World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 7.409

PHYTOCHEMICAL SCREENING, IN-VITRO ANTIOXIDANT EFFICACY & ANTHELMINTIC ACTIVITY OF CHLOROFORM- METHANOLIC EXTRACT OF DALBERGIA SISSOO

Abinash Kumar Sahu*, Sweta Shankar Pal, Ritwik Dutta, Sushant Kumar, Farbish Sahu and Godti Yamini

Department of Medicinal Chemistry. The Pharmaceutical College, Tingipali, Barpali, Bargarh, 768029, Odisha, India.

***Corresponding Author: Abinash Kumar Sahu**
Department of Medicinal Chemistry. The Pharmaceutical College, Tingipali, Barpali, Bargarh, 768029, Odisha, India.

Article Received on 21/12/2024 **Article Revised on 11/01/2025** **Article Accepted on 31/01/2025**

ABSTRACT
The total objective of our study must be to identify drugs to estimate human illness by a thorough analysis of plant Ayurveda and modern medicine techniques must be coupled in order to bring out high quality of herbal product with rapid onset of action and good bioavailability. **Aim:** The aim of the study is to investigate Phytochemical screening of Chloroform-methanolic Extract of *Dalbergia sissoo*. **Result & Conclusion:** The qualitative phytochemical analysis of Chloroform:Methanol (60:40) extract & Aqueous extract of *Dalbergia sissoo* was done with preliminary identification of different bioactive compounds such as alkaloids, glycosides, Cardiac Glycosides, flavonoids, tannins, carbohydrate, steroids and saponins are present. The content of total phenolic compounds (TPC) was expressed as mg/g of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve. Total flavonoids content was calculated as quercetin equivalent (mg/g) using the equation based on the calibration curve: $Y=0.110\ X+0.004$, $R^2=0.999$, where X is the absorbance and Y is the quercetin equivalent (QE). The amount of total flavonoids was determined with the Quercetin. Quercetin was used as a standard compound and the total flavonoids were expressed as mg/g Quercetin equivalent using the standard curve equation: $Y = 0.008X + 0.007$, $R^2= 0.999$, Where Y is absorbance at 420 nm and X is total flavonoids content in the extracts of *Dalbergia sissoo* expressed in mg/gm. DPPH scavenging activity has been used for screening the in vitro antioxidant activity of plant extracts. The absorption maximum of a stable DPPH radical in methanol was at 517nm in U.V spectrophotometer. IC50 for standard Ascorbic acid was found to be 59.69 µg/ml and for *Dalbergia sissoo* was found to be 185 µg/ml. Thus the anti-oxidant activity of sample was less than that of standard ascorbic acid. The data revealed for anthelmintic activity state that the aqueous extract has a better wormicidal effect than Chloroform: Methanolic extract with compared with the standard drug Albendazole. Further study is required to find out the novel phytoconstituents responsible for anthelmintic action against various helminthes.

KEYWORDS: Traditional use, Phytochemical screening, Antioxidant activity, Anthelmintic activity.

INTRODUCTION
All increasing technological horizons (Excluding genes) aimed at tapping myriads of techniques of altering plants around us are circumscribed by herbal technology. To harvest the abundant products that the plants produce, such as natural dyes, biofertilizers, biopesticides, and biofuel, a variety of systems have been created.[1] The first step in codifying concepts and creating scientific procedures of this new concept of profitably controlling the plants around us was Herbal Technology. Herb Technology has been at the forefront of herbal medicine development for nearly two decades. The traditional skill of herbal formulation has been refined by our team of Ayurvedic, Chinese, and Western specialists. Herb Technology professional formulae have set a standard for the therapeutic practice of herbal therapy, combining modern scientific findings with traditional knowledge. The herbal sector provides a one-of-a-kind and strategic investment opportunity, which has led to its rapid global expansion.[2]

Plant profile
Dalbergia sissoo, known commonly as North Indian rosewood or *shisham*,[3] is a fast-growing, hardy, deciduous rosewood tree native to the Indian subcontinent and southern Iran. *Dalbergia sissoo* is a large, crooked tree with long, leathery leaves and whitish or pink flowers.

Taxonomical classification

Table 01: For taxonomical classification.

