Geo-Environmental Factors and the Effectiveness of Mulberry Leaf Extract in Managing Malaria: A Study in Purulia District, West Bengal, India

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Abstract

Malaria prevalence has become medically important as well as a socioeconomic impediment for the endemic regions, including Purulia, West Bengal. Geo-environmental variables, humidity, altitude, and land use patterns are responsible for malaria. For surveillance of the endemic nature of Purulia's blocks, statistical and spatiotemporal factors analysis have been done here. Also, a novel approach for the Pf malaria treatment using methanolic leaf extract of *Morus alba* S1 has significantly reduced the parasite load. The EC_{50} value (1.852) of the methanolic extract of *M. alba* S1 with *P. falciparum* 3D7 strain is close to the EC_{50} value (0.998) of the standard drug chloroquine with the same chloroquine-sensitive strain. Further studies with an in-silico model have shown successful interaction between DHFR and the leaf extract. Both 1-octadecyne and oxirane interacted favourably, which was depicted through GC-MS analysis. The predicted binary logistic regression model will help the policy makers for epidemiological surveillance in malaria-prone areas worldwide when substantial climate variables create a circumstance favourable for malaria. From the in vitro and in silico studies, it can be concluded that the methanolic extract of *M. alba* S1 leaves is promising and may play an influential role in the *Pf* malaria treatment in the near future.

Introduction

Malaria is one of the most fatal vector borne infectious diseases that affect humans. It is clinically and economically troublesome because it is prevalent in poorer countries and regions, which significantly hampers socio-economic development. Unicellular protozoan parasites from the genus Plasmodium are the causal agents of malaria in humans and other vertebrates. While humans are the primary host for some species of Plasmodium, and some species can also infect a wide range of vertebrates, including mammals, birds, and reptiles. More than 200 species have been adequately defined of the *Plasmodium* parasite to date, and each species can infect a definite range of hosts. *Plasmodium vivax (P. vivax), Plasmodium falciparum (P. falciparum), Plasmodium ovale (P. ovale), Plasmodium malariae (P. malariae)*, and *Plasmodium knowlesi (P. knowlesi)* are the five species of this parasite which can naturally infect humans to cause malaria in various parts of the world. However, their distribution is not uniform across large areas of the world. The first four species are restricted to humans. Whereas, P. knowlesi is a species that infects macaques in Southeast Asia but can also cause human malaria and has emerged as a significant public health concern in some areas.

P. vivax and *P. falciparum* are the most common species of Plasmodium and are responsible for most malaria cases worldwide. *P. vivax* is found mainly in Asia and South America, while *P. falciparum* is found predominantly in sub-Saharan Africa.

P. malariae and *P. ovale* are less common and are found primarily in Africa, although they have also been reported in other regions worldwide.

P. falciparum is the deadliest among these five species. Mosquito is generally the insect vector, which causes *Plasmodium* species' transmission between vertebrate hosts. The vector is the definitive host, not a carrier only, which is the place of *Plasmodium* species' sexual reproduction and inside the insect body, the development of a parasite is necessary for the transmission to the following vertebrate host. The *Plasmodium* species' critical development can be supported by a whole range of insect species depending on individual host-specific parasite species, but anopheline mosquitoes are mainly responsible for the transmission of all the five species of *Plasmodium* which cause malaria in humans. Noticeable genetic flexibility is owned by *Plasmodium* species which facilitates them to adapt to environmental alterations. This also helps them by giving them the potential to develop resistance fast against therapeutics, like- antimalarials and to change the specificity of host[1].

Approximately, 247 M malaria cases are there worldwide in 84 malaria-endemic countries in 2021, which is 2 million cases plus from the year earlier including the French Guiana territory. A maximum portion of this increase comes from the WHO African Region's countries. Malaria remains endemic in tropical region of Asia and Africa[2, 3]. From 2000 to 2019, it was seen that malaria deaths were gradually reduced throughout the world. The reduction is like- from 897000 deaths in 2000 to 577000 deaths in 2015 and 568000 deaths in 2019. There was an escalation of 10% in malaria deaths in 2020 compared to 2019, which were approximately 625000 deaths. In 2021, deaths slightly declined to 619 000. At the time of the COVID-19 pandemic situation in between 2019 and 2021, disruptions in essential malaria services were responsible for about an extra 63000 malaria deaths[4, 5, 6]. When the inoculation of motile sporozoites takes place into the dermis, the life cycle starts, which travels to the liver afterwards; a hepatocyte is invaded by each sporozoite and multiplies afterwards. The asexual cycle begins when the red blood cells are invaded by thousands of merozoites released into the bloodstream after the liver schizonts burst after approximately a week. The beginning of illness is detected consequent to the total asexual parasite load reaching roughly 100 million in circulation.

The development of some parasites occurs in the form of gametocytes, i.e., sexual forms. The formation of gametocytes results within a feeding anopheline mosquito due to the form of an ookinete and an oocyst after that through sexual development after reproduction inside the gut of the mosquito. Sporozoites are liberated after the oocyst bursts, which the salivary glands receive in order to for the expected inoculation at the next blood feed. More or less, one month is needed for the entire cycle. In an adult round, about 2% of parasitaemia corresponds to 10^{12} parasite burden in a total body[7].

Environmental factors affect the transmission intensity, seasonality, and geographical distribution of malaria, and together with the vector, the human and the parasite compose the eco-system of malaria[8]. Meteorological factors, i.e., temperature, humidity and precipitation, are the primary environmental determinants of malaria. Temperature impacts vector and parasite development and thus is a significant constraint on the geographical suitability to malaria[9, 10]. The overall correlation between mosquito density and rainfall has been frequently illustrated; rainfall offers mosquito breeding locations and raises the humidity, which improves their survival[11]. The difference in climatological conditions, mostly temperature, humidity and rainfall, regulates the spatiotemporal configurations of malaria through their effects on both the Plasmodium parasite and the Anopheles vector[12].

