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Antioxidant Activity of *Dalbergia Melanoxylon* Bark Extract

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Abstract:

The bark extract of *Dalbergia melanoxylon* (Fabaceae) was assessed for its antioxidant activity by *in-vitro* methods. This activity was evaluated by using various methods like hydrogen peroxide scavenging activity, Nitric oxide scavenging activity, DPPH radical scavenging activity and reducing power assay. Quantitative analysis of antioxidative components like total phenolic content, total flavonoid content and antioxidant capacity were estimated using spectrophotometric methods. The obtained results from this method are compared with ascorbic acid acts as a standard antioxidant drug. From the results it can concluded that the plant extract contains flavonoids and related polyphenols. And the extract has shown good scavenging activity in *in-vitro* methods. So the plant extract may be responsible for antioxidant activity.

1. Introduction

From ancient times, the plants have been used as rich source of effective and safe medicines. Plants produce significant amount of antioxidants to prevent the oxidative stress caused by photons and oxygen, they represent a potential source of new compounds with antioxidant activity.

Bioactive compounds are important elements that regulate the therapeutic effects of a plant. Most important bioactive phytochemical constituents are alkaloids, glycosides, flavonoids, tannins, saponins, essential oils, lactones and terpenoids. Phenols are widely distributed in medicinal plants and they have multiple biological effects, including antioxidant, anti-inflammatory, anticancer, anti-viral, anti-bacterial and cardio-protective activity.

Dalbergia (Fabaceae) is a large genus of small to medium size trees, shrubs and lianas, with a wide distribution in southern asia (Vasudeva N et al., 2009). This genus has been shown to possess various pharmacological activities including anti giardial, antiplasmodial, antidiarrhoeal, analgesic, antipyretic, anti-inflammatory, antimicrobial, antiulcerogenic, larvicidal, mosquito repellent, anti-fertility and cancer chemopreventive activities (Ravi P et al., 1989).

This plant contains chemical constituents such as sterols, anthraquinones, isoflavones, isoflavanones, neoflavones, cinnamyl esters and triterpenes. 3-Hydroxy isoflavanones are among the rare flavonoids (Ahluwalia et al., 1963).

D melanoxylon has been used traditionally for cleaning wounds. The roots are used to alleviate abdominal pains, antihelmintic and also acts as a part in preparation for the treatment of gonorrhoea. The leaves are boiled in soup and drung to relieve pains in joints. The bark of the plant produces antimicrobial activity (Sharma A et al., 1980).

2. Material and methods

2.1 Preparation of plant extract

The dried coarsely powdered bark of *Dalbergia melanoxylon* was successively extracted using a soxhlet apparatus with solvents of increasing polarity such as hexane, ethyl acetate and methanol at 60-70°C for 18 hours. The obtained extracts were redistilled and concentrated under vacuum evaporator. This crude extract was used for testing the activity

2.2 Chemicals

Ascorbic acid, $Alcl_3$ were purchased from Finer chemicals, Ahmedabad. EDTA, Folin-cio calteu reagent from Merck specialties, Mumbai. Sodium nitroprusside, Potassium dihydrogen ortho phosphate from Molychem,

Mumbai. N-1-Napthyl Ethylene Diamine Dihydrochloride from LOBA chemie pvt LTD. Galli acid purchased from Quali Kems Fine chem pvt Ltd. 1,1-diphenyl-2-picryl hydrazyl (DPPH) was obtained from sigma Aldrich. All chemicals and solvents were used of analytical grade available commercially.

2.3 Estimation of total flavonoids

Estimation of total flavonoids was done by using Aluminium chloride colorimetric method. The plant extract (0.5ml of 1:10g ml^{-1}) in methanol was separately mixed with 1.5ml of methanol, 0.1ml of 10% aluminium chloride, 0.1ml of 1M potassium acetate and 2.8ml distilled water. It was kept at room temperature for 30 min. The absorbance was measured at 415nm with a UV/visible spectrophotometer. The calibration curve was prepared by using Quercetin solution as a standard. It was prepared at concentration of 12.5 to 100g ml^{-1} in methanol (Akinpelu DA et al., 2010, Ghosh T et al., 2009).

