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Research Article

# *INVITRO* ANTIOXIDANT AND PHYTOCHEMICAL EVAULATION OF *DESMODIUM TRIANGULARE* (RETZ.) MERR. ROOT

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**ABSTRACT**
The present study was aimed to investigate the antioxidant potential of the root of *Desmodium triangulare* (Fabaceae). 70% Methanolic extract was screened by using *in vitro* by antioxidant activity screening models such as (DPPH) radical scavenging activity, ABTS radical scavenging activity, Hydroxyl radical scavenging activity, superoxide radical scavenging activity and inhibition of lipid peroxidation. The extract showed significant antioxidant activity in all the models studied. Preliminary phytochemical analysis of the extract were also carried out and revealed the presence of alkaloids, steroids, glycosides, saponins, fixed oils and fatty acids,
**Keywords**: Antioxidant and phytochemical evaluation.

## INTRODUCTION

Free radicals are chemically unstable atoms or molecules that cause extensive damage to cells as a result of imbalance between the generation of Reactive oxygen Species (ROS) and the antioxidant enzymes. Reactive oxygen species are different forms of activated oxygen such as superoxide anion (O2•) and hydroxyl (HO•) radicals and non-free radical species such as $H_2O_2$ and singled oxygen (1O2). [5, 15, 3]. ROS are produced by all aerobic organisms and can easily react with most biological molecules including proteins, lipids, lipoproteins and DNA. Thus, ample generation of ROS proceed to a variety of pathophysiological disorders such as arthritis, diabetes, inflammation, cancer and genotoxicity [10, 2.] Free radical formation is checked naturally by various beneficial compounds known as antioxidants. An antioxidant is any substance which when present at low concentrations as compared to that of an oxidizable substrate prevents the oxidation of that particular substrate. These are our first line of defence against free radical damage and are critical for maintaining optimum health and well-being [13]. A critical balance usually exists between the generation and detoxification of free radicals in the cells. But certain endogenous or exogenous factors could lead to excess load of free radicals in the body causing an imbalance between the oxidants (free radicals) and antioxidants. This imbalance creates an oxidative stress that has been suggested to be the main cause of various diseases such as atherosclerosis, stroke, diabetes, cancer and neurodegenerative diseases such as Alzheimer's and Parkinson's [4.] Research in the recent past has accumulated enormous evidences revealing that the enrichment of body systems with natural antioxidants may prevent, delay or ameliorate many of the disorders caused due to oxidative stress[7.] The present investigation was aimed at examining the antioxidant activity of 70% methanolic extracts of *Desmodium triangulare* root through various *in vitro* models and the major class of phytochemical present in the extract.

## COLLECTION AND AUTHENTICATION

*Desmodium triangulare* were obtained from Peramangalam, Thrissur, Kerala, India and were authenticated by Dr. N Sasidharan, Scientist, NWFP Division, Kerala Forest Research Centre, Peechi, Kerala, India.

### General chemicals and instruments

All chemicals and solvents used in the study were of analytical grade 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) were purchased from Sigma-Aldrich (India). Nitroblue tetrazolium (NBT), naphthylethylene diamine dihydrochloride, thiobarbituric acid (TBA), potassium persulfate from SRL P. Ltd (India) All other reagents used were of analytical quality. UV Vis Spectrophotometer, centrifuge, weighing balance and pH meter were the instruments used for the study.

### Preparation of plant extract

About 100g of air dried powdered material was taken in a soxhlet apparatus and extracted by using 70% methanol (merck india ) as solvent, till colour disappeared. After that extract was concentrated by distillation and solvent was recovered. The final solution was evaporated to dryness. The dried extract was redisolved in distilled water and used for further studies.

### Qualitative phytochemical analysis

The methanolic extract of root of *Desmodium triangulare* were subjected to qualitative examination for different phytoconstituents like Alkaloids, Carbohydrates, Flavonoids, Proteins, Saponins, Terpenoids and Steroids by using standard methods[8,9.]

