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IN-VITRO ANTIPLASMODIUM ACTIVITY OF DIFFERENT EXTRACTS *BAUHINIA VARIEGATA*

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ABSTRACT: Malaria remains a major global health concern due to increasing resistance of *Plasmodium falciparum* to existing antimalarial drugs. The present study evaluates the *in-vitro* anti-plasmodial activity and cytotoxicity of methanolic and aqueous leaf extracts of *Bauhinia variegata*. Extracts were prepared using Soxhlet and maceration methods and subjected to phytochemical screening. Anti-plasmodial activity was assessed against chloroquine-sensitive (3D7) and resistant (Dd2) strains using a microfluorimetric DNA-based assay, while cytotoxicity was evaluated on PBMCs using the trypan blue exclusion method. The methanolic extract exhibited potent activity with low IC_{50} values (1.32 and 1.54 µg/mL) and high selectivity indices, along with low cytotoxicity. The aqueous extract showed moderate activity. Hemolytic studies confirmed minimal toxicity to erythrocytes. These findings suggest that *B. variegata*, particularly its methanolic extract, holds promise as a potential source of novel antimalarial compounds.

INTRODUCTION: Malaria remains one of the most significant global public health challenges, particularly in tropical and subtropical regions [1]. It is primarily caused by protozoan parasites of the genus Plasmodium, with *Plasmodium falciparum* being the most virulent species responsible for severe morbidity and mortality [2]. Despite advances in chemotherapy and vector control strategies, the emergence and spread of drug-resistant strains of Plasmodium have complicated malaria management. This growing resistance to conventional antimalarial drugs has created an urgent need for the discovery and development of new, safe, and effective anti-plasmodial agents, especially from natural sources [3, 4]. Medicinal plants have long served as a valuable source of therapeutic agents, with several modern antimalarial drugs, such as quinine and artemisinin, being derived from plant origins. Among these, *Bauhinia variegata*, commonly known as the orchid tree, is a widely distributed medicinal plant traditionally used in various systems of medicine for its anti-inflammatory, antimicrobial, and antioxidant properties [5, 6, 7]. Phytochemical investigations have revealed that the plant contains bioactive constituents such as flavonoids, tannins, glycosides, and alkaloids, which may contribute to its pharmacological activities. However, its potential anti-plasmodial activity has not been extensively explored [8, 9].

In this context, the present study aims to evaluate the *in-vitro* antiplasmodial activity of different extracts of *Bauhinia variegata* [10, 11]. By employing suitable *in-vitro* screening methods against Plasmodium species, this research seeks to identify promising plant extracts with significant inhibitory effects on parasite growth. The findings of this study may contribute to the identification of novel plant-based antimalarial compounds and support the continued exploration of traditional medicinal

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plants as potential sources of new therapeutic agents.

MATERIALS AND METHODS:

Collection and Identification of Plant: The selection of plant material was based on the literature survey and traditional uses. Moreover, the selection of plants was on easy availability, uncomplicated cultivation, invasive nature and its pharmacological applications. Fresh and mature leaves were collected from different localities of pollution-free areas of Gormi, Bhind (M.P.). The plant samples were transferred from their original habitat to the laboratory using clean polythene bags. The leaves were separated from the main branch and other unnecessary parts were removed followed by washing with distilled water (DW) to get rid of any dust attached to it. Further, the cleaned leaves were shade dried at room temperature (RT). The dried leaves were grounded using an electric grinder and sieved to get a fine powder [12, 13].

Preparation of Extracts and Phytochemical Investigation: The powdered plant materials (50 g) were sequentially extracted using different polarity solvents by Soxhlet apparatus (Rotek). The extracts were filtered using Whatmann filter paper no. 1 and allowed for air drying to get concentrate. The obtained extracts were stored in a refrigerator until the next process. Additionally, the aqueous extract was prepared by soaking plants powder (20 g) in DW (100 mL) for about 24 hrs at RT on a magnetic stirrer [14]. At the end of extraction, the micelle was separated from marc by filtration using Whatmann filter paper No. 1. Subsequently, the micelle was then separated from the menstruum by evaporation in a hot air oven and stored at 4 ℃ until further use. The percent (%) yields of solvent and aqueous extracts were calculated according to the following formula.

