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Research Article

Potential of *desmostachya bipinnata stapf* (poaceae) root extracts in inhibition of cell proliferation of cervical cancer cell lines

Kalpana P. Rahate*[1], A Rajasekaran[2], KSG Arulkumaran[2]

[1]Karpagam University, Pollachi Main Road, Eachanari, (Post), Coimbatore, Tamilnadu, India
[2]KMCH College of Pharmacy, Kovai Estate, Coimbatore, Tamilnadu, India

ABSTRACT

Desmostachya bipinnata Stapf (Poaceae), a *Sacrificial Grass* is an important constituent of ayurvedic preparations of Tripanchamool, Kusablecha, and Kusadya-ghrita. The objective of the study was to evaluate the antioxidant and anticancer activities of *Desmostachya bipinnata* root extracts. The antioxidant activities of different concentrations of 70% methanolic extract of the roots of *Desmostachya bipinnata* were examined for different reactive oxygen species (ROS) scavenging activities including DPPH, nitric oxide, hydrogen peroxide and hydroxyl radical scavenging activities. The in-vitro cytotoxic study was performed on the human cervical cancer cell lines (HeLa), human laryngeal epithelial carcinoma cells (HEp-2) and NIH 3T3 using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide [MTT]. The extract has shown effective antioxidant activity in all assay techniques. Further *Desmostachya bipinnata* was found to be most potent scavenger of hydrogen peroxide with IC_{50} value 127.07± 6.44 µg/ml (R^2 0.982) against standard ascorbic acid 122.60± 2.14 µg/ml (R^2 0.984) respectively. The present investigation revealed that the methanolic extract of *Desmostachya bipinnata* possess significant in vitro anti-cancer activity at 400 µg/ml and showed inhibition in concentration dependant manner of range between 25 – 400 µg/ml on all the cell lines. The results obtained in the present study indicate that the roots of *Desmostachya bipinnata* are a potential source of natural antioxidants. The study indicated that *Desmostachya bipinnata* was more active against cervical cell lines.

Keywords: *Desmostachya bipinnata;* DPPH; HeLa; HEp-2; MTT; NIH 3T3

INTRODUCTION

Reactive oxygen species (ROS) are formed in living organisms in different ways, including normal aerobic respiration, stimulated polymorphonuclear leukocytes and macrophages, and peroxisomes. They are natural by-products of our body's metabolism and react easily with the free radicals produced in normal or pathological cell metabolism and become radicals themselves (Gulcin, I et al., , 2004). However, excess accumulation of the radicals of ROS is dangerous as they attack biological molecules such as lipids, proteins, enzymes, DNA and RNA, leading to cell or tissue injury. Thus the oxidative stress experienced by the cells contributes to numerous diseases including cardiovascular disease, ageing, cancer, Parkinson`s disease, autoimmune disease, stroke, ischemia, reperfusion injury, acute hypertension, hepatitis, haemorrhagic shock, Alzheimer's disease and a variety of other clinical disorders (Halliwell B et al, 1992; Knight JA et al, 1995; Smith MA et al, 1996). The reactive oxygen species are also known for activation and remodelling the extracellular matrix such as matrix metalloproteinase (e.g. collagenase) causing increased destruction of tissues e.g. collagenase damage seen in various arthritic conditions (Kim Y et al, 2004; Peter S et al. 2006). Free radical damage of the tissues is generally prevented by enzymes such as superoxide dismutase (SOD) and catalase present in the body, or compounds such as ascorbic acid, tocopherol and glutathione (Niki E et al. 1994). However, due to various pathological processes these protective mechanisms are disrupted, and antioxidant supplements are vital to combat oxidative damage. Flavonoids derived from plants can have the potential to bind with metalloenzymes thus altering or inhibiting metabolic pathways and flavonoid-metal complexes have shown potential to be SOD mimetics (Kostyuk VA et al. 2004). Recently, much attention has been directed towards the development of ethnomedicines with strong antioxidant properties but low cytotoxicities.

