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Moringa oleifera for drinking water treatment: influence of the solvent and method used in oil-extraction on the coagulant efficiency of the seed extract

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ABSTRACT

Moringa oleifera seeds contain a cationic coagulant protein that can be used either for drinking water clarification or wastewater treatment. The seeds contain 30–40% (w/w) oil, which represents a valuable resource with industrial, food and renewable energy applications. However, the oil also increases the organic matter added to water and avoids storage of treated water and its consumption within 24 h. So oil extraction is recommended as a way to purify the extract. This work proposes solvent extraction using three solvents (hexane, acetone, ethanol) and two procedures (batch and Soxhlet) and analyses for the first time the influence that these factors have on the coagulant efficiency of the extract prepared using defatted seeds. Results show that solvent-defatted seed extracts have a coagulant efficiency comparable to that observed for polyaluminium chloride coagulant (up to 88%), while non-defatted seed extract efficiency is 30% lower. Moreover, ethanol and Soxhlet method for oil extraction allows obtaining a defatted extract that requires doses of coagulant protein 5–33 times lower than hexane or acetone defatted extract to clarify turbid water, reducing in consequence the organic load added. SDS-PAGE analysis showed no influence of the solvent on the nature of the coagulant protein present in the extracts. The results of the study provide an oil extraction procedure that recovers *M. oleifera* oil for industrial purposes and enhances the defatted seeds obtaining an improved coagulant for drinking water treatment.

Keywords: Moringa oleifera; Oil; Solvent extraction; Coagulant efficiency; Water treatment

1. Introduction

Moringa oleifera is a tropical multipurpose tree that naturally grows in India, South Saharan Africa and South America [1]. Almost every part of the plant (leaves, flowers, seeds, roots and bark) can be used as food or for medicinal, cosmetic and therapeutic purposes [2].

Furthermore, *M. oleifera* seeds are widely used for drinking water clarification and wastewater treatment at household level, directly or as crude extract [1]. These seeds contain large amounts of a water-soluble cationic coagulant protein that is able to reduce turbidity in the treated water.

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M. oleifera seeds also contain 33–41% of oil (known as "Ben oil") that is traditionally used as a mechanical lubricant in fine machinery or in cosmetic preparations [3]. *M. oleifera* oil contains more than 70% oleic acid and has a high monounsaturated fatty acid ratio similar to olive oil which makes it suitable for human consumption [4].

The main concern in using *M. oleifera* as coagulant is the significant increase in organic matter of the treated water [5] that prevents storage and requires consumption within 24 h [1]. More recently [6], it has been discussed, the toxicity of insoluble fatty acidic components of *M. oleifera* seed which would remain in the water treated if it is added without any pretreatment. While some authors use crude *Moringa* seeds without removing oil [1,7,8], a few studies have reported the possible interaction of oil in the quality of the treated water [7–9] or in the coagulation process [10]. Therefore, it seems highly recommended reducing organic content of the seed extract before its use in water treatment [5,11]. One of the alternatives is oil extraction before solid–liquid extract preparation, as a way to purify the coagulant protein and thus, reducing organic matter in the treated water with *Moringa* [5,9,10].

Among the different methods for oil extraction, it is reported cold press cake [4], water solutions containing enzymes [12] or supercritical carbon dioxide [13], although the most common process is solvent extraction [8,9,11,14–17]. Solvents that have been used for *Moringa* oil extraction by previous researchers are: hexane [8,9,14,15], ethanol [11], methanol [9], petroleum ether [9,14], acetone [9,14,16,17] or methanol/chloroform mixtures [4,9], and the two most reported extraction procedures are batch and Soxhlet.

With regard to the effect of the oil extraction process on coagulant protein and its efficiency, only a few authors specifically recommend or reprove a specific method or solvent for extraction. Thus, Muyibi et al. [8] and Ali et al. [10], recommend Soxhlet method and hexane solvent because this combination does not reduce coagulant efficiency. On the other hand, Arnoldsson et al. [15], reprove the removal of oil with cyclohexane because it reduces the coagulant efficiency of the extract due to the solubility of the active compound in the solvent.

These varying results indicate that further study is needed to analyse the influence that the method and the solvent used for oil extraction would have on coagulant efficiency of the *M. oleifera* extract.

The present paper focuses on the analysis of the influence that oil extraction method and solvent used have over the coagulant efficiency of the protein for water treatment. Several solvents (ethanol, acetone and hexane) and two extraction procedures (batch and Soxhlet) have been tested with synthetic turbid water, under the same experimental conditions, in order to compare extraction yield and the coagulant efficiency. Besides, results with different extracts have been compared with the most chemical coagulant used in drinking water treatment.

