

COMPARATIVE ANTI-OXIDANT AND ANTHELMINTIC ACTIVITY OF *DALBERGIA SISSOO* ROXB EX DC AND *DALBERGIA LATIFOLIA* ROXB EX DC

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ABSTRACT

Objective: Anti-oxidant and anthelmintic activity of the ethanolic extracts of the leaves of the *Dalbergia sissoo* Roxb. ex DC and *Dalbergia latifolia* Roxb. ex DC were measured by means of the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay and Indian earth worms respectively.

Methods: A synthetic antioxidant, Butylated hydroxy anisole (BHA) was used as a standard in antioxidant assay whereas Albendazole was used as standard in anthelmintic activity.

Results: The results revealed that the ethanolic extracts of both the leaves of the *Dalbergia sissoo* and *Dalbergia latifolia*. showed maximum antioxidant activity at 300 µg/ml and highest anthelmintic activity at 200 mg/ml.

Conclusion: The present study indicates the potential usefulness of *Dalbergia sissoo* and *Dalbergia latifolia* leaves as an anti-oxidant and anthelmintic.

Keywords: Anti-oxidant, Anthelmintic activity, *Dalbergia sissoo*, *Dalbergia latifolia*, DPPH, Indian earth worms.

INTRODUCTION

Reactive oxygen species (ROS), such as superoxide anions, hydrogen peroxide, and hydroxyl, nitric oxide and peroxy nitrite radicals, are producing the oxidative stress. They are responsible for the pathogenesis of various important diseases [1]. In humans, the production of free radicals is balanced by the anti oxidative defense system; however, oxidative stress is generated when equilibrium favors free radical generation as a result of a depletion of antioxidant levels [2]. The oxidation of lipid, DNA, protein, carbohydrate, and other biological molecules by toxic ROS may cause DNA mutation or/and serve to damage target cells or tissues, and this often results in cell senescence and death. Antioxidants have been suggested for the Cancer chemo prevention [3]. It offer a good potential in providing important fundamental benefits to public health, and is now considered by many clinicians and researchers as a key strategy for inhibiting, delaying, or even reversal of the process of carcinogenesis [4].

The antioxidant present in the markets is mostly synthetic such as Butylated hydroxy anisole (BHA), Butylated hydroxyl toluene (BHT), tertiary butylated hydroquinone (TBHQ) and Gallic acid esters [5]. Such synthetic antioxidants are known to have potential side effects and toxicity in *in-vivo* condition. Hence their use is being restricted nowadays and there is increase interest in finding out safer and bioactive natural antioxidant present in plant species [6].

Helminth infections are the most common infections in man that affect large proportions of the world's population [7]. Most diseases caused by helminths are chronic and debilitating in nature. They probably cause more morbidity, greater economic and social deprivation among humans and animals than any other parasites. Helminthiasis is very common in regions with poor sanitation, poor family hygiene, malnutrition and crowded living condition. It has been estimated that about half of the world's population suffers from helminthiasis and the number is increasing. In the treatment of helminthiasis, anthelmintic drugs are used irrationally.

Anthelmintics or anti helminthics are drugs that expel helminth parasitic worms (helminths) from the body, either by stunning or killing them. They may also be vermifuges (stunning) or vermicides (killing). However, they have shown the development of resistance to some broad spectrum anthelmintics (Benzimidazoles, Levamisole, and Avermectins) and also some narrow spectrum wormers such as

the Salicylanilides (Closantel). Anthelmintic resistance is a major problem for the control of many parasitic nematode species and has become a major constraint to livestock production in many parts of the world. Due to the prevalence of parasitic infections and the developed resistance of some anthelmintic drugs is now an enclosing area in the field of research. Hence there is an increasing demand towards natural anthelmintics [8].

There have been several studies on the antioxidant and anthelmintic activities of various herbs/plants with medicinal values. The present study aims to investigate the free radical scavenging activities and anthelmintic activity of the ethanolic extract of the leaves of *Dalbergia sissoo* Roxb. ex DC and *Dalbergia latifolia* Roxb. ex DC. They are commonly used in the traditional treatment of various diseases in India.

