



Preliminary Physicochemical and Phytochemical Evaluation of Grain of Kodo Millet Flour at Western Odisha, Sambalpur.

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ABSTRACTS

Minor millets play food based approach for achieving food, nutritional security and also address life style disorders. They provide nutritious grain and fodder in a short span of time. According to traditional practice the grains of *Paspalum scrobiculatum* is used for various therapeutic benefits such as prevention of heart diseases, diabetes, migraine, cancer and gastro intestinal diseases. In this study Kodo millet was investigated for its preliminary physicochemical and phytochemical screening including fluorescence analysis, pH determination, loss on drying, extractive value (methanol soluble, water soluble, petroleum ether and benzene) Ash value (total ash, acid insoluble ash, water soluble ash and sulphated ash), and total phenolic contents were studied dry weight.

Kew words: - *Paspalum scrobiculatum*, physicochemical, phytochemical screening, diabetes.

INTRODUCTION

Drastic climatic changes and reduced irrigation facilities are heading to increase the percent of dry lands and posing threat to food production and subsequently food and nutritional security.[1] To find sustainable solution that quench's world hunger and improve the quality of life the role millets cannot be overlooked in achieving sustainable food and nutritional security.[2] *Paspalum scrobiculatum* Linn. belonging to family *Poaceae*, commonly known as 'Kodo millet', it is a tufted perennial grass, up to 120-150 cm tall, culms stout glabrous, somewhat bulbous at base, distributed in Odisha, Madhya Pradesh, Chattisgarh and Karnataka in India.[3] It is a hard crop and drought-tolerant, it is an annual millet that varies in height from 30-90cm or 4 feet and has a basal tiller. It requires 25-27°C, after 4 months crop will be ready to harvest after harvesting period the grain occurs in the hard husk which makes detrainment of grains difficult. The size of seed is small having 2mm in length, 1.5mm width and colour changes from pale brown to dark grey. [4]. The kodo millet grain is composed of many nutrients like it provides 11% protein, 37-38% dietary fiber, opposite to rice which provides 0.2/100g and 1.2/100g an adequate amount of -fiber helps to resist the feeling of hunger. The grains accommodate 66.6g of carbohydrates which provide 353kcal per 100g of grain compared to other millet and fat 3.6g/100g, the grains contain high amount minerals like (calcium 15.27mg, Phosphorus 188mg, iron 2.34mg, copper 0.26, magnesium 147mg, sodium 4.6mg, potassium 144mg, zinc 0.7mg), vitamins are thiamin 0.299mg, riboflavin 0.20mg, niacin 1.49mg.[5] It is thus recommended as an ideal food for diabetics. It also contains significant amounts of potential antioxidants like phenols, phenolic acids, and carotenoids[6]. Millets are therefore, consumed as multi-grains to reap the collective health benefits of nutrients. The kodo millet whole grain consumption has health promoting effects like prevention of insulin resistance, heart disease, diabetes, ischemic stroke, obesity, breast cancer, childhood asthma and premature death [7] because of this benefits, millets can be used in functional food and as nutraceuticals. Hence they are also called as 'nutricereals'. Millets are rich sources of phytochemicals, micronutrients and antioxidants, such as phenolic acids and glycosylated flavonoids[8]. Minor millets, with their low carbohydrate content, low digestibility and water soluble gum content (b-glucan) have been attributed to improve glucose metabolism. These grains release sugar slowly in the blood and also diminish the glucose absorption. [9] Kodo millet has around 11% protein and is found to superior to other cereals in terms of dietary fiber and antioxidant potential [10]. The present investigation deals with the studies on some important physicochemical and phytochemical characteristics of the grains of *Paspalum scrobiculatum* as whole and its powdered form.

MATERIALS AND METHODS

COLLECTION OF PLANT:

Area of collection: Kodo millet grains were collected from local village, Naxapali, Sambalpur district of Odisha, India. Grains were cleaned and milled. Flour was used to assess the quality.

Month of collection:

The Kodo millet grains were collected in the month of may- June 2023.

