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Antimalarial evaluation of selected medicinal plant extracts used in Iranian traditional medicine

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ABSTRACT

Objective(s): In an attempt to discover new natural active extracts against malaria parasites, the present study evaluated the antiplasmodial properties of selected plants based on Iranian traditional medicine.

Materials and Methods: Ten plant species found in Iran were selected and collected based on the available literature about the Iranian traditional medicine. The methanolic extracts of these plants were investigated for *in vitro* antimalarial properties against chloroquine-sensitive (3D7) and multi-drug resistant (K1) strains of *Plasmodium falciparum*. Their *in vivo* activity against *Plasmodium berghei* infection in mice was also determined. Cytotoxicity tests were carried out using the Raji cells line using the MTT assay. The extracts were phytochemically screened for their active constituents.

Results: According to the IC_{50} and selectivity index (SI) values, of the 10 selected plant species, *Citrullus colocynthis*, *Physalis alkekengi*, and *Solanum nigrum* displayed potent *in vitro* antimalarial activity against both 3D7 and K1 strains with no toxicity (IC_{50}= 2.01-18.67 µg/ml and SI=3.55 to 19.25). Comparisons between treated and untreated control mice showed that the mentioned plant species reduced parasitemia by 65.08%, 57.97%, and 60.68%, respectively. The existence of antiplasmodial compounds was detected in these plant extracts.

Conclusion: This was the first study to highlight the *in vitro* and *in vivo* antiplasmodial effects of *C. colocynthis*, *P. alkekengi*, and *S. nigrum* in Iran. Future studies can use these findings to design further biological tests to identify the active constituents of the mentioned plant species and clarify their mechanism of action.

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Introduction

The World Health Organization (WHO) lists malaria, human immunodeficiency virus (HIV), and tuberculosis as the three major infectious diseases throughout the world (1). In 2015, WHO reported 214 million malaria cases and 438000 deaths due to the disease. Unfortunately, 70% of all malaria deaths occur in children less than five years of age (2). Malaria has always been a great health issue in southeastern parts of Iran (3). Since Iran has long borders with Afghanistan and Pakistan, imported malaria cases constitute about 40% of all cases of the disease in the country (4, 5).

Previously cheap and very effective drugs like chloroquine are no longer effective due to the rapid spread of resistant strains. This is currently posing an important challenge to malaria control as the disease re-emerges in areas where it was eradicated and appears in previously unaffected areas. *Plasmodium falciparum* resistance to known antimalarial drugs has become widespread in almost all areas of its prevalence (6, 7).

Cases of chloroquine (CQ) resistance in *P. falciparum* malaria in Iran were first reported in 1983 (8).

New drugs are thus needed to combat resistance to the existing drugs while decreasing the side effects and increasing the efficacy of malaria treatment. Various plants have historically been used to fight against malaria. Quinine and artemisinin, two major antimalarial drugs, are both extracted from plant species (9, 10). Thus, plants represent a vast resource of novel molecular entities, which may be developed into new antimalarial drugs. Efforts to discover new antimalarial agents should hence evaluate the safety and efficacy of traditionally used antimalarial medicinal plants.

According to information provided by Persian

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traditional medicine and various textbook reviews, 10 medicinal plants with potential antimalarial activity were selected (11-15). From various parts of the country, the plants were collected and assessed in terms of *in vitro* and *in vivo* antimalarial effects and toxicity.

Materials and Methods

Plant Materials

Plants which used to treat febrile diseases, infectious diseases, and inflammation in different areas of Iran were selected for this study (Table 1). Fresh samples of different plant parts were collected and the botanical identity of the specimens was confirmed by Dr MR Kanani, Department of Biology, Medicinal Plants and Drug Research Institute, Shahid Beheshti University, Tehran, Iran. The specimens were then shadow dried at room temperature, powdered using an electric grinder under hygienic conditions, and stored in suitable dark containers at 4 ℃.

Plant Extraction

In the next stage, 100 g of the powdered samples were macerated in 80% methanol and constantly shaken at room temperature for 48 hr. Vacuum filtration was then performed using Whatman filter papers and the obtained methanol soluble fractions were transferred into a rotary evaporator and concentrated under decreased pressure at 35℃. The extracts were moved to air-tight vials and maintained at 4°C, away from light.