Dalbergia sissoo Roxb. ex DC (Family-Fabaceae) also called 'shisham' is used time immemorial for treatment of various ailments like burning sensations, dysentery, dyspepsia, leucoderma, and skin ailment, anti-inflammatory, memory enhancer and leaves have significant levels of flavonoids which showed antioxidant activity twice of commonly used antioxidants like vitamin C and selenium [9]. It possesses antioxidant and prevents central nervous system damage [10].

Dalbergia latifolia Roxb. ex DC (Family-Fabaceae) is a premium quality timber species internationally known as 'Indian rosewood' a large glabrous tree a single stem with characteristic smell [11]. It is distributed in Bihar, Bundelkhand and Central India. It's seeds contain dalbinol a new 12a-hydroxyrotenoid, sisafolin coumarin. Bark contains β-sitosterol, also contain dalbergichromene, lupeol, latifolin and dalbergin. Heartwood contains latinone, neo flavonoid dalcridon and Latinone, a substituted phenanthrene-1, 4-quinone. Ethanomedicinally, the stem barks contain tannin is used for treatment of leprosy, obesity and worm.

MATERIALS AND METHODS

Collection of plant material

The plants *Dalbergia sissoo* Roxb. ex DC. and *Dalbergia latifolia* Roxb. ex DC. are native to the Indian subcontinent and Southern Iran. In the present work, the plants were collected from Satpal Arbitorium,

Ponda, Goa-403 401. These plants were identified and confirmed by the botanist, Dr. M. K. Janarthanan, Head, Department of Botany, Goa University, Goa.

Extraction of plant material

The leaves of *Dalbergia sissoo* Roxb. ex DC. and *Dalbergia latifolia* Roxb. ex DC were dried under shade and powdered. The dried powdered (500 gram) was successively extracted using Petroleum ether (60-80 °C), chloroform, ethanol and water by refluxing separately. The last trace of solvent was removed under by distillation and then vacuum dried. Finally the dried crude extracts were used for the study.

Worm collection and authentication

Endrullus enguinae (earth worm) nearly equal size (6 cm) was collected from Ella-Farm, Vermi-culture department, old-Goa, Goa-403402.

Anti-oxidant activity

DPPH Free radical scavenging activity [12]

The free-radical scavenging activity of the ethanolic extracts of the leaves of *Dalbergiasissoo* Roxb. ex DC and *Dalbergia latifolia* Roxb. ex DC were measured by the decrease in the absorbance of methanol solution of DPPH. Methanolic solution (100 µl) of different concentrations (100µg/ml, 200µg/ml, 300µg/ml) of extracts and Butylated hydroxyl anisole were mixed with five milliliters of a 0.1 mM methanolic solution of 1,1-diphenyl-2-picryl hydrazyl (DPPH) solution.

After an incubation period of 20 min at 25 °C, the absorbance was measured at 517 nm. Scavenging activity was expressed as the percentage inhibition calculated using the following formula.

$$\% \text{ Free radical scavenging activity} = \frac{\text{Control Abs} - \text{Standard Abs}}{\text{Control Abs}} \times 100$$

The results were presented in Table.1

Anthelmintic activity

The anthelmintic activity was performed according to the Ghosh T et al. Method [13]. On adult Indian earth worm *Endrullus enguinae* as it has anatomical and physiological resemblance with the intestinal round worm parasites of human beings. *Endrullus enguinae* was placed in petridish containing four different concentrations (50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml,) of the ethanolic extract of the leaves of *Dalbergia sissoo* Roxb. ex DC and *Dalbergia latifolia* Roxb. ex DC separately. Each petri dish was placed with 4 worms and observed for paralysis (or) death.

The mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; the time death of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli. In the same manner albendazole was included as standard compound. The test results were compared with standard compound Albendazole (15 mg/ml) treated samples. The results are presented in table. 2.

RESULTS

Table 1: Anti-oxidant activity of ethanolic extract of the leaves of *Dalbergia sissoo* Roxb ex DC and *Dalbergia latifolia* Roxb ex DC. by DPPH free radical scavenging method

Concentration of ethanolic extract (µg/ml)	Free radical scavenging activity (%)		
	<i>Dalbergia sissoo</i>	<i>Dalbergia latifolia</i>	BHA
100	3.62	35.29	15.07
200	8.12	41.88	33.62
300	13.19	46.74	44.28

Table 2: In vitro anthelmintic activity of ethanol extracts of the leaves of *Dalbergia sissoo* Roxb ex DC and *Dalbergia latifolia* Roxb ex DC

