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Phytochemical Screening and Antioxidant Potential of *Desmostachya bipinnata* (Leaf and Roots)

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ABSTRACT

Desmostachya bipinnata is perennial herbaceous plant having multiple medicinal health benefits. *Desmostachya bipinnata is* useful therapeutic medicinal plant, commonly known as Dabh and belong to Poaceae family. Experimental plant has a good medicinal property and used as: Urinary infections, fever, dysentery and menorrhagia, and as a diuretic and a blood purifier. The primary metabolites use as nutrition and the free radicals are generated in the body as metabolic products of several reactions. These free radicals cause multiple harms to the cell, on their cell wall, to the DNA and cause number of diseases. In the present study, the plants were selected and collected from Rajasthan and were shade-dried. The powder was formed of leaves and root. The methanolic extract was prepared for further studies of primary estimation and antioxidant potential activity (enzymatic and non-enzymatic) of evaluated through their standard protocols. Antioxidants fight as anti-cancer agents in human beings.

***Key words*:** *Desmostachya bipinnata*, Primary metabolites, UV spectroscopy, In vitro study, Antioxidants

It is believed that plants have high medicinal values and they are being used for the same from decades. A large number of plants are reported as a remedy for cellular and metabolic disorders. Free radicals are generated inside the body as after product of metabolic reactions. It can also be said that the free radicals are continuously produced by aerobic life. They have generated inside the body as the response of stress. After being generated inside the body these free radicals use to damage the healthy cells by damaging their cell membrane. These free radicals generate due to smoking, excessive exposure to sunlight, because of tobacco, and prolonged exposure of the heavy metals [1].

Effect of a few specific antioxidants is limited maintained inside the body, although the antioxidants work to neutralize the side effects generated due to these free radicals. The ratio of oxidants and antioxidant necessary to be maintained inside the body, any kind of alteration in this reaction can make the free radicals get deposited inside the body and results in oxidative stress. This oxidative stress can cause major tissue damage which results in cancer [2]. Oxidative stress can be defined, that it plays a deciding role in carcinogenesis and also a very crucial role in the last stage of cancer for its progression and prevention. The oxidative stress can be defined as diligence imbalance in the antioxidant/pro-oxidant level, which results in unidirectional cell damage. The antioxidants scavenge the generated free radicals and inhibit the damage caused due to oxidative stress [3-4].

Desmostachya bipinnata is valuable medicinal plant, commonly known as Dabh and belong to Poaceae family and *Desmostachya bipinnata* is also known as *"*Halfa grass". Presently 10,000 species and more than 660 genera includes in Gramineae family. It is well known for its great economic and medicinal importance because it includes all cereals, bamboos and sugar cane. There are so many therapeutic activities of Gramineae species such as astringent and in treatment of wound, anti-emetic, diuretic [5], and in treatment of eye problems [6].

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MATERIALS AND METHODS

Selective experimental medicinal plants are searched in desert area of Rajasthan India, sample were collected of *Desmostachya bipinnata* (root and leaves) and experimental plants samples were deposited in the herbarium of Department of Botany, University of Rajasthan, collect sample and washed with distilled water and shade dried make it powdered for further experiment**.**

Primary metabolites

Primary metabolites directly involved in growth and development for the plants and uses nutrient while

secondary metabolites are not involved directly and they have been worked as biocatalysts and use as herbal medicine.

Estimation of Primary Metabolites (*Carbohydrates, proteins, phenols and lipids*)

Carbohydrate estimation

Total soluble sugar: 80% ethanol use for extraction according protocol was followed using the method of Mc-Cready *et al*. [7]. 0.1 ml of sample was mixed with 5 ml of 80% ethanol reagent. Centrifuge at 10000 rpm for 20 min then supernatant collects in test tube. Add 5ml H_2SO_4 with 1ml 5% phenol then mix by vortex. Now kept sample at room temperature for 20 minutes. Absorbance was read at (wavelength) 490 nm against a reagent blank. The analysis was performed in triplicates and the results were expressed as mg/gram dry weight sample.

Starch: The protocol was followed using the method of Loomis and Shull [8] for total soluble sugar. Take 5 ml of 80% ethanol in a test and mix with 0.1 ml plant sample, mix properly with the help of vertex and centrifuge at 10000 rpm for 20 minutes, collect pellet and mix with 1ml perchloric acid ($HClO_4$) mix by vertex. Take 1 ml sample in test tube add 5ml H_2SO_4 and 1ml 5% phenol mixing by vortex keep 20 min room temp. The absorbance was read at 490 nm against a reagent blank. The analysis was performed in triplicates and the results were expressed as mg/g dry weight.

