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Phytochemical Investigation and Evaluation of Antioxidant Activity of *Dalbergia sissoo* Bark

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ABSTRACT

Medicinal plants play a pivotal role in the health care of ancient and modern cultures. Ayurveda, the Indian system of medicine mainly uses plant-based drugs or formulations to treat various Human ailments because they contain the components of therapeutic value. Reactive Oxygen species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide play a crucial role in the development of various ailments such as arthritis, carcinoma and Parkinson's disease. Free radicals produced in the body react with various biological molecules namely lipids, proteins and deoxyribonucleic acids resulting in the imbalance between oxidants and antioxidants. Even though our body is safeguarded by natural antioxidant defense, there is always a demand for antioxidants from natural sources. Antioxidants from plant materials terminate the action of free radicals thereby protecting the body from various diseases. Hence, the present study was aimed at measuring the relative antioxidant capacities of *Dalbergia sissoo* bark. Antioxidant and free radical scavenging activities were performed by using Nitric oxide radical scavenging, DPPH radical scavenging activity, Hydrogen peroxide radical scavenging and reducing power methods. The reductive ability of DSME was more than that of standard i.e., ascorbic acid total phenolic contents, total flavonoid content was also measured by using standard procedures. Based on the above observations it can be concluded that the extract possesses potent antioxidant activities. *Dalbergia sissoo* has an ability to combat various diseases having mechanism of oxidative stress. Further research enhances the use of this herb to treat various diseases.

INTRODUCTION

Herbal drugs play an important role in curing diseases throughout the history of mankind. Now medicinal plants are recognized globally as important resources for all major systems of medicine, healthcare, nutraceuticals, phytochemicals and cosmetics. The study of medicinal plants has attracted many researchers, owing to the useful applications of plant for the treatment of various diseases in humans and animals. [1]

Currently, there has been an increased interest globally to identify antioxidant compounds that are pharmacologically potent and have low or no side effects for use in preventive medicine and food industry. The widespread use of traditional herbs and medicinal plants has been traced to the occurrence of natural products with medicinal properties.

Plant extracts and plant products such as flavonoids, carotenoids, vitamin-A and E and other polyphenolic constituents have been reported to be effective immunostimulants, radical scavengers and inhibitors. As 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.

Antioxidant refers to a compound that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions and which can thus prevent or repair damage done to the body's cells by oxygen. During the process of oxygen utilization in normal physiological and metabolic processes, approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals. All these radicals known as reactive oxygen species (ROS) exert oxidative stress toward the cells of human body. [2]

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Reactive oxygen species are constantly being generated in the body, as a result of the normal metabolic processes. Mitochondria, which consume more than 90% of the oxygen in aerobic living organisms, are the main source of ROS and free radicals. ROS can be classified into oxygen–centered radicals such as superoxide anion, hydroxyl radical, alkoxyl radical, peroxyl radical and oxygen-centered non radical derivatives such as hydrogen peroxide (H_2O_2) and

singlet oxygen. Other common reactive species are nitrogen species such as nitric oxide, nitric dioxide, and peroxynitrite. ROS cause lipid oxidation, protein oxidation, DNA strand breaks, and modulation of gene expression. ROS are involved in many diseases such as atherosclerosis, cancer, stroke, asthma, arthritis and other age related diseases.

The study was aimed at investigating the effect of Antioxidant property of *Dalbergia sissoo* bark.

2. MATERIALS AND METHODS

Collection of plant material:

Dalbergia sissoo is a large genus of small to medium size tree. Found in local areas of Tirupati (Chittoor), Andhra Pradesh (A.P). Collected bark was dried and size reduced into powder with the help of laboratory mixer and sieved.

Preparation of Plant Extract:

In the present study, the extracts were prepared by 200gm of finely powdered bark was extracted with different solvents in their order of increasing polarity *viz*., petroleum ether (60-80^{o}C), chloroform, ethyl acetate, methanol and water in soxhlet apparatus for 8 hrs. The extracts were collected evaporated under reduced pressure at low temperature (30^{o}C), until soft mass obtained and dried in a desiccator. The color, consistency and percentage yields of each extracts were recorded.

