



RESEARCH PAPER

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Antimalarial potential of leaves crude extract of *Monotheca buxifolia*

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Abstract

The human's malarial infection is transmitted mainly by *Plasmodium vivax* and *Plasmodium falciparum* where the most usual cause of malarial infection is *Plasmodium vivax* which is about 80% of all malarial cases and 10% of the deaths. So the present study was focused on the effects of methanolic crude extract of *Monotheca buxifolia* leaves at the concentration 200 µg/ml against *Plasmodium falciparum* and *Plasmodium vivax* present in human blood samples. The blood samples (n=100) were collected from both the genders of various ages from six union councils namely, Uster Zai, Dhoda, Khormato, Jerma, Bilitang and Chickar Kot of district Kohat. Out of 100 patients, 4 patients were infected by *Plasmodium falciparum* (male=3, female=1) and 96 were infected by *Plasmodium vivax* (male=70, female=26). Maximum inhibition (80%) in the growth of *Plasmodium falciparum* was exhibited by methanolic fraction at the age groups of 6-15, 16-30 and 31-50 years male and female malarial patients. While, in the case of *Plasmodium vivax*, maximum percentage inhibition was exhibited by methanolic fraction (male=80%, female=82%) at the age group of 31-50 years. The Resochine (40 µg/ml) was used as positive control exhibited 100% inhibition of malarial parasites. Moreover, distilled water was used as negative control (200µl/ml) caused no effect.

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Introduction

Plants are essential for the living of poor people, all over the globe. The majority of medicinal plants are flower producing. Ten percent plants are medicinal out of about 320000 angiospermic floras (Prance, 2001). The phytochemical study indicates that the plant is naturally enriched with anthraquinones, flavonoids, reducing sugar, terpenoids, tannins, saponins, and cardiac glycoside. Fruits of the plant are the source of food and traditionally, it is used as expectorant, refrigerant and purgative (Sidra and Khan, 2012). The human's malarial infection is transmitted mainly by *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium knowlesi* (Singh *et al.*, 2004). The most usual cause of malarial infection is *Plasmodium vivax* which is about 80% of all malarial cases and 10% of the deaths (Mueller *et al.*, 2007). The *Plasmodium* spp. also infects other animals like monkeys, birds, reptiles, chimpanzees and rodents (Mendis *et al.*, 2001).

Currently, malarial infections are increasing at a dangerous rate, specifically in Africa where 250 to 450 million cases every year cause 1.6 to 2.6 million deaths (Escalante and Ayala, 1994).

Presently, for the cure of cardiac disease, high BP, fever, lung infection and some other diseases, plants derivatives are used commercially and traditionally. For example, *Ephedra* which contains Ephedrine-an energetic constituent is used for lungs problem, asthma and other respiratory tract diseases (Shaw, 1998).

Monothea buxifolia of family Sapotaceae is locally known as Gurgura in Pashto language. *Monothea buxifolia* is a large shrubs or a spiny evergreen tree with woody stem, up to 10m height and with white pubescent flowers. Leaves are simple, dark-green and fully developed. Fruits are berry, globose, fleshy and 1-seeded. Seeds almost round with ruminant endosperm. It is commonly distributed in North West Pakistan, Afghanistan, Oman, Somalia and Ethiopia (Malik, 2011).

The anti- malarial drug resistance is the main problem for malarial control, particularly of *Plasmodium falciparum*, the most dangerous malarial parasite. The most important problem is the failure of inexpensive drugs such as sulphadoxine, pyrimethamine and chloroquine while new drugs are 6-60 times costly (Rodriguez-Morales *et al.*, 2006).

Materials and methods

Collection of plant material

The leaves of plant were collected from the mountains of Dara Adam Khel, Kohat, Khyber Pakhtunkhwa. The fresh plant leaves were washed by distilled water and put below shadow at room temperature for drying.

