International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 15-08-2021, Accepted: 30-08-2021, Published: 16-09-2021
Volume 6, Issue 5, 2021, Page No. 586-589

Assessment of *In vitro* antioxidant activity of two different varieties of pearl millet (*Pennisetum glaucum*-Kambu) and its formulation

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Abstract

Antioxidant activity of petroleum extracts of two different varieties and its formulation were carried out for proving its utility in free radical mediated diseases including diabetic, cardiovascular, cancer etc. In the current study, the petroleum extracts of *Pennisetum glaucum* varieties (powder of C010, Nattu kambu and flake of C010, Nattu kambu) and its formulation (1:1 ratio of C010, Nattu kambu powder and 1:1 of ratio C010, Nattu kambu flake) were screened for *in vitro* antioxidant activities by DPPH scavenging, total antioxidant and nitric oxide scavenging activity at different concentrations and compared with standard as ascorbic acid. Throughout the studies Pearl millet extracts showed dose dependent marked antioxidant activity The order of antioxidant activity was observed as C010: Nattu kambu flake, C010: Nattu kambu powder, C010 flake, Nattu kambu powder and C010 powder variety. The observed effects may be associated with the presence of phenolic and flavonoids. Among the various *Pennisetum glaucum* varieties and its formulation, Nattu kambu flake variety and C010: Nattu kambu flake formulation has potential antioxidant activities were observed. Overall, the *Pennisetum glaucum* extracts is a source of natural antioxidants which might be helpful in preventing the progress of various oxidative stress mediated diseases including diabetic.

Keywords: antioxidant activity, pearl millet, *Pennisetum glaucum*, reactive oxygen and nitrogen species scavenging assay

Introduction

The adverse effects of oxidative stress on human health have become a serious issue. Under stress, our bodies produce more reactive oxygen species (ROS) (e.g., superoxide anion radicals, hydroxyl radicals and hydrogen peroxide) than enzymatic antioxidants (e.g., superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase) and non-enzymatic antioxidants (e.g., ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione, carotenoids, and flavonoids). This imbalance leads to cell damage (Bhatia *et al*., 2003; Peuchant *et al.*, 2004) [1, 2] and health problems (Steer *et al.*, 2002) [3]. A lack of antioxidants, which can quench the reactive free radicals, facilitates the development of degenerative diseases, including cardiovascular diseases, cancers, neurodegenerative diseases, Alzheimer's disease and inflammatory diseases (Velavan, 2011; Alma *et al*, 2003) [4, 5]. Natural and synthetic antioxidants are beneficial to free radical mediated diseases. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have been widely used as antioxidants in the food industry and may be responsible for liver damage and carcinogenesis (Grice, 1988) [6] for this reason, interest in the use of natural antioxidants has increased. Millets are nutritious food and they are rich in phytochemicals, fiber and minerals. Magnesium in millet can help to reduce the effects of migraines and heart attacks. Niacin (vitamin B3) in millet can help to lower cholesterol. Phosphorus in millet helps with fat metabolism, body tissue repair and creating energy. Fiber from whole grains has been shown to protect against breast cancer and childhood asthma. It contains a wide range of phenolics, phytic acid and tannins which are good sources of natural antioxidants and contribute to boost health, prevent ageing and metabolic diseases. A wide variety of traditional foods and beverages are produced in countries where millets are grown for consumption. Millet foods produced from meal or flour include flat bread (fermented or unfermented), couscous and porridges, in addition to snack foods (Anoma, 2011) [7].

Pearl millet (Kambu) is recognized as being the most widely grown of all the millet types and is grown extensively in the tropics and a staple food for the low income groups in some countries of the world. Globally the millet production is more concentrated in the Asian and African countries. It is the basic staple food in the poorest countries and used by the poorest people. For human consumption it can be used in a variety of ways including both leavened and unleavened breads, in porridges, and can also be boiled or steamed (Anu Sehgal and Kawatra, 2006) [8]. With this background and abundant source of unique active components harbored in millet. Present study the chosen millet namely *Pennisetum glaucum* belonging to Poaceae family and the antioxidant activity of *Pennisetum glaucum* was not evaluated. Therefore, the present study were to investigate the antioxidant activity of *Pennisetum glaucum* through the DPPH scavenging, total antioxidant and nitric oxide scavenging assay.

Materials and Methods

Chemicals

Nitro blue tetrazolium (NBT), ethylene diamine tetra acetic acid (EDTA), sodium nitroprusside (SNP), trichloro acetic acid (TCA), thio barbituric acid (TBA), potassium hexa

cyano ferrate [$K_3Fe(CN)_6$], and L-ascorbic acid were purchased from Sisco Research Laboratories Pvt. Ltd., India. All other chemicals and solvents used were of analytical grade available commercially.

