Research Article

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Antiplasmodial potential of *Eucalyptus obliqua* leaf methanolic extract against *Plasmodium vivax*: An *in vitro* study

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Abstract: Malaria is an intraerythrocytic parasitic disease caused by the genus *Plasmodium* of which *Plasmodium vivax* and *Plasmodium falciparum* are the major species. The high cost and associated side effects of antimalarial drugs triggered research about medicinal plants to develop alternative and low-cost drugs with lesser side effects. Therefore, this study was designed to investigate the antiplasmodial activity of the *Eucalyptus obliqua* L'Hér. leaf extract against *P. vivax* and its phytochemicals in *in vitro*. The methanolic extract of *E. obliqua* was prepared and different concentrations of the crude extract and phytochemicals were used against *P. vivax*. The methanolic extract of *E. obliqua* showed profound antiplasmodial activity (LD_{50} 0.084 mg/mL; 80.04%) at 0.1 mg/mL concentration after 24 h. Alkaloids, flavonoids, saponins, and tannins were found in the *E. obliqua* methanolic extract. Only alkaloids at the concentration (0.1 mg/mL) exhibited 60.93% inhibition of *P. vivax*. The methanolic extract of *E. obliqua* exhibits antiplasmodial activity *in vitro*. However, *in vivo* efficacy is an important aspect in the testing of medicinal

plants against parasitic infections and should be evaluated in future.

Keywords: *Plasmodium vivax*, *Eucalyptus obliqua*, antiplasmodial activity, *in vitro*

1 Introduction

Malaria is a parasite-borne intraerythrocytic infection that affects more than half of the world population [1,2]. It is caused by hemoparasites of the genus *Plasmodium* and is transmitted by the female anopheles' mosquito [3]. It is estimated that 60% of the total population of Pakistan resides in areas with high malaria incidence [4]. In Pakistan, *Plasmodium vivax* is responsible for 64% of malaria incidence [5]. The resistance of malarial parasites to many available antimalarial drugs is a major monetary constraint in combating malaria [6]. The current commercially available antimalarial drugs are associated with various side effects. Moreover, the expenses associated with the conventional approaches of managing malaria are reasonably high particularly for individuals living in low-income countries like Pakistan. Therefore, new drugs are required to avert the complications posed by drug-resistant *Plasmodium* strains [7].

For centuries, medicinal plants have been used to treat human diseases worldwide like stomach pain, diabetes, hyperacidity, gonorrhea, dysentery, cystitis, urethritis, laryngitis, leucorrhoea, inflammation, bronchitis, tuberculosis, wounds, and many others. More than 80% of the population of both developing and developed countries rely on traditionally synthesized herbal drugs [8]. The need and utilization of medicinal plants in China, South Africa, and India have dramatically increased in the last few years. A lot of studies have been carried out on the sustainable use and conservation of medicinal plants in these countries [9]. Some studies have been published in Pakistan on the antiplasmodial potential of medicinal plants.

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Medicinal plants offer viable alternatives with fewer side effects, are reasonably cheaper, and easily available. Pakistan has a distinctive geography and has a wide range of diversity in climatic zones due to which it is rich in the diversity of plants. It is estimated that Pakistan has more than 6,000 species of higher plants among which 12% have medicinal importance. These medicinal plants are also exported to other countries [10]. Studies in Pakistan have revealed that eucalyptus leaves collected from the Soon Valley in Khushab, Pakistan, have been traditionally used against malaria infection [11].

The family (Myrtaceae) of eucalyptus plants is a rich source of biologically active compounds, such as steroids, alkaloids, tannins, saponins, terpenes, flavones, flavonoids, polyphenolics, phenolics, triterpenoid, fatty acids, lignins, vitamin C, anthraquinone, glycosides, anthocyanin, coumarins, cardiac glycosides, and volatile oils [12,13]. *Eucalyptus obliqua* L'Hér. is a fast-growing evergreen plant, named after its oblique leaves. *E. obliqua* is well known for the isolation of antimalarial compounds extracted from its leaves and is also used widely for draining swamps due to its water-absorbent ability [14,15].

The local names of *E. obliqua* are Sufaida and Laachi in Pakistan [16]. *E. obliqua* has potential antimalarial ability as it is being traditionally used as folk medicine in many parts of the world [17]. It is also reported that oils and secondary metabolites of *E. obliqua* exhibit anti-microbial and antifungal properties. Most of the species of *Eucalyptus* are also known for their medicinal importance and are used in the treatment of malaria, microbial infections, and dysentery [18,19]. There is a dire need to assess medicinal plants for antiplasmodial agents which may be a source of an alternative antiplasmodial drug(s). Therefore, this study aimed to investigate the antiplasmodial activity of *E. obliqua* leaf extract and phytochemicals against *P. vivax* *in vitro*.

