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Phytochemical Estimation of Katarni Rice Grown in Bhagalpur and Banka District

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Abstract: *In Bihar, aromatic rice (Oryza sativa) is cultivated throughout the state. Basmati, Tulsi Manjari, Badhsabhog etc. are the predominant aromatic rice cultivated in Bihar. The root, leaf and seed of katarni rice were used for qualitative analysis in the traditional aromatic rice growing region of Bhagalpur and Banka district, where varieties such as Tulsi Manjari, Katarni are mostly common. For the determination of the active phytochemical, an alcoholic and aqueous extract of root, leaf and seed was used. The results shows that katarni rice contains much amounts of various active ingredients. From the results we conclude that aqueous & alcoholic extract of katarni rice contains various bioactive phytochemical compounds.*

Key Words: *Oryza sativa, Aromatic, Katarni, Phytochemical, Extract.*

1. INTRODUCTION:

Rice is the seed of the Oryza sativa (Asian rice) or Oryza glaberrima grass species (African rice). As a cereal grain, for a large part of the world's human population, especially in Asia, it is the most widely consumed staple food. It is the world's third-highest agricultural product (rice, 741.5 million tonnes in 2014), after sugarcane (1.9 billion tonnes) and maize (1.0 billion tones).

Rice, a monocot, is typically grown as an annual plant, although it can thrive as a perennial in tropical areas and can yield a ratoon crop for up to 30 years.[3] Rice cultivation is well suited to low labour cost and high rainfall countries and regions, as it is labor-intensive to grow and needs sufficient water. However, even on a steep hill or mountain area with the use of water-controlled terrace systems, rice can be grown practically anywhere. Although its parent species are native to Asia and some parts of Africa, in many cultures worldwide, centuries of trade and export have made it commonplace. Rice is the staple food of over half the population of the planet. For 17 countries in Asia and the Pacific, 9 countries in North and South America and 8 countries in Africa, it is the predominant dietary energy source. Rice provides 20% of the world's dietary energy supply, while 19% is provided by wheat and 5% by maize (corn).

In India, in the state of Bihar, Katarni rice (Arwa chawal in Hindi) is a special tasting, aromatic, short-grain rice. Katarni rice, grown indigenously in the districts of Bhagalpur and Banka, is not only in demand in Bihar, but throughout the country.

Katarni rice is facing the danger of extinction in spite of its uniqueness. There has been a substantial decrease in the area of Katarni rice cultivation since 1991-92, mainly due to: I increased irrigation costs, (ii) increased production of other paddy varieties; (iii) decreased demand in both local and global markets due to the market introduction of adulterated varieties. For some blocks under the Bhagalpur, Banka and Munger districts of Bihar, it has recently been labelled with Geographical Indication. It has enormous export potential thanks to unique quality characteristics. Including phenolic compounds, rice is a good source of natural antioxidants [9]. Both phenolic and flavonoid compounds have the ability to serve as free radical scavengers, reduction agents, and metal ion chelators as antioxidants, leading to the benefits of human health.

2. MATERIALS AND METHODS:

Collection of Plants

Plant was collected from field. After the plants are collected from field they have to be processed for cleaning in order to prevent the deterioration of phytochemicals present in plants. The collected plant sample were washed with distil water.

Drying

The main purpose of drying is to remove the water content from plants so that the plants can be stored. Two different methods were adopted for drying.

Natural Process

Natural process of drying includes the plant material exposed to shade for making them air dry. A total of 48 hours were provided for making them air dry.

Artificial Drying

Artificial drying were performed with the help of artificial driers. This process reduced the drying time to several hours or minutes.

Powdering

After complete drying of plants they were subjected to grinding to prepare powder.

3. METHODS OF EXTRACTION :

Plant Tissue Homogenization

Dried and wet, fresh samples of plants are grinded into fine particles in a blender, placed in a certain amount of solvent and shaken vigorously for 5-10 min and left for 2-5 days after filtering the extract. The filtrate was then centrifuged at 10000 rpm for 5 min then dried on water bath [10].

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Soxhlet Extraction

Soxhlet extraction is needed only where there is limited solubility of the desired compound in a solvent and the impurity is insoluble in that solvent. If a solvent is highly soluble in the desired compound, a simple filtration may be used to isolate the compound from the insoluble product. The benefit of this scheme is that only one batch of solvent is recycled instead of several parts of warm solvent being transferred through the sample. For thermo-labile compounds, this method cannot be used as prolonged heating can lead to compound degradation [11].