Kingdom	Plantae
Division	Magnoliophyta
Phylum	Tracheophyta
Order	Fabales
Family	Fabaceae/ Leguminosae
Sub Family	Faboideae
Tribe	Dalbergieae
Genus	*Dalbergia*
Species	*Sissoo*
Binomial Name	*Dalbergia Sissoo* DC.
Domain	Eukaryota

Vernacular names

English	Indian rosewood,
Hindi	Sitsal, Shisham
Kannada	Beete
Malayalam	Kariveeti, Cholaveetti, Veeti, Eeti
Tamil	Tawadi, Thodagathi, Eravad, Eeti
Telugu	Chittegi, Nalla yarugudu chettu
Marathi	Kalarukh, Sheesham
Sanskrit	Simsapa, Krishna-sinsapa

Leaves are leathery, pinnately compound, alternate leaflets, petiolated leaf stalk, measures about 15 cm long, each leaflet widest at the base, to 6 cm long with a fine pointed tip.[4]

Flowers are whitish to pink, fragrant, nearly sessile, and in dense clusters.

Pods are oblong, flat, thin, strap-like 4–8 cm long, 1 cm wide and light brown. They contain 1–5 flat bean-shaped seeds 8–10 mm long. It has a long taproot and numerous surface roots which produce suckers. Young shoots are downy and drooping, stems have light brown to dark grey bark up to 2.5 cm (0.98 in) thick, shed in narrow strips, large upper branches support a spreading crown.[5]

Seeds

Seeds are 6-8 x 4-5 mm, kidney shaped, thin and flat, light brown. The fruit is dry and hard. The sapwood is white to pale brown in colour and the heartwood is golden to dark brown in colour. It develops a long taproot from an early age, and numerous lateral ramifying roots.[6-7]

Geographical distribution

Exotic range: Afghanistna, Bangladesh Bhutan, India, Malaysia, Pakistan.[8]

Native range: Cameroon, Cyprus, Ethiopia, Indonesia, Iraq, Israel, Kenya, Mauritius Nigeria, Sudan, Tanzania, Thailand, Togo, US, Zimbabwe.

Traditional uses: Various parts of *Dalbergia sissoo* are traditionally used in treating different diseases and are mentioned below.[9]

Seeds: *Dalbergia sissoo* oil is used to treat blue itching, burning on the skin, and scabies.

Leaves: Finely ground paste of 8-10 leaves of *Dalbergia sissoo* and 25gm of palm candy taken in the morning alleviates profuse menstruation. 50-100ml decoction of the leaves taken thrice in a day is useful in Painful micturition and to cure boils and pimples. 10-15 ml juice (leaves) taken thrice in a day helps in eliminating pus in urine and in treating jaundice. The leaves warmed and tied on breast, and consuming the decoction of the leaves removes swelling of the breast.

Bark: 3-6gm powdered bark or decoction of the leaves is helpful in gonorrhea. Decoction of the bark and leaf is given in leprosy. Make a decoction of 10gm *Dalbergia sissoo* bark with 500gm of water and it should be boiled

till the liquid reduces to half. Mix the juice of the bark and consume for forty days every morning which helps in leprosy.

Chemical constituents

Sissoo nectar: Take 20gm of *Dalbergia sissoo* nectar, 320gm water, and 160 gm milk. Boil it till only milk remains. Consume 3 times a day. This milk cures any type of fever.

Leaves: Sissotrin and Isoflavone-O-Glycoside.[10]

Flowers: Biochanin A, Tectorigenin, 7, 4-Dimethyl Tectorigenin and 7-O-Methyl tectorigenin.

Green pods: Meso-Inisitol, 7-O-Methyltectorigenin and 4´-Rhamnoglucoside.

Mature pods: Isocaviumin, Tectorigenin, Dalbergin, Biochanin A, 7-Hydroxy-4-Methyl Coumarin, 7-O-Glucosides of Tectorigenin, Caviunin and Tannins.

Stem bark: Dalberginone, Dalbergin, Methyldalbergin, 4-Phenylchromene, Dalbergichromene and Isotectorigenin.

Heartwood: Dalbergin, Nordalbergenones, Dalbergichromene 3, 5-Dihydorxytrans- Stilbene, Biochanin A, Allylphenol of Latifolin Type – Dalbergiphenol and Fixed Oil.

USES

Timber

It is the best-known economic timber species of the rosewood genus sold internationally, but it is also used as fuel wood and for shade and shelter. After teak, it is the most important cultivated timber tree of Bihar, which is the largest producer of *shisham* timber in India. In Bihar, the tree is planted on roadsides, along canals, and as a shade tree for tea plantations. It is also commonly planted in southern Indian cities such as Bangalore as a street tree.

