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EVALUATION OF ANTIMALARIAL ACTIVITY OF *PLUMERIA ACUTIFOLIA* BARK

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ABSTRACT

In vitro antimalarial activityof plant *Plumeria acutifolia* bark *has been evaluated by performed serial dilution methods.* The different extracts *viz.* Pet ether, chloroform, methanol and water extract were used. They were tested by using *Plasmodium falciparum parasite. Out of these, the chloroform extract of* IC_{50} *of* 27.3 ± 1.453µg/ml to 100 µg/ml *showed significant results against the parasite in vitro study.* The above used extracts were tested against *Plasmodium berghei* parasite was used to evaluate antimalarial activity using *in vivo methods. In this experiment found that* chlorofrom extract of *Plumeria acutifolia bark* was active against used parasite as in the chloroform extract used in in vitro study. The chloroform extract of the dose 400 mg/kg showed significant results against the *Plasmodium berghei*. Hence this studies authenticates traditional uses of the plant using both methods.

KEYWORDS: *Plumeria acutifolia; Plasmodium bergheii;* Antimalarial activity; Apocynaceae.

1. INTRODUCTION

Malaria is an infective insect borne disease, widespread all over the world. The World Health Organisation (WHO) estimates that a large quantity of people (between 300 to 500 million) are tainted with malaria and about two million people die annually.[1] The re-emerging of malaria in several parts of the World is due to rapid enlarging in thrash about to available antimalarial drugs and resistance of the vector to insecticide.[2] The *Plasmodium* strains are also found resistant to many conventional antimalarial drugs and quite difficult to treat. Currently a lot of drugs have been seen to possess side effects which are used for prophylaxis and treatment of malaria and have been seen to possess side effects. *Plasmodium falciparum* and *P vivax* are the predominant species worldwide with an estimated incidence of 207 million and 8·5 million cases respectively in 2016.[1] The ancient history has revealed that a plant have been considered as source of medicine. In malaria-endemic countries, the several plants are used as traditional medicine for the treatment of malaria. The antimalarial potential of compounds that are derived from plants is demonstrated. The great majority of *P. falciparum* malaria occurs in sub-Saharan Africa (approximately 190 million cases) where transmission remains intense in many locations, although there is considerable variation in incidence within and between countries and the plant products used to treat disease.[2] The compound Quinine is obtained from *Cinchona* species and artemisinin from *Artemisia annua*.[3,4] The literature survey has revealed that amongst many unexplored plants in the world. The present studies have been undertaken to evaluate inhibitory effect of various extracts of unexplored plant for antimalarial activity by using *in vitro and In vivo* methods.

2. MATERIAL AND METHODS

2.1. Plant Material

The bark of *Plumeria* acutifolia were collected from campus of university in June 2010 and authenticated by Dr H.B. Singh, Head Raw Material Herbarium & Museum, Ref. NISCAIR/RHMD/Consult-2010-11/11/1413/11. A voucher specimen has been retained in Department of Pharmaceutical Science, Guru Jambheshwar University of Science & Technology, Hisar. The plant material was air-dried at room temperature and then powdered. All other chemicals used were of analytical reagent grade.

2.2. Preparation of P. acutifolia bark extract

The dried powder (3kg) of *Plumeria acutifolia* bark was exhausted successively by petroleum ether (60-80°C), chloroform and methanol by hot extraction process and then aqueous extract was prepared by maceration in distilled water for 18 hrs. The liquid extracts so obtained were concentrated in vacuum at 40°C. The extracts were stored in refrigerator at 4°C until used for experiment reported in this study.