Collection of Pearl millet and preparation of powder and Porridge flakes

Pennisetum glaucum as C010 (Bill No. 077963) and Nattu kambu (Bill No. 077964) purchased from Tamil Nadu Agricultural University, Tiruvannamalai, Tamil Nadu, India. Before the experiment, dried whole grain of pearl millet was ground into powder using a blender. In preparation of flakes, 200gms of powdered pearl millet mixed with green chillies (10gms), small onion (20 gms), cumin seeds (5gms) and curry leaves (10gms). Required amount of salts were added. Further the mixed sample was grinded, cooked and then kept in overnight. After the overnight, 20ml of buttermilk was added and mixed well. Finally put in the mould and dried the sample under sunlight to make a Porridge flake.

Extraction of Pearl millet powder and Porridge flakes

The powdered pearl millet were separately weighed by sensitive digital weighing balance and a total of 20g of each variety Pearl millet (powder of C010, Nattu kambu and flake of C010, Nattu kambu) and its formulation (1:1 ratio of C010, Nattu kambu powder and 1:1 ratio of C010, Nattu kambu flake) were macerated with petroleum ether (20 g in 150 mL) in Erlenmeyer flask for 24 hrs at room temperature (25–27°C).

The extraction process goes on for 24 hours facilitated by using shaker. After 24 hrs, the extract was separated from the marc using gauze and further filtered by Whatman filter paper No. 1.

The obtained filtrates were concentrated in a water bath set at 40°C. After drying, the amount of dry extract obtained was harvested and the dried extract was transferred into airtight bottles and stored in a refrigerator at −4°C until used.

Antioxidant Assay

The scavenging ability of the natural antioxidants of the plant extract towards the stable free radical DPPH was measured by the method of Shimada *et al.,* (1992) [9]. The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al.,* (1999) [10]. Nitric oxide radical scavenging activity was determined according to the method reported by Garrat (1964) [11].

Statistical analysis

Tests were carried out in triplicate for 3 separate experiments. The amount of extract needed to inhibit free radicals concentration by 50%, IC_{50}, was graphically estimated using a nonlinear regression algorithm.

Results and Discussion

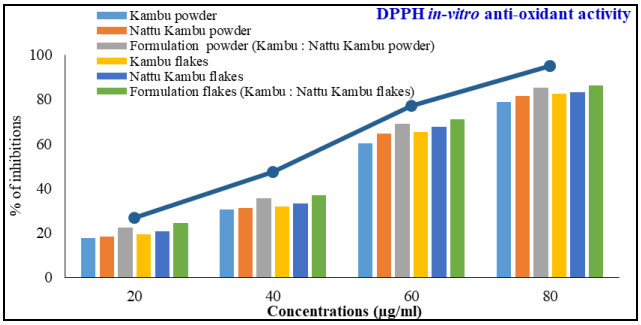
An antioxidant can be defined as any substance that, when present in low concentrations compared to that of an oxidisable substrate, significantly delays or inhibits the oxidation of that substrate‖ The physiological role of antioxidants, as this definition suggests, is to prevent damage to cellular components arising as a consequence of chemical reactions involving free radicals (Halliwell and Gutteridge, 1995) [12]. Antioxidants play an important role in the body‘s defence system against Reactive Oxygen Species (ROS), which are the harmful by-products generated during normal cell aerobic respiration (Ou *et al*, 2002) [13]. In foods, antioxidants prevent undesirable changes in flavour and nutritional quality of a product (Zielinski and Kozłowska, 2015) [14]. Cereals and millets are the most commonly consumed food items in India. They contain a wide range of phenolics which are good sources of natural antioxidants.

DPPH Assay

DPPH radical scavenging activity of Pearl millet extract and standard as ascorbic acid are presented in Figure 1. The DPPH radical was widely used to evaluate the free-radical scavenging capacity of antioxidants (Nuutila *et al.,* 2003) [15]. Recently, the use of the DPPH• reaction has been widely diffused among food technologists and researchers, for the evaluation of free radical scavenging activity on extracts from plant, food material or on single compounds. In the DPPH assay, the antioxidant was able to reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine. The molecule of 2, 2-diphenyl-1-picryl hydrazine is characterized as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole. The proton transfer reaction of the DPPH• free radical by a scavenger (A-H) causes a decrease in absorbance at 517 nm, which can be followed by a common spectrophotometer set in the visible region. The effect of antioxidants on DPPH• is thought to be due to their hydrogen donating ability (Sindhu and Abraham, 2006) [16]. The Pearl millet extract exhibited a significant dose dependent inhibition of DPPH activity. The IC_{50} was 52.95, 50.97, 50.15 and 48.89µg/ml for the powder of C010, Nattu kambu, flake of C010, Nattu kambu) while 47.21, 45.70 and 40.10 µg/ml in formulation 1:1 ratio of C010, Nattu kambu powder, 1:1 ratio of C010, Nattu kambu flake and standard ascorbic acid, respectively. The lowest IC_{50} values has highest antioxidant activity. Among the various Pearl millet varieties and its formulation, Nattu kambu flake variety and C010: Nattu kambu flake formulation has potential antioxidant activities were observed and near to standard as ascorbic acid.