North Indian rosewood is usually dried before being used in furniture manufacturing; a process commonly known as seasoning. Locally, it is left in open areas to dry under the sun for about six months. Commercially, it is dried in closed chambers with hot-air circulation for about 7 to 15 days, depending on weather conditions. The ideal moisture level is around 5 to 6% for thinner pieces and up to 11% for thicker ones, depending on use. Any level lower than this can cause sudden cracking of the final products.

North Indian rosewood is among the finest cabinet and veneer timbers. It is the wood from which 'mridanga', the Rajasthani percussion instrument, is often made. In addition to musical instruments, it is used for plywood, agricultural tools, flooring, as a bentwood, and for turning.

The heartwood is golden to dark brown; the sapwood is white to pale brownish white. The heartwood is durable (its specific gravity is 0.7 – 0.8) and is very resistant to fungi, but the sapwood is readily attacked by dry-wood termites and borers. *Dalbergia sissoo*is known to contain the neoflavonoid dalbergichromene in its stem bark and heartwood.[11]

Fuel wood

The calorific value of both the sapwood and heartwood is excellent, being reported to be 4,908 kcal/kg and 5,181 kcal/kg, respectively. As a fuel wood, it is grown on a 10- to 15-year rotation. The tree has excellent coppicing ability, although a loss of vigor after two or three rotations has been reported. The wood makes excellent charcoal for heating and cooking.

Pesticide

An ethanolic extract of the fruits of *Dalbergia sissoo* exhibited molluscicidal effects against eggs of the freshwater snail *Biomphalariapfeifferi*.[12]

Construction

The juice of this plant is a potent ingredient for a mixture of wall plaster, according to the Samarangana sutradhara, which is a Sanskrit treatise dealing with silpasastra (Hindu science of art and construction)[13]

Health benefits

Traditional medicine

The tree's seed oil and powdered wood are used in the treatment of skin ailments. *Dalbergia sissoo* may also have efficacy in the treatment of stomach and blood conditions.[14]

Teeth brushing

Traditionally, slender tree twigs (called *datun*) are first chewed as a toothbrush and then split as a tongue cleaner. This practice has been in use in Pakistan, Africa, and the Middle East for centuries. Many of India's 80% rural population still start their day with the teeth cleaning twig either with *Salvadora persica* or *Azadirachta indica*. In other parts of the world, *shisham* twigs are still collected and sold in markets for this use in rural areas.

MATERIALS AND METHODS

The different Mayer's, Hager's, Barfoed's, Benedict's and millon's reagent, Wagner's, Dragendorff's, Fehling's A & B, α-naphthol, Ferric chloride, Conc. Sulphuric acid, Pyridine, Sodium nitropruside, Acetic anhydride, were purchased from S.D. Fine Chemical, Mumbai. The solvents petroleum ether, Chloroform, and Ethanol were purchased from Hi Media Laboratories Pvt. Ltd., Mumbai. All others chemicals, solvents and reagents were of analytical grade and procured from authorized dealer.

Experimental work

Plants collection, Identification and Processing

The fresh whole plant as *Dalbergia sissoo* was collected from local area of Gandhamardana hill ranges of Bolangir district of Odisha, India in the morning hour during the month of September 2022. The plant was

authenticated as *Dalbergia sissoo* Roxb ex DC of family Leguminosae on the basis of the morphological characters of all the parts of the plant by Botanical survey of India of Central National Herbarium of Howrah having letter number CNH/Tech-II 2022/71 on dated 29-09-2022. The plant was washed properly with water to remove the mud or dust, and then it was dried in sun light for one hour and the stem bark was dried under shade and was powdered by the help of mechanical process. The coarse powder have stored in air tight container for further studies.

Extraction

The dried powder plant material was extracted with Chloroform-Methanol and Aqueous extract in a (60:40) ratio by successive cold maceration method with increasing order of their polarity. The powdered drug was extracted for 7 days with each solvent. The extract was then filtered using filter paper and the filtrate so obtained was evaporated in a distillation unit. The results thus obtained from the extraction of *Dalbergia sissoo* are shown in (Table 1).

Table 1: Yield, colour.