Parasite Culture

The protocol originally proposed by Trager and Jensen was adopted for the *in vitro* culture of the erythrocytic stages of the CQ-sensitive (3D7) and CQ-resistant strain (K1) of *P. falciparum*. The culture was performed using 4- (2-hydroxyethyl) - 1-piperazine ethanesulfonic acid (HEPES) – buffered (Roswell Park Memorial Institute (RPMI) – 1640 medium at a pH of 7.2 and in an atmosphere containing 91% nitrogen, 6% carbon dioxide, and 3% oxygen. The medium was supplemented with fresh O^+ human red blood cells (obtained from the Tehran Blood Transfusion Center), 0.2% sodium bicarbonate, 0.5% AlbuMAX, 45 µg/l hypoxanthine, and 50 µg/l gentamicin (16).

Drug dilutions

All extracts were dissolved in dimethyl sulfoxide (DMSO) to produce a concentration of 50 mg/ml and then diluted in RPMI-1640 medium to obtain a concentration of 2 mg/ml. Thereafter, all stocks were diluted in culture medium to reduce the concentration from 200 to 1.56 µg/ml. The final concentration of the solvent in the assay plates was always less than 0.1%, a concentration previously shown to have no effect on parasite growth (17, 18).

In vitro antiplasmodial assay

Synchronization of malaria parasites at the ring stage was accomplished by the sorbitol treatment (19). The supernatant was separated and discarded after the centrifugation of cell suspension at 2500 rounds per min (rpm) for five min at room temperature. The packed cells were diluted with washed fresh uninfected erythrocytes to reach a final parasitemia of 1% and a hematocrit level of 2% using the complete medium. Afterward, 150 µl of this parasitized cell suspension were added to each well of 96-well microplates containing 50 µl of each drug concentration (200–1.56 µg/ml). All microplate tests were incubated in candle jars at body temperature (37 ℃) and all experiments were run in triplicate. Parasitized erythrocytes without drug and parasitized red blood cell cultures with CQ were used as negative and positive controls, respectively. After 48 hr, Giemsa staining was performed and parasitemia was confirmed if more than 5000 erythrocytes were counted and was reported as the percent of parasitized erythrocytes. The inhibitory concentration 50 (IC_{50}) values of the prepared extracts were determined from dose-response curves drawn in Microsoft Excel (20).

In vitro cytotoxic assay

To evaluate the cytotoxic effects of extracts on host cells the 3- (4, 5- dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay was used. Raji cell line (B lymphocyte cell line) cultured in RPMI containing 10% fetal bovine serum, 0.21% sodium bicarbonate, and 50 µg/ml gentamycin were used in the MTT assay. In order to conduct the assay, cell suspensions of 10^4 cells per well were seeded

Table 1. Specific information regarding the selected plants

Species	Family	Part used	Place of collection (province)	Date of collection	Voucher number
Solanum nigrum	Solanaceae	Fruits	Guilan	September 2014	1420
Teucrium polium	Lamiaceae	Aerial parts	Tehran	July 2014	1421
Physalis alkekengi	Solanaceae	Leaves&Fruits	Guilan	August 2014	1422
Citrullus colocynthis	Cucurbitaceae	Fruits	Khozestan	July 2014	1423
Salix alba	Salicaceae	Leaves	Alborz	July 2014	1424
Achillea millefolium	Compositae	Flowers	Golestan	May 2014	1425
Gossypium herbacum	Malvaceae	Leaves	Golestan	July 2014	1426
Verbena officinalis	Verbenaceae	Flowers	Khozestan	June 2014	1427
Portulaca oleracea	Portulacaceae	Aerial parts	Tehran	August 2014	1428
Lavandula angustifolia	Lamiaceae	Flowers	Tehran	August 2014	1429

into 96-well microplates. After 24 hr, the cells were washed and different concentrations of each extract were added. The beginning concentration of each extract was 50 µg/ml in DMSO and then was serially diluted in complete culture medium (50-1.56 µg/ml). The cell culture and extracts were placed in a Co_2 incubator (at 37 °C, 5% CO_2, and 90% humidity) and incubated for 72 hr. In the next stage, 20 µl of MTT were added to each well and the microplates were gently mixed and incubated in a Co_2 incubator for 4 hr at 37°C. At the end of the incubation, the MTT solution was removed carefully and 100 µl of DMSO were added to each well.

The formation of the blue formazan products was measured colorimetrically and was read on a microplate enzyme-linked immunosorbent assay (ELISA) reader at 570 nm. Absorption was directly related to viable cells and was used to determine the number of cells not affected by the extract. All experiments were performed in triplicate and IC_{50} levels were calculated by the dose-response curves analysis. Cells viability in the presence of extracts was compared with that of control cultures without extracts. Positive control was Tamoxifen (50 to 1.56 µg/ml) (21).

