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Research Article

Evaluation of Antioxidant and Anthelmintic Properties of *Caesalpinia sappan* L. Leaves

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ABSTRACT

Caesalpinia sappan L. is a popular medicinal plant known to both the Ayurveda and Chinese traditional medicines since ancient time. The aim of this research was to study the qualitative phytochemistry and to determine the antioxidant and anthelmintic activities of *C. sappan* leaves. Phytochemical analysis indicated the presence of various plant bioactive metabolites in *C. sappan* leaves. Different solvent extracts of the crude drug were tested for their in vitro antioxidant potential using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and ferric ion-reducing antioxidant power (FRAP) assay. Total phenolic content (TPC) and total flavonoids content (TFC) of the extracts were determined by spectrophotometric method. Methanol extract and ethyl acetate extract of the leaves were evaluated for anthelmintic activity against earthworms (*Eisenia fetida*). Results of this study revealed that *C. sappan* leaves methanolic extract has significant antioxidant potential as compared with standard, vitamin C. Methanol extract also exhibited potent anthelmintic activity with paralysis time (19.13±0.340 min) and death time (54.21±0.533 min). These activities are attributed to the complex chemical nature of *C. sappan* leaves. The result of present study revealed that *C. sappan* leaves can serve as a good natural source of potent antioxidants and anthelminthiasis medicines. As per our extent of information, *C. sappan* leaves anthelmintic activity is reported for the first time in this paper. Further works are required for identification of phytochemicals and studying in vivo pharmacological activities of *C. sappan* leaves.

Keywords: *Caesapinia sappan*, antioxidant, oxidative stress, DPPH, FRAP, anthelmintic.

INTRODUCTION

Medicinal plants founded the base of therapy and diseased treatment. However, in this modern era along with availability of various synthetic drugs in the market, a revival of herbal and natural drug usages are clearly considered. About 80% of the world population primarily in developing countries depends on traditional system of medicine for their primary health care needs[1]. Researches on medicinal plants and seeking for new drugs of natural origin have an ascending movement from almost last four decades. This can be attributed to the fact that synthetic and presently available medicines are either too expensive or tend to bring out side effects. Plants secondary metabolites have provided a variety of lead structures, which serve as templates for the development of new drugs [2]. *Caesalpinia sappan* L. is a medium sized tree belongs to family Fabaceae (Leguminosae) and as a medicinal plant is very much known in folk and traditional medicine systems. In Chinese it is called Su mu, its other common names are sappan, patang, brazilwood, or sappanwood[2, 3]. *C. sappan* is a thorny and shrubby small to medium-sized tree up to 4-8(-10) m tall. It bears few prickly branches and large bipennate compound leaves up to 20 – 45(or 50) cm long and 10 – 20 cm broad. The compound leaves are stipulate, alternate and comprised of 8-16 pairs of pinnae, each up to 20 cm long. Each pinnae is composed of 10-20 pairs of oblong, 10-20 mm x 6-10 mm long, subsessile leaflets. Leaflets have oblique base and round or emarginated apex. The flowers are yellow coloured, 2 – 3 cm long, 5-merous with special fragrance and gathered in terminal and axillary panicles. Each flower are. The fruits are woody beaded pods each contains 3-4 yellowish-brown seeds [2]. *Caesalpinia sappan* L. is found in India, Peru, Malaya, etc.[2,4]. It grows wildly in mountains and is cultivated in the gardens for its large panicles of yellow flowers. In India the tree is distributed in Tamilnadu, Kerala, Karnataka, Andhra Pradesh and West Bengal[3]. The plant is used in Ayurveda for curing several ailments since very long time [4]. *C. sappan* is widely used in both Ayurveda and Chinese traditional medicine[5]. Phytochemistry and various pharmacological activities of *C. sappan* heartwood are reported by several authors [2,4 ,6-9]. The dried heartwood of *C. sappan* is used medicinally. As per literature survey *C. sappan* is used for purifying blood, quenching thirst, treatment of jaundice, cough, respiratory ailments and wounds[3,10]. *C. sappan* is reported to be useful for mitigating or curing various ailments such as; blood pressure, heart diseases, amenorrhea, dysmenorrhea, blood stasis after delivery, pricking pain in the chest and abdomen, traumatic swelling and pain. Seeds of the plant are used for stomach aches and nervous disorders. Decoction of wood and bark of the plant is used

