optimal means that the conditions for coagulation are not the optimum; the floc formed may be unsuitable for the method of clarification in use, is not removed efficiently and passes on to the filters where it may break through to appear as turbidity in the final water or seriously reduce the length of filter run (EPA, 2002).

Absolutely pure water is rarely, if ever, found in nature. The impurities occur in three progressively finer states - suspended, colloidal and dissolved matter (EPA, 2002). Water that has not yet been treated and is to be used for domestic supply is referred to as raw water, in contrast with treated water that has passed through some form of treatment (e.g. filtration, disinfection). Drinking water standards and guidelines mostly apply to treated waters (MOH, 2017).

2.4. Coagulants in water treatment

2.4.1. Overview

Coagulation and flocculation processes require addition of chemicals that are called coagulants (Fig. 2). Coagulants are substances which in solution furnish ionic charges opposite to those of the colloidal turbid particles present in water. Coagulants neutralize the repelling charges on the colloidal particles and produce a jelly-like spongy mass called a floc (Talnikar, 2017).

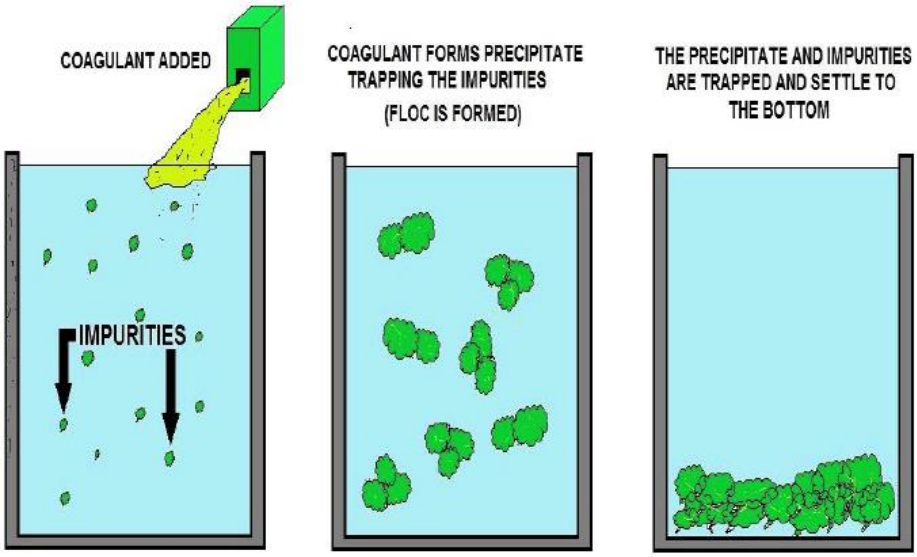

Figure 2. Basics of coagulation/flocculation process (TLC, 2018)

2.4.2. Types of coagulants

According to Sillanpaa *et al.* (2017), coagulants can be classified into metallic, polymeric or composite (hybrid) coagulants. Metallic coagulants: The most widely used metal coagulants in drinking water treatment have been aluminum (aluminum sulfate and aluminum chloride) and ferric salts (ferric sulfate and ferric chloride). Polymeric coagulants: In general, polymeric coagulants are macromolecular synthetic (inorganic and organic) or natural compounds made of repetitive chemical units (monomers) and are able to destabilize the constituents of an aqueous media, thus enhancing their flocculation. Composite (hybrid) coagulants: The main objective of developing composite coagulants is to benefit for the advantages of the previously mentioned coagulants, metallic and polymeric (synthetic or natural), while mutually overcoming their shortcomings. Many novel composite or hybrid coagulants have been developed via various combination schemes.

2.4.3. Polyelectrolytes and coagulation mechanisms

2.4.3.1. Polyelectrolytes

The term polyelectrolyte refers to a large variety of natural or synthetic, water soluble, macromolecular compounds which have the ability to destabilize or enhance flocculation of the constituents of a body of water. Polyelectrolytes are special classes of polymers containing certain functional groups along the polymer backbone which may be ionizable. If present, when the ionizable groups dissociate, the polymer molecules become charged either positively or negatively, depending on the specific functional groups present, and are thus referred to as cationic or anionic polyelectrolytes respectively. Polyelectrolytes that possess both positively and negatively charged sites are referred to as ampholytic, whereas those that possess no ionizable functional groups are termed nonionic polyelectrolytes (Bratby, 1980).

The most important characteristics of polymeric coagulants are MW and, in the case of polyelectrolytes (ionic polymer), charge density (CD). MW values range from a few thousand up to tens of millions. Conventionally, polymers are regarded as having, low, medium or high MW, corresponding to MW values in the ranges $<10^5$, 10^5–10^6 and $> 10^6$ respectively. CD can be expressed in terms of mole percent of charged groups or as milliequivalents per gram (meq/g). Broadly, polyelectrolyte CD values are regarded as low, medium or high if the mol% of ionic groups is approximately: 10%, 25% and 50–100% respectively (Bolto and Gregory, 2007).

2.4.3.2. Coagulation mechanisms

Polyelectrolytes are generally associated with two coagulation mechanisms: Bridging mechanism: Where polyelectrolyte segments are adsorbed on the surfaces of adjacent colloids thereby binding them together. Electrostatic patch mechanism: Whereby ionic polyelectrolytes, bearing a charge of opposite sign to the suspended material, are adsorbed and thereby reduce the potential energy of repulsion between adjacent colloids (Bratby, 1980).

2.4.4. Natural coagulants

Natural polymers are also called biocoagulants. Biomass derived coagulants have been produced in order to develop greener alternatives to the conventional coagulants. Thus, numerous coagulating/flocculating biochemicals (mainly polysaccharides and proteins) derived from various bioresources have been investigated as coagulants or coagulant aids, either from

terrestrial plants, marine species or microbial organisms. So far, the commercially available natural coagulants mainly include chitosan and tannin based coagulants (Sillanpaa *et al.*, 2017).

A number of effective natural coagulants have been identified from plant origin. Some of the common ones include *Moringa oleifera*, *Cactus latifaira*, *Prosopis juliflora*, beans and maize. One of the natural coagulants from animal origin is chitosan. It is a high molecular weight polyelectrolyte derived from deacetylated chitin. Chitin is cellulose like biopolymer widely distributed in nature, especially in insects, fungi, yeasts and shells of crabs and shellfish (Ghebremichael, 2004). Natural coagulants can be specified as anionic and cationic depending on its source. Moringa oleifera and chitosan are sources of cationic coagulants whereas tannin and grape seed are sources of anionic ones (Dwarapureddi and Saritha, 2016). The main advantages of those biocoagulants are their renewability, biodegradability, non-toxicity and relative cost effectiveness (Sillanpaa et al., 2017). Table 1 shows a comparison of natural coagulants over conventional chemical coagulants

Table 1. Advantages of natural coagulants over conventional chemical

No.	**Parameters**	**Natural coagulants**	**Conventional chemical coagulants**
1	Cost	Sustainable and economical	Complex and expensive
2	Toxicity	Non-toxic to environment	Highly toxic
3	Corrosiveness	Non-corrosive to the materials	Highly corrosive due to alkalinity
4	Sludge characteristics	Small amount of non-hazardous & biodegradable sludge	Large amount of hazardous & non biodegradable sludge
5	pH	Do not alter the pH of water under treatment.	More changes in pH due to metallic salts
6	Sensitiveness	Provides rapid flocculation and decantation	Provides slow flocculation and decantation
7	Fragrance	Acts as a deodorant agent forming insoluble complexes with organic species such as proteins and carbohydrates.	Offensive odor due to decomposed chemicals in the sludge

Source: Vijayaraghavan and Shanthakumar (2015)

2.4.4.1. Plant based coagulants

Plant based coagulants are popular because of their practicability and suitability for mass production. The coagulants may be derived from seeds, leaves, fruits, roots and barks. However, the most common plant part used is the seed of plants (Dwarapureddi and Saritha, 2016). Although many plant based coagulants have been reported, only four types are generally well-known within the scientific community, namely, nirmali seeds (*Strychnos potatorum*), *Moringa oleifera*, tannin and cactus (Yin, 2010).

2.4.4.1.1. *Lupinus albus, Moringa stenopetala, Trigonella foenum-graecum* and *Vicia faba* coagulants

a. *Lupinus albus*

Lupinus albus L. (white lupine) belongs to the family Fabaceae (Leguminosae) subfamily Papilionoideae. The white lupine in Ethiopia is locally known as "Gibto". It is produced by small holder farmers mainly in two regional states of Ethiopia; Amhara and Benishangul-Gumuz, the former being the largest producer. It is grown in elevations ranging between 1500 to 3000 m.a.s.l (Abdie Oumer *et al.*, 2015). White lupine production by small holder farmers in the area is targeted for its grain and soil fertility maintenance values. Its grain is used as snack and for the preparation of local alcoholic drink, Areke (Likawent Yeheyis *et al.*, 2010). To the knowledge of the author, no information was available about the coagulation potential of white lupine for water treatment.

b. *Moringa stenopetala*

Moringa stenopetala (Baker f.) Cufod., indigenous to southern Ethiopia and northern Kenya, is a member of the single family Moringaceae (Cherinet Abuye, 2003). It is a deciduous plant widely distributed at altitude range of about 1100 to 1600 meters in Ethiopia. The major growing regions are Arbaminch and surrounding areas, Negelle and Wollayeta-Sodo area. *M. stenopetala* is commonly called Shiferaw in Amharic, and Haleko in Wollaytegna and Gamugna (Yalemtsehay Mekonnen and Amare Gessesse, 1998).

The seeds of *M. stenopetala* have coagulating and antimicrobial properties. The active coagulating substances are found in the cotyledons of the seeds. *M. stenopetala* seeds have better water purifying properties than *M. oleifera* (Eyassu Seifu, 2014). Moringa extract showed appreciable turbidity removal at an optimum dose range of 20 to 80 mg/L in 6 hr of settling time.

About 99.9% of microbial load removal was observed which is comparable with chlorine disinfection (Moa Megersa *et al.*, 2016).

Moringa stenopetala is not widely used for water treatment; however, in some areas rural people know very well about the water purifying capacity of the powdered seed. As the oil is not useful for water treatment, there is no conflict with food security to use this coagulant for water treatment (Moa Megersa *et al.*, 2015).

c. *Trigonella foenum-graecum*

Trigonella foenum-graecum L. (fenugreek) plant is widely distributed throughout the world and belongs to the family Fabacecae (Leguminosae). Fenugreek seed contains 23% - 26% protein, 6% - 7% fat and 58% carbohydrates of which 25% is dietary fiber, saponins and rich in flavonoids (Lu *et al.* 2008 *cited in*: Ramamurthy *et al.*, 2012). Fenugreek leaves and seeds are consumed in different countries around the world for different purposes such as medicinal uses (anti-diabetic, lowering blood sugar and cholesterol level, anti-cancer, antimicrobial etc.), making food (stew with rice in Iran, flavor cheese in Switzerland, syrup and bitter run in Germany, mixed seed powder with flour for making flat bread in Egypt, curries, dyes, young seedlings eaten as a vegetable, etc.), roasted grain as coffee-substitute (in Africa), controlling insects in grain storages, perfume industries etc. (Kor *et al.*, 2013). Fenugreek is cultivated between altitudes of 1600 and 2300 m in Ethiopia. It grows in Amhara, Tigray, Oromia and S.N.N.P. regional states (Mebrahtu Hagos, 2011)

The ability of seed extracts of *Trigonella foenum-graecum* (*T. foenum-graecum*) to act as natural coagulants was tested using natural turbid water. Seed extracts were prepared using distilled water and NaCl (0.5 M and 1.0 M) solution. Only 1.0 M NaCl extract of *T. foenum-graecum* had coagulation capability and did not depend on pH values. The seed extract of *T. foenum-graecum* showed about 80% coagulation properties, where as the best known natural coagulants such as *Strychnos potatorum, Moringa oleifera* and chemical coagulant such as $Al_2(SO_4)_3$ showed around 90%, 65% and 95% respectively (Ramamurthy *et al.*, 2012).

d. *Vicia faba*

Vicia faba L. (faba bean) is one of the most important food legumes due to its high nutritive value both in terms of energy and protein contents (24-30 %) and is an excellent nitrogen fixer.