Extraction

After the maceration, the extraction was done. Hundred grams of *Monothea buxifolia* powder were chopped and soaked in 1000 ml of Methanol for 7 days. After continues shaking, soaked material was filtered with Whatman's filter paper No. 1.

Blood samples collection of malarial patients

From the 73 male patients of malaria, the blood samples were collected from district Kohat. Two ml of blood were collected from every patient of different ages randomly.

Medium preparation

RPMI 1640 Medium was used for the culture and growth of parasite present in the samples of blood, which involves the following steps:

In 30 ml distilled water, 0.3g (10.43g/liter of distilled water) of RPMI 1640 powder was dissolved. In 100 vials of Bijeo bottles, this culture medium was dropped, having 10% fetal bovine serum (FBS) with 5 ml of the dissolved medium supplemented.

Culturing of malarial blood samples

The cultures were maintained in the laboratory for the growth of malarial parasites using the Candle Jar technique in human RBC (blood type ^b). In 73 tubes, the infected blood samples were cultured in RPMI 1640 medium. In each tube, 5ml of RPMI 1640 medium and 2 ml of infected blood were kept in incubator at 37°C for 72 hours.

Preparation of stock solution

For preparation of stock solution, 0.25 g of methanolic crude extract was dissolved in 25 ml of distilled water.

Use of test samples against malarial parasites

Antimalarial test was performed using 100 collected samples. At the concentration of 10mg/ml, 20 μ L of every test sample was poured into 100 μ L of the infected blood samples. Control was consisted of culture medium in infected blood with no test sample.

Then every tube was incubated immediately at 37°C for 48h. After 48h, on glass slides, Giemsa-stained blood films were organized from infected blood samples of every tube. Microscopically, the surviving parasites number was observed. With respect to control, %inhibition was determined (Olliaro *et al.*, 1996) by applying the formula;

(Number of *Plasmodium spp.* in the test vial/number of *Plasmodium spp.* in control vial \times 100).

Statistical analysis

All the data were analyzed by the software STATA 12.0 applying Chi-square test.

Results and discussion*Use of methanolic fraction at the blood samples of male malarial cases*

The methanolic crude extract of *Monothecha buxifolia* was tested against *Plasmodium vivax* and *Plasmodium falciparum* and caused 80% maximum inhibition at the ages of 4, 6, 7, 14, 16, 18, 22, 24, 26, 29, 30, 31, 37, 40, 45, 53, 57, 64, 65, and 69 years and showed 67% minimum inhibition at the age of 6, 19 and 20 years.

Table 1. Use of methanolic fraction of *Monothecha buxifolia* (20 μ l/100 μ l) at the blood samples of male malarial cases.

Age (years)	Effect of extract (%)	Effect of distilled water (%)	Effect of resochine (%)
4	80	0	100
4	71	0	100
6	67	0	100
6	80	0	100
6	71	0	100
7	80	0	100
9	75	0	100
9	75	0	100
9	73	0	100
9	78	0	100
10	75	0	100
11	75	0	100
12	75	0	100
12	75	0	100
12	77	0	100
13	71	0	100
14	80	0	100
14	77	0	100
14	73	0	100
15	76	0	100
16	73	0	100
16	80	0	100
17	75	0	100
18	80	0	100
18	80	0	100
18	78	0	100
19	71	0	100
19	75	0	100
19	67	0	100
19	75	0	100
20	71	0	100
20	78	0	100

20	67	0	100
20	77	0	100
22	75	0	100
22	71	0	100
22	80	0	100
22	71	0	100
23	75	0	100
23	75	0	100
24	75	0	100
24	80	0	100
24	75	0	100
25	75	0	100
25	78	0	100
25	75	0	100
25	71	0	100
26	80	0	100
26	75	0	100
27	75	0	100
27	73	0	100
27	75	0	100
27	73	0	100
28	75	0	100
28	71	0	100
29	80	0	100
29	75	0	100
30	80	0	100
31	75	0	100
31	80	0	100
35	75	0	100
37	80	0	100
40	80	0	100
45	80	0	100
45	78	0	100
45	71	0	100
53	80	0	100
57	80	0	100
62	75	0	100
64	80	0	100
65	80	0	100
66	78	0	100
69	80	0	100

$X^2 = 233.1727$, $P \leq 0.696$.