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for curing tuberculosis, diarrhoea, dysentery, postpartum tonic and skin infections[3]. Several publications are available relating different pharmacological activities (in vitro and in vivo) of *C. sappan* heartwood and its isolated compounds[11]. *C. sappan* has shown to have antioxidant activity [3,8], anthelmintic activity[12] , hepatoprotective activity[2,4], anti-inflammatory activity[13], antidiabetic activity [2,3,4], antitumor activity [5], antiproliferativ activity, analgesic activity, anticancer activity, vasorelaxing effect, immunosuppressive activity[3], etc. *C. sappan* is one of the ingredients of Lukol[4], Vicco vajradanti and other Ayurvedic formulations. Lukol™ as different oral dosage-forms is administered in gynaecology for the treatment of non-specific leucorrhoea and bleeding following insertions of intrauterine device (IUD). Vicco vajradanti is a famous tooth paste and tooth powder used for curing bleeding of gingiva. The powerful astringent, haemostatic and healing properties of *C. sappan* wood helps to stop bleeding in gums[2]. Oxidative stress is a challenging issue to mankind health. Free radicals and other reactive oxygen species (ROS) and reactive nitrogen species (RNS) are constantly formed in the human body[14, 15]. Uncontrolled or excess production of ROS and RNS cause oxidative stress[17] and could be the onset of several dangerous ailments including cancer, rheumatoid arthritis, cirrhosis, atherosclerosis and age related degenerative diseases[15]. Oxidative stress will arise either due to excess generation of ROS and RNS or due to imbalance status of antioxidant/oxidant in the body. However, the body normally produces endogenous antioxidants and has the antioxidant defence system, but in case of excess regeneration of free radicals within the body, endogenous antioxidant system of the body will not suffice for quenching all those ROS and RNS. This failure leads to oxidative stress which can only be prevented by taking sufficient amount of exogenous antioxidants from diet or supplements. Medicinal plants are the unique natural sources of antioxidants such as phenolics, tannins, flavonoids, coumarins, and so on[14]. Heleminthiasis refers to infestation with intestinal parasite worms like roundworms (*Ascaris lumbricoides*), whipworms (*Trichuris trichuris)* or hookworms (*Nectator americanus*). The disease affects the health of large fraction of the human population including animals. The regular and long–term resistance in nematodes and schistosomes – and the continuous reliance on a small range of anthelmintic compounds has led to the development of drug dependent resistance of parasitic worms. Some types of dangerous helminthic infections like filariasis have only a few therapeutic modalities at present. Because of limited availability and affordability of modern medicines, most of the population worldwide depend extensively on traditional medicine system. Herbal remedies used in the traditional folk medicine provide an interesting and still largely unexplored source for finding out potential leads which might help to overcome the growing problem of disease management. Therefore, screening of medicinal plants in order to explore their active constituents as new drugs with novel mechanisms of action, is of great interest of today's researches[18]. Medicinal plants including *C. sappan* used in the traditional medicine system have a supportive role in providing a rich source of accessible, affordable and effective therapeutic agents to the people[19]. Drugs of medicinal plants origin have good therapeutic potential with no or less side effects than most of those made synthetically[20]. Based on both the above evidences and ethnobotanical importance of *C. sappan* leaves was selected for this study. As per our knowledge antioxidant potential and anthelmintic activity of *C. sappan* leaves are reported for the first time in this paper.

MATERIALS AND METHODS

Plant materials

Caesalpinia sappan leaves were procured from Dhanlakshami Agro Plantations and Consultancy, Tamilnadu. The plant materials were authenticated by Dr. H.B. Singh, Chief Scientist & Head of Raw Materials Herbarium & Museum (RHMD) at National Institute of Science Communication and Information Resources, New Delhi. Collected materials were shade dried and powdered to form a uniform coarse powder.

Chemicals

All the chemicals were of analytical grade procured from standard companies. For instance – methanol, hexane (E. Merck, Mumbai), ethylacetate, petroleum ether (CDH, New Delhi), ethanol (Jiangsu Huaxi International Trade Company Ltd., China), dichloromethane and hydrochloric acid (LOBA Chemie, Mumbai), etc.

Experimental animals

The earthworms of species *Eisenia fetida* were purchased from Ujjwal Ujjala farm, Amritsar.

