ASSESSMENT OF ANTI-MALARIAL POTENTIAL OF ALLIUM SATIVUM AGAINST PLASMODIUM VIVAX

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ABSTRACT

Malaria is a vector-borne protozoal disease, caused by genus Plasmodium, where female anopheles' mosquito works as a vector to transfer malarial parasite. The disease has been reported to be endemic in tropical and subtropical regions of the world. Since drug resistance of the Plasmodium species and economic burden of the disease are the major concerns associated with malaria. Thus, most of the world's population is focusing on the utilization of medicinal plants as the natural treatment for several diseases including malaria. There is limited published data available regarding anti-malarial activity of Allium sativum against Plasmodium vivax. Therefore, this study was aimed to assess the in-vitro anti-malarial activity of Allium sativum methanolic extract. The methanolic extract of Allium sativum showed significant anti-plasmodial activity (80.57%) at 0.2 mg/ml highest tested dose after 24h. The phytochemical analysis showed the presence of Flavonoids, Vinyldithiins, Ajoenes, Alliin and Allicin in the Allium sativum methanolic extract. Among the phytochemicals, only Allicin at the highest tested dose (0.2mg/ml) showed the inhibition (59.75%) of P. vivax inhibition. Allium sativum methanolic extract exhibits anti-plasmodial activities in-vitro. However, further studies are required to assess the in-vivo anti-plasmodial activity of Allium sativum methanolic extract against plasmodium vivax in future.

Key Words: Plasmodium vivax, Allium sativum¸ Parasite, Antimalarial activity, methanolic extract, in vitro.

INTRODUCTION

Malaria is a vector-borne parasitic infection, caused by intra-erythrocytic protozoa parasites of the genus Plasmodium (P). Female Anopheles mosquito transmits the parasites that can cause malaria. Malaria is mostly reported as endemic in tropical and subtropical regions of the world. Malaria affects over half of the global population(1). More than 100 known Plasmodium species have been reported around the globe, however, only five of these species including P. vivax, P. malariae, P. knowlesi, P. falciparum and P. ovale are known to significantly infect human(2). Due to Pakistan's strategic location in a geographical area where more than 60% of its population has been reported positive for Malaria. In Pakistan, Plasmodium vivax can cause about 64% of malarial cases(3). After the Second World War, the struggle to control malaria was intensively promoted(4). Millions of population is at risk of malaria but it is considered as neglected disease in the tropical regions(5). According to a recent survey in 2014, 1.2 billion people have been reported to be at high risk of malaria. Remarkably, malaria cases occur more frequently in high-risk zones, affecting 97 nations and territories, with 712,000 deaths recorded in the African region(3). Due to the drug resistance of malarial parasites, the available medicines have low effect on overcoming the Plasmodium species. The cost of the current medicines available at markets are difficult to manage in poor countries where lifestyles are below the standard level. Therefore, new medicines are required to overcome drug resistance issue.

Eighty percent of the world's population now relies on plants as the natural medicines as the main source of healthcare therapy(6). Recently, the use of antibiotics and the majority of synthetic medications have been constrained due to disagreeable side effects on the growth of pathogenic bacteria that are resistant to these

drugs, hazardous side effects, and withdrawal problems(7). To avoid such problems, the scientific world is now focusing on the traditional ways of treatment utilizing medicinal plants and their derivatives. For the treatment of numerous vector-borne diseases, including malaria, majority of the world's population is now mainly focusing on the use of herbs and plants as their daily alternative medicine(8). For several decades, medicinal plants are in continuous use to treat human diseases including stomach pain, headaches, diabetes, hyperacidity, viral infections, bacterial infection, protozoan infections, and many others.

Garlic (Allium sativum L.; Family: Amaryllidaceae) is an annual aromatic herbaceous plant. It is one of the earliest and most significant plants that have been utilized to use traditional medicine since ancient times (9,10). It is regarded as the second most widely used species of Allium family after onion (Allium cepa L.), which is used as a treatment for a number of common diseases like the common cold, the flu, snakebites, and hypertension(11). According to research conducted on humans, Allium species and their active ingredients have been shown to lower the risk of diabetes and cardiovascular disease, defend against infections by boosting the immune system, and have antibacterial, antifungal, anti-aging, and anti-cancer capabilities(12). Allium sativum contains various medical properties and elements, which can be utilized for various life purposes including treating several diseases(13). Somehow, limited pieces of information are available on the antimalarial activities of Allium sativum against P. vivax. Therefore, this study was aimed to assess the *in-vitro* antimalarial effects of Allium sativum methanolic extract against P. vivax.

METHODS

Plant collection and Preparation of garlic extract

Fresh cloves of garlic were collected at the natural environment in the western-south areas of district Bannu, Khyber Pakhtunkhwa, Pakistan with coordinates (latitude: 32.986111 and longitude: 70.604164). After washing with distilled water, methanolic extraction was performed according to the published method as discussed earlier(14), with slight modifications. The garlic cloves were grinded and plant extract was prepared by mixing 20 mg of ground garlic with 100 ml of methanol in a graduated cylinder. Before being filtered with 11µm grade cellulose filter paper, the suspension was shaken in an electric shaker for 24 hours at 28°C. The final filtered solution was placed into multiple containers for freeze-drying after the filtration procedure was repeated twice. The final product (200 g) of freeze-dried garlic powder was kept at 4°C for further processing.

