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Comparative Study of Phytochemical Analysis, Antimicrobial and Antioxidant Activity of Different Root Extracts of *Desmostachya bipinnata Stapf* (*Kush*)

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A B S T R A C T

The present study was designed to evaluate Preliminary phytochemical analysis, *in vitro* antimicrobial activity and antioxidant activity of different six extracts of *Desmostachya Bipinnata Stapf* (*Kush*). Plant roots were extracted in different six solvents viz. Hexane, ethyl acetate, acetone, Methanol, Water and Methanol: Water (90:10) through Soxtherm according to polarity gradients. The phenolics, flavonoid, tannin and other phytochemicals of the extract were also determined using standard phytochemical reaction methods. Methanol: water and methanol extracts showed the presence of Phenol and glycosides, while in acetone extract terpenoids and glycosides were found. Aiming to investigate antimicrobial activities, agar well diffusion method was followed using three pathogenic bacteria and two fungi as test organisms. The plant root extracts showed moderate antibacterial activities (zone of inhibition (ZOI): 6-9mm) which was compared with standard kanamycin, while extracts showed positive antifungal activities (ZOI: 6-12 mm) and fluconazole was used as standard antifungal agent. We assessed the antioxidant potential of all six extracts of *Desmostachya bipinnata Stapf (Kush*) using test involving inhibition of DPPH activities. The highest antioxidant activity of acetone extract was noticed at IC_{50} (Inhibition concentration at 50%) of 17.42µg /ml followed by methanol extract at IC_{50} of 25.83 µg /ml compared to those of ascorbic acid (7.5 µg /ml). Current studies indicated that plant root extracts possessed moderate antimicrobial activities and good antioxidant activity. So our findings revealed that the acetone extract of *Desmostachya bipinnata Stapf (Kush*) possess antioxidant properties and could serve as free radical inhibitors or scavenger or, acting possibly as natural antioxidants.

Keywords

Desmostachya bipinnata Stapf, Antimicrobial activity, Antioxidant activity, DPPH.

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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 (Nostro *et al*., 2000; Arokiyaraj *et al*., 2008). 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 (Cowan, 1999).

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 (Harborne *et al*., 1992).

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.*

Materials and Methods

Collection of plant material

The roots of *Desmostachya bipinnata Stapf* were collected from Junagadh region (Fig. 1). Using standard taxonomical methods, Dept. of Botany, JAU, Junagadh provided information regarding identification of the plant's parts used in this work. The samples were then separated and cleaned from impurities.

Extraction of plant material

The roots of plants were separated and washed with tap water to remove the impurities. The roots were cut into small pieces and were subjected to air dry for 10 days. The air-dried samples were then transferred into oven for drying and then were crushed. Dried powder of experimental material was extracted in soxtherm apparatus successively with hexane, ethyl acetate, acetone, methanol and water, respectively due to their nature of polarity. 130ml solvent required per 10gm dried powder of experimental material. Plant materials were extracted in the mixture of methanol and water in 9:1 ratio. Desired sample was weighted and dissolved in a reasonable amount of the corresponding solvent (typically about 1.5 ml for every 10 mg of sample). The solution was filtered through a 0.2 micron filter to ensure that no particles were present in the solution. The method for soxtherm has been selected as per the table 1.

After extraction, the hexane, ethyl acetate, acetone, methanol, water and methanol: water extracts were concentrated using rotary evaporator and dried in hot air oven at 500 ^{o}C to get the solid mass and remaining sample

weighted yield was collected after lyophilisation for further use. Extractive yield in different solvent was calculated in %.

Preliminary phytochemical screening

The extracts were screened for primary phytochemicals (Raja *et al*., 2011; Reddy *et al*., 2012) with minor modifications. Procedure for the qualitative preliminary phytochemical screening is given in table 2.

