Laboratory Practices for Purification of Polysaccharide

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ABSTRACT

The present work aims to purify the mucilage polysaccharide extracted from linseed by deproteinization using Trichloroacetic acid (TCA) and Hydrochloric acid (HCl). 10% w/v TCA deproteinized more than 80% when the pH of the polysaccharide solution was 3. TCA can be considered as a better deproteinizing agent when compared to hydrochloric acid as evidenced by the highest deproteinization efficiency (88.57%).

INTRODUCTION

Nature continuously synthesizes huge amounts of polysaccharides, which serve particularly as structural scaffolds like cellulose in plants and chitin in animals or as storage carbohydrates like starch and glycogen[1]. One important source of polysaccharides is seeds of Linseed, a well-known and easily available drug in the research laboratory. The present work has focussed on the extraction of Linseed mucilage polysaccharide by using acetone as solvent and purification using trichloroacetic acid in comparison with hydrochloric acid for the precipitation of protein by pH adjustment.

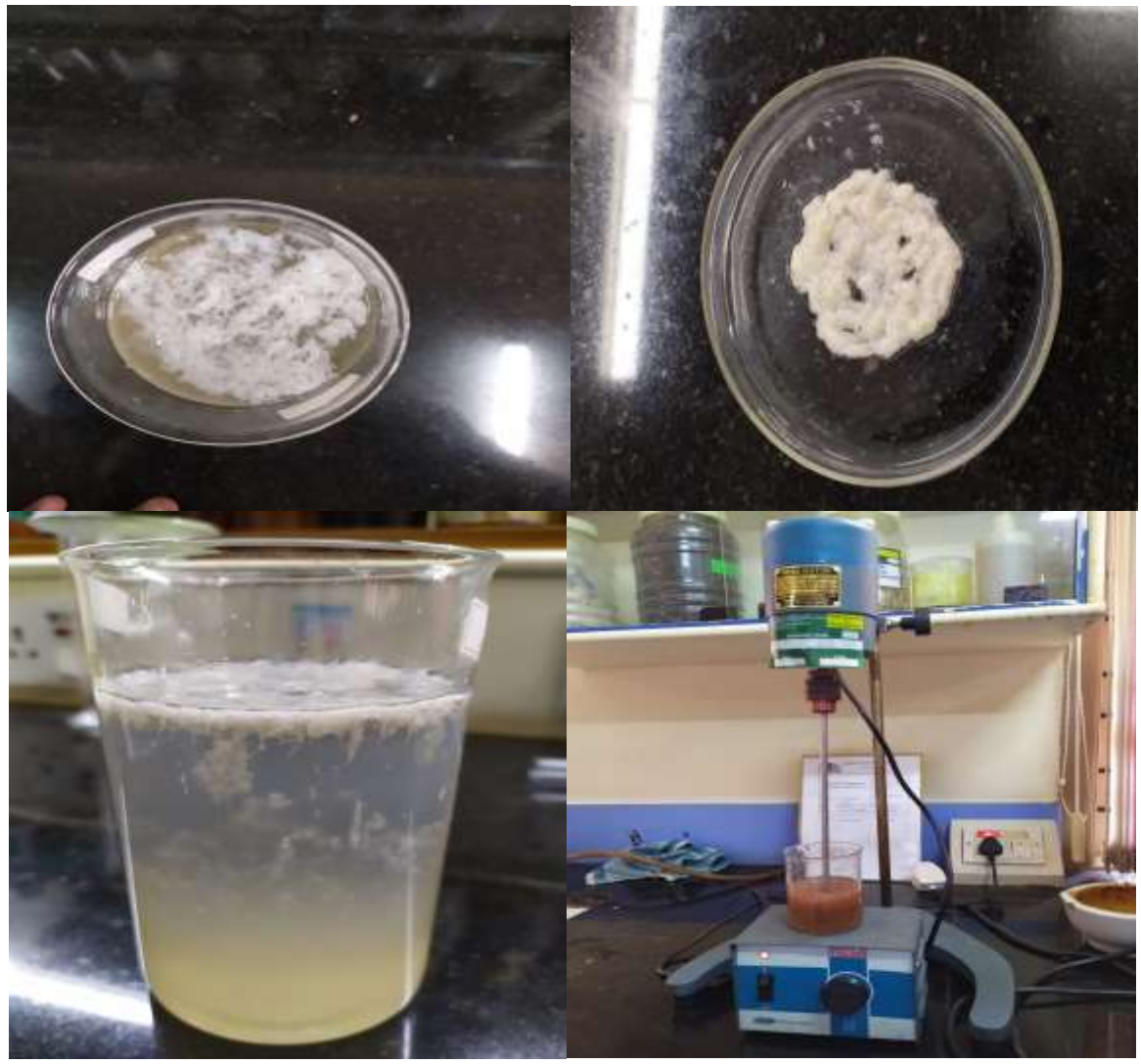
METHODS

A UV absorbance ratio at A/260/280 is used to characterize if the polysaccharide contained proteins and nucleic acids. The total protein content was assayed by the Lowry method using Bovine serum albumin as standard.

PURIFICATION TECHNIQUES

Linseed was soaked and extracted in distilled water (300 ml) by electrical stirring (Figure 1) for 24 hours and to the filtrate twofold the volume of acetone (4 times the volume of aqueous filtrate) was added for the complete precipitation of crude polysaccharide. Then the aqueous polysaccharide solution was treated with 10% (w/v) trichloroacetic acid and hydrochloric acid for adjustment of other pH values (3, 4, and 5) separately to remove any adherent protein contaminants and deproteinized.

