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In-vitro Assessment of Antioxidant Activity of *Dalbergia latifolia* Barks Extract Against Free Radicals

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Abstract: *Dalbergia latifolia* Roxb. (Family: Fabaceae) is a traditional herb, contain latinone and dalcriodain flavonoid having excellence medicinal value. Antioxidants play an important role for protecting the human body against damage by reactive free radicals. Methods: The antioxidant activities and phenolic contents of the ethanolic extract of the bark of *Dalbergia latifolia* was evaluated using *in vitro* standard procedures for the determinations of total phenol, total flavonoid. Gallic acid and rutin equivalents were used for these parameters. The antioxidant activities of the bark extract of *Dalbergia latifolia* were determined by the 1,1-Diphenyl-2-picrylhydrazyl (DPPH), nitrous oxide thiocyanate and reducing antioxidant property methods. Results: The total phenolic content was found to be 210±1.16 μg mL^{-1}; however the flavonoid content was 46±3.61 μg mL^{-1} for *D. latifolia* bark extract. DPPH, NO and thiocyanate percentage inhibition scavenging activity 92.10±1.10, 86.39±2.12 and 87.22±2.47 respectively. Conclusion: The antioxidant played an important role of protecting the human body against free radicals. The present data demonstrated that *D. latifolia* bark exerted significant anti-oxidant scavenging potential. Further, it requires identifying the exact phytoconstituents responsible for the activity.

Key words: Antioxidant · 1, 1-Diphenyl-2-picrylhydrazyl · Phenolic content · Flavonoid content · Dalbergia latifolia · Thiocyanate · Latinone

INTRODUCTION

The antioxidant activity is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers and have a potential to make chelating with metal components [1]. Plants are potential sources of natural antioxidants and produce various antioxidative compounds to counteract reactive oxygen species [2]. Reactive oxygen species, which include free radicals such as super oxide anion radicals, hydroxyl radicals and non free-radical species such as hydrogen peroxide and singled oxygen and various forms of activated oxygen. These molecules are exacerbating factors in cellular injury and aging process [3]. These reactive species exert oxidative damaging effects by reacting with nearly every molecule found in living cells [4]. The excess reactive oxygen species are not eliminated by antioxidant system. They play important roles in the pathogenesis of age related disorders such as hypertension, atherogenesis, Alzheimers disease and Parkinsons disease [5, 6]. The protective capacity of polyphenols is also supported by a number of studies indicating an effect of dietary polyphenols on coronary heart disease (CHD) [7], cancer [8]. Phenolic compounds found in both edible and nonedible plants have a capacity to scavenge free radicals and exerted multiple biological effects, including antioxidant activity [9, 10].

Dalbergia latifolia (Roxb) Family- Fabaceae [11] a larg glabrous tree a single stem with characteristic smell [12]. The tree has grey bark that peels in long fibres, compound leaves and bunches of small flowers [13]. The bark is grey, thin with irregular short cracks, exfoliating in fibrous longitudinal flakes [14]. It is distributed in Bihar, Bundelkhand and Central India [15]. It contain dalbinol a new 12a-hydroxyrotenoid [16], sisafolin coumarin from seeds, β- sitosterol, also contain dalbergichromene, lupeol, latifolin and dalbergin from bark of the tree, heartwood contains latinone, neoflavonoid dalcriodon [17] and Latinone, a substituted phenanthrene-1, 4-quinone was isolated from *Dalbergia latifolia* [18]. Ethanomedicinally, the stem barks contain tannin is used for treatment of leprosy, obesity and worm [15].

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The genus consists of 300 species and about 25 species occur in India. Many species of *Dalbergia* are important timber trees, valued for their decorative and often fragrant wood, rich in aromatic oils [19, 20]. Traditionally various species are reported to be used as aphrodisiac, abortifacient, expectorant, anthelmentic, antipyretic, appetizer, allays thirst, vomiting, burning sensation, cures skin diseases, ulcers, diseases of the blood, reduces obesity, used in leucoderma, dyspepsia, dysentery, for diseases of the eye and nose, syphilis, stomach troubles, leprosy, leucoderma, scabies and ringworm [15, 21].