2 Materials and methods

2.1 Plant collection and methanolic extract preparation

Fresh leaves of *E. obliqua* were collected from its natural habitat in the southern district of Kohat, Khyber Pakhtunkhwa, Pakistan (coordinates latitude: 33.6060705292903 and longitude: 71.46785718724671). The plant leaves were rinsed with fresh water and were identified by a taxonomist at the

Department of Botanical and Environmental Sciences, KUST, Kohat, and a sample voucher number: MEB397 was given and has been deposited. The leaves were shade dried and ground in an electric grinder. The methanolic extract was prepared as described by ref. [20] with slight modifications. Briefly, 10 × 100 mL of leaf powder was mixed in absolute methanol in a beaker. The suspension was kept in an electric shaker for 48 h at room temperature.

The suspension was filtered through Whatman's filter paper one and the filtrate was processed in a rotary vacuum evaporator (BUCHI Rotavapor R-200, Switzerland) at 40°C. The concentrated crude extract was obtained and stored at 4°C for further analysis.

2.2 Phytochemical analysis

The extract of *E. obliqua* was subjected to analysis and extraction of alkaloids, flavonoids, and saponins as described by ref. [21], and tannins as described by ref. [22].

2.3 *P. vivax* culture and maintenance

P. vivax strains (confirmed in this study) were used to maintain the culture at the Molecular Parasitology and Virology Laboratory of KUST, Khyber Pakhtunkhwa, Pakistan. A blood medium mixture of 200 µL with 2% hematocrit, consisting of McCoy's 5A medium (Life Technologies, VIC, Australia) supplemented with 20% human serum, was used for culturing. The parasites were cultured in a candle jar at 37.0°C. Incubation was deemed successful and stopped when ≥40% of the ring-stage parasites had reached the mature schizont stage (≥4 distinct nuclei per parasite). Gentamicin sulfate (5 µL) was also added to the culture. Thick blood films were prepared on glass slides and stained with 5% Giemsa solution for 30 min and examined microscopically. Daily, infected erythrocytes were inoculated into a fresh complete medium to propagate the culture [23].

2.4 *In vitro* antiplasmodial activity

The *in vitro* antiplasmodial activity of the methanolic extract and phytochemicals was carried out as previously

Figure 1: Healthy and affected schizonts/cytotoxic activity under the microscope.

described by ref. [24]. The antiparasmodial activity was performed in 96 microplates. Chloroquine and proguanil were used as positive controls while microplates with parasitized culture and without drug/plant extract served as the negative control. Five concentrations (0.02, 0.04, 0.06, 0.08, and 0.1 mg/mL) of plant extract, phytochemicals, and drugs were used [25,26]. The microplates were shaken gently and kept in a candle jar to increase the concentration of CO₂. The plates were placed in an incubator at 37°C for 24 h. After 24 h the supernatant was removed from each microplate and red blood cells were picked with a micropipette to prepare thin smears using the Giemsa stain and was observed under a microscope (Figure 1) [27].

2.5 Statistical analysis

Maturation percentage and inhibition percentage were evaluated using the following formula [24].

Maturation percentage =

$$\frac{\text{No. of developed schizonts for the experimental group}}{\text{No. of developed schizonts for control}} \times 100$$

Inhibition percentage = 100 – Maturation percentage

The mean inhibition and standard deviation were calculated using Statistix 9 software. LD₅₀ was calculated by using an online tool AAT Bioquest (<https://www.aatbio.com/tools/ld50-calculator>).

3 Results

E. obliqua crude extract exhibited 31.88, 35.54, 38.72, 58.9, and 80.04% growth inhibition at 0.02, 0.04, 0.06, 0.08, and 0.1 mg/mL after 24 h, respectively. The antiparasmodial activity of the methanolic extract was dose-dependent with LD₅₀ 0.084 mg/mL. In comparison, the antiparasmodial activity of *E. obliqua* extract was analogous to chloroquine and proguanil (Table 1).

The phytochemical analysis revealed the presence of alkaloids, flavonoids, saponins, and tannins in *E. obliqua* (Table 2). Of this, only alkaloids at the concentration (0.1 mg/mL) exhibited about 60.93% growth inhibition of *P. vivax* which was comparable to the control chloroquine (Table 3).