Drugs named Primaquine and Chloroquine are used for the treatment of uncomplicated *Plasmodium vivax* malaria. For 14 days, Primaquine is taken to resist the relapse caused by Pv malaria, and Chloroquine is taken for 3 days according to the doctors' guidance. Primaquine cannot be given to the children under one year and pregnant mothers for treatment. Primaquine also cannot be given to the patients who have G-6PD deficiency. To treat the affected patients by uncomplicated Pf infections, artesunate combination therapies (ACT), like- Artemether + lumefantrine, Artesunate + amodiaquine, Artesunate + sulfadoxinepyrimethamine (SP), etc. are used for 3 days[5]. The treatment regimen for Pf malaria involves administering a single dose of Primaquine on the 2nd day to clear the gametocyte as per the patient's body weight. If patients suffering from mixed infections of both Pv and Pf malaria, ACT is used for three days, and a daily dose of Primaquine is prescribed for 14 days as per the patient's body weight [13].

The emergence of resistance to antimalarial-drug is comparatively a recent phenomenon, although there is a long history of the use of antimalarial drugs. In Thailand, there was the first appearance of the chloroquine-resistant forms of *Plasmodium falciparum* malaria in 1957[14]. Artemisinin-resistant malaria has become a pressing issue worldwide in recent years. The first reports of resistance to artemisinin-based combination therapy (ACT) came from western Cambodia and the Thailand-Cambodia border in 2002–2004. Since then, the problem has spread to other regions, and there is growing concern about the potential impact on global malaria control efforts.[15]. The rise of artemisinin resistance in Southeast Asian countries has led to an increased treatment failure rate, as the effectiveness of both artemisinin and its partner drugs has diminished[16]. This resistance is particularly pronounced in the Greater Mekong sub-region, where it has spread widely and intensified by resistance to the partner drug, leading to the collapse of artemisinin combination therapies. This development is concerning as it undermines efforts to control and eliminate malaria in the affected regions[17]. So, there is a safe, effective, and affordable TACTs (Triple ACTs) alternative approach formed by Artemisinin and two of the existing partner drugs for tackling this global issue of resistance to ACT.

The objective of this study was to construct a binary logistic regression model that uses four climatic variables - relative relief, temperature, humidity, and rainfall - to predict the endemic status of blocks in the Purulia district, where blocks of Purulia district were considered as response variable. In the model, endemic blocks were coded as ‘1’ and non-endemic blocks as ‘0’. By leveraging these variables, the model can help to identify areas at risk of endemic malaria and provide guidance for public health officials and policymakers seeking to prevent and control the spread of the disease. A model framework that utilises four explanatory variables can help to predict the level of endemic malaria in a given block without the need for door-to-door data collection. This predictive model can help local authorities to take proactive measures to reduce malaria cases before significant climatic variables create a favourable environment for malaria transmission. By leveraging these predictive insights, administrators can take targeted actions to prevent the spread of malaria in vulnerable areas, ultimately helping to improve public health outcomes. In addition, another aim of this study is to determine the efficacy of the methanolic extract of *M. alba* S1 leaves against 3D7 strain of Pf malaria through in vitro, in silico analysis which might be helpful for public health by playing a potentially effective role in the treatment of Pf malaria.

Methods

Spatiotemporal factors. We have observed that four key climatic variables - namely relative relief, temperature, humidity, and rainfall - are widely recognised as critical determinants of malaria incidence[18–25]. The relative relief of the Purulia district was obtained from the Radiometric Terrain Correction Alos Palsar DEM with a resolution of 12.5 m. The DEM data were collected from the NASA Earth Data server. Fishnet tool of ArcMap calculated the relative relief of the Purulia District. The temperature and rainfall data used in this study were collected from the World Bio-Climatic Data Portal which is using remote sensing technology". NASA’s Climate Data Services (CDS) were the source of Humidity data for the study area.

The spatial resolution, pixel depth, and projection system (UTM) of all four geo-climatic variables (relative relief, temperature, humidity, and rainfall) were verified using data from NASA's Climate Data Services (CDS) and the World Bio-Climatic data portal. The humidity data for the study area was obtained from NASA's CDS with a spatial resolution of 1 km x 1 km. A spatial zonation map was generated using the Inverse Distance Weightage (IDW) method in ArcGIS v.10.8 and Global Mapper v.22 software, based on spatially rectified images of the geo-climatic variables [26].

Statistical Analysis. The study conducted a correlation analysis among four spatiotemporal factors: elevation, temperature, rainfall, and humidity, to identify significant correlations at 1% and 5% levels of significance. Based on the endemicity of each block in the Purulia district, a binary logistic regression model was structured with the aforementioned environmental variables as explanatory variables. This model framework can predict the endemicity of a block beforehand, provided the respective environmental variables are known.

