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Research Article

Antioxidant and Antiproliferative Activities of Methanol Extract of Leaves of *Debregeasia longifolia* Linn.

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ABSTRACT

The current study was aimed at evaluation of Antioxidant & Anti proliferative potential of the methanol extract of *Debregeasia longifolia* leaves and to elucidate its phytochemical profile using antiproliferative activity. The methanol extract of leaves of *D. longifolia* was screened for its antioxidant potential by dot-plot assay, DPPH radical scavenging assay and hydroxyl radical scavenging assay. Since the results were quite encouraging, it stimulated the investigators to proceed further to screen the phytochemical profiles by qualitative and quantitative methods which were further extended to thin layer chromatography, and antiproliferative activity. The result indicates that the methanol extract of leaves of *G. rottlariformis* showed significant radical scavenging activity, potent toxicity to liver cancer cells. Since the results are promising, *D. longifolia* could be considered as a significant source of Antioxidant and Anti proliferative agents.

Key Words: Antioxidant, DPPH, HepG2, Nilgiri plant, TLC.

INTRODUCTION

Latest trends have shown that there is an increasing demand for phyto drugs and some medicinal herbs have proven potential to cure dreadful diseases like cancer, paralysis, bronchial asthma etc. Medicinal herbs and their extracts are widely used in the treatment of liver disorders like hepatitis, cirrhosis and loss of appetite [1]. The medicinal plants contains wide array of chemical compounds called as secondary metabolites which are responsible for curing various diseases and hence called as therapeutics agents [2]. Since the synthetic drugs pose a number of side effects currently, people developed confidence on herbal remedies to prevent or cure cancer. The side effects of synthetic drugs are remarkable because liver is the organ known to filter toxins and drug particle from blood. Hence it has become a great challenge for the synthetic drug manufacturers to find out an alternative and safe treatment for liver diseases, which resulted in exploring the herbal sources [3]. The Nilgiri district popularly known as "The Blue Mountains" is a vital place for medical, ethno-botanical as well as anthropological studies. It is located in the Western Ghats and the tribes of this district are Kotas, Kurumbas, Irulas, Paniyas, and Kattunayaks [4]. Regarding the potent tribal drugs, they remain unexplored due to lack of scientific knowledge. *D. longifolia* has been reported to be effective in curing indigestion, sunburn, skin diseases and external tumors. Hence the present study is aimed to explore the efficacy of *D. longifolia* as anti-proliferative compounds. [5]

MATERIALS AND METHODS

Materials

The plant leaves of *D. longifolia* was collected from Kotagiri zone of Nilgiri district and identified by S. Aroumougame CAS in Botany University of Madras. Leaves of *D. longifolia* were washed with tap water, rinsed with distilled water and shade dried. The dried leaves were ground to obtain coarse powder and subjected to solvent extraction using methanol. [6]

Methods:

Qualitative Phytochemical Analysis: The phytochemical profile of methanol extract of leaves of *D. longifolia* (MELD) was studied by following standard procedures. [6]

Quantitative Phytochemical Estimations

Total Phenolic Content: The total phenol content was determined by Folin-Ciocalteu reagent method with slight modifications. 1 ml of MELD was mixed with 1 ml of Folin-Ciocalteu's phenol reagent. After 3 min, 1 ml of Na_2CO_3 (7%) was added to the mixture and it was made up to 10 ml by adding deionized water. The mixture was kept for 30 min at room temperature in dark. The absorbance was measured at 725 nm against the blank. The total phenolic content was expressed as mg of Gallic acid equivalents (GAE) per gram of dry extract. [8]

Total Flavonoid Content: The total flavonoid content was determined by aluminum chloride reagent method. 1 ml of MELD was mixed with 100 µl of 5% $NaNO_2$ solution and after 6 min, 500 µl of 10% $AlCl_3$ and H_2O were added. After 5 min, 0.5 ml of 1M NaOH was added. The absorbance was measured immediately against the prepared blank at 510nm.Quercetin was used as a standard and the results were expressed as mg of quercetin equivalents (QE) per g of dry extract. [8]

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Table 1: Qualitative analysis of methanol extract of leaves of D. *longifolia*

S. No	Phytochemical Constituents	Test	Result
1.	Phenol	Ferric Chloride test	+
2.	Flavonoids	Mg filaments test	+
		NaoH test	+
3.	Alkaloids	Mayer's test	+
4.	Terpenoids	Salkowski test	+
5.	Saponins	Foam test	+
6.	Glycosides	Born trager's test	+