2.1. Qualitative Phytochemical Screening:

The methanolic extract of the bark of *Dalbergia sissoo* was subjected to different qualitative phytochemical screening tests for detection and establishment of the nature of chemical composition.

2.2. TLC profile of phytoconstituents:

TLC studies are useful in resolving various phytoconstituents present in a crude extract. The solvent systems were selected by trial and error and based on previous experimental reports since herbal extracts contain a wide variety of chemicals with varied physicochemical properties. After development of TLC plates, the location of various compounds was detected by observation under UV light before and invisible light after spraying with suitable reagents.

2.3. *In-vitro* Antioxidant activity:

2.3.1. Estimation of total flavonoids:

Aluminum chloride colorimetric method was used for flavonoids determination. The plant extract (0.5 ml of 1:10 g ml^{-1}) in methanol was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. It remained at room temperature 30 min. The absorbance of the reaction mixture was measured at 415 nm with a UV/visible spectrophotometer. The calibration curve was prepared by preparing Quercetin solution at concentration 12.5 to 100 g ml^{-1} in methanol.[3]

2.3.2. Determination of total Phenolic Content:

The amount of total phenolic content of extract was determined by the method of exactly 0.5 ml of the extract was transferred to a 100 ml Erlenmeyer flask and final volume was adjusted to 46 ml by addition of distilled water. 1ml of Folin-ciocalteu reactive solution was added and incubated at room temperature for 3 min. 3 ml 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 760 nm.

HUMAN

Gallic acid was used as the standard for a calibration curve. The phenolic compound content was expressed as gallic acid equivalent. [4]

2.3.3. 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 (20-100 µg/ml) was mixed with 0.6 ml of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm against blank solution containing the H_2O_2 without plant extract. All the analysis was performed in triplicate and results were averaged, and 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. [3]

$$H_2O_2 \text{ scavenging activity} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where,

$A_{control}$ = Absorbance of control reaction and

A_{test} = Absorbance in the presence of the samples of extracts

2.3.4. Nitric Oxide Scavenging Activity:

In the present study, the extract competes with oxygen to react with NO and thus inhibits the generation of the anions. Using 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 use of Griess reagent. SNP (10mM) in phosphate buffer saline (PBS) was mixed with 1 ml of different concentration of extracts (20-100µg/ml) and incubated at 25^{o}C for 150 minutes. To 1 ml of incubated solution, 1 ml of Griess reagent (1% sulphanilamide, 0.1% naphthyl ethylenediamine dichloride and 3% phosphoric acid) was added. The absorbance of the chromophores formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthyl ethylenediamine dichloride was read at 546 nm. All the analysis was 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. [5]

$$\text{Nitric oxide scavenged} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where,

$A_{control}$ = Absorbance of control reaction and

A_{test} = Absorbance in the presence of the samples of extracts

2.3.5. Reducing Power Assay:

The reducing power was determined according to the method of Oyaizu (1986). Various concentrations of extracts (20-100 µg/ml) were mixed with 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5 ml of 10% trichloroacetic acid (W/V) was added, the mixture was centrifuged at 3000 rpm for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml distilled

water and 0.5 ml of 0.1% of ferric chloride, and the absorbance was measured at 700 nm, higher absorbance indicates higher reducing power. The assays were carried out in triplicate and the results were averaged. Ascorbic acid was used as standard. [6]

2.3.6. 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). One ml of extract and the reference compound in various concentrations (10, 20, 50, 75 and 100 µg/ml) were added to 1 ml of 0.1 mM solution of DPPH in methanol was used as control, whereas ascorbic acid was used as a reference material. All tests were performed in triplicate. Percent inhibition was calculated using equation. [7]

$$\text{Percentage inhibition} = \frac{A_{conrol} - A_{test}}{A_{control}} \times 100$$