Preparation of leaf extract. The collection of plant material, comply with relevant institutional, national, and international guidelines and legislation. Such plant samples were collected in the close coordination with Phulpahari Sericulture Complex, Midnapore. The plants voucher specimen was deposited in a publicly available central national herbarium of the Botanical Survey of India (Specimen No: P/M/PM/SP-01). Living material is growing at Phulpahari Sericulture Complex nursery for future research. The plant material was prepared from fresh and healthy collected leaves of *Morus alba* S1 from Phulpahari Sericulture Complex, Midnapore. The freshly collected leaves were subjected to thorough wash under running tap water followed by distilled water before they were cut into small bits. Then the leaves were dried under sunlight. After that, the dried leaves were finely powdered using mixture grinder and preserved in an air tight container. The dried powdered leaf sample of 20 grams was dissolved in 200 ml of methanol in amber colored bottle and stirred for 8 hours in magnetic stirrer. Then the sample was filtered using Whatman filter paper 1. The filtrate sample was concentrated with rotary evaporator at 65°C under reduced pressure. The concentrated sample was further dried in hot plate at 40°C for overnight. The dried sample was further kept in -20∘C for further use[27, 28].

GC–MS analysis. The GC-MS analysis of the methanol leaf extract was performed using GC-MSQP10 Ultra, equipped with a fused silica column, packed with Rxi-1ms capillary column. 99.99% helium gas was used as the carrier gas. The injector temperature was 310°C. The column oven temperature was set to 80°C for 2 min and increased to 150°C, 200°C and 260.0°C subsequently each for 4 min and the final temperature rose to 310°C for 18 minutes. The injection mode was split and the total flow was 10 ml/min where the column flow was 0.64 ml/minute. The purge flow was 3ml/ min with split ratio of 10.0. The spectral detection gained at 1.03 kV + 0.00 kV of ionisation energy. The acquisition mode of the sample is scan with 0.30 scan time and 3333 scan speed; the fragments range from 50 m/z to 800 m/z. The test sample constituents were analysed by considering their retention time (minute), peak height, peak area and mass spectral patterns with the spectral patterns present in the standard database.

In vitro antimalarial activity. *P. falciparum* strain 3D7, sensitive to the standard drug chloroquine (CQ), was cultured in RPMI media supplemented with AB^{+ve} human serum (10%) and A^{+ve} erythrocyte at 5% hematocrit. Synchronisation of the parasite was done at the early trophozoite stage with 5% sorbitol. Briefly, a 1:9 ratio of pellet and sorbitol was kept at room temperature for 5 min[29]. The supernatant was removed by centrifugation, and the pellet was washed thrice in complete media (RPMI-1640 supplemented with 10% AB^{+ve} human serum). To this synchronised culture, complete media was added to make a 1:9 ratio of infected RBC (5% parasitaemia and 10% hematocrit) and media, and the experiment was planned. The plant extract samples were dissolved in DMSO to obtain a stock solution of 1mg/ml. The stock solutions were further diluted with RPMI-1640 to get the different concentrations of 50 µg/ml to 0.8 µg/ml in serial dilution and dispensed in the 96-well flat-bottomed microplates. The experiment was set up with a modified WHO MARKIII protocol[30]. Briefly, parasite cultures were treated with different concentrations of samples, and incubated at 37^{o}C in a gas mixture of 90% nitrogen (N), 5% carbon dioxide (CO2) and 5% oxygen (O2) for 24 hours, and incubation continued till the schizont growth was 10% in control. The blood smear was prepared from all the wells and stained with Giemsa stain. Schizonts with three or more merozoites were counted. The result was analysed using dose-response curves by non-linear regression analysis performed with HN-NonLin Regression Analysis[31].

In silico study. Studies for molecular docking were performed using Biovia Discovery Studio 2021 (v21.1.0.20298) software to determine the possible interaction modes of the target compounds in the dihydrofolate reductase (DHFR). The X-ray crystallographic structure of the enzymes DHFR was downloaded from the protein data bank (https://www.rcsb.org/structure/2BL9), PDB codes: 2BL9 respectively. While the bound substances (ligand and cofactors) and solvent molecule associated with the receptor were removed from the target protein (DHFR; 2BL9) to vacate the binding site for the interaction. The chemical structure of the molecules was drawn with ChemDraw ultra-Version 8.0 and further pre-optimized for docking. Furthermore, the prepared ligands were docked in silico with the DHFR inhibitors using Biovia Discovery Studio 2021 (v 21.1.0.20298)[32–36].

Ethics declaration

This study was allowed by Zilla Swasthya Bhawan, Purulia, Govt. of West Bengal (Memo No. 2041; Dt. 03.11.2020). The ethical approval (No. RUHECRP001) was also acquired from the Raiganj University Human Ethical Committee (RUHEC). The data were anonymized and combined at the Zilla Swasthya Bhawan without any individual patient information. All protocols were executed as per the pertinent guidelines and regulations. Informed consent was collected individually from all the participants or if the participants are < 18, from a parent and/or legal guardian.

Results

Geo-environmental externalities. Spatial distribution of the Purulia district according to the average measure of four different spatiotemporal factors, i.e. elevation, temperature, rainfall and humidity, are established and classified into five different zones very low, low, moderate, high and very high. Empirical evidence indicates that areas with elevations classified as very high (600m) to high (400m) have a higher incidence of malaria, as demonstrated in the maximum malaria endemic blocks of Jhalda-1, Bagmundi, Jhalda-II, Joypur, Arsha, and Balarampur. (Fig. 1A). The result depicts that higher elevation of Purulia district has an associations with endemic nature of these community development blocks. According to the Indian Meteorological Department of India (2021), the average annual temperature of the Purulia district varies from 21°C to 38°C. *Plasmodium falciparum* cannot complete its growth cycle in the Anopheles mosquito and thus cannot be transmitted at temperatures below 20°C (68°F). Lunde et al. showed that the optimal temperature for malaria transmission is below 30°C[37]. Based on observation, there appears to be a correlation between the average temperature and the incidence of malaria cases in certain regions. Specifically, in the Jhalda-I, Jhalda-II, Bagmundi, Bandwan, Arsha, Balarampur, and Joypur blocks, the average temperature has been found to range from very low (25°C) to low (30°C), which appears to be synchronized with a higher incidence of malaria cases (Fig. 1B).

