REPORT

ANTIMALARIAL ACTIVITY OF THREE PAKISTANI MEDICINAL PLANTS

SABA IRSHAD[1,2], ABDUL MANNAN[1,3] AND BUSHRA MIRZA[1] *

[1]*Department of Biochemistry, Quaid-i-Azam University, Islamabad, Pakistan*
[2]*Institute of Biochemistry & Biotechnology, Punjab University, Lahore, Pakistan*
[3]*Department of Pharmaceutical Sciences, COMSATS Institute of Information Technology, Abbottabad, Pakistan*

ABSTRACT

This study was conducted to determine the *in vitro* anti-malarial activity of three medicinal plants, *Picrorhiza kurroa, Caesalpinia bonducella* and *Artemisia absinthium* of Pakistan. Different extracts of various parts of these plants were prepared by maceration and percolation, and were evaluated for their antimalarial activity. Aqueous, cold alcoholic and hot alcoholic extracts of *Picrorhiza kurroa* showed 34%, 100% and 90% inhibition in growth of *Plasmodium falciparum*, respectively, at 2.00 mg/ml. While aqueous, cold alcoholic and hot alcoholic extracts of *Caesalpinia bonducella* showed 65%, 56% and 76% inhibition in growth of *Plasmodium falciparum*, respectively at same concentrations. In the case of *Artemisia absinthium*, aqueous, cold alcoholic and hot alcoholic extract of *Artemisia absinthium* showed 35%, 55% and 21% inhibition in growth of *Plasmodium falciparum*, respectively at 2.00 mg/ml. In our study, extracts of *Picrorhiza kurroa* were found good for traditional therapy with highly significant results.

Keywords: Antimalarial activity, *Artemisia absinthium, Caesalpinia bonducella, Picrorhiza kurroa.*

INTRODUCTION

Malaria, a serious disease, affected more than 247 million people worldwide while 3.3 billion people were at the risk in 2006. It is the cause of at least 1 million deaths every year mostly of children under 5 year. One hundred nine countries were endemic for malaria in 2008. Pakistan is in high-burden country. There were about 1.5 million malarial episodes in 2006 and 0.0014 million ended in death. Among episodes, about 30 % cases were due to *Plasmodium falciparum* (WHO, 2008). The rise of multi-drug resistant *Plasmodium* species and the pesticide resistance of the infection vector, the Anopheles mosquito, have made the eradication of this disease very difficult (Winstanley *et al*., 2002). Malaria has a tremendous impact on both economic and social development (Sachs and Malaney, 2002).

Many developing countries still depend on traditional antimalarial medicine as a source for the treatment of the Malaria. But, it is very difficult to assess the efficacy of these herbal remedies. However, recognition and validation of these traditional medicinal practices could lead to new plant-derived drugs, e.g. artemisinin from *Artemisia annua* of Chinese traditional medicine (Ridley, 2002). Therefore, it is important that traditionally used antimalarial plants are investigated, in order to establish their efficacy and to determine their potential as sources of new antimalarial drugs.

The present study reports the *in vitro* antimalarial activity of nine extracts from three different medicinal plants such as *Picrorhiza kurroa*, *Caesalpinia bonducella* and *Artemisia absinthium*, in use as traditional antimalarial agents in Pakistan. These plants were selected because of their use in traditional medicines as febrifuge and analgesic as claimed by some Pakistani traditional healers to cure malaria.

**Corresponding author:* e-mail: bushramirza@qau.edu.pk

MATERIALS AND METHODS

Plant materials

Three plants, *Picrorhiza kurroa* (Scrophulariaceae), *Caesalpinia bonducella* (Fabaceae) and *Artemisia absinthium* (Asteraceae) were chosen from a list of medicinal plants, used as antimalarial (Gbeassor *et al.*, 1990) as shown in table 1. The plant material were collected and identified by Dr. Mir Ajab Khan and compared with voucher specimens of Herbarium of Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan.

Preparation of crude extracts

Before starting the extraction, plant materials were crushed and powdered for better isolation of secondary metabolites. Aqueous and hot alcoholic extracts of roots, seeds and leaves of *Picrorhiza kurroa, Caesalpinia bonducella* and *Artemisia absinthium* respectively, were prepared via percolation by using distilled water and alcohol in the soxhlet apparatus respectively. While cold alcoholic extracts of roots, seeds and leaves of each plant material were prepared via maceration by placing the 150g each plant material in 300ml of absolute alcohol for 7 days.

