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In-Vitro Study for Evaluation of Proximate Composition, Phytochemical & Neutraceutical Properties of Different Millet Samples

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Abstract: ***Millets are members of poaceae family are loaded with potent bioactive components like Phenols, Flavonoids tannins, etc which has been linked with potential antioxidant activities. These properties are surely believed to prevent the deterioration of human health. The present study deals with in-vitro study for evaluation of proximate composition, phytochemical and neutraceutical properties of different millets. The proximate analysis revealed the presence of Moisture Content (5.9-8.9%), Ash (2.0- 4.5%), Crude Protein (8.5-13.2%), Fat (1.8-7.1%), Carbohydrate (59.9-70.1) & Crude Fibre (2.2-11.5%). Phytochemical studies showed a considerable amount of Phenolics, Flavonoids & Tannins in all samples, whereas alkaloids and Saponins were absent in some of the millet samples. These results showed that the selected millets contains essential nutrients which favourably competes well with those staple cereals and said to be a promising neutraceutical with wide health benefits and for use in herbal medicine to fight against various diseases.***

Keywords: phytochemicals, neutraceuticals, in-vitro, Barnyard, Pearl, Proso, Little, Finger, Khodo & Foxtail millets

1. Introduction

The concepts of food are changing from a previous emphasis on survival, hunger satisfaction, absence of adverse effects on health and health maintenance to a current emphasis on the use of neutraceutical foods which promise to promote better health & well being thus reduce the risk of prolong illness. Use of plant source to cure illness was an ancient practice holds good till today. Cereal grains serves as a sources of food, fodder, medicine and other valuable neutraceuticals. Allopathic system of medicine already proved the beneficiary functions of cereal grains. In addition to being a source of basic nutrients cereal grains also works as herbal medicines. Although a variety of drugs are available but there is no medicine prepared till date that have no side effects/adverse effects *[Sukhvinder Singh Purewal 2014]***[1].** These days phytochemical analysis is in demand and attracting the attention of various researchers. Most of the developed and developing countries have adapted the standard methods to detect the presence of bioactive compounds, alkaloids and other important constituents from plants and cereal grains. Researchers have reported that the presence of dietary fiber and phenolic compounds help in the prevention of many diseases such as diabetes, cardiovascular diseases, and cataractogenesis *[Himanshu, et al.2018]***[2].** These phytochemicals are reported to have antioxidant *[Okoyomoh K., et.al 2013]***[3],** antimicrobial *[Nair et al 2005]***[4]** & anti-diabetic properties *[Singh and Gupta 2007]***[5]** also. Hence keeping in mind that millets are highly nutritious, small seeded, grassy, non-glutinous, least allergenic and not acid forming foods and are very easy to digest a study was conducted for qualitative phytochemical screening, proximate analysis with evaluation of neutraceuticals for selected millet samples.

2. Materials and Methods

Collection of plant materials – The plant materials for this present study consists of seeds of seven different samples of millets viz Barnyard Millet (*Echinochloa* spp.), Pearl Millet *(Pennisetum typhoideum*), Proso Millet (*Panicum miliaceum*), Little Millet (*Panicum sumatrense*), Finger Millet (*Eleusine coracana*), Khodo Millet (*Paspalum scrobiculatum*), and Foxtail Millets (*Setaria italica*), were collected. The samples were cleaned, crushed to coarse powder using grinder and stored in air tight bags.

Extraction - About 100g of each powdered dry sample of millet were soaked using 70% ethanol for 24hrs at room temperature. The next day contents were refluxed for 2hrs at temperature not exceeding 65^0C, cooled and filtered through four layer of cheese cloth and residues were re-extracted under same condition with 100ml of 70% ethanol. The filtrate was evaporated to dryness at low temperature. The extract were stored in desiccators and used for further study.

Proximate Analysis- The proximate analysis viz moisture, ash, crude protein, crude fibre, crude fat & carbohydrates were carried out in triplicates and results obtained were the average values.

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.

Total Fat: The total fat content was determined by extraction of 2.0- 3.0 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.

Total Protein: The total protein content in the sample is estimated by Lowry's method using BSA as standard solution (range-1 mg/ ml).

Crude Fiber: Extract 2 g of ground sample with ether or petroleum ether to remove fat (Initial boiling temperature 36-40°C and final temperature 53°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 porcelain 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 2h at 140°C. Cool the dish in desiccators. Ignite for 30 min at 600°C. Cool in desiccators.

Ash Content: Ash content was determined by dry ashing method. About 50 gms of sample 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
% Ash (Dry basis) = $M_{ash}X100 / M_{dry}$

3. Phytochemical Screening

Qualitative analysis: The ethanolic extract of the different seed materials are prepared by transferring into ethanol in (40% v/w), and keeping it for 2 hours. The ethanol is then removed and washed twice with distilled water. The extract is well dried and further subjected to analysis by dissolving 1 gm of extract /10 ml.