S. No	Extract of *Dalbergia sissoo*(Roxb.)	Percentage yield	Colour
1	Chloroform -Methanolic	11.21%	Dark black
2	Aqueous extract	9.85%	Yellowish green

Qualitative phytochemical screening[15-19]

The qualitative phytochemical analysis of Chloroform:Methanol and Aqueous extract with a ratio of 60:40 extract of *Dalbergia sissoo* was taken And a preliminary identification of bioactive compounds such as alkaloids, glycosides, Cardiac Glycosides, flavonoids, tannins, are present and carbohydrate, steroids and saponins was done as shown in **Table. 2**

Table 2: Phytochemical screening of the two extracts of *Dalbergia sissoo*.

Phytochemical test	Chloroform - Methanolic Extract	Aqueous Extract
Alkaloid test		
Mayer's test	+ve	+ve
Wagner's test	+ve	-ve
Hager's test	+ve	+ve
Dragendorff's test	+ve	+ve
Carbohydrates		
Molish's test	+ve	+ve
Fehling's test	+ve	-ve
Barfoid's test	-ve	-ve
Benidict's test	+ve	-ve
Borntrager's test	+ve	+ve
Saponins		
Foam test	-ve	+ve
Proteins & amino acid		
Millon's test	+ve	+ve
Biuret's test	+ve	-ve
Ninhydrin test	+ve	+ve
Phenolic compounds & flavonoids		
Ferric chloride test	-ve	-ve
Lead acetate test	+ve	-ve
Alkaline test	+ve	+ve
Phytosterol		
Libermann-Burchard's test	-ve	-ve

(+): Present (-) : Absent

Table 3: Powder analysis with chemical reagent.

Reagents	Color of powder
Powder as such	Light Brown
Powder + conc. HCI	Light yellow
Powder + conc. HNO_3	Yellowish
Powder + conc. H_2SO_4	brown
Powder + glacial acetic acid	Brown
Powder + dil. HCI	Brown
Powder + NaOH sol.	Light brown

Powder + $FeCl_3$	Yellowish
Powder + picric acid	Yellow
Powder + ammonia	Green
Powder +Iodine	Light Brown

Table 4: Fluorescence analysis of powder drug.

Reagent	Colour observed (naked eye)	Colour observed (U.V short wave length)	Colour observed (U.V long wave length)
Powder as such	Brown	Brown	brown
Powder + 1N NaOH in methanol	Yellowish	Light green	Dark brown
Powder + NaOH in water	Light brown	Light brown	Dark brown
Powder + 50% HCl	yellowish	Light green	Black
Powder +50% H_2SO_4	brown	Light green	Black
Powder +50% HNO_3	Light brown	green	brown
Powder + petroleum ether	Light brown	Light brown	Dark brown
Powder + chloroform	brown	green	green
Powder + picric acid	yellow	Dark brown	green
Powder + 5% $FeCl_3$	Yellow	Black	Black
Powder + 5% iodine solution	Dark	Dark green	Black
Powder + methanol	Green	brown	Dark brown
Powder + (HNO_3 + NH_3)	brown	green	brown

Estimation of total phenolic and flavanoid content[20,21]

Total phenolic content estimation

Phenolic compounds are a class of antioxidant agents which act as free radical terminators and their bioactivities may be related to their abilities to chelate metals, inhibit lipoxygenase and scavenge free radicals. The amount of total phenol was determined with the Folin-Ciocalteu reagent. The content of total phenolic compounds (TPC) was expressed as mg/g of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.008X + 0.009, R^2= 0.999, where X is the absorbance and Y is the Gallic acid equivalent (GAE). Gallic acid was used as a standard compound and the total phenols were expressed as mg/g gallic acid equivalent using the standard curve equation: Y = 0.008X+ 0.009, R^2= 0.999, Where X is absorbance at 760 nm and Y is total phenolic content in the extracts of *Dalbergia sissoo* expressed in mg/gm. **Table.05** shows the variation of mean absorbance with concentration of Gallic acid and **(Fig no.1)** shows the calibration curve of standard gallic acid. The contents of total phenols that were measured by Folin-Ciocalteu reagent in terms of gallic acid equivalent.