Desmostachya bipinnata (family: Poaceae), a perennial grass grows up to 50 cm in height, found throughout India in hot and dry places. Leaves many, long, acute, linear, with hispid margins, panicle erect, clothed with sessile spikelets; grains small, ovoid, trigonos and laterally compressed. It is commonly known as '*Sacrificial*

* Corresponding Author
Email: rahatekalpana@gmail.com
Contact: +91-9895872328
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Grass' in English and '*Darbha*' in Sanskrit. This plant pacifies pitta, asthma. In Indian traditional medicine, it is used as diuretic and is used in diarrhoea, dysentery, menorrhagia, jaundice, skin diseases, burning sensation and excessive perspiration (Dhiman et al. 2007; Kulkarni P H).

The plant showed the presence of flavonoid glycosides identified as kaempferol, quercetin, quercetin-3-glucoside, trycin, trycin-7-glucoside (Amani S et al. 2008), and 4'-methoxy quercetin-7-O-glucoside (Mohamed A et al. 2009).

It is reported to exhibit antiulcerogenic (Amani S et al. 2008), antihelicobacter (Mohamed A et al. 2009), analgesic, antipyretic and anti-inflammatory effects (Panda S et al. 2009). However, there has been no report on the antioxidant properties of this species. No reports are available on the chemopreventive action on peptic or gastric cancer of the root extracts of the plant so far. Thus, it was thought worthwhile to assess the anticancer activity of the extract i*n-vitro*.

The objective of the present study was to evaluate the antioxidant potential and free radical scavenging activity and *in vitro* cytotoxicity of a 70% methanol extract of roots of *Desmostachya bipinnata*.

MATERIALS AND METHODS

Plant material

The plant *Desmostachya bipinnata Stapf* (Poaceae) was collected in the month of October from different places of Tuticorin in Tamilnadu. The plant was authenticated by The Joint Di rector, Botanical Survey of India, Coimbatore, India.

Chemicals

1, 1-Diphenyl-2-Picrylhydrazyl (DPPH), nitroblue tetrazolium (NBT), quercetin were procured from Sigma Aldrich (St. Louis, MO, USA). Sodium nitroprusside (SNP), trichloroacetic acid, reduced nicotinamide adenine dinucleotide (NADH), Tannic acid, L-ascorbic acid, sulphanilamide, potassium ferricyanide, naphthyl ethylenediamine dihydrochloride, aluminium chloride ($AlCl_3$), phenazine methosulfate (PMS) and 2-deoxy-2-ribose, were obtained from Sisco Research Laboratories Pvt. Ltd, Mumbai, India. sodium nitrite ($NaNO_2$), gallic acid, Mannitol and Folin-Ciocalteu's reagent were purchased from Nice Chemicals Pvt. Limited, India. All the other chemicals and reagents were of pure analytical grade and obtained from local supplier.

Extraction

The roots of *Desmostachya bipinnata Stapf* (Poaceae) dried and powdered. The powder was extracted with 70% methanol in soxhlet apparatus for 18 hrs. The refluxed sample was concentrated under reduced pressure in rotating vacuum evaporator and then dried. The extractive value was calculated on dry weight basis. . The dry extract was kept at 20°C for future use.

Phytochemical screening

Phytochemical screening of the methanolic root extract of *Desmostachya bipinnata* was carried out to identify the secondary metabolites such as alkaloids (Mayer's and Draggendorff's test), flavonoids (Shinoda test), terpenoids (Salkowski test), tannins (Ferric chloride test), saponins (Frothing test), cardiac glycosides (Keller-Killiani test) and anthraquinones (Borntrager's test) according to standard phytochemical methods (Harborne JB, 1991).

