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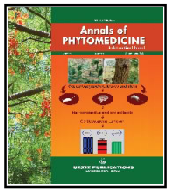

Original Article : Open Access

Phytochemical profiling, antioxidant potential and antimicrobial activities of *Dalbergia sissoo* Roxb.

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Abstract

This study was conducted to analyze the phytochemical components, antioxidant capacity and antimicrobial activity of the leaves and shoots extracts of Indian rosewood (*Dalbergia sissoo* Roxb.), a commonly growing plant of family Fabaceae. The total flavonoid, phenolic and tannin content were evaluated using aluminium colorimetric method and Folin-Ciocalteau method. The solvents used in the extraction were chloroform, methanol and petroleum ether. The antioxidant activity of methanol and chloroform was determined using DPPH assay. It was noticed that the methanolic leaves extracts of *D. sissoo* had greater antioxidant activity than the shoots. The antifungal and antibacterial effects of *D. sissoo* methanol leaf extracts were assessed against two fungal and two microbial strains. In bacterial strains, the highest antimicrobial effect of *D. sissoo* leaf extract was observed against *Bacillus subtilis* while the highest antifungal activity was reported against *Fusarium oxysporum*. All the results were compared with standard antibiotic disc and negative control used as a methanol solvent. On the basis of this study, this plant appears as a good contender to evaluate further for future herbal formulations.

1. Introduction

Plants have been important contributors to human welfare since the beginning of civilization. Besides food, shelter and clothing, they are an important source of useful chemicals that are used in the pharmaceutical industry all over the world. The most critical application of plants can be attributed to the derivation of medicines all for alleviating pains and diseases. Man over the centuries, learnt the art of manipulating the poisonous and healing properties of plants and used a large number of wild plants both in raw and processed form for treating a variety of human ailments (Sofowora *et al*., 2013).

Plants are being surveyed for a wide range of biological activity ranging from antibiotics to anticancer agents, as scientists continue their search for therapeutic plants (Alam, 2019). Bioactive constituents derived from plants can be found in all parts of the plant including the bark, leaves, flowers, roots, fruits and seeds. As a result, plants are an important part of ethnopharmacology and are directly utilized by the majority of civilizations around the world to cure a variety of health problems. Plant-based products are gaining popularity because they are less expensive, have fewer side effects and but are also the good source for safe future (Pandey *et al*., 2013; Zhang *et al*., 2015).

D. sissoo is a medium sized evergreen tree belonging to Fabaceae family. It is also a nitrogen fixing leguminous multipurpose tree that thrives well up to an altitude of 1000 m and is extensively used for timber, shelter belts and fuel wood in the sub-humid and drier areas. *D. sissoo* has been successfully introduced to other parts of the Asian subcontinent as well as Southeast Asia, the Caribbean and tropical America. In India, *D. sissoo* is widely distributed in Rajasthan, Haryana, Uttar Pradesh and Jammu and Kashmir, *etc.* (Lakshmi *et al*., 2014; Rijhwani and Bharty, 2016; Sharma *et al*., 2021).

Leaves are pinnately, leathery compounds and leaflets are alternate. They have a fine pointed tip, are ovate, acuminate, and petiolate. Flowers are 5-8 mm long racemes, ranging from pale to pink and are practically sessile. Leaves contains isoflavone-O-glycoside and sissotrin and flowers, stem bark and pods contain various chemical constituents such as biochanin A, 7-o-methyl tectorigenin, meso-ionsitol, 4- rhamnoglucoside, dalbergione, dalbergin, methyl dalbergin and isotectorigenin (Devi *et al*., 2017). *D. sissoo* has been found to have a wide spectrum of biological properties. It is also used to treat several ailments such as stomach troubles, leucoderma, dysentery, ulcers, emesis and skin diseases. Bark of this plant possesses phenolic compounds, tannins and flavonoids. It is commonly utilized to cure vata ailments including hemiplegia and sciatica (Sehra and Sharma, 2018).

