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Phytochemical Screening Antioxidant and Anti-diabetic Activity of *Dalbergia Latifolia* Leaves

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ABSTRACT

The plant *D.latifolia* belongs to the family of Fabaceae is known to exhibit various pharmacological activities like anti-bacterial, anti-inflammatory and anti-arthritic activity. In this paper, the phytochemicals, antioxidant and anti-diabetic activity of *D.latifolia* leaves were studied. The phytochemical screening of ethanolic and aqueous extract of leaves showed the presence of alkaloids, flavonoids, phenol, Saponins, terpenoids and tannin respectively. The antioxidant activity of the leaves extract were studied using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) ABTS, Nitric oxide (NO) and Superoxide (SO) Scavenging assays. The results showed that the ethanolic plant extract exhibited significant antioxidant properties compared to standard Ascorbic acid. The extract also showed inhibitory activity of α-glucosidase enzyme which is responsible for possessing anti-diabetic effect. Thus this study antioxidant,anti-diabetic effect of *D.latifolia* leaves can be a breakthrough for further studies.

Keywords: *Dalbergia latifolia*, phytochemicals, Antioxidant, Anti-diabetic activity.

INTRODUCTION

Traditional medicine still plays a vital role in the developing countries. Medicinal plants are an important source of producing valuable bioactive secondary metabolites which are of great importance for the health of individuals and societies. The medicinal values of the plants are due to the chemical substances that produce physiological actions on humans [1-3]. Some of the curative potentials of biological active substances exist in the plants such as alkaloids, tannin, flavonoid and phenolic compounds offering an untold diversity of chemical structures. The WHO estimated that approximately 80% of the traditional medicine for their primary health care needs and most of this therapy involves the use of their active component of plant extract.

D.latifolia is known as *Amerimnon latifolium*, Black Rosewood and an Indian Rosewood. Its tamil name is Etti. The plant is predominantly a single stemmed deciduous tree with a dome shaped crown of lush green foliage. The tree can become 20-40m tall, with a trunk

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diameter of 1.5 - 2m. The bark is gray, thin with irregular short cracks, peeling in fibrous longitudinal flakes. The leaves are alternately arranged of old pinnate with 5 - 7 unequal sized leaflets originating from the same rachis. Leaflets are broadly blunt-tipped dark green above and pale below. The flowers are whitish pink in color. Its crown is oval in shape. Fruit is brown and rod like in shape. Seeds contain 1 - 4 smooth brown seeds and do not open at maturity.

The plant contains flavonoids that too isoflavonoid, O-glycosides, albergione and dalbinol. *D. latifolia* is containing antioxidant, anti-diabetic and antibacterial properties [4-7]. In this study we have examined the phytochemicals present in the leaves of *D.latifolia* and also the evaluation of antioxidant and anti-diabetic potential. Therefore this study has been undertaken to overcome the effect of ethanolic leaves extract of *D. latifolia*.

MATERIALS AND METHODS

Extraction of *D.latifolia*

The leaves of *D.latifolia* were collected from the St.Joseph's college campus at Cuddalore. The leaves of *D.latifolia* were washed with water. It was shade dried at room temperature and powdered coarsely. 50gm of powder was used for extraction using soxhlet apparatus with 300ml of ethanol and water. The process was run for 48hrs after which the sample was concentrated using rotary evaporator and freeze dried to powdered form. The extracts were stored in air tight container for further studies.

Preliminary Phytochemical Screening

The secondary metabolites like alkaloids, flavonoids, saponins, Phenols,tannins, terpenes and phytosterols were screened according to the standard protocol. [8,9].

Moisture Content

About 1gm sample was weighed into a clean dry pre-washed petriplate. Then the sample was determined in the triplicate by drying at 1200C in a hot air oven and then the plates were removed and cooled in a room temperature. Weight of the sample was taken after cooling process and it is expressed in percentage using the formula.

$$\text{Moisture content \%} = \frac{W1 - W2}{\text{Weight of samples}} \times 100$$

Ash Content

About 1gm of the sample was weighed into a clean dry pre-washed silica dish. Then the sample was ignited slowly over a Bunsen flame in a fume cupboard until the fumes has been evaporated. Then the dish was transferred to muffle furnace and incinerated until it was free from black carbon particles and turns into white in colour about 3hrs. Dish was removed carefully and cooled in desiccator. Weight was taken after cooling process completed and it is expressed by using the formula.