Perecent yield (%) = Weight of plant extract after solvent evaporated / Weight of the dry plant powder

The plant crude extract was qualitatively analyzed to identify the presence of various bioactive constituents [15, 16].

***In-vitro* Anti-plasmodium Activity:**

***In-vitro* Culture of *Plasmodium falciparum*:** Two different strains of *Plasmodium falciparum* were used, the chloroquine sensitive (3D7) and chloroquine resistant (Dd2) [17]. A detailed description of the culture and synchronization methods used has been reported previously.

***In-vitro* Anti-plasmodial Activity of the Plant Extracts:** A microfluorimetric DNA-based assay was used to evaluate Plasmodium falciparum (3D7 and Dd2 strains) growth inhibition by different concentrations of plant extracts. Synchronized ring-stage parasites from stock cultures were exposed to serial dilutions of extracts (100 to 0.01 µg/mL) in 96-well plates, maintaining 2% hematocrit and 1% parasitemia. Cultures were incubated for 48 h at 37°C in 5% CO_2.

After incubation, plates were centrifuged at 600 × g for 10 min, and erythrocytes were lysed using 0.15% saponin in phosphate-buffered saline (PBS). The pellets were washed twice with PBS to remove hemoglobin and resuspended in PBS. PicoGreen reagent was added, and plates were incubated in the dark for 30–60 min. Fluorescence was measured at 485/528 nm. Chloroquine served as reference, and IC_{50} values were determined using SigmaPlot from triplicate experiments [18, 19, 20].

***In-vitro* Cytotoxicity Test of the Plant Extracts on Human Peripheral Blood Mononuclear Cells:** The cytotoxic activity of plant extracts against human peripheral blood mononuclear cells (PBMCs) was assessed using the trypan blue dye exclusion method. PBMCs were isolated from healthy donor blood using Histopaque [21]. A total of 6×10^5 cells per well were seeded in 96-well microplates and treated in triplicate with varying concentrations of plant extracts (100–0.01 µg/mL) in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin, making a final volume of 200 µL. The cells were incubated for 48 h at 37°C in a humidified atmosphere containing 5% CO_2. After incubation, equal volumes of cell suspension and trypan blue dye were mixed, and viable cells were counted. Three independent experiments were conducted in triplicate, and LC_{50} values were calculated using Sigma Plot software [22].

RESULTS AND DISCUSSION:

Extract Yield and Phytochemical Investigation: Phytochemicals were extracted from air-dried,

powdered plant leaves using various solvents. Each plant extract was labelled as shown in **Table 1**, along with the percent yield and physical properties of the extract. The methanol extract of *B. variegata* produced the most extract (13.91%) followed by aqueous (6.53%). According to the findings, methanol was found to be the most effective solvent for extracting.

TABLE 1: EXTRACTS, SOLVENT USED, PERCENTAGE YIELD AND PHYSICAL NATURE OF EXTRACTS

S. no.	Plant Name	Solvent Used	Yield (%)	Extract Physical Nature
1	*B. variegata*	Methanol	13.91	Dark green, sticky, no odour
		Aqueous	6.53	Light green with a stinking smell

The phytochemical analysis of the anti-plasmodium active plant was screened using a colour test. **Table 2** summarized the primary phytochemical analysis which revealed the presence of alkaloids, phenolics, flavonoids, glycosides, terpenoids, phytosterols, saponins and tannins in the methanol and aqueous extracts.

TABLE 2: PHYTOCHEMICAL SCREENING OF *B. VARIEGATA* LEAVES EXTRACTS

S. no.	Secondary Metabolites	Test Name	Methanol	Aqueous
1	Alkaloids	Mayer's	+	+
		Wagner's	+	+
2	Phenolics	Ferric chloride	+	-
		Gelatin	+	-
3	Flavonoids	Alkaline reagent	+	+
		Lead acetate	+	-
4	Terpenoids	Salkowski's	+	+
5	Glycosides	Bontrager's	+	+
		Legal's	–	–
6	Fixed Oils and Fats	Saponification	–	–
7	Saponins	Froth	+	+