Antimalarial activity

Antimalarial activity of all crude plant extracts was

performed by following procedure of Kerharo & Adam, (1974). The test parasites, *P. falciparum*, were obtained from the blood of the patients suffering from malarial infection. The *in vitro* antimalarial test was performed in 96 well microtiter plates. Each plant extract was dissolved in their respective solvent again with final concentration of 20mg/ml. Each test sample was applied in a series of different dilution with concentration between $2x10^3$µg and 2µg/ml along with negative control. Chloroquine was used as positive control. 50µl of test samples were dispensed into each well of microtiter plate and dried at 37°C under sterile conditions. A 100µl of the infected blood suspension without agitation and 200µl of RPMI 1640 culture medium supplemented with 23mM NaHCO3 and 25mM HEPES were added aseptically in each well. Controls consisted of infected blood in culture medium with no test sample. The content of each microtiter plate was gently mixed and then incubated without agitation for 48h at 37°C. After 48h, Giemsa-stained blood films on glass slide were prepared from blood sample of each well. Their number of survived parasites was observed, microscopically. These parasites were compared with that of control plates for the determination of percentage inhibition of the parasite growth. These results were analyzed statistically by using ANOVA at P=0.05.

RESULTS

The results of *in vitro* antimalarial activity are presented in figs. 1-3 that are statistically significant. All extracts of different medicinal plant parts were tested for their putative antimalarial activity *in vitro*. Results showed that four extracts produced more than 60% inhibition of the parasite growth when tested at 2mg/ml. The remaining five extracts inhibit the parasite growth less than 50% at same sample concentration. These results showed that more than 50% growth inhibition was observed at a concentration of 2mg/ml. The result of *in vitro* assay generally showed that these medicinal plants possessed antimalarial activity against *P. falciparum* and the ethanolic extracts seemed to have better effect than aqueous extracts.

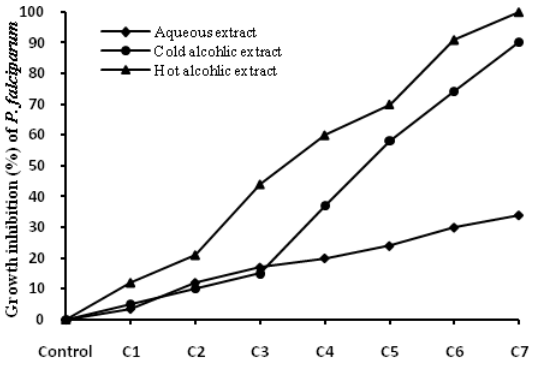

Fig. 1: Effect of *Picrorhiza kurroa* extracts on growth inhibition of *Plasmodium falciparum.*

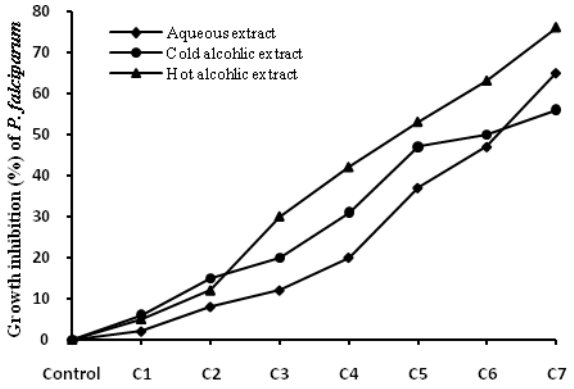

Fig. 2: Effect of *Caesalpinia bonducella* extracts on growth inhibition of *Plasmodium falciparum.*

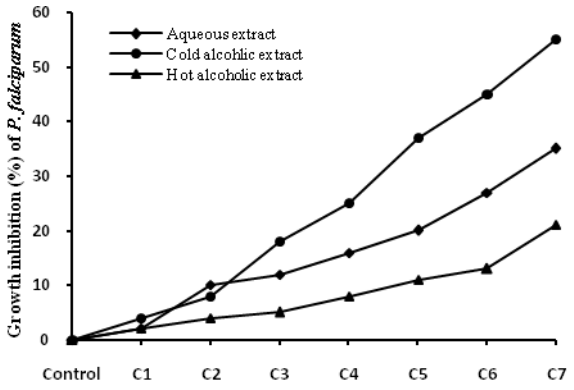

Fig. 3: Effect of Artemisia absinthium extracts on growth inhibition of *Plasmodium falciparum.*