## IN VITRO ANTIOXIDANT ASSAYS

### 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The free radical scavenging activities were determined by DPPH method with some modifications of the method proposed by[18]. DPPH dissolved in methanol (0.05 mg/ml) was prepared and a series of extract solutions with varying concentrations were prepared by dissolving the dried extracts in methanol and 0.1 ml of solutions from each extract was added to 1.4 ml of DPPH solution. The absorbance at 517 nm was recorded after 5 min of incubation at room temperature. Radical scavenging capacity of each extract has been calculated as the percent DPPH radical scavenging affect which is

% of inhibition = (Absorbance of control - Absorbance of extract / Absorbance of control x100)

**ABTS (2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)) radical scavenging activity**

The assay was carried out by interacting the extract with a model stable free radical derived from 2,2-azinobi (3-ethylbenzo thiazolic-6-sulphanic acid (ABTS). The production of radical cat ion was accomplished as described by long and [19] with some modifications. In brief a stock solution of ABTS (7mM) was prepared in water. To this solution ammonium persulphate (2.45mM final conc) was added and the solutions were allowed to react leading to an incomplete oxidation of ABTS to generate ABTS radical. The ABTS radical solution was diluted to an absorbance of 0.75 at 734nm in PBS and 10µl of different concentrations of the extract were added to 1ml of ABTS radical solution. Absorbance was measured spectrophoto metrically at 6 minutes after initial mixing, using PBS as reference.

% of inhibition = (Absorbance of control - Absorbance of extract/ Absorbance of control x 100)

**Superoxide radical scavenging activity**

The reaction mixture contained 3 mg NaCN dissolved in EDTA (6 $\mu$M), riboflavin (2 $\mu$M) NBT (50 $\mu$M) and various concentrations (10 – 1000 $\mu$g/ml) of the extract and phosphate buffer in a final volume of 3ml. The tubes containing the reaction mixture were uniformly illuminated with an incandescent lamp for 15 min and the absorbances were measured at 530 nm before and after the illumination [16]. Percent inhibition of superoxide radical was calculated using the equation

% of inhibition = (Absorbance of control - Absorbance of extract / Absorbance of control x 100)

**Hydroxyl radical scavenging activity**

Hydroxyl radical scavenging activity of the extract was measured by studying the competition between deoxyribose and test compounds for the hydroxyl radicals generated from Fe3+/ascorbate/EDTA/$H_2O_2$ system (Fenton reaction). The hydroxyl radicals attack deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances (Elizabeth and Rao, 1990). The reaction mixture contained deoxyribose (2.8 mM), ferric chloride (0.1 mM) EDTA (0.1 mM), H2O2 (1 mM), ascorbate (0.1 mM), KH2PO4-KOH (20 mM, pH 7.4) and various concentrations of the sample in a volume of 1 ml was incubated for 1hr at $37^0$C. Deoxyribose degradation was measured as thiobarbituric acid reactive substrate by the method of [17]. The inhibition produced by different concentration was calculated compared to control. Percent inhibition of hydroxyl radical was calculated using the equation:

% inhibition = (Absorbance of control - Absorbance of extract / Absorbance of control x 100)

**Lipid peroxidation assay**

The level of lipid peroxidation was measured by the method of [17]. 10 – 1000 $\mu$g/ml of extract was incubated with 0.1 ml rat liver homogenate (25%) containing 30 mM KCl, Tris-HCl buffer (0.04 M, pH 7.0), ascorbic acid (0.06 mM) and ferrous ion (0.16 mM) in a total volume 0.5 ml for 1hr. After incubation, 0.4 ml of reaction mixture was treated with 0.2 ml SDS (8.1%), 1.5 ml TBA (0.8%) and 1.5 ml acetic acid (20%, pH 3.5) distilled water were kept for 1hr in a boiling water bath at $100^0$C. After 1 h, the reaction mixture was removed from the water bath, cooled and added 5 ml of pyridine : butanol (15:1 ratio), mixed thoroughly and centrifuged at 3000 rpm for 10min. Absorbance of the clear supernatant was measured at 532 nm against pyridine : butanol. Percent inhibition of lipid peroxidation was calculated using the equation:

% inhibition = (Absorbance of control - Absorbance of extract/ Absorbance of control x 100)

**RESULTS**

The roots of *D. triangulare* were extracted using 70% methanol, The extracts obtained 12%were subjected to various phytochemical tests, to identify the active constituents. The tests revealed the presence of alkaloids, steroids, glycosides, saponins, fixed oils and fatty acids, whose results were given in table1

**DPPH (2, 2- diphenyl -1 -picryl hydrazyl) Radical Scavenging Assay**

The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants. In this method the commercially available and stable free radical DPPH (2,2- diphenyl -1 -picryl hydrazyl) soluble in methanol was used, which disappeared or reduced by the antioxidant compounds. The concentration of methanol extract of the plant showed IC50 values of 26.0 µg/ml.

