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In-vitro antioxidant activity and antibacterial assay of minor millet extracts

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ABSTRACT

In vitro antioxidant property and antibacterial assay of the three genders of minor millets (Echinochloa crus-galli, Panicum miliaceum L, Panicum sumatrense) were evaluated. IC_{50} for various extracts were determined for DPPH scavenging, bleaching of β- carotene and % inhibition of H_2O_2 and compared with standard positive controls viz. butylated hydroxytoluene (BHT) for DPPH, propyl gallate for β-carotene and ascorbic acid for H_2O_2 assay. Antibacterial assay of six extracts of three species were evaluated against bacterial strains of Staphylococcus aureus (MTCC 96), Bacillus megaterium (MTCC-428), Escherichia coli (MTCC 443) and Pseudomonas aeruginos (MTCC1688). It is concluded from the results that phytochemicals are responsible for such inhibition of multi resistance microorganisms and could be a source of new antibacterial drugs.

Keywords: Antibacterial assay; Antioxidant activity; Phenolic content; Minor millet; flavonoid

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INTRODUCTION

Echinochloa crus-galli, Panicum sumatrense, and Panicum miliaceum L. native to East African highland, India and China. *Echinochloa crus-galli, Panicum sumatrense, and Panicum miliaceum L* are considered richest sources of phenolics and flavonoids, which have high biological activity [1]. Many synthetic antioxidants are available but these compounds must be used under strict regulation due to their potential hazards [2, 3]. Therefore, new interest has been developed as purifying and characterizing safe antioxidants from natural sources. The predominant flavonoids and phenolic acids are almost exclusively present in glycosylated forms in *Echinochloa crus-galli, Panicum sumatrense, and Panicum miliaceum* [4, 5]. There have been many attempts made to determine the contents and physiological activity of phenolic compound in *Echinochloa crus-galli, Panicum sumatrense, and Panicum miliaceum* due to the apparent relationship of phenolics in minor millet with prevention of chronic diseases [6]. Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and repercussion injury of many tissues, a central nervous system injury, gastritis, cancer and type-2 diabetes [7]. Due to environmental pollutants, radiation, chemicals, toxins, deep fries and spicy foods as well as physical stress, free radicals cause depletion of the immune system, the change in gene expression and induce abnormal proteins. The oxidation process is one of the most important routs for producing free radicals in food, drugs, and even living systems. Antioxidants are important species, which possess the ability of protecting organisms from damage caused by free radical-induced oxidative stress [8]. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators [9]. A number of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been extensively added to foodstuffs, although their use has begun to be questioned because of their toxicity, so there is considerable interest in preventive medicine and in the food industry in the development of natural antioxidants obtained from botanical sources, especially herbal plants [10].

It is believed that antioxidant activity might be correlated with antibacterial activity and therefore, it is interesting to see the potential of extracted active ingredients against bacterial strains of *Staphylococcus aureus* (MTCC 96), *Bacillus megaterium* (*MTCC-428*), *Escherichia coli* (*MTCC 443*) and *Pseudomonas aeruginos (MTCC1688*).

The medicinal value of these plants lies in some chemical substances that produce a definite physiological effect on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, phenolic compounds and flavonoids [11]. Among these, flavonoids are the ubiquitous group of plant secondary metabolites demonstrating a wide range of biochemical and pharmacological effects, including anti-diabetic, antioxidant, anti-inflammatory, antibacterial, antifungal [12, 13]. In the present study, we focused on two different types of assays that are antioxidant and antibacterial using six crude extracts from three genders of minor millets were carried out and compared with standards [14, 15].

EXPERIMENTAL SECTION

2.1. Plant material

Seeds of *Echinochloa crus-galli, Panicum sumatrense, and Panicum miliaceum* were collected from local marker at Bhavnagar, Gujarat-India The collected samples were preserved in dark and dry place at ambient temperature with passive ventilation prior to extraction.

