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Proximate and Phytochemical Analysis of Seed Coat from *P. sumantranse* (Little Millet)

Sandeep Raja Dangeti[1,2]*, S Karthikeyan[2], Githa R Kumar[2] and Sarth Desai[2]

[1]Biomedical Informatics, New No: 42, 2nd Main Road, Adayar, Chennai, I600020, India
[2]School of Biological Sciences and Technology, VIT University, Vellore, Tamilnadu, 632014, India

Abstract

Little millets (*P. sumantranse*) has health benefits, and as well as economic importance. The rural farmers at Javadhu hills near Vellore district were exploited by the retailers in processing of little millets. We took this problem, in removing the seed coat from this seed in economic way using enzyme based technology. In this process, we concentrated in proximate and phytochemical analysis of the seed coat for better understanding of its chemical properties, prior to enzymatic treatment. 100 gms of seed coat is removed from the seed through mechanical treatment and we carried the proximate analysis, i.e. reducing sugar content, total carbohydrate, elemental analysis, moisture content, total fat, total protein, total fiber content and Total ash. The results found to be total carbohydrate-47.85 gms/100 gms of seed coat, total protein-6.26 gms/100 gms, total fat-2.03 gms/100 gms, ash content (by dry basis)-20.51 gms/100 gms and moisture content to be 10.16%. The phytochemical analysis showed that flavanoids 0.18 gms/100 gms and phenolics 0.32 gms/100 gms.

Keywords: Little Millet; Phytochemical analysis; Alkaloids

Background

The transformation of agriculture to more productive systems has often been accompanied by increased production of a fewer crops species. Concurrently, the area and production of a great diversity of traditional crops have declined. Yet in many parts of the world, these traditional crops play an important role in maintaining stable and sustainable forms of agriculture. One such traditional group of cereal crops is the little millets. (The millets considered here are those being cultivated in the subtropical and tropical areas of the Old World), little millet (*Panicum sumatrense*) the grains of little millets, being nutritionally superior to rice and wheat, provide cheap proteins, minerals and vitamins to poorest of the poor, where the need for such ingredients is the maximum. Practically devoid of grain storage pests, the little millets have indefinite storage life [1]. The untapped grain yield potential coupled with nutritional superiority makes the little millets potential future food crops, particularly in the more difficult rain fed areas. Little millets have high nutritional and medicinal value, high dietary fibre content, easy digestibility and other uses. It has medicinal values in the management of diabetes and lowering lipids esp. cholesterol. Best remedy for obese, diabetic, elders and flat belly aspirants. The rural farmers at Javadhu hills near Vellore district were exploited by the retailers in processing of the seeds from little millets. The retailers used to cost around 1.5 \$/kg for processing of seed. The farmers are always exploited by the middle man, as they do not have accessibility to technology. We taken this problem as a serious issue and we started developing an enzyme based technology [2-4], which could decrease the processing cost of the seeds. In the article, we are providing the chemical composition and nutrient value of the seed coat from the little millet through proximate and phytochemical analysis, by analyzing the different previous studies [5-7].

Methodology

Preparation of sample

Seeds are taken from the farm which is then cleaned, washed and dried. The seed coat of about 100 gms is collected by simple mechanical treatment of the seeds.

Proximate analysis

Estimation of reducing sugar: The reducing sugar is estimated by di-nitro salicylic acid method [8,9]. In this method, weigh 100 mg of the sample and extract the sugars with hot 80% ethanol twice (5 ml each time). Collect the supernatant and evaporate it by keeping it on a water bath at 80°C. Add 10 ml water and dissolve the sugars. Pipette out 0.5-3 ml of the extract in test tubes and equalize the volume to 3 ml with water in all the tubes. Add 3 ml of DNS reagent. Heat the contents in a boiling water bath for 5 min. When the contents of the tubes are still warm, add 1 ml of 40% Rochelle salt solution. Cool and read the intensity of dark red colour at 510 nm. Run a series of standards using glucose (0-500 μg), and plot a graph.