Table 1: DPPH scavenging activity of the petroleum ether extract of two different Pearl millet varieties and its formulation and comparison with standard drug Ascorbic acid

% of inhibitions	Concentrations (µg/ml)				IC_{50} (µg/ml)
	20	40	60	80	
C010 (Kambu powder)	17.86±1.25	30.48±2.13	60.40±4.22	78.72±5.51	52.95
Nattu Kambu powder	18.54±1.29	31.17±2.18	64.61±4.52	81.34±5.69	50.97
Formulation powder (Kambu: Nattu Kambu powder)	22.41±1.56	35.60±2.49	69.16±4.84	85.21±5.96	47.21
C010 (Kambu flakes)	19.56±1.36	31.96±2.23	65.41±4.57	82.36±5.76	50.15
Nattu Kambu flakes	20.81±1.45	33.33±2.33	67.57±4.72	83.16±5.82	48.89
Formulation flakes (Kambu: Nattu Kambu flakes)	24.45±1.71	36.97±2.58	71.10±4.97	86.34±6.04	45.70
Std. (Ascorbic acid)	26.73±1.87	47.55±3.32	77.13±5.39	94.99±6.64	40.10



Values expressed as Mean ± SD for triplicates

Fig 1: DPPH scavenging activity of the petroleum ether extract of two different Pearl millet varieties and its formulation and comparison with standard drug Ascorbic acid

Total antioxidant activity

The yield of the petroleum extract of Pearl millet and its total antioxidant capacity are given in Figure 2. Total antioxidant capacity of Pearl millet extract is expressed as the number of equivalents of ascorbic acid.

The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/ Mo (V) complex with a maximal absorption at 695 nm. The assay is successfully used to quantify vitamin E in seeds and, being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extract (Prieto *et al.*, 1999) [10]. The Pearl millet extract exhibited a significant dose dependent inhibition of total antioxidant activity. The IC_{50} was 57.35, 54.08, 51.90 and 49.56µg/ml for the powder of C010, Nattu kambu, flake of C010, Nattu kambu) while 47.34, 44.22 and 41.01µg/ml in formulation 1:1 ratio of C010, Nattu kambu powder, 1:1 ratio of C010, Nattu kambu flake and standard ascorbic acid, respectively. The lowest IC_{50} values has highest antioxidant activity. Among the various Pearl millet varieties and its formulation, Nattu kambu flake variety and C010: Nattu kambu flake formulation has potential antioxidant activities were observed and near to standard as ascorbic acid.

Table 2: Total antioxidant activity of the petroleum ether extract of two different Pearl millet varieties and its formulation and comparison with standard drug Ascorbic acid

% of inhibitions	Concentrations (µg/ml)				IC_{50} (µg/ml)
	20	40	60	80	
C010 (Kambu powder)	13.74±0.96	29.71±2.07	54.76±3.83	72.28±5.05	57.35
Nattu Kambu powder	15.96±1.11	32.37±2.26	58.98±4.12	75.83±5.30	54.08
Formulation powder (Kambu: Nattu Kambu powder)	22.61±1.58	39.91±2.79	66.51±4.65	81.81±5.72	47.34
C010 (Kambu flakes)	18.62±1.30	34.14±2.38	61.86±4.33	77.60±5.43	51.90
Nattu Kambu flakes	21.50±1.50	37.02±2.59	63.41±4.43	79.82±5.58	49.56
Formulation flakes (Kambu: Nattu Kambu flakes)	25.72±1.80	41.68±2.91	69.17±4.84	88.24±6.17	44.22
Std. (Ascorbic acid)	27.05±1.89	46.56±3.25	73.83±5.16	93.12±6.51	41.01

Values expressed as Mean ± SD for triplicate

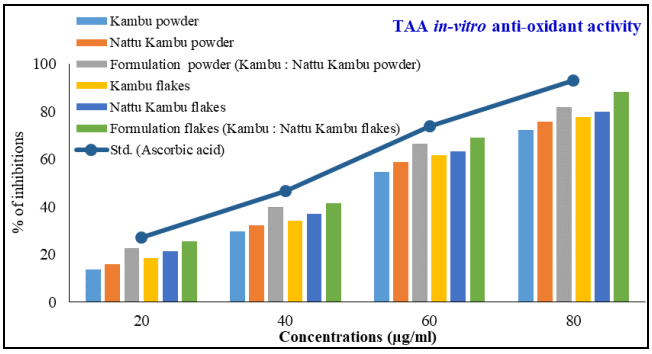


Fig 2: Total antioxidant activity of the petroleum ether extract of two different Pearl millet varieties and its formulation and comparison with standard drug Ascorbic acid