Preparation of plant extract

The coarsely powdered dried *C. sappan* leaves (100 g) were successively extracted with petroleum ether (3 L) at 60 ˚C for 8 hrs using Soxhlet apparatus. Then the plant material (marc) was dried at room temperature for overnight. The dried marc was extracted with dichloromethane (3 L) at 40 ˚C for 8 hrs. The marc was again dried at room temperature for overnight. Extraction of the same marc was repeated using ethyl acetate at 77 ˚C for 4 hrs and methanol at 78 ˚C for 3 hrs. All of the obtained extracts were timely filtered, evaporated and dried to obtain the viscose semi solid contents. The dried extracts were kept in refrigerator at 4 ºC for further use.

Preliminary phytochemical screening

Various chemical qualitative tests using standard different colour and precipitative reagents were used for detection of phytochemicals present in different extracts of *C. sappan* leaves. Qualitative chemical tests aimed to detect the presence of alkaloids, glycosides, flavonoids, phenolics, tannins, sterols, etc. were carried out as per described procedures[21,22,23]. Results are tabulated in Table1.

Determination of antioxidant activity

Total phenolic content (TPC), total flavonoid content (TFC) and antioxidant potential of *C. sappan* leaves extracts were determined. DPPH free radical scavenging assay and Ferric ion (Fe^{+3}) reducing antioxidant power (FRAP) assay were used for evaluation of antioxidant potential of the extracts.

Table 1: Data showing the results of phytochemical analysis of *C. sappan* leaf different extracts.

Compounds	Extracts			
	Pet. ether	DCM	EA	MeOH
Alkaloids	-	-	-	-
Carbohydrates	-	-	-	+
Glycosides	-	+	-	+
Flavonoids	+	+	+	+
Phytosterols	-	-	-	-
Phenols	-	-	-	+
Saponins	-	-	-	+
Tannins	-	-	+	+
Amino acids and proteins	-	+	+	+

'+' = present, '–' = absent, DCM= dichloromethane, EA= ethyl acetate, MeOH=methanol

Table 2: Data showing the absorbance of different concentrations of Tannic acid.

Conc. (µg/ml)	Ab (765 nm) of tannic acid
5	0.345±0.001
10	0.436±0.02
15	0.527±0.032
20	0.613±0.004
25	0.719±0.002

Values are the average of triplicate experiments and represented as Mean ± SEM

Determination of total phenolic content (TPC)

The total phenolic content of the extracts was determined by Folin-Ciocalteu method[24]. 1 ml of extract aqueous solution was mixed with 0.5 ml of Folin-Ciocalteu reagent. After 5 minutes of incubation at room temperature, 1.5 ml of Na_2CO_3 20% aqueous solution was added to the mixture followed by addition of 7 ml distilled water. The mixture was kept in the dark for 2 hrs. Then the absorbance was taken at 765 nm using UV-spectrophotometer (Company). Table2. shows the absorbance of tannic acid solution. Various concentrations of tannic acid aqueous solution were used to plot standard curve (Figure 1.). TPC of the samples was expressed as microgram tannic acid equivalent/mg dry weight of extract (Table 3).

Determination of total flavonoid content (TFC)

For estimation of TFC, 1ml of the extract solution was diluted with 4.3 ml of 80% aqueous ethanol containing 0.1 ml of 10% aluminium nitrate and 0.1 ml of 1 M aqueous potassium acetate. After 50 min the UV absorbance was taken at 415 nm. Various concentrations of quercetin dehydrate were used to plot standard curve (Table 4. Figure 2.). The flavonoid content of the extract was expressed as microgram quercitin equivalent/mg dry wt. of extract[24,25]. (Table 5.)

DPPH radical scavenging method

C. sappan leaf extracts were evaluated for their potential to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. DPPH radical scavenging assay was carried out as per method[14]. Purple coloured DPPH turns yellow by reacting with antioxidant compounds. The amount of 0.5 ml DPPH methanolic solution (0.5 mM) was mixed with 3.5 ml of extracts methanolic solutions having different concentrations (5 – 25 µg/ml). Ascorbic acid methanolic solutions at different concentrations were used as standard. All of the mixtures were allowed to stand in dark for 30 min at room temperature. Then their UV absorbance was measured at 517 nm. Methanol was used as blank. Methanol (3.5 ml) added 0.5 ml DPPH (0.5 mM) was used as positive control. The percentage scavenging was calculated as per the following formulae:

% scavenging = 100 X [(Ac – As) / Ac]

Where; *Ac* is absorbance of control and *As* is absorbance of sample / standard

The assay was performed in triplicate and the results (% scavenging) were recorded as mean ± SEM of the triplicates (Table 6.).