2.2. Preparations of extracts

The aerial parts of *Echinochloa crus-galli, Panicum sumatrense, and Panicum miliaceum* were cleaned with deionized water, oven dried at 40°C for 60 h and powdered in a grinder. The plant material (100 g) was extracted with three different solvents 1% acidified methanolic, 95% ethanolic, water (1500 mL) using Soxhlet apparatus for 48h at temperature not exceeding the boiling point of the respective solvents. The obtained extracts were filtered using Whatmann filter paper No.1 and concentrated under vacuum at 40°C using a rotary vacuum evaporator (Buchi Laboratoriums, Switzerland) to dryness. The extractive values of the extracts were calculated. The extraction conditions were optimized based on different percentage recovery of extracts in the different experimental conditions. The solvent was evaporated under reduced pressure to give the maximum percentage yield from *Eleusine coracana (L.)*. An aliquot of 1% acidified methanol was chromatographed by open column chromatography on silica gel, using chloroform: methanol mixtures of increasing polarity.

2.3. Chemicals and reagents

2,2-Diphenyl-1-picrylhydrazyl hydrate (DPPH), b-carotene, linoleic acid, butylated hydroxyanisole (BHA), butylatedhy droxytoluene (BHT), ferrous chloride and Folin–Ciocalteu reagent were purchased from Hi-Media Lab. Pvt. Ltd., Mumbai, India. Sodium dihydrogen orthophosphate, di-sodium hydrogen orthophosphate dehydrate and sodium acetate was purchased from E-Merck India Ltd. Other chemicals and solvents were procured from SD Fine-Chem Ltd., Mumbai, India and were of analytical grade, while Hydrogen peroxide (H_2O_2) was purchased from RFCL Limited (Rankem), Mumbai, India. Reference standard diclofenac sodium was obtained as gift sample from Unique Pharmaceuticals, Ankleshwer, India. For biological assay we used Nutrient Agar (Himedia, g/L) and nutrient broth (Himedia, g/L) while Fungus were assayed on Potato Dextrose Agar (Himedia, g/L).

2.4 Antibacterial assay

2.4.1. Microbial stain

The studied bacterial strains include *Staphylococcus aureus* (MTCC 96), *Bacillus megaterium (MTCC-428)*, *Escherichia coli (MTCC 443)* and *Pseudomonas aeruginos (MTCC1688),* were procured from IMTECH, Chandigarh and screening were carried out at Junagadh Agricultural University, JUNAGADH (India).

2.4.2 Culture Media:

Bacteria were assayed on Nutrient Agar (Himedia, g/L) and nutrient broth (Himedia, g/L) while Fungus were assayed on Potato Dextrose Agar (Himedia, g/L) were used in this study.

2.4.3 Agar disc diffusion method

Echinochloa crus-galli, panicum miliaceum L. and *panicum sumatrense* extracts (50,100 & 200 µg/mL) were prepared in six different solvents (Water, Ethyle Acetate, Acetone, 95% Ethanol, Chloroform and 1% acidified methanol). The dishes were left for 30 min at room temperature to allow the diffusion of extracts, which were incubated at 37°C for 12h to 48h for bacteria [19].

Table 1 The yields of the extracts obtained from three different minor millets in three different solvents

Solvents	*Echinochloa crus-galli*	*Panicum miliaceum*	*Panicum sumatrense*
1% Methanol	3.87gm ± 1.25	3.049 gm ± 0.85	3.02gm ± 1.13
95% Ethanol	2.45 gm ± 1.20	1.004 gm ± 1.52	1.49 gm ± 1.03
Water	2.39gm ± 1.10	1.83 gm ± 1.05	2.315gm ± 0 86

2.5. Extraction process

The yields of the extracts obtained per 100 g of dry plant material with the different solvents are given in Table 1.

The highest yield of the extract (44.0 g/100 g of dry plant material) was obtained from extraction with 1%-acidified methanol.