Purulia district is situated in a tropical savanna climate (Aw) and experiences significant rainfall during the monsoon season—the average rainfall of the district is from 1,100 to 1,500 mm, which influences the growth of malaria cases. Our study revealed that Arsha, Balarampur, Bagmundi, Barabazar, and Bandwan blocks received the highest rainfall, and remarkably, they fall under the endemic category (as shown in Fig. 1C). Anopheles mosquitoes thrive in low humidity conditions[38]. Our analysis indicated that Arsha, Bagmudi, Balarampur, Jhalda-I, Jhalda-II, and Joypur blocks have lower humidity levels, which may help Anopheles mosquitoes to survive and transmit the malaria parasite (as shown in Fig. 1D).

<Figure 1>

The correlation test between all four spatiotemporal variables is carried out and reported in the following table (Supplementary Table S1). It has been observed that all four spatiotemporal variables significantly correlated with each other.

To predict the endemic nature of a block, we have carried out binary logistic regression over any one of the above four significant spatiotemporal variables while coding 10 endemic blocks as '1' and the remaining 10 non-endemic blocks as '0' (Table 1). As the above are significantly correlated, we can consider any of the four as an explanatory variable. In this study, we have considered temperature as the explanatory variable because of easy to measure compared to other variables. The predicted binary logistic regression model and respective fitted model table are as follows:

Table 1
Binary logistic regression fitted table.

Variable	Co-efficient	Standard Error	p-value
Constant	262.2979	121.9345	0.0315*
Temperature	-10.0701	4.6759	0.0313*
N.B. * Correlation is significant at the 0.05 level (Source: Prepared by the authors, 2022 SPSS v.24)			

$$P(Y=1)=\frac{1}{1+e^{-(262.2979-10.0701*temperature)}} \quad \text{.........} (1)$$

The above model (Eq. 1) will help to find out the endemic nature of a particular block of the Purulia district in future when we can easily observe the average monthly temperature. Before substantial climate variables create a circumstance where malaria is likely to occur, this predicted model will assist the administration in reducing the number of malaria-affected cases.

GC−MS analysis of the methanolic leaf extract of *M. alba* S1 detected three different phytochemicals. The Chromatogram of GC-MS showed three visible peaks which were recognised by their corresponding peak, retention time, peak area (%), height (%) and with mass spectral fragmentation patterns as specified through the known compounds described by the library of the National Institute of Standards and Technology (NIST) (Fig. 2). The three compounds detected in GC-MS were identified as 1-Octadecyne, 6-Octen-1-ol, 3,7-dimethyl-, propanoate and Oxirane, tetradecyl-SSHexadecane,1,2-epox which in corresponds with the NIST Library search (Table 2). The structure of these three compounds was analysed and revealed in Supplementary Fig. S1. The details of the three phyto compounds of the methanolic extracts from leaves of *M. alba* S1 are presented in Table 2. Out of these three compounds, two compounds showed the highest percentage (%) of area, i.e.,1-Octadecyne (61.40%) and Oxirane, tetradecyl SSH exadecane,1,2 epox (30.67%) and were used for further analysis.

Table 2
Phyto-chemistry of the methanolic leaf extract of *M. alba* S1 as revealed through GC-MS analysis

Peak No.	Compound Name	Molecular formula	Molecular weight	Retention time (minute)	Area	Area (in %)	Height	Height (in %)
1.	Oxirane, tetradecyl-	$C_{16}H_{32}O$	240.4247	13.754	263372	30.67	118614	30.4
2.	6-Octen-1-ol, 3,7-dimethyl-, propanoate	$C_{13}H_{24}O$	212.3285	13.534	68042	7.92	39066	10.12
3.	1-Octadecyne	$C_{18}H_{34}$	250.4626	13.246	527189	61.40	228182	59.14

In vitro study. The results of in vitro antimalarial activity against CQS 3D7 strains of *P. falciparum* of plant extracts are presented in (Table 3).

Table 3
In-vitro antimalarial activity of plant extracts against CQS (3D7) strain of *P. falciparum*.

Sample Id	Polynome	EC50	EC90	EC95	EC99	R2
S1	3	1.852	23.029	37.935	47.880	0.9681
S1-D	3	1.928	29.658	39.935	47.099	0.9453
CQ with 3D7 (CQ sensitive)	3	0.998	1.849	10.883	11.915	0.9115
CQ with RKL9 (CQ resistant)	3	11.078	7.714	9.865	110.723	0.9591

In silico study. Study of molecular docking of the target compounds, which was carried out against 2BL9 enzyme and is displayed in Table 4. Compounds 1-Octadecyne **(1)**, Tetra decyl oxirane (**2**) and gallic acid (**3**) with the best binding were visualised and analysed using Discovery Studio. The binding affinity values for all the target compounds range from – 6.031 Kcal/mol to -8.029 Kcal/mol with the target site against the DHFR enzyme. The target compounds **1** (-8.029 Kcal/mol), **2** (-7.997 Kcal/mol) and **3** (-6.031 Kcal/mol) showed remarkable binding affinity, which was more significant than the binding affinity of recommended drugs; chloroquine **(std)** (-7.813 kcal/mo) while gallic acid shows lesser binding affinity than the other two tested compounds and recommended drug.

Table 4
Molecular docking study of the target compounds with DHFR.