Nitric Oxide Scavenging activity assay

Nitric oxide (NO) released from sodium nitro prusside (SNP) has a strong NO^{+} character which can alter the structure and function of many cellular components. The extract of Pearl millet exhibited good no scavenging activity leading to the reduction of the nitrite concentration in the assay medium. The NO scavenging capacity was concentration dependent. Pearl millet in SNP solution significantly inhibited the accumulation of nitrite, a stable oxidation product of NO liberated from SNP in the reaction medium with time compared to the standard ascorbic acid. The toxicity of NO increase when it reacts with superoxide to form the peroxynitrite anion (ONOO-) which is a potential strong oxidant that can decompose to produce OH and NO_2 (Pacher *et al.*, 2007) [17]. The Pearl millet extract exhibited a significant dose dependent inhibition of Nitric oxide scavenging activity (Figure 3). The IC_{50} was 57.92, 56.27, 54.20 and 51.94µg/ml for the powder of C010, Nattu kambu, flake of C010, Nattu kambu) while 48.58, 46.22 and 41.45µg/ml in formulation 1:1 ratio of C010, Nattu kambu powder, 1:1 ratio of C010, Nattu kambu flake and standard ascorbic acid, respectively. The lowest IC_{50} values has highest antioxidant activity. Among the various Pearl millet varieties and its formulation, Nattu kambu flake variety and C010: Nattu kambu flake formulation has potential antioxidant activities were observed and near to standard as ascorbic acid.

Table 3: Nitric oxide scavenging activity of the petroleum ether extract of two different Pearl millet varieties and its formulation and comparison with standard drug Ascorbic acid

% of inhibitions	Concentrations (µg/ml)				IC_{50} (µg/ml)
	20	40	60	80	
C010 (Kambu powder)	15.43±1.08	32.75±2.29	50.23±3.51	71.96±5.03	57.92
Nattu Kambu powder	17.95±1.25	34.64±2.42	51.49±3.60	73.07±5.11	56.27
Formulation powder (Kambu: Nattu Kambu powder)	22.51±1.57	41.57±2.90	60.47±4.23	80.94±5.66	48.58

CO10 (Kambu flakes)	18.26±1.27	38.26±2.67	53.70±3.75	74.33±5.20	54.20
Nattu Kambu flakes	20.31±1.42	39.52±2.76	55.59±3.89	77.32±5.41	51.94
Formulation flakes (Kambu: Nattu Kambu flakes)	23.93±1.67	42.51±2.97	65.82±4.58	82.83±5.79	46.22
Std. (Ascorbic acid)	27.71±1.93	45.98±3.21	72.44±5.07	90.70±6.34	41.45

Values expressed as mean ± SD for triplicate

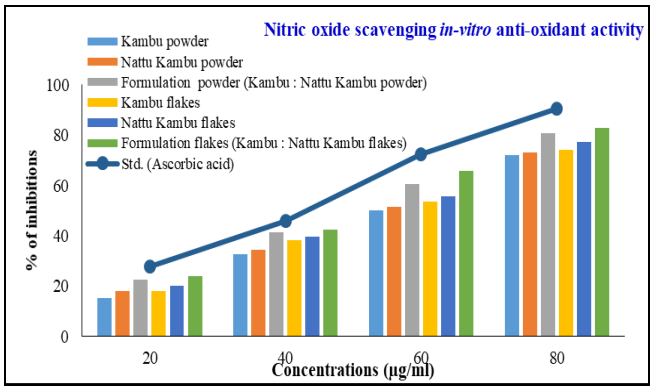


Fig 3: Nitric oxide scavenging activity of the petroleum ether extract of two different Pearl millet varieties and its formulation and comparison with standard drug Ascorbic acid

Conclusion

The results of the present study showed that the extracts of *Pennisetum glaucum* which contains of flavonoids and polyphenols. These phytochemicals are exhibited the greatest antioxidant activity DPPH scavenging, total antioxidant and nitric oxide scavenging activity which participate in various pathophysiology of diseases including diabetic, cancer etc. This work has gathered experimental evidence on the *Pennisetum glaucum* extract as natural antioxidant for its capacity to scavenge reactive oxygen and nitrogen species and protect cells/organism from oxidative damage and thus could be an effective against oxidative stress. In addition, the *Pennisetum glaucum* extract found to contain a noticeable amount of total phenols which plays a major role in controlling antioxidants. Thus, it can be concluded that *Pennisetum glaucum* extract can be used as an accessible source of natural antioxidants with consequent health benefits.

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