2.6. Determination of total phenols and flavonoids

Total phenolic content was determined according to Folin–ciocalteu method. The results are expressed as grams of gallic acid equivalents per 100 g of dry extract. The concentration of phenolic compounds was calculated according to the following Eq. (1) obtained from the standard Gallic acid (5–50 μg) curve.

$$Absorbance = 0.016 \times GAE \text{ in } \mu g + 0.029 \ (R^2 = 0.996) \quad (1)$$

Flavonoid content in the various extracts was determined by a colorimetric method. The results are expressed as grams of quercetin equivalents per 100 g of dry extract. The concentration of flavonoid compounds was calculated according to the following Eq. (2) obtained from the standard quercetin (20–100 μg) curve [20].

$$Absorbance = 0.00001 \times Quercetin \text{ in } \mu g + 0.011 \ (R^2 = 0.994) \quad (2)$$

2.7. DPPH radical scavenging assay

In this assay, free radical scavenging activity of crude extract was determined by measuring the bleaching of purple-colored methanol solution of DPPH. The radical scavenging activity was determined as described elsewhere. One millilitre from a 0.5 mM methanol solution of the DPPH radical was mixed to 2.0 mL of different concentrations of 95% ethanol; methanol and water extract and were added 2.0 ml of 0.1 M sodium acetate buffer (pH 5.5). The mixtures were well shaken and kept at room temperature in the dark for 30 min. The absorbance was measured at 517 nm using a UV Spectrophotometer. BHT was used as positive control, whereas methanol was used as negative one. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation-3.

$$\% RSA = [(A_0 - A_S)/A_0] \times 100 \quad (3)$$

Where A_0 and A_S are the absorbance of the control (containing all reagents, except the test compound) and test compound respectively [21].

2.8. Reducing sugar power

The reducing power of crude extract was determined using the method as described previously. Different concentrations of extracts (20 to 100 µg/mL) were mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1%). The mixture was incubated at 50°C for 20 min. Aliquots (2.5 mL) of 10% trichloroacetic acid were added to the mixture. The above mixture was then centrifuged at 1036 x g for 10 min. The upper layer of the solution (2.5 mL) was mixed with 2.5 mL of distilled water and 2.5 mL of 1% ferric chloride solution. The absorbance was measured at 700 nm in a UV spectrophotometer. Increased absorbance of the reaction mixture indicated increased reducing power and compared with those of standard antioxidant BHA and BHT [22].

2.9. H_2O_2 Assay

The ability of all seeds extracts to scavenge hydrogen peroxide was determined according to the. A solution of hydrogen peroxide (2 mM) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm. Extracts samples (10 to 50 µg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of scavenging of hydrogen peroxide of both extracts and standard compounds are calculated by using following equation 5 [23].

$$\% Scavenged\ H_2O_2 = ([A_0 - A_1]\ A_1) \times 100 \quad (5)$$

Where A_0 was the absorbance of the control, and A_1 was the absorbance in the Presence of the extracts and standards.

2.10. β-carotene belching

Antioxidant activity was determined using β-carotene bleaching test. 1 mL of β -carotene solution (0.2 mg/mL in chloroform) was added to 0.02 mL of linoleic acid and 0.2 mL of 100% Tween 20. (shon et al , 2003) The mixture was evaporated at 40 ºC for 10 min using rotary evaporator to remove chloroform. The resultant mixture was immediately diluted with 100 mL of distilled water to form emulsion. 5 mL of the emulsion was transferred into different test tubes containing 0.2 mL of samples in 70% ethanol at different concentrations (500, 250,100 and 50 µg/mL). 0.2 mL of 70% ethanol in 5mL of the above emulsion was used as control. Standard (propyl gallate) at the same concentration as samples was used for comparison. The tubes were gently shaken and placed at 45 ºC in a water bath for 60 min. The absorbance of the samples, standard and control was measured at 470 nm using a UV Spectrophotometer against a blank, consisting of an emulsion without β-carotene. The measurement was carried out at initial time (t = 0) and successively at 30 and 60 min [24]. All samples were assayed in triplicate and averaged. The antioxidant activity (AA) was measured in terms of successful bleaching of β-carotene using equation-4.

$$\%Inhibition = [1-(A_{S\,(0)} - A_{S\,(60)} / - (Ac_{\,(0)} - Ac_{\,(60)})] \times 100 \qquad (4)$$

Where $A_{S(0)}$ the initial absorbance of the sample at $A_{S(60)}$ the absorbance of the sample at 60 min, $Ac_{(0)}$ the absorbance of the negative control at 60min. The extract concentration providing 50% antioxidant activity (IC_{50}) was calculated from the graph of antioxidant activity percentage against extract concentration.