***In-vitro* Anti-plasmodial and Cytotoxic Activity of the Plant Extracts Studied: Table 3** presents the anti-plasmodial activity and cytotoxicity values of *B. variegata* extracts against the chloroquine-resistant Dd2 and chloroquine-sensitive 3D7 strains of *Plasmodium falciparum*. The methanolic extract of *B. variegata* exhibited potent anti-plasmodial activity with IC_{50} values of 1.32 ± 0.032 µg/mL (Dd2) and 1.54 ± 0.014 µg/mL (3D7), indicating high activity. It also showed low cytotoxicity with an LC_{50} value of 387.5 ± 23.28 µg/mL, resulting in high selectivity indices (SI) of 312.5 (Dd2) and 222.6 (3D7). In contrast, the aqueous extract displayed moderate activity with an IC_{50} of 6.9 ± 0.076 µg/mL against the 3D7 strain and significantly lower activity against the Dd2 strain (75 ± 0.84 µg/mL). The LC_{50} of the aqueous extract was 268.5 ± 16.54 µg/mL, resulting in SI values of 38.2 (Dd2) and 43.6 (3D7).

TABLE 3: ANTI-PLASMODIAL ACTIVITY AND CYTOTOXICITY VALUES OF PLANT EXTRACTS

Scientific Name	Extract	IC_{50} Dd2 (µg/mL)	IC_{50} 3D7 (µg/mL)	Antiplasmodial Activity	Cytotoxicity LC_{50} (µg/mL)	SI (Dd2)	SI (3D7)
B. variegata	Methanol	1.32 ± 0.032	1.54 ± 0.014	High	387.5 ± 23.28	312.5	222.6
	Aqueous	75 ± 0.84	6.9 ± 0.076	Good	268.5 ± 16.54	38.2	43.6

IC_{50}: 50% lethal concentration against Plasmodium falciparum. The IC_{50} values are expressed as the mean ± SD of three different determinations per experiment, ‡LC_{50}: 50% lethal concentration in human peripheral blood mononuclear cells. The LC_{50} values are expressed as the mean ± SD of three different determinations per experiment, §SI=LC_{50}/IC_{50}. IC_{50}: 50% inhibitory concentration, SD: Standard deviation, LC_{50}: 50% lethal concentration, SI: Selectivity index

The methanolic extract of *B. variegata* demonstrated strong anti-plasmodial activity against both resistant and sensitive strains of *P. falciparum*, as indicated by its low IC_{50} values and high selectivity indices, suggesting a high therapeutic potential with minimal cytotoxic effects. These findings imply that methanol may be more effective in extracting bioactive phytochemicals with anti-plasmodial properties, such as flavonoids, alkaloids, and phenolic compounds, which have been previously reported in *B. variegata*. In contrast, the aqueous extract

showed moderate to low activity, particularly against the Dd2 strain, possibly due to the limited solubility of key bioactive constituents in water or the degradation of heat-sensitive compounds during extraction. Despite lower activity, the aqueous extract still demonstrated a reasonable safety margin with SI values above 30, indicating selective toxicity towards the parasite. These results support the traditional use of *B. variegata* and suggest its potential as a source of natural antimalarial compounds, particularly in its methanolic form. Further bioassay-guided fractionation and mechanistic studies are recommended to isolate and identify the active constituents responsible for the observed activity. According to the WHO guidelines, anti-plasmodial activity is classified as follows: high activity at IC_{50}<5 µg/mL, good activity at 5–10 µg/mL, moderate activity at 11–50 µg/mL, and inactive at >50 µg/mL. The selectivity index (SI) of each extract is also presented in Table 3. The SI is defined as the ratio of the LC_{50} value in PBMCs to the IC_{50} value against P. falciparum.

Hemolytic Effects of the Active Plant Extracts: To evaluate the effects of the active extracts on the structural integrity of erythrocytes, the hemoglobin concentration was determined in samples of red blood cells incubated with each extract with high anti-plasmodial activity (methanol extract and aqueous) for 48 h at 37°C. Compared with the control treatment without extract, none of the extract treatments showed hemolytic activity against healthy erythrocytes, as shown in **Table 4** which suggests low toxicity for the extracts against erythrocytes.