4.3 AUTHENTICATION:

The herbarium was prepared one was sent to H.O.D. of Botany, Government Autonomous College. Angul, Odisha for proper authentication. The sample was identified to be *Paspalum scrobiculatum* Linn. belonging to family *Poaceae*

Fluorescence Analysis:

The fluorescence characteristic of powder drug were studied under U.V. light after treating with different chemical reagents and reported. [11]

pH Determination:-

Materials:

- A) Powder drug
- B) 100 ml of distilled water
- C) Filter paper
- D) P^H meter with standardized glass electrode (Elico)

Method for 1% Solution:-

1 gm of powdered drug was accurately weighed and macerated with 100 ml distilled water and filtered. P^H of the filtrate was checked with a PH meter having standardized glass electrode.

Method for 10% Solution:

10 gm of powdered drug was accurately weighed and macerated with 100 ml distilled water and filtered. PH of the filtrate was checked with a PH meter having standardized glass electrode.

PHYSICAL EVALUATION

LOSS ON DRYING:

Materials:

- a) Powder drug 5 gm
- b) Glass stoppered shallow weighing bottle
- c) Hot air oven
- d) Desiccator

Procedure:

A glass stoppered shallow weighing bottle was dried and weighed. 5gm of the powdered drug was transferred to the bottle. The bottle covered with stoppered and weighed. The sample was then distributed as evenly as possible by gentle side wise shaking to a depth not exceeding 10mm. The loaded bottle was kept in hot air oven, the stopper was removed and left in the oven. The powdered drug was dried for 30 minute at a temperature of 105°C. After drying was completed the hot air oven was opened and the bottle was closed promptly, allowed to cool at room temperature in a desiccator before weighing. The bottle and the contents were then weighed. The procedure was continued until constant weight is obtained. [12]

EXTRACTIVE VALUE: [13]

Methanol soluble extractive:

Materials:

- a) Powder drug 5 gm
- b) Stoppered conical flask (250ml)
- c) 90% Methanol
- d) Flat bottom shallow dish

Method:

5 gm of the air dried drug was coarsely powdered, taken in a stoppered conical flask and macerated with 100 ml of methanol (90%) for 24 hours. It was shaken frequently during the first 6 hrs and allowed to stand for 18 hrs. There after it was filtered rapidly taking precaution against loss of methanol and

then 25ml of the filtrate was evaporated in tarred flat bottom shallow dish, dried at 105°C and weighed. The percentage of methanol soluble extractive was calculated with reference to the air dried drug.

Water Soluble Extractive:

Materials:

- A) Powder drug
- B) Stoppered conical flask 250ml
- C) Triple distilled water
- D) Chloroform (Merck)
- E) Flat bottom shallow dish.

Method:

5 gm of the air dried drug was coarsely powdered, taken in a stoppered conical flask and macerated with 100 ml of chloroform water for 24 hours. It was shaken frequently during the first 6 hrs and allowed to stand for 18 hrs. There after it was filtered rapidly taking precaution against loss of chloroform water and then 25ml of the filtrate was evaporated in tarred flat bottom shallow dish, dried at 105°C and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drug.

Petroleum Ether Soluble Extractive:

Materials:

- A) Powder drug
- B) Stoppered conical flask 250ml
- C) Triple distilled water
- D) Petroleum Ether (RFCL Ltd)
- E) Flat bottom shallow dish

Method:

5 gm of the air dried drug was coarsely powdered, taken in a stoppered conical flask and macerated with 100 ml of petroleum ether for 24 hours. It was shaken frequently during the first 6 hrs and allowed to stand for 18 hrs. There after it was filtered rapidly taking precaution against loss of petroleum ether and then 25ml of the filtrate was evaporated in tarred flat bottom shallow dish, dried at 105°C and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drug.

Benzene Soluble extractive:

Materials:

- A) Powder drug.
- B) Stoppered conical flask 250ml.
- C) Triple distilled water.
- D) Benzene.
- E) Flat bottom shallow dish.

