

Antimalarial Potential of Plants Used as Chewing Sticks for Oral Hygiene in Rural Areas of Rajasthan, India

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ABSTRACT

The present study was planned to evaluate antiplasmodial activity of two plants *Azadirachta indica* and *Acacia nilotica* regularly used in primary health care and oral hygiene in rural areas of Rajasthan, India. The aim was to corroborate how traditional practices using chewing sticks (Fresh twigs of medicinal plants in place of tooth brush) in relation to control of malaria. The *In vitro* antimalarial activity of fresh twigs of *Azadirachta Indica* (neem) and *Acacia nilotica* (Babul) were evaluated against chloroquine sensitive *Plasmodium falciparum* 3D7 strain by light microscopy using Giemsa-stained smears. *Azadirachta indica* most commonly used in the area showed promising antiplasmodial activity with IC_{50} of 39.86 $\mu\text{g/mL}$ as compared to *Acacia nilotica* the IC_{50} value ranged beyond 50 $\mu\text{g/mL}$ (IC_{50} 77.78 $\mu\text{g/mL}$). Both the plants studied under present investigation are known for their medicinal properties, regular use may provide a solution for better management of the disease in malaria risk areas.

Keywords- *Azadirachta Indica*, *Acacia nilotica*, *Plasmodium falciparum*, Antimalarial, Medicinal plants.

INTRODUCTION

Malaria is one of the most severe public health problems worldwide, though it is entirely preventable and treatable mosquito borne illness. According to the World Health Organisation's World Malaria report 2013 and Global Malaria Action plan, 3.4 billion people (half of the world's population) live in areas of malaria transmission in 106 countries and caused an estimated 207 million clinical episodes, and 627,000 deaths¹. Traditional medicine

occupies a central place among rural communities of developing countries for the provision of health care in the absence of an efficient public health care system. According to World Health Organization's report, over 80% of the world population relies on traditional medicine for their primary health care². For many traditional herbal medicines may be the only source of treatment available³.

Rajasthan is well known for its biodiversity richness of diverse cultural mosaic. The state is located in the North

western part of India. Geographically, it lies between 23°3' to 30°12' longitudes and 69°30' to 78°17' latitudes. Southern part of Rajasthan is the tribal belt comprising Banswara, Chittorgarh, Dungarpur, Udaipur and Rajsamand districts. Therefore, an attempt has been made during 2013-2014 to collect information about plants used by tribes and local people (aboriginal) in their traditional health care system for Rajsamand district. The plants growing around them form an integral part of their culture. The local communities use their indigenous experiences and knowledge in categorizing various medicinal plants when dealing with different ailments. The region was frequently visited with regards to collection of medicinal plants.

The cultural system tends to favor practices which minimize the risk of diseases and maximize health care⁴. Traditional use of plants to improve dental health and promote oral hygiene had been known since antiquity. Some of the selected plants used for such practices viz; *Acacia catuchu*, *jatropha curcus*, *Thevetia peruvina*, *Nerium indicum*, *Acacia nilotica* and *Azadirachta indica*. The subjects reported a decrease in malaria attacks using *Azadirachta indica* in various forms, though the diagnosis of malaria was not clinically confirmed. In the present investigation two plants *Azadirachta indica* and *Acacia nilotica* were selected for antiplasmodial activity against *Plasmodium falciparum* 3D7 strain.

Azadirachta indica (Meliaceae) is well known in India as one of the most versatile medicinal plants having a wide spectrum of biological activity. Every part of the tree has been used as traditional medicine for house hold remedy against various human ailments and still regarded as a village dispensary in India^{5,6}. Biological and pharmacological activities that attributed to different parts of *A. Indica* includes antiplasmodial, anti-trypanosomal,

anti-oxidant, anti-cancerous, anti-bacterial, antiviral, larvicidal and fungicidal activities⁷. The neem leaf extract reported for both schizonticidal and gametocidal activities against *Plasmodium falciparum*⁸.

Another plant selected for antiplasmodial activity was *Acacia nilotica* (Family: Fabaceae and sub-family: Mimosoideae), a spiny tree native to the Indian subcontinent and most parts of Africa, tender twigs are used as tooth brush⁹. The plant is widely used in folk medicine to treat a variety of diseases with potential antioxidant activity. The gum, bark, leaves and fruits of this tree were used medicinally for cold, bronchitis, pneumonia, diarrhoea, hemorrhage, antibacterial and antiplasmodial activity¹⁰. *Acacia nilotica* had also been proved as an effective medicine in the treatment of malaria, sore throat (aerial parts) and tooth ache bark^{11, 12}. Besides young stem and twigs of the neem and Babul the leaves of Papaya (*Carica papaya*), Tulsi (*Ocimum sanctum*), mint (*Menthe spicata*), curry leaves (*Murraya koenigii*) and leaf of mango (*Mangifera indica*) are chewed and kept in mouth for sometime as mouth freshener with remarkable medicinal properties¹³. In view of this, the present study was planned to evaluate the antimalarial activity and to correlate how traditional practices aids important inputs in control and better management of the disease.