DPPH Radical scavenging assay

Free radical scavenging capacity was evaluated *in vitro* by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay (Brand-Williams et al. 1995; Mothana et al.2008; Spanou et al.2008). Sample stock solutions (1.0 mg/ml) were diluted to final concentrations of 50, 40, 30, 20, 10 µg/ml, in ethanol. One ml of a 0.3 mM DPPH ethanolic solution was added to 2.5 ml of sample solutions of different concentrations, and allowed to react at room temperature. After 30 minutes the absorbance values were measured at 518 nm and percentage of antioxidant activity (AA) was calculated using the following formula:

$$\mathbf{AA\ \% = 100 - \{ [(ABS_{SAMPLE} - ABS_{BLANK}) \times 100] / ABS_{CONTROL}\}}$$

Ethanol (1.0 ml) plus compound solution (2.5 ml) was used for blank. DPPH solution (1.0 ml; 0.3 mm) plus ethanol (2.5 ml) was used for negative control. Quercetin was used as positive control. Average percent of antioxidant activity from three separate tests were calculated.

Nitric oxide scavenging assay

Aqueous sodium nitroprusside generates nitric oxide at physiological pH which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions (Sangameswaran B et al. 2009).

2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of methanolic extract at various concentrations (50 – 250 µg) and the mixture incubated at 25°C for 150 min. From the incubated mixture 0.5 ml was taken out and added into 1.0 ml sulfanilic acid reagent and incubated at room temperature for 5 min. Finally, 1.0 ml 1% w/v naphthyl ethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min. The pink chromophore generated during diazotization of nitrite ions with sulphanilamide and subsequent coupling with naphthyl ethylenediamine dihydrochloride was measured spectrophotometrically at 546 nm against a blank sample. Ascorbic acid was used as a standard.

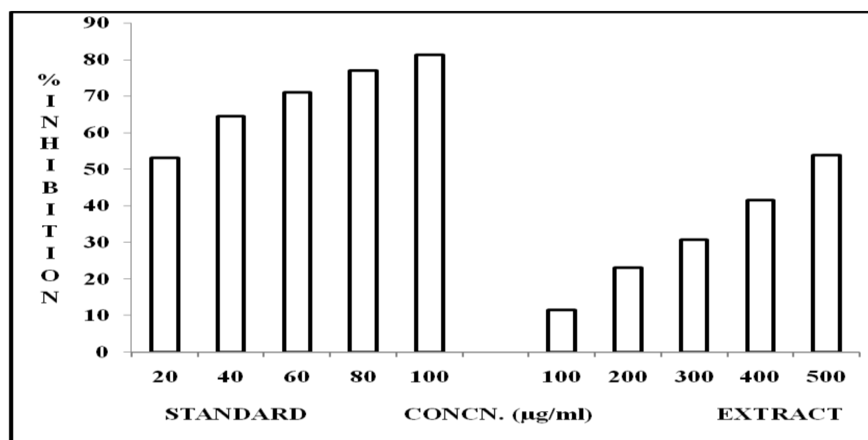

Figure 1: DPPH Radical Scavenging Activity of *Desmostachya bipinnata* and standard (quercetin)