Compound codes	Binding Affinity (Kcal/mol)	Hydrogen bond		Hydrophobic bond Amino acid	Neutral amino acid
		Amino acid	Bond length (A°)		
1	-8.029	-	-	TYR A: 167, LEU A:162,ILEA:10, PHEA:171,ALA A:12, TYR A:179, ILE A:13, ILE A: 172,LEUA:45, ALAA:15,TRP A:47, ALA A:12	NDPA: 1239, CP A:61240
2	-7.997	-	-	ALA A:15, CYS A: 14,TYRA:179, ALA A:12, ILE A: 172, VAL A: 112, LEU A: 158, ILE A:10, LEU A: 162, CYS A: 170, VAL A:110	NDPA: 1239, CP A: 61240
3	-6.031	CP A: 6124	2.30	ILEA:172,ALA A:12, ILE A:13	-
		TYR A: 11 TYR A:179	3.10 2.75		
		TYR A: 11	2.85		
std	-7.813	ILE A:13	2.93	ILE172,ILE188, ILEA:10,CYS A:182, ALA A: 12, VAL A: 178, TYR A :179, TYR A: 56	-
N.B. "-" indicates no interactions					

Compound **1** (Fig. 3) accounts for no hydrogen bond with the target site. While it formed a hydrophobic bond with TYR A: 167, LEU A:162, ILEA:10, PHE A:171, ALA A:12, TYR A:179, ILE A:13, ILE A: 172, LEU A:45, ALA A:15, TRP A:47, and ALA A:12 of the target site. Compound **1** formed neutral interaction with the target site CP A:61240, and NDP A:1239. Compound **2** (Fig. 3) formed no hydrogen bond with the target site while it interacts with ALA A:15, CYS A: 14, TYR A: 179, ALA A:12, ILE A: 172, VAL A: 112, LEU A: 158, ILE A:10, LEU A: 162, CYS A: 170, and VAL A:110 to formed a hydrophobic bond with the target site. Similarly, tetra decyl oxirane formed neutral interaction with the target site CP A:61240, and NDP A:1239. Compound **3** (Fig. 3) formed four hydrogen bonds with the target site. The –C = O group and OH of the compound acted as a hydrogen bond acceptor and formed four hydrogen bonds with CP A: 6124, TYR A: 11, TYR A:179, and TYR A: 11 of the targets. Compound **3** formed hydrophobic bonds with ILE A:172, ALA A:12, and ILE A:13 of the target sites.

The recommended drugs, chloroquine (std) (Fig. 4**)**, account for one hydrogen bond (**ILE A:13**) while hydrophobic bonds were observed with ILE172, ILE188, ILE A:10, CYS A:182, ALA A: 12, VAL A: 178, TYR A :179, and TYR A: 56 and account no hydrophilic interaction with the target site of DHFR.

Discussion

The Purulia district has been classified into five different zones from very low to very high in malaria-endemic based on various spatiotemporal factors. Maximum malaria-endemic areas are in extremely high to high elevations, including Jhalda-1, Bagmundi, Jhalda-II, Joypur, Arsha, and Balarampur. Although below 30°C is the ideal temperature for malaria transmission, The average temperature varies between very low and low in the Jhalda-I, Jhalda-II, Bagmundi, Bandwan, and Arsha, Balarampur, Joypur blocks, which coincides with a high number of malaria case. Those blocks are also identified as high rainfall and low humidity zone, making malaria-prone endemic blocks. According to such endemic and non-endemic nature, the binary logistic regression model has been framed by considering temperature as a significant spatiotemporal factor. The predicted model will help the administration in minimising the number of cases of malaria before significant climatic variables lead to a situation where malaria is likely to arise.

The GC-MS analysis of the methanolic extract of leaves has revealed two reproducible major peaks with retention time of 13.246 (min) and at 13.754 (min). The GC-MS reference catalogue analysis identified the compounds as 1-Octadecyne (peak 1 with molecular weights of 250) and Oxirane (peak 3 with molecular weights of 240).

The detailed result of GC-MS analysis showed in Fig. 2, Supplementary Fig. S1 and Table 2. The studies found these compounds of other origins already have potent antimalarial and antioxidant activities [39, 40]. 1- Octadecene was reported to have therapeutic applications representing potent antioxidants and antibacterial activities[40]. The synthetic derivatives of Oxirane were found to be promising antiplasmodial compounds which mechanistically might inhibit serine protease of *P. falciparum*[39]. Therefore, the leaf of *M. alba* contained both of these antimalarial compounds together, which could be utilised in potential therapeutic applications in antimalarial drug developments.

As a standard in the *in vitro* study to co-relate the efficacy of the said methanolic extract of *M. alba* S1 leaves against malaria, the CQ-sensitive strain 3D7 and the CQ-resistant strain RKL9 were used. Here CQ was used as a standard drug. The result was obtained from the experiment, which was designed with the modified WHO MARKIII protocol in the 96-well flat-bottomed microplates (Supplementary Fig. S2A)[30]. From the Giemsa-stained blood smears (Supplementary Fig. S2B) of all the wells, these results were evaluated and analysed through dose-response curves by non-linear regression analysis using HN-NonLin Reg. Analysis[31]. Here, half maximal effective concentration of the (EC50) value of CQ with 3D7 strain (CQ sensitive) was 0.998 and 1.852 was the EC50 value of our test drug, i.e., *M. alba* S1 methanolic extract from leaves, which was quite close to CQ. Again, EC99 value of CQ with RKL9 strain (CQ resistant) was 110.723 and 47.880 was the EC99 value of the test drug that is more effective than the EC99 value of CQ with RKL9 strain. From the R^2 value of the methanolic leaf extract, which was 0.9681, it can be suggested that the said methanolic leaf extract of *M. alba* S1 leaves has a significant effect against Pf malaria (Table 3). To sum it up, the result shown by the methanolic extract of *M. alba* S1 leaves is efficient against the 3D7 strain of Pf malaria parasite. The activity can be improved by creating better extraction conditions. Also, microscopically, parasite clearance was found after treatment with the methanolic leaf extract, which demonstrates the methanolic leaf extract's potential efficacy against malaria (Supplementary Fig. S2C).