Estimation of total carbohydrate: The total carbohydrate present in seed coat is estimated by phenol- sulphuric acid method [8,9]. In this method, weigh 100 mg of the seed coat (sample) into a boiling tube. Hydrolyze by keeping it in a boiling water bath for three hours with 5 ml of 2.5 N HCl, and cool to room temperature. Neutralize it with solid sodium carbonate, until the effervescence ceases. Make up the volume to 100 ml and centrifuge. Pipette out 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard into a series of test tubes. Pipette out 0.1 and 0.2 ml of the sample solution in two separate test tubes. Make up the volume in each tube to 1 ml with water. Set a blank with 1 ml of water. Add 1 ml of phenol solution to each tube. Add 5 ml of 96% sulphuric acid to each tube and shake well. After 10 min, shake the contents in the tubes and place in water bath at 25-30°C for 20 min. Read the colour at 490 nm. Calculate the amount of total carbohydrate present in the sample solution using the standard graph.

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Moisture content: The moisture content of these seeds was determined in triplicate by drying at 120˚C to constant dry weight in a hot-air oven [8].

Total fat: The total fat content was determined by extraction of 2.0-2.5 g of dry ground sample for 12 h in a Soxhlet with petroleum ether, and removed the solvent by rotary evaporator, then dried the sample in hot air oven at 100˚C for about 1 h to allow the ether evaporate [4,8].

Total protein: The total protein content in the seed coat is estimated by Lowry's method. Different dilutions of BSA solutions are prepared by mixing stock BSA solution (1 mg/ ml) and water in the test tube, as given in the table. The final volume in each of the test tubes is 5 ml. The BSA range is 0.05-1 mg/ ml. From these different dilutions, pipette out 0.2 ml protein solution to different test tubes and add 2 ml of alkaline copper sulphate reagent (analytical reagent). Mix the solutions well. This solution is incubated at room temperature for 10 mins. Then add 0.2 ml of reagent Folin Ciocalteau solution (reagent solutions) to each tube, and incubate for 30 min. Zero the colorimeter with blank and take the optical density (measure the absorbance) at 660 nm. Plot the absorbance against protein concentration to get a standard calibration curve. Check the absorbance of unknown sample, and determine the concentration of the unknown sample using the standard curve plotted above [8,10].

Estimation of crude fiber: Extract 2 g of ground material of seed coat with ether or petroleum ether to remove fat (Initial boiling temperature 35-38˚C and final temperature 52˚C). If fat content is below 1%, extraction may be omitted. After extraction with ether, boil 2 g of dried material with 200 ml of sulphuric acid for 30 min with bumping chips. Filter through muslin and wash with boiling water, until washings are no longer acidic. Boil with 200 ml of sodium hydroxide solution for 30 min. Filter through muslin cloth again and wash with 25 ml of boiling 1.25% H_2SO_4, three 50 ml portions of water and 25 ml alcohol. Remove the residue and transfer to ashing dish. Dry the residue for 2 h at 130 ± 2˚C. Cool the dish in desiccators. Ignite for 30 min at 600 ± 15˚C. Cool in desiccators [8].

Ash content: In this, dry ashing method is used. Dry ashing procedures use a high temperature muffle furnace capable of maintaining temperatures of between 500 and 600˚C. The seed coat sample of 50 gms is taken and weighed before keeping for drying in muffle furnace, and after ashing to determine the concentration of ash present. The ash content can be expressed on either a dry basis [8].

$$\%ASH\,(DRY\,BASIS) = Mash \times 100 / Mdry$$

Elemental analysis: Samples are prepared as by taking 2 gms of samples (seed coat obtained by different solvent treatment), and add 10 ml of conc. nitric acid. Transfer them in to crucibles and keep them inside furnace at 200 degrees. Take out the ash formed in the crucibles. Dilute them with water and filter the supernatant. Standards are prepared as per, and readings are noted with the help of Atomic absorption spectroscopy [8].

Phytochemical analysis

Qualitative analysis: The methanolic extract of the seed coat is prepared by transferring in to methanol in (40% v/w), and keeping it for 2 hours. Now, the methanol is removed and washed twice with distilled water. Now, the extract is dried and dissolved at 1 gm/10 ml and analysis was carried out [8].