2.3 Cultivation of malaria parasite

The malaria parasite of *Plasmodium falcipuram* was cultivated by using one pocket media of RPMI-1640

[Sigma] which contains 25mM of N-2-hydroxyethylpiperazine-N-2-ethane-sulfonic acid (HEPES) buffer and is dissolved in 960 ml of triple distilled water followed by addition of 2 g of glucose and 40 µg/ml of gentamycin sulphate to avoid contamination. The solution was sterilized by passing through a Millipore filter of 0.22 microgram porosity and stored at 40°C as 960 ml aliquots in glass media bottle. The parasites were maintained in continuous culture on human erythrocytes in 1640 medium supplemented with 10% human Ab^+ serum, 25 mM HEPES, 25 mM sodium bicarbonate and 40 µg/ml gentamycin sulphate. The culture was incubated at 37°C in an atmosphere of 91% N_2, 6% CO_2, and 3% O_2.[5,6]

2.4 In vitro anti-plasmodial (Schizont Maturation Inhibition) assay

The plant extracts were assessed by using modified parasite lactate dehyrogenase method. In this, parasite culture was synchronized to the ring stage by treatment with 5% D-Sorbital. The dried extracts were dissolved in DMSO and a stock solution of 1mg/ml was prepared. These extracts were first dissolved in 0.1 ml of DMSO and then diluted with incomplete medium (without serum) to achieve required concentration. Then stock solution was diluted in media RPMI-1640 (Sigma) to obtain different concentrations. The serial dilutions of the extracts were dispensed in triplicates in 96-well flat bottomed micro-plates. The negative control wells contained culture medium only. The synchronized cultures with parasitemia of 1% and 5% final haematocrit were aliquotted into the plates and incubated at 37°C. The growth was monitored after 24-30 hrs of culture, after confirmation of schizont maturation and then blood smears were prepared from all wells. The slides were fixed in methanol and stained with Giesma stain. The number of schizonts was counted per 200 asexual stage parasites. The values were compared with test well and control wells. The inhibition was calculated as:

Inhibition = 100 – A,

A	No. of schizonts in test well	X
	No. of schizonts in control well	100

Where 100 No. of schizonts in control well, A is No. of schizonts in test well.

Finally the *In vitro* antimalarial study of the samples was expressed as the IC_{50}.[7] It was calculated from the graph of the probit of chloroquin activity and Icg of drug concentration by linear regression analysis.

3 In Vivo study

3.1 Animals

Swiss albino mice (25-30 g) of either sex were used for the study. They were fed with standard diet and *water ad libitum*. The animals were housed in standard cages and acclimatized for a period of 10 days. The approval for the study was obtained from the Animal Ethics Committee, Guru Jambheshwar University of Science & Technology Hisar [Endst. No. 69-77 dated 04-01-2011, 20th Meeting held on 15th Nov. and 17th Dec 2010.]

3.2 Parasite inoculation

The chloroquine-sensitive *Plasmodium berghei* strain was obtained from the National Institute of Malaria Research (NIMR), Dwarka, New Delhi and was maintained in mice. The inoculums consisted of 5 x 10^7 *P. berghei* parasitized red blood cells per millilitres and diluting the blood with isotonic saline. Each mouse was inoculated intraperitoneally on day 0 with 0.2 ml of infected blood containing 1 x 10^7 *P. berghei* parasitized red cells per millilitres. This was done by determining both the percentage of parasitaemia and red blood cell count of the donor mice using a hemocytometer and stained with Giemsa.

3.3 Drug administration

The drug and extracts used in this study were orally administered with the aid of a feeding cannula to mice.

3.4 *Evaluation of suppressive activity on early infection (4-day test)*

The suppressive activity of the *P. acutifolia bark* extract was evaluated, using the method.[8] In the classical 4-day suppressive test, each animal (*Swino albino* mice) was inoculated on the first days (day 0), intraperitoneally with 0.2 ml of infected blood containing about 1x10^7 *Plasmodium berghei* parasitized erythrocytes. Different dilutions of test compounds were prepared with suspending the compound in saline containing 0.5% tween80. After inoculation, the animals were divided into five groups of six mice each and orally administrated extracts *viz.* chloroform, methanol and water (100, 200 and 400mg/kg/day), chloroquine (10 mg/kg) and 0.2 ml normal saline (plus 0.5% tween-80) to one negative control group, for 4 consecutive days (day 0 to day 3), respectively. On the fifth day (4 day), thin films were prepared from the tail blood of each mouse and the parasitemiamia level was determined by counting the number of parasitized erythrocytes out of 200 erythrocytes in random fields of the microscope. Average percentage suppression was calculated as 100 (A – B/A), where A is the average percentage parasitemia in negative control group and B, average percentage parasitemia in the test group.