Table 05: **Preparation of calibration curve of Gallic acid.**

Sl. No.	Concentration (µg/ml)	Absorbance (Mean) λ max=760 nm
1	25	0.740
2	50	0.855
3	75	0.954
4	100	1.050
5	125	1.151

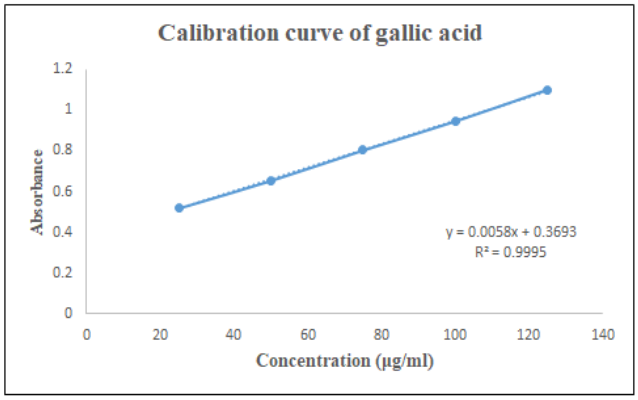

Fig. No. 1: Calibration curve of Gallic acid.

Total flavonoids content estimation

Total flavonoids content was calculated as quercetin equivalent (mg/g) using the equation based on the calibration curve: Y=0.110 X+0.004, R^2=0.999, where X is the absorbance and Y is the quercetin equivalent (QE). Flavonoids as one of the most diverse and wide spread group of natural compounds are probably the most important natural phytoconstiuent.

The amount of total flavonoids was determined with the Quercetin. Quercetin was used as a standard compound and the total flavonoids were expressed as mg/g Quercetin equivalent using the standard curve equation: Y = 0.008X + 0.007, R^2= 0.999, Where Y is absorbance at 420 nm and X is total flavonoids content in the extracts of *Dalbergia sissoo* expressed in mg/gm.

Table no.06 shows the variation of mean absorbance value with different concentration of Quercetin reagent and (**Fig no.02**) shows the calibration curve of Quercetin. The contents of total Flavonoids were measured by $AlCl_3$ reagent in terms of Quercetin equivalent.

Table no.04 shows the Total Phenolic and flavonoids content in *Dalbergia sissoo* plant extracts and it was observed that the total phenol content 36.45 ± 0.025 mg/g in the extracts. The total flavonoids varied from 42.36±0.01 mg/g in the extracts.

Table 06: **Preparation of calibration curve of quercetin.**

Sl. No.	Concentration (µg/ml)	Absorbance (Mean) λmax=420 nm
0	0	0
1	25	0.184
2	50	0.358
3	75	0.558
4	100	0.739
5	125	0.906

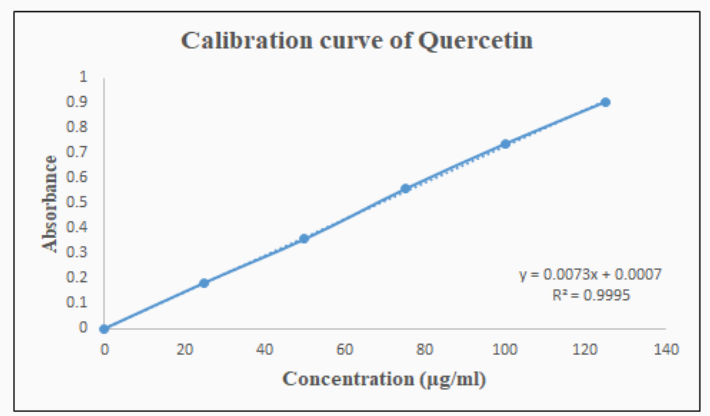

Fig. No. 02: Calibration curve of quercetin.

Table no.07 shows the total Phenolic and flavonoids content in *Dalbergia sissoo* plant extracts and it was observed that the total phenol content 36.45 ± 0.025 mg/g in the extracts. The total flavonoids varied from 42.36±0.01 mg/g in the extracts.

Table No. 07: Total Phenolic and flavonoids content in of *Dalbergia sissoo*.

Sl. No	Plant extract	Total phenol (mg/gm)	Total flavonoids (mg/gm)
1.	*Dalbergia sissoo*	36.45 ± 0.25	42.36 ± 0.01

In-vitro antioxidant activity of chloroform-methanolic extract

Results of In *Vitro* free radical scavenging Activity of DPPH

DPPH scavenging activity has been used by various researchers as a rapid, easy and reliable parameter for screening the in vitro antioxidant activity of plant extracts. DPPH is a stable free radical and accepts an electron to become a stable diamagnetic molecule. The absorption maximum of a stable DPPH radical in methanol was at 517nm in U.V spectrophotometer. It was observed that with the increase of concentration, there is decrease of absorbance value. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidants molecules and radical, progresses, which results in the scavenging of the radical by electron donation.

