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Antioxidant, Cytotoxic, and Antimicrobial Activities of *Pterocarpus santalinus* Leaves

Sumiya Sharmin Mou[1,2], Fatema-Tuz-Zohora[2], Seagufta Afrin[3], Md. Solaiman Hossain Tuhin[4,5], Md. Abdul Muhit[4], Akash Kumar Bhawmick[6] and Firoj Ahmed[7]

[1]Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka, Dhaka, Bangladesh.
[2]Department of Pharmacy, University of Asia Pacific, Dhaka, Bangladesh.
[3]Independent University, Bangladesh, Department of Pharmacy, Bashundhara, Dhaka, Bangladesh.
[4]Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka, Bangladesh.
[5]Department of Pharmacy, State University of Bangladesh, South Purbachal, Kanchan, Dhaka, Bangladesh.
[6]Department of Botany, Jagannath University, Sadarghat, Dhaka, Bangladesh.
[7]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka, Bangladesh.
*Corresponding Author E-mail:firoj72@du.ac.bd

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This study evaluates the chemical and biological properties of Pterocarpus santalinus leaf methanolic extract to establish its scientific basis for potential therapeutic applications. The phytochemical analysis revealed the presence of 595.04 mg/g flavonoids (quercetin equivalent) and 69 mg/g phenolic compounds (gallic acid equivalent), underscoring its antioxidant potential. The extract exhibited DPPH radical scavenging activity with an IC50 of 337.79 μg/ml, indicating moderate antioxidant capacity. Additionally, mild antimicrobial activity was observed against Escherichia coli and Aspergillus niger. The cytotoxic effects of the extract were assessed in HeLa cells, demonstrating a concentration-dependent relationship. At concentrations of 100 μg/ml and 200 μg/ml, cell viability remained above 95%, indicating non-toxic effects. However, at 500 μg/ml, a significant decrease in cell viability to 20-30% was observed, with an LC50 value of 365.77 μg/ml, indicating substantial cytotoxicity at higher concentrations. These results suggest that while lower concentrations are safe, higher doses may induce cell death, necessitating the establishment of safe dosage ranges for therapeutic applications. The findings support the pharmacological potential of P. santalinus leaf extract, highlighting its relevance in antioxidant, antimicrobial, and anticancer research, and warrant further investigation to refine its medicinal applications.

Keywords: Antioxidant; Antimicrobial Activities; Cytotoxic; Pterocarpus santalinus Leaves.

Pterocarpus santalinus belongs to the Fabaceae family and is commonly known as Rakta Chandan (Red Sandalwood) or Rakta Chandal. [1] This Asian species is native to the southern Eastern Ghats Mountain range of South India and can also be found in China, Pakistan, Sri Lanka, and Taiwan. [2] In Bangladesh, notable habitats for *P. santalinus* include Dinajpur, Rajshahi, and Natore. Due to

over-exploitation of its timber in South India, it is listed in both the CITES appendix II and the IUCN's list of threatened species. [3-4] It has been used traditionally as an anthelmintic, diaphoretic, antipyretic, anti-inflammatory, aphrodisiac, and anti-hyperglycemic drug. [5] It has been stated that wood is prescribed in conjunction with other medications to treat poisonous incidents such as scorpion stings and snake bites. About herbal and ayurvedic treatment has been noteworthy. [6-7] So many studies have found parts of this plant or the compounds from this plant to be potential therapeutic agents against Diabetics, liver diseases, cancer treatment, and so on. [8] We are motivated to investigate the various chemical and biological characteristics of the methanolic extract of *P. santalinus* leaves to confirm their ethnopharmacological application and establish their important therapeutic capabilities in Bangladesh, based on prior traditional use and scientific research. Various scientific investigations recognized *P. santalinus* as a highly impressive indigenous herb. As it has been a hot cake for researchers for the last 2 decades. [9] In most cases, the concentration was on the wood especially. Red sandalwood leaves are of great interest in the field of ethnopharmacology, particularly in the Indian region. This has led to a surge in research on this topic in South Asian countries. All the species of red sandalwood are of Indian origin. It is important to consider the Bangladeshi species in order to identify medicinal properties that could contribute to the development of phytochemicals in this region.[10]

In our investigation, we are going to focus on how the leaves can be used in the medicinal sector, especially in Bangladesh. Thus, finding out some essential properties of the methanolic extract of *P. santalinus* that was obtained from the northern part of Bangladesh, will justify the main objective of our study and will be a basis to justify other studies.