Antimicrobial activity

The antimicrobial activity of the crude extracts were determined by the agar well diffusion method (Bauer *et al*., 1966) against the microbial strains given in table 5 whereas Kanamycin (30 μg/ml) and fluconazole (30 μg/ml) were used as the standard for antibacterial and antifungal respectively. The extracts were dissolved separately in DMSO concentration of 100 μg/ml and carefully load into the well. The plates were then incubated at 37°C for 24 h to allow maximum growth of the organisms. The test material having antimicrobial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the well. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm.

Collection of microorganism

Escherichia coli, Salmonella, Vibrio, Aspergillus niger and *Aspergillus flavus* were provided by Department of Biotechnology, Junagadh Agricultural University, Junagadh. Microorganisms were stored at 4°C on Nutrient agar slant and potato dextrose agar slant before use.

In vitro antioxidant assays

The DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) free radical scavenging activity was measured by the modified method of McCune and Johns (2002). The reaction mixture (3.0 ml) consisted of 1.0 ml DPPH in methanol (0.3 mM), 1.0 ml methanol and 1.0 ml different concentrations of the extracts were incubated in dark for 10 min, after which the absorbance was measured at 517 nm against blank. For control, 1.0 ml of methanol was used in place of extract. Ascorbic acid was used as positive control (Yamaguchi *et al*., 1998). Percentage of inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = (A_0 - A_1 / A_0) \times 100$$

Where, A_0 is the absorbance of control and A_1 is the absorbance of sample.

In order to calculate IC_{50} value, plant extract solution in methanol was further diluted and tested for DPPH assay to find out 50% inhibition. IC_{50} value was calculated by graph method.

Results and Discussion

Extractive yield

Many researchers reported influence of different extraction solvents on the content of natural compounds in extracts. Efficiency of solvents and methods are strongly dependent on plant matrix used (Das *et al*., 2010). The extractive Yields of dried root powder of plants are given in table 3. Highest solubility of metabolites was found in water extract followed by methanol: water and methanol.

Preliminary phytochemical analysis

Maximum amount of phenol, glycosides, Steroids and flavanoids were found in, respectively present in moderate amount in Methanol: Water (90:10) extracts and methanol extracts. Saponins, terpenoids, triterpenoids and fat were absent in Methanol: Water (90:10) extracts. Water extracts of

Desmostachya bipinnata Stapf had moderate amount of Tannins, alkaloids, and carbohydrates. Acetone extracts had maximum amount of glycosides, terpenoids. terpenoids, triterpenoids, phenol, glycosides and steroids were present in moderate amount in ethyl acetate extract. Hexane extract had maximum amount of Fat and fixed oils. The phytochemical screenings of different extracts of *Desmostachya bipinnata Stapf* are listed in table 4.

Table.1 Soxtherm method set up for extraction in different solvents

Solvent	Temperature (°C)	T- Class Value	Extraction time (Hrs)
Hexane	75	200	6:30
Ethyl Acetate	85	200	6:30
Acetone	65	200	6:45
Methanol	73	300	11:30
Water	105	300	10:30

Table.2 Procedure for the qualitative preliminary phytochemical screening

Sr. No.	Phytochemical	Procedure	Nature of color change	Inference
1.	Flavonoids	Substance + 10 % NaOH	Green brown	Present
2.	Saponin	Substance shake in water	Frothing present	Present
3.	Steroids	0.5 ml of extract + 1 ml conc. H_2SO_4	Wine red color	Present
4.	Quinone	Substance + conc. HCl	Green color	Present
5.	Cellulose	Extract +Iodine followed by H_2SO_4	Brown color	Present
6.	Terpenoids	Substance + 2 ml chloroform + conc. H_2SO_4	Reddish brown color at the interface	Present
7.	Triterpenes	0.5 ml of extract + few drops of acetic anhydride + 1 ml conc. H_2SO_4 from the side of test tube	Red ring at the junction	Present
8.	Cardiac glycosides	Substance + 2 ml glacial acetic acid + 1 drop of $FeCl_3$ + 1 ml of conc. H_2SO_4 from the wall of test tube	Reddish brown ring at the junction of the two solvents	Present
9.	Phenol	Substance + alcohol + $FeCl_3$	Greenish yellow	Present
10.	Tannin	0.5 g substance + 20 ml H_2O is boiled. + 0.1 % $FeCl_3$	Brownish green	Present
11.	Alkloids	2 ml test solution + 2 N HCl + Mayer's reagent	Yellowish orange precipitate	Present
12.	Lignans	0.5 ml extract + 2 ml of 2 % (V/V) furfuraldehyde	Red color	Present
13.	Carbohydrate	Crude extract + shake + 2 ml conc. H_2SO_4 from the side of test tube	Violet ring at the junction	Present
14.	Amino acid, Protein	Crude extract boiled with 2 ml 0.2 % ninhydrin	Violet color	Present
15.	Fat and fixed oil	Substance + Sudan III	Shining orange color	Present