Selectivity index (SI)

The SI was used to compare the toxicity of extracts against human cells line (Raji cells) and the plasmodium parasite. It is computed by dividing the cytotoxicity of extracts against Raji cells by their antiplasmodial activity.

In vivo antimalarial assay

The four-day suppressive test against the CQ-sensitive strain of *P. berghei* (ANKA) was performed to assess the *in vivo* antimalarial activity of the active extracts (22, 23). Parasite stock was stored in liquid nitrogen (-80 °C). Murine malaria parasite was preserved by serial passage of blood from infected donor mice to uninfected ones. Parasitemia was monitored regularly. In order to perform the experiments female Swiss albino mice, (weight: 18–20 g) were intraperitoneally inoculated with 1×10^7 infected erythrocytes in saline suspensions of 0.2 ml. Active extracts were dissolved in 18% dimethyl sulfoxide then diluted with RPMI-1640 medium to obtain the required concentrations. The mice were randomly allocated to groups of five per cage and received intraperitoneal injections of different concentrations of plant extracts in a dose volume of 0.2% for four consecutive days. The experiments included two control groups. The first control group (positive control) received 25 mg/kg CQ and the second group received only normal saline as placebo. On the fifth day, blood samples were collected from the caudal vein and thin blood smears were prepared. After Giemsa staining, the blood smears underwent microscopic examination. The parasitemia detected in the infected control and test animals were recorded at each dose and the percentage suppression of parasitemia was computed based on the obtained values. The mean survival time was determined for each group. After collecting *in vivo* antimalarial activity data, one-way analysis of variance (ANOVA) and two-tailed Student's *t*-test were applied to compare average parasitemia. All analysis was performed using SPSS 15.0 (SPSS Inc, Chicago, IL, USA). The study procedure was designed based on the Guide for the Care and Use of Laboratory Animals and approved by the Ethics Committee of Pasteur Institute of Iran (Tehran, Iran).

Phytochemical analysis

Qualitative phytochemical analysis of plant extracts was performed to screen for known antimalarial compounds (24-27).

Alkaloids: In order to evaluate the presence of alkaloids, 10 ml of ammoniacal chloroform solution was mixed with 2 ml of each plant extract. The extracts were then treated with 10% H_2SO_4 (10 drops) and a few drops of Meyer's reagent. The existence of alkaloids was confirmed if a white precipitate was formed.

Anthraquinones

The existence of anthraquinones was confirmed if a bright pink color occurred after adding 2 ml of chloroform and 10% NH_4OH to 2 ml of each plant extract solution.

Flavonoids

The presence of flavonoids was confirmed if a pink-red color was seen after adding a few fragments of magnesium ribbon and a few drops of concentrated hydrochloric acid to two milliliters of each plant extract solution.

Steroids

The presence of steroids was confirmed if a blue-green ring was observed following the addition of 2 ml of ethanoic anhydride solution followed by 1 ml of concentrated H_2SO_4 to 2 ml of each plant extract.

Coumarins

One gram of ground plant was placed in a small petri dish, moistened with an aliquot amount of water, and then covered with a filter paper soaked in 10% NaOH solution. The filter paper was exposed to UV light (360 nm) for several minutes. The presence of coumarins was confirmed if a yellow-green fluorescence was seen.

Glycosides

The organic extracts (2 ml) were added with equal amounts of chloroform and 2 ml of concentrated H_2SO_4.The development of a reddish brown color indicated the presence of glycosides in the form of a steroidal ring (which is a glycine portion of a glycoside).

Table2. *In vitro* bioassays on *Plasmodium falciparum* 3D7 and K1 strains and toxicity assay of the selected plants

No	Species	Extracts IC_{50} 3D7 (µg / ml)	Extracts IC_{50} K1 (µg / ml)	Raji cells CC_{50} (µg / ml)	SI CC_{50}/3D7	SI CC_{50}/K1
1	*Solanum nigrum*	**10.29**	**18.67**	**66.3**	**6.44**	**3.55**
2	*Teucrium polium*	>200	>200	ND	ND	ND
3	*Physalis alkekengi*	**11.31**	**13.08**	**47.3**	**4.18**	**3.61**
4	*Citrullus colocynthis*	**2.01**	**6.9**	**38.7**	**19.25**	**5.6**
5	*Salix alba*	164.49	>200	ND	ND	ND
6	*Achillea millefolium*	**34.36**	142.36	**69.7**	**2.02**	ND
7	*Gossypium herbacum*	146.56	159.36	ND	ND	ND
8	*Verbena officinalis*	>200	>200	ND	ND	ND
9	*Portulaca oleracea*	136.57	172.39	ND	ND	ND
10	*Lavandula angustifolia*	173.26	>200	ND	ND	ND

ND: Not Determined

Terpenoids

In order to evaluate the presence of terpenoids, 20 mg of the ground plant were extracted with 10 ml of chloroform for 15 min. After gravity filtration, 2 ml of concentrated H_2SO_4 and 2 ml of acetic anhydride were added to the filtrate. A blue-green ring appeared on top of the mixture in the presence of terpenoids.

