Antimalarial activity of Curcuma caesia against 3D7 and K1 strains of Plasmodium falciparum

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Research

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Abstract

Background: Malaria is one of the severe tropical disease and majority of deaths occurred due to *Plasmodium falciparum*. Lack of a vaccine and the widespread resistance to antimalarial drugs have resulted in emphasis on novel antimalarial drugs development. The purpose of the study was to evaluate *in vitro* and *in-silico* antiplasmodial potential of *Curcuma caesia* extracts against *P. falciparum*.

Methods: Ethyl acetate and methanol extracts of *C. caesia* were prepared and analysed for their antiplasmodial activity against Chloroquine sensitive (3D7) and resistant (K1) strains of *P. falciparum* using fluorescence-based SYBR Green assay. The cytotoxicity tests were carried out using the vero cell lines by MTT assay. The phosphoethanolamine methyltransferase enzyme ((PfPMT) essential for growth of *P. falciparum* was used as protein target for *in-silico* study.

Result: *C. caesia* ethyl acetate extracts showed the potent antiplasmodial activity with IC_{50} values of 3.37 µg/ml and 1.53 µg/ml against 3D7 and K1 strain respectively. The IC_{50} values of methanol extract were reported, 8.57 µg/ml against 3D7 and 18.29 µg/ml against K1 strains The cytotoxicity assay revealed that the extracts were not toxic against vero cell lines as the CC_{50} values were less than $IC_{50.}$ Docking results show that β-selinenol an oxygenized sesquiterpene present in *C. caesia* had the free binding energy of -6.76 Kcal/mol.

Conclusion: The compounds β-selinenol, α-eudesmol, α –acorenol, boldione and xanthinin

present in the *C. caesia* extract possess antimalarial potential being inhibitor of PfPMT. The present findings, however preliminary in nature. Further studies are needed to identify the active compounds and *in vivo* mechanism to prove the antimalarial efficacy of *C. caesia* in the development of antimalarial drugs.

Background

Malaria remains an important global health concern in tropical and subtropical regions and antimalarial drug resistance has emerged as alarming concern for discovery of novel antimalarial compounds. However, the increase of insecticide-resistant Anopheles mosquitos triggers not only malaria expanding to new areas but its re-emergence in places where it has historically been eradicated. In addition, emergence of resistance to antimalarial drugs of *P. falciparum* is major concern. An estimated 228 million cases of malaria occurred in 2018 with 405 000 deaths worldwide. In India 429928 cases of malaria were reported in 2018. About 67% children under 5 years of ages were affected with malaria. According to WHO report *Plasmodium falciparum* is the most rampant malaria parasite accounting for 99.7% of estimated malaria cases in 2018 [1]. Traditionally, in various cultures, medicinal plants were used to sustain their primary health care needs. The use of different extracts from medicinal plants for malaria treatment has a long and successful tradition [2]. As an example quinine from Cinchona, and

artemisinin isolated from Qinghaosu [3]. Many recent studies concentrate on natural and herbal product in search of antimalarial drugs [4,5].

caesia is one of the traditional tribal medicinal plants which is used in India for treatment of fever and other ailments. It belongs to Zingiberaceae family which is commonly called as black turmeric. Plants of the family Zingiberaceae have been documented as having antimalarial properties [6]. It is a perennial plant with bulky bluish color rhizome. The objective of the present study was to analyse antimalarial potential of *C. caesia* ethyl acetate and methanol extracts against the *P. falciparum* strains along with the molecular docking study to search out the possible metabolites which interfere with Plasmodium enzyme.

Methods

Plant extracts preparation and GCMS analysis: The extract of *C. caesia* was prepared in ethyl acetate and methanol solvent by following the method of our previous study [7].

