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IN VIVO ANTI-MALARIAL EVALUATION OF *OCIMUM SANCTUM* LINN. AND *O. BASILICUM* LINN

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ABSTRACT

Different extractives from leaves and roots of *Ocimum sanctum* Linn. and *O. basilicum* Linn. have been evaluated for *in vivo* anti-malarial activity using Peter's 4-day suppressive test against *Plasmodium berghei* in mice. Ethanol extracts of roots of both plants exhibited maximum anti-malarial activity amongst various extracts viz., petroleum ether, chloroform, ethanol and water at the dose of 800 mg/kg in mice. The results were observed on the 4th and 7th day. Ethanolic extract of *O. sanctum* roots showed maximum antiplasmodial activity of 3.2 ± 0.74 at dose of 800mg/kg on day 4th. However, *O. basilicum* root extract showed maximum activity of 4.9 ± 0.96 at dose 800mg/kg on day 4th. Maximum activity of ethanoic extract was observed on day 4th.

Keywords: *Ocimum sanctum*, *O. basilicum*, anti-malarial activity, *Plasmodium berghei*.

INTRODUCTION

Malaria is a public health problem in more than 90 countries inhabited by 2.4 billion people. It is responsible for >500 million clinical cases and 1.5–2.7 million deaths per year, most of whom are children under 5 years of age and pregnant women (WHO, 1996; Schwartlander, 1997). Every year, 10% of the global population is infected with malaria, and many (99.4%) of them survive after 10–20 days of illness. *Plasmodium* species are protozoan parasites responsible for malaria, an illness killing about millions of people per year (WHO, 2005). With the absence of an operational vaccine for malaria or leishmaniasis in the immediate horizon, chemotherapy and chemoprophylaxis remain the main methods for disease control. Current anti-protozoal drugs are inadequate due to parasite resistance, toxicity, lack of efficacy and inability to eliminate all stages of parasites from the host (Tasdemir *et al*., 2005). However, with the increase in cases of drug resistance and failure, there is an increase in the use of herbal medicine. Approximately 80% of the people in the developing countries depend on traditional medicine for the management of disease conditions (Phillipson and Wright, 1991).

The discovery of quinine and artemisinin from *Cinchona succiruba* (Rubiaceae) and *Artemisia annua*, respectively, followed by their development into powerful anti-malarial drugs represent milestones in the history of anti-parasitic drugs from plants (Kayser *et al*., 2003).

Today's researchers are exploring the plant kingdom to lay hands on the bioactive phyto-moieties, which can be used to cure malaria. *Ocimum sanctum* Linn. (Family Labiatae) commonly known as 'Sacred Basil' or 'Holy Basil' (Tulsi in Hindi) is an herbaceous annual plant indigenous to India. *O. sanctum* has been utilised as a general promotor for health in herbal medicine (Rai, 1993) and most of its properties like antistress (Ashok and Vaidya, 1997), adaptogenic (Sembulingam *et al*., 1999), anticancer (Aruna *et al*., 1992), anti-inflammatory (Chattopadhyay *et al*., 1994; Singh *et al*., 1996), anti-hyperlipidemic (Rai and Mani, 1997), antihyper-cholesteremic (Sarkar *et al*., 1994), hepatoprotective (De, S. Ravishankar *et al*., 1993), radioprotective (Uma Devi *et al*., 1998) and antimicrobial (Rajendhran and Arun, 1998) have been examined scientifically. It has been used traditionally to cure malarial fever (Chopra *et al*., 1956; Usha Devi *et al*., 2001). The present study has been undertaken with an objective to evaluate leaves and roots of *O. sanctum* and *O. basilicum* for their i*n vivo* anti-malarial activity in mice.

MATERIALS AND METHODS

Plant material and extraction: The leaves and roots of *O. sanctum* and *O. basilicum* used for the present study were collected from the plants grown locally in the Medicinal Plants Garden of the University Institute of Pharmaceutical Sciences (UIPS), Panjab University, Chandigarh. The leaves and roots of *O. sanctum* and *O. basilicum* were dried in shade. Each portion was reduced to moderately coarse powder (# 10) and separately extracted with petroleum ether (60-80^{o}C), chloroform, ethanol and distilled water successively using soxhlet apparatus. All the extracts were dried under reduced pressure.