This study has been done with the objective to investigate the phytoconstituents, antioxidant, and antibacterial activities of a common plant from the Fabaceae family.

2. Materials and Methods

2.1 Collection of plant material

The leaves and shoots of *D. sissoo* were collected from the Banasthali Vidyapith Campus (Tonk) in Rajasthan. The reference specimens were gathered and deposited at the Banasthali University Rajasthan India (BURI). The authentication number of the reference specimen is BURI-1400/2022.

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2.2 Extraction

20 g of powdered leaves and shoots were taken in a thimble prepared using Whatman filter paper (No. 1) and placed in the extraction chamber of the Soxhlet assembly for 12 h with 300 ml of three different extraction solvents were poured in the boiling flask. The Soxhlet apparatus was assembled in the figure. The flow of ice cold water was maintained at a constant level in the condenser part of the assembled Soxhlet apparatus. Temperature was maintained at 40-45°C in the boiling flask with the help of heating mantle. Between extraction chamber containing boiling flask and thimble, a cyclic flow of the extraction solvent was detected. This cyclic flow was sustained for 20- 25 cycles until the solvent in the extraction chamber became colorless (Zwawi *et al*., 2020)

Following that, conventional calculations were used to identify and quantify the secondary metabolites present in the extracts. Both qualitative (alkaloids, amino acids, fixed oils and fats, carbohydrates, flavonoids) and quantitative (total phenolic, flavonoid and tannin contents) determination was done (Banu and Cathrine, 2015; Agbadi *et al*., 2018; Ajuru *et al*., 2017).

2.3 Quantitative analysis

2.3.1 Total phenolic content

Using the Folin-Ciocalteau technique, the total phenolic content was determined and represented as gallic acid standards Mean ± SD (Rekha *et al*., 2012; Vats, 2014).

2.3.2 Total flavonoid content

The total flavonoid content was determined using the aluminium chloride colorometric methods (Chandra *et al*., 2014). To create the stock quercetin, 5 mg quercetin was dissolved in 1.0 ml methanol. 1ml of plant extract was taken and methanol (3 ml), 0.2 ml aluminium chloride (10%), 1 M potassium acetate (0.2 ml) and then added double distilled water to it. The mixture of the samples was vortexed and it was kept under dark for 60 min at room temperature then OD was taken at 420 nm. As standard or reference, quercetin was used. All of the tests were performed with three replicates at same concentrations to calculate the mean ± SD.

2.3.3 Total tannin content

The total tannin content was determined according to Dey *et al*. (2014) with some modifications. As the standard or reference, tannic acid was used. Separately 1 ml of each sample and standard solutions were taken, add 0.5 ml folin-ciocalteau reagent (1:1), cover the sample with foil paper and left for 4-5 min at room temperature, add 7.5 ml double distilled water, 1 ml of 35% Na_2CO_3 solution were added to it. The solution was properly vortexed and left for 30 min under dark conditions. The absorbance was taken at 700 nm.

2.4 Antioxidant activity

2.4.1 DPPH free radical scavenging assay

Antioxidant efficacy of all different plant extracts was estimated by non-enzymatic assay "DPPH (1, 1- diphenyl 2-picryl-hydrazyl)" free radical scavenging assay according to Blois *et al*. (1958) and Chandra *et al*. (2014) with some minor modifications. Ascorbic acid was used as standard (20-100 µg/ml). Plant extract with same concentration (20-100 µg/ml) was taken, then 1ml of 0.3 mM DPPH was added to it and incubated for 30 min in dark conditions. Absorbance was taken at 517 nm against methanol used as a blank. Experiments were performed in three replicates and mean value was recorded.