Review of literature on *Pterocarpus santalinus*

Pterocarpus santalinus, or Red Sanders, is a tree native to India that possesses significant pharmacological properties and has a long history of use in Ayurveda. Current research confirms its medicinal efficacy across several domains.

Extracts from *P. santalinus* have been demonstrated to inhibit cancer cell proliferation and induce apoptosis, suggesting its potential as an anticancer agent. The plant also reduces pro-inflammatory cytokines, alleviating pain and swelling in conditions such as arthritis.

Additionally, *P. santalinus* exhibits antibacterial and antifungal activities against various pathogens, indicating its role as a natural antimicrobial agent. Its strong free radical scavenging properties may help prevent oxidative stress-related diseases, including cancer and cardiovascular conditions.

Moreover, the plant supports liver health by lowering elevated liver enzymes and promoting regeneration, while also enhancing cognitive function and providing neuroprotection through its antioxidant effects. So, *Pterocarpus santalinus* shows significant promise in treating various diseases and merits further research to explore its full therapeutic potential.[11, 12, 13]

MATERIALS AND METHODS

Methods of collection and plant material preparation

The plant sample was collected from the Natore district in 2010 and was authenticated by Mr. Mustafizur Rahaman, Associate Professor, Department of Botany, University of Rajshahi, Bangladesh. Fresh green leaves were collected and dried in a shaded area. The dried form was used to make powder, and then maceration was done for 7 days. During this step methanol was used using occasional stirring. Filtration was done in the next phase to eliminate the residual particles. Evaporation will be done to have a semisolid extract (MELM) (yield 8.5% w/w).

Estimation of Total Phenolic Content

The phenolic content was estimated using the Folin-Ciocalteu reagent (FCR) with gallic acid as the reference standard. The crude extract was dissolved in methanol, diluted, and mixed with FCR. After adding Na_2CO_3 and incubating the mixture, the absorbance at 750 nm was measured to determine the phenolic content in Gallic acid equivalent (GAE) per gram of extract. Absorbance = 0.006x + 0.039 (x is the Gallic acid equivalent). [14]

Quantitative analysis of total flavonoid content

The calorimetric aluminum chloride technique was used to determine flavonoids.

Quercetin was used as the measure of total flavonoid content. The concentration of flavonoid content was calculated using the equation Absorbance = 0.0067x + 0.0132, where x denotes the Quercetin equivalent and Y denotes absorbance. [15-16]

DPPH free radical scavenging activity

A stable radical called DPPH was used to test our sample's scavenging activity. The radical gets reduced in the presence of antioxidants, and its absorbance changes. Different concentrations of the extract were prepared, and their absorbance was measured. The percent inhibition was also calculated. The inhibition percentage is determined using the formula I% = (1 " Asample/Acontrol) × 100. The desired concentration for inhibiting 50% was determined from a calibration curve plotting %inhibition against different concentrations. [17]

Total phenolic and flavonoid content

The total phenolic contents of the *P. santalinus* crude extract are 69 mg/g, measured as gallic acid equivalent. The presence of flavonoids and phenolics in the plant may be related to its biological activity. These chemicals give the plant its antioxidative qualities, making it valuable as an herbal medicine. [18]

Antimicrobial activity

The agar disc diffusion method was employed for the determination of antimicrobial activities. [19]

Cytotoxic activity

The Center for Advanced Research in Sciences used commercial services to evaluate the cytotoxic effect on HeLa cells. The cells were seeded onto 96-well plates and incubated at 37ºC with 5% CO_2. After 48 hours, cytotoxicity was assessed using an inverted light microscope. Duplicate wells were utilized for each sample. [20-21]

Table 1. Antimicrobial activity of *P. santalinus*

Test organism	Zone of inhibition of Standard (mm) Disc of Ciprofloxacin 30 µg/ml	Zone of inhibition of Sample (mm) (200µg/ml)	Zone of inhibition of Sample (mm) (400µg/ml)
E. coli	40	7	7.4
Aspergillus niger	40	6.5	6.8