**ABTS radical scavenging activity**

The ABTS radical scavenging ability of the *D triangulare* extract showed an $IC_{50}$ value of 19.0 µg/ml.

**Superoxide radical scavenging activity**

Superoxide anion derived from riboflavin reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. The extract *of D triangulare* was found to scavenge the superoxide radical generated by photo reduction of riboflavin. The concentration of the extract required for 50% ($IC_{50}$) inhibition of superoxide generation was found to be 23.0 µg/ml.

**Hydroxyl radical scavenging activity**

The degradation of deoxyribose to TBARS by hydroxyl radicals generated from $Fe^{3+}$/ ascorbate/EDTA/$H_2O_2$ system was markedly decreased by *D trianulare* extract. The $IC_{50}$ value was found to be23.6 µg/ml.

**Lipid peroxidation**

The extract was found to inhibit lipid peroxides generated by induction of $Fe^{2+}$ /ascorbate in rat liver homogenate. Concentration of methanolic extract needed for 50% inhibition of lipid peroxide was found to be 36.0 µg /ml.

**Table 1**: **Qualitative Phytochemical analysis of methanolic extract of root of *D. triangulare***

| Phytoconstituents | Methanol extract |
|---|---|
| Alkaloids | + |
| Saponins | + |
| Glycosides | + |
| Tannins | - |
| Flavanoids | + |
| Steroids | + |
| Fixed oils and fatty acids | + |

(+) presence of compound, (-) absence of compound

**Table**: **2 Free Radical Scavenging Activity of *D. triangulare***

| Antioxidant assays | % of inhibition ($IC_{50}$ value*) |
|---|---|
| DPPH radical scavenging activity | 26 µg/ml |
| ABTS radicals scavenging activity | 19µg/ ml |
| Superoxide radical scavenging activity | 23 µg/ml |
| Hydroxyl radical scavenging activity | 23.6 µg/ml |
| Lipid peroxidation | 36µg/ml |

*$IC_{50}$ value is the concentration of the extract needed to scavenge 50% of Radicals produced in the reaction mixture

## DISCUSSION

The present investigation revealed that, the preliminary phytochemical analysis of the *D.triangulare* root extracts are bestowed with the presence of several bioactive compounds *viz* alkaloids, steroids, glycosides, saponins, fixed oils and fatty acids, the *D.triangulare*. root extract therefore encourages antioxidant studies.

The free radicals can also be generated in biological systems in the form of reactive oxygen species (ROS) [6]. These reactive ROS cause destructive and irreversible damage to the components of a cell, such as lipids, proteins and DNA [11].Although normal cells possess antioxidant defense systems against ROS, the continuous accumulation of damage to the cells induces diseases such as cancer diabetes, cardiovascular disorders, rheumatoid arthritis and aging [1, 12]. The continuous antioxidant dose also plays a preventive role against these diseases by removing the ROS in biological systems [14]. Hence, the antioxidant property of the extract was evaluated using various in vitro models such as, DPPH radical, ABTS radical, superoxide radical, hydroxyl radical scavenging activities and lipid peroxidation inhibition assay. The IC50 values obtained for each assay was very low showing the high antioxidant potential of the extract. The inhibition of radicals was found to increase with increase in concentration of extract. The scavenging of DPPH, ABTS and highly reactive superoxide radical, hydroxyl radical, and inhibition of lipid peroxidation by extract also proves its ability to protect DNA, lipids and proteins from oxidative damage.

## CONCLUSION

This study reported the antioxidant activity of *D .triangulare* 70% methanolic extract is capable of scavenging free radicals such as superoxide radical, ABTS radicals, hydroxyl radical and DPPH radicals.The extract also inhibited the tissue lipid peroxidation in liver.