Results

In vitro antiplasmodial assay

This study investigated the *in vitro* antiplasmodial activity of all prepared plant extracts against the CQ-sensitive and resistant strains (3D7 and K1, respectively). Of ten extracts tested, methanolic extracts of *Citrullus colocynthis* showed promising antiplasmodial activity against 3D7 and K1 (IC_{50} = 2.01 and 6.9 µg/ml, respectively). *Solanum nigrum* (IC_{50} = 10.29 µg/ml) and *Physalis alkekengi* (IC_{50} = 11.31 µg/ml) showed promising activity against the sensitive strain, but had moderate activity against the resistant strain (IC_{50} = 18.67 and 13.08 µg/ml, respectively). *Achillea millefolium* showed moderate anti-3D7 activity (34.36 µg/ml) but its IC_{50} value against the resistant strain was disappointing (Table 2).

Cytotoxic activity assay

In cytotoxicity assessments, high, moderate, and mild cytotoxicity were defined as 50% cytotoxic concentration (CC_{50}) of < 1, 1 – 10, and 10-30 µg/ml respectively. CC_{50} values larger than 30 µg/ml indicated nontoxicity (28). According to these classifications, plant extracts with promising or moderate activity were not toxic to the Raji cell line. Since the SI is obtained by dividing the CC_{50} values of the Raji cells to the antiplasmodial activity of the extracts (IC_{50}), higher SI values directly related to clinically safer therapy of the extracts (29) (Table 2).

In vivo antimalarial assay

The percentage of growth inhibition was determined as:

100 - [(mean parasitemia treated/mean parasitemia control) × 100]

Differences between the means at the 5% level (P<0.05) were considered significant. Plant extracts with promising and moderate activity were selected for *in vivo* antiplasmodial evaluation against *P. berghei* infection in mice. The experiments showed that three extracts (*S. nigrum, P. alkekengi, and C. colocynthis)* significantly suppressed the growth of parasitemia (P<0.05). This suppression ranged between 57.97% and 65.08 %. Ten days post infection *C. colocynthis* extract gave 90%, but *S. nigrum, P. alkekengi* gave 100% mouse survival (Table 3).

Table 3. Suppressive activities of plant extracts against *Plasmodium berghei*

Species	Dose (mg/kg)	Mean parasitemia (SD)	Suppression Parasitemia (%)	Survival (%) on day 10	*P*-value (*P*<0.05)
NC		2.95(0.13)		100	
Solanum nigrum	100	1.16 (0.04)	60.68	100	0.014
Physalis alkekengi	100	1.24 (0.18)	57.97	100	0.032
Citrullus colocynthis	50	1.03 (0.09)	65.08	90	0.008
Chloroquine	25	0		100	

NC Negative control

Table 4. Constituents in the selected plant extracts

Species	Alkaloids	Anthraquinones	Flavonoids	Steroids	Coumarins	Glycosides	Terpenoids
Solanum nigrum	+	-	+	-	+	+	+
Teucrium polium	-	-	+	+	-	+	+
Physalis alkekengi	+	+	+	-	-	+	+
Citrullus colocynthis	+	-	+	+	+	+	+
Salix alba	+	-	+	-	-	-	+
Achillea millefolium	+	-	+	+	-	-	+
Gossypium herbacum	+	-	+	+	-	+	-
Verbena officinalis	+	-	-	+	-	+	-
Portulaca oleracea	+	-	+	+	-	-	+
Lavandula angustifolia	-	-	+	-	-	-	-

+: present, -: absent

Phytochemical analysis

Phytochemical screening revealed the presence of some vital antiplasmodial constituents including alkaloids, flavonoids, glycosides, and terpenoids in the most active plant extracts. While anthraquinone was detected in *P. alkekengi*, *C. colocynthis* contained coumarins and steroids (Table 4).

