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Original Article

Antimalarial Effect of Alcoholic Extract of Curcuma longa and Heracleum persicum on Cultivated Plasmodium falciparum 3D7 Strain

Haleh Hanifian [1], Somayeh Yaghmori [1], Afsaneh Motevalli Haghi [1*], Mehdi Nateghpour [1,2*], Bahman Rahimi Esboei [3,4], Leila Farivar[1], Effat Souri [5]

[1] Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

[2] Research Center of Quran, Hadith and Medicine, Tehran University of Medical Sciences, Tehran, Iran

[3] Department of Parasitology and Mycology, School of Medicine, Tonekabon branch, Islamic Azad University, Tonekabon, Iran

[4]Toxoplasmosis Research Center, Mazandaran University of Medical Sciences, Sari, Iran.

[5]Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

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Correspondence
Afsaneh Motevalli Haghi
Email:amh.mot@gmail.com
Mehdi Nateghpour
Email:nateghpourm@tums.ac.ir

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Abstract

Introduction: Plasmodium falciparum causes the most fatal form of malaria in humans. At present, the common treatments are not effective enough and the incidence of drug resistance is increasing in malarious areas. Therefore, presenting novel methods for therapeutic purposes assumes significant importance. Recent studies have indicated that aqueous or alcoholic extracts of Curcuma longa and Heracleum persicum show a broad spectrum of anti-microorganisms activity. In this (in vitro) study, the effects of C. longa and H. persicum extracts were assessed on P. falciparum since there has been limited clinical research into their effectiveness on Malaria.

Materials and Methods: The alcoholic extracts of H. persicum and C. longa were prepared in 10^{-1}, 10^{-3}, and 10^{-5} mg/ml dilutions. These solutions were tested on P. falciparum with 10% parasitemia in RPMI 1640 medium with 10% hematocrit. Each of the dilutions was examined in triplicate and the inhibitory effect of the solutions on parasites was measured via determining the average parasitemia and their schizont rate. Finally, the results were analyzed using SPSS software.

Results: The rate of parasitemia declined in three different dilutions of both H. persicum and C. longa. The mean of antiplasmodial inhibitory activity of herbs was 83.23±2.47% in H.persicum and 99.91±٠,٠% in C.longa. Moreover, all dilutions of both H.persicum and C.longa showed a significant effect on decreasing the percentage of schizont in comparison with the control group (P-value<0.05).

Conclusions: The present study indicated that alcoholic extracts of C. longa and H. persicum possess acceptable antiplasmodial effects and can be developed as valuable alternatives to ineffective antimalarial drugs. These results support the claims of recent studies that C. longa and H. persicum, have considerable antimicrobial activities. Considering the notable in vitro antiplasmodial efficacy of C.longa and H.persicum, further studies with in vivo method are recommended.

Keywords: Plasmodium falciparum; Heracleum persicum; Curcuma longa; in vitro; Iran.

1. Introduction

Malaria is one of the most life-threatening mosquito-borne diseases caused by Plasmodia spp. parasites. This disease is prevalent in tropical and subtropical regions, given the suitable environmental factors such as frequent rainfalls, high humidity, and desirable temperature for both vectors and parasites. In 2019, approximately 228 million new cases of Malaria and 405,000 deaths were reported globally, majority of the cases being from Africa and more than 67% of deaths among under 5-years old children group [1]. The infection may result in a wide variety of symptoms from an asymptomatic form or very mild symptoms to severe illness and even death. Among the parasites causing malaria, Plasmodium falciparum (P. falciparum) has been associated with a more severe and sometimes fatal form of the disease [2].

The rate of parasite resistance to Chloroquine (CQ) – the golden treatment choice for Malaria- is increasing in the world١. Traditional medicines besides industrial chemical drugs have been used in the treatment of malaria for years and interestingly some of the herbal medicines administered in endemic regions for malaria treatment such as Artemisia annua have been approved by the WHO [3]. Nowadays, artemisinin therapy is considered a basic therapeutic line in the treatment protocol of malaria all over the world [4]. Towards this attitude, research on natural local plants can play a pivotal role not only in the investigation of new effective anti-malarial agents but also in controlling and treatment of the disease, accordingly. For instance, H. persicum and C. longa, have recently been considered by some researchers and many studies have also reported their antibacterial and anti-fungal effects[5-10]. C. longa (Zingiberaceae) is a perennial herb that is widely cultivated in tropical regions of Asia. Its rhizome, generally used for flavoring foods and as a powder called turmeric, is utilized as a therapeutic agent for various medical goals. It has been explained that the extract of C. longa has an anticancer impact in colon, liver, pancreas, and prostate cancers [11-12]. Curcumin derived from C. longa is also used as an anti-inflammatory, anti-parasitic, laxative, and a drug for chronic liver disease [13-14].