In one of our studies, we found the gallic acid to be an essential compound in mulberry leaf extract, which extract was analysed for the antibacterial activity[41]. It was reported previously that gallic acid has adequate antimalarial activity[42]. We also saw in this study that gallic acid has antimalarial activity in molecular docking through hydrophobic bonds with the target sites (Table 4). Here, our two target compounds, i.e., 1-Octadecyne and Tetra decyl oxirane, had shown remarkable binding affinities which were greater than the binding affinity of recommended drugs, such as chloroquine (Table 4, Fig. 3, & Fig. 4). This indicates that the said methanolic extract of *M. alba* S1 leaves has significant antimalarial activity.

Conclusion

In conclusion, the prevalence of malaria in Purulia district, West Bengal, India, is influenced by various geo-environmental factors such as temperature, humidity, rainfall, vegetation, and topography. Anthropogenic activities such as deforestation, urbanisation, and agricultural practices also play a significant role in spreading the disease. Effective measures to control the incidence of malaria in this region would require a comprehensive understanding of the interactions between these factors and their impact on the disease. All these are tried to analyse in this study which will help the policymakers to strengthen the epidemiological surveillance of malaria. This study also demonstrates the chemico-biological efficacy of *Morus alba* S1 leaf extract against the 3D7 strain of *Plasmodium falciparum* malaria through both *in-vitro* and *in-silico* analysis, as revealed by a positive interaction between participating target components of the *M. alba* S1 leaf extract. This was also proved through GC-MS analysis and molecular docking studies, as the common interacting target components were found to be same in both studies. It demonstrates that Mulberry leaf extract is potentially effective against Pf malaria, which will further open a new avenue for public health advancement for the treatment of Pf malaria.

Declarations

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Author contributions

S.P., S.H., S.R., S.M., P.D., R.M., A.G., T.B., and S.S contributed to the epidemiological investigation and collected the data. W.S.S., S.K.M., S.R, A.B., K.C.P., S.S., K.V., T.K.D, P.K.K., S.M., D.B., M.S., A.B.M., A.K.M. and I.A.I. accessed, verified, analysed data and drafted the manuscript. All authors contributed to the interpretation of results and critical revision.

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Ethics declarations

Competing interests

The authors declare no competing interests

Data availability

A complete de-identified patient dataset will be available to the researcher upon request. Individuals wishing to access the data should send a request to the tkdolai@hotmail.com or amitmandal08@gmail.com, or ikbal.agah.ince@gmail.com.