Table 2: Quantitative analyses of methanol extract of leaves of *D. longifolia*

S. No	Phenol GAE/g	Flavonoid QE/g
1	113.19	52.70

Table 3: DPPH radical scavenging assay of methanol extract of leaves of *D. longifolia*

S. No	Concentration (µg/ml)	% RSA
1.	10	22.44
2.	20	38.65
3.	30	43.75
4.	40	51.08
5.	50	63.86
6.	60	75.63

Table 4: Hydroxyl radical scavenging assay of methanol extract of leaves of *D. longifolia"*

S. No	Concentration (µg/ml)	% HRSA
1.	10	17.24
2.	20	21.84
3.	30	34.51
4.	40	42.08
5.	50	51.04
6.	60	58.77

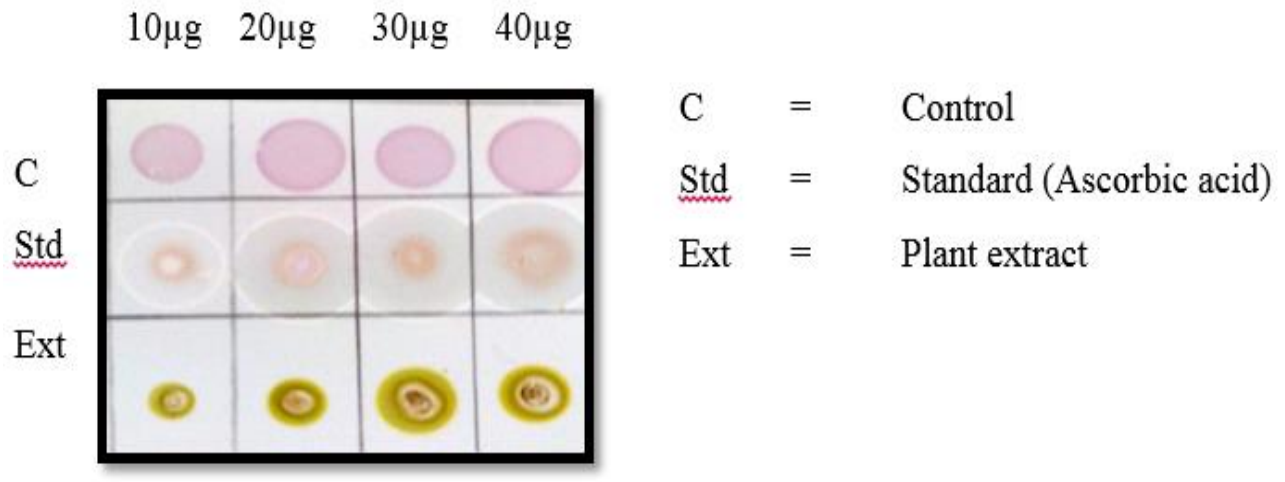

Fig 1: Dot Plot analysis of methanol extract of leaves of D. longifolia

Antioxidant Activity: The antioxidant activity of the MELD was assessed by dot-plot assay as well as radical scavenging assays.

Dot-Plot Rapid Assay: Aliquots of MELD (3µl) of various concentration were spotted carefully on TLC plates and dried for 3 minutes. The sheet bearing the dry spots was placed upside down for 10 sec in a 0.4 mM DPPH methanol solution and the layer was dried. The stained silica gel plate containing active compounds was observed for any significant color change from purple to yellow. [7]

DPPH Scavenging Effects of Extracts: The DPPH radical

Table 5: IC50 of methanol extract of leaves of *D. longifolia* on radical scavenging assay

S. No	Assay	IC_{50} (µg/ml) *D. longifolia*	Standard value
1.	DPPH	39.15	4.58 (quercetin)
2.	OH·	48.98	5.14 (ascorbic acid)

Table 6: Cell viability of methanol extract of leaves of *D. longifolia* on HepG2 cell line

S. No	Concentration (µg/ml)	Cell viability (%)	Cytotoxicity (%)
1	100	78.77	21.23
2	200	69.19	30.81
3	300	65.28	34.72
4	400	54.33	45.67
5	500	58.83	41.17

Table 7: IC_{50} of methanol extract of leaves *of D. longifolia* on cytotoxic activity in cell line

