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Evaluation of antioxidant potential of grains of *Paspalum scrobiculatum* Linn.

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ABSTRACT

Antioxidants play a vital role in protection against various diseases and disorders. In search for new antioxidants, nutraceuticals are extensively studied for their preventive effects. *Paspalum scrobiculatum* Linn., commonly known as Kodo millet, is a minor millet found in India having pharmacological importance. Whole grains of kodo millet were evaluated for its antioxidant potential. Five different extracts of kodo millet, i.e. 70% ethanol, 70% methanol, ethyl acetate, chloroform and petroleum ether extracts were screened for their reducing capacity and free radical scavenging capacity. 1, 1-diphenyl-2-picrylhydrazyl (DPPH) Assay was performed using ascorbic acid as standard at 515 nm. The reducing capacity was measured at 700 nm with ascorbic acid as reference standard. Highest radical quenching was observed in ethanol extract, 64.609 ± 0.131µg/ml Ascorbic Acid Equivalent (AAE). The reducing capacity was found maximum in ethanol extract, followed by methanol. All the extracts showed antioxidant potential and the hydro-alcoholic extracts, i.e. methanol and ethanol showed high reducing and radical scavenging capacity respectively. The results of preliminary study indicate that the grains of *Paspalum scrobiculatum* have good antioxidant potential and these can be used as potent Nutraceutical against target diseases which can be studied further.

Keywords: DPPH, Kodo millet, Nutraceutical, *Paspalum scrobiculatum*, Reducing Power.

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INTRODUCTION

Free radicals are the product of metabolic processes occurring in the human body or formed due to external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants, and industrial chemicals. [1] Reactive Oxygen Species (ROS) like superoxide ($O_2^{-\bullet}$), hydroxyl ($OH^{\bullet}$), hydroperoxyl ($HOO^{\bullet}$), peroxyl ($ROO^{\bullet}$), and alkoxyl ($RO^{\bullet}$) radicals or Reactive Nitrogen species (RNS) like nitric oxide ($NO^{\bullet}$) and peroxynitrite anion ($ONOO^{\bullet}$), interact with various metabolic processes in our body and are a part of natural cellular processes, but at high concentration, they cause damage leading to various disorders like neurodegeneration, cancer and ageing. [2, 3] To combat this, antioxidants play a vital role. Antioxidants are chemicals that interact with and neutralize free radicals, thus preventing them from causing damage. Antioxidants are also known as free radical scavengers. The body makes some of the antioxidants it uses to neutralize free radicals. These antioxidants are called endogenous antioxidants. [4]

Phytochemicals can be active as antioxidants and show beneficial role in protecting against oxidative stress. [5] Several studies have revealed that natural antioxidants, such as vitamin E, vitamin C and beta-carotene, may help in scavenging free radicals generated during the initiation and progression of diseases. [6, 7] Therefore, there has been significant increase in interest in plant phytochemicals with antioxidant property as potential agents to prevent diseases. [8]

Paspalum scrobiculatum Linn (*P. scrobiculatum*) belongs to family *Poaceae*. It is commonly known as 'Kodo millet'. It is a tufted perennial grass, up to 120-150 cm tall. 9 It is an extremely drought resistant crop. [10] It is salt resistant and is grown in saline soils also. [11] This species thrives even in very poor soils. [12] Kodo millet is a minor grain crop grown throughout India, but to a greater extent in the Deccan and South India. [5] In India, it is distributed in Madhya Pradesh, Chattisgarh and Karnataka. [9]

Kodo millet is reported to be rich in proteins when compared to other minor millets. [5] Flavonoids and phenolic acids were reported in Kodo millet acetone extract. [13] Quercetin was the flavonol present. The phenolic acids located were vanillic acid, syringic acid, cis-ferulic acid, p-hydroxy benzoic acid and melilotic acid. [11] Ferulic and p-coumaric acids were the major hydroxycinnamic acids. [13] Kodo millets are rich sources of phenolics, tannin and phytates, which can be active as antioxidants and show beneficial role in protecting against oxidative stress and maintaining blood glucose response. [14] Kodo millet extract (Methanolic) was found to be a potent drug in the treatment of skin wounds. [11, 15] The crude extracts and the pure isolates were reported to have nutritive, anti-fungal effects. [16]

The present study investigates the antioxidant potential of five extracts of *P scrobiculatum* grains. Previous study conducted on kodo millet for its antioxidant potential was done using methanol solvent, in which high radical quenching was observed. [17] Ethanol has been found to be an effective solvent for extracting millet phytochemicals, therefore in this study, apart from methanol, ethanol was also used to access its antioxidant potential.

