INVITRO ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENT IN EXTRACTS FROM THE BARK OF *Dalbergia sissoo* Roxb.

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Abstract

Extract of bark of *Dalbergia sissoo* Roxb (Fabaceae) was assessed for its antioxidant activity by invitro methods. Antioxidant activity was studied using hydrogen peroxide scavenging activity, reducing power assay. Antioxidant activities compared with ascorbic acid as standard antioxidant .Quantitative analysis of antioxidative components like total phenolic content, total flavonoid content, total antioxidant capacity were estimated using spectrophotometric methods. Results showed that extracts exhibited significant antioxidant activity. From the results, it is concluded that flavonoids, and related polyphenols present in Ddalbergia sissoo extract may be responsible for the antioxidant activity.

Key words*: Dalbergia sissoo,* antioxidant, total phenolic content, total antioxidant capacity.

INTRODUCTION

Plants have always been a part of medical science from the beginning of human civilization to the present modern world of synthetic medicines. Even in the presence of variety of effective synthetic drugs, use of medicinal plants for maintaining human health has acquired a lot of importance in present era [1]. There is a global interest in non-synthetic, natural drugs derived from plant sources, because of low cost, non toxic nature and availability. Many plants with antioxidant potential possess flavonoids and Phenolic compounds [2]. Free radical reactions have been implicated in the pathology of many human disease including atheroscelorosis, ischemic heart disease, the ageing process, inflammation, diabetes and other conditions [3].

Dalbergia sissoo Roxb. (Fabaceae) has been used mainly as aphrodisiac, abortifacient, expectorant, anthelmintic and antipyretic. It is also used in conditions like ucers, leucoderma, dyspepsia and syphilis [4-6]. The alcoholic extract of the green branches has been reported to possess a dose dependent inhibitory effect on motility of isolated rabbit duodeneum, pronounced bronchodilation, estorgens like activities, significant antipyretic and analgesic activities. Significant anti-inflammatory activity in acute, sub acute and chronic models of inflammation has also been reported [7-8.] The various chemical constituents , which have been isolated from *Dalbergia sissoo* plant (stem bark)includes 4-phenyl coumarins such as dalbergin [9], methyl dalbergin, allyl quinone such as dalbergenone[10] and 4-phenylchromone namely dalbergichromene[11]. Many reports are available, which answered positive to antioxidant potential of the compounds possessing coumarins, chromone and flavones nucleus [12-14]. Thus the present study is aimed on exploring traditional Indian plant Dalbergia sissoo for antioxidant and free radical scavenging properties. Further an attempt has also been made to find the correlation between total Phenolic content of the extracts and antioxidant activity.

2.MATERIALS AND METHODS

Plant collection and Authentication

The fresh bark of the plant *Dalbergia sissoo* was collected from Komaneri, Tuticorin district, Tamil Nadu, India and it was botanically identified and authenticated by Dr.V.Chelladurai, Research Officer-Botany (Scientist-C), Central Council for Research in Ayurveda and Siddha, Government of India. The barks were pulverized to coarse powder using mechanical grinder. The sieved powder was used for evaluation and extraction purpose.

Preparation of plant extract

The dried coarsely powdered plant material of bark of *Dalbergia sissoo* Roxb were successively extracted using a soxhlet apparatus with solvents of increasing polarity such as Hexane, Ethyl acetate, Ethanol at 60-70°C for 18 hours. All the extracts were redistilled and concentrated under rotary vacuum evaporator. This crude extract was used for further investigation for potential antioxidant properties.

Total phenolic content (Folin-ciocalteu assay) [15]

Total phenolic content of extract were determined using Folin-ciocalteu assay. Briefly 0.5ml extract solutions were mixed with 2.5ml of 10 fold diluted folin-ciocalteu reagent and 2.5ml of 7.5% sodium carbonate (NA_2CO_3). After incubation at 40°C for 30 minutes, the absorbance of the reaction mixtures was measured at 765nm a spectrometer. Three replicates were made for each test sample. Gallic acid was a standard and the extract were expressed in milligram .