$$\text{Ash \%} = \frac{\text{Weigth of Ash}}{\text{Weight of samples}} \times 100$$

Total Phenolic Content

Folin-Ciocalteau reagent method was used to estimate the total soluble phenolic content. The standard phenolic compound is used here asgallic acid. 1ml of stock solutions of aqueous and ethanolic extracts was prepared (g/ml). The aliquots were pipetted out into test tubes and the volume was made up to 3 ml with distilled water, then added freshly prepared Folin-Ciocalteau reagent and the test tubes were kept in 3 min, then added 2 ml of 20% sodium carbonate solution, each tubes were mixed thoroughly and placed in boiling water bath for one minute, cooled and the absorbance was measured at 650 nm against a reagent blank. The concentrations of the total phenolic compounds in the extracts were obtained and its concentration of total phenols was expressed as mg/g of dry extract.

Total Flavonoid Content

Aluminum chloride colorimetric method was used to estimate the total flavonoid content for both aqueous and solvent extracts [10]. Take 0.5ml of stock solution (g/ml) of the extract, added 1.5 ml methanol and 0.1ml potassium acetate (1M) to the test tubes and the volume was made up to 5 ml with distilled water and kept at room temperature for 30 min; the absorbance was measured at 415 nm. Total flavonoid content was calculated by extrapolating the absorbance of reaction mixture on standard curve of rutin. The experiment

was repeated thrice and the total flavonoid content was expressed as equivalent to rutin in mg / g of the extracts.

In-vitro Antioxidant Assays

Determination of DPPH scavenging activity

DPPH scavenging activity of ethanolic extract was determined according to the standard protocol[11]. DPPH solutions were prepared using 95% of methanol. From the stock plant extract solution 250, 500, 750 and 1000 µg/ml concentrations were taken in five test tubes and to this 0.5ml of DPPH reagent were added. The mixture was shaken vigorously and incubated for 10min in the dark at room temperature. The absorbance was measured at 517nm using UV spectrophotometer. Ascorbic acid was used as a positive control. DPPH free radical scavenging activity% was calculated using the formula.

Determination of ABTS assay

ABTS radical scavenging activity of ethanolic extract of *D.latifolia* was determined according to the method of Re et al., 1999. Briefly, ABTS radical cation ($ABTS^{\bullet+}$) was produced by mixing ABTS stock solution (7 mM in water) with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. Then, $ABTS^{\bullet+}$ solution was diluted with ethanol and the absorbance is read at 734 nm. To 3.0 ml of diluted $ABTS^{\bullet+}$ solution, different concentration 250, 500, 750 and 1000µg/ml of leaves extract in ethanol was added and after 1 min, the decrease in absorbance was measured at 734nm spectrophotometerically.

Determination of NO radical scavenging activity

Sodium nitroprusside (5 mM) in phosphate buffer pH 7.7 was incubated with 250, 500, 750 and 1000µg/ml concentrations of drug dissolved in a suitable solvent (alcohol) and tubes were incubated at 25°C for 120 minutes. At intervals, 0.5ml of incubation solution was removed and diluted with 0.5 ml of Griess reagent. The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and subsequent N-napthylethylenediamine was measured at 546 nm [12-13] respectively.

Determination of Superoxide anion (So) radical scavenging assay

The Superoxide radical scavenging activity of *D.latifolia* was measured [14]. In this method, the activity is measured by reduction of riboflavin/light/ NBT (Nitro blue tetrazolium). The 1 ml of reaction mixture contained Phosphate buffer, NADH, NBT and various Concentrations of sample solution. The method is based on generation of superoxide radical by autooxidation of riboflavin in the presence of light. The Superoxide radical reduces NBT to a blue coloured formation that can be measured at 560 nm.

Anti-Diabetic Activity

Alpha Glucosidase inhibition assay

The α-glucosidase inhibitory activity was performed with a set of eppendorf tubes which was labelled as blank, control and samples at different concentrations were arranged in a stand. The reaction mixture containing 150µl of 0.1M PBS (pH 7.4), 75µl of 20mM p-nitrophenyl α-D-glucopyranoside (PNPG) solution and 25µl of the sample were added and the mixture was pre-incubated at 37ºC for 10 mins. The reaction was initiated by adding 50µl of rat intestinal α-glucosidase enzyme solution (0.17U/ml, sigma) and incubated at 37ºC for 10mins. The reaction was terminated by adding 1ml of 0.1M Na_2Co_3 and the amount of p-nitrophenol (PNP) released was determined by measuring the absorbance at 405nm. For blank the reagents was added in reverse order. The control samples were free of test solution. A set of colour control was done for the test samples without enzymes and PNPG but maintain the volume with buffer. The absorbance of all the samples was measured at 405nm using UV visible spectrophotometer **[15],[16]**. The percent inhibitions of enzyme activity by the test samples were calculated.