3.5 *Evaluation of schizontocidal activity on established infection (Curative or Rane test)*

Evaluation of curative potential of *P. acutifolia bark* extracts was done using a similar method.[9] The mice were infected intraperitoneally with standard inoculums of 1x10^7 *Plasmodium berghei* infected erythrocytes on the first day (day 0). Seventy two hours later, the mice were divided into five groups of six animals each. The groups were orally administrated with bark extracts *viz.* Pet ether, chloroform, methanol and water (100, 200 and 400 mg/kg/day), chloroquine (10mg/kg) and 0.2 ml normal saline (plus 0.5% tween80) a negative control.

The drug/extract were given once daily for 5 days. Thin films stained were prepared from the tail blood of each mouse daily for 5 days and stained with Giemsa stain to monitor the parasitemia level. The mean survival time for each group was determined arithmetically by the average survival time (day) of the mice (post-inoculation) in each group over a period of 30 days (day 0 to day 29).

Statistical analysis

The results are expressed as Mean ± SEM and ANOVA was used to assess statistical significance.

4. RESULTS

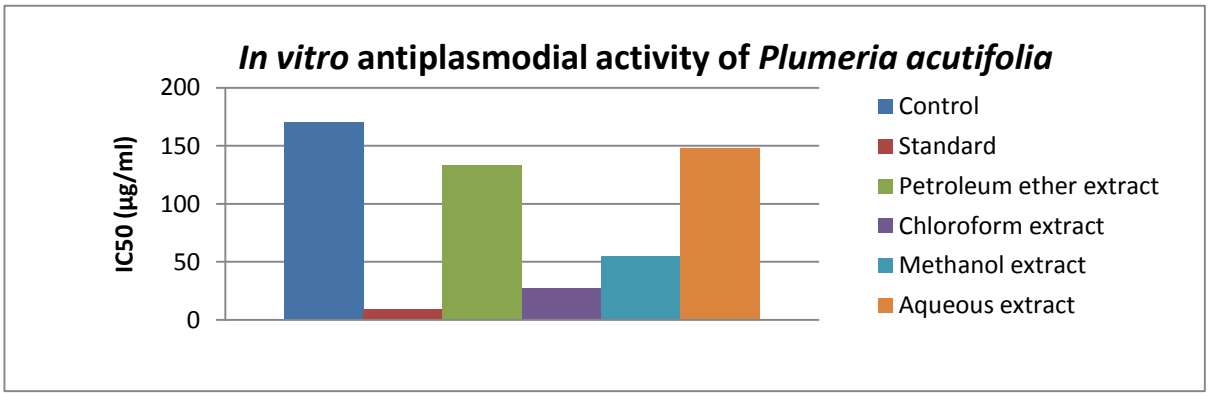
4.1 In vitro Study

In this, *in vitro* investigation of *Plumeria acutifolia* Poir extracts revealed prominent results against the parasite *Plasmodium falciparum* used as shown in Table 1. The chloroform extract showed good response against the parasite at the concentration of 27.3 ± 1.453 µg/ml to 100µg/ml. The extracts of pet ether, methanol and aqueous were either less effective or not effective against the parasite used and only chloroform extract showed a significant response against *Plasmodium falciparum*.

Table 1: *In vitro* antiplasmodial activity of *Plumeria acutifolia* bark by modified parasite lactate dehydrogenase method.

Sr. No.	Extracts	IC_{50} (µg/ml)
1	Pet ether	133.6 ± 1.856
2	Chloroform	**27.3 ± 1.453**
3	Methanol	54.6 ± 1.667
4	Aqueous	148.33 ± 4.256
5	Control	170.0 ±1.155
6	Standard	9.0 ± 0.5744

IC_{50} values are mean ± Standard deviation from three independent experiments.