MATERIALS AND METHODS

Plant material

Plants were collected from district Rajsamand located between latitudes 24°46' to 26°01' N and Longitudes 73°28' to 74°18' N of the state Rajasthan, India. A field survey was carried out randomly during the period January 2013- June 2014. Fresh twigs of *Azadirachta indica* and *Acacia nilotica* were collected between April-May, 2013, The plants were authenticated by Botanist

Dr. Pratibha Cheturvedi, Haffkine Institute and Voucher specimen were deposited for future reference.

Preparation of extracts

The fresh twigs of both the plants were washed twice with distilled water, crushed and extracted with water using standard extraction procedures¹⁴. Solvents were removed under reduced pressure by using a rotary vacuum evaporator. The percent yield of the extract was determined and the crude extracts were stored at 4° C prior to antimalarial assay. Preliminary phytochemical screening was done using standard procedures¹⁵.

Parasite cultivation

The *Plasmodium falciparum* 3D7 strain was procured from Indian Institute of Technology (IIT), Mumbai, India, was maintained in continuous culture by the modified method of Jensen and Trager¹⁶ (1980) in O+ human red blood cells at a 5% hematocrite in RPMI 1640 medium, supplemented with L-glutamine (4.2mM), HEPES (25 mM), NaHCO₃ (25 mM) hypoxanthine (6.8 M), 0.5% AlbumaxII (Invitrogen) and 50µg/ml Gentamicin. Cultures were incubated at 37°C in an atmosphere of 5% CO₂, 91% N₂, and 3% O₂. Chloroquine was used as positive control.

In vitro antiplasmodial assay

The *In vitro* activity was evaluated against *Plasmodium falciparum* by means of the Mark III test, as developed by the WHO¹⁷. Briefly, the parasite cultures, prior to experimentation, was synchronized to the ring stage of treatment with 5% D-sorbitol¹⁸. Each extract was tested in duplicate in two independent experiments. Extracts were filter sterilized and different concentrations (150, 100, 50, 25, 12.5, 6.25 and 3.125 µg/mL) were incorporated in 96 well tissue culture plate with 1-2% parasitemia and 2% hematocrite. The plates were incubated at

37° C in CO₂ incubator and inhibition of parasite was determined microscopically after 48 h by using Giemsa-stained smears¹⁹. The control parasite culture freed from extracts was referred to as 100% growth. The IC₅₀ values, the extract concentration required to inhibit parasite growth by 50% was determined for each extract-parasite combination.

RESULTS

The present study investigated with fresh aqueous extracts derived from the twigs of *A. indica* and *A. nilotica* against chloroquine sensitive *Plasmodium falciparum* 3D7 strain by *In vitro* method. The results are summarized in table 1. The basic measurement of antimalarial activity used in this study was the reduction in the number of parasitized erythrocytes in extract treated test cultures as compared to control without drug at 48 h incubation period. The parasitemia decreased with increasing concentration of the extract, reflecting an inhibitory activity of parasite replications. Figure 1 (slides a-f) represents drug induced inhibition of parasitaemia. Figure 1-a represent negative control (without drug) showing very high parasitaemia as compared to the positive control (chloroquine- 3.0 µg/mL) showing almost nil parasitaemia (figure 1-b). Figure 1-c and 1-d represents activity of the highest (100 µg/mL) and lowest concentration (3.125 µg/mL) of *A. indica* extracts. The results clearly demonstrated promising inhibitory activity of parasite replication in higher concentration as compared to lowest concentration. Figure 1-e and 1-f represents the results of *A. nilotica* extract showing inhibition of parasitaemia in both the concentrations, but significantly low as compared to *Azadirachta indica*. From the literature antimalarial activity of the extracts was defined according to the IC₅₀ (Extract concentration that gives 50% inhibition of

parasitemia) values obtained. An extract showing an IC_{50} value ≤ 50 $\mu\text{g/mL}$ was classified as active. Extracts having activity beyond this range were considered inactive²⁰. Based on this classification *A. indica* exhibited promising antimalarial activity with IC_{50} of 39.86 $\mu\text{g/mL}$ as compared to *Acacia nilotica* the IC_{50} value ranged beyond 50 $\mu\text{g/mL}$ (IC_{50} 77.78 $\mu\text{g/mL}$).