Alkaloids: For the detection of presence of Alkaloid, Wagner's was performed, where initially solvent free extract 50 mg was mixed with few ml of dilute hydrochloric acid and then filtered, the filtrate is used for testing the presence of alkaloids. To a few ml of filtrate, a few drops of Wagner's reagent were added by the side of the test tube. A reddish brown precipitate indicates the presence of alkaloids [8].

Glycosides: 50 mg of extract was mixed with few ml of conc. hydrochloric acid for 2 hours on water bath and then filtered; the filtrate was used for testing the presence of glycosides by legal's test. 0.5 ml of filtrate was dissolved in pyridine, and then sodium nitroprusside solution was added and made alkaline using 10% sodium hydroxide. Pink color indicates the presence of glycosides [8].

Saponins: The extract (50 mg) was diluted in distilled water, and then made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. A 2 cm layer of foam indicates the presence of saponins [8].

Oil: A small quantity of extract was pressed between two filter papers; oil stain on the paper indicates the presence of fixed oil [8].

Phenolics and tanins: The extract (50 g) was dissolved in 5 ml of distilled water and filtered. Filtrate obtained was used for ferric chloride test. To 0.5 ml of filtrate, few drops of neutral 5% ferric chloride solution were added. A dark green color indicates the presence of phenolic compounds [8].

Lead acetate: The extract was dissolved in distilled water and to this, 3 ml of 10% lead acetate solution was added, a bulky white precipitate indicates the presence of phenolic compounds [8].

Gum test: The extract (100 mg) was dissolved in 10 ml of distilled water, and to this solution, 25 ml of absolute alcohol was added with constant stirring. White or cloudy precipitate indicates the presence of gums [8].

Erpenoids: Salkowski Test: To 5 ml of each extract was mixed in 2 ml of chloroform and 3 ml of conc. H_2SO_4 was carefully added form the sides of test tube. A reddish brown coloration of the interface was formed to show positive results for the presence of terpenoids [8].

Steroids: Liebermann test: To 2 ml of acetic anhydride was added to 0.5 g methanolic extract of each sample with 2 ml H_2SO_4. The color changed from violet to blue or green in some samples, indicating the presence of steroids [8].

Quantitative analysis

Determination of total phenolic contents: The total phenol content in the methonolic extract of seed coat extract was determined spectrophotometrically using Folin-Ciocalteu method as described by Kujala et al. [9]. An aliquot of 1 ml extract was mixed with 5 ml of Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml of Sodium carbonate (7% w/v), and shaken. The solution was allowed to stand for 30 min in dark at room temperature, after which absorbance was measured at 765 nm using a spectrophotometer. Gallic acid was used as a standard compound, in the range of 50 to 200 μg/ml concentration to construct a standard curve. The amount of total phenolic was expressed as gallic acid equivalent (GAE) in milligram per gram dried extract [8].

Determination of flavanoids: The total flavonoids were determined using a colorimetric method, as described by Shiva et al. [10]. Briefly 0.1 ml of the methonolic extract was diluted with 0.9 ml of methanol. Aliquots of diluted extracts (0.5 ml) were added to test tubes and mixed

with 0.1 ml of 10% aluminum nitrate, 0.1 ml of 1 M aqueous potassium acetate and 4.3 ml of methanol. After standing for 40 min at room temperature, the absorbance of the reaction mixture was measured at 415 nm. Quercetin was used as a standard compound in the range of 50-200 μg/ml concentration to construct a standard curve. The amount of total flavonoids was expressed as quercetin equivalent in milligram per gram of dried extract [8,11,12].