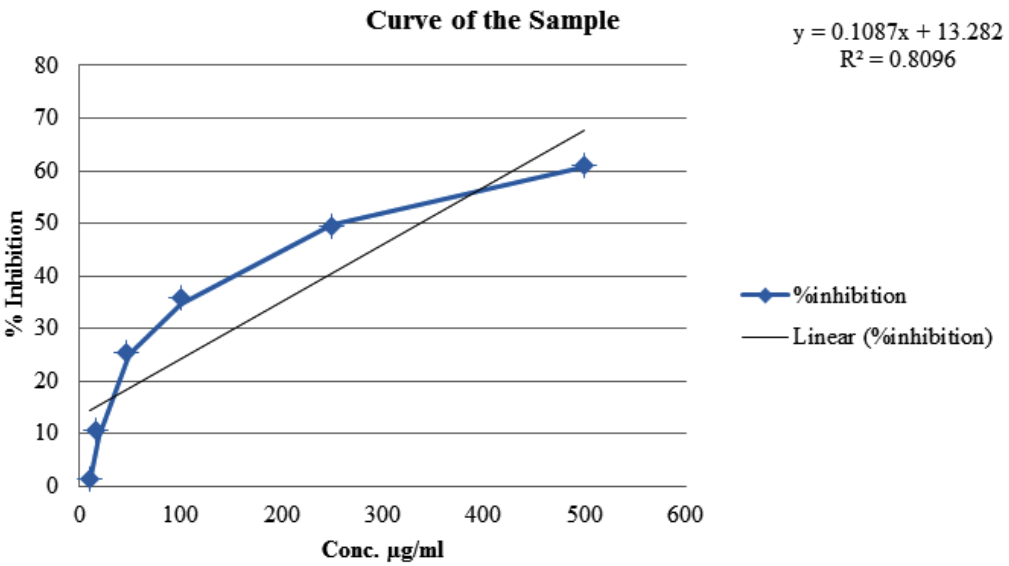

Fig. 1. Plot of % Inhibition vs Concentration of test sample to find IC_{50}

RESULTS

Antioxidant activity

The aluminum chloride spectrophotometric method revealed that the amount of flavonoids in the plant extract is 595.0447 mg/g in methanolic extract. Research has shown that flavonoids affect membrane permeability and inhibit membrane-bound enzymes, contributing to the plant extract's antioxidative action.

From The Graph above we got an equation. By Plotting 50 at the place of Y, we can easily find our desired IC_{50}, which can indicate the Conc. required Inhibiting 50%. (Figure 1).

Antimicrobial activity

Two portions of the methanolic extract were used in the experiment. The sample (200µg/ml and 400µg/ml) had a zone of inhibition of 7 mm & 7.4 mm and 6.5 & 6.8 mm against *E. Coli* and *Aspergillus niger*, respectively. Maximum activity of the leaf extract was seen against *Escherichia coli*, while *Aspergillus niger* exhibited only minimal activity. Activity that depends on concentration is observed (Table 1 & Figure 2,3). Therefore, some of the molecules that give it its antibacterial activity may be present

Cytotoxic activity

There are previous data about the ethanolic extract of *P. santalinus* about its effect on tumour growth. When looking at how methanolic extract affected Hela cells, it was discovered that over 95% of cells survived when the crude extract was present; however, when the extract was used at a greater concentration of 500 µg/ml, the survival

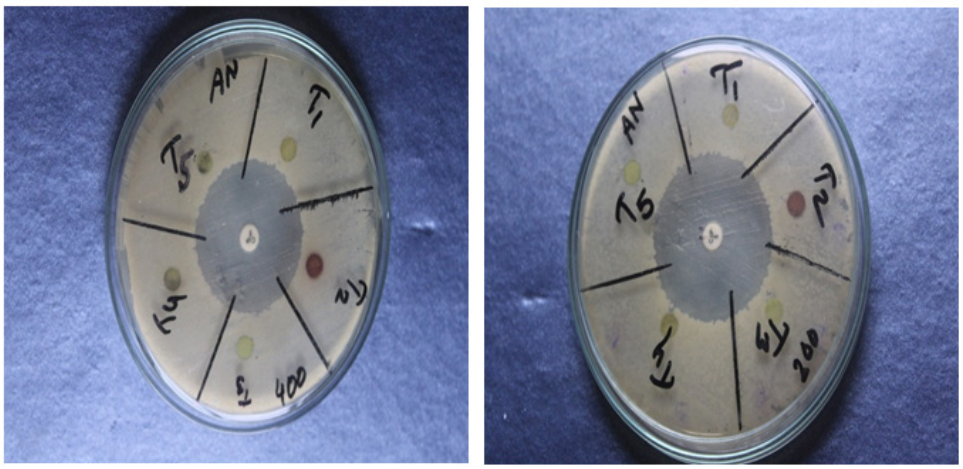

Fig. 2. Antimicrobial sensitivity test on *Aspergillus Niger* with 400µg/ml & 200µg/ml of sample