Discussion

Since drug resistance is a significant challenge in the fight against malaria, developing new drugs to control falciparum malaria is absolutely critical. In all malaria endemic countries, herbal medicines have been traditionally used to treat the disease. In fact, such herbal products have an undeniable situation in the development of antimalarial chemotherapeutic drugs. Therefore, plants are considered as a strong source for the development of future effective antimalarial agents.

This study selected 10 plants based on traditional Iranian medicine and evaluated their antiplasmodial activity. According to previous studies antiplasmodial activities of plant extracts were categorized as promising activity ($IC_{50} \leq 15$ µg/ml), moderate activity (IC_{50} = 15-50 µg/ml), weak activity ($IC_{50} > 50$ µg/ml), and inactivity ($IC_{50} > 100$ µg/ml) (30-32). The obtained results (IC_{50} = 2.01-18.67 µg / ml and SI = 3.55-19.25) indicated the significant antiplasmodial activity of *C. colocynsis, P. alkekengi,* and *S. nigrum* against K1 and 3D7 strains. *A. millefolium* showed moderate anti- 3D7 activity (IC_{50} = 34.36 µg / ml, SI= 2.02), but had no *in vitro* activity against K1 (Table2). Following *C. colocynsis, P. alkekengi,* and *S. nigrum* administration, the suppression rates of parasitemia in female Swiss albino mice were 65.08%, 57.97%, and 60.68%, respectively (Table 3).

In traditional medicine, *S. nigrum* is used to treat various conditions such as hepatomegaly, edema, pain, inflammation, enteric diseases, and fever (13). It is particularly administered as a painkiller and anti-inflammatory agent in Iranian traditional medicine (33-35). The results from the current study revealed that plants in Solanaceae family showed potentially antileishmanial activity against *L. guyanensis*, *L. major,* and *L. donovani* (IC_{50} = 51, 61 and < 16 µg / ml) (36). In another study, the crude methanol extract of *S. nigrum* showed significant antiparasitic activity against *Haemonchus contortus* and larval stages of the *Fasciola hepatica* (37, 38). In our study, *S. nigrum* showed significant activity against 3D7 and K1 strains (IC_{50} = 10.29 and 18.67 µg/ ml, SI = 6.44 and 3.55 respectively). Based on *in vivo* tests, percentage suppression of parasitemia was 60.68% following *S. nigrum* administration. Several studies have attributed the antiplasmodial properties of plants to their alkaloids, flavonoids, terpenoids, anthraquinones, and glycosides contents (39). The phytochemical analysis in this study confirmed the existence of alkaloids, flavonoids, coumarins, glycosides, and terpenoids in *S. nigrum*. Previous qualitative screening of phytochemical constituents revealed the presence of tannins, well-known antimicrobial agents, in *S. nigrum* extracts (40). Tannins can inhibit the growth of various types of fungi, yeasts, bacteria, and viruses (41). Therefore, the antimalarial effects of *S. nigrum* can be attributed to either one or a combination of the mentioned compounds. Our study was the first to highlight both the *in vitro* and *in vivo* antiplasmodial effects of *S. nigrum* in Iran.

Physalis angulata is used to cure malaria in Congo and Taiwan (42, 43). There have been reports about the *in vivo* and *in vitro* antiplasmodial activity

of methanolic extracts of *P. angulata* against both CQ-sensitive and resistant *P. falciparum* strains (IC_{50} = 1.27 and 3.02 µg/ml, respectively). The chloroform extract of the plant had similar effects, as well (IC_{50} = 1.96 and 2 µg/ml, respectively) (43). Another study confirmed the *in vitro* antiplasmodial activity of the hydroalcoholic extract of *P. angulata* against the CQ-resistant *P. falciparum* (IC_{50} = 4.6 µg/ml) (44). *P. angulata* is of American origin and is not a native plant in Iran (45). Another species belonging to the Solanaceae family, *P. alkekengi* is distributed in Asia (including Iran) and Europe. In Iranian herbal medicine, *P. alkekengi* has been used for the treatment of febrile diseases, inflammation, arthritis, rheumatism, and general edema (14). However, this is the first study in Iran to evaluate the antiplasmodial activity of *P. alkekengi*. This plant showed significant activity against the 3D7 strain (IC_{50} = 11.31 µg/ml and SI= 4.18) and the K1 strain (IC_{50} = 13.08 µg/ml and SI = 3.61) without any evidence of toxicity. According to *in vivo* test results, *P. alkekengi* suppressed parasitemia by 57.97%. Our study confirmed the promising antiplasmodial activity of *P. alkekengi* (which was of course lower than that of *P. angulata*). A Recent study confirmed the antimalarial activity of Physalin F purified from *P. angulata* (46). Based on phytochemical analysis, the antiplasmodial activity of *P. alkekengi* may be attributed to the presence of antimalarial compounds, such as alkaloids, anthraquinones, flavonoides, glycosides, and terpenoids in this plant.