RESULT AND DISCUSSION

Phytochemical Screening

Table 1: Phytochemical screening of various extract of *D.latifolia* leaves

S.No	Tests	Ethanol	Water
1	Alkaloids	+	-
2	Phenols	+	+
3	Flavonoids	+	+

4	Tannins	+	-
5	Saponins	+	+
6	Glycosides	+	+
7	Terpenoids	+	+
8	Steroids	-	_
9	Protein	_	_
10	Gum and mucilage	_	_

(+) indicate presence; (-) indicate absence

Table-1 shows the results of preliminary Phytochemical screening of *D.latifolia* extract. This confirms that the majority of the Phytochemicals present such as alkaloids, flavonoids, phenol, saponins, terpenoids, tannin and absence of fat, proteins, gum, mucilage and phytosterols respectively.

Table 2: Quantitative Analysis of Moisture Content

S.NO	MOISTURE CONTENT				
1	**LEAF**	**T1**	**T2**	**T3**	**MEAN±S.D**
2	FRESH	62.7	61.7	56.7	60.36±3.21
3	DRY	8.3	7.5	7.2	7.66±0.56

Table-2 shows the quantitative analysis of moisture content. This confirms that the moisture content was found to be high in fresh leaves when compared with the dried leaves.

Table 3: Quantitative analysis of Ash Content

S.NO	ASH CONTENT				
1	**LEAF**	**T1**	**T2**	**T3**	**MEAN±S.D**
2	FRESH	76.5	73.8	81.7	77.33±4.01
3	DRY	65.2	52.4	73.6	63.73±10.67

Table-3 shows the quantitative analysis of Ash content. In this Ash content was found to be high in fresh leaves when compared with the dried leaves.

Table 4: Total Phenol and Flavonoid Content in different Extracts of *D.latifolia*

Test	*D.latifolia* leaf	
	Aqueous Extract	**Ethanol Extract**
Total phenolic content	70±0.71	110 ±1.06
Total Flavonoids content	12±1.10	30±3.61

Table-4 shows the quantitative analysis of total phenol and flavanoids. This confirms that the ethanol extract has high content of phenol and flavonoids compared with aqueous extract.

Antioxidant activity

In the present study, the antioxidant potential of *D.latifolia* leaves extract is examined by four methods such as DPPH, ABTS, No and Superoxide radical scavenging assays.

Figure 1: DPPH Scavenging Activity of *D.latifolia* Leaves Extract

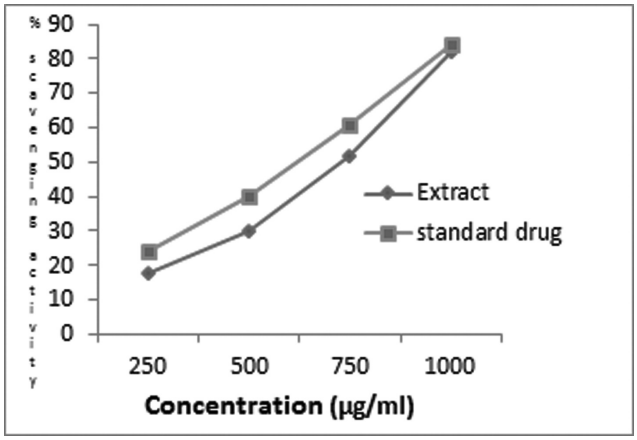


Figure 1 shows the scavenging activity of the plant extract at 82%and the scavenging activity of standard drug at 84%.