DISCUSSION

Three type of extracts i.e. aqueous, hot alcoholic and cold alcoholic extract of *Picrorhiza kurroa* were evaluated in vitro. Two extracts, i.e. hot alcoholic and cold alcoholic extracts showed very good antimalarial activity. 100% and 90% inhibition of *Plasmodium falciparum* growth at 2mg/ml was achieved which is comparable to the activity of *Artemisia annua* and some *Simasoubaceae* plants (Mukherjee, 1991). The activity was higher than that of ethanolic extract of *Artemisia absinthium*. It was reported that *Picrorhiza kurroa* roots were effective against intermittent fevers. In China and Malaya the rhizome of *Picrorhiza kurroa* is a favorite remedy for bilious dyspepsia accompanied by fever (Oliver-Bever, 1986). The root of *Picrorhiza kurroa* contains glycosides, bitter principal picrorrhizin (Kutkin), non bitter product kurin, vinillic acid, kutkiol and kutkisterol (Gbeassor *et al.*, 1990; Oliver-Bever, 1986). Hot alcoholic extract obtained by soxhlet extraction method showed 100% inhibition at 2mg/ml and more than 90% at even its half concentration i.e.1mg/ml. Similar results were obtained in Commelina and elliptica extract (Munos *et al.*, 2000).

Table 1: Plant species used for antimalarial evaluation

S. No.	Species	Family	Parts Used	Local name	Type of extracts used
1	*P. kurroa*	Scrophulariaceae	Roots	Kutki	1: Aqueous extract 2: Cold alcoholic extract 3: Hot alcoholic extract
2	*C. bonducella*	Fabaceae	Seeds	Fever nut,	1: Aqueous extract 2: Cold alcoholic extract 3: Hot alcoholic extract
3	*A. absinthium*	Asteraceae	Leaves	Worm wood	1: Aqueous extract 2: Cold alcoholic extract 3: Hot alcoholic extract

The results of *in vitro* assay show that all extracts i.e. aqueous, cold ethanolic, hot ethanolic of *Caesalpinia bonducella* at their highest concentration (2mg/ml) possess activity against early stages of *Plasmodium falciparum*. Aqueous, cold ethanol and hot ethanol extracts of *Caesalpinia bonducella* showed 65%, 56% and 76% growth inhibition of *P. falciparum* respectively. This revealed that active compounds of *Caesalpinia* are soluble in both solvents i.e. distilled water and alcohol but solubility increased with the rise in temperature and it may also be probably due to fresh proportion of solvent coming in contact with the sample after an interval of time. Munos *et al.* (2000) also obtained similar results while working on extraction of essential oils with soxhlet apparatus.

Artemisia absinthium showed poor activity as compared to *Picrorhiza kurroa* and *Caesalpinia bonducella*. The aqueous extract and hot alcoholic extract of *Artemisia absinthium* had low antimalarial activity even at highest concentrations of these extracts i.e. 2mg/ml. They possessed less than 50% inhibitory effect on the growth of *plasmodium*. Another species of this genus i.e. *Artemisia annua* is famous for their antimalarial activity (Neill *et al.*, 1985). In the present study, crude ethanlolic extract obtained by maceration possessed higher antiparasitic activity as compared to aqueous and hot alcoholic extracts of *Artemisia absinthium*, that is in contrast with the results of Baquar (Baquar, 1989), who had found that hot water and ethanolic extract of *Artemisia annua* had no antimalarial effect. The antimalarial activity exhibited by the crude ethanolic extract was perhaps due to the presence of some active principal, which might have higher solubility in alcohol. It might be due to the precipitation of carbohydrates and protein with ethanol, which allow easy penetration and subsequent extraction of tissues (Sauza and Gloria, 1998).

Among aqueous extracts of *A. absinthium*, *C. bonducella* and *P. Kurroa*, only *Caesalpinia* showed less activity *Picrorhiza kurroa* proved the best plant among these, as its ethanol extracts (hot ethanol and cold ethanol) showed 100% and 90% growth inhibition of *P. falciparum* at 2mg/ml concentration, cold ethanol extract of A. absinthium and cold ethanol and hot ethanol extracts of *C. bonducella* showed only 55%, 56% and 76% growth inhibition of *P. falciparum*. These extracts will be investigated further, in an attempt to isolate and identify their active constituents.

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