Phytochemicals analysis

The phytochemical analysis of Allium sativum was performed according to previously published protocol with slight modifications for the presence of medically important phytochemicals(15).

Identification of Plasmodium vivax through Microscope

Thin blood smears were made from the collected blood samples and observed under Light Microscope (100X) magnification lens for the presence of P. vivax.

Culturing of Plasmodium vivax

Plasmodium vivax strains were cultured at the Molecular Laboratory in the Department of Zoology University of Science and Technology Bannu, Khyber Pakhtunkhwa, Pakistan in a candle jar. For culturing, a blood medium mixture of 200 µl having 2.0% hematocrit, in a liquid sterile McCoy's 5A medium provided with human serum 20%, was used. The plasmodium strains were cultivated in a glass jar and kept on incubation at 37 °C. At around 40% of the adult schizont ring stage, the incubation was completed. The culture was supplied with Gentamicin sulfate (5 µl). Thin blood smears stained with 5% Giemsa stain were prepared and observed under microscope. In order to propagate the culture the infected red blood cells were further used for inoculation in a fresh medium(16).

In vitro anti-plasmodial activity

The plant extract and phytochemicals were tested for their in vitro anti-plasmodial activity in as previously reported (17). The anti-protozoan activity was carried out in 90 microplates. The positive controls utilized were Chloroquine and Nevaquine, whereas the negative controls employed parasitized culture on microplates without the use of any medicine or plant extract. Six different concentrations (0.02, 0.04, 0.06, 0.08, 0.10,

and 0.2 mg/ml) of plant extract, phytochemicals, and drugs were used as reported earlier (18,19). The concentration of CO2 was increased by gently shaking the microplates in a candle jar. For 24 hours, the plates were kept at 37°C in an incubator. After 24 hours, each microplate's supernatant was discarded, and red blood cells were extracted using a micropipette to create thin smears that were stained with the Giemsa dye and examined under a microscope as given in Figure 1.

Statistical analysis

The percentage of maturation and inhibition was calculated by using the following formula:

$$\textbf{Inhibition(\%)} = \frac{\text{No. of developed schizonts in experimental group}}{\text{No. of developed schizonts in control group}} \times 100$$

$$\textbf{Maturation (\%)} = 100 - \text{Inhibition \%}$$

RESULTS

Phytochemicals analysis of Allium sativum

The phytochemicals analysis was performed and showed the presence of Alliin, Allicin, Ajoenes, Vinyldithiins and Flavonoids in Allium sativum methanolic extract given in Table 1. Our data is correlated with the already published report(20). Herein our data suggest that due to presence of various compounds could modulate the fast growth of P. vivax over RBC of humans.

Anti-plasmodial potential of Allium sativum

Next we examined the anti-plasmodial potential of Allium sativum extract by comparing with slandered anti-malarial drugs concentration LD50 0.082 mg/ml such as Nevaquine and Chloroquine. The in-vitro anti-plasmodial potential of A. sativum extract was analogous and comparable to Chloroquine and Nevaquine as shown in Table 2. The methanolic extract of A. sativum showed maximum 80.57% inhibition of P vivax at 0.2 (mg/ml) tested dose.

By comparing the Anti-plasmodial activity of different phytochemicals present in Garlic (Allium sativum) with control group such as Chloroquine on Plasmodium vivax.

The phytochemicals were tested for the inhibition among which only Allicin at the highest tested dose (0.2 mg/ml) exhibited 59.75% growth of P. vivax, which was almost analogues and comparable to Chloroquine as shown in Table 3.

Table 1: Represents the photochemical constituents of Garlic (Allium sativum) in methanolic extract

S. No.	Phytochemicals	Methanolic crude extract
1	Allicin	+
2	Alliin	+
3	Ajoenes	+
4	Vinyldithiins	+
5	Flavonoids	+

Figure 1: Represents the anti-malarial effects of Allum sativum on Schizonts of P. vivax using Microscopic Examination

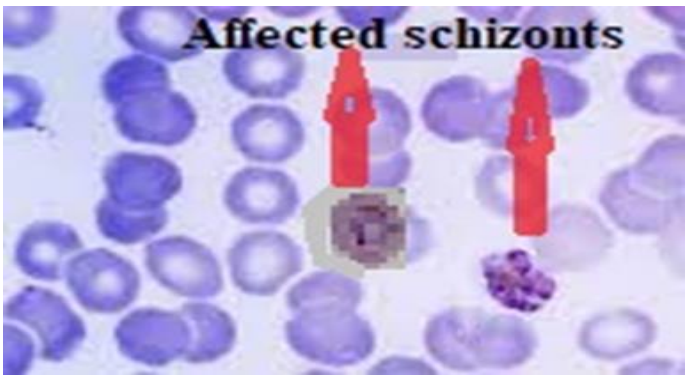

Table 2: Represents the in-vitro activity of the extract on Plasmodium vivax.