H. persicum is a perennial herbaceous flowering plant that belongs to the family Apiaceae, native to Iran (Persia). It grows wild in mountainous regions in Iran and contains chemical compounds such as acetyl acetate, methylated butyrate, butyric acid as well as fats, minerals, salts, and various types of vitamins 7-8. Due to the presence of these elements, H. persicum is commonly used for controlling tympanites, dyspepsia, appetite disorders, and similar enterogastric problems. Moreover, essential oils and hydroalcoholic extracts of this plant have anti-inflammatory and antimicrobial effects. H. persicum contains volatile oils of flavonoids that substantially contribute to strong immune responses [9-10].

Consumption of H. persicum and C. longa as medical agents has a long history; however, the effect of H. persicum against P. falciparum has not been studied so far. Even though the inhibitory effect of C.longa has recently been proved on the growth of P. falciparum [6], no similar research has been conducted yet to study the effect of the Persian strain of this plant on Plasmodia. Turmeric, C. longa, displays a certain level of both phenotypic and genotypic variation all over the world. For instance, there are 40 to 50 species of turmeric in India and 30 to 40 species in Thailand. Moreover, other wild species are reported from different Asian countries. Concisely, considering the growth of C.longa in various geographical regions and their probable different efficacies, due to their variant genotypes, it

is of importance to assess the effect of Persian C.longa on P.falciparum. The results of this study can lead to preparing an efficient antimalarial medicine in order to control and treat infections caused by P. falciparum[15-16].

2. Materials and Methods

The wound fluid (seroma) samples were collected from BC patients. Seroma was collected from the drain after 24 hours (h) of surgery. In this method, seroma was gathered directly from the wound healing surgery site. The seroma was allowed to gradually be collected in the drain during 24h and then was all gathered. All samples were obtained from different patients with different molecular and pathological signature who underwent BCS in the Shohada hospitals (Table 1). The gained seroma was collected in protease inhibitor solutions and was transferred under icepack to the laboratory. Seroma was centrifuged (300g in 5min), sterile filtered (0.22 and 0.45 µm) and stored at -80^{0}C.

Procedures:

2.1 Cultivation of P. falciparum and preparing complete culture medium (CCM)

Plasmodium falciparum 3D7 strain was cultivated according to the protocol described by Trager & Gensen with some modifications 17. To prepare a complete culture medium (CCM), RPMI 1640 medium containing HEPES and glucose (Gibco, USA), supplemented with Hypoxanthine 50 mg/L (Sigma-Aldrich, Germany), Gentamycin 50 mg/L (Sigma-Aldrich, Germany), and 10% AB+ human inactive serum was used. Following the cultivation stage, two drops of infected blood sample were added to 4.5 ml of prepared CCM with a one-minute swirling process. In order to provide 10% hematocrit, non-infected O+ blood samples were washed three times by RPMI-1640, centrifuged at 2,000 rpm for 5 minutes, and added (pH= 7.2-7.4). The cell flasks were incubated at 37 ºC with a combination of O2 (5%), CO2 (5%), and N2 (90%) gas mixture. The parasites were sub-cultured for every 24 hours up to reach 10% of parasitemia.

2.2. Preparing alcoholic extracts of plants

H. persicum (aerial parts) and C. longa (roots) were collected from mountainous areas of Mazandaran province in the north and Sistan-and-Baluchestan province in the southeast of Iran, respectively. The plants (500 g) were dried at room temperature under shade and powdered by electric shredder. The powders were then mixed in 1500 ml of ethanol (Merck, Germany) for 72 hours. As the next step, the solvents were completely evaporated using a rotary evaporator (Hans Heidolph Gmbh, USA), until the time when a totally solid substance was obtained. Afterwards, 10-1, 10-3, and 10-5 mg/ml dilutions were prepared from final product, using normal saline as the solvent.

2.3. In vitro anti-plasmodial assay

The anti-plasmodial activity of the methanol extracts of H. persicum and C. longa were completed in triplicate in a 96-well microlitre plate, according to the method described by WHO and based on assessing the inhibition of schizont maturation 18. The blood with 10% parasitemia (consisting of 80% ring form) was transferred into a sterile Falcon tube. After centrifugation, the supernatant was removed and the final hematocrit fixed on 50% using CCM. Fifty microliters of final the mixture was added to each well. Ten microliters of each herbal extract dilution were added to each well and incubated at 37 °C for 24 to 30 hours. Chloroquine-added and normal saline-added wells were considered positive and negative controls, respectively. Afterwards, smears were

prepared and stained with the Giemsa method and the percentage of infected RBCs was measured by determining infected RBC per 5000 erythrocytes, while the percentage of schizonts was calculated as non-sexual parasites per 200 counted parasites. Finally, the percentage of the inhibition of the parasite was calculated for each group through the following formula and compared to the control group:

$$\text{Inhibition}(\%) = \frac{\text{Mean \% parasitemia of untreated group} - \text{Mean \% parasitemia of treated group}}{\text{Mean \% parasitemia of untreated group}} \times 100$$