C. colocynthis traditionally is used to treat malaria in India and Yemen (47, 48). In Iranian traditional medicine, *C. colocynthis* is known as a potent phlegmatic purgative and is administered for the treatment of diabetes, arthritis, sciatica, gout, bleeding, and inflammation (11, 15, 49). Recent studies have highlighted the anti-inflammatory, antibacterial, and antifungal activity of *C. colocynthis* (50, 51). *C. colocynthis* showed antileishmanial activity against *leishmania tropica* promastigotes (52). Curcubitacin E and Curcurbitacin L 2- O- ß-glucoside, two pure isolated compounds from *citrullus spp*., revealed strong potent antigiardial activity (53). In our study, *C. colocynthis* extract showed excellent antiplasmodial activity against 3D7 and K1strains (IC_{50} = 2.01 - 6.9 µg/ml; SI = 19.25 and 5.6 respectively). Moreover, the hydroalcoholic extract of this plant had significant *in vivo* antimalarial activity (65.08%). Phytochemical analysis indicated that *C. colocynthis* contained compounds such as alkaloids, flavonoids, steroids, coumarins, glycosides, and terpenoids, which could be responsible for its antimalarial activity.

In a previous study it was found that a number of extracts were involved in the inhibition of hemozoin formation (54). It was envisaged that some of the extracts in the current study would react in the same way and accumulate in the parasite food vacuole (unpublished data). We believe, however, that the remaining extracts involve other mechanisms in addition to the inhibition of hemozoin formation. Further study is planned to confirm this theory.

Conclusion

There is ongoing research aiming at the identification of effective plant extracts to treat malaria. This study reported significant results in this regard and was actually the first to highlight the antiplasmodial activity of *P. alkekengi, C. colocynthis,* and S. *nigrum* extracts against both CQ- sensitive and resistant strains of *P. falciparum*. Our findings also confirmed the *in vivo* activity of mentioned extracts against *P. berghei*. Furthermore, we reported the preliminary phytochemical profiles of these extracts. Further research would undoubtedly be required to isolate the active compounds of the extracts and clarify their mechanisms of action.

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References

1. World Health Organization. World malaria report 2010. Availabe at: http:// WWW.who.int/ malaria/world-malaria-report-2010/en/.
2. World Health Organization. World malaria report 2015. Geneva 2015. Availabe at: http:// http://www.who.int/malaria/publications/world-malaria-report-2015/.
3. Sargolzaie N, Salehi M, Kiani M, Sakeni M, Hasanzehi A. Malaria epidemiology in Sistan and Balouchestan province during April 2008-March 2011, Iran Zahedan. J Resh Med Sci 2011; 16: 41-43.
4. World Health Organization . Iran Malaria Profile. World Malaria Report 2005. Geneva: WHO; 2005.
5. World Health Organization.World malaria report 2011. Geneva 2011. Availabe at: http:// WWW.who.int/malaria/world-malaria-report-2011/en/.
6. Maregesi S1, Van Miert S, Pannecouque C, Feiz Haddad MH, Hermans N, Wright CW, Vlietinck AJ, Apers S, Pieters L. Screening of Tanzanian medicinal plants against Plasmodium falciparum and human immunodeficiency virus. Planta Med 2010; 76: 195-201.
7. World Health Organization. Guidelines for the treatment of malaria: World Health Organization; 2006.
8. Raeisi A, Gouya MM, Nadim A, Ranjbar M, Hasanzehi A, Fallahnezhad M, *et al.* Determination of malaria epidemiological status in Iran, s malarious areas as baseline information for implemention of malaria elimination program in Iran. Iran J Publ Health 2013; 42:326-333.
9. World Health Organization. The use of antimalarial drugs, part II: Artemisinin and its derivatives 2001.
10. Mutabingwa TK. Artemisinin-based combination therapies (ACTs): best hope for malaria treatment