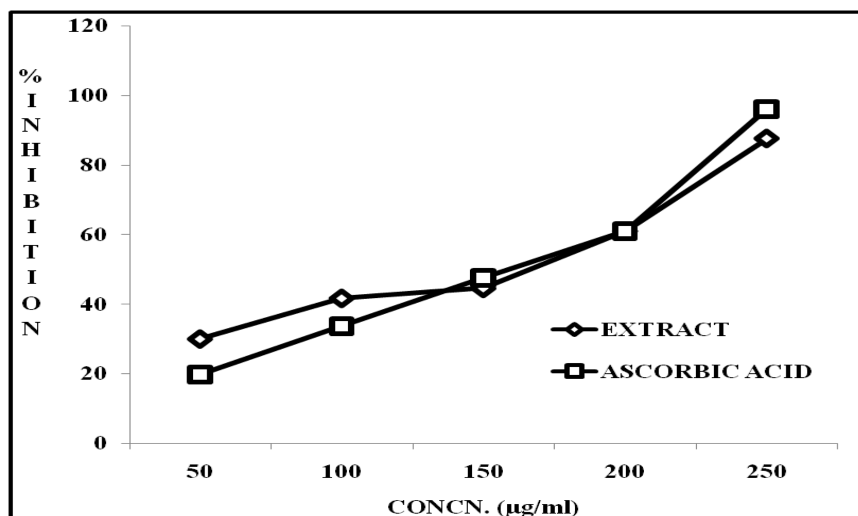

Figure 2: Nitric oxide scavenging activity of *Desmostachya bipinnata*

Hydrogen peroxide scavenging assay

The ability of root extract of *Desmostachya bipinnata* to scavenge the initiation of lipid peroxidation by H_2O_2 was studied (Ogunlana et al.2009). **E**xtract of various concentrations (50 -250 µg/ml) was added to 0.6 ml of 4 mM H_2O_2 solution in phosphate buffer solution (pH 7.4). Absorbance of the solution was measured at 230 nm after 10 min. Ascorbic acid was used as reference standard.

Hydroxyl radical scavenging assay

Hydroxy radical generated from Fe^{2+}-ascorbate-EDTA-H_2O_2 system which attack the deoxyribose & the series of reactions that result in formation of pink chromagen with TBA. Manittol was used as reference standard. All the solutions were freshly prepared (Mandal P et al).

500 µl of methanolic root extract of *Desmostachya bipinnata* (100-500 µg/ml) in phosphate buffer, 100 µl of 28 mM 2-deoxy-2-deribose in phosphate buffer, 200 µl 1.4 mM EDTA & 200 mM $FeCl_3$, 100 µl 1.0 mM H_2O_2 & 1.0 mM ascorbic acid was mixed to form a reaction mixture. After incubation at 37^0C for 1 hr. the extent of deoxyribose degradation was measured by addition of 1.0 ml 1% TBA &1.0 ml 2.8% TCA. The solution was further incubated at RT for 15 min. and absorbance was measured at 532 nm against reagent control.

Cytotoxocity screening

A] Tumour cell lines

The human cervical cancer cell line (HeLa), Human laryngeal epithelial carcinoma cells (HEp-2) and NIH 3T3 mouse embryonic fibroblasts were obtained from National Centre for Cell Science (NCCS), Pune. The HeLa and HEp-2 cells were grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS) and NIH 3T3 fibroblasts were grown in Dulbeccos Modified Eagles Medium (DMEM) containing 10% FBS.

B] Determination of cytotoxicity using Microculture tetrazolium (MTT) assay

The cells were seeded into 96-well plates in 100µl of medium containing 10% FBS and incubated at 37^0C,5% CO_2, 95% air and 100% relative humidity for 24 hr prior to addition of extracts. The medium was then replaced with respective medium of extract containing 1% FBS at various concentrations (50, 100, 200, 300 and 400 µg/ml) and incubated at 37^0C, 5% CO_2, 95% air and 100% relative humidity for 48 h. 10µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each

