Study of Antimalarial Activity of *Dalbergia sissoo* Roxb. ex DC. Through β-Hematin Inhibition Assay

Nitin Balkrishna Aher*[1], Laxmikant B. Borse[2], Sandhya L. Borse[3]

[1]Department of Pharmacognosy, Sandip Foundation's Sandip Institute of Pharmaceutical Sciences, Nashik, Maharashtra, India- 422213, Savitribai Phule Pune University, Pune.
[2]Department of Pharmacology, Sandip Foundation's Sandip Institute of Pharmaceutical Sciences, Nashik, Maharashtra, India- 422213, Savitribai Phule Pune University, Pune.
[3]Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Nashik, Maharashtra, India-422213, Sandip University, Nashik.

Abstract

Background: Globally Malaria known as major infectious disease, which causes significant illness and death, particularly in tropical and subtropical regions. As a result of the increasing resistance to traditional antimalarial drugs, there is a need to find new antimalarial drugs from natural sources, such as *Dalbergia sissoo* Roxb., which has been traditionally used to treat Snake bite, fever, infections, and inflammation.

Material and Methods: This study examines the antimalarial potential of *Dalbergia sissoo* leaf extract by assessing its capacity to inhibit beta-hematin formation, a critical process in the detoxification pathway of *Plasmodium falciparum* parasites. The research tis done in-vitro antimalarial testing using UV spectroscopy and FTIR methods.

Results: Due to the presence of phenolic compounds, flavonoids, tannins, and other bioactive substances suggests potential antiplasmodial activity. Since flavonoids and phenolics are known to interfere with hemozoin formation, *Dalbergia sissoo* may be a valuable source of antimalarial compounds.

Conclusion: The findings of this study could support the development of effective antimalarial treatments derived from plants.

Keywords: Malaria, *Dalbergia sissoo* , Antimalarial activity , β-Hematin Inhibition assay , Hemozoin , Flavonoids , Phytochemicals etc.

Introduction

Malaria is a life threatening parasitic disease caused by protozoan parasites of the genus Plasmodium and transmitted to humans through the bites of infected female Anopheles mosquitoes . Despite significant advances in treatment, malaria remains a major public health concern , particularly in tropical and subtropical regions of the world. The disease is primarily caused by plasmodium falciparum and Plasmodium vivax , although P. malariae , P.ovale , P.knowlesi also contribute to the global disease burden . common clinical manifestations include fever , chills , headache , fatigue , nausea , vomiting , and anemia , while severe malaria may lead to cerebral complications, renal failure , multi-organs dysfunction and death [1][2] The increasing emergence of resistance to conventional antimalarial drugs , including chloroquine and artemisinin based combination therapies , poses a serious challenge to malaria control programs worldwide . Consequently , there is a growing need to indentify and develop novel antimalarial agents with improved efficacy and safety profiles [3][4] Medicinal plants have historically served as valuable sources of antimalarial drugs such as Ocimum sanctum, which contains bioactive compounds such as alkaloids, flavonoids, and phenols, has demonstrated the strongest antimalarial activity among the Ocimum species by inhibiting Plasmodium falciparum.[5] Azadirachta indica (Neem) is a significant source of azadirachtin, nimbolide, gedunin, and quercetin, which have been shown to inhibit parasite growth and protein synthesis.[6] Therefore the exploration of plant-derived bioactive compounds remains an important strategy in antimalarial drug discovery.

Dalbergia sissoo roxb. (family fabaceae) is one of the medicinal plant contain bioactive compounds such as flavonoids , phenolic compounds , fatty acids , and terpenoids. These contribute to its anti-inflammatory , antimicrobial , antioxidant and antidiabetic properties making it a valuable source for developing phytopharmaceutical agents.[7][8]

Material and method

Plant material

The plant material leaves used in the present study was collected from the area near Makhmalabad villege, near Nashik

Drying , Pulverization and defatation

These leaves were subjected to shade drying in a well-ventilated area at room temperature for one week, protected from direct sunlight to prevent the degradation of heat- and light-sensitive compounds and to avoid fungal growth. Once completely dry, the leaves were pulverized using a mechanical grinder to obtain a coarse powder, which was stored in an air tight container to protect it from moisture and contamination. Finally, the coarse powder was subjected to defatting using a suitable non-polar solvent, typically petroleum ether, for 4–5 days to remove non-

polar constituents such as fats, waxes, and chlorophyll. This step enhances the efficiency of subsequent extraction by improving the penetration of polar solvents and preventing interference from lipophilic impurities. After the completion of defatting, the solvent was removed, and the defatted marc was dried and used for further extraction.