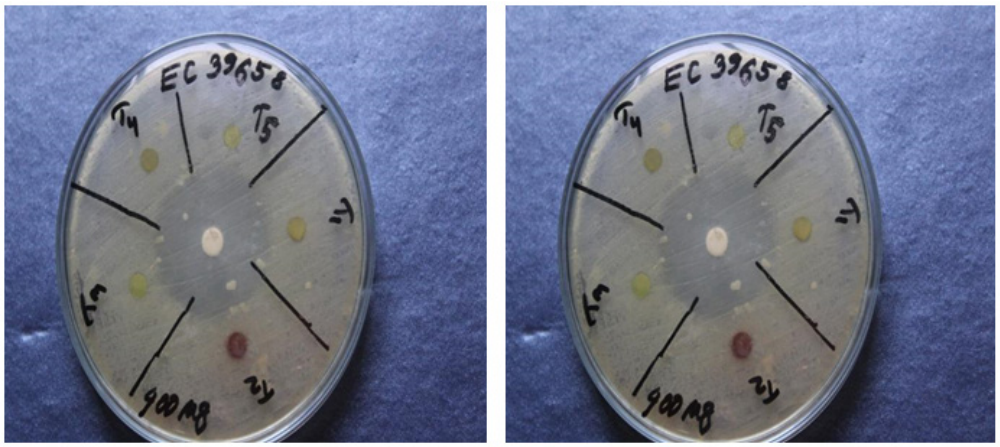

Fig. 3. Antimicrobial sensitivity test on *E. coli* with 200µg/ml & 400µg/ml of sample extract

rate drastically changed(Table 5). By increasing the concentration, the survival rate dropped to between 20 and 30 percent. Few studies indicate that a cellular survival rate of less than 40% indicates a markedly cytotoxic environment. This indicates that the methanolic extract has cytotoxic properties, as seen by its killing rate of 70–80% (Figure 5). As a result, after considering the data and analyzing the study's findings, it is possible to conclude that the substance has cytotoxic properties.

According to this study, the extract may have some effect on HeLa cells. A human cervical cancer cell line was kept in Dulbecco's Modified Eagle's Medium (DMEM), which contained 10% fetal bovine serum, 0.2% gentamycin, and 1% penicillin-streptomycin (1:1). Cell cytotoxicity was observed for sample 500µg on HeLa cell line and the calculated LC_{50} is 365.77µg/ml (Table 3 & Figure 4, 5).

Statistical Summary

Mean Survival Rate: The average survival rate across the three concentrations is 71.67%.

Standard Deviation

The variation in survival rates across concentrations is 33.04%, indicating that the survival rate varies considerably due to the higher concentration.

Table 3. The data obtained from laboratory analysis on Hela cell line

Sample	Conc. (µg/ml)	Survival of HeLa cell	LC_{50} (µg/ml)	Cytotoxic effect
P. Santalinus	100	>95%	365.77	No
	200	>95%		No
	500	20-30%		Yes