Groups	Drug	Concentration (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
Group-1	Albendazole (Reference)	15 mg/ml	37±0.2	53±0.3
Group-2	Ethanol extracts of the <i>Dalbergia sissoo</i>	50 mg/ml	27±0.3	62±0.2
Group-3		100 mg/ml	20±0.4	42±0.5
Group-4		150 mg/ml	15±0.3	30±0.4
Group-5		200 mg/ml	13±0.6	25±0.2
Group-6		Ethanol extracts of the <i>Dalbergia latifolia</i>	50 mg/ml	25±0.3
Group-7	100 mg/ml		20±0.4	45±0.5
Group-8	150 mg/ml		13±0.3	33±0.4
Group-9	200 mg/ml		9±0.6	27±0.2

Results are expressed as mean±Standard error mean (n=4).

DISCUSSION

The DPPH test provides information on the reactivity of the test compounds with a stable free radical. DPPH gives a strong absorption band at 517 nm in the visible region. When the odd electron becomes paired off in the presence of a free radical scavenger, the absorption reduces and the DPPH solution is decolourised as the colour changes from deep violet to light yellow. The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract [14]. The Ethanol extracts the leaves of *Dalbergia latifolia* Roxb ex DC were more active antioxidant than *Dalbergia sissoo* Roxb ex DC in all the three concentrations (100µg/ml, 200µg/ml, 300µg/ml). However *Dalbergia latifolia* Roxb ex DC appeared to be as potent as BHA with a maximum inhibition of 46.74% at 300 µg/ml which is comparable to 44.28% for BHA at the same concentration.

Anthelmintics are drugs that may act locally to expel worms from the GIT or systemically to eradicate adult helminthes or development forms that invade organs and tissues. Helminthiasis or infections with parasitic worms are pathogenic for human beings [15]. Immature forms of the parasites invade human beings via the skin or gastrointestinal tract (GIT) and evolve into well differentiated adult worms that have characteristic tissue distribution. Tannins produce anthelmintic activity by binding to free protein in the gastrointestinal tract of the host animal[16] or glycoprotein on the cuticle of the parasite[17] and phenolic compounds by uncoupling oxidative phosphorylation hinder the energy production in helminth parasites[18] and may cause death. Alkaloids may act on the central nervous system and cause paralysis of the earth worm [19]. The effect would be due to presence of the steroidal alkaloid oligoglycosides which may suppress the transfer of sucrose from the stomach to the small intestine together with its

antioxidant effect which is capable of reducing the nitrate generation which could interfere in lical homeostasis which is essential for the development of helminthes[20]. Phytochemical analysis of the ethanol extracts the leaves of *Dalbergia sissoo* Roxb ex DC and *Dalbergia latifolia* Roxb ex DC. revealed the presence of tannins, alkaloids and phenolic as compounds.

The progress of Anthelmintic activity of both the ethanol extracts of *Dalbergia sissoo* Roxb. ex DC and *Dalbergia latifolia* Roxb. ex DC at four different concentrations such as 50 mg/ml, 100 mg/ml, 150 mg/ml and 200 mg/ml in earth worms are presented in Table.2. From the Table, it is clear that the both the higher concentration (200 mg/ml) of extract produced paralytic effect much earlier and the time to death was shorter for all worms. The results are compared with that of the standard Albendazole. The ethanol extracts of *Dalbergia sissoo* Roxb. ex DC and *Dalbergia latifolia* Roxb. ex DC showed anthelmintic activity in the dose-dependent manner giving the shortest time of paralysis and death.

CONCLUSION

The present study revealed significant anti-oxidant and anthelmintic activities of the ethanol extract of *Dalbergia sissoo* Roxb. ex DC and *Dalbergia latifolia* Roxb. ex DC. Both activities were dose dependent. The *Dalbergia latifolia* Roxb. ex DC was the better antioxidant than *Dalbergia sissoo* Roxb. ex DC. Whereas *Dalbergia sissoo* Roxb. ex DC was the better anthelmintic than *Dalbergia latifolia* Roxb. ex DC. The results obtained provide support for the traditional use of both the plant for curing wounds and cuts which confirm the presence of active chemical compounds related to these activities. However, further investigations are required to isolate the active constituents responsible for these activities and to elucidate the exact mechanisms of action.

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CONFLICT OF INTERESTS

Declared None

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