S. No	Cell line	IC_{50} (µg/ml)
1	HepG2	437.92

scavenging activity of MELD was determined according to the method of Hossain *et al*, (2012). Various concentrations of MELD (1 ml) were mixed with 0.1 mM of DPPH solution in methanol (1 ml). The mixture was shaken vigorously and left to stand for 3 0 min, at room temperature in dark and the absorbance was measured at 517 nm. The percentage inhibition was calculated by the following formula. [8-10]

% RSA = {control – sample / control} X 100

Hydroxyl Radical Scavenging Assay**:** The hydroxyl radical scavenging activity the MELD was determined according to the method of Chang W. Choi *et al*, (2002). Various concentrations of the MELD were taken in different test tubes and evaporated to dryness. One milliliter of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of EDTA (0.018%), and 1 ml of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) were added to these tubes, and the reaction was initiated by adding 0.5 ml of 0.22% ascorbic acid. Test tubes were capped tightly and heated on a water bath at 80-90 °C for 15 min. The reaction was terminated by the addition of 1 ml of ice-cold TCA (17.5% w/v). Three milliliters of Nash reagent (75.0 g of ammonium acetate, 3 ml of glacial acetic acid, and 2 ml of acetyl acetone were mixed and raised to 1 L with distilled water) was added to all of the tubes and left at room temperature for 15 min for color development. The intensity of the yellow color formed was measured spectrophotometrically at 412 nm against reagent blank. The percentage hydroxyl radical scavenging was calculated by the following formula [8-9]

% HRSA = {control – sample / control} X 100

Cytotoxic Activity on HepG2 Cell Line: The cytotoxic activity of MELD was carried out by MTT assay method. HepG2 cell line was used and effective doses were calculated from dose-response curve.

Cell Culture on Liver Cancer Cell: HepG2 cells obtained from NCCS (National Centre For Cell Science, Pune) were cultured in Rose well Park Memorial Institute medium (RPMI), supplemented with 10% fetal bovine serum, penicillin/streptomycin (250 U/ml), gentamycin (100µg/ml) and amphotericin B (1mg/ml) were obtained from Sigma Chemicals, MO, USA. All cell cultures were maintained at 37° C in a humidified atmosphere of 5% CO_2. Cells were allowed to grow to confluence over 24 h before use.

Chemicals and Reagents: MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) Invitrogen, USA. Acridine orange were obtained from Sigma, USA. All other fine chemicals were obtained from Sigma–Aldrich, St. Louis.

Cytotoxic Activity by MTT Assay Method: Cell viability was measured with the conventional MTT reduction assay, as described previously with slight modification. Briefly, HepG2 cells was seeded at a density of 5×10^3 cells/well in 96-well plates for 24 h, in 200ul of RPMI with 10% FBS. Then culture supernatant was removed and RPMI containing various concentrations (0.11–100 µg/ml) of test compound was added and incubated for 48 h. After treatment cells were incubated with MTT (10µl, 5mg/ml) at 37 ◦C for 4 h and then with DMSO at room temperature for 1 h. The plates were read at 595nm on a scanning multi-well spectrophotometer. Data represented the mean values for six independent experiments. The cell viability was calculated by the following formula. [11-12]

Cell Viability (%) = 1- (OD of Treated Cells – OD of Control Cell) X 100

Thin Layer Chromatography: TLC plate of Slica gel 60 F_{254} (5x1.5cm) was used to carried out thin layer chromatography method. MELD was spotted on the TLC plate 0.5 cm above from the bottom. The solvents chloroform and methanol were used as the mobile phase. Slica gel supported aluminum sheet was used as the stationary phase. The spotted silica gel plate was placed upside down in the TLC chamber containing mobile phase.