In vitro antimalarial activity screening: The *in vitro* cultures of both Chloroquine - resistant (K1) and sensitive (3D7) strains of *P. falciparum* were performed using RPMI-1640 medium of pH 7.2 supplemented with 25mM HEPES, 0.5% ALBUMAX-II, 0.2% D-glucose and 0.21% sodium bicarbonate [8]. The stock (5mg/ml) solution of test samples were prepared in DMSO and required dilutions were prepared in culture medium. The maximum concentration of extract used was 50.0µg/ml. 1.0% parasitized cell suspension containing 0.8% parasitaemia (Asynchronous culture with more than 80% ring stages) were incubated in 96 well plate with two-fold serial dilutions of test sample. The plates were kept in CO_2 incubator at 37^0C with an atmosphere of 5% CO_2 and air mixture. After 72 hours, 100µl of lysis buffer with 2x concentration of SYBR Green-I (Invitrogen) was added in each well and kept at 37^0C for one hour [9]. The plates were examined at 485±20nm of excitation and 530±20nm of emission for relative fluorescence units (RFUs) per well, using the fluorescence plate reader (FLX800, BIOTEK). The IC_{50} values (concentration that inhibits 50% growth of *P. falciparum*) were calculated by Logit regression analysis of dose-response curves. Chloroquine diphosphate (SIGMA) was used as the reference drug.

Cytotoxicity assay: The cytotoxicity of the test samples was conducted against Vero cell line (C1008, Monkey kidney fibroblast cells) using MTT assay [10]. The cells were incubated with test sample dilutions for 72h and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was used as reagent for detection of cytotoxicity. The highest concentration of test sample used was 200µg/ml. MTT dye turned from yellow to purple color after cellular enzymatic reduction. CC_{50} value (concentration that reduced the cell viability by 50%.) was calculated using dose-response curves. Podophyllotoxin (SIGMA) was used as the reference drug for cytotoxicity.

Macromolecules and ligand structure retrieval: The target protein a multifunctional enzyme phosphoethanolamine methyltransferase (PfPMT) (PDB ID: 3UJ9) was selected on the basis of the literature study. This enzyme catalyzes the methylation of phosphoethanolamine to phosphocholine

which is necessary for membrane synthesis. Hence, it is vital for survival and growth of *Plasmodium falciparum.* As this enzyme is absent in humans, so it's good drug target in search of new anti-malarial drug [11]. Fifty-three metabolites were obtained from a previous study [7] by GCMS analysis of methanol and ethyl acetate extract of *Curcuma caesia* were used as a ligand for docking study. Ligand structures were downloaded in SDF format from the Pubchem database and were converted into PDB format by PyMol software.

***ADMET prediction*:** Before docking study all ligand molecules were subjected to computational ADMET analysis by using online software Swiss ADME [12] and ADMET-SAR [13].

***Docking and visualization*:**AutoDock 4.2 was used to study protein and ligands interaction. AutoDock is the most widely used fully automated software for the docking study [14]. Polar hydrogen and Kollman charges were added to the protein molecule [15]. Grid box having dimensions X, Y, and Z were set to 120*120*120. Lamarck genetic algorithm was used to analyze the output for ligand conformations. The least negative ΔG specifies a strong binding and favourable conformation between ligand and protein [16]. The 3D visualization of docked structures was achieved by a graphical user interface, and Discovery studio [17].

Results

In vitro antimalarial activity screening and cytotoxicity assay

In vitro antimalarial activity of *Curcuma caesia* ethyl acetate and methanol extracts were analyzed for both Chloroquine-resistant (K1) and sensitive (3D7) strains of *P. falciparum.* IC_{50} values of ethyl acetate extract were 3.37 µg/ml against 3D7, and 1.53 µg/ml against K1 strain. The methanol extract possesses IC_{50} values of 8.57 µg/ml against 3D7 and 18.29 µg/ml against K1 strains (Table 1). The IC_{50} value for promising lead compounds was considered equal to or less than 10.0 µg/ml.