Ferric ion (Fe^{3+}) reducing antioxidant power (FRAP) assay

Solutions *C. sappan* leaf extracts at different concentrations of 5 – 25 µg/ml in methanol were prepared. 1ml of prepared solutions were separately mixed with 2.5 ml of phosphate buffer (0.2M, pH=6.6) followed by 2.5 ml of 1% potassium Ferro-cyanide. The mixture was incubated at 50 °C for 20 minutes, and then 2.5 ml of trichloroacetic acid (10%) was added to the mixture. The mixture was centrifuged at 12000 rpm for 10 minutes and 5 ml of the supernatant was taken and mixed with 3 ml of distilled water and 0.5 ml $FeCl_3$ (0.1%). The absorbance of the final mixture was measured at 700 nm. The higher absorbance indicates a higher reduction capability (Table 7.). Ascorbic acid was used as a standard.

Determination of anthelmintic activity

Table 3: Data showing the total phenolic content of C. sappan leaves various extracts as TAE µg/mg extract.

Plant extract	Total phenolic content (µg/mg)
Dichloromethane	10.09±0.01
Ethylacetate	6.32±0.02
Methanol	14.05±0.023

Values are the average of triplicate experiments and represented as Mean ± SEM

Table 4: Data showing the absorbance of various concentration of standard Quercetin dehydrate.

Conc. (µg/ml)	Absorbance (415 nm) of Quercetin
3	0.189
6	0.267
9	0.342
12	0.421
15	0.520
18	0.611
21	0.710

Values are the average of triplicate experiments and represented as Mean ± SEM

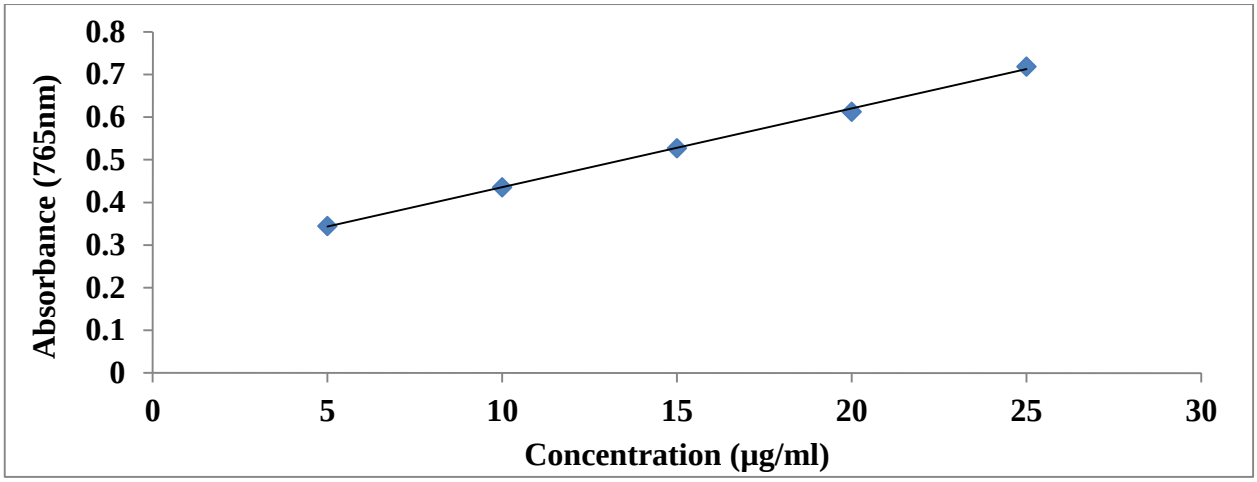

Figure 1: Graph showing the calibration curve of standard phenolic tannic acid.

