Antioxidant and HPTLC fingerprinting analysis of *Desmostachya bipinnata*

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***Abstract*:** In current investigation, a popular ayurvedic crude drug *Desmostachya bipinnata* also known as darbha was studied for in-vitro antioxidant activity. The drug has been identified by HPTLC fingerprint analysis. The dried rhizome and roots were pulverized into fine powder. Required quantity of powder was employed for successive extraction by using soxhlet apparatus. The successive extracts were used for HPTLC fingerprint investigations, while the Hydro-alcoholic extract was prepared separately for antioxidant studies. A silica gel $60F_{254}$ TLC precoated aluminium plates (10 x 10 cm, layer thickness 0.2mm, Merck, Mumbai) were employed as a stationary phase. About 1µL of the sample was applied on the TLC plate under a stream of nitrogen using a semiautomatic spotter and the bands were detected employing a deuterium lamp. The chromatographic conditions were optimized and estimations were performed on a stationary phase, pre coated silica gel60 F_{254} aluminum sheets (10 x10) which were prewashed with the n-hexane (for n-hexane extract), chloroform (for chloroform extract) and methanol (for methanol extract) and dried in air. Then the plated were saturated with the respective mobile phases for 30 minutes and the migration distance allowed was 72mm. The wave length scanning was performed at 254nm for n-hexane extract and 366nm for chloroform and methanol extract. The source of radiation was deuterium lamp emitting a continuous UV spectrum between 190-400nm. The samples were spotted and developed at 25±2°C. The HPTLC finger prints of n-hexane, chloroform and methanol extracts were developed employing following mobile phases respectively. a) Hexane: ethyl acetate: acetic acid (8:2:0.5 v/v) at 254 nm. b) Toluene: ethyl acetate: acetic acid (8:2:0.2 v/v,) at 366 nm. c) Toluene: ethyl acetate: acetic acid (8:2:0.2 v/v) at 366 nm. The results of the antioxidant study revealed that the plant extract possess effective antioxidant activity. HPTLC fingerprints were developed for rapid identification, authentication and control of adulteration of this crude drug, which is used as one of the ingredient in several ayurvedic formulations.

Keywords: *Desmostachya bipinnata*, HPTLC fingerprinting, Antioxidant activity, DPPH.

I. INTRODUCTION

At present, herbal medicine represents one of the most important fields of traditional medicine all over the world. To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants having folklore reputation in a more intensified way. A huge number of the world's population have exclusively been used medicinal plants for centuries as remedies for human diseases [1] Knowledge of the chemical constituents of plants is desirable because such information will be value for the synthesis of complex chemical substances. Phytochemical screening of plants has revealed the presence of numerous chemicals including alkaloids, flavonoids, tannins, steroids, glycosides and saponins. Secondary metabolites from plant serve as defense mechanisms against predation by many microorganisms, insects, herbivores and oxidative stress [2]. Oxidative stress induced ROS and free radicals are believed to be major cause of physiological disorders like Alzheimers, Parkinson's, arthritis, atherosclerosis, coronary heart diseases, emphysema, gastric ulcer, diabetes mellitus, cirrhosis, aging and cancer. Presence of a multitudes of vitamins, polyphenols, flavonoids, tannins and phenolic acids in natural extracts of vegetables, fruits, herbs, spices and medicinal plants and inverse relationship between these natural antioxidants and the risk of oxidative diseases has caused spurt in extensive research and have been described to possess biological activities such as antioxidant, anti-inflammatory, oestrogenic, cytotoxic, antitumor [3]. *Desmostachya bipinnata* Stapf (Family: Poaceae) locally named English name- Sacrificial Grass (smaller var.), Ayurvedic name- Kusha, Suuchyagra, Yagyabhuushana, Kshurapatra, Siddha/Tamil name-Tharubai, that is widely distributed throughout the plains of India in hot and dry places. The roots of plant are cooling, diuretic, galactagogue, emollient, aphrodisiac, astringent, used for menorrhagia, diarrhea, dysentery, skin disease, renal and vesical calculi, asthma, jaundice, dysurea, bleeding piles, burning

sensation, cystitis, dispesis, vaginal discharges and erysipelas (The Ayurvedic Pharmacopoeia of India, Government of India Ministry of Health and Family Welfare Department of Ayush). In present research work, we have made an attempt to examine the preliminary phytochemical test, antimicrobial and antioxidant activity of the different six extracts of roots of *Desmostachya bipinnata* Stapf.

II. MATERIAL AND METHODS

Plant materials and preparation of the extracts:

The plants were collected from in and around Ibrahimpatnam, R.R Dist, Andhra Pradesh, India and authenticated by Botanist in Osmania University, Hyderabad, India. The dried rhizome and roots were pulverized into fine powder and stored in an air tight container. Required quantity of powder was used for successive extraction in soxhlet apparatus using solvents of increasing polarity. The successive extracts were used for HPTLC fingerprint studies, while the Hydro-alcoholic extract was prepared separately for antioxidant studies.

Antioxidant studies:

DPPH free radical scavenging activity: The free radical scavenging activities of the plant extracts were measured employing the modified method of Blois [4].

Nitric oxide radical scavenging: Griess Illosvoy reaction was utilized for quantification of nitrite ions produced on reaction of nitric oxide generated from aqueous sodium nitroprusside (SNP) solution with oxygen at physiological pH.