The formula for calculating percentage inhibition is given below:

%Inhibition = (Absorbance control – (Absorbance sample/ Absorbance control)*100

Table 1: Qualitative analysis of *D. sissoo* leaves and tender shoots in three different solvents

S. No.	Phytoconstituents	Leaf extracts			Shoot extracts		
		Methanol	Chloroform	Petroleum ether	Methanol	Chloroform	Petroleum ether
1.	**Alkaloids**						
	Mayer's test	++	+	-	++	+	-
	Wagner test	++	+	-	++	+	-
2.	**Flavonoids**						
	Alkaline reagent test	+++	++	+	++	+	-
3.	**Phytosterols**						
	Lebermannburchard test	+++	+	-	+	+	-
	Salkalowski test	++	+	-	+	+	-
4.	**Amino acids**						
	Ninhydrin test	++	+	-	+	+	-
5.	**Carbohydrates**						
	Benedict's test	++	+	-	+	+	-
6.	**Phenolics**						
	Lead acetate test	+++	++	+	++	+	-

+++ (highly present), ++ (moderately present), + (low present), - (Absent).

2.5 Determination of antimicrobial activity

The leaf extracts of *D. sissoo* were assessed for antifungal and antibacterial potential was observed using the method of disc diffusion according to Mostafa *et al*. (2018). Potato dextrose agar and nutrient media were used for the preparation of media. Both the media were sterilized and poured in petri-dishes in a laminar air flow. The filter paper discs (6 mm in diameter) were individually impregnated with 20 µl of the extract solutions. Bacterial and fungal petri-dishes were inoculated with bacteria at 37°C for 24 h and 28°C for 48 h for the fungal strains. All the tests were performed in triplicates and zone of the inhibition was measured in mm. The zone of the inhibition was compared against the positive control and negative control. Chloramphenicol and clotrimazole were used for the bacterial and fungal strains as positive control. Methanol was used as negative control.

2.6 Statistical analysis

The data are shown as averages of three replicates (n=3). The IBM SPSS Statistics 20 software was used to analyze all of the data. Between the identified variables, three-way interactions were tested. Multiple-comparison analysis is performed on each and every result variable. To compare the variance of data, the $p<0.05$ post hoc test was used in Turkey. Graphs were plotted and statistical calculations were carried out using Origin Pro-8 and Microsoft excel.

3. Results

Qualitative phytochemical study of different extracts of *D. sissoo* leaves and shoots revealed that the methanolic extracts included significant levels of flavonoids, phytosterols, carbohydrates, and amino acids. While the extracts prepared in chloroform has moderate presence of the specified variables and lesser amount of carbohydrates and alkaloids were detected. Both the extracts prepared in petroleum ether showed less traces of the bioactive compounds. Finally, the best results were obtained in the methanol extracts (Table 1).

Table 2: Quantitative analysis of *D. sissoo* leaf extract in different solvents

Variable	Methanol	Chloroform	Petroleum ether
Total phenolic content	177.32 ± 0.97^{c} mg/gGAE	91.17 ± 0.69^{b} mg/gGAE	19.07 ± 0.12^{a} mg/gGAE
Total flavonoid content	64.0 ± 0.39^{c} mg/g QE	31.05 ± 0.19^{b} mg/g QE	05.03 ± 0.01^{a} mg/g QE
Total tannin content	62.07 ± 0.49^{d} mg/g tannic acid equivalents	33.07 ± 0.26^{c} mg/g tannic acid equivalents	09.06 ± 0.04^{a} mg/g tannic acid equivalents

The data are the means and standard deviation of n=3 separate experiments.

Table 3: Quantitative analyses of *D. sissoo* shoot extract in different solvents

Variables	Methanol	Chloroform	Petroleum ether
Total phenolic content	93.0 ± 0.72^{c} mg/g GAE	62.75 ± 0.32^{a} mg/gGAE	14.25 ± 0.07^{a} mg/gGAE
Total flavonoid content	45.15 ± 0.25^{b} mg/g QE4	27.01 ± 0.14^{a} mg/g QE	03.01 ± 0.01^{a} mg/g QE
Total tannin content	8.07 ± 0.32^{d} mg/g tannic acid equivalents	27.14 ± 0.24^{b} mg/g tannic acid equivalents	09.0 ± 0.02^{a} mg/g tannic acid equivalents

The data are the means and standard deviation of n=3 separate experiments.