Results

Proximate analysis

At 510 nm, the absorbance of total reducing sugar using DNSA method found to be zero, which indicates absence of reducing sugar in the seed coat. At 600 nm, the OD for total carbohydrate is found to be 0.574 and concentration was observed to be 47.85 gms/100 gms, as shown in the figure 1A. The total protein as per Lowry's method shows OD of 0.67 at 600 nm. This result in the concentration of protein in seed coat is found to be 6.26 gms/100 gms, as shown in figure 1B. The ash content of the seed coat is observed with the help of dry basis, it showed the mass of ash in dry basis as 10.25 gms for 50 gms, and percentage of ash on dry basis is found to be 20.51%. The total fat observed by
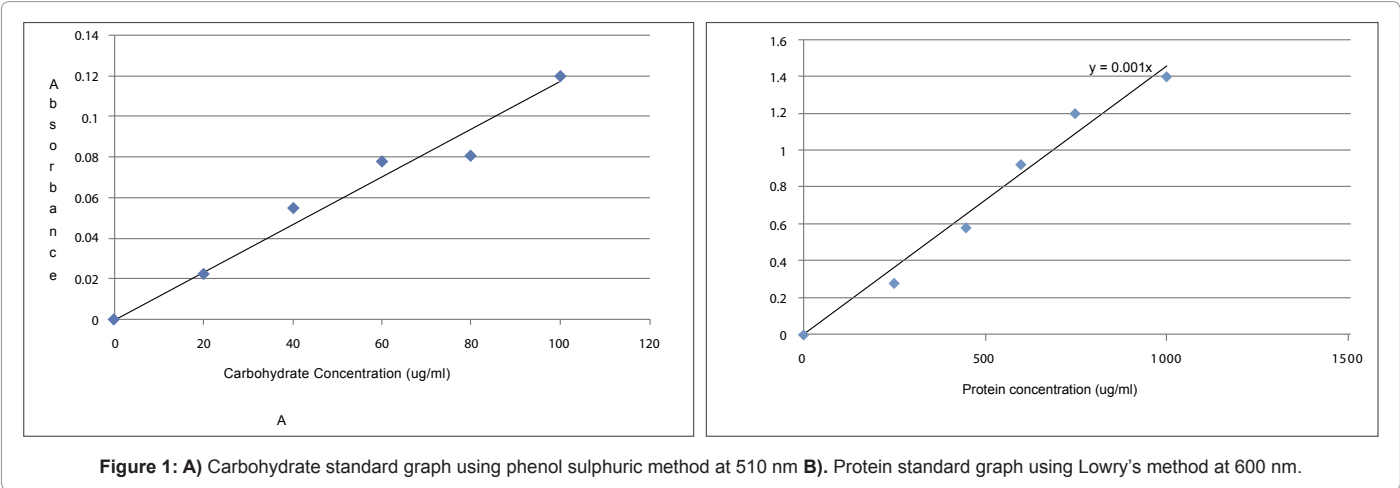
soxhlet apparatus is 2.04 gms/100 gms. The mineral analysis using Atomic absorption spectroscopy, shows Iron (Fe)-0.116 mg/1 g, Zinc (Zn)-0.01mg/1 g, Nickel (Ni)-0.048 mg/1 g, Copper (Cu)-0.05 mg/1 g, Calcium (Ca)-0.05 mg/1 g and Phosphorus (P)-0.11 mg/1 g. And crude fiber of seed coat is estimated to be 13.08 gms/100 gms. The complete proximate analysis is plotted in table 1.

Phytochemical analysis

In the qualitative analysis of seed coat found, Terepenoids, Alkaloids, Gums and Saponins, but it indicated the presence of Starch, Protein, Glycosides, Phenolics and Flavanoids. The detail study is given table 2.

The quantitative analysis of the phenolics using shows the total phenolic content present is found to be Folin-Ciocalteu method is found to be 2.12 mg/gm, as shown in the figure 2A, and total flavanoids by Quercetin equivalent is found to be 1.3 mg/gm, as shown in the figure 2B.