Reducing power: The Fe^{3+}-reducing power of the extract was determined by the method of Oyaizu with a slight modification [5].

HPTLC studies:

The dried successive n-hexane, chloroform and methanol extracts were dissolved in the respective solvents to get a final concentration of 1000µg/ml. The extracts were filtered through a whatman filter paper. A silica gel $60F_{254}$ TLC precoated aluminium plates (10 x 10 cm, layer thickness 0.2mm, Merck, Mumbai) were used as a stationary phase. A CAMAG HPTLC system (Switzerland) comprising a CAMAG Linomat IV semiautomatic sample applicator, a CAMAG TLC scanner, a CAMAG twin through chamber (10 x 10), a CAMAG CATS 4 software, a Hamilton syringe and a ultra sonicator were used. About 1µL of the sample was applied on the TLC plate under a stream of nitrogen using a semiautomatic spotter and the bands were detected using a deuterium lamp [6].

The chromatographic conditions were optimized and estimations were performed on a stationary phase, pre coated silica gel60 F_{254} aluminum sheets (10 x10) which were prewashed with the n-hexane (for n-hexane extract), chloroform (for chloroform extract) and methanol (for methanol extract) and dried in air. Then the plated were saturated with the respective mobile phases for 30 minutes and the migration distance allowed was 72mm. The wave length scanning was performed at 254nm for n-hexane extract and 366nm for chloroform and methanol extract. The source of radiation was deuterium lamp emitting a continuous UV spectrum between 190-400nm. The samples were spotted and developed at 25±2°C [10].

Results and Discussion: The *in vitro* antioxidant activity of *D.pinnata* using DPPH radical nitric oxide radical and reducing power was tested with different concentrations of hydro-alcoholic extract and the results are given in table 1, 2 and 3 respectively. The HPTLC finger prints of n-hexane, chloroform and methanol extracts were developed using following mobile phases respectively. a) Hexane: ethyl acetate: acetic acid (8:2:0.5 v/v) at 254 nm. b) Toluene: ethyl acetate: acetic acid (8:2:0.2 v/v,) at 366 nm. c) Toluene: ethyl acetate: acetic acid (8:2:0.2 v/v) at 366 nm.

Table 1: DPPH Assay of *D.pinnata* and Ascorbic acid

Sl. No.	Concentration	*D. pinnata* extract	Ascorbic acid
		% inhibition (mean±SEM)	% inhibition (mean±SEM)
1	5µg/mL	58.7±0.21	28.7±0.24
2	10µg/mL	59.1±0.11	37.9±0.26
3	25µg/mL	65.3±0.01	44.5±0.15
4	50µg/mL	68.1±0.24	58.7±0.19
5	100µg/mL	72.84±0.15	61.4±0.12

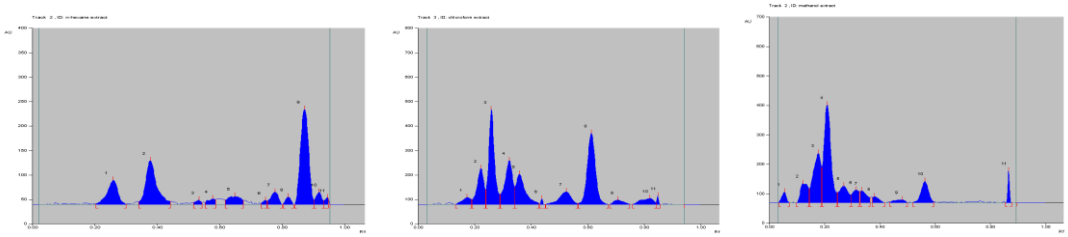
Table 2: Nitric oxide scavenging assay of *D.pinnata* and Rutin

Sl. No.	Concentration	*D. pinnata* extract	Rutin
		% inhibition (mean±SEM)	% inhibition (mean±SEM)
1	5µg/mL	34.0±0.21	7.4±0.15
2	10µg/mL	35.3±0.14	13.4±0.10
3	25µg/mL	40.1±0.09	21.9±0.04
4	50µg/mL	43.0±0.15	34.7±0.19
5	100µg/mL	47.0±0.24	52.7±0.20

Table 3: Reducing power assay of *D.pinnata* and Butylated hydroxy toluene

Sl. No.	Concentration	Reducing Power Absorbancy
		D. pinnata (mean±SD)
1	5µg/mL	0.0972±0.014
2	10µg/mL	0.1976±0.011
3	25µg/mL	0.3942±0.004
4	50µg/mL	0.4971±0.015
5	100µg/mL	0.5176±0.008
6	BHT	0.728±0.001

a) n-Hexane extract b) Chloroform extract c) Methanol extract



Conclusion: The results of the antioxidant study revealed that the plant extract possess effective antioxidant activity. HPTLC fingerprints were developed for rapid identification, authentication and control of adulteration of this crude drug, which is used as one of the ingredient in several ayurvedic formulations such as Ashmarihar kashay churna, Brahma rasayan, Brahmi vati, Karpuradyarka, Mutravirechan kashay churna, Stanyajanana kashay churna, Traikantak ghrita and Trinapanchmool kvath churn.

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