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IN VITRO STUDY ON THE ANTIPLASMODIAL POTENTIAL OF METHANOLIC EXTRACT OF LEAVES OF SALVADORA PERSICA L

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ABSTRACT

Malaria is one of the major global health problem that is caused by Plasmodium parasites and spread by Anopheles mosquitoes. According to an estimate by WHO in 2019, about 227 million cases of malaria with a total death toll of 558,000 has been recorded. The number increased to 241 million with 667,000 deaths in 2020. The main cause of the malaria is *P. falciparum* (60%) followed by *P. vivax* with a percentage of 40. But the report cases for *P. vivax* is increasing each year. In Pakistan, about 64% of malarial cases is due to *plasmodium vivax*. Assessments of transmission and occurrence at such passion is a grave problem. The key challenges to control *P. vivax* malaria is because of the dearth of treatment options to clear hypnozoites, the resistancy of malarial parasites to several existing antimalarial medications, the expenses associated in managing malaria in low income countries and its associated side effects is a major problem in combating malaria. Due to these challenges, there is an inadequate advancement in the production of experimental study tools, thus hindering production of new drugs and vaccines. New drugs are required to avert the Plasmodium strains. Therefore, in this study we investigated the antiplasmodial activity of *Salvadora persica*, also known as Miswak. The leaves methanolic extract of *S. persica* was formulated and different concentrations were used against the malarial parasite *Plasmodium* vivax. *S. persica*. Our results revealed that methanolic extract of *S. persica* leaves possess antiplasmodial activity and the inhibition has been found to be dose dependent and has displayed maximum growth inhibition of about 66.53%. Our results indicated that *S. persica* can be used as strong antimalarial agent.

INTRODUCTION

Malaria is amongst the main public health snag related to high morbidity and mortality. The causal agent of malaria is plasmodium parasite that is transmitted by Anopheles mosquitoes (WHO, 2024). Currently, malaria holds 2.6% of the total world disease burden. Major cause of the malaria is *P. falciparum* which account for 60% of the disease followed by *P. vivax* (40%). While the cases for *P. vivax* are increasing day by day (Haileselassie *et al.,* 2022). And this is the main cause substantial morbidity and mortality, particularly in sub-Saharan Africa (Eboh & Adebayo, 2023). 20 species of Plasmodium are known so far which mostly parasites on primates. However, 4 species cause infections in humans, *Plasmodium vivax, Plasmodium falciparum*, *Plasmodium malariae* and *Plasmodium ovale* of which *P. vivax* is considered as deadly (Okonko *et al.,* 2009).

One of the major reason of spreading of this disease is resistance of the parasite to the conventional drugs (chloroquine) and partial resistance to artemisinin-based therapies (Rosenthal *et al.,* 2024). Besides, the ideal environment for mosquito breeding is the stagnant water after heavy rainfalls. *P. vivax* infection is the major cause as *P. falciparum* cases are few. The *P. vivax* peak ranges from June to September. And the infection rises again April to June. The infection period for *P. falciparum* is listed between August and December (Khattak, *et al*., 2013). In Pakistan, malaria ranked the 2nd utmost clinically suspected infection following acute respiratory disease affecting 1.5 million peoples. Which is leading to about 50,000 losses each year. About 2.7 million confirmed cases of malaria have been filed across all Pakistan in 2023. And a total of 15.65 million malarial suspects were analyzed at such health care facilities. Majority of the cases were reported for *P. vivax* 64.2% (1.75 million) followed by *P. Falciparum* (PF) 32.1% (0.8 million) and Mixed cases 3.7% (99,690). Provincial data demonstrated that maximum number of cases was reported from Sindh 49.4% (1,346,210). Balochistan counts for about 37.9% (1,034,112). The cases in Khyber Pakhtunkhwa were about 8% (240,872) followed by Punjab 0.1% (3,202), merged Areas 3.7% (101,922) and AJK 0.0% (165) (Malaria Technical Unit, Pakistan, 2024).