RESULTS AND DISCUSSION

3.1 Antibacterial assay of the extracts

Antibacterial assay of extracts of three minor millets were carried out and zone of inhibition for each extract was measured at three concentrations [25]. Table-2 represents the data for zone of inhibition at 200 µg/mL concentration of each extract.

Table 2 Minimum zone of inhibition (mm) of six extracts at 200 µg/mL concentration of three millets

Echinochloa Crus-galli					
		Gram Positive		**Gram Negative**	
		B. megaterium	***S. aureus***	***E. Coli***	***P. Aeruginosa***
1	Water	8.3334±0.9	11±2.1602	8.6667±2.8674	12±4.242
2	Ethyl acetate	11±0.816	13.6667±3.298	11±3.5590	11.3334±2.054
3	Acetone	4.334±1.24	7.3334±1.2472	9.3334±2.6246	11.3334±2.494
4	95% Ethanol	6.0±1.8856	11.3334±3.299	10.3334±2.624	8.0±0.4714
5	Chloroform	8.667±4.98	9.3334±2.0548	7.3334±1.6996	7.6667±0.9428
6	1% Methanol	11.6667±5	10.6667±2.867	16±5.0990	13.3334±0.471
Panicum miliaceum L.					
1	Water	10±1.6321	13.0±2.160	9.0±1.4142	10.0±1.6329
2	Ethyl acetate	9.334±2.05	10.6667±1.69	10.0±2.160	8.667±3.0912
3	Acetone	12.0±1.414	16.334±4.1899	12.6667±5.312	11.0±5.6568
4	95% Ethanol	9.0±2.1602	11.0±4.9665	8.334±0.9428	11.0±2.449
5	Chloroform	8.667±2.86	9.6667±1.699	7.334±1.2472	8.0±0.4714
6	1% Methanol	12.334±3.3	16.0±2.9439	13.6667±3.299	14.334±1.6996
Panicum sumatrense					
1	Water	8.3334±0.9	12.0±3.2659	8. 667±2.6246	7.667±1.885
2	Ethyl acetate	10.0±1.414	14.0±2.1602	9. 667±3.2998	12.0±2.828
3	Acetone	12.34±0.94	17.667±1.2472	16.67±4.7140	16.0±2.8284
4	95% Ethanol	11.34±3.29	11.3334±2.624	10.67±2.6246	13.67±3.2998
5	Chloroform	10.34±1.24	9. 667±2.4944	10.3334±4.189	10.3334±0.942
6	1% Methanol	13.0±2.160	12.334±2.0548	13. 667±3.681	16.334±0.9428

Antibacterial activity was manifested by the 1% acidified methanol extract of seed of *echinochloa crus-galli E .coli* with maximum zone of inhibition of 16 mm, while seed extract of *panicum miliaceum L* in acetone showed good activity against *S. aureus* with 16.4 mm zone of inhibition. Acetone extract of *panicum sumatrense* showed good activity against *S. aureus* with 17.7 mm zone of inhibition [26].Moreover, it was observed that acetone extract of *panicum sumatrense* showed good activity against both gram-positive and gram-negative bacterial strains.

The results obtained in this study indicate differences in antimicrobial assay between extracts depending on the species of minor millets as a function of the type of extracting solvents. Other authors [27] have observed similar differences. These results were in agreement with the suggestion of Oloke et al [28] those phytochemicals of any medical plant have different solubility in different extracting solvents. The reason for weak activity of aqueous extracts was that phytochemicals present in the species of minor millets were insoluble or poorly soluble in water.

The results also indicate that the efficacious interaction effect may be dependent on the precise concentrations of certain phytochemicals in an extract. In spite of the fact that most independent plant-derived extracts have shown weak potency against pathogenic bacteria compared to antibiotics, plants usually, fight infections successfully in their natural environment.