Table.3 Extractive yield (%w/w) of roots of *Desmostachya bipinnata Stapf (Kush)*

Plant / Solvents	*Desmostachya bipinnata Stapf (Kush)*
Hexane	0.5%
Ethyl acetate	0.493%
Acetone	0.28%
Methanol	8.49%
Water	10.71%
Methanol: Water (90:10)	9.14%

Table.4 The qualitative preliminary phytochemical screening of *Desmostachya bipinnata Stapf (Kush)*

No. of tests	Solvents / Tests	HEXANE	ETHYLE ACETATE	ACETONE	METHANOL	WATER	METHANOL:WATER (90:10)
1	FLAVONOIDS	-	+	+	++	+	+
2	SAPONINS	-	-	-	-	+	-
3	STEROIDS	+	++	+	-	-	++
4	QUINONE	-	-	-	+	-	+
5	CELLULOSE	-	-	-	-	-	-
6	TERPENOIDS	+	++	+++	-	-	-
7	TRITERPENOIDS	+	++	++	-	-	-
8	GLYCOSIDES	+	++	+++	+++	+	+++
9	PHENOLS	+	++	++	+++	++	+++
10	TANNINS	-	-	-	+	++	+
11	ALKALOIDS	-	-	-	-	+	+
12	LIGNANS	-	-	-	-	-	-
13	CARBOHYDRATES	-	-	-	+	++	-
14	PROTEINS & AMINO ACIDS	-	-	-	+	-	+
15	FAT & FIXED OILS	+++	+	-	-	-	-

Presence = +, Moderate Presence = ++, considerable amount = +++, and Absent = –

Table.5 Antimicrobial activity of different extracts of *Desmostachya bipinnata Stapf*

Plant root Extracts	ZONE OF INHIBITION (mm)				
	Escherichia coli	***Salmonella***	***Vibrio***	***Aspergillus niger***	***Aspergillus Flavus***
Hexane	-	-	7	-	-
Ethyl acetate	-	7	6	6	-
Acetone	7	-	-	8	-
Methanol	9	-	-	11	-
Water	-	-	-	12	-
Methanol: water (90:10)	-	-	-	11	8
Kanamycin	14	13	13	-	-
Fluconazole	-	-	-	14	12

Table.6 The antioxidant activity of various extracts of *Desmostachya bipinnata Stapf*

Plant	Solvent name	IC_{50} (µg/ml)
Kush (*Desmostachyabipinnata Stapf)* rootExtract	Hexane	-
	Ethyl acetate	-
	Acetone	**17.42**
	Methanol	25.83
	Water	-
	Methanol: water (90:10)	-
Standard	Ascorbic acid	7.5