Extract/drugs	Concentration (mg/ml)	Schizonts in experimental group (mean ± SD)	Schizonts developed in control group (mean)	Maturation %	Inhibition %	LD50 mg/ml
Allium sativum	0.02	184.44 ± 1.22	258.44	71.36	28.64	0.082
	0.04	180.44 ± 1.22	258.44	69.81	32.19	
	0.06	159.22 ± 1.21	258.44	61.60	38.4	
	0.08	140.22 ± 1.19	258.44	54.25	45.75	
	0.10	93.44 ± 2.98	258.44	36.15	63.84	
	0.2	50.22 ± 2.82	258.44	19.43	80.57	
Chloroquine	0.02	100.67 ±1.70	258.44	40.11	59.89	0.070
	0.04	80.74 ± 1.79	258.44	31.24	68.76	
	0.06	62.89 ± 2.87	258.44	25.20	75.8	
	0.08	35.44 ± 2.90	258.44	13.71	86.29	
	0.10	27.11 ± 2.43	258.44	12.83	87.17	
	0.2	13.00 ± 2.42	258.44	5.03	94.48	
Nevaquine	0.02	124.67 ± 1.76	258.44	48.23	51.77	0.077
	0.04	104.67 ± 1.69	258.44	40.50	59.32	
	0.06	79.31 ± 0.89	258.44	30.68	69.32	
	0.08	59.33 ± 2.41	258.44	22.95	77.05	
	0.10	27.60 ± 0.79	258.44	10.67	89.33	
	0.2	**13.22 ± 0.89**	**258.44**	**5.11**	**93.89**	

Table 3. Shows the in-vitro anti-plasmodial activity of different Phytochemicals of Garlic (Allium sativum) methanolic extract on Plasmodium vivax

Concentration (mg/ml)	Inhibition percentage					
	Chloroquine	Allicin	Alliin	Ajoenes	Vinyldithiins	Flavonoids
0.02	62.76	26.76	2.50	2.05	1.08	2.10
0.04	64.76	30.76	3.07	2.20	1.15	2.20
0.06	67.8	34.8	4.03	2.90	1.30	2.35
0.08	75.1	41.1	6.06	3.12	1.45	2.50
0.10	83.17	48.17	8.07	3.25	1.93	2.75
0.2	90.75	59.75	11.10	4.12	2.42	2.95

DISCUSSION

Over long periods, the world has been focusing on the use of medicinal plant remedies as a natural treatment for several infectious diseases. Due to the advancement in research activities on medicinal plants, many different kinds of anti-plasmodial drugs have been discovered but due to cost effect of the available drugs, most

of the world's population is focusing on the natural remedies of medicinal plants. The current research study has explored the anti-plasmodial potential of Allium sativum against P. vivax. The methanolic extract of Allium sativum inhibited the P. vivax activity at the highest concentration of dose (80.57%). The assessment of anti-plasmodial effect of the methanolic Allium sativum extracts depends on the concentration of tested dose. This study supports the previously published study as discussed previously who reported the anti-plasmodial potential of Allium sativum against different human protozoan's parasites(21). The phytochemicals analysis revealed Alliin, Allicin, Ajoenes, Vinyldithiins and Flavonoids. Allicin was the major compound that inhabited (59.75%) growth of P. vivax in vitro, which was comparable to the effect of Chloroquine and Nevaquine. The anti-plasmodial activity of Allium sativum is supported by the presence of several bioactive compounds which are identified in the current research study. Numerous studies have recommended that the polarity of the solute of interest should be taken into consideration when selecting the solvents to be utilized for extracting biomolecules from plants. The solute will dissolve correctly and with a high yield in a solvent with similar polarity to the solute(21). For most of the plant extraction, because of its polar character, which facilitates the extraction of various naturally occurring, biologically active chemicals present in plants, methanol is the suitable solvent(22). Since in animal's research studies the in vivo studies are more expensive, time-consuming, and subject to ethical debates as compared to *in-vitro* procedures. The *in-vivo* studies of testing the effects of medicinal plants are an important assay in research studies, which should be carried out in future. Moreover, further studies are required to isolate the pure biological active compounds of the mentioned classes in Allium sativum extract and to evaluate the in-vivo anti-plasmodial activities against P. vivax in future.

CONCLUSION

From the current study, it has been concluded that the methanolic extract of Allium sativum has strong anti-plasmodial activity in an in-vitro manner. An essential factor in evaluating the benefits of medicinal plants is their *in-vivo* potential. Therefore, further research studies are required to assess the *in-vivo* anti-plasmodial activities of Allium sativum against P. vivax.

Conflict of interest:
Authors declare no conflict of interest
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This study was approved by Local Ethics Committee.

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