2.4 Determination of total phenolic content

The total phenolic content was determined by the method of Susanta et al., 2006 which exactly 0.5ml of the extract was transferred to a 100ml Erlenmeyer flask and final volume was adjusted to 46ml by addition of distilled water. 1ml of Folin-ciocalteau reactive solution was added and incubated at room temperature for 3 min. 3ml of 2% sodium carbonate solution was added and the mixture was shaken on a shaker for 2 hr at room temperature. The absorbance was measured at 760nm (Susanta et al., 2006).

The calibration curve was prepared by using gallic acid as a standard, The phenolic compound content was expressed as gallic acid equivalent (Aqil F et al., 2006).

2.5 Hydrogen peroxide scavenging activity

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (SH) groups. Plant extract prepared in various concentrations (20-100µg/ml) was mixed with 0.6ml of 4mM H_2O_2 solution prepared in phosphate buffer (0.1M pH 7.4) and incubated for 10min. The absorbance of the solution was taken at 230nm against blank solution containing the H_2O_2 without plant extract (Chacha M et al., 2005). All the analysis was performed in triplicate and results were averaged. Ascorbic acid used as a positive control treated in the same way with H_2O_2 solution. The percentage inhibition was measured by comparing the absorbance of control and test (Indu PK et al., 2006).

$$H_2O_2 \text{ scavenging activity} = \frac{\text{Acontrol-Atest}}{\text{Acontrol}} \times 100$$

Where,
Acontrol= Absorbance of control
Atest= Absorbance of extract sample

2.6 Nitric oxide scavenging activity

NO is an important chemical mediator generated by endothelial cells, macrophages etc., Excess NO concentration is associated with several diseases. Oxygen reacts with excess NO thus generating nitrate and peroxynitrite anions, which act as free radicals. In the present study the extract competes with oxygen to react with NO and thus inhibits the generation of the anions. Sodium nitroprusside (SNP) in aqueous solution at physiological pH spontaneously generates NO which interacts with oxygen to produce nitrite ions that can be estimated by the Griess reagent. SNP(10mM) in phosphate buffer saline was mixed with 1ml of different concentration of extracts (20-100µg/ml) and incubated at 25°c for 150 min. To 1ml of incubated solution, 1ml of Griess reagent (1% sulphanilamide, 0.1% naphthylethylenediamine di chloride and 3% phosphoric acid) was added. The absorbance of chromophores formed durig the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine dichloride was read at 546nm. All the analysis were performed in triplicate and results were averaged and ascorbic acid used as a positive control treated in the same way with Griess reagent. The percentage inhibition of nitric oxide generated was measured by comparing the absorbance of control and test (Kuroda M et al., 2009, Stankovic, 2011).

$$\text{Nitric oxide scavenged} = \frac{\text{Acontrol-Atest}}{\text{Acontrol}} \times 100$$

Where,
Acontrol= Absorbance of control
Atest= Absorbance of extract sample

2.7 Reducing power assay

The reducing power was determined according to the method of Oyaizu (1986). Different concentrations of extracts (20-100µg/ml) were mixed with 2.5ml of 0.2M phosphate buffer (pH 6.6) and 2.5ml of 10% trichoroacetic acid (W/V) was added, the mixture was centrifuged at 3000rpm for 10min. The upper layer (2.5ml) was mixed with 2.5ml distilled water, 0.5ml of 0.1% of ferric chloride and the absorbance was measured at 700nm, higher absorbance indicates higher reducing power. The assays were carried out in triplicate and the results were averaged. Ascorbic acid was used as standard (Nagulendran et al., 2007).