Negative control Distilled water (D.W) had neutral effects. However, the Resochine (positive control) caused complete inhibition. The extract inhibition was effective for the age group of 6-15 years (Table 1, Fig. 1-3).

Use of methanolic fraction at the blood samples of female malarial cases

The maximum inhibition results of methanolic fraction of *Monothecha buxifolia* against *Plasmodium* (*P. vivax* and *P. falciparum*) were 82% in the parasite of blood samples of female patients of 47 and 63 years and showed 67% (minimum) inhibition at the age of 20 and 65 years. The negative control (D.W) exhibited neutral effect on *Plasmodium* species. The positive control (Resochine) caused cent-percent inhibition (Table 2, Fig. 4 & 5).

Current research was carried out to explore the medicinal significance of *Monothecha buxifolia* against two selected species of *Plasmodium*. Although, *Monothecha buxifolia* has vast therapeutic importance but antimalarial activity of this plant has not been studied. From results of the study, it was noticed that 20µl/100µl dosage of methanol extract caused 80% inhibition of malarial parasites. The positive control (Resochine 40µg/ml) caused complete inhibition of the malarial parasites and negative control (D.W) caused no inhibition. In the past, the genus *Monothecha* has not been used for anti-malarial activity whereas other biological activities have been documented.

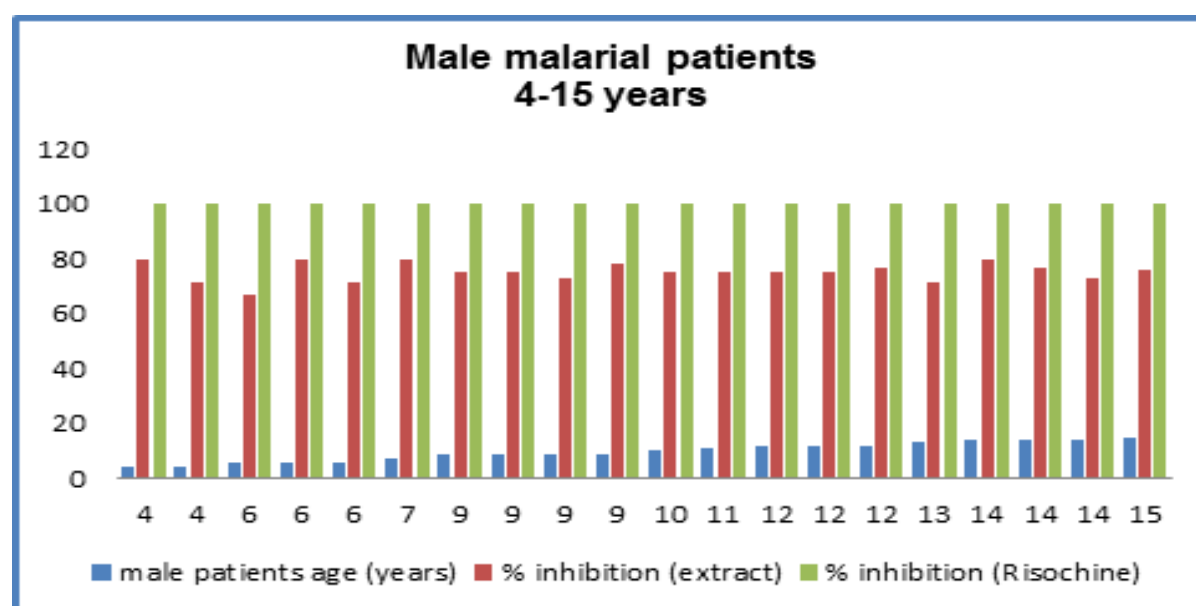
Table 2. Use of methanolic fraction of *Monothecha buxifolia* (20µl/100µl) at the blood samples of female malarial cases.