MATERIALS AND METHOD

Collection of plant material

The grains of *Paspalum scrobiculatum* used for experiment were collected from Mandla district of Madhya Pradesh, and authenticated by Dr. Zia-ul-Hassan, Dept. of Botany, Safia Science College, Bhopal, Madhya Pradesh having authentication number- 519/Bot./Safia/2015.

Chemicals & Reagents

The chemicals used for the antioxidant assays were Methanol (CH_3OH), Ethanol (C_2H_5OH), Petroleum ether, Ethyl acetate ($CH_3COOC_2H_5$), Chloroform ($CHCl_3$), 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Potassium ferricyanide $K_3Fe(CN)_6$, Phosphate buffer, Trichloroacetic acid (CCl_3COOH), Ferric chloride ($FeCl_3$), water, etc. All the chemicals were of analytical grade.

Preparation of crude extract

The grains of Kodo millet were washed, dried in shade, coarsely powdered in a grinder, weighed and then kept in a closed jar. For extraction process, Maceration technique was used, in which solvents of different polarity were added successively to the kodo grains for a period of 2 to 7 days. In this polarity based solvent extraction process, petroleum ether, chloroform, ethyl acetate, 70% ethanol and & 70% methanol were used in the order of increasing polarity. The millet grains were kept in Petroleum ether for 2 days, 4 days in chloroform and 7 days each in ethyl acetate, ethanol and methanol, with occasional shaking/ stirring. [18] The marc obtained after each solvent extraction was heated at $< 60\ ^{o}C$ till all the solvent evaporated. The concentrated mass obtained, i.e. the crude extract for the five solvents was weighed and kept in a refrigerator for further experimental procedure.

Experimental Procedure

To determine the antioxidant potential of *Paspalum scrobiculatum* grains, two assays were performed- DPPH Assay and Reducing Power Assay. The five extracts of the studied plant were used for the assays as given herein:

1, 1-diphenyl-2-picrylhydrazyl (DPPH) Assay [19]

The antioxidant activities of plant extracts and the standard were assessed on the basis of the free radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity. The diluted solutions of the plant extracts were prepared in methanol. Ascorbic acid (100µg/ml) was used as standard. 0.1 mM DPPH was prepared in methanol and 2ml of this solution was mixed with 2ml of test sample solution and standard solution separately of varying concentrations (10-100 µg/ml). A mixture of 1mL methanol and 2ml DPPH solution was used as control. The solutions were kept in dark for 10 min and absorbance was measured at 515 nm by spectrophotometer. Methanol (2ml) with DPPH solution (2ml) was used as blank. The absorbance was recorded and % inhibition was calculated using the formula given below:

Percent Inhibition= *Absorbance (control) – Absorbance (sample) x100/ Absorbance (control)*

A curve was then plotted between % inhibition and concentration to get the line of regression, which is used to estimate IC50. The IC50 value indicates the concentration of tested sample required to reduce the free radical concentration by 50%.

Reducing Capacity [20]

Different concentrations of test samples were prepared. To 0.5 mL of different sample concentrations, 0.2 M Phosphate buffer (pH 6.6) and 0.5 mL Potassium ferricyanide (1% w/v) were added and incubated at 50 ^{o}C for 20 min. After cooling, 1.5 mL Trichloroacetic acid (10% w/v) added to reaction mixture to terminate the reaction. To this, 0.5mL Ferric chloride (0.1% w/v) was added and absorbance measured at 700 nm using ascorbic acid as reference standard. A curve was then plotted between absorbance and concentration. Increased absorbance of the reaction mixture indicates increase in reducing power.

Statistical analysis

All data were reported as mean ± standard deviation (Mean ± SD) of three replicates.

RESULTS AND DISCUSSION

The DPPH radical scavenging activity using ascorbic acid as standard showed an increasing value of IC50, with the increase in polarity of the solvents. Ethanolic extract of *Paspalum scrobiculatum* showed highest quenching capacity of 64.609 ± 0.131µg/ ml AAE while Petroleum ether extract showed minimum scavenging activity of 627.459 ± 18.918 µg/ ml AAE. (Refer Table 1)

Table 1: Free radical scavenging activity of five different crude extracts of *P. scrobiculatum* grains in terms of IC50