2. Materials and methods

2.1. Materials

M. oleifera seeds were collected during November from the surroundings of Ressano Garcia village with geographical coordinates (25° 26′ 34′′ South, 31° 59′ 43′′ East) in Mozambique. Pods and shells were removed manually and then seeds were dried at 80°C in a laboratory oven (Selecta SA) until a constant weight was achieved. After, the kernels were ground in a domestic blender (Elma) and sieved through a 600 μm stainless steel sieve.

The solvents selected for oil extraction were 95% ethanol (Et), hexane (Hx) and acetone (Acet) (Panreac Quimica).

2.2. Oil extraction procedure

The following two procedures were used with the three solvents mentioned before, in six experimental tests.

2.2.1. Batch (B)

The procedure used was described by [5,9,11,15]. Grounded seeds were defatted using one of the organic solvents selected (ethanol, hexane or acetone) in a 5% (w/v) suspension (5 g of seed per 100 mL of solvent), by mixing with a magnetic stirrer for 30 min. The supernatant was separated by centrifugation (3,000 rpm, 45 min), and the settled powder was dried at room temperature for 24 h.

2.2.2. Soxhlet (Sx)

Fifty grams of *M. oleifera* crushed seeds were fed to a lab-scale Soxhlet extractor fitted in a 500-mL round bottom flask with 350 mL of solvent (ethanol, hexane or acetone). Extraction time was 6 h, and 20 cycles were performed. Each test was repeated three times for each solvent.

The extracted oil yield was expressed as a percentage (% w/w), which is defined as the weight of oil extracted with that of the *M. oleifera* dried seed.

2.3. Coagulant protein extraction

A protein extract was prepared with crude or defatted seeds (oil extracted with the procedure of point 2.2) and surface river water in a 5% (w/v) suspension (5 grams of seed per 100 mL of water), which was mixed with a magnetic stirrer for 60 min and left to settle for 20 min. The *M. oleifera* crude extract was then filtered through a 0.45-μm cellulose acetate filter (Spartan 30 B, VWR International).

2.4. Coagulant efficiency test

Jar tests were conducted in 1 L beakers to determine the effective dosage of coagulant able to reduce the turbidity of the sample. Conditions for Jar test were high stirring speed of 100 rpm during 2 min, followed by low stirring speed of 30 rpm for 15 min and settling time of 20 min. Supernatant was collected from each beaker, and the turbidity was measured using a D 112 turbidimeter (DINKO Instruments).

The residual turbidity was used as a basis for comparing the coagulant efficiency (in percentage) calculated with Eq. (1):

$$\text{Coagulant efficiency}(\%) = \frac{(\text{Initial turbidity} - \text{Residual turbidity})}{\text{Initial turbidity}} \times 100 \quad (1)$$

Jar tests were performed with turbid synthetic water made of 10 g/L kaolin (Merck) in distilled water, with an initial turbidity of 200 ± 4 NTU.

Polyaluminium chloride (PAC) was selected as a control in all experiments due to its extended use as coagulant in drinking water treatment plants. A 10-g/L commercial solution (PAX XL 18) of PAC was prepared. Non-defatted seed extract (OCE) was also included as a blank in all the tests.

Fig. 1 shows a block diagram of the experimental procedure.

2.5. Protein content

Samples of crude extract were also analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using an 18% gel following the protocol of Laemmli [18]. The protein content of the extract was estimated by the Bradford method [19] using bovine serum albumin as a standard. The same protein mass was loaded for each sample in the gel, and calculated using Eq. (2):

$$\text{Protein mass added}\,(\mu g) = \text{Protein content}\left(\frac{\mu g}{\mu L}\right) \times \text{extract volume}\,(\mu L) \quad (2)$$

Coomassie blue dye (Sigma–Aldrich) was used to visualise the protein bands. The molecular marker used was Page Ruler™ (Fermentas).

3. Results and discussion

3.1. Extraction method: oil extraction yield

In order to evaluate the most effective method to extract oil from *M. oleifera* seeds, oil extraction yield has been considered.

The influence of the procedure used for oil extraction was analysed for two extraction methods (batch and Soxhlet) and three solvents (acetone, hexane and ethanol). The average results are summarised in Table 1. Mean relative error was below 2.2% for all tests.