In the present investigation, Free radical scavenging of DPPH, percentage of different concentration of standard

ascorbic acid in shown in the **table no.08** and that of *Dalbergia sissoo.*

Table No. 8: Result of *in vitro* free radical scavenging activity of standard.

% Inhibition		
Conc.	**Ascorbic acid**	***Dalbergia sissoo***
10	49.4	33.25
20	58.6	36.73
40	68.1	50.30
60	76.1	53.78
80	88.9	61.58
100	90.6	66.50
IC 50	17.70	50.72

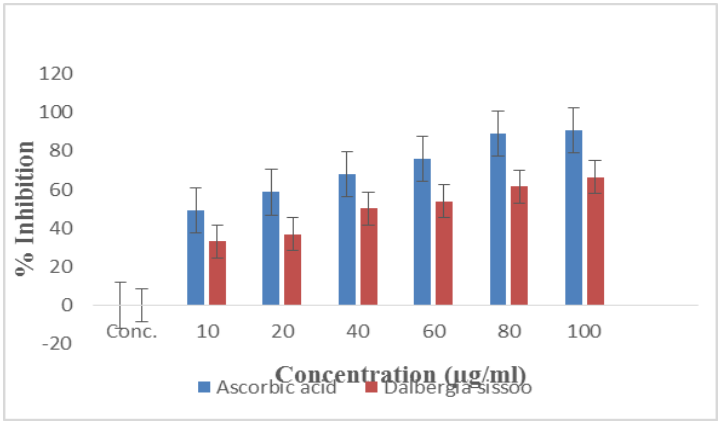

Figure 03: DPPH radial scavenging assay of *Dalbergia sissoo.*

IC_{50} for standard Ascorbic acid was found to be 17.70µg/ml and for *Dalbergia sissoo* was found to be 50.72µg/ml. Thus the anti-oxidant activity of sample was less than that of standard ascorbic acid.

Free radicals are the cause for several major disorders. So, evaluation of antioxidant activity in plants could result in the discovery of natural antioxidants with pharmacological and food value. The importance of phenol compounds in plants as natural antioxidants and their use as substitutes to synthetic antioxidants in food additives is well known. Therefore there observation can be used in pharmaceutical to explore new drugs. Thus the present aim is to assess the antioxidant activity of conventional and non conventional species of curcuma by DPPH method and also to compared the their % antioxidant activity with standard ascorbic acid.

Determination of anthelmintic activity[22-27]

The anthelmintic study was done by using one in-vitro species adult earthworms Pheretima posthuma. Earthworms were collected near the swampy water in our locality. The average size of the round worm was 5-7 cm; average size of the earthworm was 8-9 cm. These earthworms were identified and services of veterinary practioner were utilized to confirm the identity of worms. The suspensions of various extracts were prepared in 2% gum acacia solution to obtain 1, 2.5 and 5% concentrations. Solutions of similar concentrations of the standard drug albendazole were also prepared in distilled water.

Two ml of each concentration of various extracts of *Dalbergia sissoo* and standard drug albendazole were diluted to 10 ml separately with normal saline and poured in petridishes. 2ml of 2% gum acacia solution was diluted to 10ml with normal saline to serve as control. Six earthworms of nearly equal size were placed in each Petridis at room temperature. Time was recorded at the time of releasing the earthworms to each concentration. The time taken (minutes) for the complete paralysis and death were recorded. The mean paralysis time for each sample was recorded. The anthelmintic activity was evaluated on adult Indian earthworm Pheritima posthuma due to its anatomical and physiological resemblance with the intestinal round worm parasites of human beings. Paralysis was said to occur when the worms did not revive even in normal saline. Death was concluded when the worms lost their motility followed by fading away of their body colour.

Table 9: Anthelmintic effect of *Dalbergia sissoo* extracts.

Group	**Concentration of Extract (%)**	**Time in minutes (Mean ± SEM)**	
		Paralysis time(Min)	**Death time(Min)**
Albendazole (standard)	10 mg/ml	16min,18 sec ±17	14min,18 sec ±42
	30 mg/ml	11min,21 sec ±12	12 min,12 sec ±10

	50 mg/ml	9 min,12 sec ±14	10min,40 sec ±11
Chloroform-Methanol extract	15 mg/ml	24min,16 sec ±17	28min,15 sec ±48
	30 mg/ml	20min,26 sec ±12	27 min,26 sec ±12
	50 mg/ml	20 min,48 sec ±14	23 min,48 sec ±14
Aqueous extract	15 mg/ml	10min,19 sec ±17	18min,15 sec ±48
	30 mg/ml	10min,26 sec ±12	14 min,26 sec ±12
	50 mg/ml	11 min,48 sec ±14	12min,14 sec ±10
Control	-	-	-

Results are expressed as mean ± SEM from six observations, *Control worms were alive upto 24 hrs. of observation,* N/A= No Activity shown within 24 hours.