Total flavonoid content [15]

Total flavonoid contents was determined by aluminum chloride colorimetric method, using quercetin as a standard. Briefly, the test samples were individually dissolved in DMSO. Then, the sample solution (150µl) of 2% AlCl3. After 10 min of incubation at ambient temperature , the absorbance of the supernatant was measured at 435nm by using a spectrometer. Three replicates were made for each test sample. The total flavonoids content was expressed as quercetin equivalent in milligram per gram extract (mg QRT/ g extract).

ANTIOXIDANT ACTIVITY [16]

Reducing power Assay

The reducing power assay was determined according to the method of Oyaizu. Various concentrations of plant extracts were mixed with 1ml of 200mmol/l sodium phosphate buffers (pH6.6) and 1ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20mins. To this 1ml of 10%trichloroacetic acid(w/v) was added, the mixture was centrifuged at 2000 rpm for 10mins. The upper layer solution (2.5ml) was mixed with 2.5ml of deionised matter and 0.5ml of fresh ferric chloride (0.1%). The absorbance was measured at 700nm; a higher absorbance value indicates a higher reducing power.

Hydrogen peroxide scavenging assay

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of (Ruch). A solution of hydrogen peroxide (2mmol/l) was prepared in phosphate buffer (pH7.4). Various concentration of extracts were added to hydrogen peroxide at 230nm was determined after 10min against a blank solution containing phosphate buffer without hydrogen peroxide.

% scavenging activity = Abs (control)-Abs (standard)/ Abs control) x 100.

RESULTS AND DISCUSSION

Determination of total phenolic and flavonoid contents of the ethyl acetate, ethanolic extracts of Dalbergia sissoo were done by Folin-Ciocalteu colorimetric and AlCl3 methods, respectively. Total polyphenol contents were estimated with Folin-Ciocalteu colorimetric method. This reagent is a mixture of phosphotungstic and phoshomolybdic acids. It is reduced during the oxidation of phenols in a mixture of blue oxides of tungsten and molybdenum. The color produced, whose absorption maximum is between 700 and 750nm,is proporionall to the amount of polyphenols present in plant extracts. The total phenolic contents were reported as mg gallic acid equivalent per gram of dry extract. The ethyl acetate crude extract of Dalbergia Sissoo bark shown a high phenolic compounds compared to ethanolic extract, as presented in Table 1.

In AlCl3 colorimetric method, aluminium chloride forms stable complex with keto or the hydroxyl groups in the A or C ring of flavoinoids. The total Flavonoid content was reported as mg Quercetin equivalent per mg of dried extract. The results are presented in Table 2, show that the ethyl acetate extract of bark of Dalbergia sissoo contained high flavonoid content compared to ethanolic extract.

There are numerous antioxidant methods for evaluation of antioxidant activity. For in vitro antioxidant screening , DPPH, nitric oxide, hydrogen peroxide and reducing power assay are most commonly used. H_2O_2 is highky important because of its ability to penetrate biological membranes. H_2O_2 is highly important because of its ability to penetrate biological membranes. H_2O_2 itself is not very reactive, but it can sometimes be toxic to cell because of it may give rise to hydroxyl radical in the cells. The results showed that extracts of Dallbergia sissoo bark had an effective hydrogen peroxide scavenging activity.

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants. In this assay, the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of each compound .presence of reducers causes the conversion of ferricyanide

complex used in this method to ferrous form. The results Table 3. showed that extracts of Dallbergia sissoo bark had an effective reducing power activity.

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CONCLUSION

In this work, we studies the antioxidant activity of extracts of bark using two methods. The reducing power assay and hydrogen peroxide scavenging activity. The results obtained show that this plant contain high enough levels of phenolics and flavonoids compounds responsible for antioxidant activity.