Ethiopia is the third largest producers of faba bean in the world, next to China and Egypt and its share is only 6.96% of world production and 40.5% of Africa. Faba bean is grown on 370,000 hectares in Ethiopia with an annual production of about 450,000 tones (Fekadu Alemu, 2012).
Faba bean is cultivated in Ethiopia in Weynadega zones (mid-altitudes 1800-2200 m.a.s.l, average annual rainfall of 740 mm, mean daily temperature of 18-22^{o}C) and Dega zones (high altitude, above 2200 m.a.s.l, average annual rainfall of 900 mm, mean daily temperature of 10-18 ^{o}C). Faba bean is tightly coupled with every Ethiopian life. It is mainly used as an alternative with peas to prepare flour called 'Shiro' which is used to make 'Shiro wot' (a cook almost ever-present in Ethiopian dishes) (Behailu Mulugeta, 2016).

Kukic *et al.* (2015) reported that faba bean coagulant was used as coagulant in water purification. The coagulant was obtained by extraction, with distilled water or NaCl solutions, from grinded seeds and was applied as coagulants, in various doses, in synthetic water having different initial turbidities and pH values. In addition, Sivaranjani and Rakshit (2016) also reported that faba bean powder was used for drinking and cooking water purification.

2.4.4.1.2. Processing steps

The general processing steps involved in production of plant based coagulants can be divided into three major stages: primary, secondary and tertiary (Fig. 3). The primary processing step is very straightforward and most research studies and domestic applications utilize only this processing step to simulate the traditional method of drying and subsequent pulverizing of plant parts into fine powder generally used by local communities in the absence of sophisticated processing equipment. In the secondary processing stage, extraction of the active agents can be performed via different solvents (organic, water or salt solution). Tertiary processing is rarely done in the case of plant based coagulants and is presently restricted to academic research on purification of *M. oleifera* extracts since it apparently increases the overall processing cost. Preliminary studies suggest that lyophilization, ion-exchange and dialysis are feasible purification methods for *M. oleifera* extracts which can be incorporated into a scaled-up setup for treatment of turbid water. Such methods have not been extensively applied to other plant-based coagulants and this presents opportunities for other research (Yin, 2010).

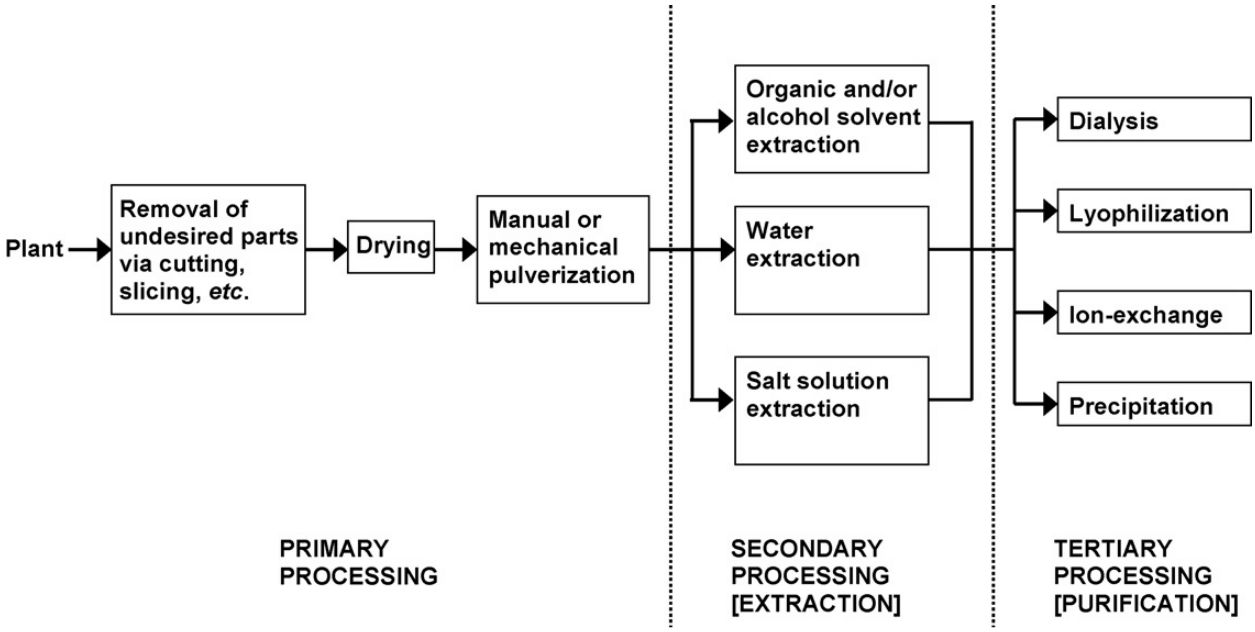

Figure 3. General processing steps in preparation of plant based coagulants (Yin, 2010)

2.4.4.1.3. Protein purification methods

Proteins vary from each other in size, shape, charge, hydrophobicity, solubility and biological activity. Purification processes make use of these properties to get good quality products using efficient procedures. Some of the commonly used purification methods include: precipitation, ion exchange chromatography, hydrophobic interaction, affinity chromatography, gel filtration, electrophoresis and ultrafiltration (Ghebremichael, 2004).

Chromatography is a separation process in which the sample mixture is distributed between two phases in the chromatographic bed (column or plane). One phase is stationary whilst the other passes through the chromatographic bed. The stationary phase is either a solid, porous, surface-active material in small-particle form or a thin film of liquid coated on a solid support or column wall. The mobile phase is a gas or liquid. If a gas is used, the process is known as gas chromatography; the mobile phase is always liquid in all types of liquid chromatography, including the thin-layer variety (Meyer, 2004). Biomolecules are purified using chromatography techniques that separate them according to differences in their specific properties, as shown in Table 2.

Table 2. Separation principles in chromatographic purification

No.	Property	Technique
1	Charge	Ion exchange chromatography (IEX)
2	Size	Size exclusion chromatography (SEC), also called gel filtration (GF)
3	Hydrophobicity	Hydrophobic interaction chromatography (HIC) Reversed phase chromatography (RPC)
4	Biorecognition (ligand specificity)	Affinity chromatography (AC)

Source: GE Healthcare (2004)

2.4.4.1.3.1. Protein purification by Ion exchange method

Ion exchange chromatography is the most commonly used chromatographic technique in protein purification. It separates molecules on the basis of differences in their net surface charge. Molecules vary considerably in their charge properties and will exhibit different degrees of interaction with charged chromatography media according to differences in their overall charge, charge density, and surface charge distribution. Proteins, which are built up of many different amino acids containing weak acidic and basic groups, net surface charge will change gradually as the pH of the environment changes, that is, proteins are amphoteric (GE Healthcare, 2004).

A stationary phase capable of ion exchange has electric charges on its surface. Ionic groups such as SO_3^{2-}, COO^-, NH_3^+ or NR_3^+ are incorporated in the resin. Charges are neutralized by mobile counter ions. The mobile phase contains ions and ionic sample molecules compete with these for a place on the surface of the stationary phase. A resin with SO_3^- groups is a strong cation exchanger and a COO^- resin is a weak cation exchanger. An anion exchanger contains NR_3^+ (strong) or NR_2H^+ or NH_3^+ (weak) groups. It forms a bond with negatively charged anions (Meyer, 2004).

Ion exchange experiments are mainly performed in five steps: equilibration, sample loading, adsorption, elution and regeneration. The ion exchange matrix is first equilibrated to have the starting pH and ionic strength that allow binding. The sample is then loaded and the proteins start to adsorb to the matrix. Once the proteins are adsorbed, the matrix is washed with the

equilibration buffer to remove non-adsorbed proteins. This is followed by elution to collect the proteins of interest. The final step is to regenerate the ion exchange matrix by removing substances not eluted in the previous experimental conditions and to equilibrate the matrix for the next purification (Ghebremichael, 2004).

2.4.4.1.4. Determination of protein concentration

Protein determination is necessary to estimate the amount of protein in the sample. Depending on the amount of sample, accuracy and presence of interfering agents, one needs to decide on the method to be used. For accurate quantification, the sample protein is compared with a known amount of a standard protein which could either be the commonly used bovine serum albumin (BSA) or it could sometimes be immunoglobulin G (IgG) (Wenk and Fernandis, 2007). There are a number of different protein assays available for the routine measurement of protein in a sample. Some of them are: Biuret, Lowry, Bradford and Spectrophotometric (A_{280}).

Most proteins have relatively intense ultraviolet light absorption centered at 280 nm. This is due to the presence of aromatic tyrosine and tryptophan residues in the protein. However, the amount of these amino acid residues varies in different proteins. If certain precautions are taken, the value of a protein solution is proportional to the protein concentration (Boyer, 2012). The assay is non-destructive as the protein in most cases is not consumed and can be recovered. Secondary, tertiary and quaternary structures all affect absorbance; therefore, factors such as pH, ionic strength, etc can alter the absorbance spectrum (Wenk and Fernandis, 2007).

3. MATERIALS AND METHODS

3.1. Experimental design

The experiment was designed in Completely Randomized Design (CRD) fashion (Amagloh and Benang, 2009). Coagulant type, sampling time, settling time, coagulant dose and optical density (OD) were variables. Each treatment effect on the optical density of raw water (response) was carried out in triplicate (Table 3).

Table 3. Experimental design for the coagulation efficiency test

	Coagulant concentration (mg/L)						
	X	NC 0	5	10	15	20	PE 5
Settling time (min)	0	$T_0C_0R_1$ $T_0C_0R_2$ $T_0C_0R_3$	$T_0C_5R_1$ $T_0C_5R_2$ $T_0C_5R_3$	$T_0C_{10}R_1$ $T_0C_{10}R_2$ $T_0C_{10}R_3$	$T_0C_{15}R_1$ $T_0C_{15}R_2$ $T_0C_{15}R_3$	$T_0C_{20}R_1$ $T_0C_{20}R_2$ $T_0C_{20}R_3$	$T_0C_5R_1$ $T_0C_5R_2$ $T_0C_5R_3$
	90	$T_{90}C_0R_1$ $T_{90}C_0R_2$ $T_{30}C_0R_3$	$T_{90}C_5R_1$ $T_{90}C_5R_2$ $T_{90}C_5R_3$	$T_{90}C_{10}R_1$ $T_{90}C_{10}R_2$ $T_{90}C_{10}R_3$	$T_{90}C_{15}R_1$ $T_{90}C_{15}R_2$ $T_{90}C_{15}R_3$	$T_{90}C_{20}R_1$ $T_{90}C_{20}R_2$ $T_{90}C_{20}R_3$	$T_{90}C_5R_1$ $T_{90}C_5R_2$ $T_{90}C_5R_3$
	180	$T_{180}C_0R_1$ $T_{180}C_0R_2$ $T_{180}C_0R_3$	$T_{180}C_5R_1$ $T_{180}C_5R_2$ $T_{180}C_5R_3$	$T_{180}C_{10}R_1$ $T_{180}C_{10}R_2$ $T_{180}C_{10}R3$	$T_{180}C_{15}R_1$ $T_{180}C_{15}R_2$ $T_{180}C_{15}R_3$	$T_{180}C_{20}R_1$ $T_{180}C_{20}R_2$ $T_{180}C_{20}R_3$	$T_{180}C_5R_1$ $T_{180}C_5R_2$ $T_{180}C_5R_3$
	270	$T_{270}C_0R_1$ $T_{270}C_0R_2$ $T_{270}C_0R_3$	$T_{270}C_5R_1$ $T_{270}C_5R_2$ $T_{270}C_5R_3$	$T_{270}C_{10}R_1$ $T_{270}C_{10}R_2$ $T_{270}C_{10}R_3$	$T_{270}C_{15}R_1$ $T_{270}C_{15}R_2$ $T_{270}C_{15}R_3$	$T_{270}C_{20}R_1$ $T_{270}C_{20}R_2$ $T_{270}C_{20}R_3$	$T_{270}C_5R_1$ $T_{270}C_5R_2$ $T_{270}C_5R_3$

Key: T = Settling time, C = Coagulant concentration, R = Replicate, NC = Negative control PE = Polyelectrolyte

3.2. Seeds collection and extraction

Lupinus albus, Trigonella foenum-graecum and *Vicia faba* seeds were purchased at local markets in Addis Ababa and Debre Markos. *Moringa stenopetala* seeds were obtained from Ethiopian Environment and Forest Research Institute (EEFRI). These plant seeds were selected based on scientific literature and indigenous knowledge. The raw seeds were ground to fine powder by using grinding mill. The fine powder was sieved through BSS 20 (British Standard Sieve 20). From each plant, 5 g fine powder was added to 50 mM ammonium acetate solution to produce a final volume of 100 ml in a beaker, stirred for 30 min and settled for 2 hrs. *Lupinus albus* slurry was filtered through filter paper (Whatman filter paper no. 3), paper towel and 0.45 μm sterile membrane filter (AquaSafe, United Kingdom). *Moringa stenopetala* slurry was filtered through filter paper (Whatman filter paper no. 3) and 0.45 μm sterile membrane filter (Ghebremichael *et al.* 2005). Finally, *Trigonella foenum-graecum* and *Vicia faba* slurries were filtered through filter paper (Xinxing Qualitative Filter Paper 102, Moderate, Ø 125 mm), paper towel and 0.45 μm sterile membrane filter.