Figure 2: ABT Scavenging Activity of Ethanolic Extract of *D.latifolia* Leaves

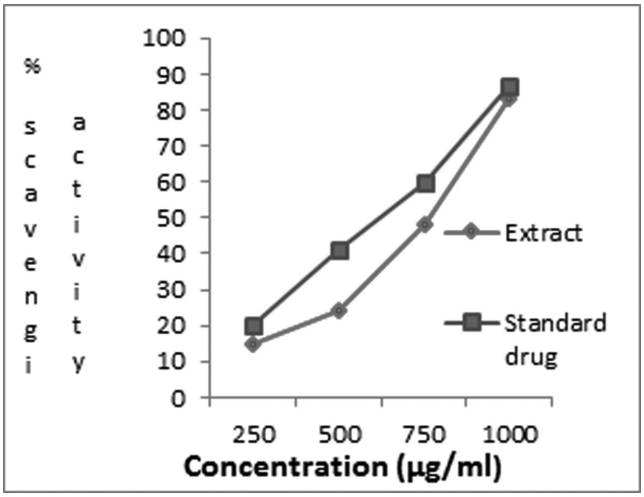


Figure 2 shows the scavenging activity of the plant extract at 83% and the scavenging activity of standard drug at 87%.

Figure 3: Superoxide Radical Scavenging Potential of Ethanolic Extract of *D.latifolia* Leaves

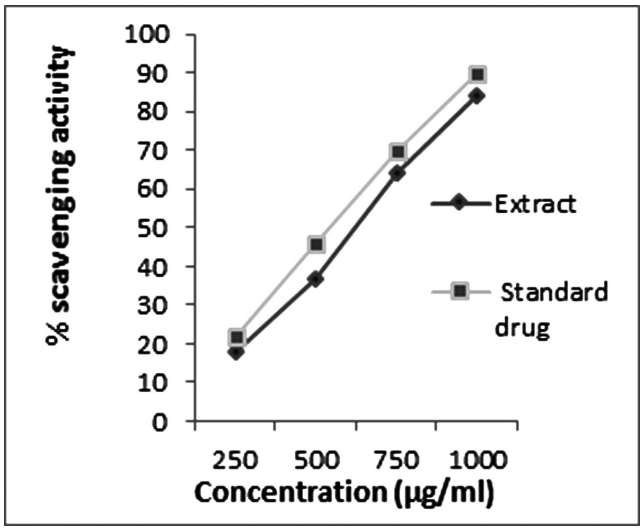
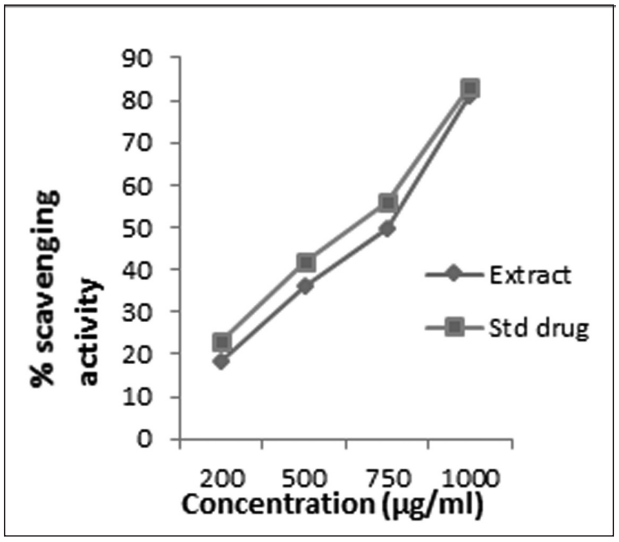


Figure 3 shows the scavenging activity of the plant extract at 84% and the scavenging activity of standard drug at 90%.

Figure 4: NO Radical Scavenging Potential of Ethanolic Extract of *D.latifolia* Leave



. Figure 4 shows the scavenging activity of the plant extract at 81% and the scavenging activity of standard drug at 83%.

The ability of natural compounds to scavenge the DPPH radical can be expressed as its magnitude of anti-oxidative ability. DPPH radical in alcoholic solution provide deep purple in color with an absorption peak at 515 nm. The principle of DPPH assay is based on the DPPH radical on accepting a hydrogen atom from the scavenger molecule i.e., antioxidant, results in reduction of unpaired valence electron at one atom of nitrogen bridge in DPPH, then change of purple color to yellow with concomitant decrease in absorbance at 515 nm[11]. The change in color from deep purple turn to yellow or the decrease in intensity signifies the antioxidant activity of the test compound.

ABTS assay involves reduction of the color intensity of ethanolic solution containing pre-formed radical monocation of ABTS;it is produced by oxidation of ABTS with potassium persulfate due to the radical scavenging activity of antioxidants (Re et al., 1999). The change in color intensity is proportional to the antioxidant efficiency of compounds.