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Chemical study: Different extractives obtained above were tested chemically for the presence or absence of alkaloids, saponins, sterols, triterpenoids, proteins, flavonoids, carbohydrates and tannins (Evans, 1996; Farnsworth, 1966).

Animals: Adult Swiss mice BALB /c strain (25-28 g) of either sex, bred in the Central Animal House of Panjab University, Chandigarh were used. Animals were fed on the standard diet and water *ad libitum*.

Tested material: Petroleum ether, Chloroform, ethanol and water extractives of leaves and stems of *O. sanctum* and *O. basilicum.*

Vehicle and preparation of doses: Tween 80 (2.5 %) in distilled water was used as control (vehicle). The extractives obtained were suspended in distilled water using suspending agent, i.e, Tween 80. The doses were so adjusted as to administer 0.25 ml in each mouse; chloroquine diphosphate in vehicle was used as standard.

Antimalarial activity

4 days suppressive test model

The 4-day test developed by Peter's was used to determine *in vivo* antimalarial activity (Peters, 1975). The mice were randomly divided into three different [control (1), standard (2) and test (3)] groups of 5 animals each. On day 0 the test animals in all the groups were inoculated with $1x10^7$ *Plasmodium berghei* infected RBC's. The animal in-group 3 was treated with the test substance on all the four days, while animals of group 1 and 2 received the vehicle and chloroquine diphosphate, respectively, at the same time on the similar days. Blood smears from all the animals were prepared on day-4 and percentage parasitaemia was recorded and compared with that of control animals. On day 4 thin blood smears were prepared from the tail vein of all the animals and stained with Giemsa's solution to monitor the parasitaemia and the reduction of parasitaemia was calculated. Any mortality within 24h of drug administration was considered as toxicity of the drug. The percent parasitaemia was calculated using the following expression:

$$\% \text{ Parasitaemia} = \frac{\text{Number of parasitized cells}}{\text{Total number of cells}} \text{ x } 100$$

Statistical analysis

Results were expressed as mean±S.E.M. and all the extractives were compared with chloroquine diphosphate (standard) and control separately using one way analysis of variance (ANOVA) followed by Dunnett's test. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

After removal of solvents from various extracts in vacuo, the percentage of various extractives obtained was obtained (Table 1). Phytochemical screening gave positive tests for saponins, sterols, triterpenoids, carbohydrates, tannins, proteins and flavonoids.

Table 1. Yield of various extracts.

Extract	Yield (% w/w)			
	Ocimum sanctum		*Ocimum basilicum*	
	Leaf	Root	Leaf	Root
Petroleum ether	6.34	0.97	5.88	1.12
Chloroform	5.42	2.18	6.15	1.98
Ethanol	6.61	2.65	6.52	2.16
Water	7.18	3.97	6.85	3.10

Antiplasmodial activity was observed for the leaves and roots extracts of *O. sanctum* and *O. basilicum* (Table 2 and 3). All the extracts i.e., PE, CE, EE and WE were given to the infected mice at doses of 100, 200, 400 and 800mg/kg using chloroquine diphosphate as positive control. Ethanolic extract of leaves and roots showed maximum antiplasmodial activity on the 4^{th} and 7^{th} day at two dose levels of 400 and 800 mg/kg. Ethanolic extract of *O. sanctum* leaves exhibited maximum antiplasmodial activity of 7.8 ± 1.54 (400 mg/kg) on 4^{th} day and 25 ± 3.97 (400 mg/kg) on 7^{th} day (Table 2). However, maximum activity of 6.2 ± 1.32 on 4^{th} day and 20.2 ± 3.24 (800 mg/kg) on 7^{th} day was observed at a dose of 800mg/kg. Ethanolic extract of *O. sanctum* roots exhibited maximum antiplasmodial activity of 5.8 ± .87 (400 mg/kg) and 19.0 ± 3.39 (400 mg/kg) on 4^{th} day and 7^{th} day, respectively. At a dose of 800mg/kg maximum antiplasmodial activity of 3.2 ± 0.74 on 4^{th} day followed by 15.7 ± 5.00 (800 mg/kg) on day 7^{th}. All other extracts were found to be inactive.