Extraction

The defatted coarse powder of *Dalbergia sissoo* leaves was subjected to the extraction using the soxhlet apparatus. A measured quantity of the plant material was placed in a thimble and extracted with suitable solvent ethanol which show high level of bioactivity as compare to other solvent [9][13]. The Soxhlet extraction was carried out for several cycles until the solvent in the siphon tube became colorless , indicating exhausting extraction of phytoconstituents.During the process , the solvent was repeatedly evaporated , condensed , and passed through the plant material , ensuring continous extraction of the solution compounds .extraction was continued for 48 hours or until the extract color fades. Extract was filtered after the extraction was completed and concentrated to semi solid sticky mass by evaporating the remaining solvent.[10][14]

EXPERIMENTAL PROCEDURE

A mixture containing 50 µL of 0.5 mg/mL hemin chloride freshly dissolved in 0.1 M NaOH, 100 µL of 0.5 M sodium acetate buffer (pH 4.4), and 50 µ L of the synthesized compound potential anti- malarial drug solution and Positive control used was chloroquine diphosphate, whereas the negative control was distilled water, was put in microtube and incubated at 37 ºC for 18hours. The tube was then centrifuged for 08 minutes at 4000 rpm. The supernatant was removed and the pH of reaction was measured. The final pH of the mixture should be between (5.0-5.2). It is important that the solutions be added to the plate in this order. The solution mixture in the wells were washed with 200 µL DMSO per well to remove free hemin chloride. The plate was centrifuged again, discharging the supernatant afterwards. The β –hematin remaining was then dissolved in 200 µL of 0.1 M NaOH to form an alkaline hematin that can be measured spectrophotometrically. Finally, the absorbance read at 405 nm. Lastly percent inhibition of hematin by compounds was calculated by using following formula. [11][12]

Percent inhibition = Reading of control - Reading of treated cells / Reading of control X 100.

Result and discussion

Percentage yield of Dalberigia sisso roxb

The percentage yield of *Dalbergia sissoo* Roxb. extract was determined to evaluate the efficiency of the extraction process. The obtained extract showed a satisfactory yield, indicating the presence of appreciable amounts of phytoconstituents in the plant material.

Percentage Yield (%) = Weight of Extract / Weight of Sample) x 100

Percentage Yield= (20.49/250) × 100 = 8.198%

pharmacological study

Table No 1. Anti-Malarial Activity of Plant Extract

Compound	Concentration (µg/mL)	Absorbance (OD) 1	Absorbance (OD) 2	Absorbance (OD) 3	Mean OD	% Inhibition
Control	—	0.808	0.926	0.932	0.888	—
Standard (Chloroquine diphosphate)	250	0.281	0.272	0.241	0.264	70.21
Standard (Chloroquine diphosphate)	500	0.156	0.163	0.166	0.161	81.80
Standard (Chloroquine diphosphate)	1000	0.112	0.101	0.103	0.105	88.14
Plant Extract	250	0.425	0.428	0.433	0.428	51.76
Plant Extract	500	0.356	0.366	0.364	0.362	59.26
Plant Extract	1000	0.325	0.321	0.324	0.323	63.61

The anti-malarial activity of *Dalbergia sissoo* roxb. extract increased with increase in concentration, indicating dose-dependent inhibitory activity against β-hematin formation. The plant extract showed moderate anti-malarial potential when compared with the standard drug chloroquine diphosphate. The observed activity may be due to the presence of phytoconstituents such as flavonoids, tannins, saponins, and terpenoids present in the extract.

These bioactive compounds are known for their antioxidant and anti-plasmodial properties, which may contribute to the inhibition of malarial parasite growth.