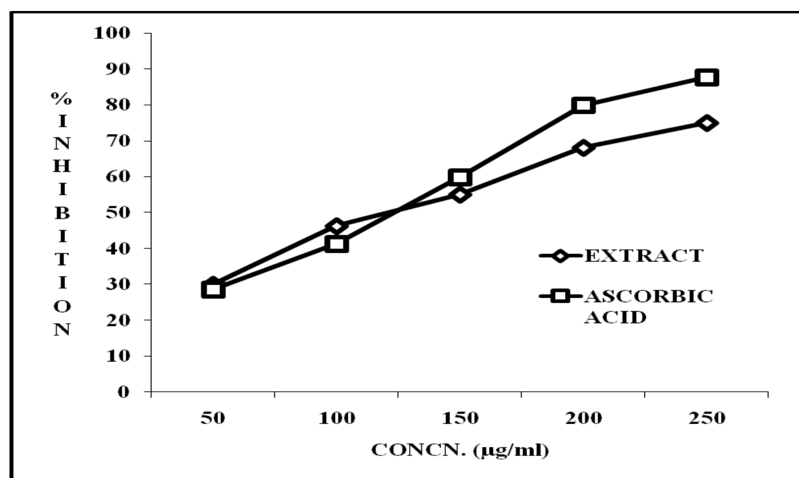

Figure 3: H_2O_2 scavenging activity of *Desmostachya bipinnata*

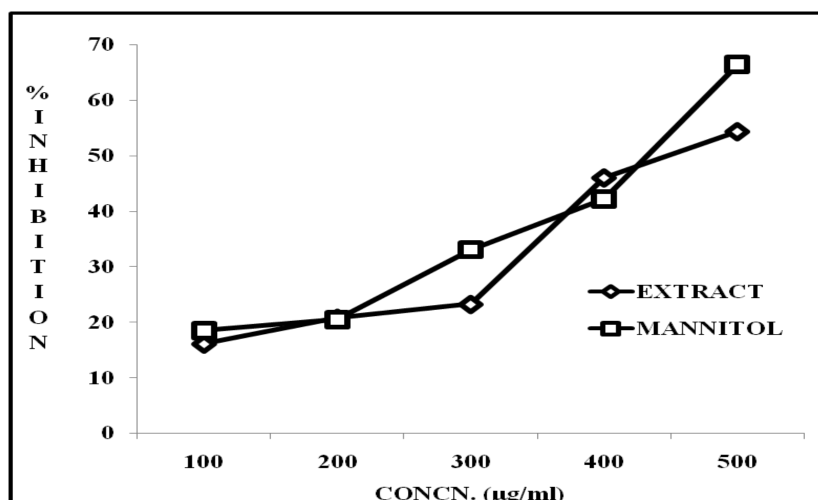

Figure 4: Hydroxyl radical scavenging activity of *Desmostachya bipinnata* and standard (manittol)

well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in DMSO and then measured the absorbance at 570 nm using micro plate reader (Moongkarndi P et al. 2004; Osama Y et al. 2009).

Statistical analysis

The results are expressed as the mean±SD for three replicates. Linear regression analysis was used to calculate IC_{50} values.

RESULTS

Phytochemical analysis

The yield of the methanol extract of *Desmostachya bipinnata* was found to be 10.37%. It showed the presence of carbohydrates and glycosides, phytosterols, phenolic compounds/tannins, flavonoids, proteins, amino acids and volatile oils. The results are shown in Table 1.

1,1Diphenyl-2-picrylhydrazyl radical scavenging activity

1, 1-Diphenyl-2-picrylhydrazyl radical scavenging assay is the most widely used method for screening antioxidant activity, since it can detect active ingredients at low concentration (Piao XL et al. 2004; Yu L et al. 2002). The methanolic extract of *Desmostachya bipinnata* showed DPPH radical scavenging activity in a concentration– dependent manner (Fig 1), The IC_{50} value for DPPH radical-scavenging activity in extract was 471.1± 7.11 µg/ml (R^2 0.995), while the value for quercetin was 89.20± 1.08 µg/ml (R^2 0.966) (Table 2).

Nitric oxide radical scavenging activity

The effect of the methanolic extract of roots of *Desmostachya bipinnata* on nitric oxide radical scavenging activity is shown in Fig 2. The compound, SNP decompose in pH (7.4) producing NO which reacts with oxygen to produce nitrate and nitrite. Here, methanolic extract of roots of *Desmostachya bipinnata* exhibited potent nitric oxide radical scavenging activity. The IC_{50} value of the methanolic extract (163.07± 6.39 µg/ml, R^2 0.909) was found to be comparable with the IC_{50} value of ascorbic acid(152.76± 0.77 µg/ml, R^2 0.947) (Table 2).