$A_{control}$ = Absorbance of control reaction and

A_{test} = Absorbance of sample

3. RESULTS:

Table 1: Percentage yield and physical appearance of different extracts

Sr. No.	Extract	% Dry weight	Colour	Consistency
1.	Petroleum ether(60- 80^{o}C)	0.37%	Yellowish Brown	Resinous
2.	Chloroform(60^{o}C)	1.03%	Brown	Solid
3.	Ethyl acetate(77^{o}C)	0.95%	Brown	Solid
4.	Methanol(64^{o}C)	10.75%	Brown	Resinous
5.	Water(100^{o}C)	2.05%	Brown	Resinous

Table 2: Phytochemical evaluation of *Dalbergia sissoo* bark

Name of the test	Methanol extract
Carbohydrates	
Molisch's test	+
Fehling's test	+
Benedict's test	+
Barfoed's test	_
Proteins, Amino acids	
Millon's test	_
Biuret test	_
Ninhydrin test	_
Flavonoids	
Shinoda test	+
Alkaline test	+
Phenolics, Tannins	
Ferric chloride test	+
Lead acetate test	+
Bromine water test	+
Iodine test	+
Alkaloids	
Dragendorff's test	+
Mayer's test	+
Wagner's test	+
Hager's test	+
Steroids-terpenoids	
Salkowski test	+
Liebermann-burchard test	+
Glycosides	
Legal test	+
Borntrager's test	+
Foam test	+

present (+); (-) absent

Table 3: TLC Analysis for Various Phytoconstituents

Test	Solvent	Detection
Alkaloids	Ethyl acetate(EA)–methanol –water (100:13.5:10)	Dragendorff's reagent
Terpenoids	Toluene –chloroform- methanol (20:2:5)	Antimony chloride in chloroform
Flavonoids	Chloroform-Glacial Acetic Acid (GAA)-water (90:45:6)	Detection under UV 256 nm (5% Ethanolic PEG reagent)
Saponins	Chloroform- Glacial Acetic Acid (GAA)-methanol-water (64:32:12:8)	Vanillin- sulphuric acid reagent
Steroids	Chloroform-methanol-water (70:30:4)	Vanillin- sulphuric acid reagent
Glycosides	Ethylacetate(EA)-formic acid(FA)- Glacial Acetic Acid (GAA)- water (100:11:11:26)	5% Ethanolic PEG reagent (detection under UV 256 nm)

Table 4: TLC profile of methanolic extract of *Dalbergia sissoo* bark

Phytoconstituents	Methanolic extract
Alkaloids	+
Flavonoids	+
Glycosides	+
Saponins	+
Steroids	+
Terpenoids	+

Figures corresponding to Thin Layer Chromatography (TLC) of *Dalbergia sissoo* bark

Chloroform-GAA-water

(90:45:6)

Toluene -chloroform-methanol

(20:2:5)

Chloroform-GAA-methanol-water

(64:32:12:8)

EA-methanol-water

(100:13.5:10)

EA-FA-GAA-water

(100:11:11:26)

Estimation of Total Flavonoids:

