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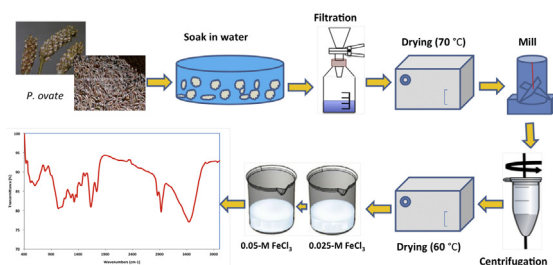
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A novel method for extraction of a proteinous coagulant from *Plantago ovata* seeds for water treatment purposes

Bahman Ramavandi^{*}, Seyedenayat Hashemi, Raheleh Kafaei

Environmental Health Engineering Department, Faculty of Health, Bushehr University of Medical Sciences, Bushehr 7518759577, Iran

GRAPHICAL ABSTRACT



ABSTRACT

Several chemicals have been applied in the process of coagulant extraction from herbal seeds, and the best extraction has been obtained in the presence of KCl or NaNO₃ [1–3], and NaCl [4]. However, the main challenge posed to these methods of coagulant extraction is their relatively low efficiency for water treatment purposes and the formation of dissolved organic matter during the treatment process. In these methods the salts, which have a one-valance metal (Na⁺ and K⁺), are deposited in the internal structure and the pore of the coagulant, and may be useful for the coagulation/flocculation process. In this research, we found that modified methods produced more dense protein. Therefore, the modified procedure was better than the older one for removal of turbidity and harness from the contaminated water. Here we describe a method where:

- According to the Hardy–Schulze rule, we applied the Fe³⁺ ions instead of Na⁺ and K⁺ for the extraction of protein from *Plantago ovata* seeds.
- The method was narrowed to extract protein by ethanol (defatting) and ammonium acetate and CM-Sepharose (protein extraction).
- Two consecutive elutriations of crude extract was directly performed using 0.025-M FeCl₃ and 0.05-M FeCl₃ according to the basis of the ion-exchange processes.

^{*} Corresponding author. Tel.: +98 9363311903; fax: +98 77334550134.

E-mail addresses: ramavandi_b@yahoo.com, b.ramavandi@bpums.ac.ir (B. Ramavandi).

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ARTICLE INFO

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Method details

1. Dry *Plantago ovata* seeds
2. Ammonium acetate
3. Sodium hydroxide
4. Hydrochloric acid
5. Ferric chloride
6. Calcium chloride
7. Ethanol (98%)
8. CM-Sepharose
9. Distilled water
10. Membrane filter (0.42 μm)

Extraction and purification processes of coagulant from *Plantago ovata* seeds

Extraction of the crude coagulant from *P. ovata* seeds was done as follows:

- The seeds were soaked in distilled water for 1 day.
- The obtained gelatinous material was passed through 0.42 μm membrane filters and dried at 70 °C in an oven and then milled in a domestic blender (VARING).
- The obtained powder was defatted by mixing it with ethanol 98% using a magnetic stirrer for 60 min. Then, the supernatant was separated by centrifugation (3500 rpm, 40 min), and the settled powder was dried overnight in an oven (at 60 °C).
- The crude coagulant was extracted from the oil-free powder using 10-mM ammonium acetate in 5% w/w. The mixture was stirred for 50 min using a magnetic stirrer, and the supernatant, namely the crude extract, was separated by centrifugation (3500 rpm, 40 min).

The purification of the coagulant protein from the crude extract was carried out as follows:

- CM-Sepharose ion exchange was equilibrated using a 10-mM ammonium acetate solution.
- Equilibrated CM-Sepharose ion exchange was added to the crude extract in 10% (v/v) proportion and mixed using a blade stirrer for 50 min.
- Finally, the absorbed coagulant protein was eluted by different concentrations of an FeCl₃ solution.

The purification procedure was performed directly with 0.025-M FeCl₃ first and then with 0.05-M FeCl₃ elution according to the basis of the ion-exchange processes [4,5]. In the first elution, proteins that presumably did not lead to a higher coagulation performance (efficiency less than 45%) and simply added dissolved organic carbon (DOC) (about 3.5 mg/L) in the treated water were removed. Consequently, the second elution produced a more purified coagulant as it contained only the active coagulant proteins. The elution stages were repeated three times to recover as much coagulant as possible. Further, after the both purification of extraction by FeCl₃, the resultant coagulant was rinsed many times with double distilled water until Fe ions not detectable in rinsed water. The coagulant

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