Table 4: Antioxidant (DPPH) activity of *D. sissoo* leaf and shoot extracts in two different solvents

Extracts	Methanol	Chloroform
Leaf extracts	61.14 ± 1.26^{c} µg/ml	48.32 ± 0.25^{c} µg/ml
Shoot extract	50.01 ± 0.31^{b} µg/ml	43.05 ± 0 .19^{b} µg/ml

Values are expressed as mean ± SD.

Table 5: Antibacterial activity of *D. sissoo* methanolic leaf extracts

S. No.	Bacterial species	Zone of inhibition (mm)		
		PE	Ab	Control
1.	*Escherichia coli*	14.0 ± 0.09^{b}	22 ± 0.19^{c}	10 ± 0.05^{a}
2.	*Bacillus subtilis*	18 ± 0.23^{b}	25 ± 0.46^{c}	12 ± 0.08^{a}

PE (leaf extract of *D. sissoo*), Ab (Chloramphenicol), Control (Methanol).

Table 6: Antifungal activity of *D. sissoo* methanolic leaf extracts

S.No.	Fungal strains	Zone of inhibition (mm)		
		PE	Ab	Control
1.	*Aspergillus niger*	17 ± 0.23^{b}	23 ± 0.30^{c}	10 ± 0.08^{a}
2.	*Fusarium oxysporum*	19 ± 0.23^{b}	22 ± 0.20^{c}	08 ± 0.05^{a}

PE (leaf extract of *D. sissoo*), Ab (Clotrimazole), Control (Methanol).

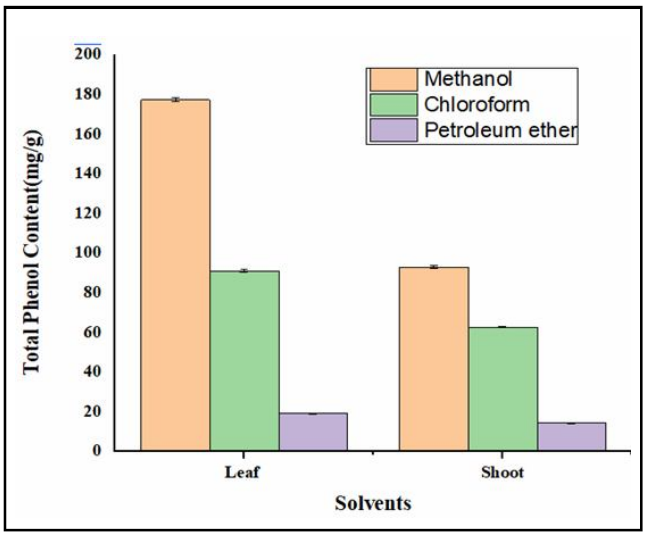

Figure 1: Total phenolic content of *D. sissoo* leaf and shoot extract in different solvents.

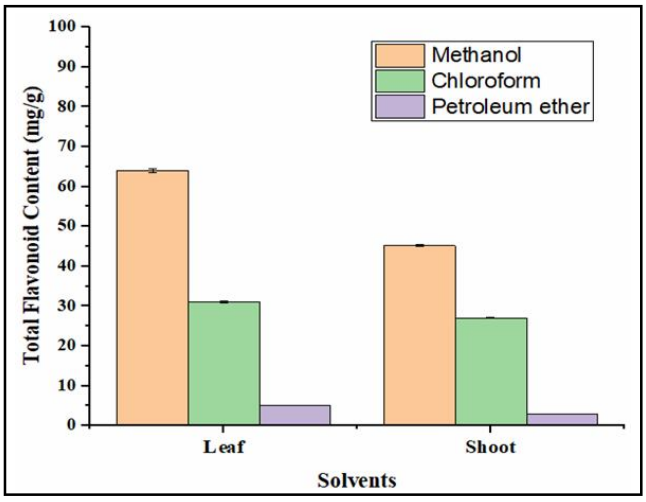

Figure 2: Total flavonoid content of *D. sissoo* leaf and shoot extracts in different solvents.

