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DETECTION OF PROTEINS FROM PLANT LEAVES USING NEAR-INFRARED SPECTROSCOPY

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ABSTRACT

One of the major primary metabolites of plants is proteins. They can be purified easily from plants using precipitation. The purpose of this research was to investigate the protein content present in the leaves of three plants namely, *Cissus quadrangularis*, *Coleus amboinicus* and *Aloe vera*. In case of leaf and stem samples 30%-70% of ammonium sulphate gave maximum yield of protein while flower samples yielded maximum protein at 80% ammonium sulphate. The extracted fractions were further analysed using near infrared spectroscopy.

Keywords : *Cissus quadrangularis*, *Coleus amboinicus*, *Aloe vera*, Protein extraction, protein fractionization

Introduction

The prominent source of protein is our diet. Protein from our diet get broken down or digested into amino acids. These amino acids are further reassembled into other new proteins by translation leading to protein synthesis (Young and Pellett, 1994; Hu, 2003). Protein foods can be divided into groups depending on the amount of protein they contain namely; high protein foods that contain about 20% protein (meats, fish, eggs, nuts, seeds, milk products, beans, spirulina etc), Medium protein foods that are incomplete proteins contain 6-14% protein (rice and other grains) and low-protein foods containing less than 5% protein which includes fruits, vegetables and juices (Okwu, 1999, 2001; Hill, 1952). Nearly 80% of world population still rely on traditional medicines (Owolabi *et al*., 2007; Jain, 1968; Okigbo *et al*., 2008)

Proteins or any organic compound can be estimated by invasive or destructive methods or by non invasive or non destructive methods. One such non destructive method is the use of Raman spectroscopy to detect proteins. It helps to find the protein structural information from Raman vibrational bands such as: amide I, amide II and amide III bands (Goormaghtigh *et al*., 1994a; Goormaghtigh *et al*., 1994b; Goormaghtigh *et al*., 1994c). All other protein conformations help in detecting the proteins (Siebert, 1995; Jackson and Mantsch, 1995). The present study was carried out to isolate and fractionate the proteins using ammonium sulphate from leaves, flower and stems of *Cissus quadrangularis*, *Coleus amboinicus* and *Aloe-vera* and to confirm their presence using NIR spectroscopy.

Material and Methods

The present work was undertaken to understand the protein content at various fractions of ammonium sulphate precipitation which will be the basis for further research on plant proteins.

Plant Materials

Three plants were selected for the study namely, *Cissus quadrangularis, Coleus amboinicus* and *Aloe vera* (Fig. 1, 2, 3). They were collected from the University campus. The leaves of each plant were used for the investigation.

A B C

Fig. 1 : Leaves of *Cissus quadrangularis*

A B C

Fig. 2 : Leaves of *Coleus amboinicus*

A B C

Fig. 3 : Leaves of *Aloe vera*

Extraction of proteins

The plant samples were collected and about 3 grams of leaf are weighed and were ground using a pestle and mortar with Phosphate buffer pH 7.0. The protein sample was prepared by centrifuging at 10, 000 rpm for 10 min and collecting the supernatant. The supernatant was used as the crude extract for the precipitation of protein.

Ammonium sulphate precipitation

To the supernatant of each sample solid ammonium sulphate was added to precipitate the protein. The concentration of ammonium sulphate used ranged from 0-70% for each sample. The supernatant was taken in a beaker and the required percentage of ammonium sulphate as mentioned in the protein fractionization table was added. The contents were stirred well using a magnetic stirrer for 20 min and then centrifuged at 10, 000 rpm for 10 min. After centrifugation the supernatant was collected separately and the precipitate was suspended in 500 µl of phosphate buffer and stored at 4º C. The supernatant was used for the next precipitation step till 70% saturation of ammonium sulphate was achieved.

Determination of protein content

Protein was estimated using Bradford method (1976).

Determination of protein content using non-invasive method

The protein content was determined by a non invasive method using near infrared spectroscopy. About 10 ml of the protein fraction was taken in a beaker for detecting the protein content.

Results and Discussion

The protein from *Cissus quadrangularis*, *Coleus amboinicus* and *Aloe vera* was extracted and fractionated using different concentrations of ammonium sulphate precipitation.

The protein was estimated using Bradford method and the results are tabulated in Tables 1, 2 and 3. It was found that significantly more proteins were precipitated at lower percentages of ammonium sulphate when compared to the leaves and stems of all three plants. In case of leaves and stem maximum amount of protein was precipitated between 30% and 70 % in all the three plants analyzed. The protein fractions obtained will used to identify individual proteins in our next study.