Cytotoxicity was carried out against Vero cell line (C1008; Monkey kidney fibroblast cells). IC_{50} and CC_{50} values of both extracts against the *P. falciparum* sensitive and resistant strains with positive and negative control values along with Selectivity index (SI) has been given in Table 1. SI was calculated by dividing the CC_{50} value with IC_{50} and the criteria for selection is that SI value should be equal or more than 50. Higher SI value is preferable for a drug to have favourble safety and efficacy [10]. Interestingly, ethyl acetate extract of *Curcuma caesia* exhibited profound *in vitro* antimalarial activity against 3D7 and K1 strains in comparision to methanol extract with higher SI index against K1strain.

Table 1: *In vitro* antimalarial and cytotoxic activity of *Curcuma caesia* extracts

Test Samples	IC_{50} (µg/ml)		CC_{50} (µg/ml)	Selective Index (SI)	
	3D7	K1		3D7	K1
C. caesia ethyl acetate extract	3.37	1.53	13.92	4.13	9.09
C. caesia methanol extract	8.57	18.29	91.20	10.64	4.98
QC-diphosphate*	0.005	0.303	175	35,000	577.5
Podophyllotoxin*	na	na	6.4	na	na

*Values are in µM

ADMET prediction

All the compounds from the GCMS study of both extracts were selected for ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) prediction. From the ADMET result only thirty-one compounds were selected which follows Lipinski rule with no violation and having bioavailability value of 0.55 for docking study. Additional file1, Table S1 shows the bioactivity radar for rapid evaluation of drug-likeness of thirty-one metabolites.

Docking analysis of compounds

Molecular docking studies were carried out to reveal the interactions of phosphoethanolamine methyltransferase enzyme (PfPMT) of *Plasmodium falciparum* with thirty-one compounds identified from *Curcuma caesia* methanol and ethyl acetate extracts. Binding energy of all thirty-oneligands against the enzyme is depicted in Additional file 2, Table S2. The higher negative value of binding energy corresponds to a more stable interaction of ligand to the enzyme. Among 31 ligands, β-Selinenol (-6.76 Kcal/mol), α-Eudesmol(-6.59 Kcal/mol), α –Acorenol (-5.85 Kcal/mol), Boldione (-5.55 Kcal/mol) and Xanthinin (-5.40 Kcal/mol) showed the highest binding energy (Table 2). The 2D and 3D interactions docking results were attained by introducing our result into the Discovery Studio Visualizer, enabling us to recognize important interactions between the ligands and the binding site of the receptor. Fig. 1 shows the 2D interaction of all top five compounds with PfPMT which helps in identifying the amino acids which are involved in the interaction. There are 2 amino acids involved in conventional hydrogen bond interaction between β-Selinenol and PfPMT,GLU226 and LYS 225, shown in Fig.2(a) and with a bond length of 2.73 and 2.95. Fig. 2(b) shows the surface view of the hydrogen bond.

Table 2: Binding energy of lead five ligand with PfPMT

Name	Binding energy (Kcal/mol)	No. of H-bond	Amino acid	Bond length (Ångstrom)
β-Selinenol	-6.76	2	GLU226, LYS 225	2.73Å, 2.95Å
α-Eudesmol	-6.59	2	ASN101,ASN74	2.55Å, 3.23Å
α –Acorenol	-5.85	1	GLN212	2.91Å
Boldione	-5.55	1	LYS165	3.09Å
Xanthinin	-5.40	3	PHE116,TRP148, GLU143	3.32 Å, 3.17Å, 3.18Å

Discussion

Artemisinin was the backbone of antimalarial research for a decade and due to artemisinin resistance, it is necessary to identify newer antimalarial agents with higher activity and lower toxicity. Therefore, this study was conducted to explore the antimalarial activity of *Curcuma caesia*. *Curcuma caesia* belongs to the Zingiberaceae family which contains many plantswith reported antimalarial activity like *Aframomum latifolium*, *Kaempferia marginata* [18], *Curcuma longa, Zingiber officinale* Roscoe, etc. Previous studies reveal that the antiplasmodial effect of *Zingiber officinale* Roscoe methanol extract against *P. berghei* in a dose-dependent manner[19]. Duker-Eshum et al.[20] showed the significant antiplasmodial activity of the extracts of *A. latifolium* and *A. sceptrum*. The supercritical extract of *Curcuma longa* also showed the high antiplasmodial activity [21].