Table 5: 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

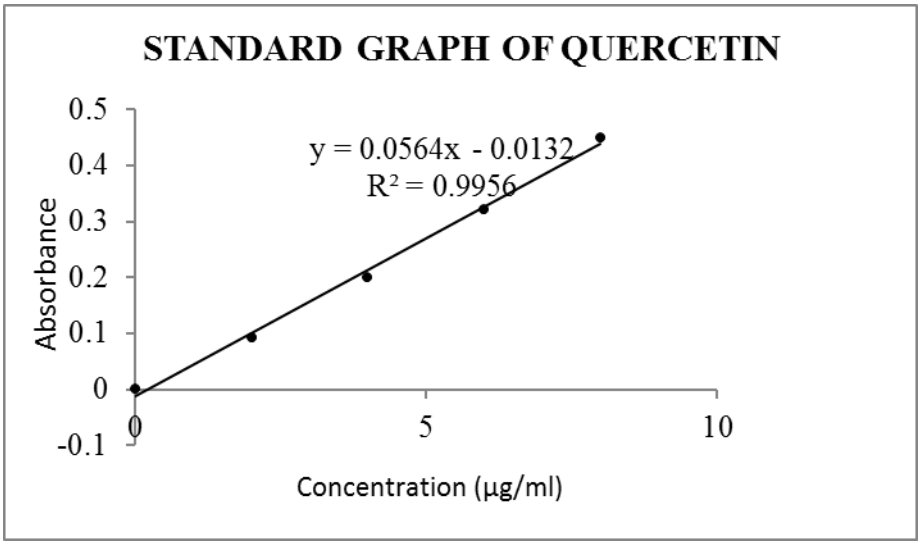

Figure 1: Standard graph of Quercetin

Determination of Total Phenolic content

Table 6: 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
1 mg extract	0.049

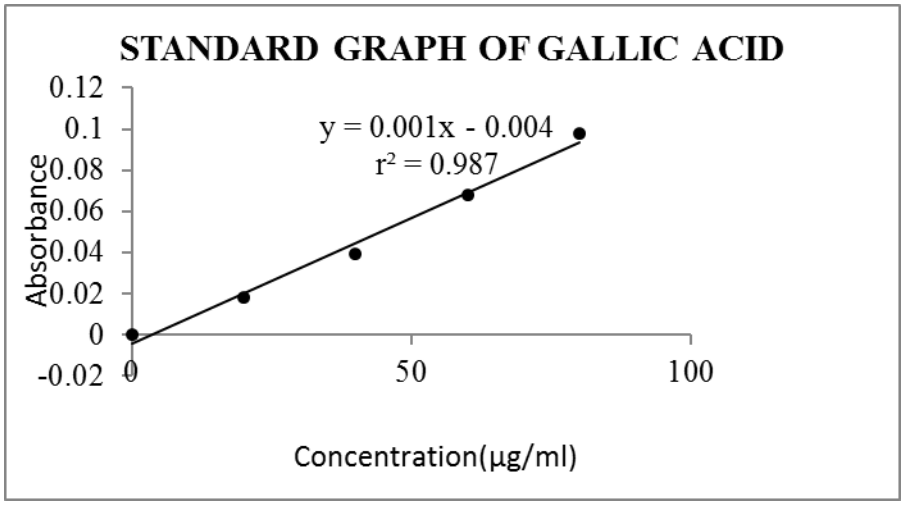

Figure 2: Standard graph of Gallic acid

***In-vitro* Antioxidant activity:**

Table 7: Effect of DSME on Hydrogen peroxide radicals

Concentration (µg/ml)	Percentage inhibition	
	Ascorbic acid	DSME
0	0	0
10	16.46 ± 0.351	12.66 ± 0.152
25	30.53 ± 0.455	25.136 ± 0.025
50	37.43 ± 0.017	30.19 ± 0.121
75	43.61± 0.060	36.25 ± 0.232
100	52.07 ± 0.020	48.33 ± 0.325
IC_{50} (µg/ml)	27.74	33.14