The leaves of ethanolic extract of *D.latifolia* scavenge DPPH and ABTS. The extract of *D.latifolia* significantly and concentration dependently reduced DPPH and ABTS radicals. However at a concentration of 1000µg/ml, the extract significantly scavenged 82 % of DPPH radicals and 83 % ABTS radicals. This results were confirmed the DPPH and ABTS radical in a concentration dependent manner.

The leaves of ethanolic extracts of the *D.latifolia* were markedly a more potent scavenger of superoxide anion compared to other extracts. Superoxide anions play an important role in the formation of the ROS such as hydrogen peroxide, hydroxyl radical, and singlet oxygen, which induce oxidative damage in proteins, lipids and DNA. The test extract exhibited a maximum of 84% superoxide scavenging activity with a significant extent at a concentration of 1000 µg/ml. The leaves of ethanolic extracts of the *D.latifolia* were expressed at a concentration of 1000 µg/ml also quenched 81% NO released by a NO donor.

Table 5: Anti diabetic Activity of Ethanolic Extract of *D.latifolia* Leaves

Concentration in µg/ml	OD at 405nm	% of inhibition
250	0.2845	4.849498
500	0.264	11.70569
750	0.2105	29.59866
1000	0.204	31.77258
2000	0.133	55.51839

Figure-4 Anti-diabetic Activity of Ethanolic Extract of *D.latifolia* Leaves

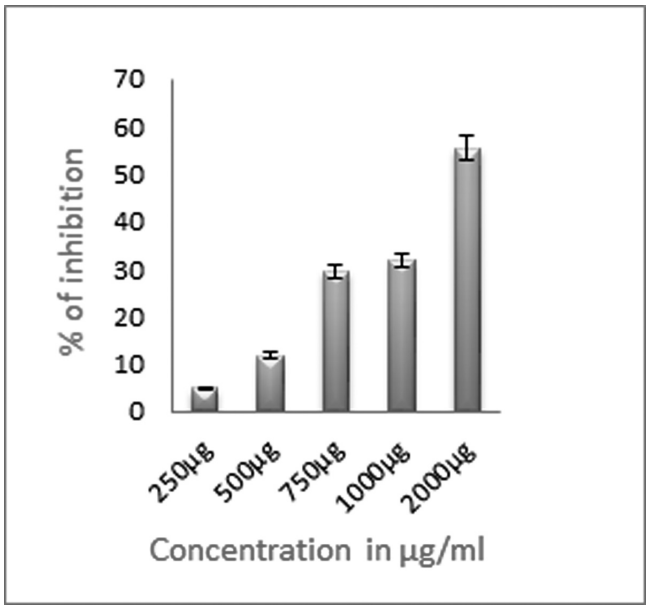


Figure-4 shows the Anti-diabetic activity of ethanolic extract of *D.latifolia* leaves. In this inhibition α-glucosidase was found to be high in 2000µg/ml concentration which reveals that it could act as most effective approaches to control diabetes.

α-glucosidase is a membrane bound enzyme located on the epithelium of the small intestine, catalyzing the cleavage of disaccharides to form glucose. The natural inhibitors from dietary sources are strong inhibitory activity against α-glucosidase [17]. Inhibitors can retard the uptake of dietary carbohydrates and suppress post-prandial hyperglycemia. So the inhibition of α- glucosidase by extract of *D.latifolia* leaves were the most effective in control of diabetes.

CONCLUSION

The ethanolic extract of D.latifolia leaves could be evaluated as major source of alkaloids, flavanoids, terpenoids, tannins, phenols, carbohydrates, glycosides and saponin. The antioxidant activity of the ethanolic extract of leaves showed potential activity when compared to the synthetic antioxidants. This study concluded that leaves of *D. latifolia* have antioxidant property and the activity was found due to the presence of phytochemicals present in it. Then vitro assays of the present study indicated that ethanolic leaves extract possess good anti-diabetic activity were the glucose uptake was found to increase in a dose dependent manner. The ethanolic extract of *D.latifolia* exhibited significantly higher activity at all glucose concentrations showing the maximum level. The result of this research contributes to the body of knowledge on the use of *D.latifolia* exhibit hypoglycemic agent and acts as a good source in the development of new and effective therapy in the management of diabetes and its complications. In future, it will be a good alternative source of drugs for various diseases.

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