4.2 In vivo study

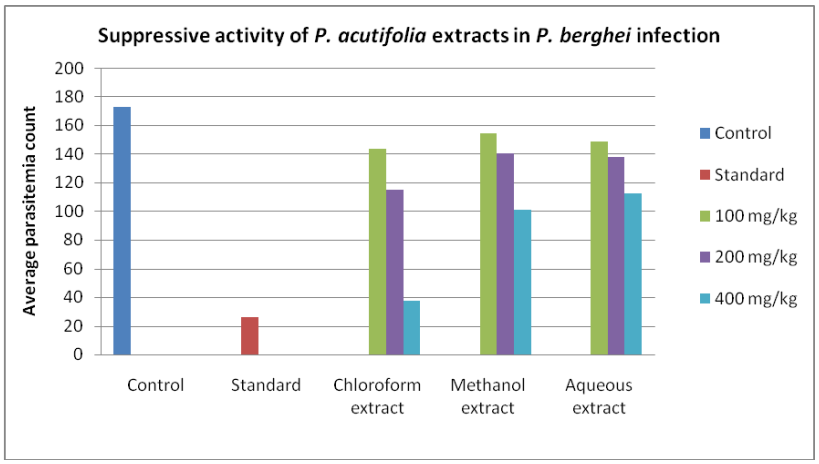
In vivo study, the chloroform extract of *P. acutifolia* bark showed activity at the dose of 400 mg/kg and significantly decreased *Plasmodium* count in mice, when compared with normal control mice. The results of the studies are presented in Table 2 and Table 3. The chloroform extract possesses schizontocidal activity in early and established infection of *Plasmodium berghei*. The average parasitemia count and chemosuppression observed in methanol, chloroform and aqueous extract were found to be 101.14 ± 1.565* with 41.08%, **37.33 ± 1.145** with 57.28%** and 112.5 ± 2.202* with 20.75% at the dose level of 400 mg/kg/day. The parasitemia count of methanol, chloroform and aqueous extract treated group at the dose of 200 mg/kg was found to be 140.33 ± 4.863* with 21.38%, 114.66 ± 2.472* with 33.26% and 138.0 ± 2.436* with 18.55% suppression. When parasitemia count with chemosuppression was observed at the dose of 100 mg/kg, it was found to be 154.33 ± 2.261 with 10.56%, 143.33 ± 2.777* with 16.68% and 148.66 ± 3.192 with 12.60% suppression was methanol, chloroform and aquous extracts. When compared to the standard drug chloroquine (10 mg/kg/day) which exhibited parasitemia count of 25.83 ± 1.302 with 84.76% suppression. Hence the present study showed that chloroform extract gave highest curative activity followed by methanol extract and aqueous extract which indicate that it holds great promise for the control of malaria as compared to control groups and standard group with use of mean survival time.

Table 2: Suppressive activities of various extracts of *P. acutifolia* bark during early *P. berghei* infection in mice.

Extract	Dose (mg/kg)	Average Parasitemia count	% Average Suppression
Methanol extract	400	101.14 ±1.565*	41.08
	200	140.33 ±4.863*	21.38
	100	154.33 ±2.261	10.56
Chloroform extract	**400**	**37.33 ±1.145****	**57.28**
	200	114.66 ±2.472*	33.26
	100	143.33±2.777*	16.68
Aqueous extract	400	112.5 ±2.202*	20.75

	200	138.0 ±2.436*	18.55
	100	148.66 ±3.192	12.60
Control (Normal saline)	0.2 ml	172.83 ±2.725	_
Std (Chloroquine)	10	25.83 ±1.302**	84.76

*P<0.05, **P<0.001 when compared with control (Dunnett's t-test after analysis of variance). Results are mean±SEM (n=6).