Historically, medicinal plants played a vital role in controlling contagious diseases (Sifuna, 2022; Gras *et al.,* 2021). About 80% of the world population is dependent upon the old-fashioned medicines and health care needs (World Health Organization, 2023). Countless plant extracts have been proven to possess antiplasmodial activity in experimental designs (Vergara *et al.,* 2022; Tajbakhsh *et al.,* 2021).

Salvadora persica L. is an evergreen shrub or small tree of Salvadoraceae family, also known as Arak (in Arabic) and Peelu (in Urdu) commonly used as tooth or chewing sticks (Miswak). Ten species of *Salvadora* were reported in Africa and Asia regions (Anthoney *et al.,* 2015).

S. persica L. has many names in the world. Arak, miswak, siwak as in Arabic, merge, pilau in India, Caday in Somalia, omungambu (Southern Africa) and while in English, it is the toothbrush tree (Quattrocchi, 2016). Every part of the plant (bark, leaves, flowers, stem, fruits seeds and roots) have been used to cure many conditions related to various physiological systems in humans including, motor, circulatory, excretory and digestive systems (Aumeeruddy *et al.,* 2017). Currently, the fruit extract has been shown to possess antimicrobial activity against various bacterial mutants (Al Bratty *et al.,* 2020).

Fresh leaves of *S. persica* were collected locally from Bannu, Khyber Pakhtunkhwa, Pakistan. The plant identification was carried out at the department of Botany, University of Science and Technology (UST) Bannu. The leaves were cleaned with fresh water and then shade dried for two weeks. The leaves were then ground to fine powder using an electric grinder. The

Aims and Objectives of Study

The aim of this study was to investigate the antimalarial effect of *Salvadora persica* L. extract at various doses against *Plasmodium vivax.*

MATERIALS AND METHODS

Collection and Extraction of Plant Material

methanolic extract was prepared as defined by Shaa *et al.,* (2011). 10gm of leaf powder was taken and diluted in 80% methanol. The sample was placed on a shaker for 72 hours at room temperature. After 72 hours, using Whatman's filter paper, the suspension was filtered out. Later, filtrate was administered in rotary vacuumed evaporator (BUCHI Rotavapor R-200, Switzerland) at 40°C. The final result was the concentrated crude extract. The extract was kept at 4°C for additional investigation.

Collection of Blood samples

Blood samples were collected from Khalifa Gul Nawaz (KGN) Hospital, Bannu Town, Khyber Pakhtunkhwa, Pakistan. The blood samples were taken from patients which came to the laboratory for diagnostic tests. 5 ml of the blood sample was taken using a syringe.

***In vitro* antiplasmodial activity**

Donkor *et al.,* 2015 method was used for *In vitro* antiplasmodial activity of *S. persica* leaves extract. The activity was performed in 96 well-microliter plate. Two drugs were used as positive control, as Chloroquine and Proguanil. The microplates with parasitized culture and without drug or plant extract were treated as negative control. For culturing, 200 µL of blood medium mixture supplemented with 2%

Statistical analysis

Maturation and inhibition %age were assessed by the formula described by (Donkor *et al.,* 2015). Using Statistics-9 software, mean

Culture preparation and maintenance

P. vivax study was performed at the Molecular Parasitology and Virology Laboratory at Kohat University of Science and Technology (KUST), Khyber Pakhtunkhwa, Pakistan. 10.50 g of Roswell Park Memorial Institute powder was diluted in 960 ml of dH_2O. 0.5 ml of gentamicin was added. The solution was filtered and kept at 4°C (Radfar *et al.,* 2009).

The plant methanolic crude extract was dissolved in dH_2O to produce 1 mg/ml of the solutions. The 1 mg/ml solution was further diluted in 9 ml malaria culture medium to prepare a stock solution of 100 µg/ml. Sterilized was done using filtration membrane (Millipore). The extract was tested in 5 serial dilutions in duplicates (0.02, 0.04, 0.06, 0.08, and 0.1 mg/mL) (Clarkson *et al.,* 2003).