Hydrogen peroxide scavenging assay

Hydrogen peroxide initiates lipid peroxidation weakly. It generates highly reactive hydroxyl radical through the Fenton reaction (Namiki M et al. 1990). Methanolic

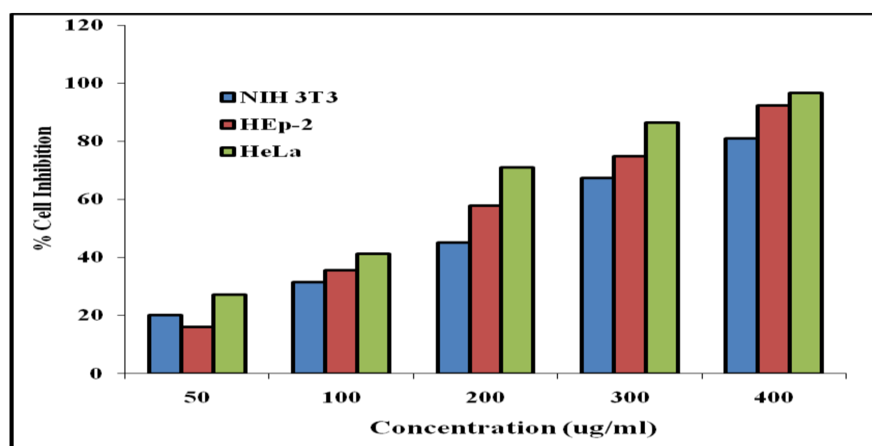

Figure 5: *In vitro* cytotoxic activity of *Desmostachya bipinnata*

Table 1: Phytochemical and antioxidative components of the methanolic extract of *Desmostachya bipinnata*

Parameters studied	Methanolic extract of *Desmostachya bipinnata*
Extractive yield (%)	10.37 ± 0.84
Phytoconstituents	Carbohydrates and glycosides, phytosterols, phenolic compounds/tannins, flavonoids, proteins, amino acids and volatile oils

Values represent in the results are mean±SD of three replicates

Table 2: Scavenging of reactive oxygen species (IC50 values) of the methanolic extract of *Desmostachya bipinnata*

	IC_{50} (µg/ml) & R^2			
Methanolic extract of roots of *D. bipinnata*	**DPPH**	**Nitric oxide**	**H_2O_2**	**Hydroxy radical**
	471.1± 7.11 0.995	163.07± 6.39 0.909	127.07± 6.44 0.982	434.62± 4.17 0.898
Standard	**Quercetin**	**Ascorbic acid**	**Ascorbic acid**	**Mannitol**
	89.20± 1.08 0.966	152.76± 0.77 0.947	122.60± 2.14 0.984	419.58± 2.65 0.909

All presented values are mean±SD of three replicates and reported with ±95% confidence limits.

extract of roots of *Desmostachya bipinnata* scavenged hydrogen peroxide in a concentration-dependent manner. The roots of *Desmostachya bipinnata* showed strong H_2O_2 scavenging activity (Fig. 3). The IC_{50} value for the methanolic extract and ascorbic acid was found to be 127.07± 6.44 µg/ml (R^2 0.982) and 122.60± 2.14 µg/ml (R^2 0.984) respectively (Table 2).

Hydroxyl radical scavenging assay

Figure 4 shows the percent inhibition shown by the methanolic extract of roots of *Desmostachya bipinnata.* As shown in the Table 2, IC_{50} value of the methanolic extract is 434.62± 4.17 µg/ml (R^2 0.898), while the value for mannitol is 419.58± 2.65 µg/ml ($R^2$0.909). It is clear from the result that the extracts have shown concentration dependent radical scavenging activity.

In vitro anticancer activity

The root extract of *Desmostachya bipinnata* was screened for its cytotoxicity against HeLa, Hep 2 and NIH 3T3 cell lines at different concentrations. The percentage growth inhibition was found to be increasing with increasing concentration of test compounds (Table 3 and 4). At 400 µg/ml concentration, maximum % cell inhibition was observed. Among the three cell lines tested, *Desmostachya bipinnata* extract showed IC_{50} value as 109.8 µg/ml for HeLa cell lines, which was less than the other two (figure 5).