3.2. Total phenolic content

Phenolics are aromatic secondary plant metabolites and called high-level antioxidants because of their ability to scavenge free radicals and active oxygen species such as singlet, superoxide free radicals and hydroxyl radicals. Natural polyphenols have chain-breaking antioxidant activities and are believed to present many degenerative diseases, including cancer and scene diseases**.**

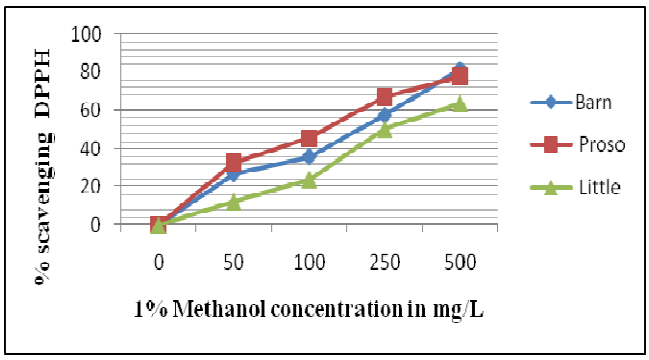
The crude extracts obtained from aerial parts of *Echinochloa crus-galli, Panicum sumatrense, and Panicum miliaceum* were characterized by their contents of total phenolics expressed as gallic acid equivalent (GAE) for *Echinochloa crus-galli, Panicum sumatrense, and Panicum miliaceum* seeds were use in three different solvents viz. 95% ethanol, 1% acidified methanol and water. Lowest total phenolics were obtained from the water extract of Panicum sumatrense 45.49 GAE/g and highest total phenolics were obtained from the 95% ethanolic extract of Panicum miliaceum 845. 33 GAE/g. The calculations of total phenolics were carried out by using standard GA curve with least square regression. Similarly, total flavonoid content equivalent to quercetin (QU. E) for crude of *Echinochloa crus-galli, Panicum sumatrense, and Panicum miliaceum* was carried out. The obtained yield was calculated from standard curve of QU with least square regression. The phenolic contents in different extracts varied significantly in both plants and as well as solvents used for extraction. The order of phenolic content in three solvents was 1% methanol > 95% ethanol > water for aerial parts of three gender of millets. Variations in phenolic contents of various extracts were attributed to polarities of different compounds present in the various parts of millet used for present study and such differences have been reported elsewhere. Similarly, the order of flavonoid content in three solvents was 95% ethanol> 1% methanol > water for this three spices of millet. The results of total phenolics and total flavonoids are summarized in Table 3.

Table 3 Total phenolic and total flavonoid contents in seeds of *Minor millets*

Millets	Total phenolic contents	Total flavonoids contents
1 % Methanol (30 µg/mL)		
Echinochloa crus-galli	245.33±2.49	1.2083±0.013
Panicum miliaceum	155.81±8.55	0.2083±0.014
Panicum sumatrense	157.61±4.13	0.1361±0.007
95% Ethanol (30 µg/mL)		
Echinochloa crus-galli	845.33±2.49	1.078±0.014
Panicum miliaceum	164.50±3.52	0.830±.006
Panicum sumatrense	171.70±4.13	0.186±0.008
Water (30 µg/mL)		
Echinochloa crus-galli	345.33±7.45	0.302±0.0078
Panicum miliaceum	145.49±6.28	0.623±0.0103
Panicum sumatrense	108.65±7.16	0.230±0.0086

3.3 2, 2 diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

DPPH is a stable free radical in aqueous or methanol and ethanol solution and accept an electron or hydrogen radical to become a stable diamagnetic molecule. It is usually used as a substrate to evaluate the antioxidative activity of antioxidants. The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517 nm. The scavenging effect of extracts in the range of 50–500 µg/mL on the DPPH radical increased with an increasing concentration of each extract is depicted in figure 1.