Hematocrit (Life Technologies, Australia) appended with 20% human serum. The culture was done at 37°C. Incubation was considered successful when ≥40% of the parasites at ring stage matured to schizont stage. 5µl Gentamicin sulfate was also mixed to the culture. Glass slides were used to prepare thick blood films. The blood films were stained for 30 min with 5% Giemsa solution and observed under the microscope (Figure 1).

inhibition and standard deviation were calculated. LD50 was evaluated using an online tool, AAT Bio-quest (https://www.aatbio.com/tools/ld50calculator).

$$\text{Inhibition} = \frac{\text{Mean control parasitaemia} - \text{Mean test parasitaemia}}{\text{Mean control parasitaemia}} \times 100\%$$

RESULTS

S. persica crude extract was analyzed 24 hours after the treatments. Growth inhibition of the crude extract was noted as 32.65, 37.95, 40.81, 51.20 and 66.53% at concentrations of 0.02, 0.04, 0.06, 0.08, and 0.1 mg/ml as shown in Figure 02. Our results revealed that the methanolic extract antiplasmodial efficacy is dose-dependent with LD50, 0.070mg/ml. In comparison, the antiplasmodial activity of *S. persica* extract was related to Arthemeter (Table 1). *P. vivax* growth inhibition shown by alkaloids was about 60.93% at a concentration of 0.1mg/ml that was similar to the control, Arthemeter (Table 1).

Table 1: *In vitro* activity of the extract of *Salvadora persica* on *Plasmodium vivax*

Extract/drugs	Concentration (mg/ml)	Schizonts in experimental group (mean ± SD)	Schizonts developed in control group (mean)	Maturation %	Inhibition %	LD50 mg/ml
Salvadora persica	0.02	165.45 ± 1.22	245.20	67.38	32.65	0.070
	0.04	152.44 ± 1.22	245.20	62.04	37.95	
	0.06	145.22 ± 1.21	245.20	59.18	40.81	
	0.08	122.22 ± 1.19	245.20	49.79	50.20	
	0.1	82.01 ± 2.82	245.20	33.46	66.53	
Arthemeter	0.02	124.67 ± 1.76	245.20	50.61	49.38	0.040
	0.04	106.67 ± 1.69	245.20	43.26	56.73	
	0.06	86.31 ± 0.89	245.20	35.10	64.89	
	0.08 72.24	68.33 ± 2.41	245.20		27.75	
	0.1 91.42	21.22 ±0.89	245.20		8.57	