The preliminary phytochemical study reveals that the tested medicinal plants contain a variety of phytochemical constituents (terpenoids, alkaloids, saponins and glycosides). Each of these phytochemicals is known for various protective and therapeutic effects²¹.

DISCUSSION

Traditional medicines occupy a central place among rural communities of Rajasthan. People residing in the tribal belt of Rajasthan rely significantly on local plant resources for their primary health care. A large number of plants and plant parts are used for dental and oral hygiene, according to individual preference and their contained medicinal property. Neem and Babul tree are largely populous in India and commonly grow along the roadside and around houses every few meters distance in this region. Bhasin²² carried out a detailed survey of oral health behavior among the Bhils of Rajasthan (the third largest tribal group of India) and also reported various traditional methods and herbal remedies used by them for dental care and oral diseases. More than any other Indian herb neem proved useful in helping the body resist diseases and restore the proper balance to the body's system²³. The tribe and local people had a strong perception that the use of medicinal plants for dental and everyday health care keeps the body healthy and protect them from malaria fever and other common illnesses. This prompted us to test the antiplasmodial

activity of twigs (chewing sticks) of two plants *Azadirachta indica* and *Acacia nilotica*, scientifically by *In vitro* method.

Both these plants are well known for their medicinal properties with a complex chemical makeup²⁴. Regular use of these plants for a long time facilitates absorption of a part of active compounds internally. Though, the concentration of active compounds may not be enough to directly inhibit the parasites unless some other mechanisms are involved. These may include immune memory acquisition, inhibiting or inducing enzymatic processes affecting drug metabolism²⁵. Sometimes the medicinal plants may act as antipyretics or may enhance the immune system, rather than having direct anti-parasitic activity²⁶ and thus, protecting from the severity of the disease and enhance quick recovery.

Another reason that reduces chances to get malaria may be because daily use of chewing sticks to clean the teeth with great efficiency may alter the chemical profile of the breath and that in turn reduce attraction of mosquito towards such people. Previous research reported that unique combination of chemicals that make up an individual's breath may play a role in repelling or attracting mosquito²⁷. Gametocytes in the peripheral blood attracts mosquitoes for their own survival and continuation of the lifecycle²⁸. The Neem plant had been reported for its excellent gametocidal activity⁷. That may prevent mosquitoes to focus their bites on such people and thereby preventing re-infection of mosquitoes and man²⁹.

Regular use of medicinal plants boosts the immunity. A person with strong intrinsic immunity may get infected with malaria, but recovered quickly and such repeated infection may develop naturally acquired immunity against malaria³⁰. The dual action of neem on the parasite and stimulation of the host immune system provides a major factor in the effectiveness

of neem against malaria³¹. The promising antiplasmodial activity of *Acacia nilotica* was reported by Tahir³² and higher analgesic activity further signifies its suitability as antimalarial agents.

The *in-vitro* results demonstrated antimalarial activity by direct action on the parasites. On the other hand using these plants for oral hygiene and primary health care, the mechanism of action may be on the hosts that keep the body healthy to fight the infection when it is really required³³. Even though no toxicity test has yet been performed, the use of these plants in traditional medicine for a long time, are considered safe and non-toxic.

CONCLUSION

Current global search for plant derived natural anti-malarial mainly focused on direct anti-parasite activity and novel mode of action. However, natural compounds targeting the hosts remains a largely unexplored but an attractive area of future research for the treatment of malaria. Plants studied under present investigation provided important inputs in malaria control. Further scientific evaluation is needed to develop a practical tool for proper management of the disease in malaria risk areas.