Preparation of Leaf Powder

The *Oryza sativa (leaf, root, seeds)* was collected, washed and cut into small pieces and dried at room temperature in shaded place or in hot air oven and made in to powder for further analysis.

Isolation of pigments

5 gram of plant tissue was ground in 50 ml of 80% acetone. The homogenate was filtered, centrifuged at 10,000 rpm for 10 minutes and the supernatant was taken as the pigment source.

Absorption spectra

Absorbency of the pigments were read between wave lengths 400-720 nm in a spectrophotometer (Systronics-104). Absorption spectra were constructed by plotting wavelength on x-axis and absorbance on y-axis. [14]

Quantification of pigments: Arnon's method (1949)

From specific absorptions obtained the pigments were quantified based on Arnon's formula.

Mg Chlorophylla/g tissue = $12.7(A_{663})$-$2.69(A_{645})$ * V/ (1000*W)

Mg Clorophyll b/g tissue = $22.9(A_{645})$ -$4.68(A_{663})$ * V/ (1000*W)

Mg total chlorophyll /g tissue = $20.2(A_{645})$ + $8.02(A_{663})$ *V/ (1000*W)

Phytochemical Analysis: [16]

Prepared plant extracts were analyzed for the presence of alkaloids, carbohydrates, glycosides, saponins, proteins, fixed oils, steroids, terpenoids, tannins, flavanoids etc. (The presence of phytochemicals extracted in various solvents was confirmed by standard protocols)[4,5,6,7,12,13].

Test for Alkaloids:Kokate CK, Purohit A P and Gokhale S B (2002)

Mayer's Test

To the 3 mL of extract, 1 ml of Mayer's reagent (Potassium Mercuric Iodide) was added. The appearance of white precipitate indicates the presence of Alkloid.

Wagner's Test:

To 3 ml of filtrate, 1 ml of Wagner's reagent (iodine in potassium iodide) was added. The appearance of reddish brown precipitate indicates the presence of alkaloids.

Hager's Test:

To 3 ml filtrate, 1 ml of Hager's reagent (saturated picric acid solution) was added. Yellow colour precipitate indicates the presence of alkaloids.

Test for Tannins:Ferric chloride Test, Kokate CK, Purohit A P and Gokhale S B (2002)
To the extracts were added a few drops of 10% ferric chloride solution. Appearance of a green or blue color indicates the presence of tannins.
Detection of Flavanoids: Shinoda *Test,* Kokate CK, Purohit A P and Gokhale S B (2002)
To the dry extract (2g), 5 ml of ethanol, 5 drops of hydrochloric acid and 0.5 g of magnesium turnings were added. Appearance of pink colour indicates the presence of flavonoids.
Test for Saponins: Foam Test, Kokate CK, Purohit A P and Gokhale S B (2002)
2g of the extracts were diluted with 20 ml of distilled water, shaken vigoursly and was observed for a stable persistent froth.
Test for Phenolic Compounds: Ferric Chloride Test, Kokate CK, Purohit A P and Gokhale S B (2002)
2 ml of diluted extracts, 2 ml of 5% v/v Ferric chloride solution. Appearance of a Blue-Black indicate the presence of phenol like compounds.

Test for carbohydrates: Sadasivam, S and Theymoli Balasubramanian (1985)

Molishs Test
Add two drops of Molisch's reagent (5% 1-napthol in alcohol) to about 2 mL of test solution. Add about 1 mL of conc. H_2SO_4 alongside of tube. Red cum violet ring appears indicates presence of carbohydrate.

Fehlings Test
To the extract add equal amount Fehling's reagent, mixed well and heated gently. Formation of a yellow or brownish-red precipitate indicates presence of reducing sugar.

4. RESULTS:
Phytochemical screening
The results of different phytochemical assay is shown in table 1.0. For the test of alkaloids, three different tests were performed viz. Mayer's Hager's and Wagner's. All the test confirms the presence of alkaloids in seed, root and leaf samples prepared in alcoholic, aqueous and pet. Ether solvents. Flavonoids were detected in the alcoholic, aqueous and pet. Ether solvent extract of roots only. Saponins were detected in the leaf extracts prepared in alcohol, aqueous and pet. ether solvents. Tannin was not detected in any of the plant samples in all the three different solvents taken for extraction purpose.