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Figures

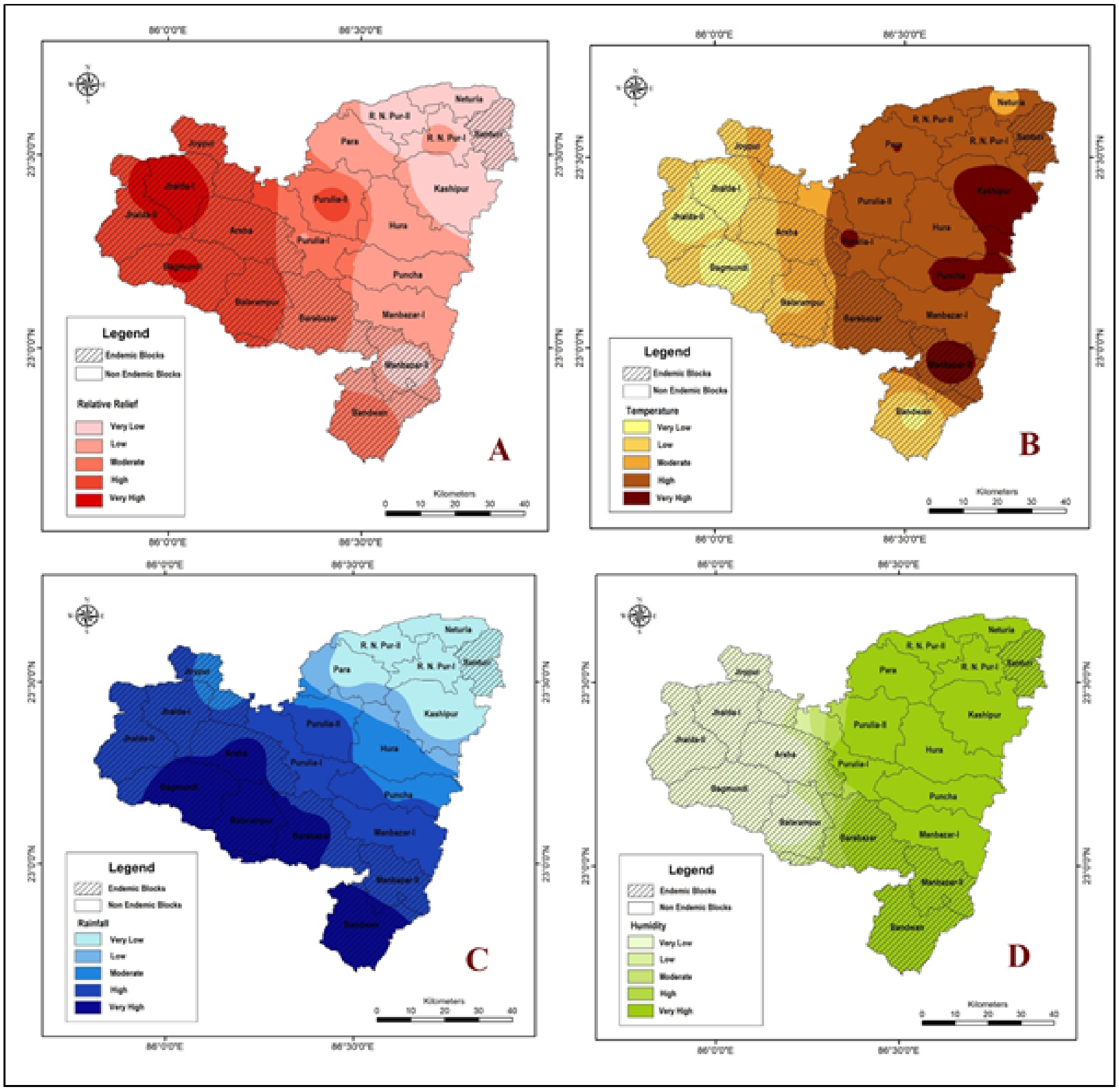

Figure 1

Spatial zonation of spatiotemporal variables (A. elevation, B. temperature, C. rainfall and D. humidity (Source: generated using ArcMap v.10.8, 2022).

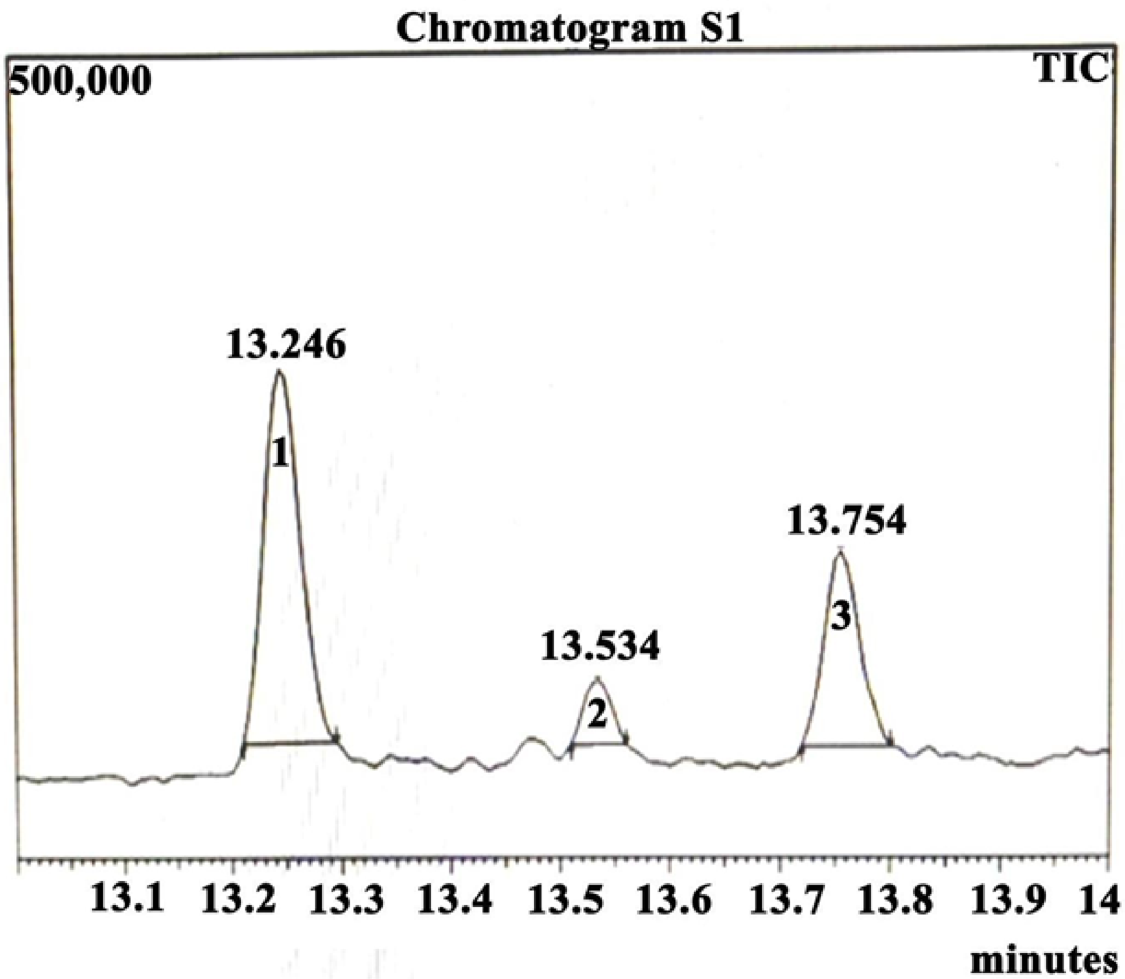

Figure 2

GC-MS chromatogram of the methanolic leaf extract of *Morus alba* S1.