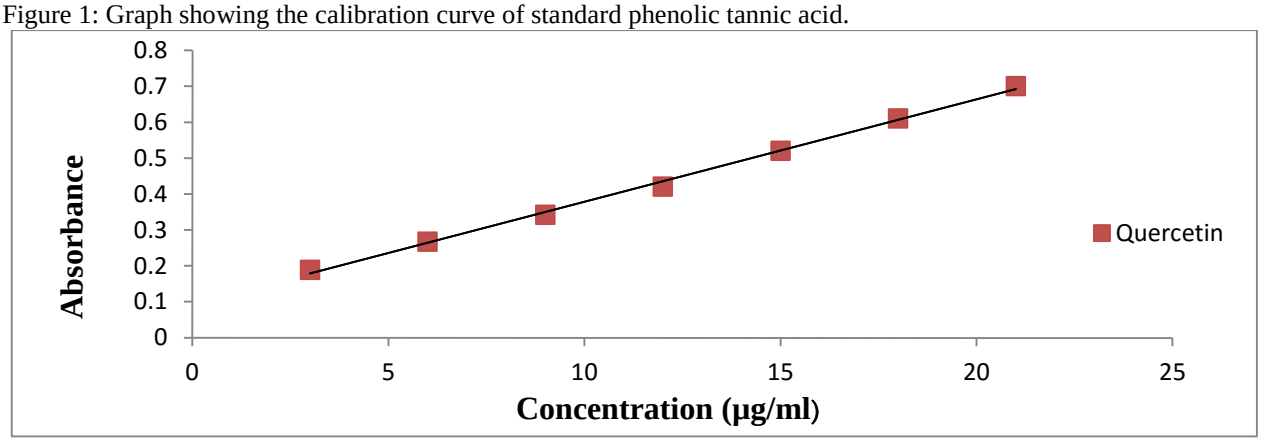

Figure 2: Graph showing the calibration curve of Quercetin dehydrate.

Pet ether extract and methanol extract of *C. sappan* leaves were tested against the earthworms (*Eisenia foetida*). Accurately weight amount of the extracts were dissolved first in small amount of dimethylformamide (DMF) then the volume was adjusted with normal saline to prepare three concentrations of 20 mg/ml, 40 mg/ml and 60 mg/ml. Albendazole solution (10 mg/ml) in normal saline was prepared as reference standard. Normal saline without extract/standard drug was used as negative control[12]. Groups of 6 worms (of 3 – 5 cm length and 0.1 – 0.2 cm width) placed in petriplates were separately treated with 10 ml solutions of sample extracts, negative control (vehicle) and standard drug (Table 8.). The worms were individually observed for recording the time taken for their paralysis and death. The time of paralysis was confirmed when no movement of any sort was observed except the worms were shaken vigorously. Time of death was noted after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water (50 ºC) and their body colour faded off. The experiment was carried out in triplicate and the data was reported as mean± SEM of the triplicates (Table 9.). Results of the extracts anthelmintic activity were compared with that of standard drug Albendazole and the data was statistically analysed using one way analysis of variance (ANOVA) followed by Dunnet test ($p< 0.05$).

RESULTS AND DISCUSSION

Table 5: Data showing the total flavonoid content of *C. sappan* leaves extracts.

Plant extract	Total flavonoid content (µg/mg)
Petroleum ether	2.18±0.043
Dichloromethane	12.60±0.052
Ethylacetate	9.19±0.057
Methanol	14.30±0.057

Values are the average of triplicate experiments and represented as Mean ± SEM

Qualitative analysis

Caesalpinia sappan leaf extracts were qualitatively analysed as per described methods[21-23]. As shown in Table 1 performed qualitative tests in present study revealed the presence of carbohydrates, flavonoids, glycosides, phenols, tannins, amino acids and proteins but absence of phytosterols, alkaloids and saponins in the crude drug. However, these preliminary tests are not rejecting presence of other components that were not found in this study.

More recently, presence of resin, terpenoids, quinone, steroids and alkaloids along with tannins and flavonoids are reported in aqueous extract of *C. sappan* leaves.

TPC and TFC results

Caesalpinia sappan leaf extracts total phenolics content (TPC) and total flavonoids content (TFC) were measured using spectrophotometric methods[24, 27]. The results of TPC and TFC estimation are shown in Tables 4, and 5. Phenolic

Table 6: Data showing the percentage free radical scavenging of *C. sappan* extracts by DPPH free radical scavenging method.

Conc. (µg/ml)	% scavenging				
	PE	DCM	EA	M	AA
5	17.4±0.0	54.3±0.01	50.1±0.06	60.3±0.04	62.3±0.01
10	22.2±0.01	61.7±0.0	56.3±0.07	68.5±0.01	70.5±0.01
15	28.6±0.01	66.3±0.01	61.7±0.01	74.3±0.01	78.5±0.01
20	33.6±0.0	73.5±0.0	66.3±0.01	79.5±0.01	85.1±0.02
25	37.6±0.02	79.7±0.0	70.7±0.01	82.9±0.02	89.5±0.01

PE = petroleum ether extract, DCM = dichloromethane extract, EA = ethyl acetate extract, M=methanol extract of leaves, AA = standard Ascorbic acid.