2.4 Statistical analysis

The obtained data were analyzed using SPSS software v.24 (SPSS, Chicago, IL, USA). Continuous variables were reported as mean ± Main Average Deviation (MAD) and discontinuous data were reported as frequency (number/percentage). An independent sample t-test was used to compare the continuous data with the untreated control group, and also Fisher's exact test was utilized to compare nominal variables. P-value less than 0.05 was considered statistically significant in reducing the parasitemia rate.

3. Results

The results of our study demonstrated that alcoholic extracts of both C. longa and H. persicum significantly reduced the growth of P. falciparum in comparison with the control group (P-value<0.05). However, it was also observed that the alcoholic extract of C. longa lowered the parasitemia in P. falciparum more than H. persicum.

As Fig 1 shows, the mean percentage of infected RBCs in negative control sample (only normal saline-added) was 11.77±1.58 % and the mean rate of schizont form in the same sample was 3.88±0.79 %. Whilst in positive control (Chloroquine-added) well, there was no evidence of parasitemia, expectedly. All dilutions of H. persicum including 10-1mg/ml, 10-3 mg/ml, and 10-5 mg/ml showed significant antiplasmodial activity against P.falciparum, with the averaged 83.23 ± 2.47 % inhibitory rates (Table 1).

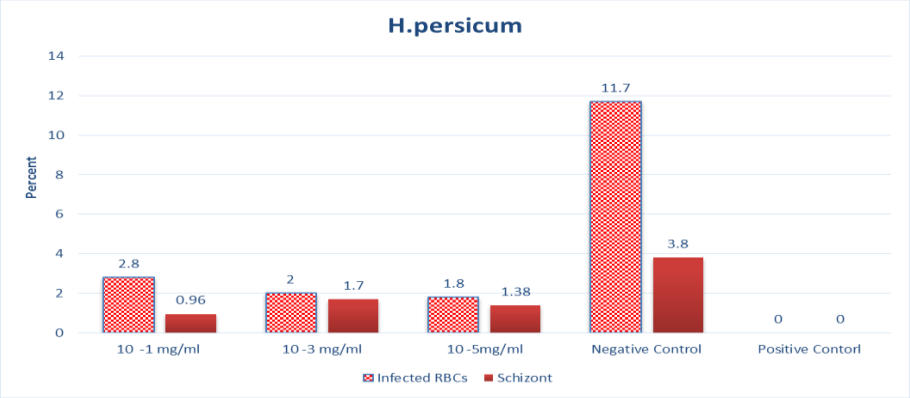

Figure 1.The comparison of infected RBCs and schizont percentages based on different dilutions of *H.persicum* versus the control group

Results of the effect of C. longa on P.falciparum based on the average of different steps and dilutions are summarized in Table 2 and Figure. 2. According to the obtained results, C.longa showed a significant antiplasmodial effect with the

the average 99.91%±0.0 % inhibition rate in all dilutions.

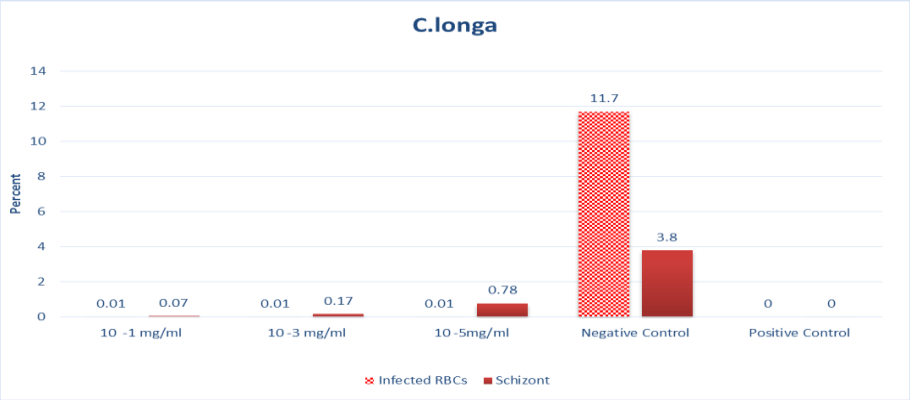

Figure 2. The comparison of infected RBCs and schizont percentages based on different dilutions of *C.longa* versus the control group