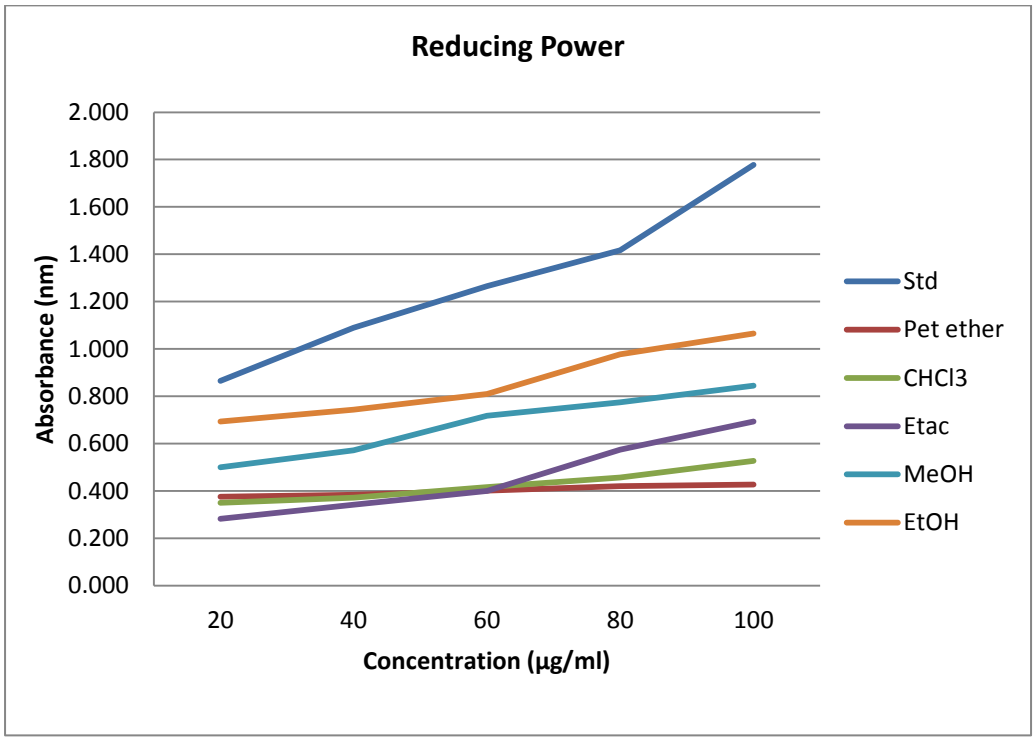
Plant extract	IC50 (µg/ ml AAE)
Pet ether	627.459 ± 18.918
$CHCl_3$	265.676

Etac	178.153 ± 2.112
MeOH	142.692 ± 2.744
EtOH	64.609 ± 0.131

*DPPH radical scavenging activity of *P. scrobiculatum* extracts expressed as Ascorbic acid equivalent. Pet ether = petroleum ether; $CHCl_3$ = Chloroform, Etac = Ethyl acetate; MeOH = Methanol; EtOH = Ethanol.

The DPPH method is a fast method and it can be helpful in investigation of novel antioxidants for a rapid estimation and preliminary information of radical scavenging abilities. The method is sensitive and requires small sample amounts. [21] The antioxidant potential of ethanol extract was found to be greater than the methanol extract; therefore ethanol is a better solvent for extracting bioactive components from kodo millet.

In Reducing Power assay, an increasing trend was observed in graph between concentration v/s absorbance for all the crude extracts of kodo millet grains. (Refer Graph 1) This indicated an increasing antioxidant capacity. Highest capacity was observed for ethanol extract, which was comparable with reference standard. This was followed by methanol. A higher antioxidant capacity is generally observed for more polar solvent extracts, which was observed in the experiment as well.



Graph 1: Reducing capacity of five extracts of *P. scrobiculatum* grains

*Std= Standard (Ascorbic acid); Pet ether= (Petroleum ether); $CHCl_3$= Chloroform; Etac= Ethyl acetate; MeOH= Methanol; EtOH= Ethyl alcohol.

CONCLUSION

Antioxidants are known to have an important role in neutralization the harmful effects of oxidative stress and fight against various diseases like, cancer, cardiovascular and neurodegenerative. [22, 23] Many plant species have been investigated in the search for novel antioxidants, but there is still a demand of more information concerning their antioxidant potential. [24] Foods having pharmacological importance, i.e. Nutraceuticals are also extensively studied these days for finding novel compounds as antioxidants. Most of the studies are conducted on crude extracts, and scientific evidence is lacking to establish bioactive component responsible for the said activity. One or more bioactive components are considered to be present in plants and if they are used as pure compound they may be very useful as antioxidant. Kodo millet is found to have high dietary fiber content, wound healing capacity, and is anti-diabetic. *P. scrobiculatum* could be promoted as a food crop by bringing it in the main stream agriculture. The presence of antioxidant activity in this millet has opened up a new horizon for further studies like extraction and characterization and can find wide industrial applications. The presence of high antioxidant potential in *P. scrobiculatum* and its efficacy as potent drug against various diseases make this millet a potential Nutraceuticals.

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