MATERIAL AND METHODS

Extract Preparation: Plants were air dried at room temperature for 3 weeks to get consistent weight. The dried plants were later ground to crude powder. Two hundred grams of crude powder was shaken separately in petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts for 48 hrs on an orbital shaker at room temperature. Extracts were filtered using a Buckner funnel and Whatman No 1 filter paper. Each filtrate was concentrated to dryness under reduced pressure at 40°C through rotary evaporator [22].

Chemicals: Folin-Ciocalteus's phenol reagent and sodium carbonate were from Merck chemical supplies (Damstadt, Germany). Ascorbic acid, gallic acid, 1, 1-Diphenyl-2-picrylhydrazyl (DPPH), ferric thiocyanate, nitric oxide, aluminium chloride and sodium nitrite rutin from purchased Qualigens. All the other chemicals used including the solvents, were of analytical grade purchased from SD fine and Fischer scientific Ltd.

Determination of Total Phenolic Content: The total phenolic contents of the *D. latifolia* bark extracts were determined by the modified Folin-Ciocalteu method [23]. Absorbance was measured at 765 nm using UV-VIS spectrophotometer. Total phenolic content was calculated by the equation based on the calibration curve: $y = 0.002x$, $R^2 = 0.994$, where x was the absorbance and y was the tannic acid equivalent (µg/g).

Determination of Total Flavonoid Content: The total flavonoid concentration was determined through method [24]. After one hour at room temperature, the absorbance was measured at 420 nm. Total flavonoid content was calculated as rutin (mcg/ml) using the equation based on the calibration curve: $y = 0.001x$, $R^2 = 0.991$, where x was the absorbance and was the rutin equivalent (µg/g).

Determination of *In-vitro* Antioxidant Activities

DPPH Free Radical Scavenging Activity: The DPPH assay is based on the measurement of the scavenging ability of an antioxidant using the stable DPPH free radical [25]. The free radical DPPH is purple in colour in ethanol and is reduced to the corresponding hydrazine, which is yellow in colour, when it reacts with a hydrogen donor. It is a discoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in ethanol and the decrease in absorbance is measured at 490 nm.

$$\% \text{ Inhibition} = [(A0-A1) / A0 \times 100]$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

Nitric Oxide Scavenging Activity: The ethanolic extract was dissolved in PBS in different concentration and sodium nitroprusside was at added in each test tube were incubated at 30°C for 5h [26]. After 5h, 0.5ml of incubation solution was removed and diluted with 0.5 ml of Griess reagent. The absorbance was measured at 546 nm.

Reducing Potential Scavenging Activity: The reducing capability of the sample extracts was measured by transformation of Fe^{+3} to Fe^{+2} in the presence of the extracts at 700 nm [27]. After adding the chemical reagents the absorbance was measured at 700 nm.

Ferric Thiocyanate Scavenging Activity: The ferric thiocyanate method in a linoleic acid emulsion was used [28] for determination of thiocyanate scavenging activity. At regular intervals during incubation, add 0.05 ml of the mixture was diluted with 2.85 ml of 75% ethanol, followed by the addition of 0.05 ml of 30% ammonium thiocyanate. The absorbance of the red-colour portion of the sample was measured at 500 nm.

Statistical Analysis: The experimental results were expressed as mean ± standard error of mean (SEM) of three replicates. IC_{50} was calculated.

RESULT AND DISCUSSION

Preliminary Phytochemical Studies: The qualitative phytochemical analysis of *D. latifolia* bark extract were found to be in petroleum ether (0.13%), chloroform (0.60%), ethyl acetate (0.36%), ethanol (5.32%) and aqueous (5.25%) extracts respectively (Table 2).