Table 1. Quantitative estimation of Phenolic content

Concentration (µg/ml)	**Absorbance**
20	0.14
40	0.17
60	0.19
80	0.23
100	0.26
Ethyl acetate	0.22
Ethanol	0.18

Table 2 . Quantitaive estimation of Flavonoid content

Concentration (µg/ml)	**Absorbance**
2	0.06
4	0.10
6	0.13
8	0.15
10	0.19
Ethyl acetate	0.17
Ethanol	0.16

Fig 1. Calibration curve of Total Phenolic content

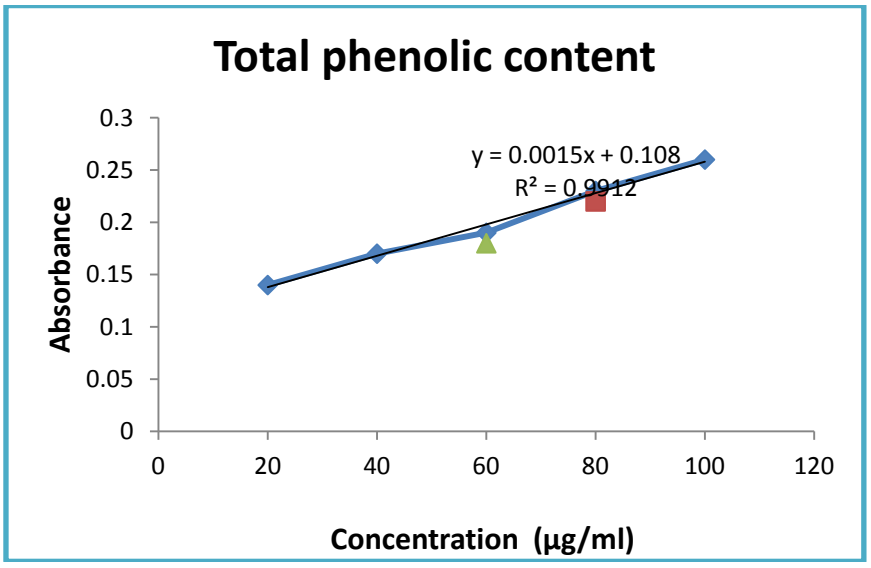


Fig.2 calibration curve of Total Flavonoid content

TABLE.3.REDUCING POWER ASSAY

S.No	Conc (µg/ml)	% Inhibtion		
		Standard	Ethyl acetate	Ethanol
1	10	14.12	13.94	10.04
2	20	26.56	24.54	22.12
3	30	42.13	40.03	33.13
4	40	60.23	56.39	53.45.
5	50	80.12	76.14	73.89

TABLE.4.HYDROGEN PEROXIDE SCAVENGING ASSAY

S.No	Conc (µg/ml)	% Inhibition		
		Std(Ascorbic acid)	Ethyl acetate	Ethanol
1	10	13.34	13.24	12.62
2	20	26.44	22.76	20.07
3	30	48.91	47.72	46.42
4	40	59.19	58.65	57.40
5	50	71.25	70.02	62.29