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REFERENCES

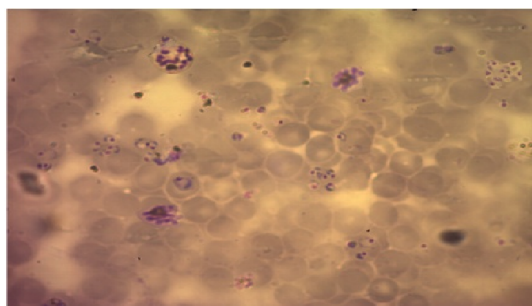
1. WHO. World Malaria Report. Geneva, World Health Organization 2013.
2. WHO .Traditional medicine strategy 2002-2005. Geneva WHO 2002.
3. Willcox ML and Bodekar G. Traditional herbal medicine for malaria. *Brit Med J* 2004; 329; 1156-1159.
4. Wright CW. Traditional antimalarial and the development of novel antimalarial drugs *J Ethnopharmacol* 2005; 100: 67-71.
5. Koul O, Isman MB, Ketkan CM. Properties and uses of *Azadirachta indica*. *Canadian J Botany* 1990; 68: 1- 11.
6. Stix G. The village Pharmacy. The neem tree yields products from pesticides to soap *Sci Am.* 1992; 132.7.
7. Ravikumar S, Jacob S, suganth LP. *In vitro* antiplasmodial activity of ethanolic extracts of South indian medicinal plants against *Plasmodium falciparum*, *Asian Pac J Trop Dis* 2012; 80-83.
8. Udeimya J I, Shu EN, Quakyi I, Ajavi FO, 2008. An antimalarial neem leaf extract has both schizonticidal and gametocytocidal activities. *Am. J. Ther* 15: 108-10.
9. Fagg CW. *Acacia nilotica* Pioneer for drug lands. Department of plant Science, University of Oxford U. K. 1990; 28-38.
10. Ali A, Akhtar N, Alikhan B, 2012. *Acacia nilotica*: A plant of multipurpose medicinal uses with potential antioxidant activity. *Jour Med Plants Res* 6: 1492-1496.
11. Joshi P. Ethnomedicine of Tribes Rajasthan- An Over view. In: Pushpangadan *et al* (Eds.), *Glimpses of India Ethnopharmacology*, TBGRI, Thrunanthapuram, Indian: 1994; 147-162.
12. Ballal AD, Bobbala SM, Qudri M, Moller D, Kompe O, Nasir A. Antimalarial effect of gum Arabic. *Malaria Journal* 2011; 10: 139.
13. Bairwa R, Gupta P, Gupta VK, Srivastava B, 2012. Traditional medicinal plants: used in oral hygiene. *Int. J. Pharma Chem Sci* 2012; 1: 94.
14. Kokate C K. Practical pharmacognosy Vallabh Prakashan 2000; 218.
15. Harbone JB. Phytochemical methods, A guide to modern techniques of plant analysis. Chapman and Hall London 1998; 54-84.
16. Jensen JB, Trager W. Cultivation of erythrocytic and exoerythrocytic stages of *Plasmodium* in malaria. Academic Press.1980; 2: 280.
17. WHO. *In vitro* micro test (mark III) for the assesment of the response of plasmodium falciparum to chloroquine, mefloquine, quinine, and artemisinin. Division of control

- of Tropical Disease Review 2001; 2 CTD/MAL/97.20.
18. Lambros C, Vanderberg J P. Synchronization of Plasmodium falciparum erythrocytic stages in culture. *Journal of Parasitology* 1979; 65: 418-420.
 19. Le Bras J, Deloron. *In vitro* study of drug sensitivity of Plasmodium falciparum: evaluation of a new semi-microtest. *Am J of Trop Med and Hyg* 1983; 274:1421-1422.
 20. Ramazani, A, Zakeri, S, Sardari, S, Khodakarim, N. Djadid, ND. *In vitro* and in vivo anti-malarial activity of Boerhavia elegans and Solanum surattense. *Malaria Journal* 2010; 9: 1-8.
 21. Saxena S, Pant N, Jain DC, Bhaluni RS. Antimalarial agents from plant sources. *Cur Sci* 2003; 85: 1314-1326.
 22. Bhasin V. Oral health behavior among Bhils of Rajasthan. *Journal Soc Sci* 2004; 8: 1-5.
 23. Dey AC, Indian medicinal plants used in *Ayurvedic preparations Dehra Dun India*; 1980: 165-166.
 24. Anonymous. The Wealth of India, Raw Material, Vol 1, CSIR, New Delhi, 2004, 11.
 25. Steven maranz. An alternative paradigm for the role of antimalarial plants in Africa. *The Scientific World Journal* 2012; 2012: 9.
 26. Phillipson, JD, Wright CW. Medicinal plants against protozoal diseases. *Trans of the Royal Soc of Trop Med and Hyg* 1991; 85: 155-165.
 27. Mukabana WR, Takken W, Killeen GF. Allomonal effect of breath contributes to differential attractiveness of human to the African malaria vector Anopheles gambiae. *Malaria journal* 2004; 29: 3(1), 1.
 28. Ronaud Lacroix, Mukabana WR. Malaria infection increases attractiveness of humans to mosquito *Journal PLOS/Biology* 2005.
 29. Bowman, W.C.; Rand, M.J. Chemotherapy of protozoan infections. In *Textbook of Pharmacology*; Publication Blackwell Scientific: Oxford, UK, 1980; pp. 36.1-36.5.
 30. Denise L Doolan Carlota Dobano J Kevin baird. Acquired immunity to malaria. *Clinical Microbiol Rev* 2009; 22: 13-36.
 31. Contrick J. Neem: The ultimate herb, Lotus Press 1st edition 2001.
 32. Tahir AEI, Gwira M, H Satti and Sani A Khalia. Antiplasmodial activity of selected Sudanese medicinal plants with emphasis on *Acacia nilotica*. *Phytotherapy Research* 1991; 13: 475-478.
 33. Benoit VF. Ethnomedicine in malaria treatment. *I Drugs* 2005; 8 (1):45-52.