Phytochemicals

		Alcohol			Aqueous			Pet. Ether		
		Seed	Root	Leaf	Seed	Root	Leaf	Seed	Root	Leaf
Alkaloids	Mayer's	+	+	++	+	+	++	+	+	++
	Hager's	+	+	++	+	+	++	+	+	++
	Wagrner's	+	+	++	+	+	++	+	+	++
Flavanoids		-	+	-	-	+	-	-	+	-
Saponnins		-	-	+	-	-	+	-	-	+
Tannins		-	-	-	-	-	-	-	-	-

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Carbohydrates

Presence of carbohydrates was detected through three different methods in order to know the status of the major biomolecule. The results were similar for Molish's, Iodine and Fehling's test of leaf and seed extracts respectively (Table 2.0).

Table 2.0: Carbohydrate estimation in leaf and seed samples

	Molish's test	Iodine test	Fehling's test
Leaf extract	+	+	+
Seed extract (Alcoholic)	++	++	++
Seed extract (Aqueous)	++	++	++

Different phytochemical estimation methods and procedures are depicted through Fig. 1.0 to 5.0. figure 1.0 shows the samples for alkaloid estimation and fig. 2.0 for flavonide estimation. Saponine and tannin estimation methods is depicted through fig. 3.0 and 4.0 respectively. Fig 5.0 shows the method of carbohydrate estimation. Absorbance range of chlorophyll is depicted through fig. 6.0