3.3. Purification

Lupinus albus, Moringa stenopetala, Trigonella foenum-graecum and *Vicia faba* crude extracts were purified using column-based protein separator and fractionator (AKTApurifer^{TM} UPC 100). The wave length of the separator was set at 280 nm. Buffer solution was homogenized and degassed using sonicator (BANDELIN electronic UW 2070, Germany). The sample was injected using injection needle into sample loop (column). The column was equilibrated with 50 mM ammonium acetate solution, pH 7 (solution A) (Ghebremichael *et al.* 2005). A flow rate of 1 ml/min was applied to load the sample to the column. Linear gradient elution with 1M ammonium acetate, pH 7 (solution B) was performed to elute cationic proteins (Ghebremichael *et al.* 2005 with modification). After separation based on net charge of the protein, 2 mL of fractionated proteins were automatically pumped out to the automatic fraction collector.

3.4. Protein extracts concentration

The concentration of *Lupinus albus, Moringa stenopetala, Trigonella foenum-graecum* and *Vicia faba* protein extracts was measured using spectrophotometer (Cary Series UV-Vis-NIR Spectrophotometer). It was determined using direct UV measurement at 280 nm. Bovine serum albumin (BSA) was used as a standard. The following method was taken from Harisha (2007)

with modifications. A series of protein standards using BSA diluted with 1M ammonium acetate to final concentrations of 0, 250, 500, 750 and 1500 μg BSA/mL were prepared. The spectrophotometer was adjusted to a wavelength of 280 nm and 0 BSA was used for a blank. Each standard and protein extract sample was added in quartz glass cuvette and read using spectrophotometer. The absorbances of the standards versus their concentration were plotted and the concentrations of protein extract samples were calculated.

3.5. Polyelectrolyte solution preparation

The polyelectrolyte (Polydiallyldimethyl ammonium chloride (polyDADMAC), cationic polymer in solution) was obtained from Addis Ababa Water and Sewerage Authority (AAWSA), and it was used as positive control. Polyelectrolyte solution was prepared based on the working procedure as used at Legedadi water treatment plant. One gram polyelectrolyte was added and dissolved in a beaker containing one- liter distilled water. A 5 mg/L concentration was applied for this experiment.

3.6. Sample site description and raw water sampling

Legedadi water reservoir (dam) is located 30 km east of Addis Ababa and the reservoir has a capacity of 42.17 million cubic meters (Fig. 4). The catchment area is 207.3 km^2. It was constructed in 1967. Legedadi water treatment plant treats raw water supplied from the Legedadi water reservoir (Fig. 5). The plant was designed for a maximum production of 150,000 m^3/ day (Birhanu Hailu, 2017). Representative raw water sample was randomly taken using clean plastic jerry can at the inlet of water treatment site. On the same date of sampling, the water sample was transported to Biomedical Laboratory in the Department of Microbial, Cellular and Molecular Biology, Addis Ababa University. Raw water samples were collected in April and August, 2019. The two sampling times were selected to represent the Belg, a short rainy season that occurs between February to May and Kiremt, which is the main rainy season that extends from June to September, according to the National Meteorology Agency climatic report (NMA, 2019).

Figure 4. Legedadi water reservoir

Figure 5. Legedadi water treatment plant

3.7. Coagulation efficiency test

Coagulation efficiency test using small sample volume and coagulant dose (Ghebremichael, 2004 and Marobhe, 2008) was conducted to evaluate the coagulation performance of *Lupinus albus, Moringa stenopetala, Trigonella foenum-graecum and Vicia faba* protein extracts. Raw water sample was mixed using magnetic stirrer for 5 min. Each falcon tube was filled with 50 ml water sample. A 5 mg/L, 10 mg/L, 15 mg/L and 20 mg/L protein extracts, and 5 mg/L polyelectrolyte (positive control) were added in labeled falcon tubes after removing equivalent doses of water sample. Raw water was used as negative control. The sample was mixed using vortex mixer for a minute. A 3 ml supernatant was pipetted out in falcon tube at t = 0 min (initial time), 90 min, 180 min and 270 min, and transferred to a plastic cuvette. Finally, optical density (absorbance) was measured at 500 nm (Ghebremichael, 2004 and Marobhe, 2008) using a spectrophotometer (Cary Series UV-Vis-NIR Spectrophotometer).

3.8. Data analysis

The percentage of coagulation efficiency was calculated using the following formula: $((t_0-t)/t_0)*100$, where t_0 is the A_{500} measured instantly after the sample has been homogenized (i.e. initial time) and t is the A_{500} measured after 90 min, 180 min and 270 min (Bodlund, 2013). One-way analysis of variance (ANOVA) was performed using SPSS statistics V. 26 software (IBM, California). Multiple mean comparisons using Least Significant Difference (LSD) were computed. A significance level of $p<0.05$ was used (Amagloh and Benang, 2009).

4. RESULTS AND DISCUSSION

4.1. Concentration of *Lupinus albus, Moringa stenopetala, Trigonella foenum-graecum* and *Vicia faba* protein extracts

Trigonella foenum-graecum achieved the highest protein concentration per liter as compared to others (Table 4). But, it had wide range. On the other hand, *Moringa stenopetala* scored lowest protein content per liter with narrow range.

Table 4. Concentration of protein extracts

No.	Protein extract type	Protein extract concentration (mg/L) (Mean ± SD)
1	*Lupinus albus*	516±69
2	*Moringa stenopetala*	509±13
3	*Trigonella foenum-graecum*	2763±377
4	*Vicia faba*	1064 ±63

4.2. Coagulation efficiency of *Lupinus albus, Moringa stenopetala, Trigonella foenum-graecum* and *Vicia faba* protein extracts

4.2.1. Coagulation efficiency of *Lupinus albus* protein extract

At the initial time, the percentage of optical density reduction was zero as a result of the initial time was used as reference for itself (Fig. 6). It was also used as reference for other settling times. At 90 min settling time, the coagulation efficiency were 3%, 3%, 3% and 5% at 5 mg/L, 10 mg/L, 15 mg/L and 20 mg/L respectively. The reaction rate of LAPE was very slow at all doses. At time of 180 min, OD reduction was increased from 5 to 50% with the increment of dose from 5 to 20 mg/L. At the time of 270 min, LAPE achieved 10 to 71% OD reduction with the increment of dose from 5 to 20 mg/L. Similarly, OD reduction of negative control (raw water) and polyelectrolyte (positive control) was increased from 0 to 10% and from 0 to 94% with the increment of settling times from initial time (0 min) to 270 min respectively. Comparative analysis using ANOVA showed that at 270 min settling time, there was significant ($p<0.05$) mean difference between 20 mg/L, negative control and polyelectrolyte on optical density in the water sample collected in April, 2019.

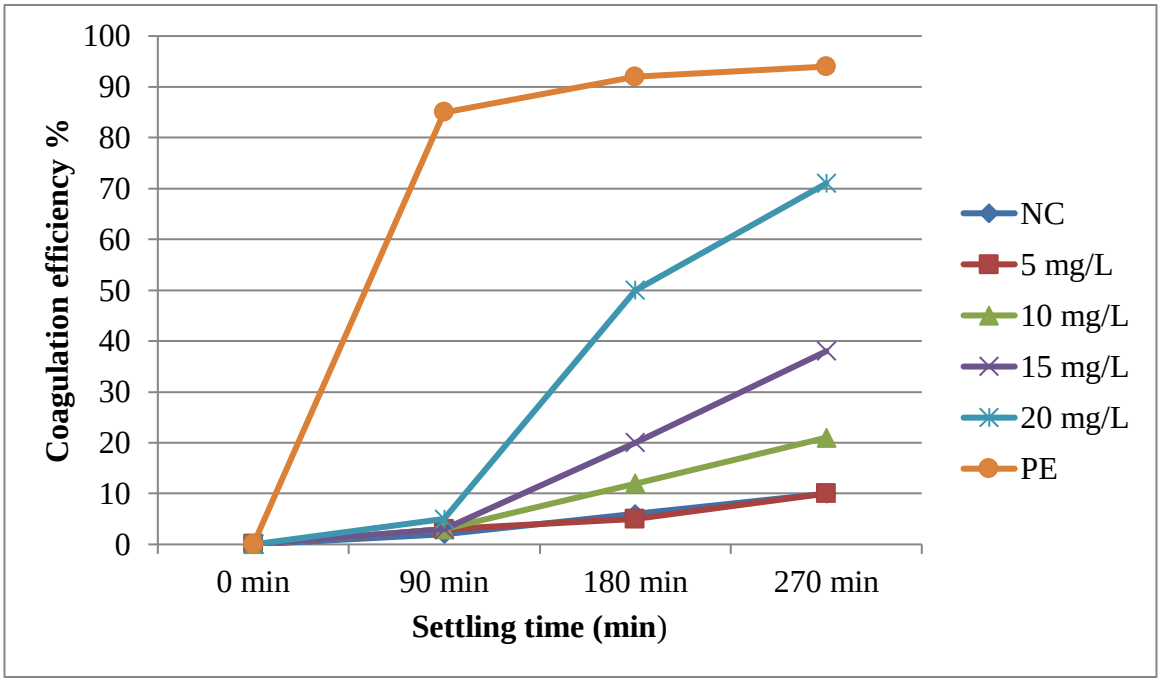

Figure 6. Coagulation efficiency of *Lupinus albus* protein extract on the water sample collected in April, 2019

The result indicated that at 90 min settling time, LAPE scored 1%, 1%, 1% and 2% coagulation efficiency at 5 mg/L, 10 mg/L, 15 mg/L and 20 mg/L respectively (Fig. 7). At time of 180 min, OD reductions were 6%, 6%, 3% and 2% at 5 mg/L, 10 mg/L, 15 mg/L and 20 mg/L respectively. At the time of 270 min, LAPE achieved 17%, 12%, 12% and 11% OD reductions at 5 mg/L, 10 mg/L, 15 mg/L and 20 mg/L respectively. On the other hand, OD reduction of negative control (raw water) and polyelectrolyte (positive control) was increased from 0 to 7% and from 0 to 90% with the increment of settling times from initial time to 270 min respectively. Comparative analysis showed that at 270 min settling time, there was no significant variation between 5 mg/L and negative control on optical density in the water sample collected in August, 2019.