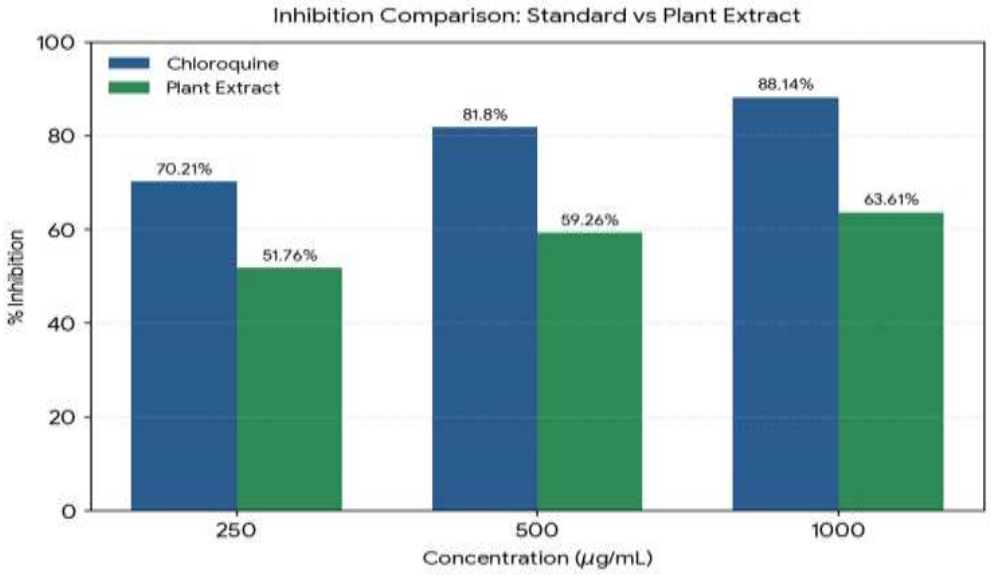

Figure No. 1 Inhibition Comparison standard versus Plant Extract

UV-Visible Spectral Analysis

The UV-Visible spectrum of the plant extract was recorded in the wavelength range of 200–400 nm. The spectrum showed two significant absorption peaks at 257 nm and 355 nm.

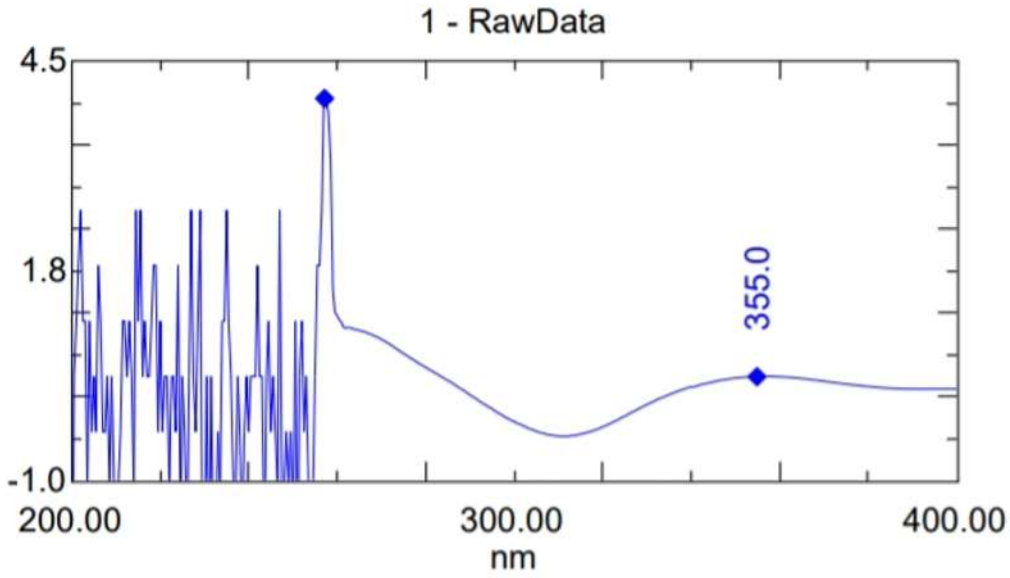

Figure No. 2. UV – Visible spectrum of the plant extract

Table No. 2. Interpretation of Wavelenght

Wavelength (nm)	Absorbance	Interpretation
257 nm	4.000	Presence of aromatic compounds, conjugated double bonds, flavonoids, and phenolic constituents
355 nm	0.361	Presence of extended conjugation, flavonoids, tannins, and polyphenolic compounds

The strong absorption peak observed at 257 nm indicates the presence of π→π* electronic transitions, which are commonly associated with aromatic rings and phenolic compounds. Such compounds are frequently reported in medicinal plants possessing antioxidant and antimalarial activity.

The absorption peak at 355 nm suggests the presence of conjugated systems, particularly flavonoids and polyphenolic constituents. Flavonoids are known to contribute significantly to antimalarial activity through antioxidant mechanisms and inhibition of parasite growth.

Therefore, the UV spectral analysis confirms the presence of bioactive phytoconstituents such as flavonoids, phenols, and conjugated aromatic compounds in the plant extract, which may be responsible for its preclinical antimalarial potential.

FTIR Spectral Analysis

FTIR spectroscopy was performed to identify the functional groups present in the plant extract.