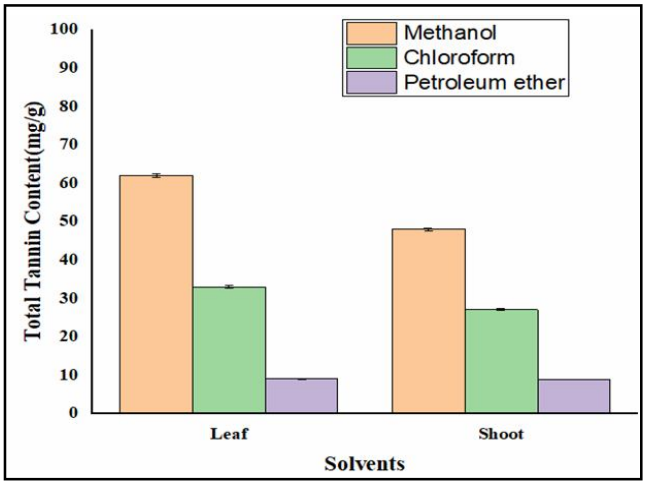

Figure 3: Total tannin content of *D. sissoo* leaf and shoot extract in different solvents.

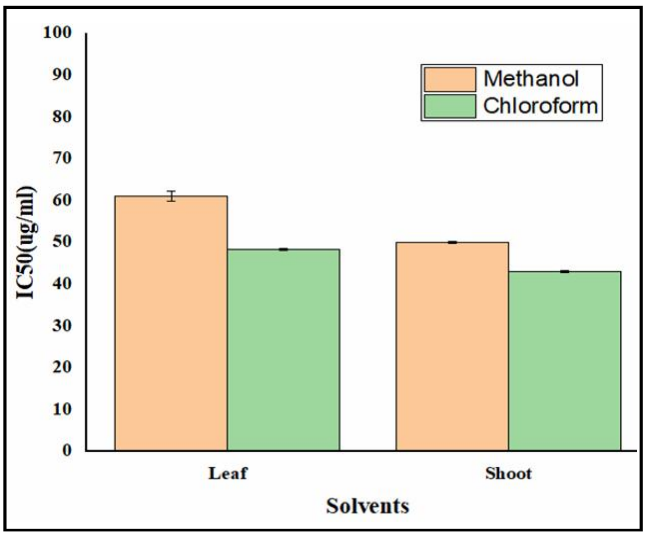

Figure 4: Free radical scavenging activity (DPPH) of *D. sissoo* leaf and shoot extract in different solvents.

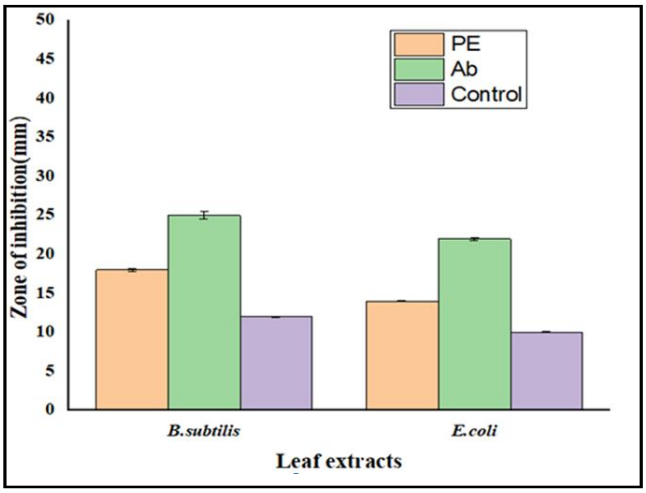

Figure 5: Antibacterial activity of *D. sissoo* leaf extracts in two different bacterial cultures.