The preliminary phytochemical screening showed the presence of alkaloids, steroids, glycosides, saponins, fixed oils and fatty acids, from the present study it can be concluded that antioxidant activity of *D. triangulare* due to the presence of these compounds.

## REFERENCES


1. Ajitha, M., Rajnarayana, K., Role of oxygen free radicals in human diseases. Indian Drugs. 2001; 38, 545–554.
2. Gulcin I, Buyukokuroglu ME, Oktay M,on the in vitro antioxidant properties of melatonin. *Journal of Pineal Research*. 2002; 33: 167–171.
3. Gulcin I, Oktay MO, Kufrevioglu OI, Determination of antioxidant activity of lichen *Cetraria islandica* (L.) Ach. *Journal of Ethnopharmacology*. 2002 b; 79: 325–329.
4. Halliwell B, Gutteridge JM and Cross CE Free radicals, antioxidants and human disease: Where are we now? *Journal of Laboratory and Clinical Medicine* (1992).119 598-620.
5. Halliwell B, Gutteridge JM. *Free Radicals in Biology and Medicine*. Oxford: Oxford University Press; 1999,
6. Halliwell, B., Aeschbach, R., Löliger, J., Aruoma, O.I.,The characterization of antioxidants. Food and Chemical Toxicology., 1995;33, 601– 617
7. Havsteen BH The biochemistry and medical significance of the flavonoids. *Pharmacology and Therapeutics* 96 67-202. nc *Biochemistry* 2002; 165 215-219
8. Kokate CK, Purohit AP, Kokhale SB.Qualitative chemical examination text book of pharmacognosy. Nirali publications,2003; 2 ed:108-9
9. Kokate, C.K., "Practical Pharmacognosy" p: 107-114, 123-125, 130. 2002: 1st edition;2002; 405-406, 426-489.
10. Kourounakis AP, Galanakis D, Tsiakitzis K. Synthesis and pharmacological evaluation of novel derivatives of anti-infl ammatory drugs with increased antioxidant and anti-infl ammatory activities. Drug Development Research.1999; 47: 9–16.
11. Lopaczyski, W., Zeisel, S.H., Antioxidants, programmed cell death, and cancer. Nutrition Research 2001; 21, 295–307.
12. Matés, J.M., Sánchez-Jiménez, F.M., Role of reactive oxygen species in apoptosis: implications for cancer therapy. *The International Journal of Biochemistry and Cell Biology* 2000 32, 157–170.
13. Percival M Antioxidants. *In: Clinical nutrition insights*. Advances Nutrition Publication 1998.
14. Sgambato, A., Ardito, R., Faraglia, B., Boninsegna, A., Wolf, F.I., Cittadini, A.,Resveratrol, a natural phenolic compound, inhibits cell proliferation and prevents oxidative DNA damage. Mutation Research2001; 496, 171–180.
15. Yildirim A, Mavi A, Oktay M, Comparison of antioxidant and antimicrobial activities of tilia (*Tilia argenta Desf Ex DC*), sage (*Salvia triloba* L.) and black tea (*Camellia sinensis*) extracts. *Journal of Agricultural and Food Chemistry*.2000; 48: 5030–5034.
16. McCord JM, Marecki JC. Superoxide dismutases. In: Sipes IG, Mc-Queen CA, Gandolfi AJ, Guengerich FP, eds. 1997;*Comprehensive Toxicology*,vol. 3, *Biotransformation*. New York: Elsevier Science,:199–216
17. Ohkawa, H., Ohishi, N. and Yagi, K. Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. *Annal. Biochem.* 1979; 95, 351-358.
18. Coruh, N, A.G Sagdicolglu Celep and F Ozgokce,. Antioxidant properties of Prangos ferulacea (L.) Lindl, Chaerophyllum macropodum Boiss. and Heracleum persicum Desf. from Apiaceae family used as food in Eastern Anatolia and their inhibitory effects on glutathione-S-transferase. *Food Chemistry,* 2007, 100 (3): 1237-1242.
19. Long L H and Halliwell B Antioxidant and prooxidant abilities of foods and beverages. *Methods in Enzymology* 2001; 335, 181–190.


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