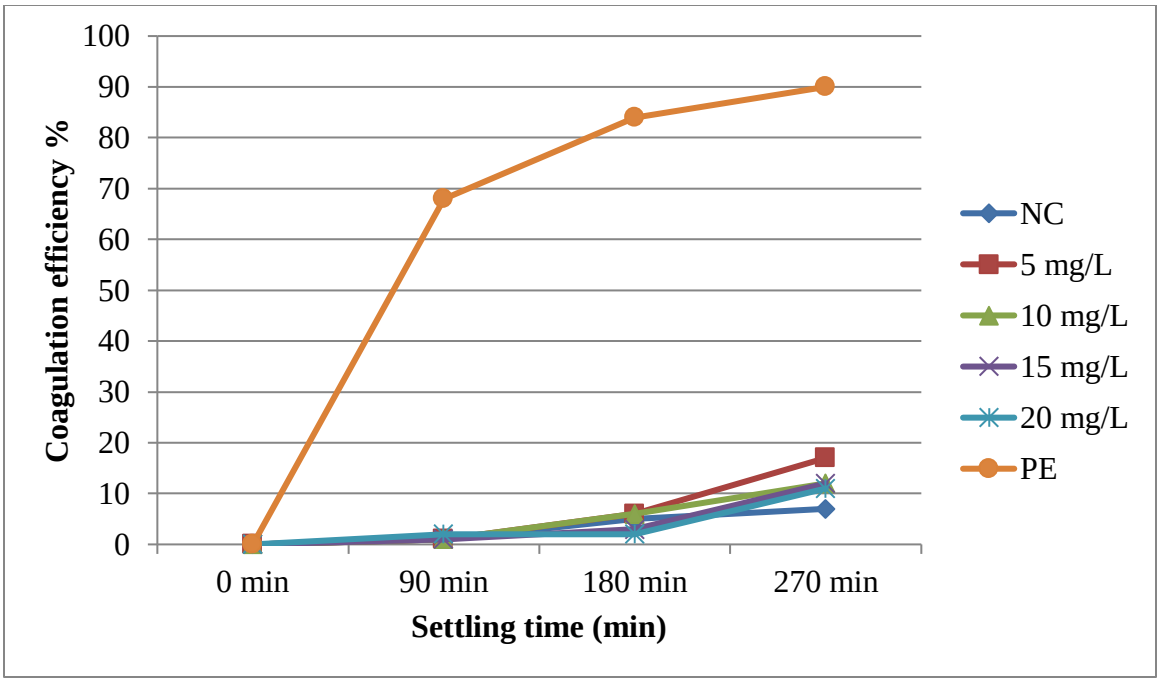

Figure 7. Coagulation efficiency of *Lupinus albus* protein extract on the water sample collected in August, 2019

The coagulation efficiency was increased when the dose of *Lupinus albus* protein extract (LAPE) was increased from 5 to 20 mg/L at 180 min and 270 min settling times in April, 2019. This increment in reduction of optical density or absorbance is due to increment of the concentration of active protein extract. Thus, 20 mg/L achieved the maximum optical density reduction at 270 min settling times among the other doses tested in April, 2019. It was 71%. On the other hand, the OD reduction potential of LAPE was low in the water sample collected in August, 2019. This is mainly due to the presence of high concentration of particles (particulate matter) in the water sample. Moreover, 5 mg/L achieved the highest optical density reduction at 270 min settling time among the other doses tested in August, 2019. It was 17%. The two sampling times revealed that LAPE was more efficient on low optical density water sample.

4.2.2. Coagulation efficiency of *Moringa stenopetala* protein extract

At 90 min settling time, *Moringa stenopetala* protein extract (MSPE) achieved 2 to 60% OD reduction with increment of dose from 5 to 20 mg/L (Fig. 8). At time of 180 min, OD reduction was increased from 11 to 85% with the increment of dose from 5 to 20 mg/L. At the time of 270 min, the coagulation efficiency was increased from 13 to 89% with the increment of dose from 5

to 20 mg/L. Similarly, OD reduction of negative control (raw water) and polyelectrolyte (positive control) was increased from 0 to 7% and from 0 to 89% with the increment of settling times from initial time to 270 min respectively. Comparative analysis revealed that at 270 min settling time, there was no significant mean difference between 20 mg/L and polyelectrolyte on optical density in the water sample collected in April, 2019.

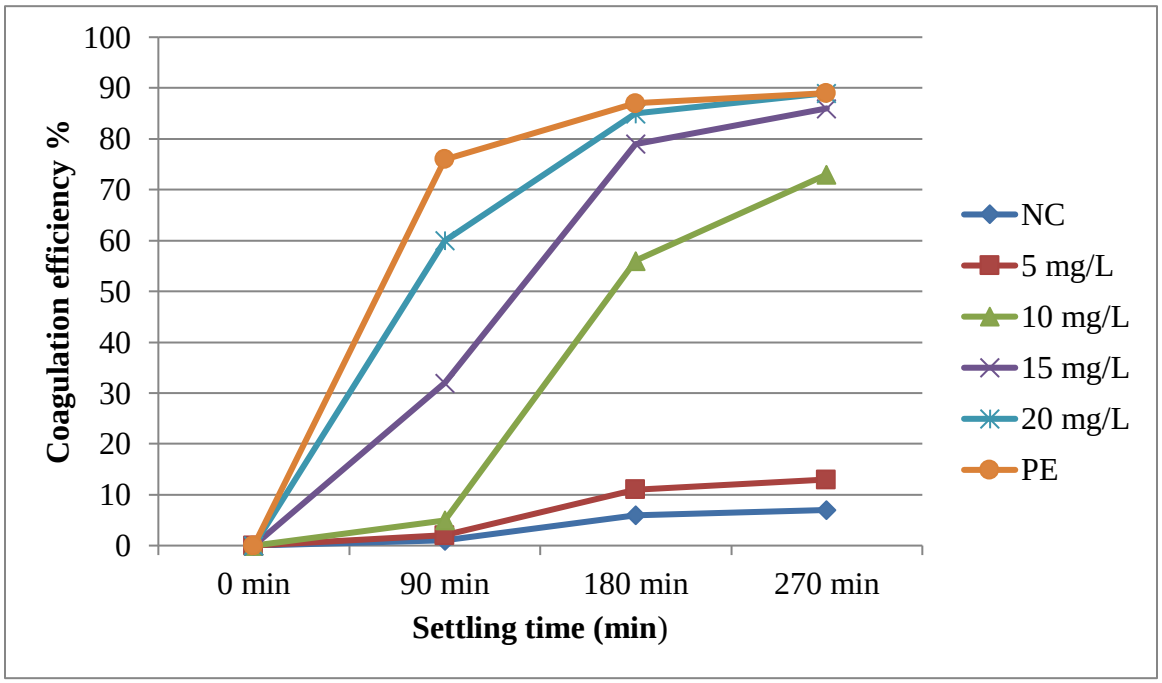

Figure 8. Coagulation efficiency of *Moringa stenopetala* protein extract on the water sample collected in April, 2019

The result showed that at 90 min settling time, the coagulation efficiency of MSPE was increased from 2 to 75% with increment dose from 5 to 20 mg/L (Fig. 9). At time of 180 min, OD reduction was increased from 7 to 85% with the increment of dose from 5 to 20 mg/L. At the time of 270 min, MSPE achieved 10 to 92% OD reduction with the increment of dose from 5 to 20 mg/L. Similarly, negative control (raw water) and polyelectrolyte's (positive control) OD reductions were increased from 0 to 8% and from 0 to 91% with the increment of settling times from initial time to 270 min respectively. Comparative analysis showed that at 270 min settling time, there was no significant variation between 20 mg/L and polyelectrolyte on optical density in the water sample collected in August, 2019.

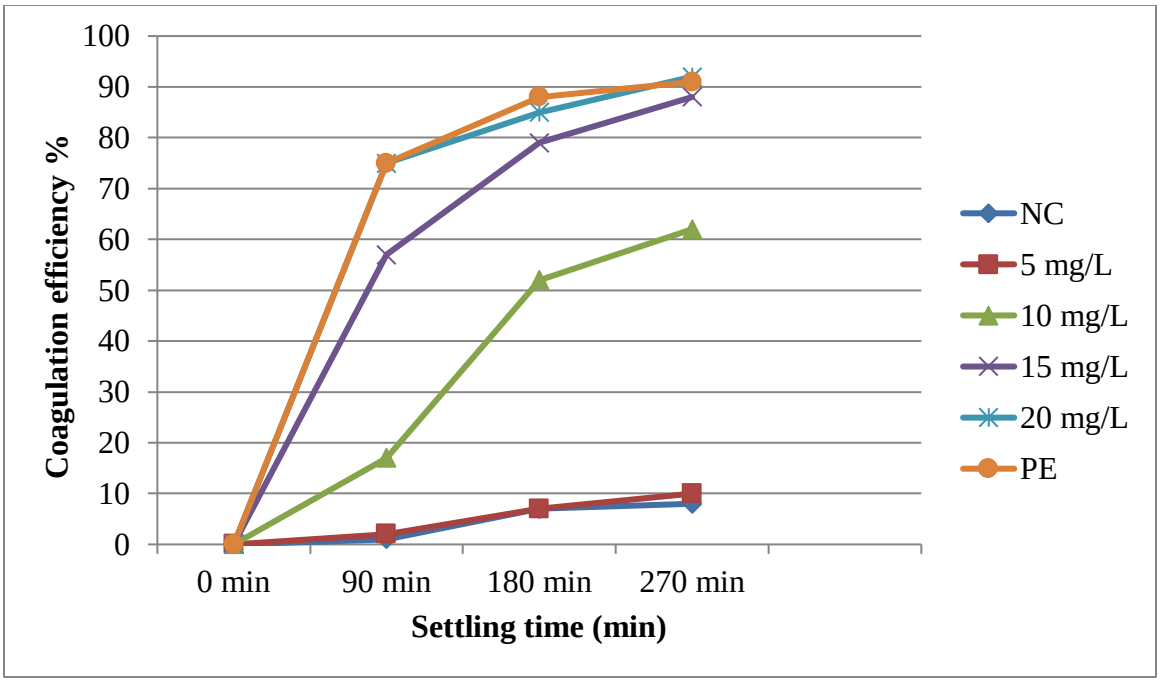

Figure 9. Coagulation efficiency of *Moringa stenopetala* protein extract on the water sample collected in August, 2019

The coagulation efficiency was increased when the dose of *Moringa stenopetala* protein extract (MSPE) was increased from 5 to 20 mg/L at all settling times tested in April and August, 2019. This increment in reduction of optical density or absorbance is due to increment of the concentration of active protein extract. Thus, 20 mg/L achieved the maximum optical density reduction at 270 min settling time among the other doses tested in April and August, 2019. It was 89% and 92% in April and August, 2019 respectively. Moa *et al.* (2016) have conducted coagulation experiment using *Moringa stenopetala* extract on the five river waters having initial turbidities of 20, 45, 46, 80, and 195 NTU with the doses of 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 mg/L. Appreciable turbidity removal (up to 98 %) was achieved at an optimum dose range of 20 to 80 mg/L in 6 hrs of settling time. Ghebremichael (2004) has reported the coagulation efficiency of *Moringa olifera* coagulant protein (MOCP) and crude extract on kaolin clay suspension using small volume coagulation test. The coagulation efficiency of MOCP and crude *Moringa olifera* extract were similar to alum for high turbidity (250 -300 NTU) clay suspension. In this study, the coagulation efficiencies of MSPE (20 mg/L) and polyelectrolyte were also similar at 270 min settling time in the water samples collected in April and August, 2019.