Protein estimation: 10% TCA use for protein extraction according here methodology of Osborne [9] was followed. Take 0.1 ml of sample mixed it with 3ml 10% TCA, centrifuge at 15000 rpm for 10 minutes, now take pellet add 10 ml 5% TCA mix it by vortex. Now take in a test tube and incubate at 80 ^oC for 30 minutes, after incubation cool it and take 1 ml sample from it and add 5 ml alkaline solution with 1 ml Folin and Ciocalteu's reagent and incubated again for 10 minutes at 37°C or room temperature. Absorbance was read at 750 nm (wavelength) against 10% TCA reagent blank. The analysis was performed in triplicates and the results were expressed mg/g dry weight sample.

Lipid estimation: Distilled water is used for lipid extraction according extraction methodology of Jayram [10] will be followed. Take 0.3 gm sample with 10 ml distilled water and crush it with the help of mortar and pestle. Add 20 ml chloroform ($CHCl_3$) with 10 ml methanol (CH_3OH) for 20 min kept on room temperature will filter it after 20 min. Now add 20 ml $CHCl_3$ with 2ml distilled water then proper mixing. Take in separating flask and collect lower layer. Dry it here blank weight less from dry weight and take result. The analysis was performed in triplicates and the results were expressed mg/g dry weight sample.

Phenol estimation: 80% ethanol is used for extraction total phenol content in each sample was estimated by spectrophotometer method of Bray and Thorpe [11]. Take 0.2 gm sample with 4 ml 80% ethanol crush it with the help of mortar and pestle. Centrifuge at 10000 rpm for 10 minutes and collect supernatant and take 1 ml of sample added 1 ml of Folin and Ciocalteau reagent and incubated at room temperature for 3 minutes. After three minutes 2 ml of 20% sodium carbonate (Na_2CO_3) was added, mixed well and incubated the tubes in boiling water bath for 1minute. Cooled rapidly and read absorbance at 750 nm (wavelength) against reagent blank. The analysis was performed in triplicates and the results were expressed as mg/g sample.

Antioxidant Activity: In future, experimental plant *Desmostachya bipinnata* and its useful medicinal properties are good sources of natural antioxidant, experimental plant may be helpful in preventing the progress of various oxidative stresses and as more possibility use food supplement or use in pharmaceutical industry.

FRAP radical scavenging activity: FRAP was carried out by the method reported by Benzie *et al.* [12]. The stock solution was prepared using 300mM of acetate buffer with pH 3.6, TPTZ solution (2, 4, 6- tripyridyl-s-triazine) in HCL 40 mM and FeCl3 20 mM solution. From stock solution, working solution was prepared fresh by 2.5 ml TPTZ in 25 ml of acetate buffer and 2.5ml of $FeCl3.6H_2O$ solution. The working solution was kept at (RT) 25°C. Different solutions of plant extracts of both the plants were taken and mixed in 2850 ml of FRAP solution; the mixture was kept in dark for 30 minutes for incubation. The color was observed and OD was measured at 593 nm. The results were expressed on the basis of antioxidant concentration with the ability to reduce ferric ion.

Catalase: The antioxidant potential through catalase was measured by Aebi *et al.* [13] 50 µl of lysate of herbal extracts was taken and mixed with 2 ml of phosphate buffer and 1 ml H_2 O_2. The activity of catalase was measured spectrophotometrically and OD was measured at 240nm for the duration of 1 minute. For determination of the activity of catalase molar extinction capacity was measured as unit/mg of protein [13].

Lipid peroxidation activity: Lipid peroxidase was measured by Dudonne *et al.* [14] in the herbal extracts of both the plants. 0.1 ml of herbal extracts, with deionized water and homogenate of egg in 0.2 M of PBS was taken. $FeSO_4$ was added into the mixture and left for 37°C for incubation. In this incubation time, lipid per-oxidation was allowed to induce. The tubes were further filled with a mixture of 20% acetic acid, 20% TCA and 0.8% TBA and the mixture was heated for 60 minutes in boiling water bath. The mixture was further allowed to cooling, after cooling 5ml butane-1-ol was added and the tubes were centrifuged for 3000rpm, the supernatant was removed safely in a separate test tube and the OD of the supernatant was measured spectrophotometrically at 532 nm. Control was taken without herbal extracts. IC 50 value and inhibition percentage were calculated through the graph [14].

Peroxidase: Estimation of hydrogen peroxide was done by Ruch *et al.* [15]. The 06 ml of H_2O_2 solution was taken and herbal methanolic extracts were mixed in it. 40mM of H_2O_2 was prepared by the help of phosphate buffer, the pH was set neutral 7.4 and the solution was 0.1 M. Total volume of the mixture was made up to 3 ml and the absorbance was taken at 340 nm. To perform the test phosphate buffer with H_2O_2 was taken as blank [15].