Several terpenes were identified as having antimalarial activity from different plant sources. For example, Artemisinin (sesquiterpene lactone) and paclitaxelare used clinically for malarial treatment. Goulart et al. [22] demonstrated that farnesol and linalool both are terpenesthat can inhibit the biosynthesis of many intermediates and end products of the isoprenoids pathway of *Plasmodium falciparum*. Limonene hinders the protein isoprenylation in the intra-erythrocytic stages of *P. falciparum* [23]. Kenmogne et al. [24] isolated Zambisiacolactone B labdane, aditerpenoids from *A. zambesiacum* which was the most active against chloroquine-resistant strain *P. falciparum* with an IC_{50} of 4.97 µM. Curcumin from *Curcuma longa* has antimalarial potential.GCMS analysis of *C. caesia* extracts revealed the presence of many sesquiterpene.

Modern studies have opened the way to identifying new drug target in search of effective and novel antimalarial drug with less time consumption. The phosphoethanolamine methyltransferase, PfPMT, of the human malaria parasite *Plasmodium falciparum*, a member of a novely identified family of phosphoethanolamine methyltransferases (PMT) found exclusively in nematodes, protozoa and plants. It is involved in the synthesis of the major membrane phospholipid, phosphatidylcholine. But PMT from these organisms use multiple methyltransferase domains for the S-adenosylmethionine (AdoMet) reactions which is a three-step S-adenosylmethionine-dependent methylation of the nitrogen atom of phosphoethanolamine to phosphocholine. In *P. falciparum*, this enzymatic reaction is a limiting step in

the pathway of synthesis of phosphatidylcholine from serine, hence it is potent new drug target for antimalarial drug discovery.and plays an important role in the development, replication and survival of the parasite within human red blood cells [11, 25].

To explore the metabolite involved in the antimalarial activity of *Curcuma caesia* in silico study against phosphoethanolamine methyltransferase enzyme (PfPMT) of *Plasmodium falciparum* was done. In molecular docking the least binding energy revealed the stronger docking berween the ligand and parasitic target. The docking results showed that β-Selinenol and α-Eudesmol present in *C. caesia* having least binding energy. Both are the isomeric form. β-selinenol displays considerable in vitro antiplatelet activity, antiangiogenic activity, anti-hepatotoxic effects and antitumoral effects [26]. Earlier reports suggest many sesquiterpene as a antiparasitic effect like artemisinin, lactucin, lactucopicrinetc [27]. Antimalarial activity of *C. caesia* may be due the presence of sesquiterpenes.

Conclusion

The development and identification of new malaria molecules is a growing need for multi-drug resistant malaria. Current study is designed for search of the new and effective antimalarial drug by *in vitro* antimalarial activity of *Curcuma caesia* extracts against Chloroquine sensitive (3D7) and resistant (K1) strains of *P. falciparum* strains. Although *Curcuma caesia* has been used in traditional medicine systems for fever and various infections, the antimalarial activity has been established for the first time. Further studies are required to proven the antimalarial efficacy *C. caesia* by isolating the active compounds and evaluate its potential as antimalarial drug.

Declarations

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Authors' contributions

Monika Chaturvedi has prformed experimentation work, data collection, and drafted the manuscript. Reena Rani has made significant involvement in the interpretation of data and revising the manuscript. Dushyant Sharma participated in the design of the study and performed the statistical analysis. Jaya Parkash Yadav helped in designed the study and manuscript.All authors read and approved the final manuscript.