Test for Phenolics: 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.*

Test for Tannins: 50 mg of the extract 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 tannins.*

Test for Alkaloids: For the detection of presence of Alkaloid, Wagner's test 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.*

Test for 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.*

Test for Saponins: 50 mg of the extract 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.*

Test for Flavonoids
To 1 ml of extract 1ml of aqueous lead acetate (10%) was added. *The formation of yellow colored precipitates indicates the presence of Flavonoids in the extracts.*

Test for Carbohydrates
Presence of carbohydrates in the different extracts millet was determined by following tests.

Fehling's test: To 5 mL extract, equal volume of Fehling`s solutions A & B was added and heated on Bunsen burner. *Appearance of red precipitate showed the presence of reducing sugars.*

Benedict`s test:
To 5 mL of extract 1 mL of Benedict's reagent was added and heated and allowed to cool. Compare the change in colour of extracts with the colour mentioned on the Benedict's reagent bottle.

4. Quantitative Analysis

Determination of total phenolic contents: The total phenolic content of each extract were quantified spectrophotometrically using Folin-Ciocalteu method taking Gallic acid as standard compound, in the range of 50 to 200 µg/ml concentration to construct a standard curve described by *Kujala et al, 2000***[6]** . 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. The amount of total phenolic was expressed as Gallic acid equivalent (GAE) in milligram per gram dried extract.

Determination of Flavonoids: The total Flavonoids were determined using a colorimetric method by taking Quercetin as a standard compound in the range of 50-200 µg/ml concentration to construct a standard curve described by *Shiva et al. 2007* **[7].** Briefly 0.1 ml of the ethanolic extract was diluted with 0.9 ml of ethanol. Aliquots of diluted extracts (0.5 ml) were added to test tubes and mixed with 0.1 ml of 10% aluminium 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. The amount of total Flavonoids was expressed as Quercetin equivalent in milligram per gram of dried extract.

Determination of reducing power activity- The reducing power of the sample was determined as described by *Oyaizu, 1986* **[8].** Reducing power activity is based on the reduction of ferric cyanide (Fe3+) in stoichiometry excess relative to the amount of antioxidants. 50µl of sample with different concentrations were mixed with 0.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 0.5 ml of 1% potassium ferric cyanide (w/v) and incubated at 50^{o}C for 20 min. After incubation, 2 ml of 10% trichloroacetic acid (w/v) were added to the mixture, followed by centrifugation at 30, 000 rpm for 10 min. The upper layer (2.5 ml) was mixed with 2 ml of deionized water and 0.5 ml of 0.1% ferric chloride (w/v), and the absorbance of the resultant solution was measured at 700 nm. Ascorbic acid was used as standard.

Determination of Water soluble protein content – the water –soluble protein content of each sample was

determined by Lowry's method as described by *Sadasivam et al 1996**[9].***

Determination of Reducing Sugar content: The reducing sugar is estimated by di-nitro salicylic acid method by *odoemelam et al 2009* [**10].** 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).

Determination of Total Carbohydrate: The total carbohydrate present in seed extract is estimated by phenol-sulphuric acid method as described by *AOAC, 1995* **[11].** In this method, weigh 100 mg of the 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- 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.

5. Result and Discussion

The Proximate, Qualitative & Quantitative phytochemical analysis results are tabulated as below:

Proximate analysis

The result of proximate analysis shows variant concentration of biochemical's & other contents. The moisture content in each species is different. Considering the overall percentage of moisture composition, it was highest in finger millet (8.9%) followed by foxtail (8.5%) & Khodo millet (8.0%) while the other had comparatively lesser composition. This indicates that the seed with less moisture has a good shelf life; hence it can be stored for long term without spoilage. Moisture content is among the most vital and mostly used measurement in the processing, preservation and storage of food *Onwuka, 2005* **[12].**

Considering the result obtained from carbohydrate analysis, finger millet (70.1%) & pearl millet (68.2%) had prominent levels compared to other millet extracts. From the results obtained after protein analysis, Foxtail Millet (13.2%) showed high protein content and a minimum was seen in Finger Millet (8.5%).While analyzing the crude fibre contents in the selected seven samples of millets, the results showed that Barnyard millet had highest concentration as compared to other samples. Crude fibre in food is an indication of the level of non-digestible carbohydrate and lignin. According to the results revealed, The Khodo Millet (4.5%) had high ash content and minimum in Proso Millet (2.0%). The overall caloric value of millet samples ranges from 410kcal-308kcal/g. The complete proximate analysis is plotted in table 1.