4.2.3. Coagulation efficiency of *Trigonella foenum-graecum* protein extract

The result showed that at 90 min settling time, *Trigonella foenum-graecum* protein extract (TFPE) achieved 3%, 4%, 4% and 2% OD reduction at 5 mg/L, 10 mg/L, 15 mg/L and 20 mg/L respectively (Fig. 10). At time of 180 min, OD reductions were 7%, 6%, 4% and 6% at 5 mg/L, 10 mg/L, 15 mg/L and 20 mg/L respectively. At the time of 270 min, the coagulation efficiencies were 8%, 6%, 7% and 12% at 5 mg/L, 10 mg/L, 15 mg/L and 20 mg/L respectively. Similarly, OD reduction of negative control (raw water) and polyelectrolyte (positive control) was increased from 0 to 9% and from 0 to 92% with the increment of settling times from initial time to 270 min respectively. Comparative analysis showed that at 270 min settling time, there was no significant difference between 20 mg/L and negative control on optical density in the water sample collected in April, 2019

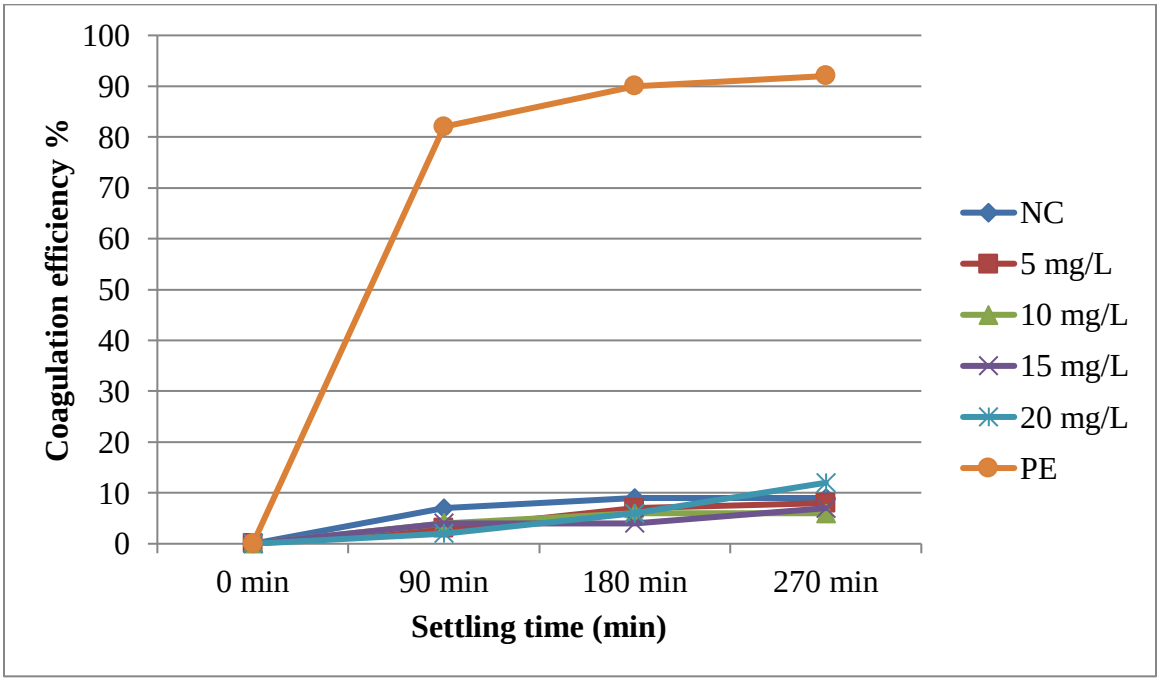

Figure 10. Coagulation efficiency of *Trigonella foenum-graecum* protein extract on the water sample collected in April, 2019

The optical density reduction performance of *Trigonella foenum-graecum* protein extract (TFPE) was low in April, 2019. This was probably due to more protein extract requirement of the water sample to enhance coagulation efficiency and/or short settling time and/or low charge density of TFPE. As result in April, 2019 anticipated, high optical density even after the application of

TFPE was expected in August, 2019. Thus, the coagulation efficiency of TFPE was not conducted from the water sample collected in August, 2019. A 20 mg/L achieved maximum coagulation efficiency at 270 min settling time. It was 12%. OD reduction of TFPE is not much better than negative control, putting TFPE as the least performing protein extract with regard to coagulation efficiency. Ramamurthy *et al.* (2012) have reported the coagulation efficiency of *Trigonella foenum-graecum* extract on pond water using jar test. The coagulation efficiency was about 80% at 10 ml/L and 6 hrs settling time. However, crude extract is not suitable for use in large water treatment system due to it contains compounds other than proteins such as carbohydrates, lipids and other organic and inorganic that may be released to the water being treated (Ghebremichael, 2004).

4.2.4. Coagulation efficiency of *Vicia faba* protein extract

At 90 min settling time, *Vicia faba* protein extract (VFPE) achieved 3%, 1%, 1% and 1% OD reductions at 5 mg/L, 10 mg/L, 15 mg/L and 20 mg/L respectively (Fig. 11). At time of 180 min, OD reduction was increased from 5 to 39% with the increment of dose from 5 to 20 mg/L. At the time of 270 min, the coagulation efficiency was increased from 6 to 67% with the increment of dose from 5 to 20 mg/L. Similarly, OD reduction of negative control (raw water) and polyelectrolyte (positive control) was increased from 0 to 6% and from 0 to 91% with the increment of settling times from initial time to 270 min respectively. Comparative analysis using ANOVA showed that at 270 min settling time, there was significant ($p<0.05$) mean difference between 20 mg/L, negative control and polyelectrolyte on optical density in the water sample collected in April, 2019.

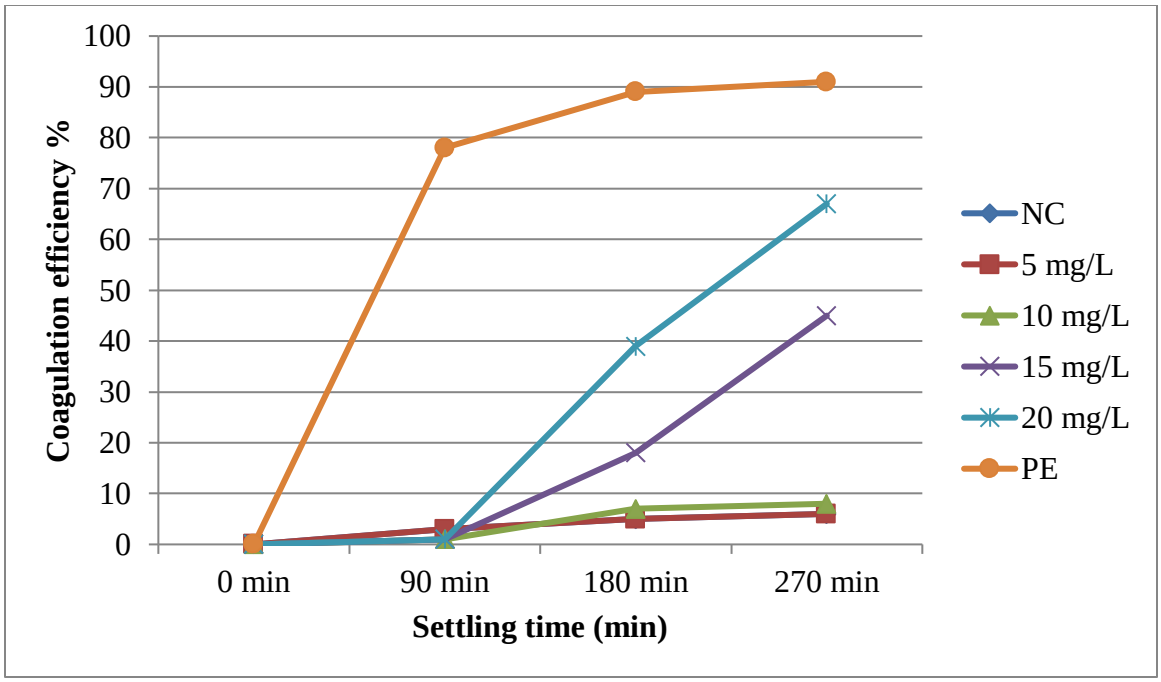

Figure 11. Coagulation efficiency of *Vicia faba* protein extract on the water sample collected in April, 2019

The result indicated that at 90 min settling time, VFPE achieved 1%, 4%, 2% and 5% at 5 mg/L, 10 mg/L, 15 mg/L and 20 mg/L respectively (Fig. 12). At time of 180 min, the three doses except 10 mg/L showed similar coagulation efficiency. OD reductions were 8%, 6%, 8% and 8% at 5 mg/L, 10 mg/L, 15 mg/L and 20 mg/L respectively. At the time of 270 min, the coagulation efficiency was 9%, 12%, 10% and 10% at 5 mg/L, 10 mg/L, 15 mg/L and 20 mg/L respectively. On the other hand, OD reduction of negative control (raw water) and polyelectrolyte (positive control) was increased from 0 to 9% and from 0 to 94% with the increment of settling times from initial time to 270 min respectively. Comparative analysis showed that at 270 min settling time, there was no significant variation between 10 mg/L and negative control on optical density in the water sample collected in August, 2019

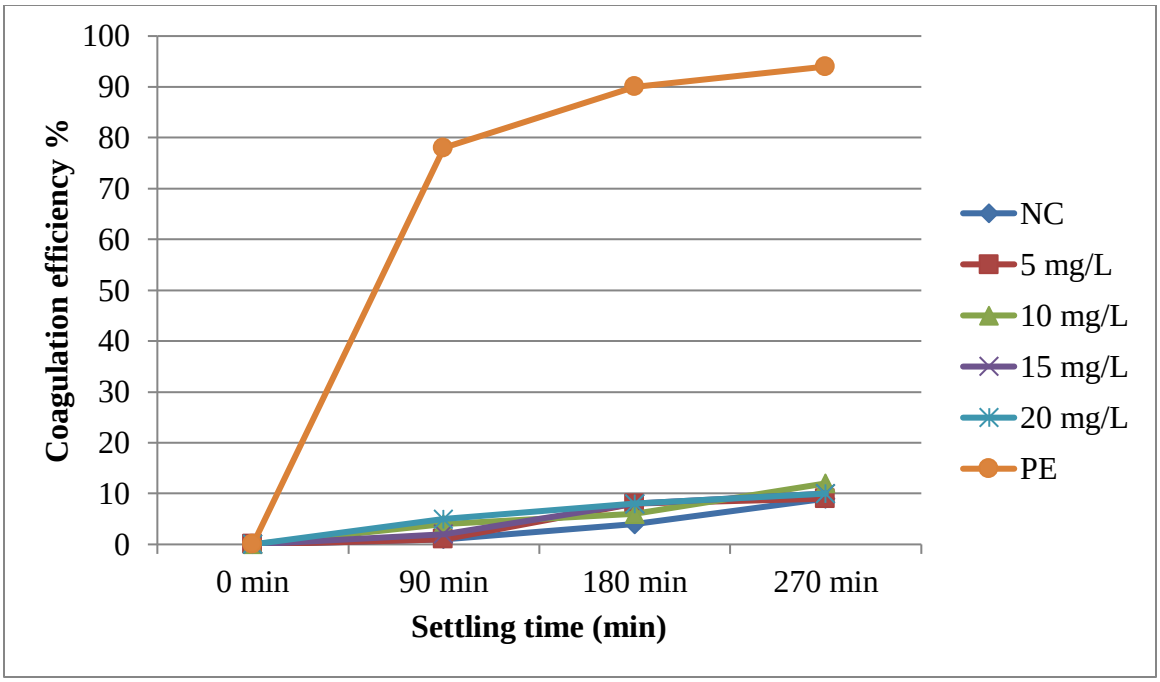

Figure 12. Coagulation efficiency of *Vicia faba* protein extract on the water sample collected in August, 2019

The coagulation efficiency was increased when the dose of *Vicia faba* protein extract (VFPE) was increased from 5 to 20 mg/L at 180 and 270 min settling times in April, 2019. This increment in reduction of optical density or absorbance is due to increment of the concentration of active protein extract. Thus, 20 mg/L achieved the maximum optical density reduction at 270 min settling times among all doses tested in April, 2019. It was 67%. On the other hand, the OD reduction potential of VFPE was low in the water sample collected in August, 2019. This is mainly because of the presence of high concentration of particles (particulate matter) in the water sample. A 10 mg/L achieved the highest optical density reduction at 270 min settling time among the other doses tested in August, 2019. It was 12%. The two sampling times revealed that VFPE was more efficient in low optical density water sample. Kukic *et al.* (2015) have reported the coagulation efficiency of *Vicia faba* extract on synthetic water sample using jar test. The coagulation efficiency was 54% at 0.25 ml/L and 1 hr in water with initial turbidity of 45 nephelometric turbidity unit (NTU).