The oil extraction yield was higher for the Soxhlet method (32–34.4%) than for the batch method (16–18.6%) for all tested solvents. The higher yield values obtained for Soxhlet extraction can be due to the fact that solid contacts with pure solvent after each stage, which favours mass transfer and results in the extraction of more amount of oil. However, no significant difference in oil yield is shown between solvents for the same procedure performed. The values obtained for the Soxhlet experiments were in agreement with those obtained previously by other authors for hexane [8,14] and acetone [14]. No values have been reported in the literature for batch extraction yield using *Moringa* seeds.

According to these results, tests with batch method are not included in the following sections.

3.2. Influence of the oil extraction method and solvent on the coagulant efficiency

Soxhlet procedure was used to defat seeds used to prepare protein extracts for carrying out the coagulant efficiency test. Extracts prepared with crude *Moringa* seeds (OCE) and PAC were also included. It also was included a blank (turbid water without coagulant).

Fig. 2 presents average results of coagulant efficiency evolution with the increase of coagulant concentration added. Coagulation concentration has been calculated considering that all the seed added for extract preparation is solved [5]. In each experimental series, high dosages of coagulant caused overdosing,

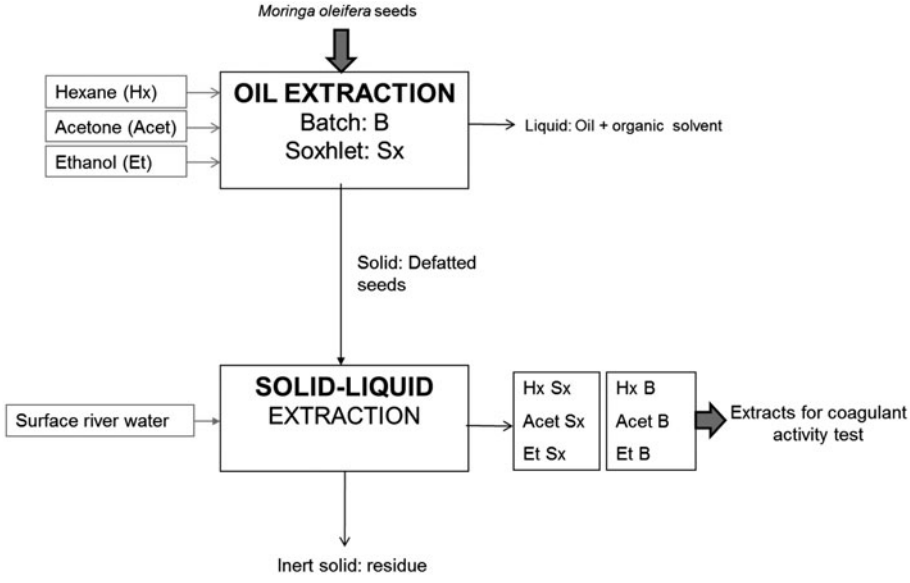

Fig. 1. Schematic diagram of the samples preparation for coagulation tests.

Table 1
Oil extraction yield for both methods and solvents

	Oil extraction yield (w/w) (%)		
Method	Hexane	Ethanol	Acetone
Batch	17.18	15.99	18.56
Soxhlet	34.37	31.99	32.73

which reverses the charge of destabilised particles [20]. As a result, residual turbidity increases [21] and coagulant activity decreases. Experimental point is selected as the best in each series is the first with the highest coagulant efficiency and the lowest coagulant concentration added.

The blank is not shown in the figure since it represents a constant value of 2.5% on average as a consequence of the natural settling of the kaolin as no coagulant is added in this case.

As it can be observed, PAC showed maximum coagulant efficiency of 92.71% for optimum dosages of 20 mg/L. Comparing the three solvents used for oil extraction, it is shown that in the case of ethanol the maximum coagulant efficiency value is 91.63% for