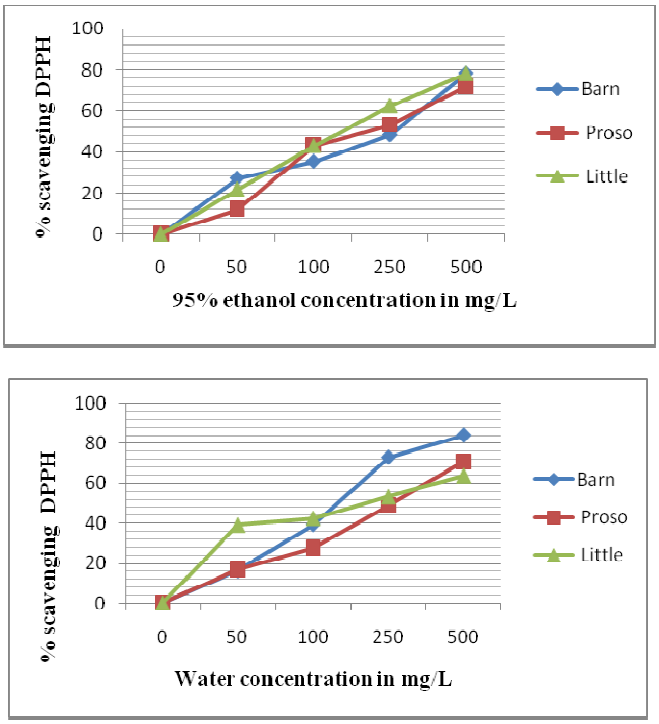


Figure 1 Percentage scavenging of DPPH in 1%methanol, 95% ethanol and water extracts of Minor millets

The scavenging of by hydrogen donation caused the decrease in absorbance of DPPH radical caused by antioxidant. DPPH assay shows that extracts of in water had highest impact with 39.47% scavenging at low concentration of seed extract. IC_{50} of extracts of *Echinochloa crus-galli*, *Panicum miliaceum* and *Panicum sumatrense* are summarized in table 3. The degree of discoloration indicates the scavenging capacity of the extract. The effect of antioxidants on the DPPH radical scavenging was thought to result from their hydrogen donating ability. IC_{50} value for DPPH scavenging by water extract of was lowest, which was found to be much higher than the standard butylated hydroxy toluene (BHT). Similarly, many authors reported that some plant extracts showed lower activity against the DPPH radical compared with butylated hydroxy toluene (BHT). In addition, there was a statistically significant correlation between the amount of phenolic compounds and DPPH scavenging activity in all the extract.

3.4 Reducing power

The results showed that the reducing power of all the three extracts was concentration dependent. Five different concentrations ranging from 20 µg /mL to 100 µg /mL were used to see the effectiveness of concentrations of all three extracts in three solvents as shown in figure 2. The results were compared against the standard BHT and BHA solutions. Water extract of millet showed significantly higher reducing power than other extracts in three different solvents. In addition, it was reported that the antioxidant activity of reductions is believed to break radical chains by donation of a hydrogen atom, indicating that the antioxidative properties are concomitant with the development of the reducing power. Various authors have reported that the reducing power of bioactive compounds (mainly low and high molecular phenolics), extracted from peanut hulls and stem bark of Indian laburnum, was associated with antioxidant activity, specifically scavenging of free radicals. Reductions are also reported to react with certain precursors of peroxide thus, preventing peroxide formation. The results obtained by us do support these claims and there were positive correlation between total antioxidant activity and reducing power of all five extracts in three solvents. Different studies have indicated that the reducing power of bioactive compounds is associated with antioxidant activity. Therefore, the antioxidant activity of the tested samples might partially be a result of their reducing power. The reducing capacity of tannins prevented liver injury by inhibiting the formation of lipid peroxides. Furthermore, the reducing compounds can react directly with peroxides and with certain precursors and thereby prevent peroxide formation. The reducing capacity of various examined extracts might be due to their hydrogen donating ability. Therefore, the examined samples might contain reducing compounds, which can react with free radicals to stabilize and terminate radical chain reactions.