Method:

5 gm of the air dried drug was coarsely powdered, taken in a stoppered conical flask and macerated with 100 ml of benzene for 24 hours. It was shaken frequently during the first 6 hrs and allowed to stand for 18 hrs. There after it was filtered rapidly taking precaution against loss of benzene and then 25ml of the filtrate was evaporated in tarred flat bottom shallow dish, dried at 105°C and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drug.

ASH VALUE:

Total Ash:

Materials:

- A) Powder drug

- B) Silica crucible
- C) Muffle furnace
- D) Ash less filter paper.

Method:

2gm of air dried drug was weighed accurately in a silica crucible and incinerated at a temperature not exceeding 450°C, until free from carbon, cooled and weighed. As carbon free ash cannot obtain in this way then the charred mass was exhausted with hot water. The residue was incinerated with filter paper until the ash is white. Then the filtrate was added, evaporated to dryness and incinerated at a temperature not exceeding 450°C. The percentage of ash with reference to air dried drug was calculated. [14]

Water soluble ash**Materials:**

- A) Powder drug
- B) 25ml distilled water
- C) Silica crucible
- D) Muffle furnace
- E) Ash less filter paper

Method:

2gm of air dried drug was weighed accurately in a silica crucible and incinerated at a temperature not exceeding 450°C. The ash obtained was weighed and boiled for 5 minutes with 25ml of distilled water and insoluble matter was collected on the ash less filter paper, washed with hot water and incinerated for 15 minutes at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash; the difference in weight represents the water soluble ash. The percentage of water soluble ash with reference to air dried drug was calculated.

Acid insoluble ash**Materials:**

- A) Ash of 2gm Powder drug.
- B) 2M Hydrochloric acid (25ml).
- C) Silica crucible.
- D) Muffle furnace.
- E) Ash less filter paper.
- F) Desiccator.

Method:

The ash was boiled for 5 minutes with 25 ml of 2 m hydrochloric acid and the insoluble matter was collected on ash less filter paper, washed with hot water, incinerated, cooled in desiccators and weighed. The percentage of acid insoluble ash with reference to the air dried drug was then calculated.

Sulfated ash**Materials:**

- A) Powder drug (1gm)
- B) Sulphuric acid
- C) Silica crucible
- D) Muffle furnaces
- E) Desiccators

Method:

A silica crucible was heated to redness for 10 minutes, allowed to cool in desiccator and weighed. 1gm of the powdered drug was then transferred to the crucible and the content along with the crucible is weighed accurately. At first it was incinerated until the substance is thoroughly charred. The residue was then cooled and moistened with 1 ml of concentrated sulphuric acid, heated gently until the white fumes are no longer evolved and incinerated at

800°+- 25°C until all black particles have disappeared. The incineration was conducted in a place protected from air currents. The crucible was then allowed to cool, few drops of concentrated sulphuric acid was added and heated. Incineration was done as before, allowed to cool and weighed. The operation was repeated until two successive weighings do not differ by more than 0.5 mg.

PHYTOCHEMICAL INVESTIGATION

DRYING AND PULVERIZATION:

The collected plant material was shade dried at room temperature, and then they are pulverized in mixer grinder to coarsely powdered drug and pass through mesh size 40 sieves.

Percentage yield of Extracts

Sl. No.	Extracts	% Yield
1.	Methanol	3.8
2.	Chloroform	1.8
3.	Petroleum ether	2.5
4.	Water	4.9

QUALITATIVE PHYTOCHEMICAL EVALUATION:

All the extracts and powder were subjected to qualitative chemical tests. [15, 16, 17]

Detection of carbohydrates

Small quantities of different extracts were dissolved in distilled water separately and filtered. The filtrates were taken for various tests to detect the presence of carbohydrates.

(A) Molisch's Test-

The filtrates were treated with 2-3 drops of 1% alcoholic α -naphthol and 2ml of concentrated sulphuric acid was added along the sides of the tube. A brown ring was formed in the filtrates of methanol and aqueous extract showed the presence of sugar.

(B) Fehling's Test-

Small portion of filtrates were treated with equal volume of Fehling's solution A and B and then heated. Brick red ppt. was formed in methanol and aqueous extracts indicating the presence of reducing sugar.