Table 1: Proximate composition of different millet samples

Nutritional contents in 100g of dry grains of Millets	Moisture (g)	Carbohydrates (g)	Crude Protein (g)	Crude Fibre (g)	Ash (g)	Fat (g)	Energy (kcal)
Barnyard millet	6.9	60.2	9.5	11.5	4.4	4.0	308
Pearl Millet	7.4	68.2	13.0	2.2	2.1	5.7	410
Proso millet	6.2	62.5	11.5	5.5	2.0	3.5	340
Little millet	5.9	67.5	12.5	6.1	4.0	6.0	341
Finger millet	8.9	70.1	8.5	3.9	2.8	1.8	352
Khodo millet	8.0	59.9	9.0	7.0	4.5	3.5	356
Foxtail millet	8.5	64.5	13.2	5.7	3.4	7.1	350

Phytochemical Analysis

The phytochemical analysis of ethanol extract of different millets revealed the presence of various phytochemicals in varying proportions. The qualitative analysis of extracts found not only the presence of Phenolics, Tannins, Alkaloids, Saponins, but also indicated the presence of Carbohydrates, Protein, Glycosides, Fibres and Flavonoids. The detail study is given Table 2.

Table 2: Qualitative test results for presence of Phytochemicals in different millet samples

Constituents	Barnyard Millet	Pearl Millet	Proso Millet	Little Millet	Finger Millet	Khodo Millet	Foxtail Millet
Phenolics	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+
Alkaloids	-	-	+	-	+	+	+
Flavonoids	+	+	+	+	+	+	+
Saponins	-	-	+	-	+	+	+
Carbohydrates	+	+	+	+	+	+	+
Proteins	+	+	+	+	+	+	+
Fibres	+	+	+	+	+	+	+
Glycosides	-	-	-	-	+	-	+

The quantitative analysis of extracts for determination of total Phenolics and Flavonoids content reveals that Khodo Millet (11.35%) shows highest phenolic content followed by Little millet (10.3%) and Barnyard Millet (10.1%) respectively whereas the minimum phenolic content was recorded by Foxtail Millet(5.5%). The total Flavonoids analysis revealed that, Barnyard Millet has maximum Flavonoids content (49.5%) compared to other samples. The fibre contents present in millets ranges from 13.3%-10.2%. This fibre content analysis reveales that millets are healthy sources for people with Diabetes. The results are shown in Table 3.

Table 3: Total Phenolics, Flavonoids & Fibre content found in different millet samples

Millet	Total Phenolics (mg/g)	Total Flavonoids (mg/g)	Fibre Content (%)
Barnyard millet	10.1±0.03	49.5±2.98	11.2.±1.57
Pearl Millet	07.3±0.52	38.6±0.62	10.9±1.15
Proso millet	03.4±0.58	22.5±0.56	11.5±0.56
Little millet	10.3±1.29	44.8±1.52	10.6±0.58
Finger millet	07.2±0.57	37.2±0.57	10.2±0.57
Khodo millet	11.3±1.15	20.3±1.15	13.3±1.15
Foxtail millet	05.5±0.56	16.5±0.56	12.5±0.56

Table 4 represents the Reducing Capacity, Reducing Sugar & Soluble Protein content found in different millets. As for as Reducing property is concerned, the Finger Millet showed highest reducing property with a least seen in Proso Millet. This property helps in reducing the risk of oxidative damage by reactive species. Among the seven millets tested, reducing sugar contents was seen maximum in Finger Millet (230.6%) and minimum in Khodo Millet (120.4%). The water soluble protein content ranges between 300.45-177.2% in different millet samples with a maximum in Foxtail Millet and least in Proso Millet.

Table 4: Reducing Capacity, Reducing Sugar & Soluble protein content found in millets

Millet	Reducing Capacity (%)	Reducing Sugar (mg/g)	Soluble Proteins (mg/g)
Barnyard millet	6.9±0.57	172.1±0.57	187.2±0.15
Pearl Millet	6.8±1.15	132.3±1.15	255.50±1.58
Proso millet	3.2±0.58	195.6±1.15	177.2±0.57
Little millet	6.2±0.58	135.3±0.58	190.3±1.15
Finger millet	8.1±1.15	230.6±1.76	229.12±1.73
Khodo millet	5.5±1.73	120.4±2.30	229.12±4.58
Foxtail millet	6.9 ±1.15	195.6±2.88	300.45±2.55

6. Conclusion

Millets can serve as a staple diet for nearly one- third of world's population. They occupy an important place in the world's food & economy. Milet can be grown in varied environmental conditions. Considering all these points the proximate analysis of the seed extracts with evaluation of qualitative & quantitative analysis for phytochemical properties was planned.

This gave an idea for its chemical composition, which has significant amount of fiber and carbohydrate & protein content. These preliminary studies will be even helpful in comparing the nutrient properties of the different seed extracts. Results obtained from the above study clearly indicate the presence of various types of phytochemicals in different Millets. The presence of huge amount of nutrient components in these millets might be helpful for the production of various neutraceuticals.

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