Conclusion

The proximate analysis of the seed coat gave an idea for its chemical composition, which has significant amount of fiber and carbohydrate. The protein content is also considerable in preparing the enzyme assay for the removing the seed coat. These preliminary studies will be even helpful in comparing the nutrient properties of the seed, without seed coat after enzymatic treatment. Based on this proximate and phytochemical analysis, it would easy to design an optimal cocktail of enzyme assay that can break the seed coat, which allow processing the seed coat from *P. sumantranse*. For example, the fiber is the one of the major component in the seed coat. The proximate analysis shows 13.08% of fiber content. From this study, the composition will help in designing an optimal enzyme assay, which can hydrolyze the fiber content and help in seed coat processing. Similarly, we have got chemical composition of proteins, carbohydrates and fats, which forms the major part of the seed coat. Our aim is to design an optimal cocktail of enzyme assay, based on the above chemical compositions, which can break seed coat and help in processing the seed coat, by retaining the original properties of the seed as such. In this study, we have considered little millet from Javadhu hills, Tamilnadu, as only source because the plant is majorly grown only in this area of Tamilnadu. Later, once we designed the prototype of this technology using this source, we would like to optimize technology, but collecting more samples from other states of India, although, the little millets grow in similar conditions.

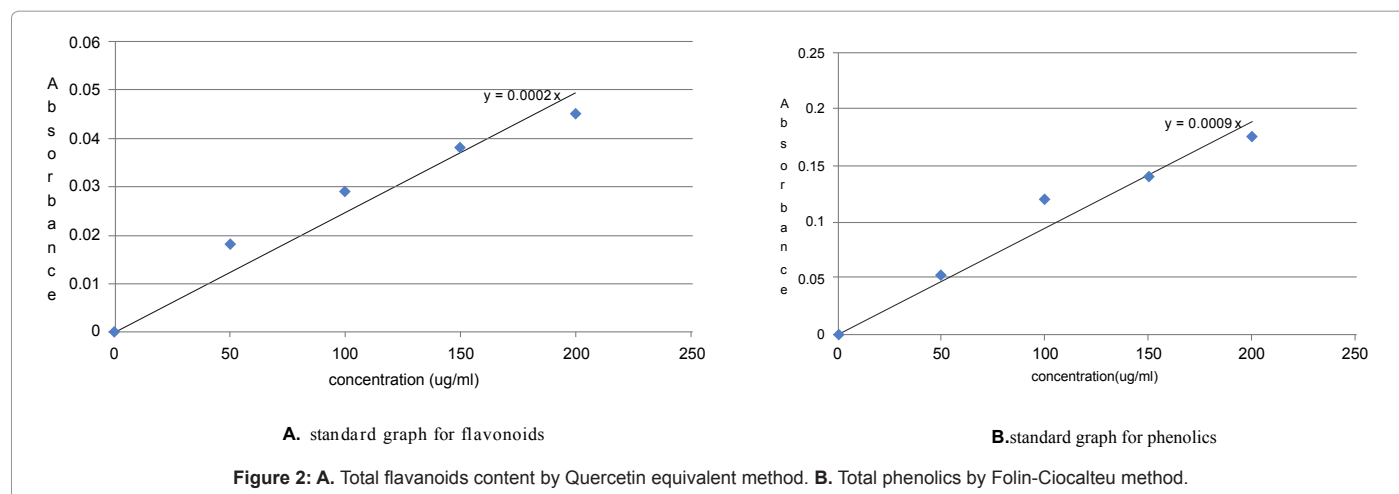| Test	Results (gms/100 gms)
Ash(dry method)	20.51
Crude fat	2.04
Crude protien	6.26
Crude fibre	13.08
Carbohydrate	47.85
Moisture	10.16
Iron(Fe)	0.116
Calcium(Ca)	0.05
Phosphorus(P)	0.11
Nickel (Ni)	0.01
Zinc (Zn)	0.048
Copper (Cu)	0.05

Note: Results above shows concentration.

Table 1: Proximate analysis of little millets (*P. sumantranse*).

Sample	Flavonoids	Glycosides	Starch	Terpenoids	Saponins	Phenolics	Alkaloids
Little millet	+	+	+	-	_	+	_

Table 2: Qualitative Phytochemical analysis of seeds from *P. sumantranse*.

Figure 1: A) Carbohydrate standard graph using phenol sulphuric method at 510 nm **B).** Protein standard graph using Lowry's method at 600 nm.

A. standard graph for flavonoids

B. standard graph for phenolics

Figure 2: A. Total flavanoids content by Quercetin equivalent method. **B.** Total phenolics by Folin-Ciocalteu method.

Acknowledgement

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