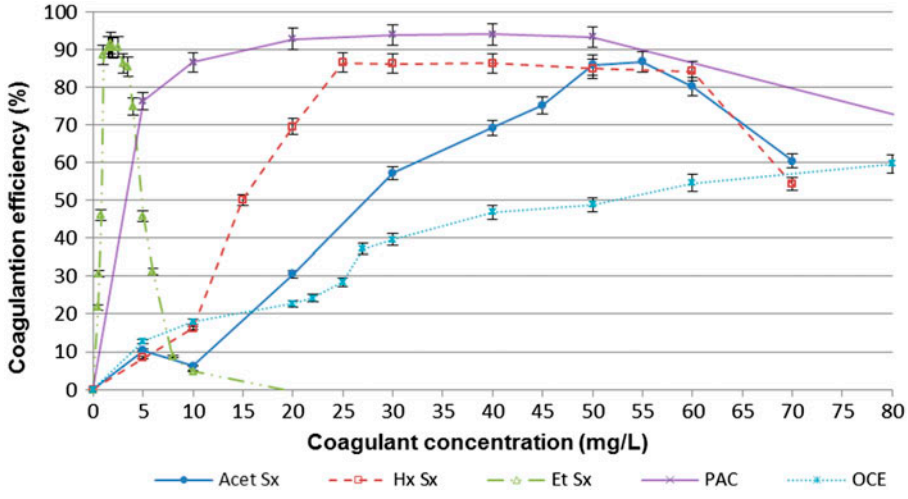

Fig. 2. Coagulant efficiency test for the Sx extracts.

Table 2
Protein extract concentration and mass of protein needed to achieve maximum coagulant efficiency

	Protein concentration (μg/μL)	Maximum coagulant efficiency (%)	Protein mass added for maximum coagulant efficiency (μg)
Acet Sx	12.95	85.79	7,122.5
Hx Sx	12.58	86.43	3,145.0
Et Sx	11.92	91.63	214.2
OCE	10.41	59.67	8,328.0

2 mg/L of the extract, while in the case of hexane and acetone values of maximum coagulant efficiency are lower (86.43 and 85.79%, respectively) at higher coagulant concentration doses (25 and 50 mg/L, respectively).

With regard to the presence of oil, it is clearly shown that the coagulant efficiency of the crude extract (OCE) was seriously reduced by the presence of oil, reaching values of 59% of coagulant efficiency for 80 mg/L of extract. So, the presence of oil in *M. oleifera* seed extract reduces significantly the coagulant efficiency of the seeds. It seems that the oil could form an emulsion or a layer bound to the protein that interferes with the suspended solids that are responsible for water turbidity, as observed by previous authors [8].

3.3. *Interaction of the solvent in the effectiveness of the* M. oleifera *extract*

Table 2 shows average values of protein concentration and protein mass added, calculated according to Eq. (2), for acetone, hexane and ethanol, Soxhlet oil defatted seed extracts and OCE. Mean relative error was below 3.5% for all the tests.

Results of Table 2 show that there is not an important difference in the protein concentration present in the extracts, being slightly lower for OCE. With regard to the influence of oil in coagulant efficiency, it is observed that maximum coagulant efficiency for OCE is near 60%, which represents a 30% lower than for the defatted extracts. Moreover, the amount of protein needed to achieve maximum coagulant efficiency is the highest one of all the extracts studied. This result confirms that oil interferes in the coagulant capacity of the protein by reducing floc formation, so its extraction is recommended to enhance turbidity removal as recommended previously by [8].

When solvent is used for oil extraction, protein extracts used in water treatment shows values of maximum coagulant efficiency similar for all extracts, with no appreciable influence of the type of solvent used to extract the oil. However, the amount of protein added to reach maximum coagulant efficiency varies significantly for each extract: 214.2 micrograms of protein for Et, 14 times higher for Hx and 33 times higher dose of protein for Acet. Therefore, it seems that Et increases protein coagulant effectiveness in comparison with the other two solvents.

In order to check if each solvent extract has the same type of protein from seeds, SDS-PAGE analysis was performed.

Fig. 3 shows results for SDS-PAGE under reducing conditions for *Moringa* crude extracts.

A band below 6.5 kDa is observed in all coagulant extracts analysed. This band corresponds to the main protein present in *Moringa* seeds, which is responsible for the coagulant capacity in accordance to previous studies [9,11]. Moreover, no difference in the molecular weight of protein was observed between OCE and the defatted seed extracts regardless of the solvent used for oil extraction. Therefore, the differences on the coagulant effectiveness (in terms of added protein) should be found in the nature of the solvent used for oil extraction and the influence of its polarity over the

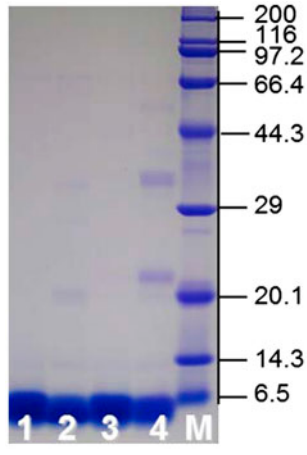

Fig. 3. SDS-PAGE analysis for proteins prepared from *M. oleifera*.
Notes: (1) Et Sx, (2) Ac Sx, (3) Hx Sx, (4) OCE and (M) molecular weight marker (kDa).