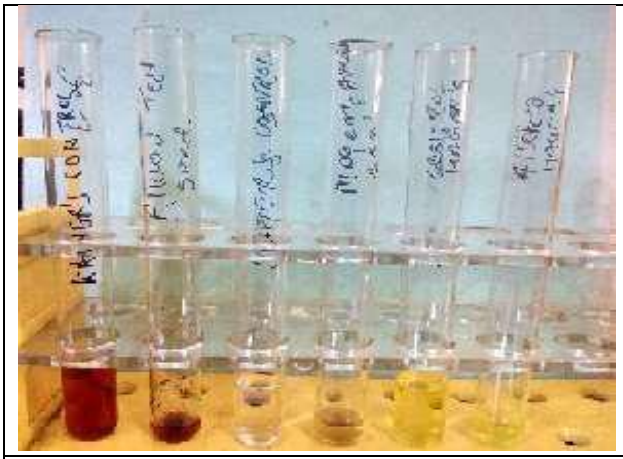

Fig. 1.0 Alkaloid estimation

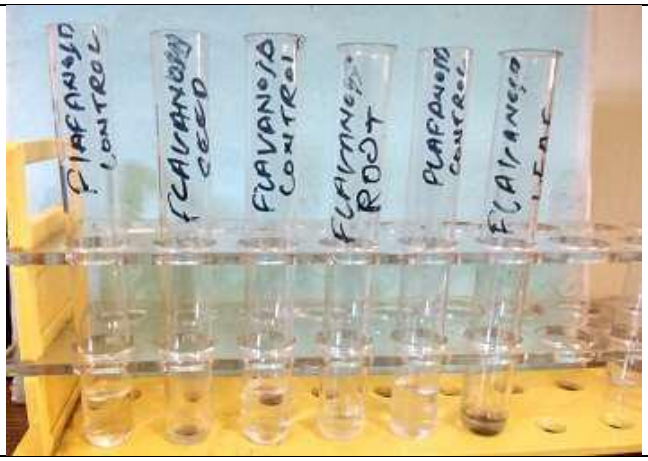

Fig. 2.0 Flavanoid estimation

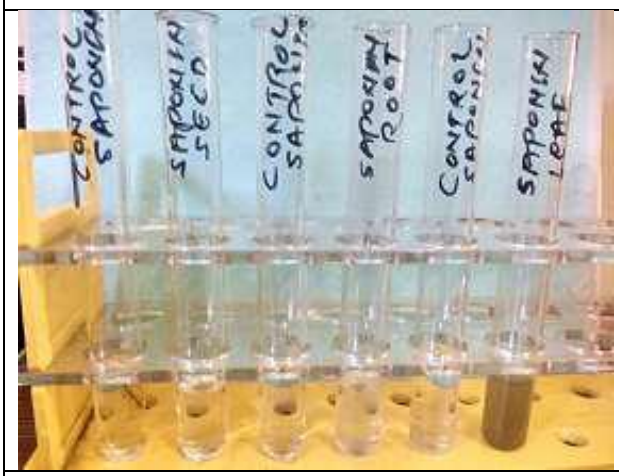

Fig. 3.0 Saponin mestimation

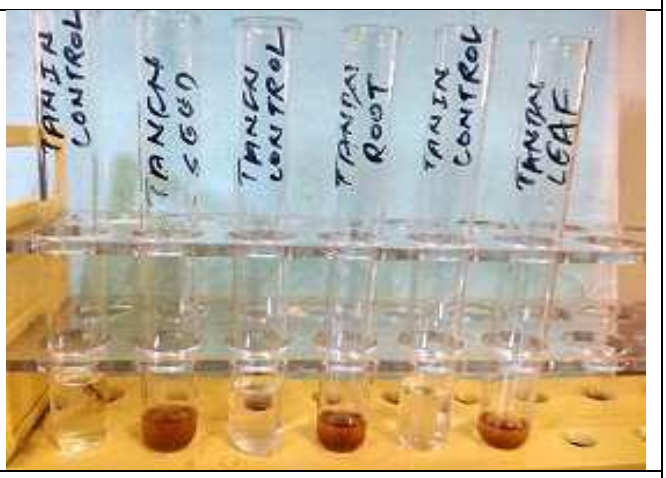

Fig. 4.0 Tanin estimation

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Fig. 5.0 Carbohydrate estimation

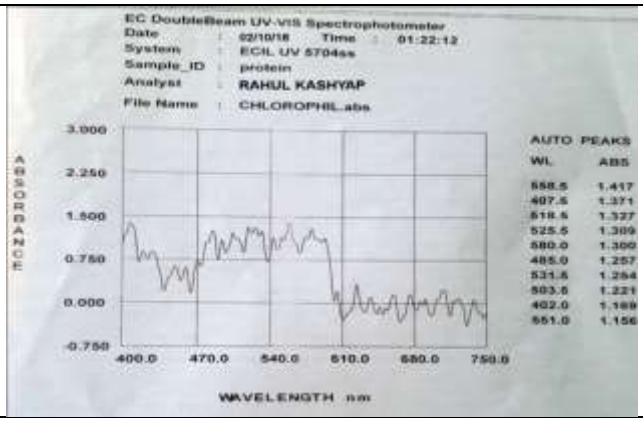

Fig. 6.0 Absorbance range of Chlorophyll

Chlorophyll /pigment concentration calculation: (Arnon, 1949):
Calculation of Chlorophyll a:

mg Chlorophylla/g tissue = $12.7(A_{663})-2.69(A_{645})$ * V/ (1000*W): where A_{663} = 0.222, A_{645} =0.097.
Therefore, 12.7 (0.222) - 2.69 (0.097) X 5 / (1000 X 5000)
= (2.82 – 0.26) X 5 / 5 $x10^6$
=12.8 / 5 $x10^6$
= 2.56 $x10^{-6}$ g
= 2.56 mg.
1 g of young fresh leave contain 2.56 mg of chlorophyll a.

Calculation of Chlorophyll b:
mg Chlorophyll b/g tissue = $22.9(A_{645})$ -$4.68(A_{663})$ * V/ (1000*W) : where A_{663} = 0.125, A_{645} =0.000
Therefore, 22.9(0.000)-4.68(.125) ×5/ (1000×5000)
= (0-0.58) ×5/5×10^6
= 2.9X10-6g.

= 2.9x10-3mg.

Calculation of total Chlorophyll:
mg total chlorophyll /g tissue = $20.2(A_{645})$ + $8.02(A_{663})$ *V/ (1000*W)
20.2(0.35) + 8.02(0.097)* V/ (1000*W)
=7.07 + 0.78 X 5/ (1000X5000)
=39.25$X10^{-6}$g.
= 39.25$X10^{-3}$ mg.

5. DISSCUSSION:

Phytochemicals are regarded as the active biomomecules present in plants and their prepared extracts (Emmanuel et al., 2017; Jain and Argal, 2014; Banu and Cathrine, 2015). Phytochemicals are assumed to have different functions with regard to metabolic activities, immunomodulation and antimicrobial activities (Kawale et al., 2010; Howes and Simmonds, 2014; Kibe et al., 2017). The present findings showed similarity in the presence of phytochemicals in rice with the works of Issac et al., 2012. The presence of saponin was detected in leaves while the prersence of flavonoids was detected in roots. The present findings are in accordance with the works of Bacco et al., 2017; Ali et al., 2018. The result of tannin was found negative which was absent in the findings of Bacoo et al., 2017. Alcoholic, aqueous and pet ether solvents showed similar kinds of results for phytochemicals present in seeds, leaves and roots of katarani rice. The estimation of chlorophyll in leaves were found to have similarity with the works of Zhang et al., 2009 and Vinsri et al., 2017

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