2.8 DPPH radical scavenging activity

The free radical scavenging activity of the different fractions of extract was measured using DPPH, employing the method of Blois (1958). 1ml of extract and the reference compound in various concentrations (10,20,50,75 and 100µg/ml) were added to 1ml of 0.1mM solution of DPPH in methanol was used as control, whereas ascorbic acid was used as a reference material. All tests were performed in triplicate.

Percentage inhibition was calculated using equation (Kaldhone et al., 2009).

$$\text{Percentage inhibition} = \frac{\text{Acontrol-Atest}}{\text{Acontrol}} \times 100$$

Where,
Acontrol= Absorbance of control
Atest= Absorbance of extract sample

2. Results and discussion

The present investigation shows the antioxidant capacity of various concentration of DMME.

3.1 Total flavonoid content

Total flavonoid content of DMME (1mg) equivalent to 5.9µg respectively of quercetin was detected. Standard graph of quercetin depicted in table 1, figure 1.

3.2 Total phenolic content

The total phenolic content of DMME (1mg) equivalent to 42.8µg respectively of gallic acid was detected. Standard graph of gallic acid depicted in table 2, figure 2.

3.3 Hydrogen peroxide scavenging activity

The scavenging activity of hydrogen peroxide by DMME exhibited lower than ascorbic acid.
IC50 value of ascorbic acid and DMME was found to be 27.74, 33.14µg/ml respectively.
Results were depicted in table 3, figure 3.

3.4 Nitric oxide scavenging activity

IC50 values of ascorbic acid, DMME was found to be 19.47, 30.46µg/ml respectively.
Results were depicted in table 4, Figure 4.

3.5 Reductive ability

The reducing power increased as the extract concentration increased, reductive ability of DMME was more than that of standard i.e., ascorbic acid. Absorbance of different concentrations of DMME and standard were depicted in table 5, figure 5.

3.6 DPPH radical scavenging activity

DPPH is a relatively stable free radical and the assay determines the ability of extract to reduce DPPH radical to the corresponding hydrazine by converting the unpaired electrons to paired once. In the present study dose dependent inhibition of DPPH radical indicates that extract cause reduction of DPPH radical. IC50 values of ascorbic acid, DMME was found to be 10.22,13.35µg/ml respectively. Percentage scavenging of DPPH radical examined at different concentrations DMME was depicted in table 6, figure6.

Table 1: Standard graph of Quercetin

Concentration(µg/ml)	Absorbance
0	0
2	0.092
4	0.220
6	0.320
8	0.450
10	0.60
1 mg extract	0.310

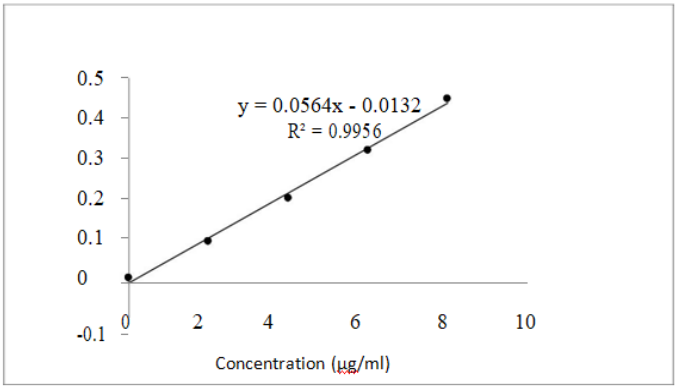

Figure 1: Standard graph of Quercetin

Table 2: Standard graph of Gallic acid

Concentration(µg/ml)	Absorbance
0	0
20	0.012
40	0.039
60	0.068
80	0.098
100	0.132
1mg extract	0.049