Table 3
Health and safety guidelines and environmental hazard of the solvents selected

Solvent	Health and Safety products based on Safe Data Sheet. European Regulation 1907/2006	Extraction solvents used in the production of foodstuffs and food ingredients. European Directive 2009/32/EC
Hexane	Highly flammable liquid and vapour Causes skin irritation. Long term exposure may cause damage to organs. Suspected of damaging fertility. May cause drowsiness or dizziness May be fatal if swallowed and enters airways Toxic to aquatic life with long lasting effects	With conditions of use Maximum residue limits of 1 mg of solvent per kg of oil obtained
Acetone	Highly flammable liquid and vapour Causes serious eye irritation Repeated exposure may cause skin dryness or cracking May cause drowsiness or dizziness	No limitations Its use results only in the presence of residues or derivatives in technically unavoidable quantities presenting no danger to human health
Ethanol	Highly flammable liquid and vapour	

structure of the protein. It is reported that proteins in non-aqueous environments, as organic solvents, can modify its structure and change its properties, as their activity, increasing it [22]. In this case, the contact between the protein and the solvent during oil extraction seems to affect the *Moringa* protein, increasing its effectiveness when is used as extract in the coagulation process. As a consequence, it is observed that seeds defatted with ethanol (polar protic solvent) require 14 and 33 times less dose of protein than acetone (aprotic solvent) or hexane (non-polar) defatted seeds. It seems that the less polarity of the solvent used in oil extraction, the highest dose of coagulant protein to reach maximum coagulation efficiency.

3.4. Analysis of solvent adequacy

According to the previous results, ethanol is the best solvent for oil extraction from *M. oleifera* seeds since coagulant efficiency of the *M. oleifera* extract is similar to PAC (synthetic coagulant) with the lowest coagulant dosage in comparison to the other tested solvents.

As the aim of the extract application is to produce drinking water, it is important to consider the toxicity, human danger and environmental hazard of the selected solvent.

Table 3 summarises the hazards according to European regulations for production of foodstuffs using solvents and health and safety information based on its physicochemical properties.

Comparing three solvents, it can be seen that ethanol has lower hazards related to health and safety for humans. As *M. oleifera* seeds are used for preparing coagulant extracts later applied to produce drinking water for human consumption, residues of solvent used for oil extraction must not show danger to human health. According to European Directive 2009/32/EC, ethanol has no limitations in the presence of residues.

This confirms ethanol as the best solvent for oil extraction from *M. oleifera* seeds used in coagulant preparation for drinking water treatment.

Finally, Al-Anizi et al. [6] reported recently that main toxicity of *Moringa* is from insoluble fatty acidic components of the seed. Oil removal using Soxhlet process and ethanol, as we have discussed in this work, would contribute to reduce toxicity of the seed and to the use of this natural coagulant widespread for drinking water treatment.

4. Conclusions

The results of this study demonstrate the influence of the solvent and the procedure used for oil extraction on the effectiveness of the coagulant protein of the extracts:

(1) Soxhlet extraction procedure performs higher yield in oil extraction than batch method (about 50%).
(2) Crude extracts prepared from defatted seeds are more effective than non-defatted seeds, as coagulant efficiency is on average 30% higher. The coagulant efficiency obtained for all *Moringa* extracts prepared from defatted seeds is similar to that observed for PAC.
(3) Ethanol-defatted seed extract has shown the best results on efficiency tests as coagulant doses required are significantly lower than those for the extracts prepared from acetone or hexane defatted seeds.

(4) It has been determined that all the extracts have the same protein concentration. However, the protein mass added to achieve maximum coagulant efficiency with the ethanol extract is 5–33 times lower than for the acetone- or hexane- defatted seeds extracts. This shows the higher efficiency of the protein in the extract obtained with ethanol as solvent.
(5) SDS-PAGE analysis showed no influence of the solvent on the nature of the coagulant protein present in the extracts.
(6) Ethanol is an allowed solvent for the production of foodstuff with no limitations of using according to the European regulations. Thus, the presence of traces of this solvent in the crude extract will not represent a danger to human health.

For all these reasons, it can be concluded that *Moringa* oil extraction using ethanol before extract preparation is recommended to improve coagulant efficiency of the extract, and to achieve maximum coagulant efficiency with lower doses of protein. Furthermore, oil extraction is dual advantageous because it allows oil recovery for industrial and food applications, and decreases organic load added to water when defatted seeds are used for drinking water treatment.

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