5. CONCLUSIONS AND RECOMMENDATIONS

The following conclusions have been drawn from the study:

- The study revealed that *Lupinus albus*, *Moringa stenopetala*, *Trigonella foenum-graecum* and *Vicia faba* protein extracts have achieved the highest coagulation efficiency at 270 min settling time and 20 mg/L concentration in the water sample collected in April, 2019. Similarly, LAPE, MSPE and VFPE have exhibited maximum optical density reduction at 270 min settling time and, 5 mg/L, 20 mg/L and 10 mg/L concentration in the water sample collected in August, 2019 respectively.
- The result showed that *Moringa stenopetala* protein extract has achieved the best coagulation efficiency among the four protein extracts and reduced the optical density similar to the polyelectrolyte at 270 min settling time and 20 mg/L in the water samples collected in April and August, 2019.

The following recommendations have been made for future consideration:

- The physical, chemical and anti-microbial properties of protein extracts should be studied. In addition, the physical, chemical and microbiological qualities of water treated with protein extracts should be examined.
- The coagulation mechanisms and storage duration (shelf life) of protein extracts should be investigated as well as the degradation (biodegradation) of protein extracts with respect to time in treated water should be studied.
- The active agent responsible for coagulation should be identified.
- The protein extracts should be tested in Bega (sunny and dry weather situation) for water purification.

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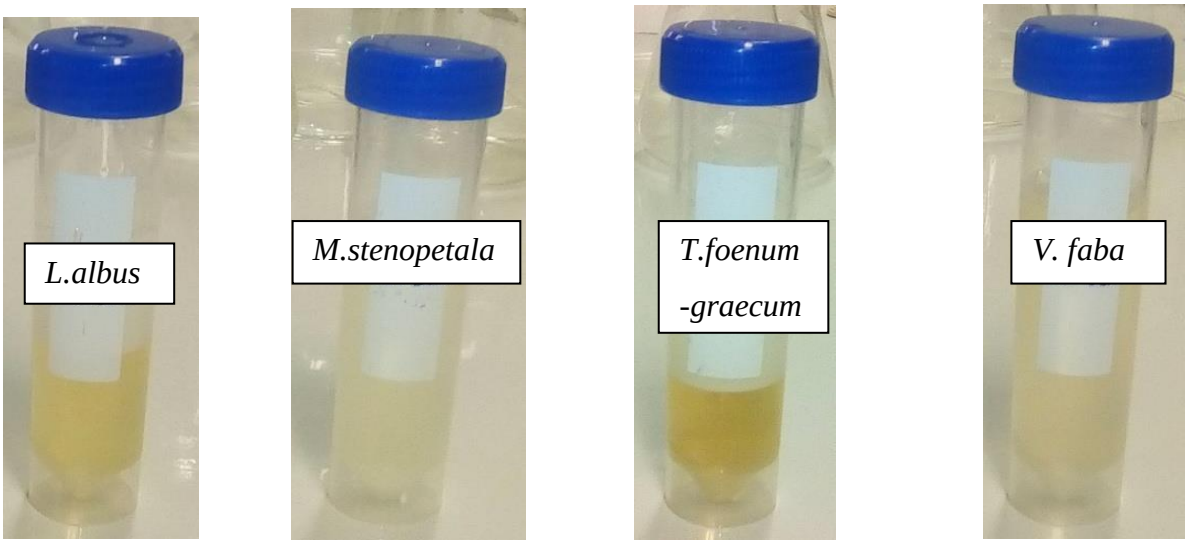
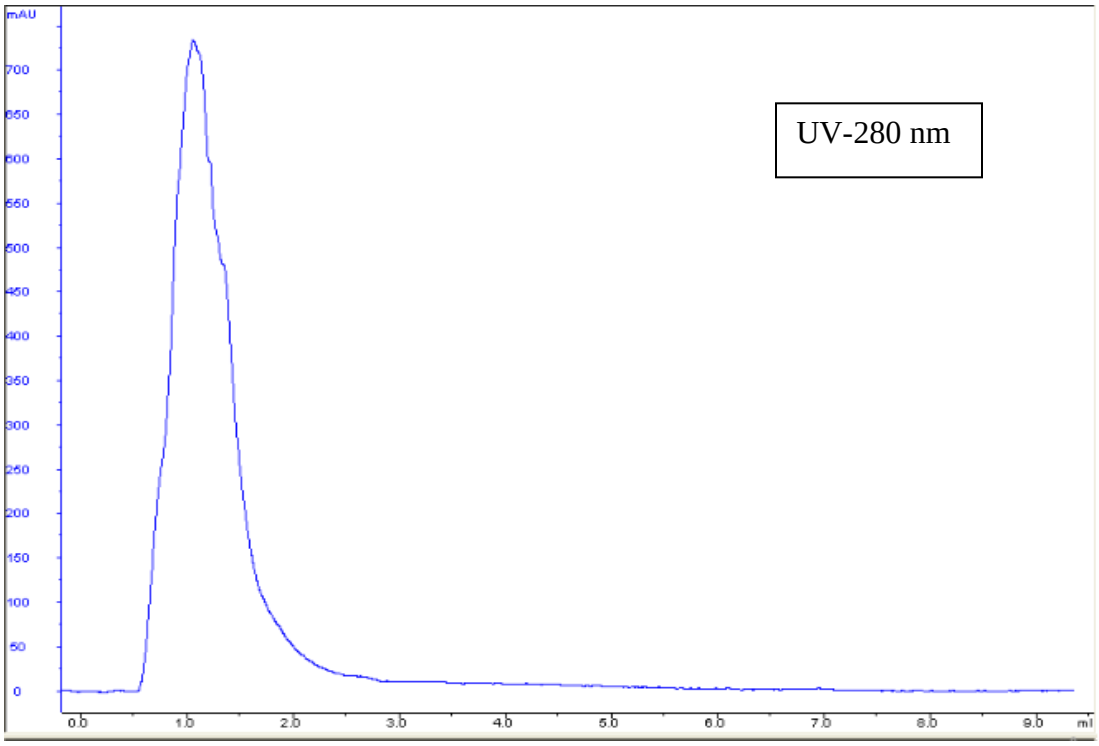
7. APPENDICES

Appendix 1. Crude ammonium acetate extracts (CAAE) of *Lupinus albus, Moringa stenopetala, Trigonella foenum-graecum* and *Vicia faba*

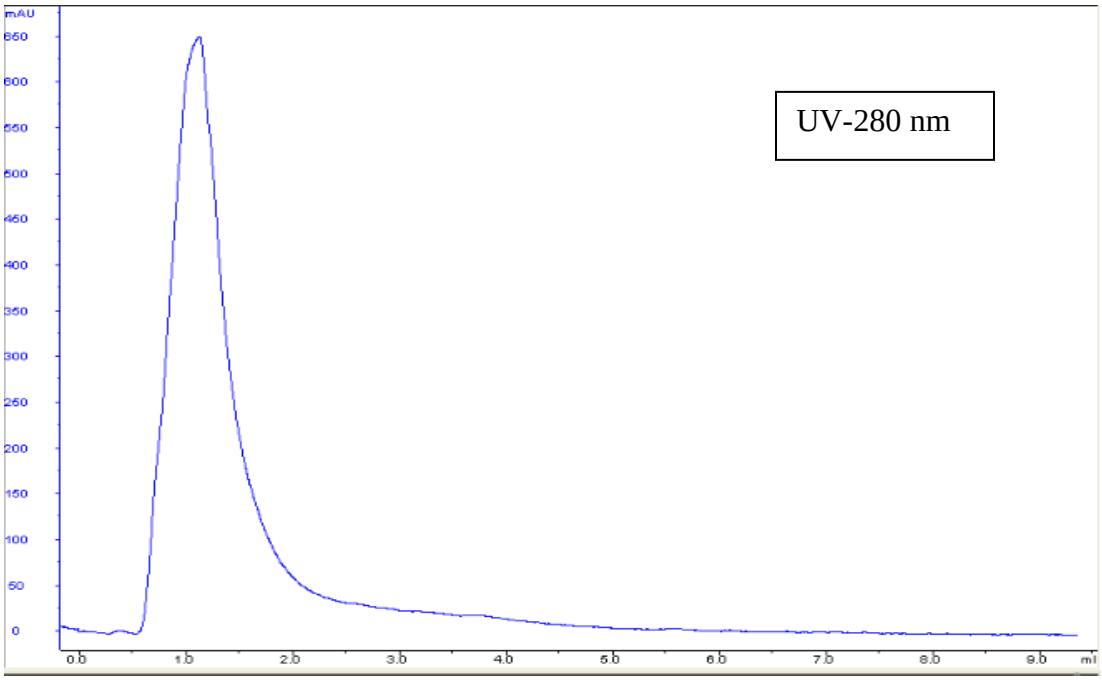


Appendix 2. Sample injection into protein purifier

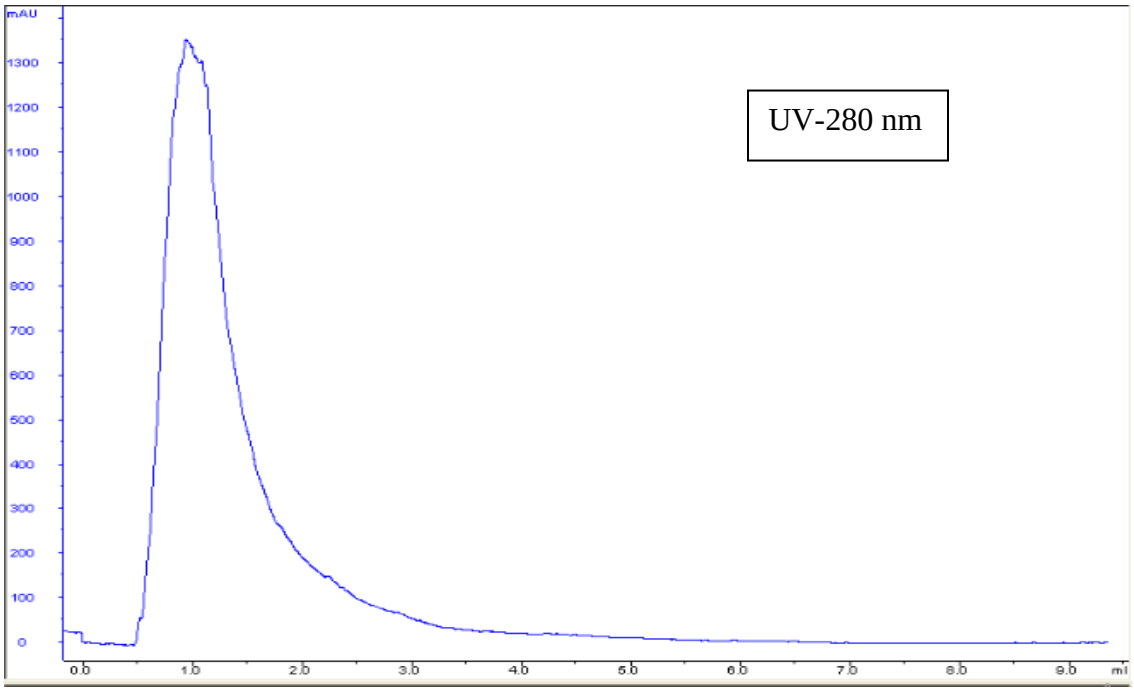
Appendix 3. Chromatogram of *Lupinus albus* protein extract