Age in Years	Effect of extract (%)	Effect of distilled water (%)	Effect of resochine (%)
3	78	0	100
5	80	0	100
8	78	0	100
12	71	0	100
16	71	0	100
20	71	0	100
20	67	0	100
21	71	0	100
21	73	0	100
21	80	0	100
26	78	0	100
30	80	0	100
32	78	0	100
34	80	0	100
36	75	0	100
38	75	0	100
39	78	0	100
40	73	0	100
41	71	0	100
45	75	0	100
47	82	0	100
48	80	0	100
57	80	0	100
63	75	0	100
63	82	0	100
65	67	0	100
66	80	0	100

$\chi^2 = 127.2536$, $P \leq 0.600$.

The other genera of the same family (Sapotaceae) have been tested for antimalarial activity and were found effective in curing the infection. Adewoye *et al.*, 2010 reported that the *Chrysophillum albidum*

(Sapotaceae) extract has the potent antiplasmodial substances which help to control the parasitaemia erythrocyte destruction during disease.

**Fig. 1.** Antimalarial effect of methanolic crude extract in male patients (4-15 years).

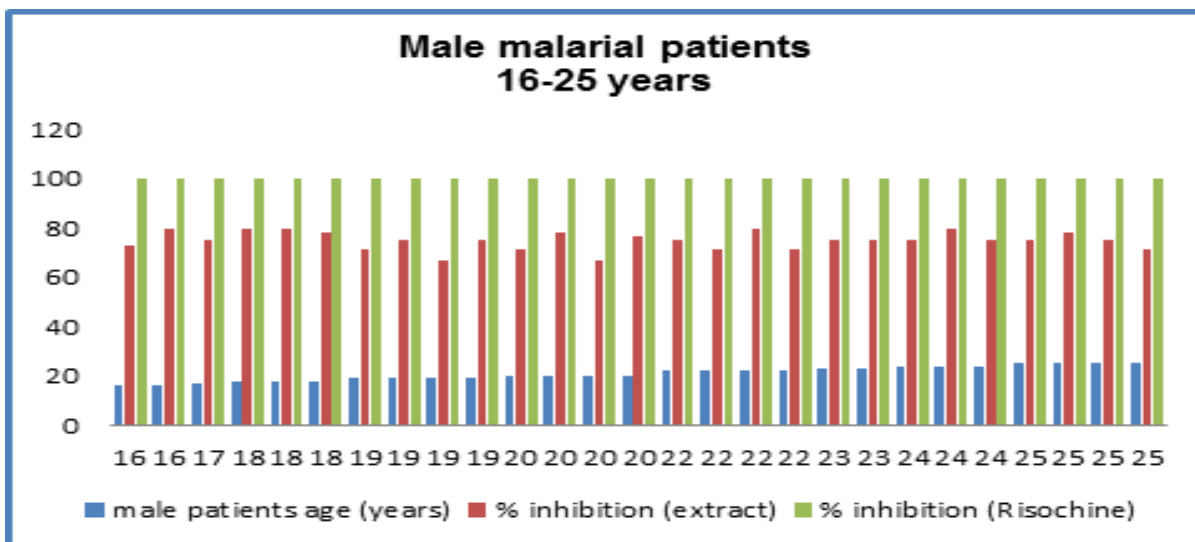


Fig. 2. Antimalarial effect of methanolic crude extract in male patients (16-25 years).

Baidya (2011) reported the phytochemical analysis of *Monotheca buxifolia* and *Polyalthia longifolia*. Both contain alkaloids, anthraquinones, flavonoids, reducing sugars, terpenoids, tannins, saponins,

carbohydrates and cardiac glycosides which showed good antileishmanial activity against promastigotes of *Leishmania donovani*.