(C) Benedict's test-

Small portion of various filtrates were treated with equal volumes of Benedict's reagent. Yellow precipitate was formed in aqueous extract showed presence of carbohydrates.

(D) Barfoed's Test-

Small portion of different extracts were treated with Barfoed's reagent. Red precipitate was formed in the methanol extract indicating the presence of reducing sugar.

(E) Test for Starch –

A small amount of various extracts was treated with dilute iodine solution. A bluish black colour was observed in none of the extracts showed the presence of starch.

Test for Gum and Mucilage

Ruthenium test:-Take small quantity of dried powder, mount it on a slide with ruthenium red solution and observe it under microscope. No pink colour develops, indicates absence of mucilage.

Test for Proteins and Amino acids

Small quantities of different extracts were dissolved in few ml of distilled water and subjected to ninhydrin, biuret, millon, xanthoproteic test, test with tannic acid and heavy metals.

A) Ninhydrin Test-

The methanol extracts were treated with ninhydrin reagent (0.1% solution) and boiled. Purple colour was observed indicating the presence of proteins.

B) Biuret Test-

To a portion of the prepared extracts, equal volumes of 5% w/v sodium hydroxide and 4 drops of 1% w/v copper sulphate solution were added. No violet colour was formed indicating the absence of protein.

C) Million's Test –

To the prepared extracts Million's reagent was added. White precipitate was formed in any extract showed the presence of protein.

D) Xanthoproteic Test –

To 3 ml of the above prepared extracts 1ml of concentrated nitric acid was added, boiled for one minute, cooled and concentrated ammonia was added till alkaline. Orange colour was formed in the water extract showed the presence of protein.

E) Test with Tannic acid-

To the above prepared extracts 10% tannic acid solution was added. While precipitate was not formed in methanol extract showed the absence of protein.

F) Test with heavy metals-

The above prepared extracts were treated with different heavy metals. Precipitate was observed in chloroform extract indicating the presence of proteins.

Test for Alkaloids

Small amount of solvent free various extracts were separately stirred with a few ml of dilute hydrochloric acid and filtered. The filtrates were tested with various alkaloidal reagents such as Mayer's, Drangendroff's, Wagner's and Hager's reagent, Phosphomolibdic acid and Tannic acid.

A) Dragendroff's Test –

To the filtrates few drops of dilute dragendroff's reagent (potassium bismuth iodide solution) was added. No Reddish brown precipitate was observed indicating that absence of alkaloids.

B) Mayer's Test –

To the filtrates few drops of Mayer's reagent (Potassium mercuric iodide solution) was added. No cream color precipitate was observed in petroleum and chloroform extract indicating absence of alkaloids.

C) Wagner's Reagent Test –

To the filtrates few drops of Wagner's reagent (iodine-potassium iodine solution) was added. No reddish brown precipitate was not observed in methanol and chloroform extract indicating that absence of alkaloids.

D) Hager's Reagent Test –

To the filtrates few drops of Hager's reagent (saturated solution of picric acid) was added. Yellow color precipitate was not observed in chloroform extracts indicating the absence of alkaloids.

E) Tannic acid Test –

To the filtrates few drops of tannic acid solution was added. Buff color precipitate was not observed in all extracts indicating the absence of alkaloids.

Test for glycosides

A small amount of different extracts were dissolved separately in 5ml of distilled water and filtered. Another portion of the extracts were hydrolyzed with hydrochloric acid for one hour on a water bath and hydrolysate was subjected to Legal's, Baljet's, Borntrager's, killer killani's test and for the presence of cyanogenic glycoside.

A) Legal's Test –

To the hydrolysate, one ml of pyridine and few drops of sodium nitroprusside solution was added and then made alkaline with sodium hydroxide solution. Pink color was not observed in petroleum ether and water extracts indicating the absence of glycosides.

B) Baljet's Test –

To a section of plant materials sodium picrate solution was added. No yellowish orange color was observed indicating the absence of glycosides.

C) Borntrager's Test –

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. No pink color was observed in ammoniacal layer in none of the extracts indicating the absence of glycosides.