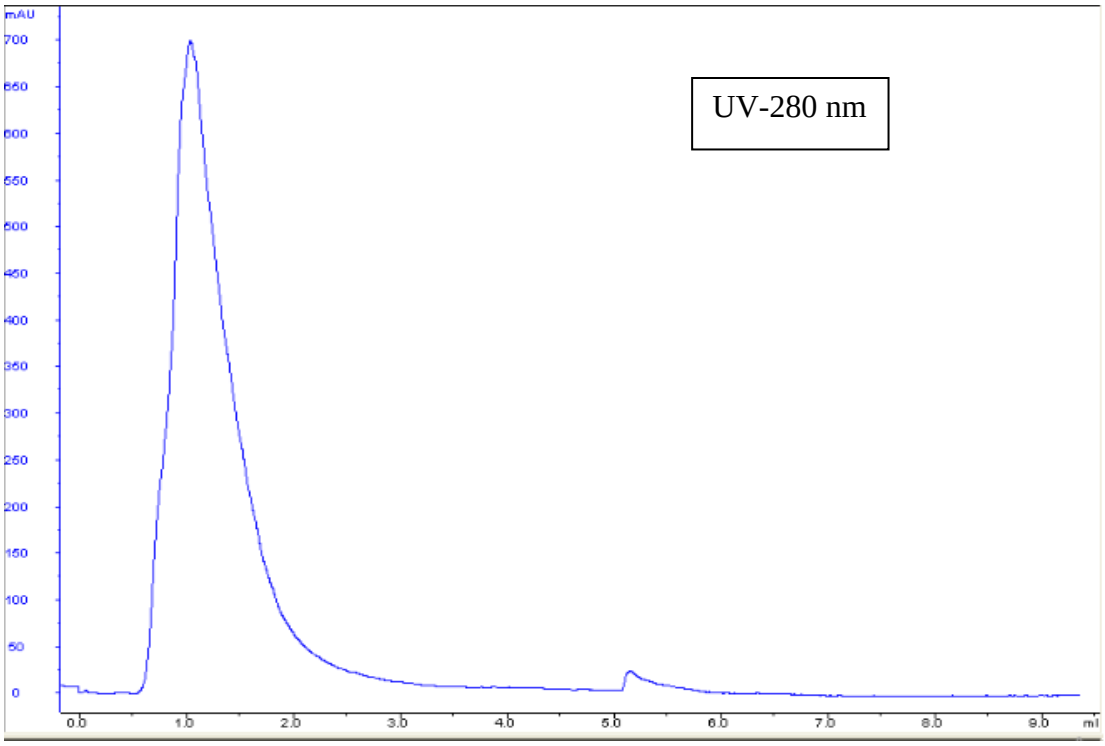
Appendix 4. Chromatogram of *Moringa stenopetala* protein extract



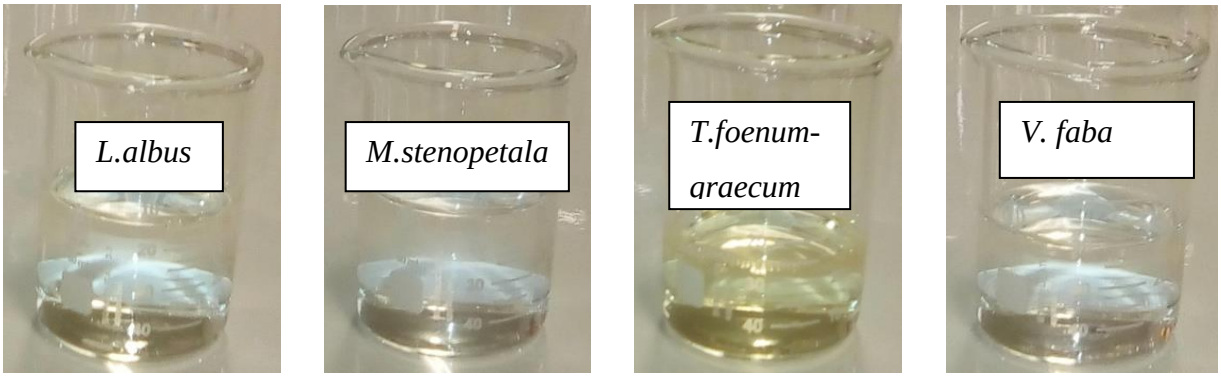
Appendix 5. Chromatogram of *Trigonella foenum-graecum* protein extract



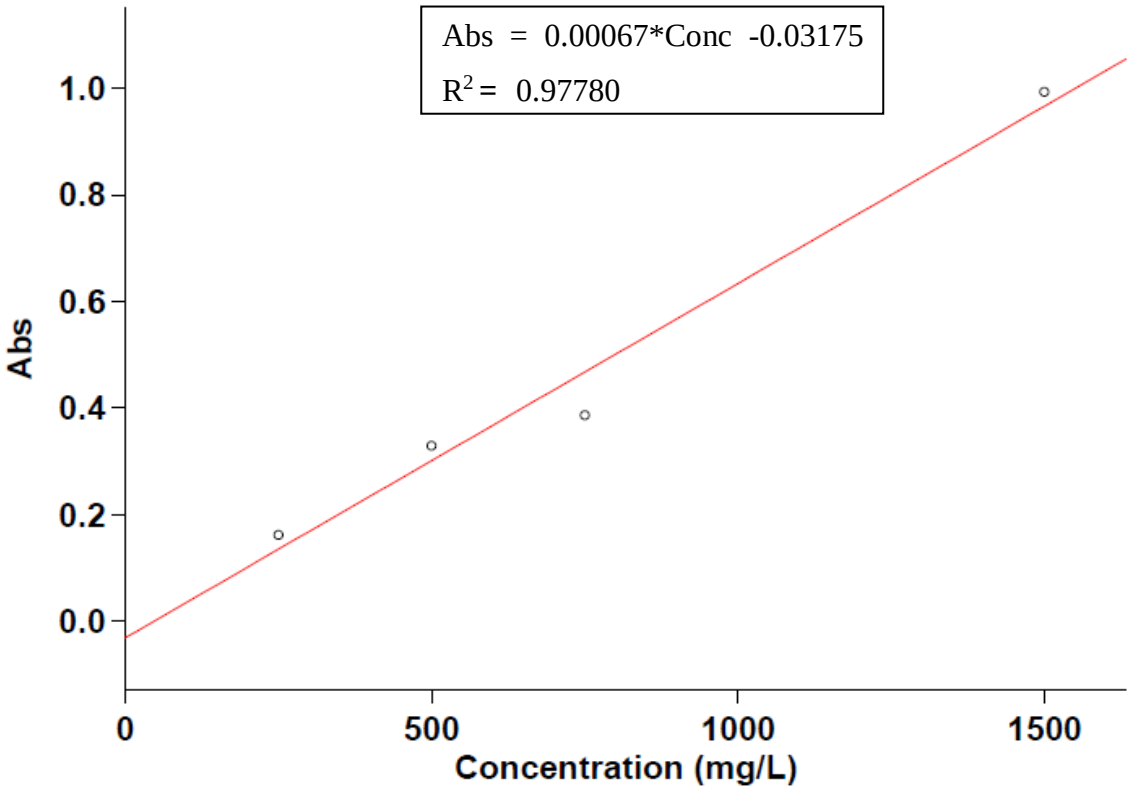
Appendix 6. Chromatogram of *Vicia faba* protein extract



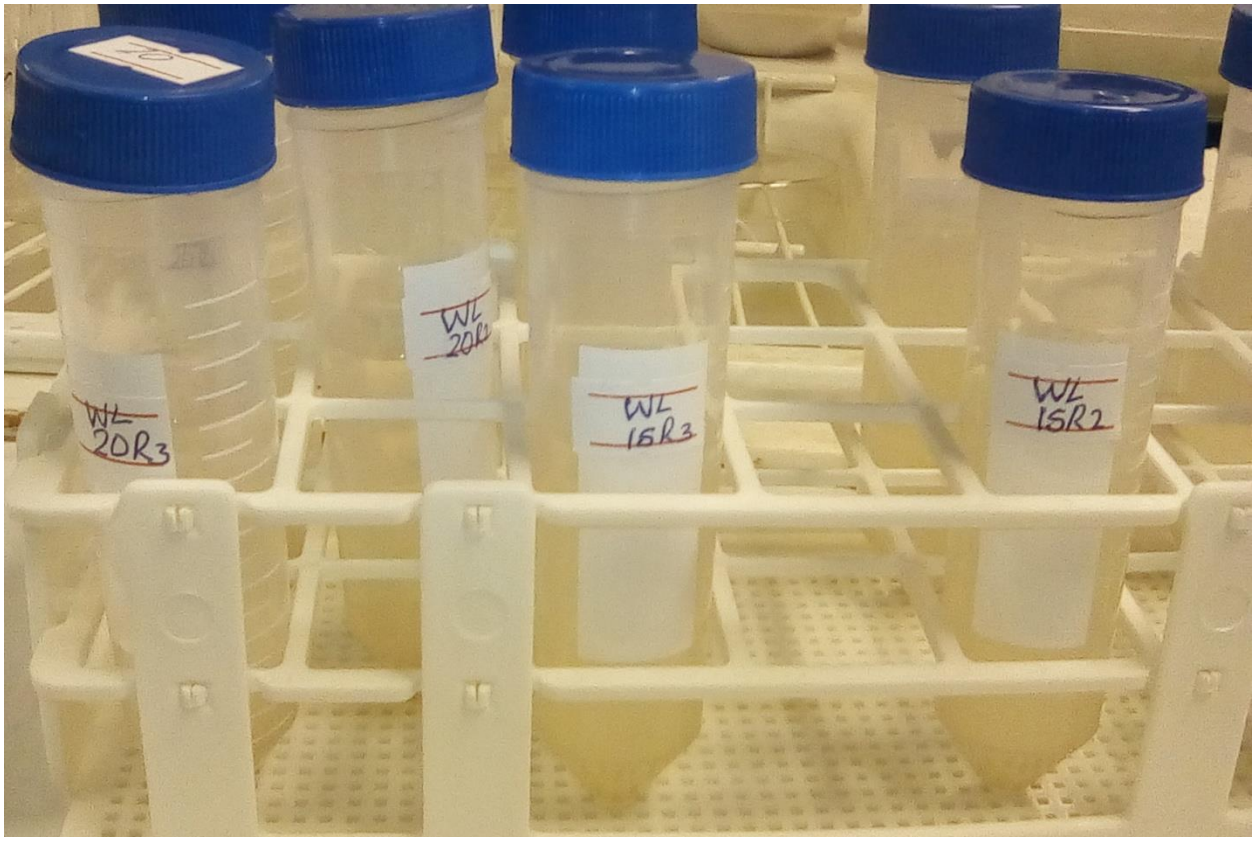
Appendix 7. Protein extracts of *Lupinus albus*, *Moringa stenopetala*, *Trigonella foenum-graecum* and *Vicia faba*