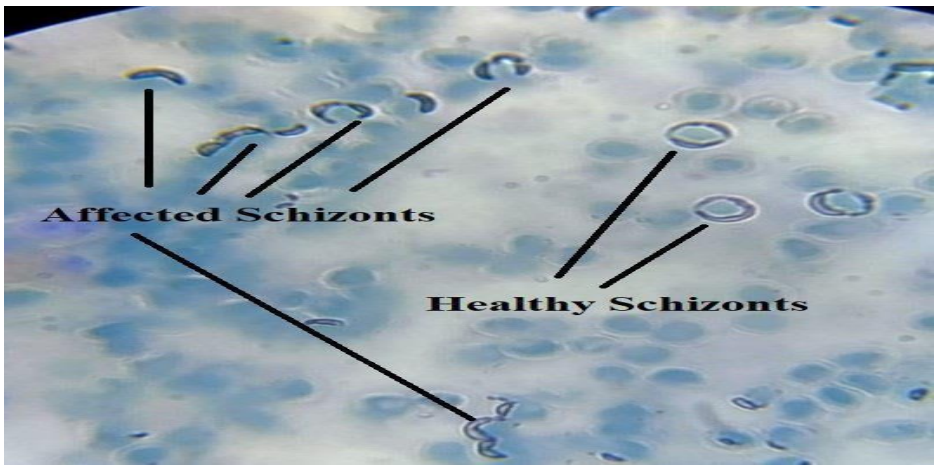

Figure 1: Microscopic observation of healthy and affected schizonts

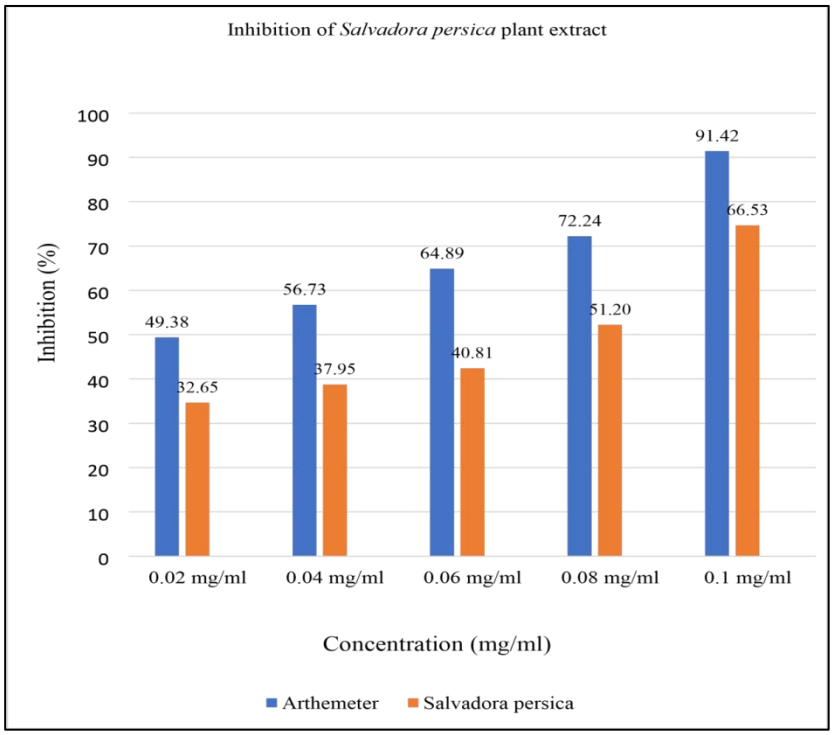

Fig 2: Represents the *in vitro* activity of the extract of *Salvadora persica* on *Plasmodium vivax*

DISCUSSION

Medicinal plants played a pivotal role in discovery and development of anti-malarial drugs (Batista *et al., 2009*). The most suitable solvent for plant extraction is methanol. Because of its polar nature, it is able to release various bioactive compounds from plants (Ali *et al.,* 2020/2021). Scientists has stated that solvents with high polarity should be use to abstract bioactive compounds as it gives high level of accuracy (Altemimi *et al.,* 2017). The effectivity of the active compounds obtained from plants rely mainly upon the solvent that is used for herbal formulation (Ali *et al.,* 2021).

This study explored the *in vitro* anti-plasmodial potential of *Salvadora persica* methanolic extract against *P. vivax. P. vivax* has been inhibited profoundly by the *Salvadora persica* leaf methanolic extract. The inhibition was found at each dose but maximum inhibition was recorded when high dose of the plant extract was applied. This showed that the extract wedged the parasite in a dose-dependent mode and showed 66.53% inhibition of *P. vivax* at the highest tested concentration. Previously, antimalarial potential of *Salvadora persica* plant has been confirmed. (Ali *et al.,* 2002 stated that *S. persica* plant possess antimalarial activity. Our results are aligned with previous findings where many plant species have been recorded to exhibit antiplasmodial potential (Jansen *et al.,* 2010; Panda and Luyten, 2018). Further, aqueous and methanolic extracts of *P. amarus* have shown to have great antimalarial potential (Aliyu *et al.,* 2021). Consequently, *S. persica* could be considered a potential antimalarial agent and is recommended for further in-depth analysis

CONCLUSION

According to traditional knowledge, *S. persica* has many medicinal potential and the present study concluded that the *S. persica* leaves methanolic extract exhibit antiplasmodial activity *In vitro*. Further, *In vivo* effectiveness is also needed to be tested as it is an significant feature in the testing of medicinal plants against parasites and should be appraised in future. Nevertheless, further studies are required to isolate the active compounds from the plant which can be used to standardize plant materials so as to install a reproducible herbal medicine.

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ETHICAL STATEMENT

This research was carried out in accord with ethical standards.

CONFLICT OF INTEREST

Authors claims no conflict of interest to declare it.

malaria-control/
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