D) Test for Cardiac glycoside (Killer killani's Test)

To the different extracts 10ml of 70% alcohol were added, boiled on a water bath, filtered. The filtrates were diluted with distilled water; one ml of strong lead acetate solution was added and filtered. The filtrates were extracted with an equal volume of chloroform. The chloroform layer was pipette and 3 ml of 3.5% of ferric chloride in glacial acetic acid was added, left for one minute and then transferred to a test tube. To the side of the test tube 1.5ml of sulphuric acid was added carefully, which formed a separate layer at the bottom and kept for few minutes. Brown color at the interface and pale green color in the upper layer was not observed in none of the extracts indicating the absence of cardiac glycosides.

E) Test for cyanogenic Glycosides –

A small amount of different extracts were moistened with water, a piece of sodium picrate paper was kept above by the help of cork and kept for 30 minutes. No brick red color was observed on the paper in none of the extracts indicating the absence of cyanogenic glycoside.

Test for phytosterols:

All the extracts were refluxed with 0.5N alcoholic potassium hydroxide until the saponification was complete. The saponification mixture was diluted with distilled water and extracted with petroleum ether. The ethereal extract was evaporated and unsaponification matter was subjected to Liebermann's, Liebermann-Burchard's and Salkowski's Test.

(A) Liebermann's Test –

The residue was dissolved in concentrated sulphuric acid and a few drops of aqueous sodium nitrate were added. Red color was observed on dilution in chloroform and ether extract indicating the presence of phytosterols.

(B) Liebermann – Burchard's Test –

The residue was dissolved in chloroform. To this Liebermann – Burchard's reagent was added. Green color was absent in methanol and chloroform extracts indicating the absence of phytosterols.

(C) Salkowski's Test –

A few drops of concentrated sulphuric acid were added to chloroform solution. The lower layer of the solution not turned in to brownish red colour with methanol and chloroform extract indicating the absence of phytosterols.

Test for flavonoids

The different extracts were separately dissolved in methanol and then subjected to the following tests.

(a) Ferric chloride test - To a small quantity of the methanolic solution, few drops of neutral ferric chloride was added. Blackish brown colour was not observed. Indicating the absence of flavonoids.

(b) Shinoda's Test –

To the alcoholic solution a small piece of magnesium ribbon was added along with concentrated hydrochloric acid. A magenta color was formed in methanol extract indicating the presence of flavonoids.

(c) Fluorescence Test –

Alcoholic solutions were seen under U.V light. No green fluorescence was observed in methanol, chloroform, water extracts indicating the presence of flavonoids.

Test for tannins and phenolic compounds

The extracts were dissolved in distilled water and filtered. The filtrates were treated with various reagents.

(a) Few ml of filtrates were treated with 5% ferric chloride solution. A bluish black color was observed in methanol extract indicating the presence of phenolic compounds.

(b) Few ml of filtrates were treated with lead acetate solution. White precipitates were not produced in all extracts indicating the absence of tannins.

(c) Few ml of filtrates were treated with strong potassium dichromate solution. Precipitate was not produced in ethyl acetate extracts indicating the absence of tannins.

(d) Few ml of the filtrates were treated with potassium ferricyanide followed by ammonia. A deep red color was observed in methanol extracts indicating the presence of phenolic compounds.

Test for saponins

Foam Test – The extracts were diluted with 20 ml of distilled water and agitated in a graduated cylinder for 15 minutes. Layer of foam was formed in petroleum ether extracts indicating the presence of saponins.

Phenol Content Analysis

Total phenolic content of methanolic and water extract of grains was carried out with the Folin-Ciocalteu reagent using standard Gallic acid following the method described by Kamtekar S et al. [18] The intense blue color was developed after incubation. The absorbance was measured at 760 nm using UV-visible spectrophotometer taking gallic acid as standard (11–62 µg/mL in water). The total phenolic content was expressed as mg of gallic acid equivalent (GAE)/g of distilled water.