RESULTS AND DISCUSSION

Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Herbal formulations have growing demand in the world market. It is an attempt made to establish the herbal gel containing *Dalbergia sissoo* leaves extract at 1% concentrations. Compounds obtained from *Dalbergia sissoo* like an isoflavone, biochanin is a potent chemotherapeutic cancer preventive agent. It possesses various Pharmacological activities to be conducted to investigate the unexploited potential of the plant.

The qualitative phytochemical analysis of Chloroform:Methanol (60:40) extract of *Dalbergia sissoo*. Was done by preliminary identification of bioactive compounds such as alkaloids, glycosides, Cardiac Glycosides, flavonoids, carbohydrate, saponins, steroids, tannins, are present.

The results of extractive value were showed the Chloroform Methanolic Extract in a (60:40) ratio has 11.21%w/w (Table-1) and extract of Aqueous in a (60:40) ratio has 9.85%w/w respectively. From the finding of extractive value of the extracts has been selected for further studies.

The content of total phenolic compounds (TPC) was expressed as mg/g of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: $Y = 0.008X + 0.009$, $R^2 = 0.999$, where X is the absorbance and Y is the Gallic acid equivalent (GAE).

Gallic acid was used as a standard compound and the total phenols were expressed as mg/g gallic acid equivalent using the standard curve equation: $Y = 0.008X + 0.009$, $R^2 = 0.999$, Where X is absorbance at 760 nm and Y is total phenolic content in the extracts of *Dalbergia sissoo* expressed in mg/gm.

Total flavonoids content was calculated as quercetin equivalent (mg/g) using the equation based on the calibration curve: $Y=0.110 X+0.004$, $R^2=0.999$, where X is the absorbance and Y is the quercetin equivalent (QE). The amount of total flavonoids was determined with the Quercetin. Quercetin was used as a standard compound and the total flavonoids were expressed as mg/g Quercetin equivalent using the standard curve equation: $Y = 0.008X + 0.007$, $R^2 = 0.999$, Where Y is absorbance at 420 nm and X is total flavonoids content in the extracts of *Dalbergia sissoo* expressed in mg/gm.

DPPH scavenging activity has been used by various researchers as a rapid, easy and reliable parameter for screening the in vitro antioxidant activity of plant extracts. DPPH is a stable free radical and accepts an electron to become a stable diamagnetic molecule. The absorption maximum of a stable DPPH radical in methanol was at 517nm in U.V spectrophotometer. IC_{50} for standard Ascorbic acid was found to be 59.69 µg/ml and for *Dalbergia sissoo* was found to be 185 µg/ml. Thus the anti-oxidant activity of sample was less than that of standard ascorbic acid.

The results (Table-9) depict the time taken for paralysis and death of earthworms after the treatment with the test extracts at the selected concentrations. The data revealed that the aqueous extract has a better wormicidal effect than Chloroform:Methanolic extract with compared with the standard drug Albendazole. Further study is required to find out the novel phytoconstituents responsible for anthelmintic action against various helminthes.

ACKNOWLEDGEMENTS

The Authors is highly grateful to extend my special thanks to Mr. Narendra Kumar Hota, Chairman, G.B of The Pharmaceutical College, Barpali for his constant encouragement & support throughout the work. The authors is also extend my sincere thanks to Mr. Himanshu Mohan Deo, President, Dr. Arnabaditya Mohanty, Principal & Dr.Ranjan Kumar Sahoo, Director of The Pharmaceutical College, Barpali for providing all kind of facilities for this work.

Conflict of interest

None.

Source of funding

Nil.