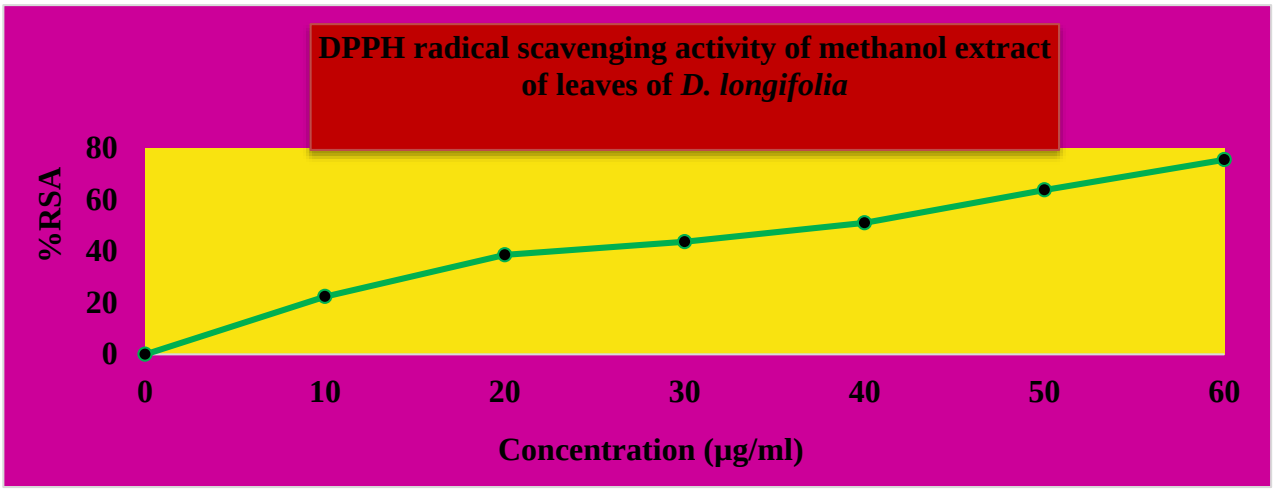

Fig 2: DPPH radical scavenging assay of methanol extract of leaves of D. longifolia

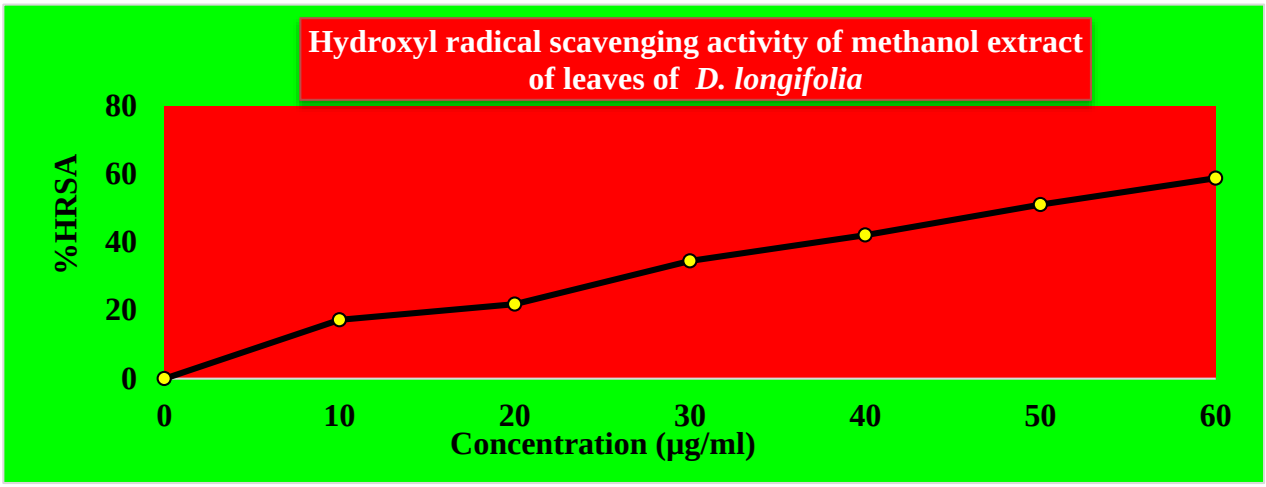

Fig 3: Hydroxyl radical scavenging assay of methanol extract of leaves of D. longifolia

The mobile phase to move through the adsorbent of silica gel containing plant extract of the plate. The TLC plate was removed from the TLC chamber. After reaching the mobile phase up to 80% of the TLC plate. The eluted spots were observed under UV (250 nm) as well as in iodine. [17]