Table 1: Polyphenol content of *Dalbergia latifolia* bark extract

Parametres	Ethanolic fraction (µg/mL)
Total Phenolic	210±1.56
Total Flavonoid	46±3.61

Table 2: Successive extraction of *Dalbergia latifolia* bark

S. No.	Solvents Fractions	Values
1	Petroleum ether	0.13%,
2	Chloroform	0.60%,
3	Ethyl acetate	0.36%,
4	Alcoholic	5.32%
6	Aqueous	5.25%
7	Total Phenolic	641.83±2.47 (µg mL^{-1})
8	Total Flavonoid	38.69±2.21 (µg mL^{-1})

Table 3: Qualitative Chemical analysis of *Dalbergia latifolia* bark extract

S.No.	Test	Pet ether extract	Chloroform extract	Ethyl acetate extract	Alcohlic extract	Aqueous extract
1	Carbohydrate	-	-	-	+	+
2	Glycoside				+	+
3	Alkaloid	-	-	-	-	-
4	Protein				+	-
5	Tannin	-	-	-	-	-
6	Flavonoid	-	-	-	+	+

(+) Present, (-) Absent

Table Preliminary analysis showed the presence of the carbohydrates, glycoside, tannins, amino acid and flavonoids in ethanolic and aqueous extracts (Table 3) [29, 30].

Total phenolic and flavonoids contents: Table 1 indicated that the total phenolic and flavonoids content in *D. latifolia* bark of ethanolic extract had higher level of phenolic compounds. The maximum absorbance of 0.73 was observed at a concentration of 210±1.51 µg/ml of extract. Gallic acid used as a standard which gave a maximum absorbance of 0.92 nm was observed at a concentration of 100 µg/ml and flavonoid observed in this plant 46±3.61 µg/g which is equivalent to 175 µg/ml of rutin standard. The antioxidant activity mainly due to the redox properties [31], which showed an important activity in adsorbing and neutralizing free radicals, entrapments of singlet and triplet oxygen, or oxidising peroxides. The results from this study suggested that phenolics are important components of these plants.

DPPH Free Radical Scavenging Activity: DPPH radical scavenging activity of *D. latifolia* bark of ethanolic extract compared with ascorbic acid. The *D. latifolia* bark extract had DPPH % inhibition activity was 92.10±1.10% at 1.5 mg/ml (Fig. 1) and IC_{50} was 0.17 mg/ml of the ethanolic extract. DPPH activity of antioxidants is thought to be due to the donating ability of hydrogen [32]. The DPPH scavenging activity of the extracts were significantly lower than those of ascorbic acid but it was evident that the extract did show the proton-donating ability and could serve as free radical inhibitors possibly as primary antioxidants. The solubility of the extract in different testing system has been reported to affect the capacity of extracts to react and entrap different radicals [33]. This study was showed that the capability of the extracts to different scavenging free radicals in different systems, indicating that they may be useful therapeutic agents for treating radical-related pathological damage.

Nitric Oxide Scavenging Activity: Nitrous oxide scavenging potential of *D. latifolia* bark extract might contain compounds able to inhibit nitric oxide and offers scientific evidence for the indigenous system in inflammatory condition. The nitric oxide scavenging activity of *D. latifolia* bark of ethanolic extract was showed 86.39±2.12% at 150 mg/ml (Fig. 2) and IC_{50} was 62.5 mg/ml. Nitric oxide showed a potent mediator in physiological process mainly smooth muscle relaxant,