REFERENCES

1. A report on the National Seminar on Herbal Technology. News Current Science, conducted at the Department of Botany, M.S. University of Baroda, Vadodara, 2005; 88(9,10).
2. Maity P, Hansda D, Bandyopadhyay U and Mishra DK: Biological activities of crude extracts and

chemical constituents of Bael, *Aegle marmelos* (L.) Corr. Indian J Exp Biol, 2009; 47(11): 849-861.
3. Bhattacharya M, Singh A, Ramrakhyani C: Dalbergia sissoo-An Important Medical Plant. Journal of Medicinal Plants Studies Journal of Medicinal Plants Studies Year, 2014; 2(2): 76-82.
4. Asif M, Khan I, Hussani MH, Khan SN.Journal of Natural Science, Biology and Medicine, 2011; 2(1): 76-80.
5. Hari Shankar Lal and Sanjay Singh. Ethnomedicinal uses of *Dalbergia sissoo* Roxb in Jharkhand, International journal of ayurvedic and herbal medicine, 2012; 2(1): 198-2.
6. *Dalbergia sissoo* [online]. 2012 Dec 9; Available from: URL:http://en.wikipedia. org/ wiki/ *Dalbergia_sissoo* Indian J Pharmacol, 2000; 32: 357-60.
7. E: *Dalbergia sissoo*Indian Rosewood, Classification of Indian Rosewood, Medicinal properties of Indian Rosewood Eco India.
8. Sheikh MI. A quick guide to useful nitrogen fixing trees from around the world, NFT Highlights, NFTA 89-07, December 1989.
9. Bhattacharya M, Singh A, Ramrakhyani C: Dalbergia sissoo - An Important Medical Plant. Journal of Medicinal Plants Studies Journal of Medicinal Plants Studies Year, 2014; 2(2): 76-82.
10. Dalbergia sissoo Indian Rosewood, Classification of Indian Rosewood, Medicinal properties of Indian Rosewood Eco India.
11. S. K. Mukerjee; T. Saroja & T. R. Seshadri. "Dalbergichromene : a new neoflavonoid from stem-bark and heartwood of Dalbergia sissoo". Tetrahedron, 1971; 27(4): 799–803. doi:10.1016/S0040-4020(01)92474-3.
12. "Make A Neem Toothbrush (Neem Tree Home Remedies)". Discover Neem. Birgit Bradtke. Retrieved, 2013; 16.
13. Adenusi A. A. &Odaibo A. B. "Effects of varying concentrations of the crude aqueous and ethanolic". African Journal of Traditional, Complementary and Alternative medicines, 2009; 6(2). abstract, PDF.
14. Nardi, Isabella The Theory of Citrasutras in Indian Painting. Routledge, 2007; 121. ISBN 978-1134165230.
15. Vogel HG. Drug Discovery and Evaluation, Pharmacological Assays. New York: Springer-VerlagBerlin Heidelberg, 2002; 670-675.
16. Sofowora A. Phytochemical Screening of Medicinal Plants and Traditional Medicine in Africa. Nigeria: Spectrum Books Ltd, 1993.
17. Harborne JB, 1984, Phytochemical Method, A Guide To Modern Technique of Plant Analysis, Chapman and Hall, London, Chapman and Hall, London and New York, 1973; 2: 182-89.
18. Kelkar GM, Phalnikar NL, Bhinde BV. Fatty oil from the seeds of *Argyreia speciosa* Sweet. *J. Indian Chem*. Soc, 1947; 24: 83-86.
19. Agarwal SR, Rastogi RP. Pharmacognostical and Preliminary Phytochemical Studies of Argyreia nervosa Burm. *Indian J.Pharmacol*, 1974; 35: 118-119
20. Fejes S, Blázovics A, Lugasi A, Lemberkovics E, Petri G, Kéry A. In vitro antioxidant activity of Anthriscus cerefolium L.(Hoffm) extracts. J Ethnopharmacol, 2000; 69: 259–65.
21. Meir S, Kanner J, Akiri B, Philosoph-Hadas S. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. J Agric Food Chem, 1995; 43: 1813–9.
22. Manjunath KP, Shivakumar H, Prakash T, Patil KS, Veeranagouda A, Jayakumarswamy BHM, Venkatesh JS and Rao NR. *Indian Journal of Natural Products*, 2006; 22, 1: 8-10.
23. Saikat D, Anup M, Mintu K, Subhash CM, Dhaka Univ. *J. Pharm. Sci*, 2007; 6**,** 2: 121-123.
24. Ajaiyeoba EO, Onocha PA, Olarenwaju OT. In-vitro Anthelimintic properties of Buchholziacoriaceae and Gynandropsisgynanndra extract. Pharm Biol, 2001; 39: 217-20.
25. Dash GK, Suresh P, Sahu SK, Kar DM, Ganapaty S, Panda SB. Anthelimintic activity of *Cissus quadraangularis* Linn Stem. J Nat Rem, 2002; 2(2): 182-185.