RESULTS AND DISCUSSION

Table 1 Primary metabolites extracts from *Desmostachya bipinnata* leaf and root (mg/gram dry weight)

		Leaf result	Root result
Carbohydrates	Total soluble sugar	0.162	0.41
	Starch	2.28	2.72
Proteins		16.93	11.93
Phenols		2.2	3.7
Lipids		16.6%	10.0%

Table 2 In-vitro non-enzymatic antioxidant activity of methanolic extract prepared from dry powder of *Desmostachya bipinnata* leaf and roots

Assay	OD (nm)	*Desmostachya bipinnata*	
		Leaf	Root
FRAP	593	0.492	0.362
LPO	532	27.59	20.46

Note: The values were obtained by independent determination in triplicate where n= 3, one way ANOVA was applied for statical calculation the mean was taken and the values showed significant difference where $p<0.005$.

Note: FRAP – Ferric-Reducing Antioxidant Power assay; LPO – Lipid Peroxidase

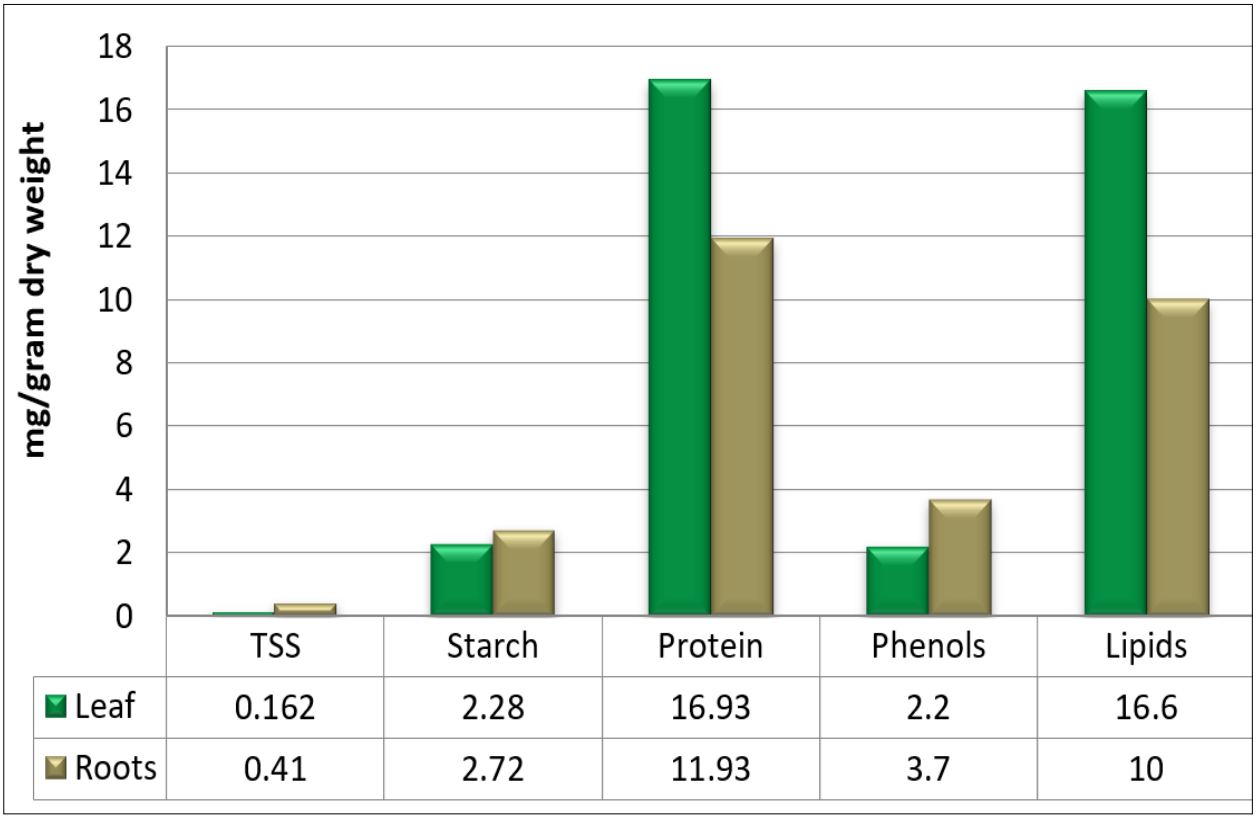


Fig 1 Primary metabolites from selective medicinal plant parts *Desmostachya bipinnata* leaf and root