Fig.1. Polysaccharide extracted from Linseed and its aqueous solutions



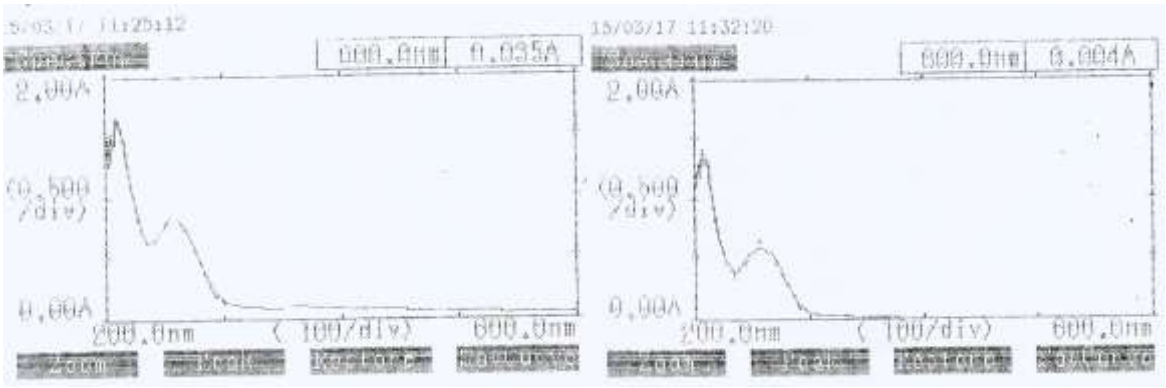


Fig.2 (a & b). Showing UV spectra of polysaccharide before deproteinization

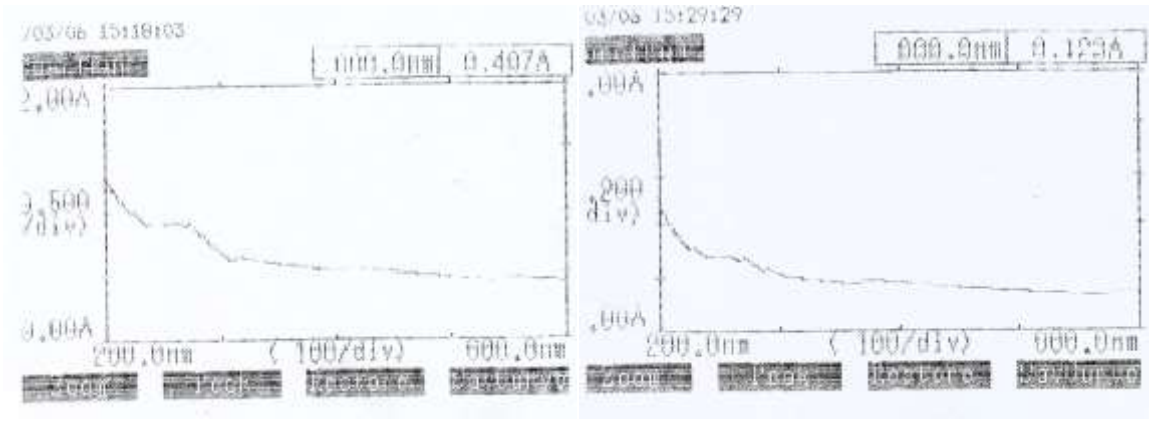


Fig.3 (a & b).UV spectra of polysaccharide after deproteinization by Hcl & TCA at pH 3

Determination of protein concentration and deproteinization efficiency

The concentration of protein was calculated according to the standard calibration curve. The percentage of deproteinization efficiency was calculated by Co-Ce/Co x 100 where Co and Ce were the protein concentration before and after deproteinization by trichloroacetic acid and hydrochloric acid respectively.

Table 1. Concentration of protein after treatment with Hcl at different pH

S.No.	pH	Protein (µg/ml)	Deproteinization efficiency (%)
1	6.5	140 (BD)	----
2	5	120	54.28
3	4	95	72.14
4	3	80	82.85

BD – Before Deproteinization

Table 2. Concentration of protein after treatment with TCA at different pH

S.No.	pH	Protein (µg/ml)	Deproteinization efficiency (%)
1	6.5	140 (BD)	----
2	5	110	61.42
3	4	87	77.85
4	3	72	88.57

BD – Before Deproteinization

RESULTS AND DISCUSSION

Nucleic acids and proteins were detected as indicated by absorption at 260-280 nm in the UV spectrum. The result obtained is presented in Figure 2 (a & b) which shows the UV spectra of Linseed polysaccharide. Before deproteinization, the total protein content of the aqueous polysaccharide solution used in the precipitation assay was 140 µg/ml using BSA as standard.