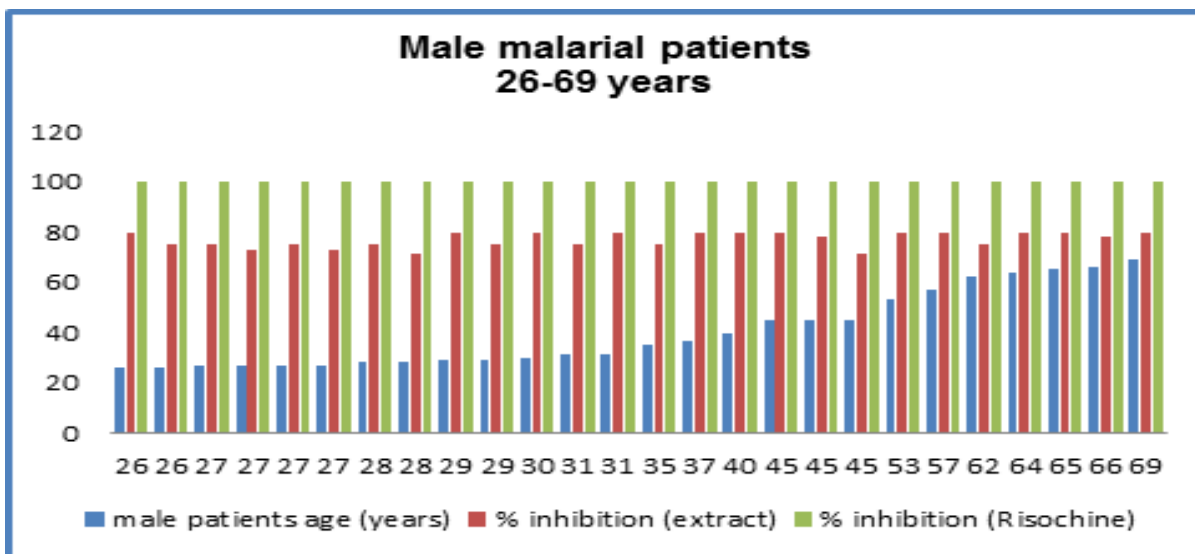


Fig. 3. Antimalarial effect of methanolic crude extract in male patients (26-69 years).

The fruits of *Monotheca buxifolia* are used as food and traditionally it is used as folk medicine for refrigerant, purgative and expectorant (Riaz *et al.*, 2010). Ethnobotanically, fruits of *Monotheca buxifolia* are laxative, digestive and used in urinary tract diseases (Rashid and Khan, 2009; Sidra and Khan, 2012).

Phytochemical screening of the leaves of *Monotheca buxifolia* showed that the leaves of the plants are

naturally enriched with cardiac glycosides, flavonoids, tannins, anthraquinones, saponins, terpenoids and reducing sugars.

On the basis of these compounds, it may be considered as a rich source of antioxidant activity (Javeria *et al.*, 2013). In the light of above reports, *Monotheca buxifolia* can be considered as potent antimalarial plant.

