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Phytochemical and In Vitro Antioxidant Activities of *Kolakhar*: A Locally Used Herbal Soda of Assam, India.

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ABSTRACT

Kolakhar is a traditional food additives of Assam. Due to its alkaline in nature, people use it to wash clothes and locally it is used as a antibacterial agents. This work has investigated its phytochemical properties as well as its antioxidant activity taking quercetin as a standard. *Kolakhar* is found to contain different phytochemical constituents and significant antioxidant activities in a dose dependent manner. Thus, this traditional food additive has potential pharmacological activities and proper investigation may give different therapeutic active agent which can used to treat different types of diseases.

Keywords: *kolakhar*, Musa balbisiana, quercetin, phytochemical, antioxidant.

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INTRODUCTION

Every community having some traditional food item. In Assam (India), Assamese peoples are using a soda (alkali) to prepare different food dishes. This soda or alkaline substance is locally known as *kolakhar*(KK) [1]. Normally, this KK is used as a boiling agent instead of marketed baking soda powder. Traditionally, root of *Musa balbisiana* or whole plant of *Musa balbisiana* cut into small pieces and dried under sunlight over several weeks. After drying, dry material is burnt into ashes. Then ashes are extracted with normal water. The filtrate after extraction is called as *kolakhar* [2,3]. Without knowing the phytochemical and pharmacological knowledge, peoples using this product. Our aim of work is to investigate phytochemical and antioxidant activity of *kolakhar.*

After biochemical and metabolism process, free radical are the fundamental bi-product. Different factors are triggers the production of free radicals eg. UV radiations, smoke etc . Excessive production of free radicals leads to Oxidative stress. Oxidative stress may be the reasons for formation of new factors, which are mainly responsible for generation of cancer, inflammation, diabetes, liver cirrhosis, cardio vascular disease, Alzheimer's, Aging and acquired immunodeficiency syndrome. The diseases associated with the ROS mainly depend on the balance of the pro-oxidant and the antioxidant concentration in the body. Pro-oxidant conditions dominate either due to the increased generation of the free radicals or due to the excessive oxidative stress of the depletion of the dietary antioxidant [3,4,5].

A great number of aromatic and other medicinal plants contain chemical compounds that exhibit antioxidant properties. Sources of natural antioxidants are primarily, plant phenolics and flavonoids that may occur in all parts of plants such as fruits, vegetables, nuts, seeds, leaves, roots and barks. But in the research work, ashes of *Musa balbisiana* is used to estimate phytochemical and antioxidant properties [6].

METHODS AND MATERIALS

Plant material

The whole plant of *Musa balbisiana* was collected locally from village Ischadagharia,Kamrup, Assam, India. The plant materials were identified and authenticated taxonomically by an expert taxonomist of Guwahati University, Assam(Authentication No- 17891).

Preparation of extract-

A mixture of 25 gm dry ash and 500ml of distilled water taken in a one litre conical flask was stirred magnetically for one hour. After filtering, the residue washed with distilled water. The filtrate (light yellow colour) is known as *kolakhar*. Then the *kolakhar* is concentrated by evaporation. Dried extracts were kept in refrigerator and used for further study.

Preliminary Phytochemical Test [7]

Test for Tannin

Ferric Chloride Test: The test solution was treated ferric chloride solution, dark color appears which shows the presence of Tannin.

Gelatin Test: The test solution was treated with 1% Gelatin solution containing 10% NaCl, a white ppt forms which shows the presence of Tannin.

Test for Flavonoid

Ferric Chloride Test: Ttreat the test solution with ferric chloride solution, the intence green colour will show the presence of Flavonoid.

Shinoda Test: Treat the test solution with few fragments of Mg ribbon & conc. HCl, Pink Scarlet Crimson colour occasionally cream to blue color shows the presence of Flavonoid.

Test for Alkaloid

Mayers Test: Treat the test solution with Mayers reagent, cream color appers which shows the presence of Alkaloid.

Wagners Test: Treat the test solution some cidic solution & Wagners reagent, brown ppt willshow the presence of Alkaloid.

Test for Fat

Solubility Test: Treat the test sample with Pet. Ether. If the sample gets dissolved then it indicates the presence of Fat.

Test for Protein

Xanthoproteic Test: Treat the test solution with Conc. HNO3 on boiling water bath, if a yellow ppt will form the it shows the presence of Protein.

Biuret Test: Treat the test solution with 40% NaOH & then add Dil. CuSO4 solution, Blue color indicates the presence of protein.

Test for Steroid

Salkuowaski Test: Treat the test solution with few drops of Conc. H2SO4, shake, allowed to stand, lower layer turns Red, indicates the presence the Steroid (5-6).

***In-vitro* anti-oxidant activity** [8-12]

In this study free radical scavenging activity of kolakhar was determined by in vitro assay models such as DPPH free radical, reducing ability. Quercetin was used as reference standard.

DPPH radical scavenging activity

Principle

DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. The color changes from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 517 nm. Radical scavenging activity increased with increasing percentage of the free radical inhibition. The degree of discoloration indicates the free radical scavenging potentials of the sample/antioxidant by their hydrogen donating ability. The electrons become paired off and solution loses colour stochiometrically depending on the number of electrons taken up.

Procedure

DPPH radical scavenging activity was measured using the method of Kiranmai et al.; with some modifications. 2 ml of reaction mixture containing 1 ml of DPPH (100 µM in methanol) 1 ml of test solution, at various concentrations of the extract fractions was incubated at 37°C for 30 min absorbance of the resulting solution was measured at 517 nm using Beckman model DU-40 spectrophotometer. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with extract) using the following equation;

$$\text{Percentage inhibition} = (1 - \text{absorbance of test/absorbance of control}) \times 100$$

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Reducing Ability

Principle

Like the antioxidant activity, the reducing power increased with increasing amount of the extract.when potassium ferricyanide react with ferric chloride in the present of anti oxidant, potassium ferrocyanide and ferrous chloride are found as a product. Presence of reducers causes the conversion of the Fe3+/ferricyanide complex used in this method to the ferrous form.