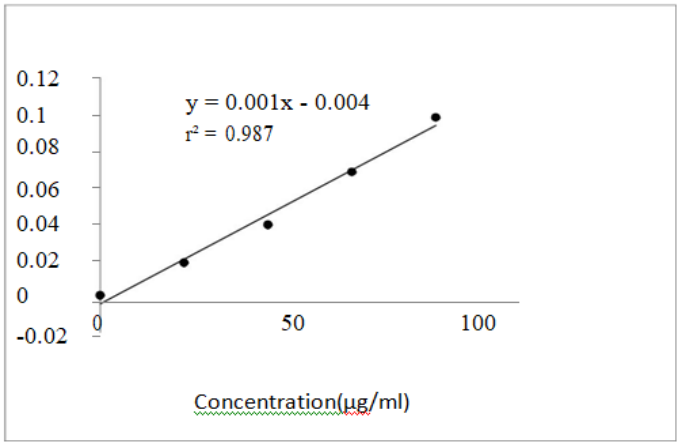

Figure 2: Standard graph of Gallic acid

Table 3: Effect of DMME on hydrogen free radicals

Concentration	Percentage inhibition	
(µg/ml)	Ascorbic acid	DMME
	0	0
	16.46 ± 0.351	12.66 ± 0.152
0	30.53 ± 0.455	25.136 ± 0.025
10	37.43 ± 0.017	30.19 ± 0.121
25	43.61± 0.060	36.25 ± 0.232
50	52.07 ± 0.020	48.33 ± 0.325
75	27.74	33.14

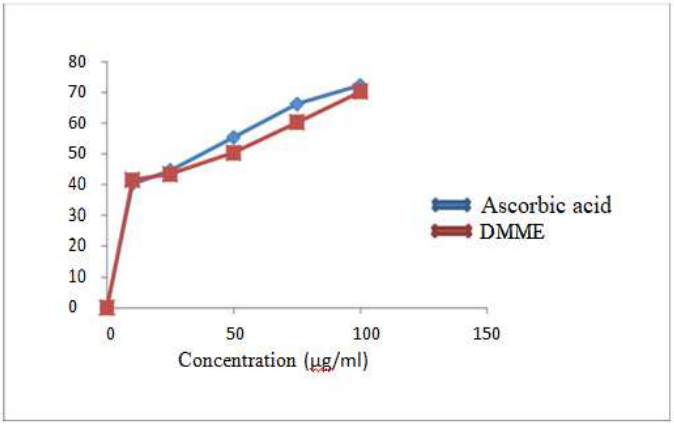

Figure 3: Effect of DMME on hydrogen peroxide radicals

Table 4: Effect of DMME on nitric oxide radicals

Concentration(µg/ml)	Percentage inhibition	
	Ascorbic acid	DMME
0	0	
20	39.3 ± 0.173	28.53 ± 0.104
40	42.06 ± 0.061	35.60 ± 0.136
60	50.75 ± 0.188	41.84 ±0.068
80	55.44 ± 0.037	48.27 ± 0.045
100	60.45 ± 0.030	58.22 ± 0.100
IC_{50}(µg/ml)	19.47	30.46

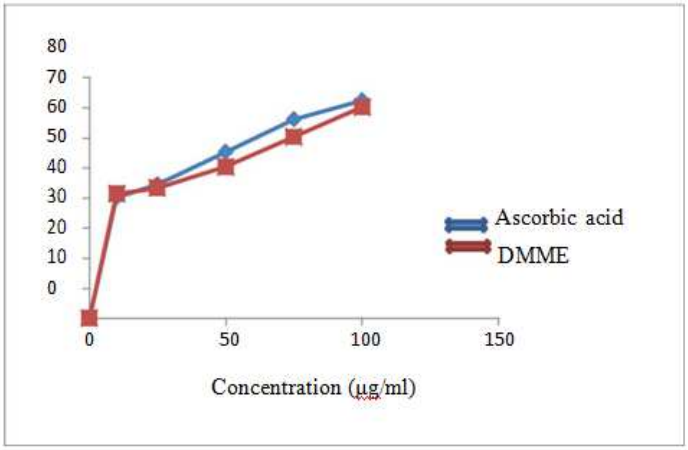

Figure 4: Effect of DMME on nitric oxide radicals

Table 5: Reductive ability of DMME

Concentration(µg/ml)	Percentage inhibition	
	Ascorbic acid	DMME
0	0	0
10	0.025 ± 0.002	0.119 ± 0.005
25	0.036 ± 0.002	0.136 ± 0.002
50	0.044 ± 0.002	0.165 ± 0.002
75	0.107 ± 0.002	0.173 ± 0.001
100	0.153 ± 0.001	0.184 ± 0.003