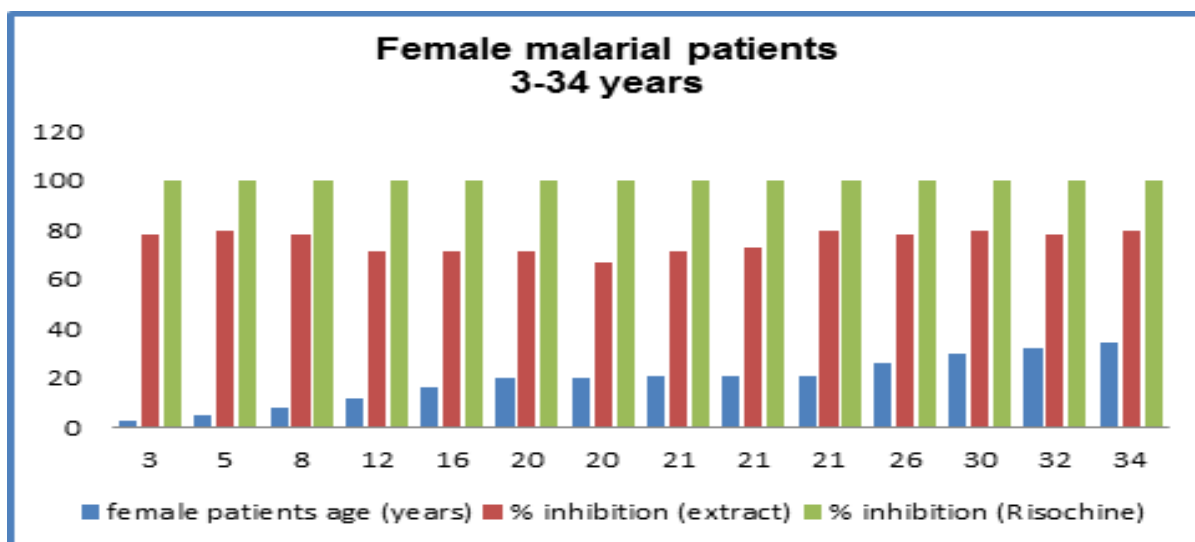


Fig. 4. Antimalarial effect of methanolic crude extract in female patients (3-34 years).

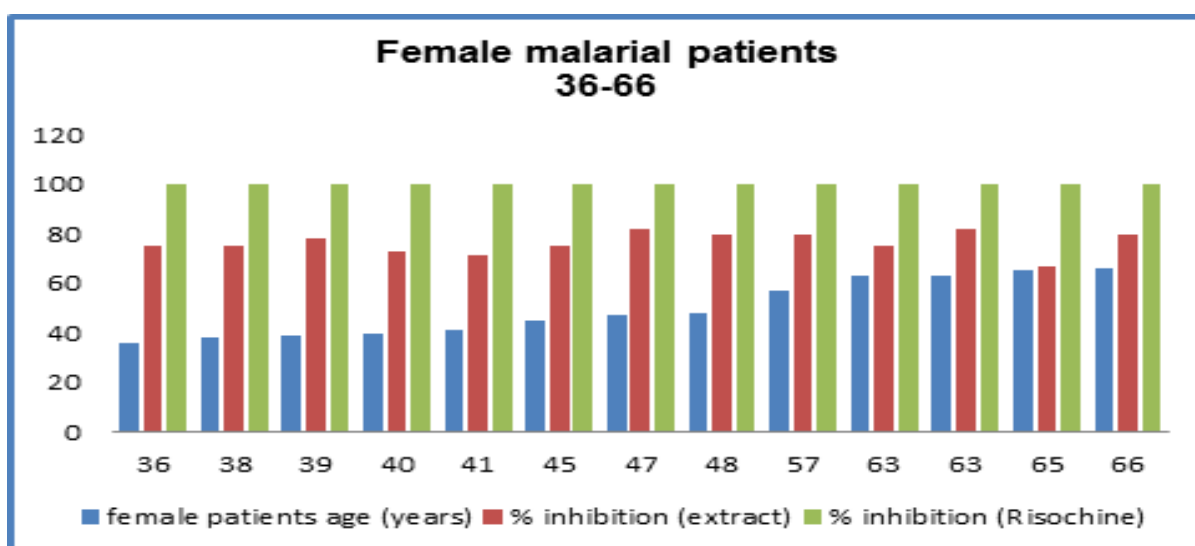


Fig. 5. Antimalarial effect of methanolic crude extract in female patients (36-66 years).

Conclusion

From the present investigation, it is clear that methanolic crude extract of leaves of *Monothea buxifolia* showed strong antimalarial potential against the two selected *Plasmodium* species. Further work is needed to check the plant crude extracts in different solvents against different *Plasmodium* species.

Potent antimalarial agents can also be isolated from its bioactive fractions.

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