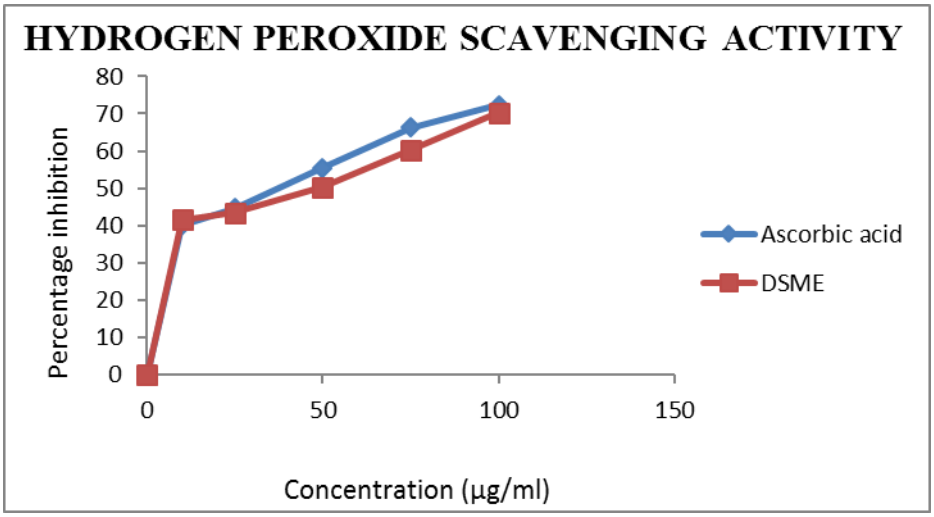

Figure 3: Effect of DSME on Hyrogen peroxide radicals

Table 8: Effect of DSME on Nitric oxide radicals

Concentration (µg/ml)	Percentage inhibition	
	Ascorbic acid	DSME
0	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

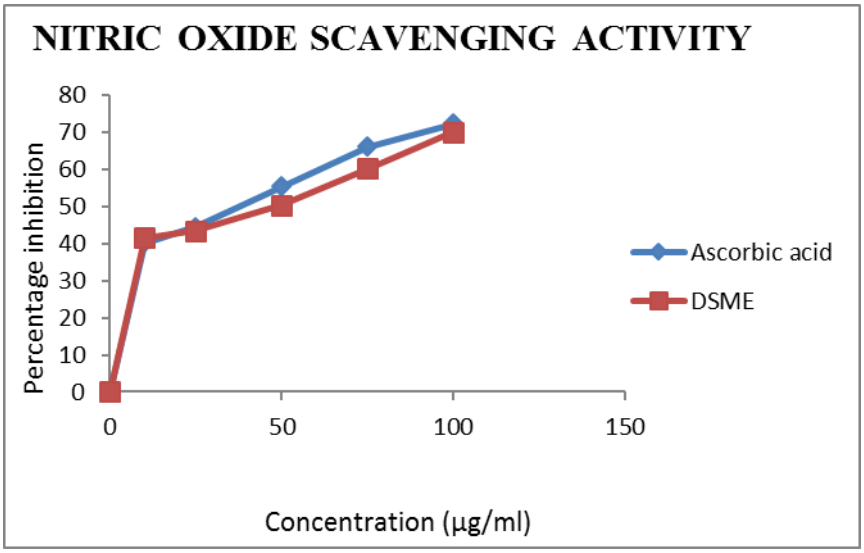

Figure 4: Effect of DSME on Nitric oxide radicals

Table 9: Reductive ability of DSME

Concentration (µg/ml)	Absorbance	
	Ascorbic acid	DSME
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