but inaccessible to the needy! Acta Trop 2005; 95:305-315.
11. Heravi M. Alabnya an al-Haghayegh al-Advia. Tehran, Iran: Tehran University;1992.
12. Jorjani L. Zakireh Kharazmshahi. Tehran: Bonyade Farhang Iran; 1992.
13. Khorasani A. Makhzan ol-Advieh. Tehran: Enghelab e Eslami Publishing and Educational Organization; 1992.
14. Zargari A. Medical plants. Tehran: Tehran University; 2003.
15. Ibn Sina. Al Qanun Fi al-Tibb.Persian translated by Sharafkandi A. Tehran, Iran: Soroush Press; 2005.
16. Trager W, Jensen JB. Human malaria parasites in continuous culture. Science 1976; 193:673-675.
17. Dagger F, Valdivieso E, Marcano AK, Ayesta C. Regulatory volume decrease in *Leishmania mexicana*: effect of anti-microtubule drugs. Mem Inst Oswaldo Cruz 2013; 108:84-90.
18. Chakrabarti R, Rawat PS, Caoke BM, Coppel RL, Patankar S. Cellular effects of Curcumin on Plasmodium falciparum include disruption of microtubules. PLoS One 2013; 8:e57302.
19. Lambros C, Vanderberg JP. Synchronization of *plasmodium falciparum* erythrocytic stages in culture. J Parasitol 1979; 65:418-420.
20. Sangian H, Faramarzi H, Yazdinezhad A, Mousavi SJ, Zamani Z, Noubarani M, *et al.* Antiplasmodial activity of ethanolic extract of some selected medicinal plants from the northwest of Iran. Parasitol Res 2013; 112:3697-3701.
21. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983; 65:55-63.
22. Peters W. The chemotherapy of rodent malaria, XXII. The value of drug resistant strains of *P. berghei* in screening for blood schizontocidal activity**.** Ann Trop Med Parasitol 1975; 69:155-171.
23. Peters W, Robinson BL. The chemotherapy of rodent malaria. XLVII. Studies on pyronaridine and other Mannich base antimalarials**.** Ann Trop Med Parasitol 1992; 86:455-465.
24. Evans WC. Treace and evans pharmacognosy. 14th ed. London: Bailiere Tindall W.B. Saunders;1996.
25. Farnsworth NR. Biological and phytochemical screening of plants. J Pharm Sci 1966; 55: 225-276.
26. George NJ, Ikot AN, Obi-Egbedi NO. Phytochemical and antimicrobial properties of leaves of *Alchonea cordifolia*. Eur J Chem 2010; 7:1071-1079.
27. Kumar GG, Gali V. Phytochemical screening of *Abutilon muticum* (del. ex dc.) and *Celosia argentea* Linn. Int J Pharm Biol Sic 2011; 2:463-467.
28. Malebo HM, Tanja W, Cal M, Swaleh SA, Omdo MO, Hassanali A. Antiplasmodial, anti trypanosomal, anti leishmanial and cytotoxicity activity of selected Tanzanian medical plants. Tanzan J Health Res 2009; 11:226-234.
29. Sarr S, Perrotey S, Fall I, Ennahar S, Zhao M, Diop YM, Candolfi E, Marchioni E. Icacina senegalensis (Icacinaceae), traditionally used for the treatment of malaria, inhibits *in vitro Plasmodium falciparum* growth without host cell toxicity. Malar J 2011; 10:85.
30. Philippe G, Angenot L, Mol PD, Goffin E, Hayette MP, Tits M, Fredrich M. *In vitro* screening of some *Strychnos* species for antiplasmodial activity. J Ethnopharmacol 2005; 97: 535-539.
31. Pink R, Hudson A, Mouries MA, Bending M. Opportunities and challenges in antiparasitic drug discovery. Nat Rev Drug Discov 2005; 4:727-740.
32. Jonville MC, Kodja H, Humeau L, De Mol P, Cao M, Angenot L, Frédérich M. Screening of medicinal plants from Reunion Island for antimalarial and cytotoxic activity. J Ethnopharmacol 2008; 120:382–386.
33. Rani P, Khullar N. Antimicrobial evaluation of some medicinal plants for their anti enteric potential against multi drug resistant *Salmonella typhi*. Phytother Res 2004; 18:670-673.
34. Acharya E, Pokhrel B. Ethno-medicinal plants used by Bantar of Bhaudaha, Morang, Nepal. Our Nat 2006; 4: 96-103.
35. Zakaria ZA, Gopalan HK, Zainal H, Mohd Pojan NH, Morsid NA, Aris A, Sulaiman MR .Antinociceptive, anti-inflammatory and antipyretic effects of *Solanum nigrum* chloroform extract in animal models. Yakugaku Zasshi 2006; 126:1171-1178.
36. Mans DR, Beerens T, Magali I, Soekhoe RC, Schoone GJ, Oedairadjsingh K*, et al*. In vitro evaluation of traditionally used Surinamese medicinal plants for their potential anti-leishmanial efficacy. J Ethnopharmacol 2016; 180:70-77.
37. Saddiqe Z, Maimoona A, Khalid S. Phytochemical analysis and anthelmintic activity of extracts of aerial part of *Solanum nigrum L*. Biologia 2013; 59:205-211.
38. Hammami H, Ayadi A. Molluscicidal and antiparasitic activity of *Solanum nigrum Villosum* against *Galba truncatula* infected or uninfected with *Fasciola hepatica*. J Helmintol 2008; 82: 235-239.
39. Batista R, De Jesus Silvanior A, De Oliveira AB. Plant-derived antimalarial agents: new leads and efficient phytomedicines. Part II. Non-alkaloidal natural products. Molecules 2009; 14: 3037-3072.
40. Gogoi P, Islam M. Phytochemical screening of *Solanum nigrum* L and *S.myriacanthus* Dunal from districts of upper Assam, India. IOSR J Pharmacy 2012; 2: 455-459.
41. Ghosh D. Tannins from food to combat diseases. Int J Pharm Res Rev 2015; 4:40-44.
42. Hsieh WT, Huang KY, Lin HY, Chung JG. Physalis anagulata induced G2/M phase arrest in human breast cancer cells. Food ChemToxicol 2006; 44:974-983.
43. Lusakibanza M, Mesia G, Tona G, Karemere S, Lukuka A, Tits M*, et al. In vitro* and *in vivo* antimalarial and cytotoxic activity of five plants used in Congolese traditional medicine. J Ethnopharmacol 2010; 129:398-402.
44. Ruiz L, Maco M, Cobos M, Gutierrez-Choquevilca Aa-L, Roumy V. Plants used by native Amazonian groups from the Nanay River (Peru) for the treatment of malaria. J Ethnopharmacol 2011; 133:917-921.
45. Rengifo Salgado E, Vargas Arana G. *Physalis angulata* L.(Bolsa Mullaca): a review of its traditional uses, chemistry and pharmacology. Bol latinoam Caribe Plant Med Aromat 2013; 12:431-45.