R_f = {Distance travelled by the solute/Distance travelled by the solvent}

RESULTS

The qualitative phytochemical analysis of the MELD showed the presence of phenols, flavonoids, alkaloids, terpenoids, saponins and glycosides (Table 1). The quantitative phytochemical analysis of MELD showed the total phenolic content was 113.918 mg of GAE equivalents per gram and the total flavonoid content was 52.70 mg of QE equivalents per gram (Table 2). The result of dot- plot assay showed (Fig 1) that the MELD changed the purple background of DPPH to yellow, it indicates that the leaves of *D. longifolia* has antioxidant active compounds. The DPPH radical scavenging activity of MELD showed (Table 3 & Fig 2) maximum activity of 75.63% at 60 µg/ml concentration. The degree of discoloration indicates that the MELD showed free radical scavenging potentials due to the hydrogen donating ability. The $OH^{\cdot}$ radical scavenging activity of MELD showed (Table 4 & Fig 3) maximum activity of 58.77% at 60 µg/ml concentration. The IC_{50} value of MELD for DPPH radical scavenging activity was 39.15 µg/ml and $OH^{\cdot}$ radical scavenging activity was 48.98 µg/ml as shown in Table 5. The cytotoxic activity of MELD on liver cancer cell line (HepG2) showed (Table 6 & Fig 4) maximum cell death of 41.17% and the cell viability was 58.83% at 500 µg/ml. The IC_{50} of MELD was recorded as 437.92 µg/ml against HepG2 (Table 7). The R_f value of the eluted spots for the MELD were 0.50, 0.60 0.66, 0.75 and 0.90 as exposed under UV at 254 nm, 0.41, 0.56, 0.89 and 0.92 as stained in iodine in the solvent ratio of 1:9 of methanol: chloroform showed in Table 8.

DISCUSSION

The methanol extract of leaves of *D. longifolia* have shown significant antioxidant property. Based on the above mentioned phytochemical results evident that the presence of phenols, flavonoids, alkaloids, terpenoids, saponins and glycosides. The study is further extended to the anti-proliferative property. The methanol extract of the leaves of *D. longifolia* showed higher toxicity at lower concentration in liver cancer cell line, which indicates its

Table 8: R_f (Retention factor) value of methanol extract of leaves of *G. rottlariformis* by TLC method"

S. No	Methanol : Chloroform (1:9)	
	UV	iodine
1.	0.50	0.41
2.	0.60	0.56
3.	0.66	0.89
4.	0.75	0.92
5.	0.90	-

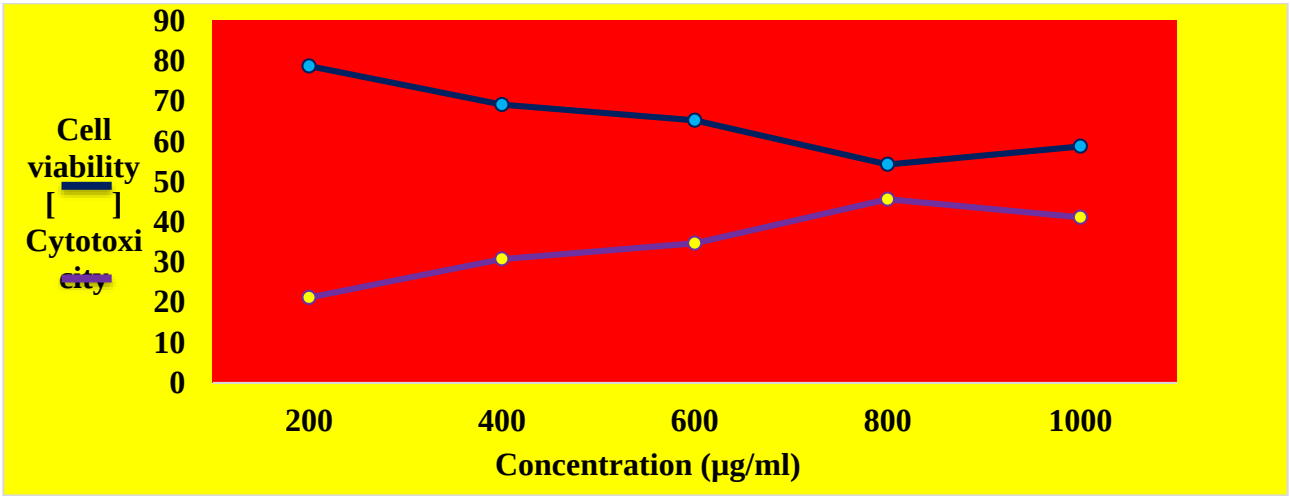

Fig 4: Cytotoxic activity of methanol extract of leaves of D. longifolia on HepG2 cell line

safety in administration as drug.

CONCLUSION

Antioxidant studies of *D. longifolia* indicate its remarkable role in free radical scavenging activity also the possibilities of possessing anti-cancerous therapeutic property. The methanol extract of leaves of *D. longifolia* in *in vitro* liver cancer cell line due to the presence of potent bioactive compound against cancer. The results stimulates that further extended through *in vivo* studies to ascertain its antiproliferative property. The present study has provided a platform for further researches to probe scientifically on plant *D. longifolia* to bring out potent drug for liver cancer.

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