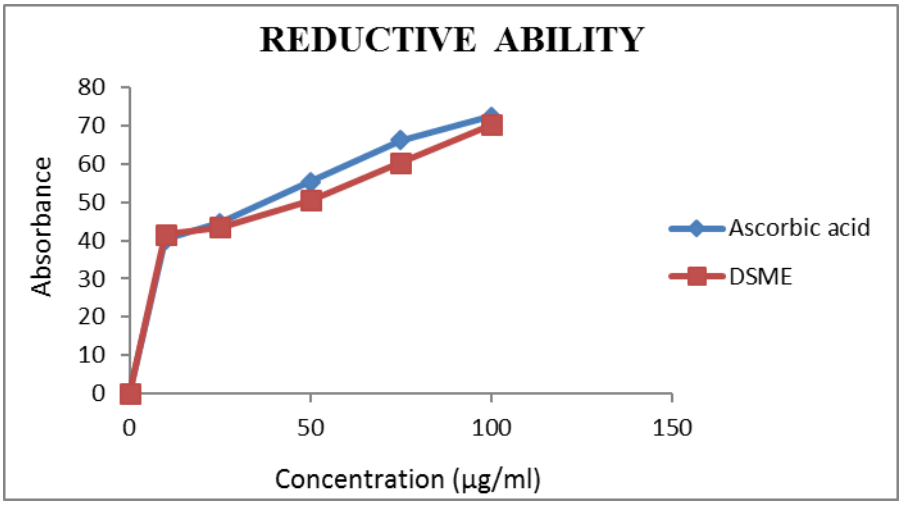

Figure 5: Reductive ability of DSME

Table 10: Effect of DSME on DPPH radicals

Concentration (µg/ml)	Percentage inhibition	
	Ascorbic acid	DSME
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

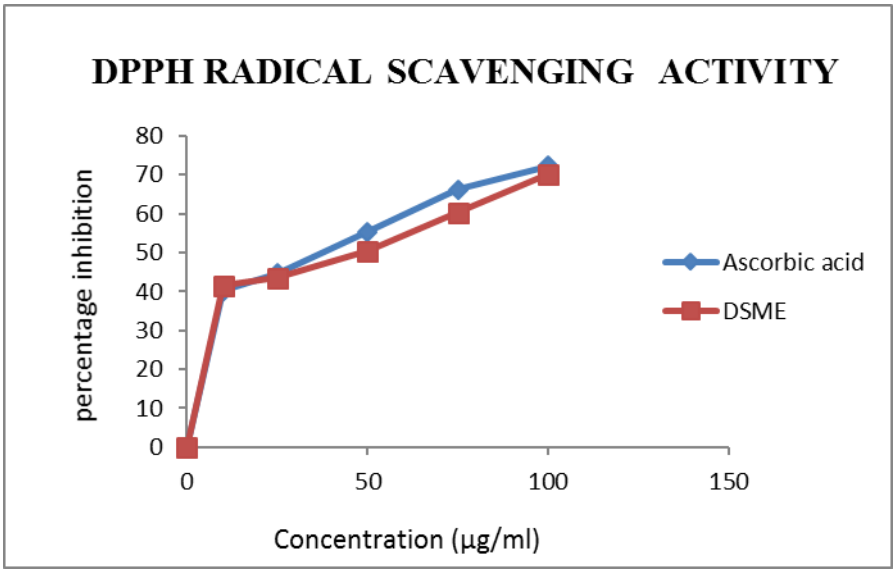

Figure 6: Effect of DSME on DPPH radicals

DISCUSSION

The bark of *Dalbergia sissoo was* collected, dried, powdered and extracted using standard procedure and the extract was subjected to physicochemical investigations in which we carried out phytochemical tests, thin layer chromatography for identification of different phytoconstituents.

Antioxidant and free radical scavenging activities were performed by using Nitric oxide radical scavenging, DPPH radical scavenging activity, Hydrogen peroxide radical scavenging and reducing power methods. The reductive ability of DSME was more than that of standard i.e., ascorbic acid total phenolic contents, total flavonoid content was also measured by using standard procedures.

The IC_{50} values of standard, DSME for nitric oxide radical scavenging was found to be 19.47, 30.46 µg/ml, DPPH radical scavenging activity was found to be 10.22, 13.35 µg/ml and hydrogen peroxide radical scavenging activity was found to be 27.74, 33.14 µg/ml respectively.

Based on the above observations it can be concluded that the extract possesses potent antioxidant activities. *Dalbergia sissoo* has an ability to combat various diseases having mechanism of oxidative stress. Further research enhances the use of this herb to treat various diseases.

CONCLUSION

The results expressed in this study are the first information on the antioxidant activities of *Dalbergia sissoo* bark. It has shown free radical scavenging activity when tested in different models. The scavenging effect on DPPH and superoxide radicals represents the fraction direct radical scavenging activity. It is well documented that free radicals are responsible for several diseases. The present result confirms the free radical scavenging activity of the plant which can be accounted for the traditional uses of the plant in treating several diseases.

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