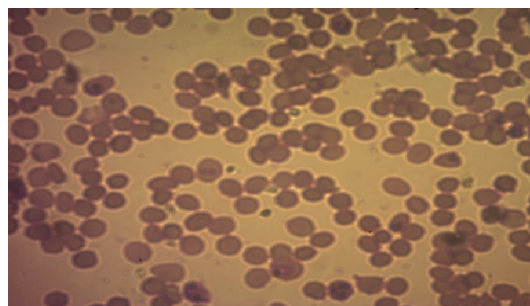
Table 1. Percent inhibition of parasitemia and IC₅₀ value of *Azadirachta indica* and *Acacia nilotica* against *Plasmodium falciparum* 3D7 strain

S. No.	Plant	Percent inhibition of parasitemia Concentration of extract(µg/mL)						*IC ₅₀ (µg/mL)
		100	50	25	12.5	6.25	3.13	
1.	<i>Azadirachta indica</i>	81.25	73.25	60.56	33.21	13.76	8.09	39.86
2.	<i>Acacia nilotica</i>	63.56	32.19	25.01	11.87	5.32	2.18	77.78

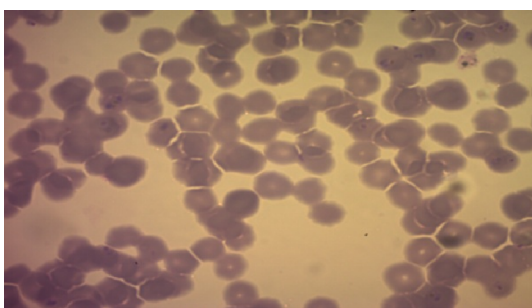
*IC₅₀ Concentration inhibiting 50% parasitemia



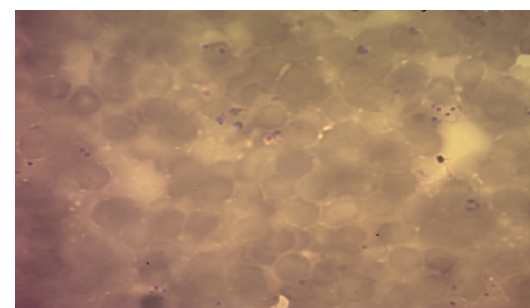
(a). Negative control (without drug)



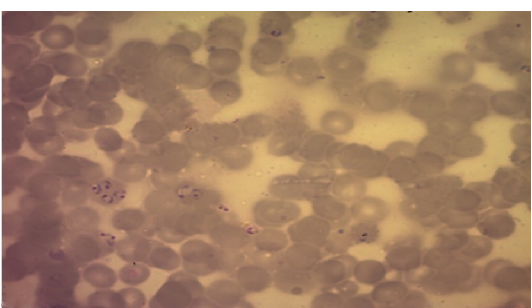
(b). Positive control: Standard drug (Chloroquine)



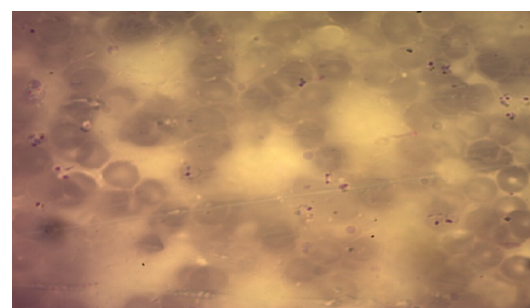
(c). *Azadirachta indica* (100 µg/mL)



(d). *Azadirachta indica* (3.125 µg/mL)



(e). *Acacia nilotica* (100 µg/mL)



(f). *Acacia nilotica* (3.125 µg/mL)

Figure 1. *In vitro* antimalarial activity of two plant extracts against *Plasmodium falciparum* 3D7