TABLE 4: PERCENTAGE OF HEMOLYSIS IN ERYTHROCYTES TREATED WITH THE ACTIVE EXTRACTS

***B. variegata* Extract**	**Concentration (µg/mL)**	**Percentage Hemolysis (Mean ± SD)**
Methanol	1	5.45 ± 0.54
	10	6.34 ± 0.73
	100	7.91 ± 0.98
Aqueous	1	5.12 ± 0.23
	10	5.75 ± 0.65
	100	6.11 ± 0.32
Positive Control	–	100
Negative Control	–	5.7 ± 0.23

*Erythrocytes were incubated with each extract at concentrations of 1, 10, and 100 µg/mL at 37°C for 48 h. Each data point represents the mean±SD of two independent experiments performed in triplicate against a positive control (100% hemolysis) and a control without treatment. The percentage of hemolysis generated by the extracts relative to that of the control (without extract) did not show statistically significant differences (P>0.05). SD: Standard deviation

The hemolytic activity assay was conducted to evaluate the safety of *Bauhinia variegata* extracts on erythrocyte membrane integrity. The results demonstrate that both methanolic and aqueous extracts exhibited minimal hemolysis across all tested concentrations (1, 10, and 100 µg/mL). The percentage hemolysis for the methanolic extract ranged from 5.45 ± 0.54 to 7.91 ± 0.98, while the aqueous extract showed slightly lower values ranging from 5.12 ± 0.23 to 6.11 ± 0.32. These values are comparable to the negative control (5.7 ± 0.23), indicating negligible damage to red blood cells. Importantly, no statistically significant difference (P > 0.05) was observed between treated and untreated groups, confirming that the extracts did not induce membrane lysis under the experimental conditions. In contrast, the positive control exhibited 100% hemolysis, validating the sensitivity and reliability of the assay. The slightly higher hemolysis observed at increased concentrations, particularly in the methanolic extract, may be attributed to the presence of certain bioactive compounds; however, the levels remain within acceptable limits. These findings suggest that both extracts, especially the methanolic extract with higher antiplasmodial activity, are safe for erythrocytes and do not compromise membrane stability. This is a crucial consideration, as an ideal antimalarial agent should selectively target the parasite without damaging host red blood cells. Overall, the low hemolytic activity supports the potential therapeutic applicability of *B. variegata* extracts and complements their observed antiplasmodial efficacy.

CONCLUSION: The present study demonstrates that *Bauhinia variegata* possesses significant *in-vitro* anti-plasmodial activity, particularly in its

methanolic extract, which showed potent inhibition against both chloroquine-sensitive and resistant strains of *Plasmodium falciparum*. The high selectivity index and low cytotoxicity observed in PBMCs indicate a favorable safety profile. In contrast, the aqueous extract exhibited comparatively moderate activity. Phytochemical analysis suggests that bioactive constituents such as flavonoids, alkaloids, and phenolic compounds may contribute to the observed effects. Additionally, the absence of significant hemolytic activity further supports the safety of the extracts. Overall, *B. variegata* represents a promising candidate for the development of plant-based antimalarial agents, warranting further investigation through isolation and characterization of active compounds.

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REFERENCES:

1. Rovira-Vallbona E, Van Hong N, Kattenberg JH and Huan RM: Efficacy of dihydroartemisinin/piperaquine and artesunate monotherapy for the treatment of uncomplicated *Plasmodium falciparum* malaria in Central Vietnam. J Antimicrob Chemother 2020; 75: 2272–2281.
2. Iwanaga S, Kubota R, Nishi T, Kamchonwongpaisan S, Srichairatanakool S and Shinzawa N: Genome-wide functional screening of drug-resistance genes in *Plasmodium falciparum*. Nat Commun 2022; 13: 6163. doi:10.1038/s41467-022-33804-w.
3. Datoo MS, Natama HM, Somé A, Bellamy D, Traoré O and Rouamba T: Efficacy and immunogenicity of R21/Matrix-M vaccine against clinical malaria after 2 years' follow-up in children in Burkina Faso: a phase 1/2b randomised controlled trial. Lancet Infectious Diseases 2022; 22: 1728–1736.
4. Kassegne K, Komi Koukoura K, Shen HM, Chen SB, Fu HT and Chen YQ: Genome-wide analysis of *Plasmodium falciparu*m isolates from Togo reveals selective signals in immune selection-related antigen genes. Front Immunol 2020; 11: 552698. doi:10.3389/fimmu.2020.552698.
5. Mulenga MC, Sitali L, Ciubotariu II, Hawela MB, Hamainza B and Chipeta J: Decreased prevalence of the *Plasmodium falciparum* Pfcrt K76T and Pfmdr1 N86Y mutations post-chloroquine treatment withdrawal in Katete District, Eastern Zambia. Malar J 2021; 20: 329. doi:10.1186/s12936-021-03859-z.
6. Gupta R, Paarakh PM and Gavani U: Pharmacognostical and phytochemical screening of *Bauhinia variegata* Linn. leaves. J Pharm Res 2009; 2(7): 1196–1198.
7. Nageshwar G, Radhakrishnaiah M and Narayana LL: Numerical chemotaxonomy of Bauhinia. Indian Acad Sci (Plant Sci) 1984; 93(6): 621–627.
8. Pandey S and Agrawal RC: Effects of *Bauhinia variegata* bark extract on DMBA induced mouse skin carcinogenesis: a preliminary study. Glob J Pharmacol 2009; 3(3): 158–162.
9. Pokhrel NR, Adhikari RP and Baral: *In-vitro* evaluation of antimicrobial activity of *Bauhinia variegata*. World J Microbiol Biotechnol 2002; 18: 69–71.
10. Rajkapoor B, Jayakar B, Murugesh N and Sakthisekaran D: Chemoprevention and cytotoxic effect of *Bauhinia variegata* against N-nitrosodiethylamine induced liver tumours and human cancer cell lines. J Ethnopharmacol 2006; 104: 407–409.
11. Rajkapoor B, Jayakar B and Murugesh N: Antitumour activity of *Bauhinia variegata* on Dalton's ascetic lymphoma. J Ethnopharmacol 2003; 89: 107–109.
12. Reddy MV, Reddy MK, Duvvuru GC, Cristelle C and Bernard B: A flavanone and a dihydrodibenzoxepin from *Bauhinia variegata*. Phytochemistry 2003; 64: 879–882.
13. Hassen J, Alemayehu GS, Dinka H and Golassa L: High prevalence of Pfcrt 76T and Pfmdr1 N86 genotypes in malaria infected patients attending health facilities in East Shewa Zone, Oromia Regional State, Ethiopia. Malar J 2022; 21: 286. doi:10.1186/s12936-022-04304-5.
14. Froeschl G, Saathoff E, Kroidl I, Berens-Riha N, Clowes P and Maboko L: Reduction of malaria prevalence after introduction of artemisinin-combination therapy in Mbeya Region, Tanzania: results from a cohort study with 6773 participants. Malar J 2018; 17: 245.
15. Lalremruata A, Jeyaraj S and Engleitner T: Species and genotype diversity of Plasmodium in malaria patients from Gabon analysed by next generation sequencing. Malar J 2017; 16(1): 1–11. doi:10.1186/s12936-017-2044-0.
16. Bruce MC, Macheso A, McConnachie A and Molyneux ME: Comparative population structure of *Plasmodium malariae* and *Plasmodium falciparum* under different transmission settings in Malawi. Malar J 2011; 10: 1–12.
17. Ryan ET, Hill DR, Solomon T, Aronson N and Endy TP: Hunter's Tropical Medicine and Emerging Infectious Diseases. Philadelphia: Elsevier 2019.
18. Jong EC and Stevens DL: Netter's Infectious Diseases. Philadelphia: Elsevier 2021.
19. Zuccala ES and Baum J: Cytoskeletal and membrane remodelling during malaria parasite invasion of the human erythrocyte. Br J Haematol 2011; 154(6): 680–689.
20. Venugopal K, Hentzschel F, Valkiūnas G and Marti M: Plasmodium asexual growth and sexual development in the haematopoietic niche of the host. Nat Rev Microbiol 2020; 18(3): 177–189. doi:10.1038/s41579-019-0306-2.
21. Smith RC and Barillas-Mury C: Plasmodium oocysts: overlooked targets of mosquito immunity. Trends Parasitol 2016; 32(12): 979–990. doi:10.1016/j.pt.2016.08.012.
22. Howes RE, Battle KE, Mendis KN, Smith DL, Cibulskis RE and Baird JK: Global epidemiology of *Plasmodium vivax*. Am J Trop Med Hyg 2016; 95(6): 15–34.

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