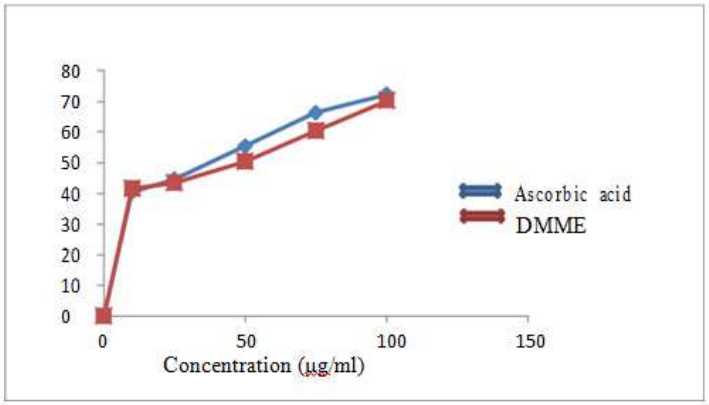

Figure 5: Reductive ability of DMME

Table 6: Effect of DMME on DPPH radicals

Concentration(µg/ml)	Percentage inhibition	
	Ascorbic acid	DMME
0	0	0
10	40.27 ± 0.075	41.53 ± 0.152
25	44.60 ± 0.045	43.43 ± 0.25
50	55.40 ± 0.020	50.39 ± 0.045
75	66.24 ± 0.045	60.34 ± 0.09
100	72.33 ± 0.125	70.22 ± 0.026
IC_{50}(µg/ml)	13.35	10.22

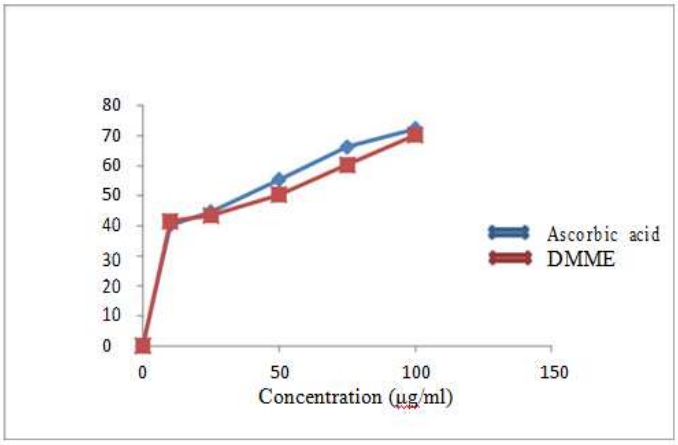

Figure 6: Effect of DMME on DPPH radicals

4. Summary and conclusion

Antioxidant activity was performed by using various methods including nitric oxide radical scavenging activity, DPPH radical scavenging activity, hydrogen peroxide radical scavenging and reducing power methods. The reductive ability of DMME was more than that of standard i.e., ascorbic acid. The concentration of total phenolic content and concentration of total flavonoid content present in the plant bark was also measured by using standard procedures.

The IC50 values of standard, DMME for nitric oxide radical scavenging was found to be 19.47, 30.46µg/ml, DPPH radical scavenging activity was found to be 10.22, 13.35µg/ml and hydrogen peroxide radical scavenging activity was found to be 27.74, 33.14µg/ml respectively.

Based on the above observations it can be concluded that the extract possess antioxidant activity. Administration of *Dalbergia melanoxylon* produces a significant stimulation of free radical scavenging activities and also it can be concluded that the antioxidant property of extract was dose dependent.

Conflict of interest

None declared

5. References

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