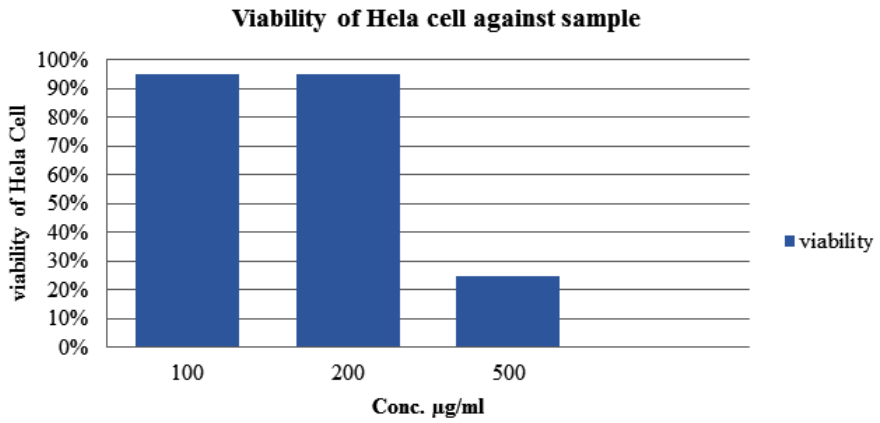

Fig. 4. Viability of HeLa cell against test sample

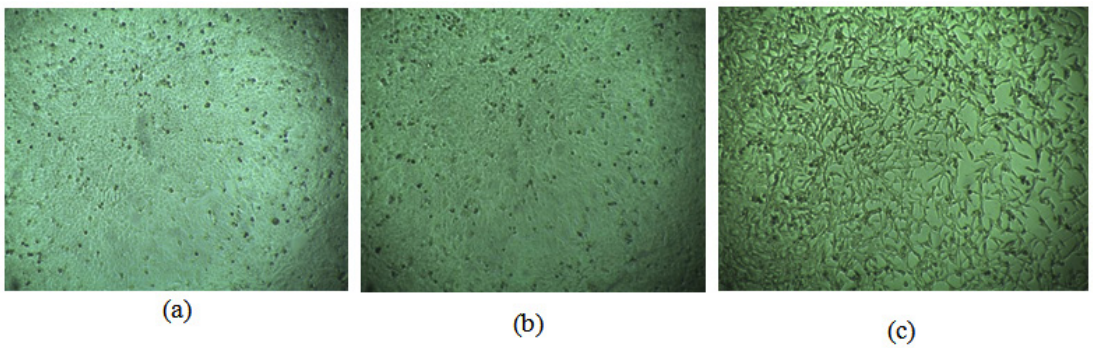

Fig. 5. Survival percentage of HeLa cells (a) > 95% of cells in the presence of solvent (b) > 95% of cells in the Absence of solvent (c) Survival of 20-30% of cell in 500µg/ml of sample

Pearson Correlation

The strong negative correlation of -0.970 suggests that the extract exhibits a clear dose-dependent cytotoxicity—higher concentrations lead to lower survival rates.

These preliminary calculations support the conclusion that *Pterocarpus santalinus* has a dose-dependent cytotoxic effect on HeLa cells.

DISCUSSION

Natural antioxidants, which are abundant in fruits, vegetables, and medicinal plants, have garnered a lot of attention and in-depth study due to their remarkable ability to scavenge free radicals. [22] This study represents a step towards the identification of natural antioxidants from *P. santalinus* leaf extracts through the use of free radical scavenging assays and assessments of anti-proliferative and antibacterial properties. Among these naturally occurring antioxidants, phenolics are unique because of their direct involvement in antioxidative activity and wide range of biological effects. [23] Our results show a strong relationship between phenolic concentration and antioxidant activity. The importance of total phenolic content in the medical area has been shown by numerous studies. [24] This study explores natural antioxidants from *P.santalinus* leaf extracts, focusing on their efficacy in scavenging free radicals and their anti-proliferative and antimicrobial properties. Phenolics are identified as a key component of these antioxidants. [25] Additionally, flavonoids, present in substantial quantities at 595.0447 mg/g equivalent to standard quercetin, demonstrate multifaceted mechanisms of action including the modulation of membrane permeability and inhibition of key membrane-bound enzymes, thereby elucidating the antioxidative potential of the plant extract. These compelling findings provide robust support for the traditional use of the plant in folk remedies for stress-related maladies and wound healing, emphasizing its significant role in cultural practices such as circumcision rites, bruises, cuts, and sores. The abundance of phenolics and flavonoids in the plant extract reinforces its therapeutic promise, warranting further investigation in both traditional and modern medicinal contexts, with future research avenues focusing on elucidating specific mechanisms of action and conducting clinical trials to validate its efficacy in treating diverse health conditions. [26-27]

The study found that *Pterocarpus santalinus* leaf and stem bark extracts have antibacterial activity against various organisms, including *Escherichia coli* and *Aspergillus niger*. The extract also holds promise as a potential treatment for cervical cancer, but further research is needed. The study also suggests the extract has potential as a treatment for cervix carcinoma, with substantial activity on HeLa cells, but further research is needed to isolate biologically active substances.

CONCLUSION

The study reveals that *Pterocarpus santalinus* leaf and stem bark extracts exhibit antibacterial properties against *Escherichia coli* and *Aspergillus niger*, and have potential for treating cervical cancer. The data indicate that *Pterocarpus santalinus* has a concentration-dependent cytotoxic effect on HeLa cells, with significant cell death occurring at 500 µg/ml. The LC_{50} value of 365.77 µg/ml provides an important benchmark for understanding the extract's potency. The results observed in vitro (cell culture) should be complemented with in vivo studies in animal models to assess the effects and safety of the extract in a living organism. Further studies are recommended to explore its mechanisms of action, efficacy at lower concentrations, and potential therapeutic uses.

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Conflict of interest

The author(s) do not have any conflict of interest

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials

Authors' Contribution

Sumiya Sharmin Mou: Conceptualization, Methodology, Writing – Original Draft; Fatema-Tuz-Zohora: Funding Acquisition; Md. Solaiman Hossain Tuhin: Methodology, Writing – Review & Editing; Md. Abdul Muhit: Conceptualization, Visualization, Supervision, Project Administration; Akash Kumar Bhawmick: Methodology, Writing – Review & Editing; Firoj Ahmed: Resources, Supervision.

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