Table 1: The *in vitro* antiparasmodial activity of *Eucalyptus obliqua* methanolic crude extract

Extract/drugs	Concentration (mg/ml)	Schizonts in experimental group (mean ± SD)	Schizonts developed in control group (mean)	Maturation %	Inhibition %	LD ₅₀ mg/mL
<i>Eucalyptus obliqua</i>	0.02	171.44 ± 1.25	251.66	68.12	31.88	0.084
	0.04	162.22 ± 1.25	251.66	64.46	35.54	
	0.06	154.22 ± 1.25	251.66	61.28	38.72	
	0.08	103.44 ± 3.40	251.66	41.1	58.9	
	0.1	50.22 ± 3.40	251.66	19.96	80.04	
Proguanil	0.02	85.78 ± 1.89	251.66	34.08	65.92	0.068
	0.04	75.89 ± 2.87	251.66	30.15	69.85	
	0.06	51.44 ± 2.94	251.66	20.44	79.56	
	0.08	30.11 ± 2.49	251.66	11.96	88.04	
	0.1	15.00 ± 2.49	251.66	5.96	94.04	
Chloroquine	0.02	100.67 ± 1.70	251.66	40	60	0.077
	0.04	76.33 ± 0.94	251.66	30.33	69.67	
	0.06	59.33 ± 2.49	251.66	23.58	76.42	
	0.08	24.67 ± 0.82	251.66	9.8	90.2	
	0.1	13.89 ± 0.94	251.66	5.52	94.48	

Table 2: The phytochemical constituents of *Eucalyptus obliqua* methanolic crude extract

S. No.	Phytochemical constituent	Methanolic crude extract
1	Alkaloids	+
2	Flavonoids	+
3	Saponins	+
4	Tannins	+

4 Discussion

Over the last hundreds of years, there has been an observed increase in research focusing on the use of medicinal plants as natural remedies for infectious diseases. A variety of novel antimalarial compounds have been discovered as a result of the extensive research on medicinal plants [28]. This study explored the antiplasmodial potential of *E. obliqua* methanolic extract and phytochemicals against *P. vivax* *in vitro*.

The *E. obliqua* methanolic extract profoundly inhibited (80.04%) *P. vivax* at the highest tested concentration. The extract impacted the parasite in a dose-dependent manner. This study is quite in agreement with the study of Sabiu and Ashafa [17] who reported the antiplasmodial potential of *E. obliqua* against the *Plasmodium* species. Previous studies have confirmed the antimalarial potential of eucalyptus plants as they inhibited more than 50% of the malarial parasite [29]. Three extracts (aqueous, n-butanol, and ethyl acetate) of *E. globulus* remarkably reduced the growth of protozoan parasites. However, the impact was dosage and extract type-dependent [30]. Scientists have analyzed and evaluated the effect of various kinds of solvents, to extract bioactive compounds from plants [31]. Methanol is the most preferred solvent for plant extraction possibly due to its polar nature that ensures the release of several bioactive compounds from plants [32,33]. It has been scientifically proven that highly polar solvents should be used to extract bioactive compounds with a high level of accuracy [31]. The effects

of the active compounds derived from plants depend mainly upon the solvent used for herbal formulation [33].

Eucalyptus oil was found effective against the asexual stages of *Plasmodium falciparum* *in vitro* [34]. Furthermore, eucalyptus plants are globally used as a traditional medicine against malarial infection [35,36]. Therefore, it could be considered a potential antimalarial agent and is recommended for further in-depth analysis.

The phytochemical analysis revealed the presence of alkaloids, flavonoids, saponins, and tannins. The alkaloids extracted from *E. obliqua* was found to be responsible for the antiplasmodial activity and it inhibited parasite growth *in vitro*. The same classes of compounds have also been identified in the ethanolic leaf extracts of *Eucalyptus citriodora* and methanolic leaf extracts of *Eucalyptus camaldulensis* [37,38]. Alkaloids and flavonoids of different plants have proven antiplasmodial activities. Alkaloids are actively involved in stopping the process of protein synthesis in the *Plasmodium* species [39]. Similarly, flavonoids have also profound efficacy against *P. falciparum* [40]. The presence of these classes of compounds validates the antiplasmodial activity of *E. obliqua* in this study. However, further studies analyzing the isolated pure compounds of the mentioned classes are highly recommended to better understand the underlying antiplasmodial activity of *E. obliqua* and related plant species.

5 Conclusion

This study concludes that the methanolic extract of *E. obliqua* exhibits antiplasmodial activity *in vitro*. However, *in vivo* efficacy is an important aspect in the testing of medicinal plants against parasitic infections and should be evaluated in future. Moreover, additional studies are also invited to elucidate and isolate antiplasmodial compound(s) from *E. obliqua* leaf extracts.

Table 3: *In vitro* antiplasmodial activity of phytochemicals against *Plasmodium vivax*

Concentrations (mg/mL)	Inhibition%				
	Chloroquine	Alkaloids	Flavonoids	Saponins	Tannins
0.02	43.48	21.57	2.90	1.86	2.61
0.04	58.84	25.22	2.72	2.84	2.96
0.06	65.22	28.41	2.78	2.78	2.90
0.08	84.06	49.10	2.61	2.72	2.72
0.1	90.49	60.93	2.03	1.04	1.28

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Data availability statement: All data generated or analyzed during this study are included in this published article and in its supplementary information files.

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