Procedure

1 ml of different concentrations (25 to 900 µg/ml) of the extract fractions were mixed with potassium ferricyanide (2.5 ml, 1%) 2.5 ml of phosphate buffer (pH 6.6). The mixture was incubated at 50°C for 20 min. 2.5 ml TCA (10%) was added to it and centrifuged at 3000 rpm for 10 min. 2.5 ml of supernatant was taken and 2.5 ml water and 0.5 ml FeCl3 (0.1%) were added to it. The absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated higher reducing power.

RESULT AND CONCLUSION

From different research work it was found that many herbal plants showing antioxidant activity. *kolakhar* is a liquer, which is made up from *Musa balbisiana* by traditional process. People of Assam not only taking *kolakhar* as a remedies for diseases, but also as a food additive to make varieties of food dishes. In the present study, we have investigated the phytochemical constituents and antioxidant activities of kolakhar. Different phytochemical constituents were found in the *kolkhar*. The result of phytochemical study is listed in table;1. The extract was checked for its antioxidant activity in two model systems i.e. in DPPH method and reductive ability. *Kolakhar* showed in vitro antioxidant activity in Dose dependant manner.

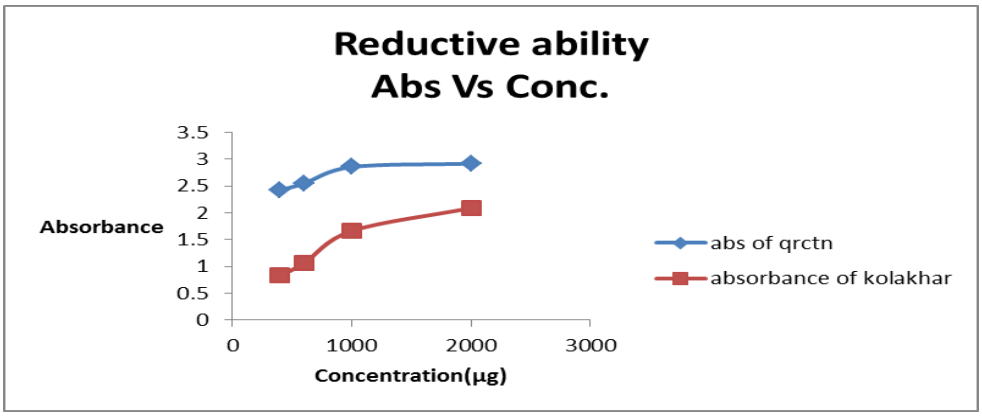

Figure 1: Reducing ability of *kolakhar* With respect to standard Quercetin.

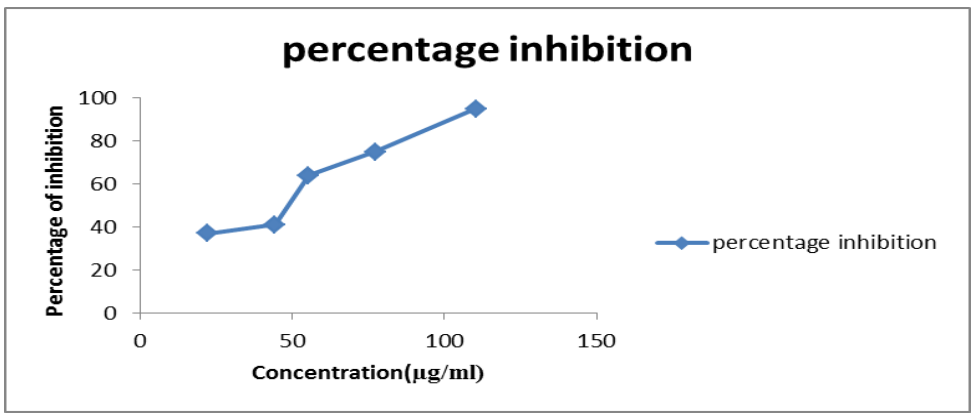

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Figure 2: DPPH free-radical scavenging activity of *kolakhar* with respect of standard quercetin.

Table 1: Phytochemical test result of the sample

Si no.	Constituents	Results
1	Tannin	+++
2	Saponin	+++
3	Carbohydrate	+++
4	flavonoids	+++
5	Fat	_ _
6	Protein	+++
7	steroid	_ _

"+" means presence and "-" means absent

Table 2 :Reducing ability of *kolakhar* With respect to standard quercetin at 700 nm

Sl no	Concentration (µg/ml)	Absorbance at 700 nm	
		Kolakhar	Quercetin
1	400	0.833	2.421
2	600	1.059	2.548
3	1000	1.662	2.855
4	2000	2.089	2.917

Table 3: DPPH Radical Scavenging Activity

Sl NO.	Concentration (µg/ml)	Absorbance at 517 nm	
		Kolakhar	Quercetin
1	22.08	1.861	2.968
2	44.16	1.412	2.392
3	55.20	1.316	1.911
4	77.28	0.976	1.252
5	110.40	0.651	0.681

Table 4: Percentage inhibition of *kolakhar*

Sl no	Concentration (µg/ml)	Percentage inhibition of *kolakhar*
1	22.08	37
2	44.16	41
3	55.20	69
4	77.28	77
5	110.40	95

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