46. Sa MS, de Menezes MN, Krettli AU, Ribeiro IM, Tomassini TC, Ribeiro dos Santos R, *et al.* Antimalarial activity of Physalis B, D, F and G. J Nat Prod 2011; 74:2269-2272.
47. AAN A, Al-rahwi K, Lindequist U. Some medicinal plants used in Yemeni herbal medicinal to treat malaria. Afr J Tradit Complement Altern Med/2004; 1:72-76.
48. Meena MC, Meena RK, Patni V. Ethanobotanical studies of *Citrullus colocynthis* (Linn.) Schrad. An important threatened medicinal herb. J Med Plants Stud 2014; 2:15-22.
49. Tonkaboni M. Tohfeh al-Momenin. Tehran: Shahid Beheshti University of Medical Sciences; 2007.
50. Marzouk B, Marzouk Z, Fenina N, Bouraoui A, Aouni M. Anti-inflammatory and analgesic activities of Tunisian *Citrullus colocynthis* Schrad. immature fruit and seed organic extracts. Eur Rev Med Pharmacol Sci 2011; 15:665-672.
51. Marzouk B, Marzouk Z, Dcor R, Edziri H, Haloui E, Fenina N, Aouni M. Antibacterial and anticandidal screening of Tunisian *Citrullus colocynthis* Schrad. from Medenine. J Ethnopharmacol 2009; 125:344-349.
52. Saeed BQ, Hassan HF, Arteen HI. Effect of some medical plant extracts on metabolism of Leishmania tropica promastigotes In vitro. J Med Microb Diagn 2014; 3:165.
53. Ahmed Hassan LE, Sirat HM, Yagil SMA, Koko WS, Abdelwahab SI. *In vitro* antimicrobial activities of chloroformic, hexane and ethanolic extracts of *Citrullus lanatus* var. Citroides (wild melon). J Med Plants Res 2011; 5:1338-1344.
54. Onyeibor O, Croft SL, Dodson HI, Feiz-Haddad M H, Kendrick H, Millington NJ, Parapini S, Phillips RM, Seville S, Shnyder SD, Taramelli D, Wright CW. Synthesis of some cryptolepine analogues, assessment of their antimalarial and cytotoxic activities, and consideration of their antimalarial mode of action. J Med Chem. 2005; 7; 2701-9.