Fig.1 Morphology and roots of *Desmostachya bipinnata Stapf*

A: Morphology

B: Root

Fig.2 Antioxidant activity of standard ascorbic acid

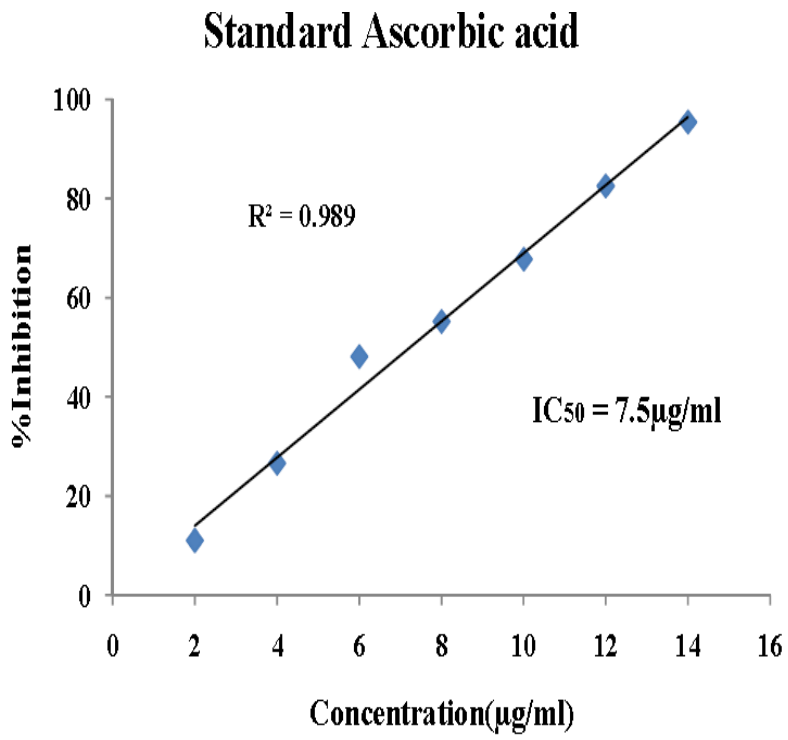


Fig.3 Antioxidant activity of acetone extract

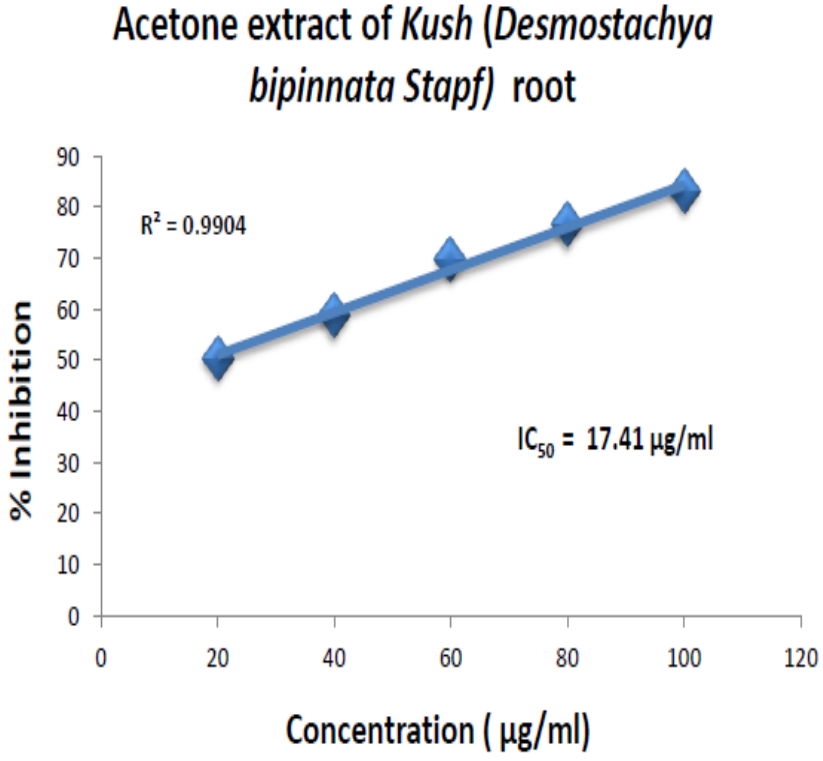
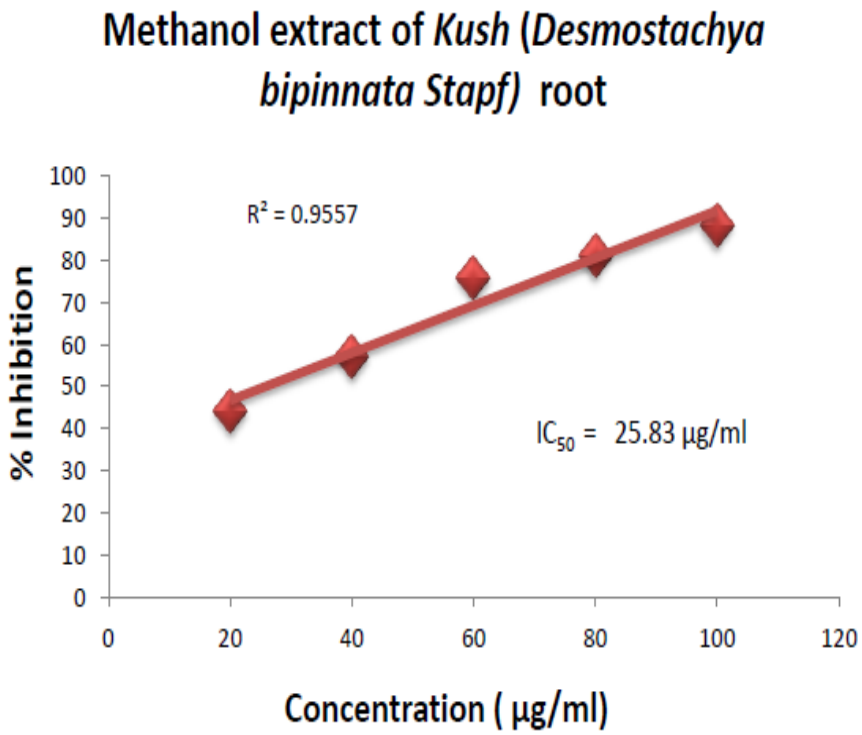