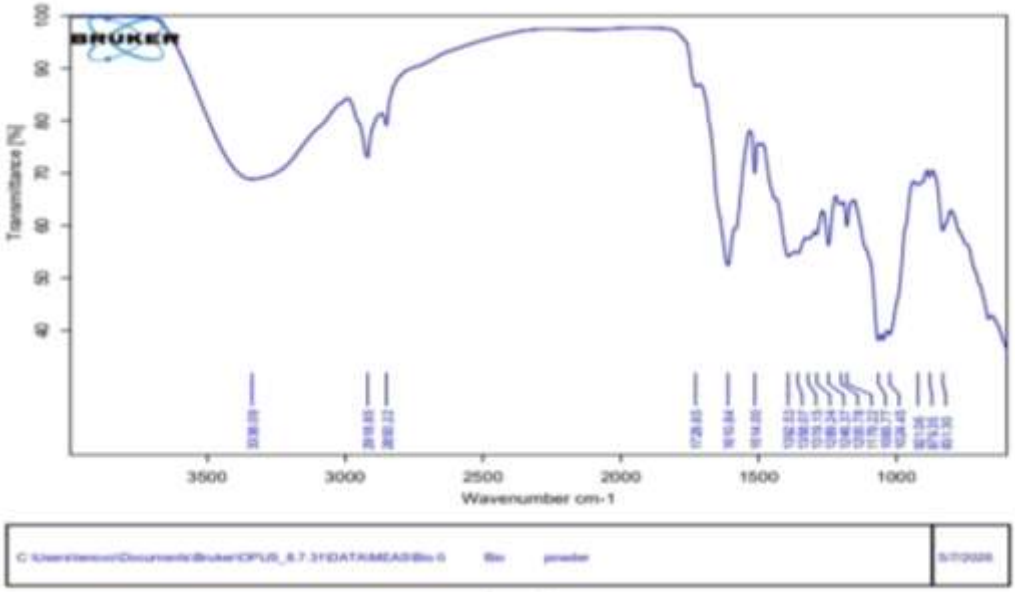

Figure No. 3. FTIR Spectroscopy

The FTIR spectrum of the plant extract exhibited several characteristic peaks at different wavenumbers, indicating the presence of various functional groups. These peaks suggest the occurrence of bioactive phytoconstituents such as phenols, flavonoids, alcohols, aromatic compounds, and carbonyl-containing compounds in the extract.

Table no. 3. Interpretation of FTIR Peaks and Functional Groups

Peak (cm^{-1})	Functional Group	Possible Compound Class
3338.09	O–H stretching	Alcohols, phenols
2918.85	C–H stretching	Alkanes
2850.22	C–H stretching	Alkanes
1728.65	C=O stretching	Aldehydes, ketones, esters
1610.84	C=C stretching	Aromatic compounds
1514.00	Aromatic ring vibration	Phenolic compounds
1392.53–1319.15	C–N / O–H bending	Amines and phenols
1289.24–1024.45	C–O stretching	Alcohols, ethers, flavonoids
921.06	=C–H bending	Alkenes
879.35	Aromatic C–H bending	Aromatic compounds
831.30	C–Cl stretching / aromatic substitution	Halogenated or substituted aromatic compounds

The broad absorption peak at 3338.09 cm^{-1} indicates the presence of hydroxyl (O–H) groups, suggesting phenolic compounds and alcohols in the extract. Phenolic compounds are well known for antioxidant and antiplasmodial activities.

The peaks at 2918.85 cm^{-1} and 2850.22 cm^{-1} correspond to aliphatic C–H stretching vibrations, indicating the presence of alkane groups.

A strong peak at 1728.65 cm^{-1} confirms the presence of carbonyl (C=O) functional groups, which may arise from aldehydes, ketones, or ester-containing phytochemicals.

The absorption bands at 1610.84 cm^{-1} and 1514.00 cm^{-1} indicate aromatic C=C stretching vibrations, supporting the presence of flavonoids and aromatic phytoconstituents.

The region between 1289.24 cm^{-1} and 1024.45 cm^{-1} corresponds to C–O stretching vibrations, indicating alcohols, ethers, and glycosidic compounds commonly found in medicinal plants.

Overall, the FTIR analysis revealed the presence of important bioactive functional groups such as hydroxyl, carbonyl, aromatic, and ether groups. These phytochemical constituents may contribute to the observed antimalarial activity of the plant extract

Conclusion

Anti-malarial Potential: The study concluded that *Dalbergia sissoo* Roxb. leaf extract shows noteworthy, dose-dependent anti-malarial activity by inhibiting \beta-hematin formation, which interferes with the malaria parasite's heme detoxification mechanism.