POWDER ANALYSIS WITH CHEMICAL AGENTS

Reagents	Color observed
Powder	Brown
Powder+ conc. HCL	Violet
Powder + conc.HNO ₃	Brick red
Powder + conc. H ₂ SO ₄	Deep red
Powder + Glacial acetic acid	Crimson yellow
Powder + Picric acid	Yellow
Powder + Ammonia	Radish brown
Powder + 5% NaOH	Wine red
Powder + 5% KOH	Creamy
Powder + 5% FeCl ₂	Blackish green

FLUORESCENCE ANALYSIS OF POWDER DRUG

CHEMICAL	Fluorescence Observed
Powder + 1N NaOH in Methanol	Blackish red
Powder + 1N NaOH in Water	Crimson Colour
Powder + 50% HCL	Light brown
Powder + 50% HNO ₃	Brown
Powder + 50% H ₂ SO ₄	Reddish brown
Powder + Petroleum ether	Crimson colour
Powder + chloroform	Light yellow
Powder + Picric acid	Yellow
Powder + 5% ferric chloride solution	Blackish green
Powder + 5% iodine solution	Reddish brown
Powder + methanol	Blackish brown
Powder + HNO ₃ + NH ₃	Reddish brown

PH OF POWDERED DRUG

Solution	1% solution	10% solution
PH	6.77	6.36

DETAILED DATA OF PHYSICAL EVALUATION PARAMETERS

SL. NO	PARAMETER	VALUES (%) W/W
1.	Loss on Drying	5%
2.	Ash Values	
	Total Ash	3.67%
	Acid Insoluble Ash	1.56%
	Water Soluble Ash	2.05%
	Sulphated Ash	0.06%
3.	Extractive Value	
	Water Soluble Extractive	3.86%
	Alcohol Soluble Extractive	0.96%
	Benzene Soluble Extractive	0.02%
	Petroleum Ether soluble Extractive	0.16%

PHYTOCHEMICAL EVALUATION

Plant constituents	Tests	Petroleum ether Ext	Methanol Ext	Water Ext	Chloroform Ext
Glycosides	Baljet's Test	-	-	-	-
	Legal test	-	-	-	-
	Borntrager's test	-	-	-	-
	Test for Cardiac glycoside (Killer killani's Test)	-	-	-	-
	Test for cyanogenetic Glycosides	-	-	-	-
Alkaloids	Drangendroff's reagent	-	-	-	-
	Mayer's reagent	-	-	-	-
	Wagner's reagent	-	-	-	-
	Hager's reagent	-	-	-	-
	Tannic acid Test	-	-	-	-
Flavonoids	Magnesium turning method	+	+	+	+
	Ferric chloride test	-	-	-	-
	Fluorescence Test	+	+	+	+
Carbohydrates	Molish test	-	+	+	-
	Fehling's test	-	+	+	-
	Benedict's test	-	-	+	-
	Barfoed's test	-	+	-	-
	Starch	+	+	+	+
Saponin	Foam test	+	+	+	+
Tannins	Ferric chloride test	-	+	+	-
Gums and mucilages	Ruthenium test	-	-	-	-
Proteins and Amino acids	NinhydrinTest	-	+	-	-
	Biuret Test	-	-	-	-
	Million's Test	+	+	+	+
	Xanthoproteic Test	+	+	+	+
	Test with Tannic acid	-	-	-	-
	Test with heavy metals	+	+	+	+
Test for phytosterols	Liebermann's Test	+	+	+	+
	Liebermann – Burchard's Test	-	-	-	-
	Salkowski's Test	-	-	-	-

+ Present, - Absent

CONCLUSION

The presence of higher levels of dietary fiber and other protective healthy nutrients like phenolic antioxidants have been found to play effective role in lowering cholesterol, blood pressure, obesity and increasing overall immunity of the system specially in the whole meal-based millet preparations may still help in the nutritional management of heart disease and diabetes. Hence to enjoy the benefit of the functional constituents, it is imperative to identify judicious processing methods for kudo millet preparations to obtain maximum health benefits from the millet through appropriate research. It could be used in formulation of nutraceuticals and functional foods which can be consumed safely without posing any of health risks. Due to readily available nutrients and energy sources in millets, scientists, agriculture industries, and food security policies are giving more attention to millet production and processing for its better utilization to mitigate hidden hunger in the world.

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