Table 1 : Protein content from different fractions of ammonium sulphate precipitation from leaves of *Cissus quadrangularis*

$(NH_4)_2SO_4$%	Protein mg/ml
20	0.576
30	0.595
40	0.646
50	0.606
60	0.704
70	1.020
80	1.140

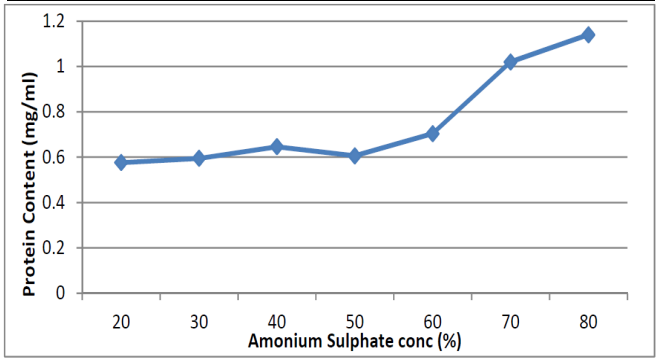

Fig. 4 : Protein fractionation of *Cissus quadrangularis*

The Fig 4 shows us the different concentration of protein at different per cent of ammonium sulphate concentration. At 20% the protein contain found to be nearly 0.576mg/ml that is very less and at 80% the protein contain found to be higher i.e. nearly 1.140mg/ml.

Table 2 : Protein content from different fractions of ammonium sulphate precipitation from leaves of *Coleus amboinicus*

$(NH_4)_2SO_4$%	Protein mg/ml
20	0.186
30	0.480
40	0.493
50	0.532
60	0.560
70	0.594
80	0.918

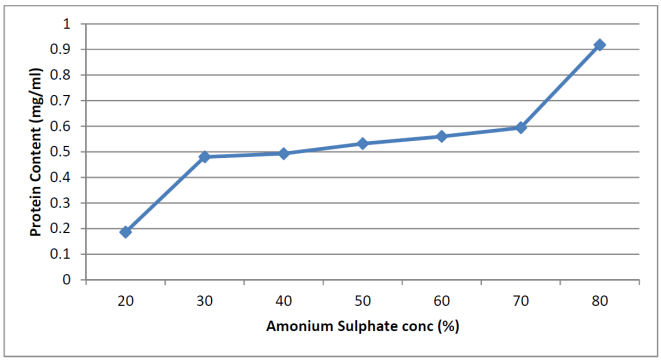

Fig. 5 : Protein estimation of *Coleus amboinicus*

Fig 5 shows us the different concentration of protein at different per cent of ammonium sulphate concentration. At 20% the protein contain found to be nearly 0.186 mg/ml that is very less and at 80% the protein contain found to be higher i.e. nearly 0.918mg/ml.

Table 3 : Protein content from different fractions of ammonium sulphate precipitation from leaves of *Aloe vera*

$(NH_4)_2SO_4$%	Protein mg/ml
20	0.688
30	0.714
40	0.760
50	0.780
60	0.858
70	1.292
80	1.444

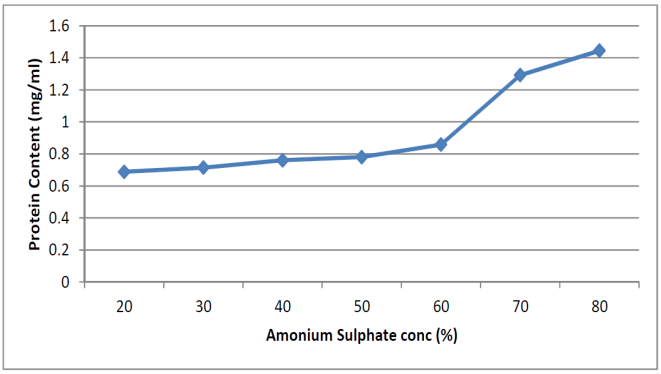

Fig. 6 : Protein estimation of *Aloe vera*

Fig 6 shows us the different concentration of protein at different per cent of ammonium sulphate concentration. At 20% the protein contain found to be nearly 0.688 mg/ml that is very less and at 80% the protein contain found to be higher i.e. nearly 1.444mg/ml.

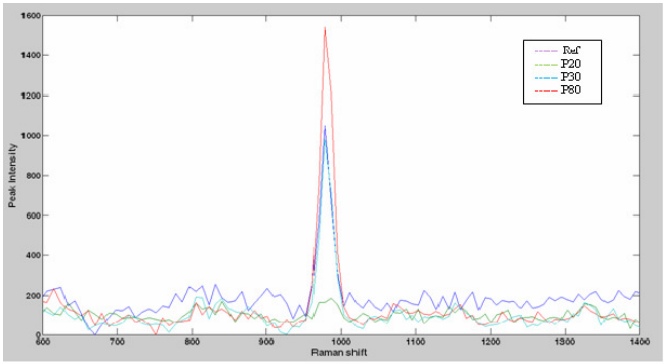

Fig. 7 : Raman spectroscopy of the protein fractions

Note: Raman spectra for protein samples (Ref, P20, P30 and P80); Laser Wavelength: 1065nm; Laser Power: 490mw; Integration Time: 1000ms; Peak Intensity wave number: 978.4 cm^{-1}

The presence of protein was determined using Raman spectroscopy. Fig 7 shows the Raman spectrum of three protein fractions of *Aloe vera*. The reference sample taken was that of BSA. The fraction containing 30% ammonium sulphate contained more proteins when compared to the other fractions.

Conclusion

There are many ways to estimate the amount of proteins. Most of them are invasive methods that are laborious. Non invasive methods are simple and can estimate the protein in the native state. This paper tells us about the invasive method of protein estimation using NIR spectroscopy. It is useful to check the presence of any organic compound in plants without destruction. We would like to explore more on this aspect in future.

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