In case, of plant *O. basilicum* (Table 3) maximum activity was observed for ethanolic extract of leaves and roots at dose of 400 mg/kg and 800 mg/kg. Leaves extract exhibit 7.8 ± 1.21 followed by 6.6 ± 0.91 on day 4^{th} at a dose of 400 and 800 mg/kg, respectively. On day 7^{th} maximum activity of 27.6 ± 4.39 (400 mg/kg) followed by 22.0 ± 6.14 (800 mg/kg). Ethanolic extracts of roots exhibited maximum growth inhibition of 5.9 ± 0.85 and 4.9 ± 0.96 at dose 400mg/kg and 800 mg/kg, respectively on day 4^{th}. While on day 7^{th} maximum antiplasmodial activity of 20 ± 3.90 (400 mg/kg) and 17.8 ± 2.95 (800mg/kg) was observed.

CONCLUSIONS

All the extracts were prepared and there *in vivo* antimalarial activity was evaluated using Swiss mice BALB /c strain. The results were observed on the 4th and 7th day. Ethanolic extract of *O. sanctum* roots showed maximum antiplasmodial activity of 3.2 ± 0.74 at dose of 800mg/kg on day 4th. However, *O. basilicum* root extract showed maximum activity of 4.9 ± 0.96 at dose 800 mg/kg on day 4th. Maximum activity of ethanoic extract was observed on day 4th.

Table 2. *In vivo* anti-malarial effect of various extracts of *Ocimum sanctum* Linn. Leaves and roots.

Treatment	Dose (mg/kg)	Mean percent parasitaemia ± S.D.			
		O. sanctum leaves		*O. sanctum* roots	
		Day 4	Day 7	Day 4	Day 7
Control	-	11.2 ± 1.90	41.4 ± 5.72	11.2 ± 1.90	41.4 ± 5.72
Standard	5	1.9 ± 0.71	5.3 ± 1.40	1.9 ± 0.71	5.3 ± 1.40
Pet. Ether Extract	100	11.4 ± 1.26	41.9 ± 2.69	11.4 ± 1.26	41.2 ± 4.58
	200	11.2 ± 2.45	39.6 ± 4.18	10.9 ± 1.68	40.8 ± 4.16
	400	10.7 ± 1.31	36.8 ± 3.90	10.5 ± 1.06	39.4 ± 2.85
	800	9.8 ± 1.25	35.9 ± 4.80	9.6 ± 1.60	38.9 ± 5.50
Chloroform Extract	100	11.6 ± 1.86	42.9 ± 5.44	11.0 ± 2.70	42.0 ± 3.43
	200	11.3 ± 1.93	41.0 ± 6.38	10.7 ± 1.82	41.6 ± 5.95
	400	10.6 ± 2.42	40.5 ± 6.89	10.0 ± 2.06	39.8 ± 2.93
	800	9.8 ± 1.96	39.1 ± 3.69	9.8 ± 1.53	37.4 ± 3.14
Ethanol Extract	100	9.7 ± 1.28	34.0 ± 2.53	9.6 ± 1.46	33.8 ± 4.70
	200	8.1 ± 1.11	30.5 ± 5.01	8.0 ± 1.30	28.9 ± 4.11*
	400	7.8 ± 1.54*	25.0 ± 3.97 *	5.8 ± 0.87*	19.0 ± 3.39*
	800	6.2 ± 1.32*	20.2 ± 3.24*	3.2 ± 0.74*	15.7 ± 5.00*
Water Extract	100	10.7 ± 1.26	38.5 ± 3.43	10.0 ± 1.05	35.9 ± 5.05
	200	9.5 ± 0.83	35.8 ± 3.59	9.3 ± 2.09	31.6 ± 3.68
	400	8.7 ± 1.39	32.5 ± 4.03	8.6 ± 1.30	31.4 ± 3.16
	800	8.3 ± 1.00	32.1 ± 3.90	8.4 ± 1.50	30.6 ± 3.35*

Table 3. *In vivo* anti-malarial effect of various extracts of *Ocimum basilicum* Linn. Leaves and roots.