Table 3 In-vitro enzymatic antioxidant activity of methanolic extract prepared from dry powder of *Desmostachya bipinnata* leaf and roots

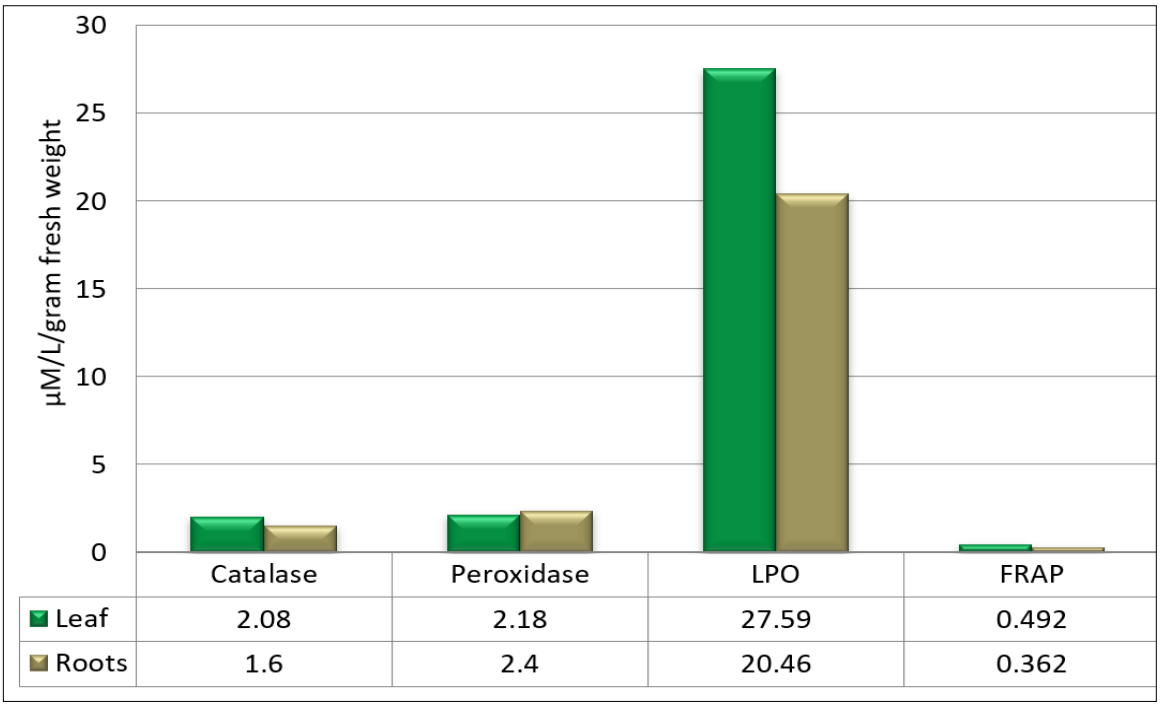
Assay	OD (nm)	*Desmostachya bipinnata*	
		Leaf	Root
CAT	240	2.08	1.6
POD	430	2.18	2.40

The values were obtained by independent determination in triplicate where n= 3, one way ANOVA was applied for statistical calculation the mean was taken and the values showed significant difference where $p<0.005$.

Note: CAT – Catalase; POD – Peroxidase

Finally found great important role of leaf and roots as potential medicinal activity, *Desmostachya bipinnata* plant parts are useful for human health, individual and communities. The medicinal values of the selective medicinal plants are lying in some chemical substances that produce a definite physiological action in the human body. Phytochemicals analyzed were importance in identifying a

new source of therapeutically and industrially valuable compounds having medicinal plants have been chemically investigated. In the present investigation primary metabolites and antioxidants activity are qualitatively and quantitatively analyzed using *Desmostachya bipinnata* plant leaf and root.



	Catalase	Peroxidase	LPO	FRAP
Leaf	2.08	2.18	27.59	0.492
Roots	1.6	2.4	20.46	0.362

Fig 2 Antioxidant assay leaf and roots from *D. bipinnata*

CONCLUSION

In the present study, quantitative analysis and free radical scavenging activities of root and leaf extract of *Desmostachya bipinnata* plant leaf and root were investigated. The extract was found to possess more primary metabolites and it exhibit radical scavenging activities, based on the results it can be concluded that, the root and leaf ethanolic extract of *Desmostachya bipinnata* which contains good amount of primary metabolites and exhibits free radical scavenging activities. In future, this plant may be proving a good source of natural compounds such as antioxidant, which may be helpful in preventing the progress of various oxidative stresses and as a possible food supplement or in pharmaceutical industry.

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