Table 7: Absorbance of various concentrations of extracts of *C. sappan* after reduction of Fe3+ to Fe+2.

Conc. (µg/ml)	Absorbance (700nm)			
	DCM	EA	M	AA
5	0.210±0.10	0.132±0.21	0.280±0.02	0.532±0.01
10	0.280±0.02	0.180±0.21	0.372±0.07	0.631±0.03
15	0.350±0.01	0.246±0.11	0.425±0.09	0.716±0.05
20	0.402±0.01	0.325±0.02	0.483±0.06	0.793±0.03
25	0.489±0.04	0.396±0.03	0.525±0.04	0.889±0.01

DCM=dichloromethane extract, EA= ethylacetate extract, M=methanol extract and AA=standard Ascorbic acid.

compounds and flavonoids are proved to have potent pharmacological activities including antioxidant activity. The phenolic compounds are known to be chain breaking antioxidants and they have scavenging ability due to their hydroxyl groups. Thus, the determination of the phenolic content of botanicals might lead to more fruitful results regarding their antioxidant potential. Theses compound along with many other phytochemicals are present in different amounts in almost every edible and medicinal plant organs[15,16]. Currently, it is claimed that that the synthetic antioxidants at certain level produces toxic effects. It is assumed that plant-derived compounds from vegetables, fruits, tea, juice, etc. may take parts in balancing the antioxidant status of the body. Recently a great interest has been dropped toward the natural antioxidants from plant origin[14]. Phytochemistry of *C. sappan* has been reported by several authors and presence of different plant secondary metabolites including flavonoids, polyphenols, alkaloids, tannins, etc. in this plant are reported in previous works[2,3,6,7,8,9]. Consumption of botanicals in daily diet plays very important role in because of their natural antioxidants such as different flavonoids, phenolics, tannins, etc. The antioxidant action of phenolic compounds is essentially due to their contributions in redox reactions by which they can absorb and neutralize free radicals such as ROS[14]. Several phenolics e.g. dibenzoxocins, flavones, homoisoflavonoids, chalcones, xanthone and brazilin are identified in *C. sappan* heartwood[16]. In present study, total phenolic content for *C. sappan* leaf was calculated and expressed as microgram of tannic acid equivalents per mg of dried extract (µg TAE/mg dry wt. ext.). TPC of methanolic extract, dichloromethane extract and ethyl acetate extract of the crude drug was measured to be 14.05±0.023, 6.32±0.02 and 10.09±0.01 µgTAE/mg respectively (Table 4). Total flavonoids content of *C. sappan* leaf pet ether extract, dichloromethane extract, ethylacetate extract and methanolic extract were expressed as quercitin equivalent (µgQE/mg dry wt. of extract) and were found to be 2.18±0.043, 12.60±0.052, 9.19±0.057 and 14.30±0.057 respectively (Table 5).

Table 8: Allocation of earthworms to various groups

Group no.	Group name	Dose(mg/ml)
I	Control group (vehicle)	-
II	Test group pet. ether extract	20
III	Test group pet. ether extract	40
IV	Test group pet. ether extract	60
V	Test group methanol extract	20
VI	Test group methanol extract	40
VII	Test group methanol extract	60
XI	Standard group (Albendazole)	10

Antioxidant activity evaluation

Free radical scavenging activity and reducing power of petroleum ether extract, dichloromethane extract, ethyl acetate extract, and methanol extract of *C. sappan* leaves were evaluated by DPPH method and FRAP method. The results were compared with that of standard i.e. ascorbic acid. As shown in Table 6. and Table 7 the highest free radical scavenging and Fe^{3+} ion reducing potential were at conc. 25µg /ml, for all of the extracts. The activities increase with increasing concentration of all the extracts, hence a dose dependent manner of activity was observed for all of the extracts. As per present study, among the tested extracts of *C. sappan* leaves, the methanol extract at dose 25µg/ml exhibited the highest % inhibition of 82.9±0.02 and highest reducing power of 0.525±0.04. Standard ascorbic acid showed % inhibition of 89.5±0.01 and reducing power of 0.889±0.01 at the same dose of 25µg/ml. DPPH assay is widely used for determination of antioxidant potential of botanicals[1,17]. DPPH is a stable free radicle. Its freshly prepared solution in methanol has dark purple colour and while reduced by antioxidants its colour turns yellow[16,28]. Antioxidant are generally the

Table 9: Data showing the anthelmintic activity of *C. sappan* leaves extracts.