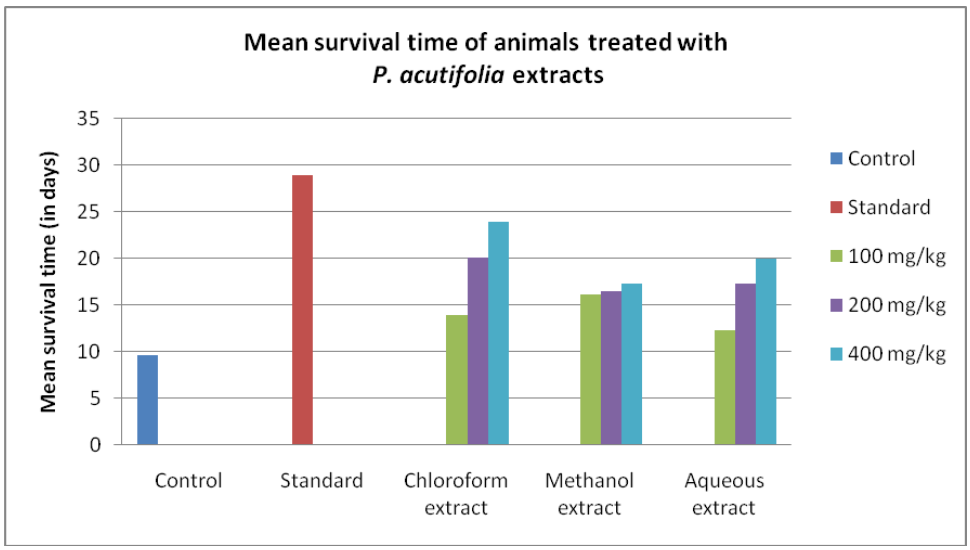


Graph 2: Suppressive activity of *P. acutifolia* extracts during early *P. berghei* infection in mice.

Table 3: Mean survival time of mice receiving dose of *P. acutifolia* bark extract during an established *P. berghei* infection in mice.

Extract	Dose (mg/kg)	Parasitemia count	Mean survival time
Methanol extract	400	120.5 ±2.030*	17.34 ±6.502
	200	128.66 ±2.944*	16.5 ±4.728
	100	119.66 ±4.412*	16.16±1.752
Chloroform extract	**400**	**45.66 ±3.204****	**24±5.381**
	200	103.0 ±2.8328*	20.16 ±2.815
	100	134.28 ±2.168	14.0 ±3.824
Aqueous extract	400	113.16 ±2.042*	20 ±8.198
	200	134.30 ±1.636*	17.34 ±7.586
	100	143.66 ±2.503	12.36 ±8.152
Control (Normal saline)	0.2 ml	174.20 ±3.342	9.68 ±4.562
Std (Chloroquine)	10	22.40 ±3.244**	29.0 ±5.476

*P<0.05, **P<0.001 when compared with control (Dunnett's t-test after analysis of variance). Results are mean±SEM (n=6).



Graph 3: Mean survival time of mice receiving *P. acutifolia* extracts during an established *P. berghei* infection.

5. DISCUSSION

In this study, *In vitro and In vivo* malaria models were used. The model used for detecting the antimalarial effects of plant extracts. The various extracts were screened for malarial activity against erythrocytic asexual stages and sexual stage. Our results reveal that chloroform extract of *P. acutifolia* possesses potential antimalarial activity which is used against *P. falcifarum in vitro* methods. These extract is further used against *P. berghei* and showed most evident results as a chemosuppression produced during the 4 day suppressive test. The method of 4 day suppressive effects were used for testing antiplasmodial activity has become most popular and extract of *P. acutifolia* was also exerted a significant curative effect in the established infection and these properties is comparable to that of the standard drug (chloroquine) as shown by the mean survival time of the extract treated group and chloroquine treated group.[10,11] Our investigation is based on folkloric use of *P. acutifolia.* Although this result is additive to suppressive activity, it is desirable that the antimalarial activities be present in such potential extract of *P. acutifolia* bark. Hence the present study has shown the efficacy of *P. acutifolia* which is traditionally used in management of malaria, by using an animal model.

6. CONCLUSION

The researcher from past few decades are keen and sincere to evaluate many ethnomedicinally used plants, due to their specific healing properties, desirable action, easy availability and less toxicity. The bark of *Plumeria acutifolia* are still used in treatment of various disorders out of which malaria is one of these, used by many populations. Hence this study clearly indicates that chloroform extract of this plant is useful for malaria control and in malaria treatment.

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CONFLICTS OF INTEREST

There is no conflicts of interest of each author.

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