Fig.4 Antioxidant activity of methanol extract



Antimicrobial activity

The extracts of the sample were tested for antibacterial activity against a three different gram positive and gram negative bacteria. Standard antibiotic disk of kanamycin at 30µg/ml was used for comparison purposes. The extracts showed antibacterial activity against limited number of the test organisms. The results of the antibacterial activity measured in terms of diameter of zone of inhibition in mm are showed in table 5. One concentration of the extracted sample 100 µg/ml was used for antibacterial activity.

The extracts of the sample were tested for antifungal activity against two fungi. Standard of fluconazole at 30 µg/ml was used for comparison purposes. The extracts Showed little antifungal activity against the test organisms. The results of antifungal activity Measured in terms of diameter of zone of inhibition (ZOI) are shown in table 5.

Antioxidant activity by DPPH method

The DPPH scavenging activity of the some extracts were significantly good compared to those of ascorbic acid and it was evident that the extract did show the proton-donating ability and could serve as free radical inhibitors as antioxidants (Kai *et al*., 2007). The antioxidant potential on plants has been found a correlation between the phenolic content and the antioxidant activity (Zahin *et al*., 2009). The antioxidant potential of acetone and methanol extract have the DPPH scavenging activity, acetone extract has maximum IC_{50} (17.42 µg/ml). Methanol extract has lower scavenging activity (IC_{50} 25.83 µg/ml) compared to acetone extract (Fig. 2–4). Study showed that the capability of the extracts to scavengering free radicals, indicating that they may be useful therapeutic agents for treating radical-related pathological damage. The antioxidant activity of various extracts of *Desmostachya bipinnata Stapf* are given in table 6.

From above study it was clearly evident that the acetone extract of *Desmostachya Bipinnata Stapf (Kush*) possess antioxidant properties and could serve as free radical inhibitors or scavenger or, acting possibly as natural antioxidants.

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