Group	Conc.(mg/ml)	Paralysis time (min.)	Death time (min.)
Control (normal saline)	-	-	-
Pet ether extract	20	22.38±0.115	74.37±0.536
	40	21.04±0.248	72.22±0.545
	60	19.69±0.194	70.12±0.283
Methanol extract	20	20.93±0.318	65.50±0.429
	40	19.94±0.282	60.03±0.268
	60	19.13±0.340	54.21±0.533
Albendazole	10	6.06±0.371	8.17±0.340

Values are the average of triplicate experiments and represented as Mean ± SEM.

compounds which neutralize free radicals or their actions[15.17]. As reported in many publications the free radical scavenging activity and reducing power of botanical extracts are well correlated with their total phenolics and total flavonoids[25,29]. In our study also a positive linear correlation was found between TPC/TFC and free radical scavenging/ion-reducing activity of *C. sappan* extracts. While methanolic extract was found the potent one among the tested extracts. Potent antioxidant activity of *C. sappan* heartwood methanol extract[30], hydro-alcoholic extract[10], ethanol 95% extract and isolated constituent of the heartwood e.g. prosappanin A, protosappanin B and brazilein[31] are reported in previous works. Ethanol extract of *C. crista* seeds is also reported to have strong antioxidant activity in vitro[1]. In another study, among five different solvent extracts of *Marrubium peregrinum* L. methanolic extract revealed high concentrations of phenolics and flavonoids and potent antioxidant property[28]. Nikolova et al (2011) have evaluated 57 methanolic extracts of 53 plants species pertaining to 30 families for their antioxidant activity using DPPH assay method[16].

Anthelmintic activity

Petroleum ether extract and methanol extract of *C. sappan* leaves were evaluated against earthworms (*Eeseinia foetida*). The methanolic extract exhibited marked anthelmintic activity. As shown in Table 9 the extracts at doses of 20 mg/ml, 40 mg/lm and 60 mg/ml exhibited dose dependent anthelmintic activity against the earthworms. The paralysis time and death time of earthworms caused by different concentration of the extracts were compared with those caused by the standard anthelmintic Albendazole (10 mg/ml). In this study, methanol extract of *C. sappan* leaves was found to have stronger anthelmintic activity than that of pet ether extract. Both paralysis and death time for methanolic extract at dose 60 mg/ml were recorded to be 19.13±0.340 and 54.21±0.533 respectively. In previous studies anthelmintic activity of ethanol extract and aqueous extract of *Caesalpinia sappan* bark against *Pheritima posthuma* was investigated and the first one is reported to be more potent[12]. Likewise methanol extract of some other botanicals are reported for their better desired effect. Mahajan et al (2014), reported prominent anthelminthic activity of *Punica granatum* fruit peel methanol extract tested in vitro against *Pheritima posthuma*[32]. It can be concluded that the potent activity of methanol extracts may be due to presence of more polar bioactive phytochemicals such as tannins, phenolics, alkaloids, glycosides, etc. in this polar solvent[28]. Commonly alcoholic extracts are reported for their potent biological activities. Although modern medicines may be available, due to socio-economical, cultural and historical reasons, herbal medicines have maintained their importance. *Caesalpinia sappan* is used traditionally for various medicinal purposes medical purposes[2,4]. As per our understanding, anthelmintic activity of *C. sappan* leaf extract is reported for the first time in this paper.

CONCLUSION

The result of this study is evident of potent antioxidant and anthelmintic properties of *C. sappan* leaves. As per present study, *C. sappan* leaf methanolic extract can serve as potent antioxidant and anthelmintic agent in preventing oxidative stress and helminthiais. Presence of various constituents e.g. phenolic, flavonoids, etc. in the crude materials can be the reason of potent pharmacological activities. Further studies on isolation of phyto-constituents and their biological activities in vitro and in vivo, and developing of new anthelmintic medicines from *C. sappan* leaves are suggested to be carried out.

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