4. Discussion

In this study, we investigated the effects of different dilutions of alcoholic extracts of two other Persian traditional medicines; C. longa and H. persicum on P. falciparum, employed in vitro method. The extensive use of herbal plants in the last decade has drawn attention to various native plants which appear to be applicable to management and treatment of malaria as new and more effective drugs[19]. For instance, according to results obtained by Feiz Haddad and colleagues, two Persian traditional medicines of Solanum nigrum and Physalis alkekengi show promising effectiveness against 3D7 chloroquine-sensitive strain of P.falciparum, with IC50s of 10.20 mg/ml and 11.31 mg/ml respectively, and also moderate activity against K1 chloroquine-resistant strain[20].

Previous studies indicate that Umbelliferon herbaceous plants have a wide spectrum of antibacterial effects [21-24]. Although H. persicum belongs to the above-mentioned plants, there are not adequate studies on its antimicrobial and anti-parasitic effects. Previous studies in Iran and the Netherlands have shown that the hydroalcoholic extract of H. persicum has strong antifungal effects on Candida spp. and antibacterial effects on Bacillus megaterium, Micrococcus sp., Pseudomonas sp. and Staphylococcus aureus [23-24]. Moreover, it has been observed that H. persicum in combination with chloroquine has antimalarial activity against P.berghei in mice[25]. Meanwhile, our study showed that the percentage of P.falciparum and the number of schizonts after exposure to H. persicum are significantly lower than the control group (P.value<0.05).

A good number of studies have been conducted on the antibacterial effects of C. longa, and all of these studies have shown that C. longa is profoundly effective on a wide range of bacteria [26-27]. It has also been shown that the effects of alcoholic extract of C. longa on bacteria isolated from burn wounds such as Pseudomonas aeruginosa, Acinetobacter, and Staphylococcus were higher than conventional antibiotics, due to their resistance to routine antibiotics [28-29]. In addition, curcumin has been shown to have an anti-inflammatory role in hepatic disorders, and a study revealed that curcumin lowers the pulmonary inflammation in mice with Klebsiella pneumoniae infection [30-31]. Indeed, anti-

inflammatory, anti-allergic and anti-cancer effects of C. longa have been published in more detail [32-34]. Our study showed that the number of parasites was significantly reduced in the treatment group with different dilutions of C. longa versus the control group (P.value<0.05). Heidarian et al. also have underscored the effects of C. longa alone and in combination with Chloroquine on Plasmodium berghei in mice. They have concluded that the extract of C. longa has a considerable antimalarial effect on P.berghei, especially in combination with chloroquine [35].

5. Conclusion

In conclusion, this study shows natural extracts derived from H. persicum and Persian C. longa, produce impressive and astonishing antiplasmodial effects and can be considered potential sources of new anti-malarial agents. Obviously, more investigations are needed to reach a reliable patent for alternation of the herbal anti-malarial medicines.

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Conflict of interest

The authors declare no conflict of interest.

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Tables:

	10-1 mg/ml dilution		**10-3 mg/ml dilution**		**10-5 mg/ml dilution**		**Negative Control (Normal Saline)**		**Positive Control (Chloroquine)**	
	Parasitemia Inhibition Rate (%)	Schizont Presence (%)	Parasitemia Inhibition Rate (%)	Schizont Presence (%)	Parasitemia Inhibition Rate (%)	Schizont Presence (%)	Parasitemia Inhibition Rate (%)	Schizont Presence (%)	Parasitemia Inhibition Rate (%)	Schizont Presence (%)
Average	**79.52**	**0.96**	**84.62**	**1.77**	**85.55**	**1.38**	**0.00**	**3.88**	**100**	**0**

Table 1. The effect of different dilutions of alcoholic extract of H. persicum on P. falciparum

	10-1 mg/ml dilution		10-3 mg/ml dilution		10-5 mg/ml dilution		Negative Control (Normal Saline)		Positive Control (Chloroquine)	
	Parasitemia Inhibition Rate (%)	Schizont Presence (%)	Parasitemia Inhibition Rate (%)	Schizont Presence (%)	Parasitemia Inhibition Rate (%)	Schizont Presence (%)	Parasitemia Inhibition Rate (%)	Schizont Presence (%)	Parasitemia Inhibition Rate (%)	Schizont Presence (%)
Average	**99.91**	**0.07**	**99.91**	**0.17**	**99.91**	**0.78**	**0.00**	**3.88**	**100**	**0**

Table 2. The effect of different dilutions of alcoholic extract of C.longa on P. falciparum