Bioactive Composition: UV spectroscopy and FTIR analysis identified secondary metabolites such as flavonoids, tannins, phenolic compounds, alkaloids, terpenoids, and glycosides, which likely contribute to its pharmacological effects.

Future Outlook: While promising as a source for novel anti-malarial agents, the study highlights the need for further research, including in vivo studies, toxicity evaluations, and the isolation of active constituents to fully validate its safety and therapeutic efficacy.

Reference

1)Fikadu, M., & Ashenafi, E. (2023). Malaria: An Overview. Infection and Drug Resistance, 16, 3339–3347.

2)Okello, G., & Aucamp, M. (2026). Malaria: A review on its current epidemiological status and management strategies. Malaria Journal, 25, Article 132.

3) Centers for Disease Control and Prevention. Drug resistance in the malaria-endemic world. Updated April 3, 2024. https://www.cdc.gov/malaria/php/public-health-strategy/drug-resistance.html

4)Cui L, Mharakurwa S, Ndiaye D, Rathod PK, Rosenthal PJ. Antimalarial Drug Resistance: Literature Review and Activities and Findings of the ICEMR Network. Am J Trop Med Hyg. 2015;93(Suppl 3):57-68. doi:10.4269/ajtmh.15-0007

5)Inbaneson SJ, Sundaram R, Suganthi P. In vitro antiplasmodial effect of ethanolic extracts of traditional medicinal plant Ocimum species against Plasmodium falciparum. Asian Pac J Trop Med. 2012;5(2):103-106. doi:10.1016/S1995-7645(12)60004-2.

6)Deshpande, P. K., Gothalwal, R., & Pathak, A. K. (2014). Phytochemical analysis and evaluation of antimalarial activity of Azadirachta indica. The Pharma Innovation Journal, 3(9), 12-16.

7) Sudhakar Johnson T, Nair S, de'Souza A, Hingorani L. *Dalbergia sissoo* Roxb. DC. Monograph. PharmacognosyReviews. 2024;18(36):137150. https://doi.org/10.5530/phrev.20242024

8) Bharath M, Tulasi ELR, Sudhakar K, Eswaraiah MC. *Dalbergia sissoo* DC. – an important medicinal plant. Int J Res Pharm Chem. 2013;3(2) *Dalbergia-sissoo*-an-important-medicinal-plant.pdf

9) Verma, S., Singh, P., & Kumar, R. (2025). *Dalbergia sissoo* ethanolic extract: A natural source of antioxidant, antibacterial, and anticorrosive agents. Chemistry & Biodiversity, 22(2), Article e202500602. https://doi.org/10.1002/cbdv.202500602.

10) Spandana, K., Rathnakar, C., & Bhavana, K. (2014). Method development and validation for the simultaneous estimation of levofloxacin and cefpodoxime proxetil by using RP-HPLC in combined tablet dosage form. Material Science Research India, 11(1), 67–73. https://doi.org/10.13005/msri/110109

11)Suhair Jaber, Saleh Abu-Lafi, Pierre Lutgen, Mutaz Qutob, Qassem Abu-Remelehand Mutaz Akkawi, Bicarbonate In-Vitro Effect on Beta-Hematin Inhibitionby Artemisia sieberi Aqueous Infusion,Journal of Pharmacy and Pharmacology 3 (2015) 63-72.

12)Deharo, E., Garcia, R., Oporto, P., Gimenez, A., Sauvain, M., Jullian, V., and Ginsburg, H. 2002. "A NonRadiolabelled Ferriprotoporphyrin IX Biomineralisation Inhibition Test for the High Throughput Screening ofAntimalarial Compounds." Exper. Parasitol,100(4):252-6.

13) Amrutkar Y, Hajare SW, Ingawale MV, et al. Anti-inflammatory activity of ethanolic leaf extract of *dalbergia sissoo* in vitro and in vivo. Adv Tissue Eng Regen Med Open Access. 2017;2(3):171–174. DOI: 10.15406/atroa.2017.02.00028

14) Shi, L., Zhao, W., Yang, Z., Subbiah, V., & Suleria, H. A. R. (2022). Extraction and characterization of phenolic compounds and their potential antioxidant activities. Environmental Science and Pollution Research, 29(54), 81112–81129. https://doi.org/10.1007/s11356-022-23337-6