Treatment	Dose (mg/kg)	Mean percent parasitaemia ± S.D.			
		O. basilicum leaves		*O. basilicum* roots	
		Day 4	Day 7	Day 4	Day 7
Control	-	11.2 ± 1.90	41.4 ± 5.72	11.2 ± 1.90	41.4 ± 5.72
Standard	5	1.9 ± 0.71	5.3 ± 1.40	1.9 ± 0.71	5.3 ± 1.40
Pet. Ether Extract	100	11.2 ± 1.19	42.4 ± 8.7	11.3 ± 1.42	41.7 ± 5.48
	200	10.9 ± 1.51	40.6 ± 8.1	10.2 ± 1.47	39.6 ± 6.26
	400	10.7 ± 1.30	39.1 ± 5.01	10.1 ± 1.00	37.5 ± 5.17
	800	9.9 ± 1.70	38.1 ± 6.06	9.3 ± 1.30	36.9 ± 6.19
Chloroform Extract	100	11.5 ± 1.34	39.3 ± 5.68	11.4 ± 1.48	40.4 ± 1.85
	200	11.1 ± 1.76	38.2 ± 4.83	9.7 ± 1.46	39.1± 2.08
	400	10.4 ± 1.55	38.1 ± 4.23	9.4 ± 1.46	39.8 ± 1.36
	800	10.1 ± 1.95	36.7 ± 5.11	8.6 ± 1.27	38.1 ± 2.60
Ethanol Extract	100	9.4 ± 1.55	33.5 ± 5.52	8.7 ± 1.65	34.2 ± 4.32
	200	8.4 ± 1.17	32.8 ± 4.77	8.3 ± 1.00	29.3 ± 4.22*
	400	7.8 ± 1.21*	27.6 ± 4.39 *	5.9 ± 0.85*	20.9 ± 3.90*
	800	6.6 ± 0.91*	22.0 ± 6.14*	4.9 ± 0.96*	17.8 ± 2.95*
Water Extract	100	11.0 ± 1.10	37.6 ± 4.53	10.9 ± 1.16	37.9 ± 4.42
	200	10.6 ± 1.07	34.1 ± 4.45	10.1 ± 1.20	32.8 ± 7.23
	400	9.3 ± 1.35	33.5 ± 2.83	9.3 ± 1.00	30.6 ± 3.54
	800	8.9 ± 1.24	31.0 ± 3.08	8.2 ± 0.87*	28.8 ± 2.66*

Since the different extractives showed reduction of parasitaemia it suggested that leaf and roots of *O. sanctum* and *O. basilicum* contain the active compounds which inhibited *P. berghei*. Phytochemical investigations demonstrated the presence of saponins, sterols, triterpenoids, carbohydrates, tannins, proteins and flavonoids.

Some of the plants such as *Brunsvigia littoralis* [Campbell *et al*., 1998), *B.* radulosa (Likhitwitayawuid *et al*., 1993), *Alstonia macrophylla* (Keawpradub *et al*., 1999) and *Peschiera fuchsiaefolia* (Federici *et al*., 2000) have exhibited antimalarial activity due to the presence of alkaloidal compounds. On the other hand *Rhus retinorrhoea* (Ahmed *et al.*, 2001) possesed antimalarial activity due to the presence of flavonoids. Therefore, the significant antimalarial activity of *O. sanctum* and *O. basilicum* extracts may be attributed to the presence of the alkaloidal and/or flavonoidal constituents. Thus, ethanolic extracts of roots and leaves of *O. sanctum* and *O. basilicum* may be used for further development.

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