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Availability of data and materials

The data supporting the conclusions of this article are provided within the article and its additional files. The original datasets analysed in this current study are available from the corresponding author upon request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

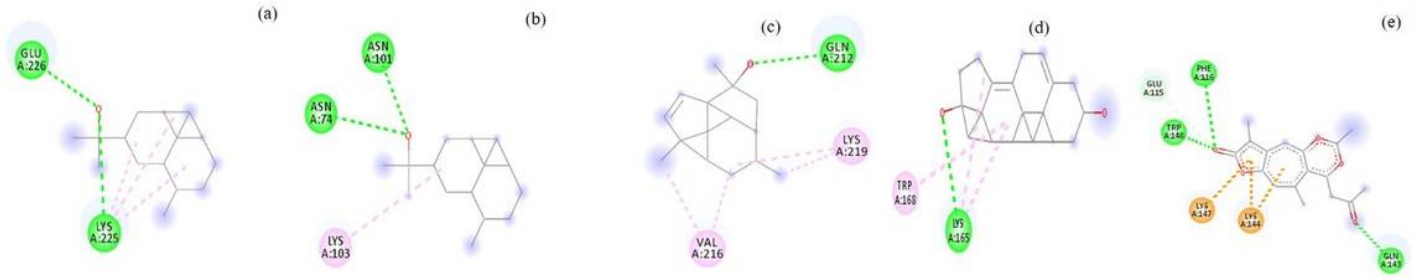

Figure 1

Showing the 2D interaction of all top five compounds with PfPMT(Phosphoethanolamine methyltransferase).

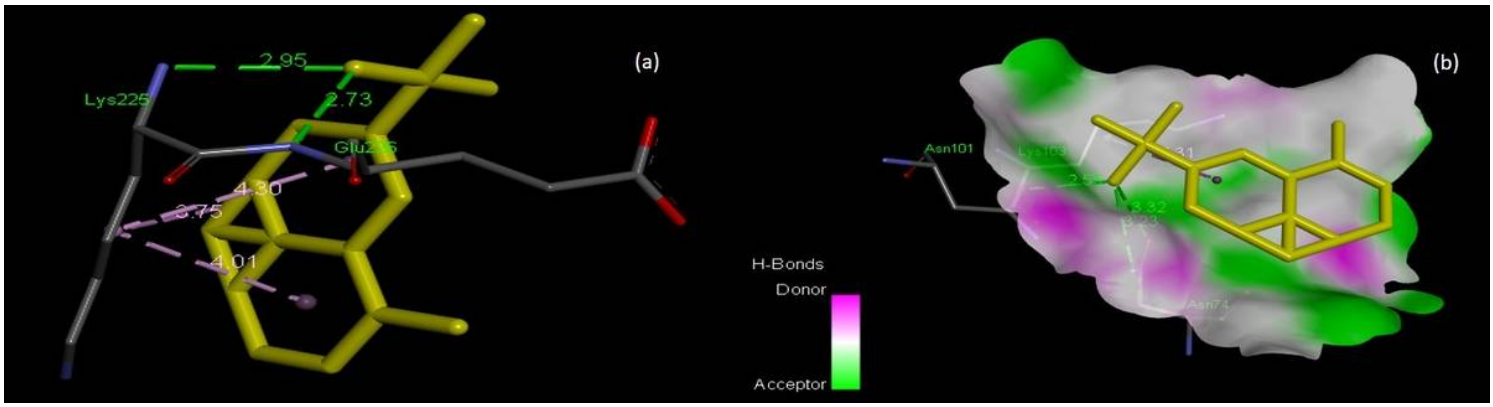

Figure 2

(a) 3D interaction β-Selinenol and PfPMT (Phosphoethanolamine methyltransferase) with bond length (b)surface view of the hydrogen bond between β-Selinenol and PfPMT(Phosphoethanolamine methyltransferase).

Supplementary Files

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