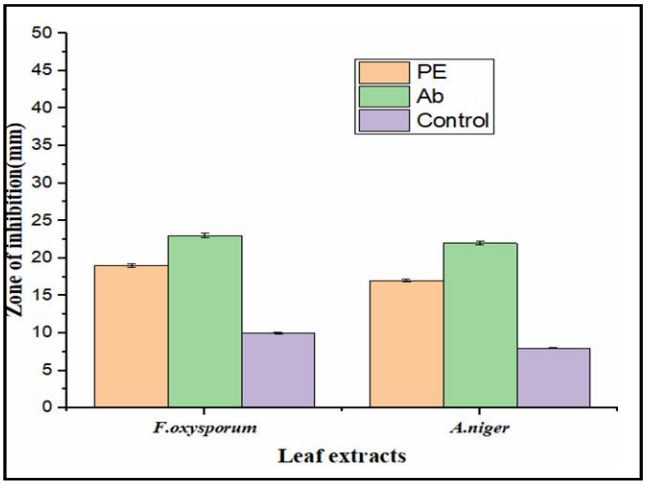

Figure 6: Antifungal activity of *D. sissoo* leaf extracts in two different bacterial cultures.

4. Discussion

Total phenolic, flavonoid and tannin content of *D. sissoo* were determined as gallic acid, quercetin and tannic acid, respectively (Tables 2, 3 and 4; Figures 1, 2, 3). The maximum total phenol content was recorded in the *D. sissoo* methanol leaf extract as compared to the shoot extracts, followed by chloroform and petroleum ether. However, the TPC of chloroform extract and petroleum ether were much lesser than the methanol extract.

The highest flavonoid and tannin concentration was found in methanol extracts for both the leaf and shoot extracts. As a result, methanol plant extracts were shown to be the best extracts to employ in the future. Petroleum ether produced minimal results due to the polarity of solvents.

4.1 Antioxidant activities

The higher total phenolic content indicates that it has good antioxidant potential. Plant phenolic chemicals are thought to be responsible for considerable free radical activities; this free radical scavenging activity is linked to their redox properties which allow them to operate as antioxidants. DPPH is a free radical and its absorbance reduces as the color changes from purple to yellow due to the antioxidant radical scavenging.

In the present work, the methanolic leaf and shoot extract of *D. sissoo* showed greater potency, followed by the chloroform extracts (Table 4; Figure 4) which is justified by the higher levels of phenol, flavonoid and tannin content.

4.2 Antimicrobial activities

The present investigation revealed that the methanolic leaf extracts obtained from *D. sissoo* have strong antimicrobial activities against selected bacterial and fungal strains. In bacterial strains, the methanolic leaf extract of *D. sissoo* showed significant effect against *B. subtilis* as compared to *E. coli* (Figure 5).

The antifungal activity of methanolic extract of *D. sissoo* showed maximum zone of inhibition against *F. oxysporum* as compared to the *Aspergillus niger* (Tables 5 and 6; Figure 6).

5. Conclusion

This study showed the presence of antioxidant compounds (phenolic acids, flavonoids and tannins) and demonstrated some level of antioxidant activities and antimicrobial activities in *D. sissoo*. Total amount of phenolic, flavonoid and tannins compounds were found maximum in polar solvent methanol. Maximum antioxidant activity was observed in methanol leaf extracts, followed by chloroform. According to research finding, *D. sissoo* is a promising source of novel bioactive compounds and helps in the discovery of new antibiotics that could serve as remedial agent against infectious diseases

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Author's contribution

BS has contributed equally in performing experiments and prepared the initial draft. AA conceptualized the research problem and guided the work done. Both the authors have finally read the manuscript and approved.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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