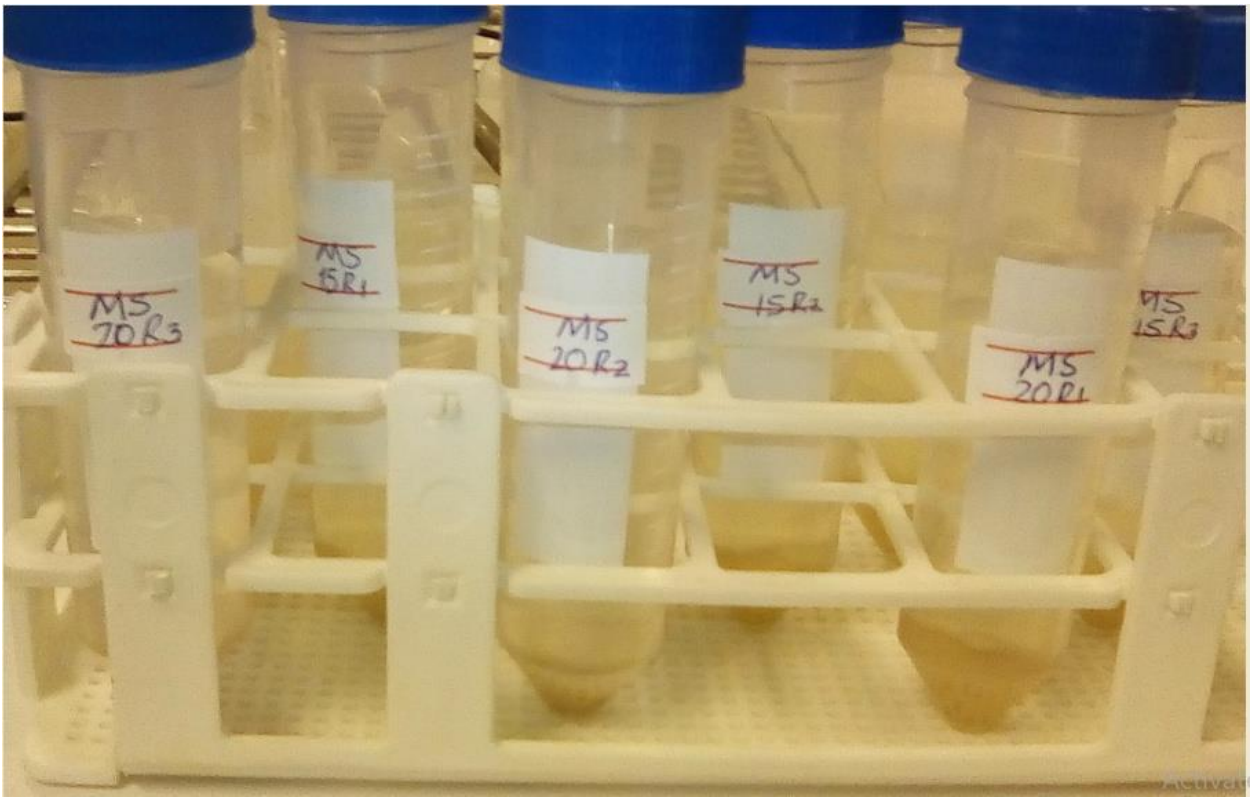
Appendix 8. Standard curve using BSA



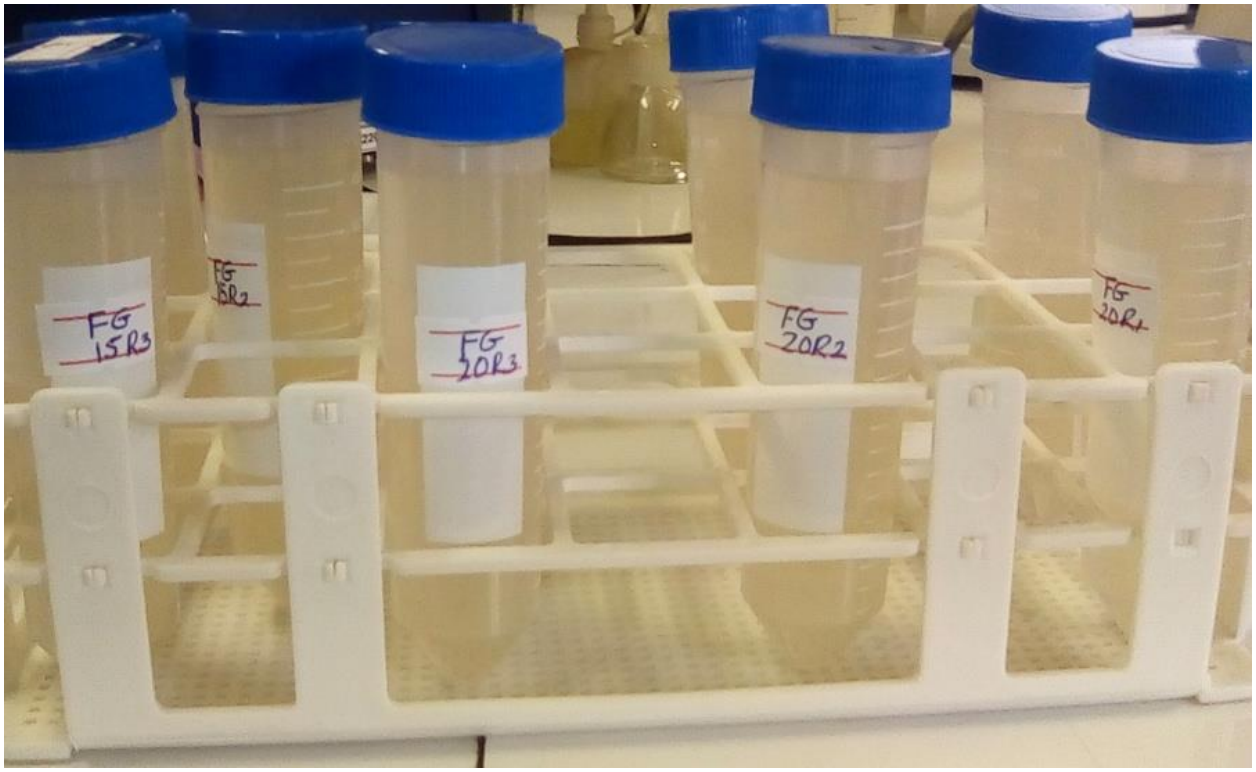
Appendix 9. Coagulation efficiency test of *Lupinus albus* (white lupine) protein extract on water sample collected in April, 2019



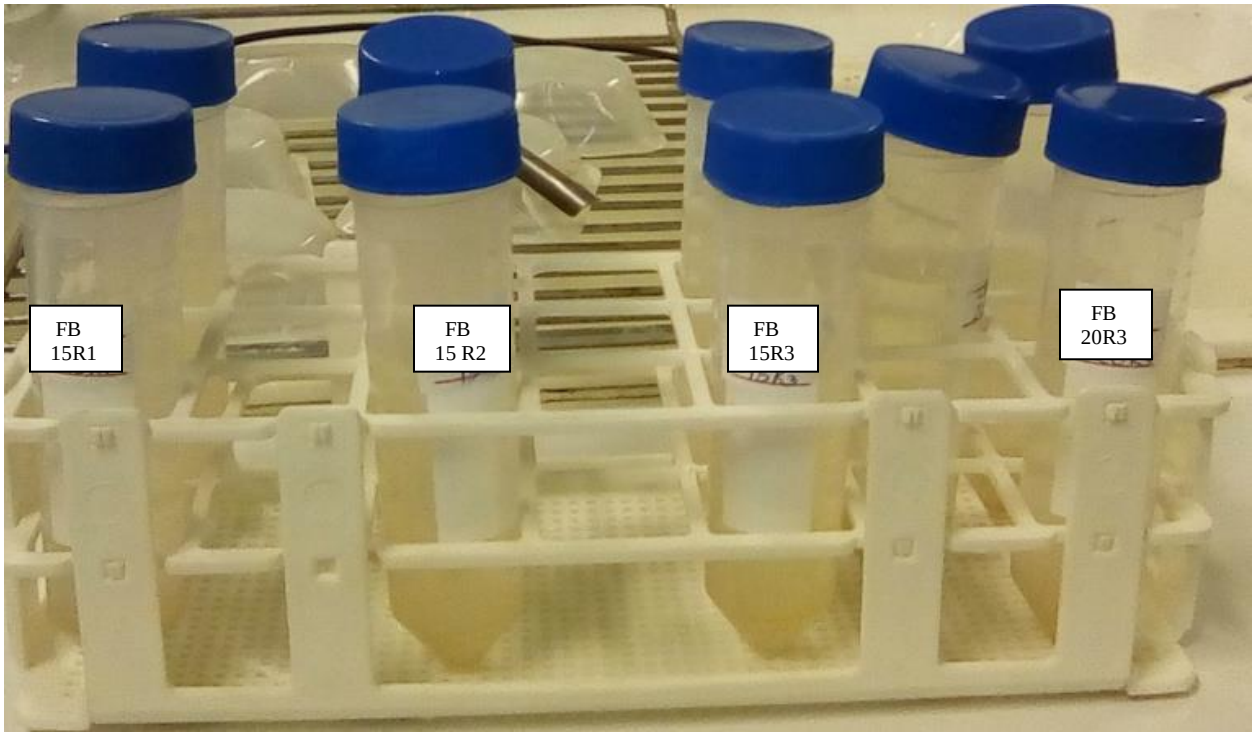
Appendix 10. Coagulation efficiency test of *Moringa stenopetala* protein extract on water sample collected in April, 2019



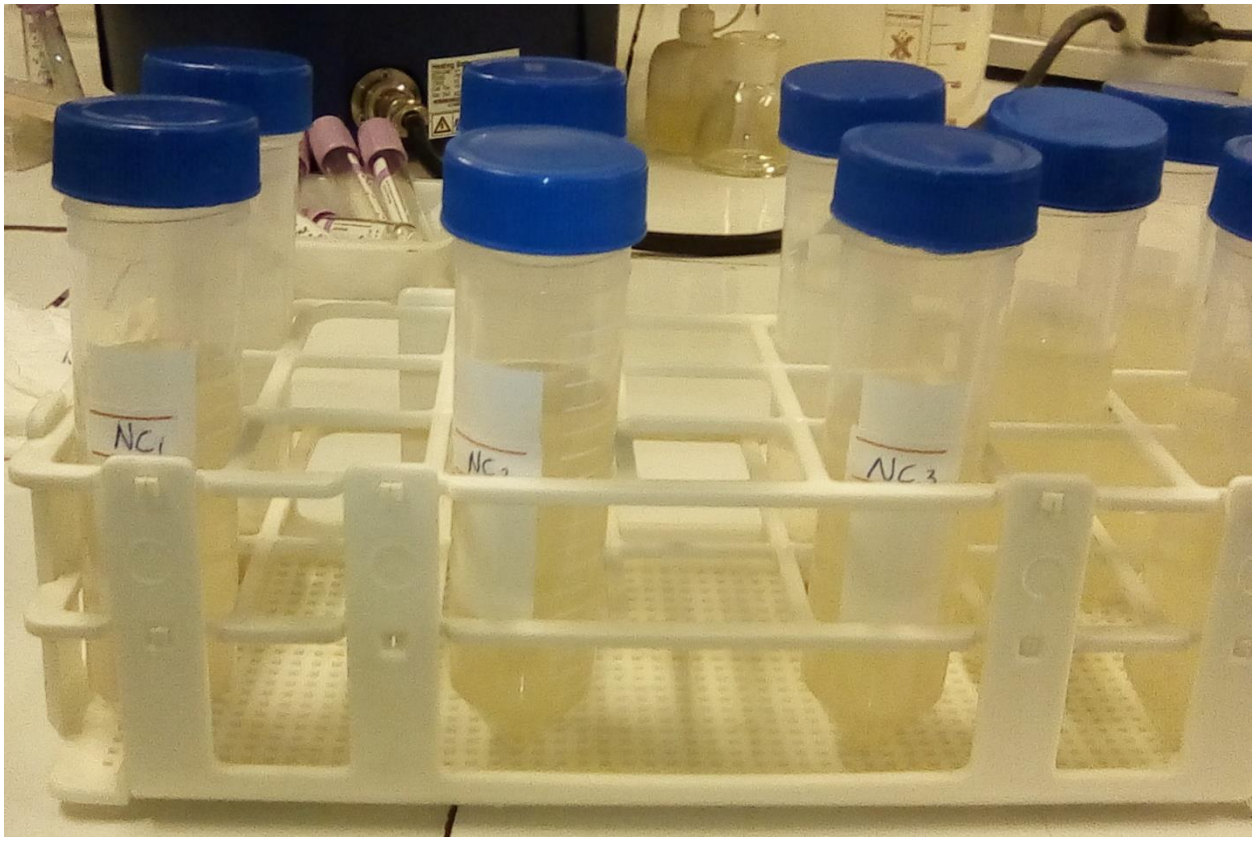
Appendix 11. Coagulation efficiency test of *Trigonella foenum-graecum* (fenugreek) protein extract on water sample collected in April, 2019



Appendix 12. Coagulation efficiency test of *Vicia faba* (faba bean) protein extract on water sample collected in April, 2019



Appendix 13. Raw water (negative control) from water sample collected in April, 2019



Appendix 14. Coagulation efficiency test of polyelectrolyte (positive control) on water sample collected in April, 2019