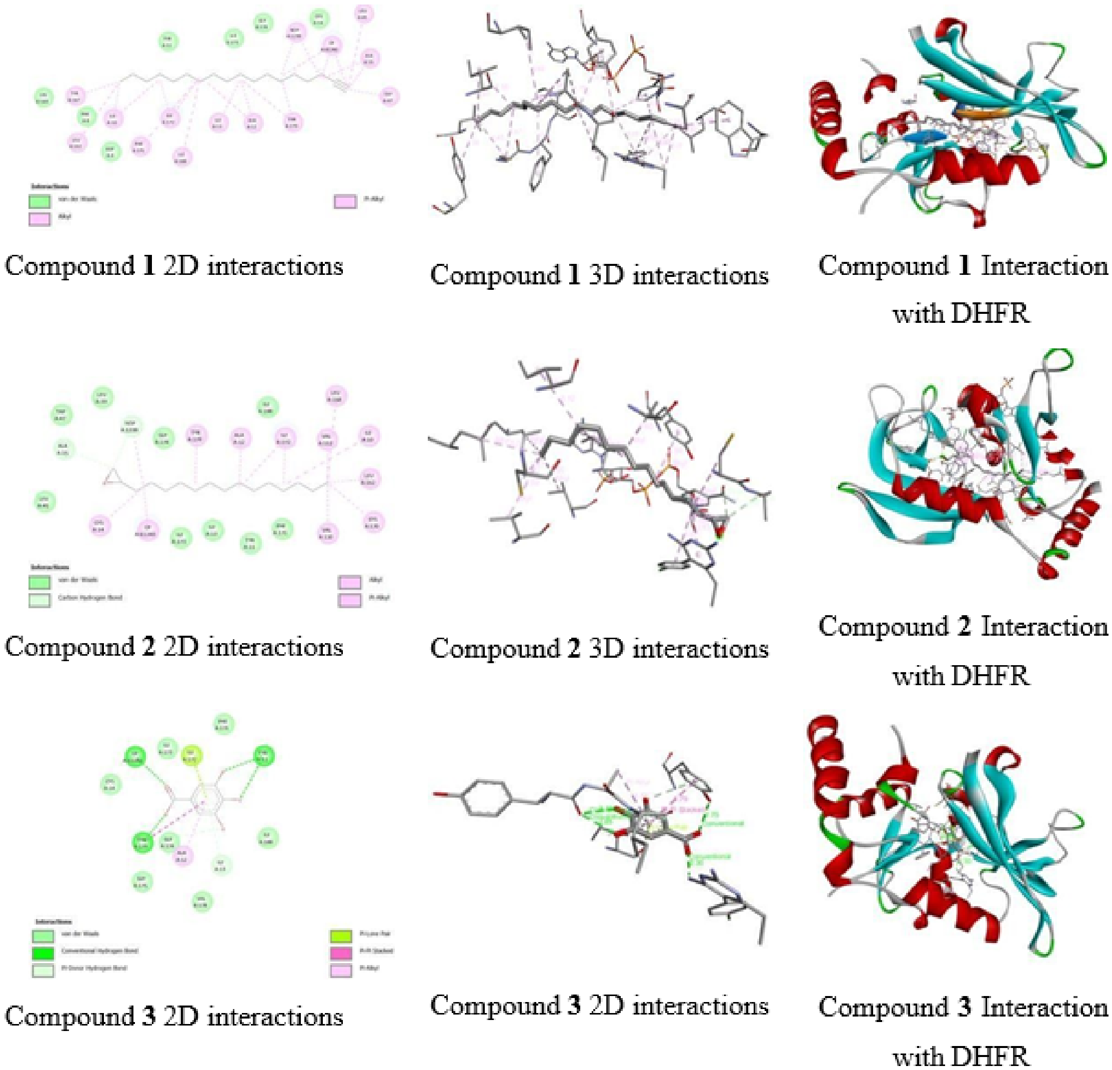

Figure 3

3D and 2D Interactions between synthesized compounds 1, 2 and 3 with the target site of the p38 MAP kinase enzyme.

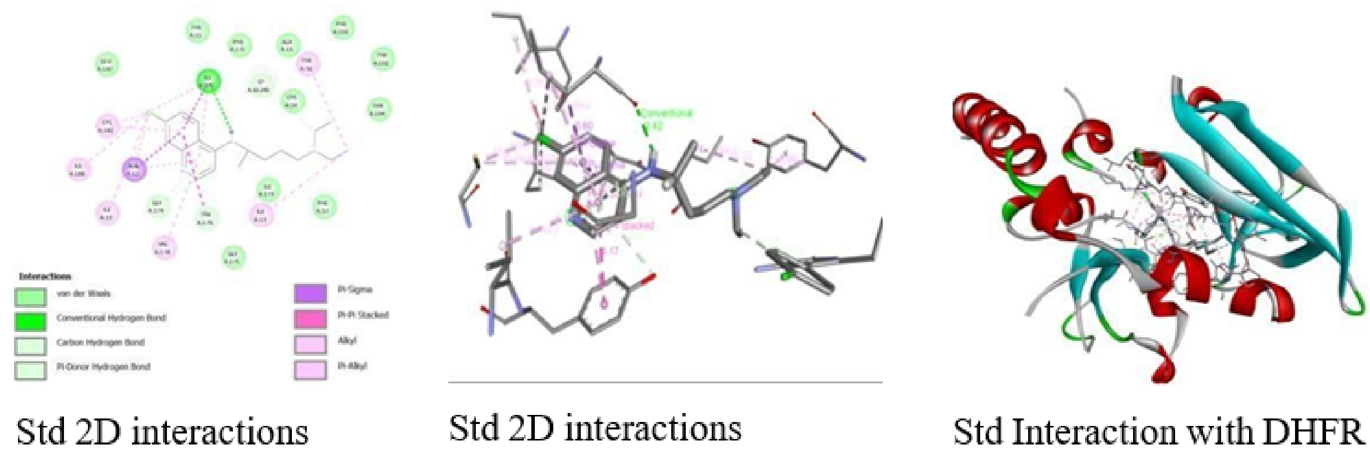

Figure 4

3D and 2D Interactions between chloroquine (std) with the target site of the DHFR enzyme.

Supplementary Files

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