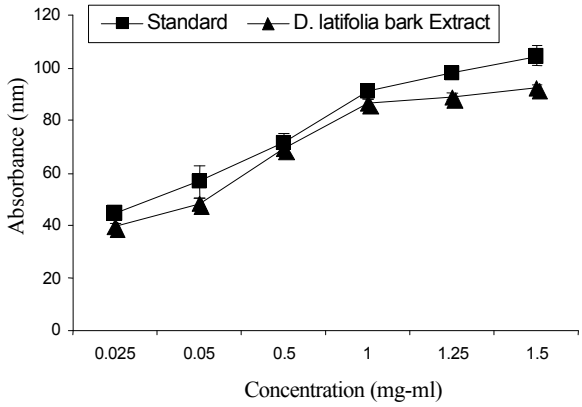

Fig. 1: DPPH free radical scavenging activity

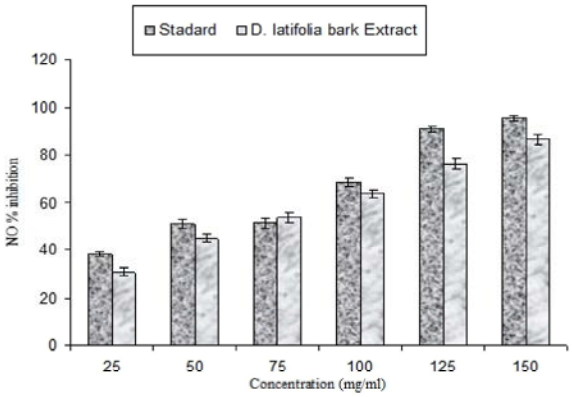

Fig. 2: Nitrous oxide scavenging activity

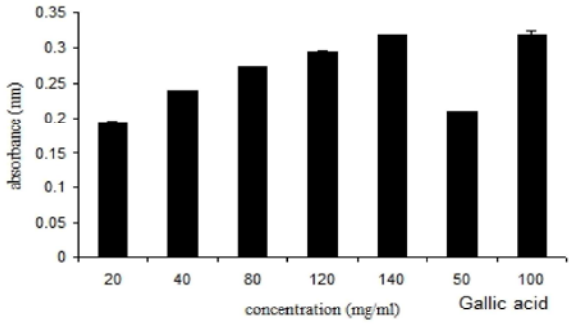

Fig. 3: Reduction potential scavenging activity

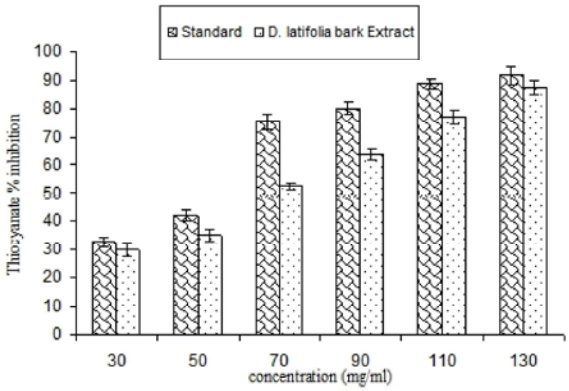

Fig. 4: Ferric thiocyanate scavenging activity

inhibition of platelet aggregation and regulation of toxicity through cell. It is a diffusible free radical which showed activity as an effectors molecule in biological systems including vasodilatation, antimicrobial and antitumor activities [34]. Although nitric oxide free radicals are involved in defence mechanism over production of these free radicals contributes to the pathogenesis of some inflammatory diseases [35].

Reducing Potential Activity: The reducing capacity of *D. latifolia* bark extract may serve as a significant indicator of its potential antioxidant activity [36]. For the measurement of the reductive activity, we investigated the Fe^{+3} to Fe^{+2} transformations in the presence of extracts. Fig. 3 had showed that the *D. latifolia* ethanolic bark extract maximum absorbance 0.318 nm was obtained at concentration 140 mg/ml 0f extract whereas gallic acid have the maximum absorbance 0.319 nm at concentration 100 mg/ml. The result indicated that the bark extract might contain compounds able to inhibit nitric oxide and offers scientific evidence for the indigenous system in inflammatory condition. The activity of antioxidants has been indicate to various mechanisms such as inhibition of chain initiation, binding of ion catalysts, decomposition of peroxides, reductive capacity and radical scavenging [37].

Ferric Thiocyanate Scavenging Activity: Ferric thiocyanate method measures the amount of peroxide value in the beginning of the lipid per oxidation. The percentage inhibitory potential of *D. latifolia* ethanolic bark extract had maximum 87.22±2.47% at 130 mg/ml concentration (Fig. 4) and IC_{50} was 72 mg/ml. The reaction between ferrous chloride and peroxide molecule to produced ferric chloride, which is reacting with ammonium thiocyanate to form ferric thiocyanate reddish colour pigment, indicates that the *D. latifolia* bark extract have antioxidant scavenging activity. The changes in absorbance of extract observed the reduction of peroxide at the initial stages of linoleic acid oxidation [38].

CONCLUSION

The present study showed that the antioxidant activities of the extracts of *D. latifolia* bark showed approximate similar activity as those of the standard drugs used in this experiment; the present results indicate that

the *D. latifolia* bark extracts possess antioxidant properties and could serve as free radical scavenging activity, acting possibly as primary antioxidants. *D. latifolia* bark extract showed maximum inhibitory concentration than other research on antioxidant scavenging potential. This study has to some extent validated the medicinal potential of the root extract of *D. latifolia.*

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