FIG.3. REDUCING POWER ASSAY

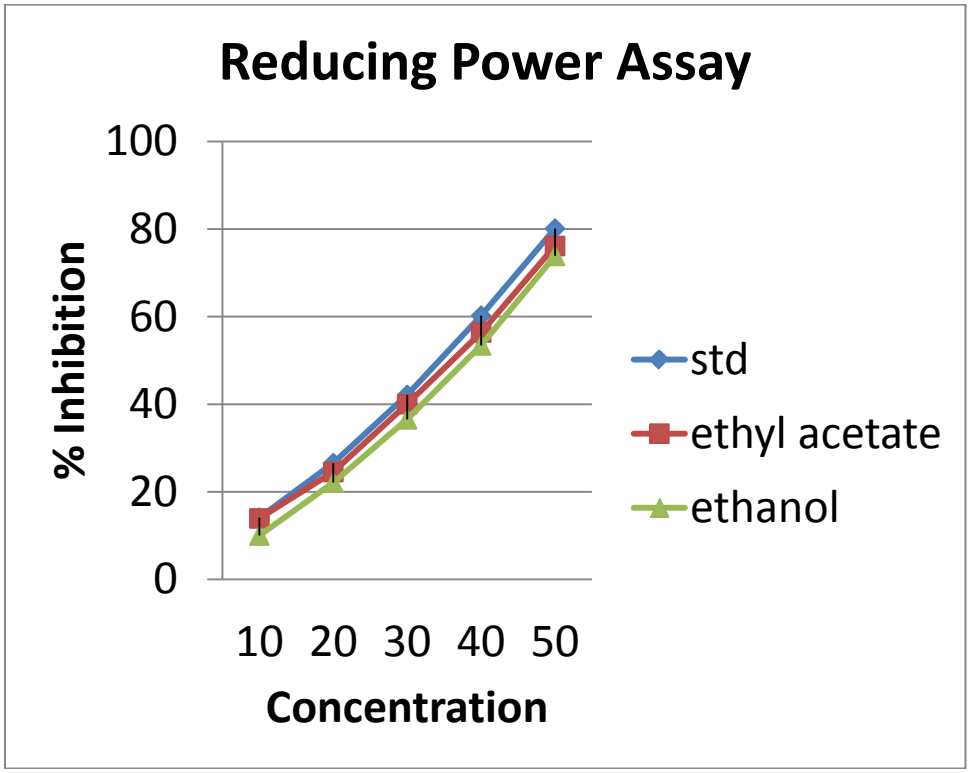
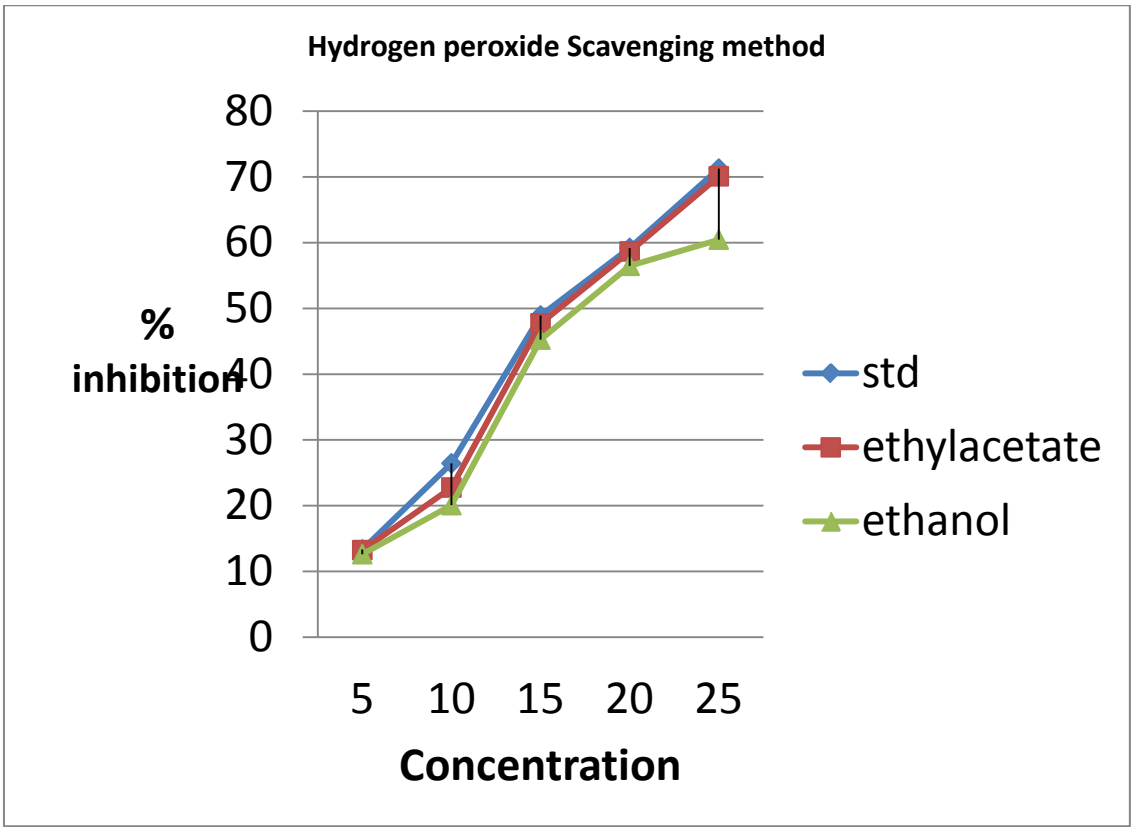


FIG.4 HYDROGEN PEROXIDE SCAVENGING ACTIVITY



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