DISCUSSION

Scavenging of the DPPH radical takes place by antioxidant extract by donating hydrogen It is visually noticeable as a colour change from purple to yellow. The phenol and flavonoid contents of this plant may be responsible for its good DPPH-scavenging activity (Ebrahimzadeh MA et al. 2010).

The methanolic extract also showed weak NO scavenging activity In addition to reactive oxygen species, NO is also implicated in inflammation, cancer and other pathological conditions (Nabavi SM et al. 2008). Plant/plant products that have the capacity to counteract the effect of NO formation may be of considerable interest in preventing the ill effects of excessive NO generation in the human body. Potent NO scavenging

Table 3: Determination of cytotoxicity of the methanolic extract of *Desmostachya bipinnata* by MTT assay

PLANT EXTRACT	CONC µg/ml	HeLa	HEp-2	NIH 3T3
Desmostachya Bipinnata	25	0.243±0.001 (4.44)	0.321±0.008 (2.82)	0.313±0.009 (3.19)
	50	0.224±0.007 (12.16)	0.277±0.001(16.03)	0.258±0.015 (20.1)
	100	0.134±0.007 (47.32)	0.212±0.007 (35.68)	0.221±0.010 (31.44)
	200	0.061±0.005 (75.94)	0.139±0.006 (57.76)	0.177±0.010 (45.05)
	300	0.024±0.002 (90.46)	0.046±0.004 (85.88)	0.106±0.022 (67.40)
	400	0.008±0.001 (96.73)	0.025±0.006 (92.34)	0.062±0.033 (80.93)
	Control	0.255±0.002	0.331±0.006	0.323±0.033

Values are mean±SD of three replicates. Values in parenthesis indicate percentage inhibition (I%)

Table 4: Comparison of IC50 of the methanol extract of *Desmostachya bipinnata* by MTT assay

CELL LINES	IC_{50} µg/ml	R^2
HeLa	109.8	0.912
HEp-2	166.8	0.98
NIH 3T3	216	0.993

ability has been reported for flavonoids (Van Acker SABE et al. 1995). In addition, scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to human health (Ebrahimzadeh MA et al. 2010).

Hela, Hep-2 and NIH 3T3 exhibited significant percent cell inhibition in a concentration dependant manner. The susceptibility of the cells to the extract was characterised by IC_{50} and R^2 values.

The methanolic extract of *Desmostachya bipinnata* was capable of scavenging hydrogen peroxide in a concentration-dependent manner. The H_2O_2-scavenging of the extract may be attributed to its phenolic contents as well as other active components such as tannins and flavonoids which can donate electrons to H_2O_2.

In most biological systems the primary free radical O_2^- is converted into more reactive species, e.g., OH^- radicals (Winterbourne et al. 2003). Free radical scavengers which can scavenge the hydroxyl radicals protect the cell membrane from damage. The methanolic extract of *Desmostachya bipinnata* was capable of scavenging OH^- radicals in a concentration-dependent manner, which was found to be comparable to manittol.

The methanolic extract of *Desmostachya bipinnata* inhibited cell proliferation in Hela, Hep-2 and NIH 3T3 cell lines markedly in a dose-dependent manner. The extract (200 and 400 µg/ml body weight) produced vacuolisation and necrosis of the tumour cells Magnitude of these effects were greater with higher dose of the extract (400 mg/kg p.o). *Desmostachya* bipinnata extract showed least IC_{50} value as 109.8 µg/ml for HeLa cell lines.

CONCLUSION

The study showed that the methanolic extract of *Desmostachya bipinnata* roots has remarkable antioxidant and antihaemolytic activities. Thus, the plant could be exploited as the source of natural antioxidants that could protect the body against damage caused by free radicals. These effects have been attributed to the presence of antioxidant components such as plant phenols and flavonoids in the plant. There is a need for further investigation of this plant in order to identify and isolate its active anticancer principle(s). The results of the study will also need to be confirmed using *in vivo* models. In addition, further investigations are needed to clarify intracellular pathways involved in the mechanism of the growth inhibition activity.

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