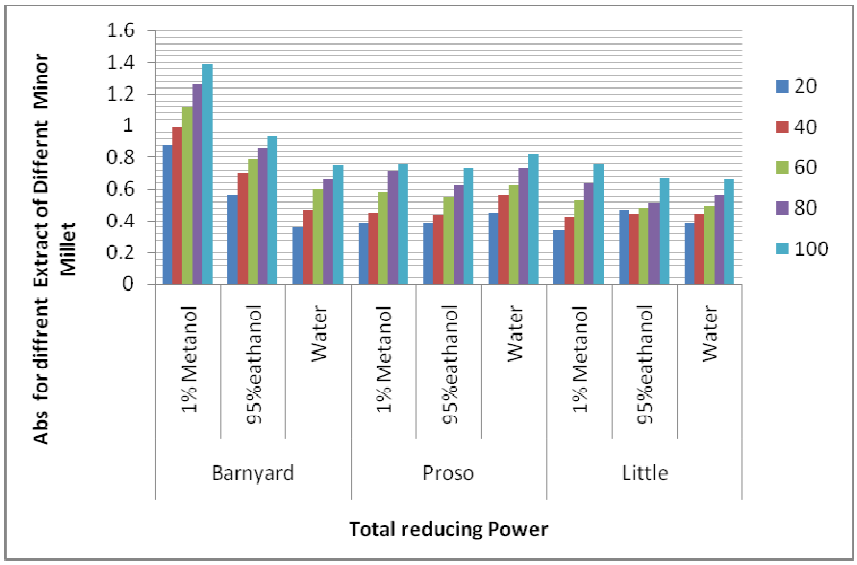


Figure 2 Reducing power of different extracts of Aerial parts of Minor Millets

3.5 H_2O_2 Scavenging Activity

The results of H_2O_2 scavenging activity indicated that all the extracts showed excellent H_2O_2 scavenging activities at a concentration range from 10µg /mL to 50µg /mL in the reaction mixture and increased steadily with the increased concentration. The percentage inhibition of standard ascorbic acid was varied between 30% to 50% at five different doses used for present study, while all five extracts in three different solvents had similar effect with percentage scavenging ranging from 5% to 49% at five different doses used for present study. IC_{50} for standard ascorbic acid was 30.9573 ± 2.306 µg /mL. IC_{50} for 95% ethanolic extract of *panicum miliaceum* was very close to standard, which indicates that water extract of *Echinochloa crus-galli* had very high impact H_2O_2 scavenging. Similarly, water extract of little has IC_{50} value 52.07 ± 0.41 µg /mL with linear regression; while 95% ethanolic extract of proso has similar kind of effect on H_2O_2 scavenging effect as IC_{50} was found 53.75 ± 0.84 µg /mL with linear regression.

3.6 β-carotene belching

The bleaching effect was measured by the peroxidation of β-Carotene and the effect of peroxidation of β-Carotene is shown in figure. Antioxidants can reduce the extent of β- Carotene destruction by neutralizing the linoleate-free radical and other free radicals formed in the system. Accordingly, the absorbance deceased rapidly in reaction mixtures without extracts, whereas in the presence of extracts the reaction mixtures retained their colour and thus absorbance for a longer time. The IC_{50} of different extracts in inhibiting the bleaching of β- Carotene suggest that bleaching can be moderately inhibited by all three extracts in three different solvents. This can be attributed to the fact that the presence of different antioxidant molecules in extracts might be responsible for inhibition of β -Carotene destruction by neutralizing the effect of linoleate-free radical and other free radicals formed in the system. The representative data are summarized in Table 3.

Table 3 IC_{50} for DPPH scavenging, β-Carotene scavenging and H_2O_2 scavenging of different extracts of Minor millets

Species	Solvent	DPPH scavenging activity (IC_{50} in µg/mL)	β-carotene bleaching effect (IC_{50} in µg/mL)	H_2O_2 (IC_{50} in µg/mL)
Echinochloa crus-galli		686.0497±8.0304	**140.165±2.498**	26.947±0.55
Panicum miliaceum	1% Methanol	**165.8623±7.3802**	237.718±6.838	24.9877±0.554
Panicum sumatrense		339.0027±12.511	377.653±21.80	**22.1303±0.244**
Echinochloa crus-galli		226.4637±13.086	**143.656±1.960**	21.5875±0.524
Panicum miliaceum	95% Ethanol	242.2003±10.947	236.527±2.693	28.7271±0.318
Panicum sumatrense		**206.876±9.04656**	227.735±31.03	**19.9343±1.542**
Echinochloa crus-galli		**199.5967±8.2838**	**130.67±4.8107**	39.624±1.5428
Panicum miliaceum	Water	300.8487±8.5480	254.625±4.056	21.4641±0.98
Panicum sumatrense		237.7147±9.01324	228.822±5.421	**16.7916±4.18**

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