In the first method of deproteinization, the initial pH 6.5 was decreased by the addition of HCl to pH 5. The same procedure was followed for the aqueous extract to other pH values (4 and 3). After deproteinization, not many nucleic acids and proteins were detected in all the aqueous solutions of different pH as indicated by less absorption at 260-280 nm by the effect of hydrochloric acid. Figure 3a. shows the UV spectra of polysaccharide after deproteinization by HCl at pH 3. The method enabled a reduction of the proteins to 80 µg/ml of the total protein content at pH 3. The result obtained is presented in table 1. The deproteinization efficiency was 82.85 %.

In the second method, the initial pH 6.5 was decreased by the addition of 10 % w/v trichloroacetic acid to other pH values. The same procedure was followed for the aqueous extract with another pH value. The result obtained is presented in table 2. Figure 3b shows the UV spectra of Linseed polysaccharide after deproteinization by TCA at pH 3. At 10 % w/v concentration, trichloroacetic acid proved to be efficient in precipitation of protein reducing to 72 µg/ml at pH 3. The deproteinization efficiency was 88.57 %. TCA can be considered as an better deproteinizing agent when compared to hydrochloric acid as evidenced by the highest deproteinization efficiency (88.57%).

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REFERENCES

1. Kogan G (2000). 1-6 beta D glucan of yeast and fungi and their biological activity. Studies in Natural Products Chemistry, 23, 107-117.