Research Article

ANTIMICROBIAL AND ANTIMALARIAL ACTIVITIES OF SOME SELECTED ETHNO-MEDICINAL PLANTS USED BY TRIBAL COMMUNITIES OF TAPI DISTRICT, GUJARAT, INDIA

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ABSTRACT

In the present study, eight medicinal plants which are traditionally used by tribal communities of Tapi District, Gujarat were studied for antimicrobial and antimalarial activity. Acetone, ethyl acetate, hexane, petroleum ether and chloroform extracts of these plants were used to check for antibacterial, antifungal and antimalarial activity. The extract from plants were examined for antimicrobial activity against two gram-positive bacterial strains and two gram-negative bacterial strains. The antifungal activity of plant extracts was checked on three fungal strains. The plant extracts were tested for antimalarial activity against *Plasmodium falciparum* strain. Acetone extract of *Bauhinia foveolata* bark and *Bridelia spinosa* bark showed most effective antibacterial activity and antifungal activity respectively in comparison with standard drugs. Ethyl acetate extracts of *Bridelia spinosa* leaf and *Bauhinia foveolata* bark showed significant antimalarial activity respectively which is superior than standard drug quinine. This study provides support for traditional uses of plants by various tribal people to cure some diseases. However, further research will be needed to isolate and characterize of active compound of these species.

Keywords: Medicinal plants, traditional uses, antimicrobial activity, antimalarial activity, Tapi District

INTRODUCTION

Traditional medicine practice has been around for centuries and still remains very common in the developing world. It is estimated that about 80% or more of the world's population rely primarily on traditional medicine for their healthcare¹. The huge reliance on traditional medicine, especially in rural areas, has been attributed to both economic and cultural factors².

Traditional herbal medicines play a prominent role in the health care systems of many countries. In India, the natives implement various herbs for curing various ailments. The preparation and administration of drugs from these plants varies from place to place³. An Ethnomedicinal study offers immense opportunities to develop new drugs. Being closer to nature, traditional societies had gained knowledge regarding the use of wild flora and fauna, mostly unknown to the urban, who are away from the natural ecosystem⁴. Though the art of herbal healing still exists through traditional folk healers, its knowledge seems to gradually perish⁵.

In the last few years, medicinal plants have attracted the attention of pharmaceutical and scientific communities as sources of antimicrobial substances⁶. Phytochemical screening, based on ethnomedicinal data, is considered an effective approach for the discovery of new therapeutic agents⁷. In the present scenario, there is necessity to search for novel and active antimicrobial⁸.

Malaria is the most widespread insect-borne disease. It kills between one and two million people every year, with as many as 300–500 million people being infected⁹. Malaria is a typical example of a disease that affects the efficiency of individuals, families and the community. The first antimalarial drug was Quinine, isolated from the bark of *Cinchona* species (Rubiaceae), and it is one of the oldest and most important antimalarial drugs

today. Chloroquine, another antimalarial drug, was synthesised, and until recently, this was the only drug used for the treatment of malaria¹⁰. The malarial parasite *Plasmodium falciparum* has become resistant to Chloroquine¹¹. In the present study, we investigate the antimicrobial and antimalarial activity of ethno-medicinal plants used by various tribes to traditionally cure various diseases from the Tapi District, Gujarat.

MATERIALS AND METHODS

Collection of plant materials

Laves, bark and whole plants of eight species of ethno-medicinal plants were collected from the forest areas of the Tapi District, Gujarat. They were identified through the Flora which are available in the Library of the Department of Botany, Bioinformatics and Climate Change Impacts Management^{12, 13, 14} where voucher specimens of each plant had been deposited.

Preparation of plant extracts

Different parts of plants were air dried at room temperature and powdered. Plant materials were extracted with five solvents namely Ethyl acetate, Acetone, Petroleum ether, Chloroform and Hexane. They were kept in a rotary shaker for 48 hours. Then all extracts were filtered and dried at room temperature.

In vitro anti-bacterial and anti-fungal activity

This work has been carried out in Microcare Laboratory and TRC, Surat, India as per the reported method of Hawkey *et al*¹⁵. The extracts from the plants were examined for antibacterial activity against two gram-positive bacterial strains (*Staphylococcus aureus* MTCC 96, *Streptococcus pyogenes* MTCC 442), two

gram-negative bacterial strains (*Escherichia coli* MTCC 443, *Pseudomonas aeruginosa* MTCC 8). The accuracy of the extract concentrations was checked by the MIC value of the control organism. The MIC is defined as the lowest concentration of the antibiotic. The amount of growth from the control tube before incubation (which represents the original inoculum) was compared. Subcultures might show: similar number of colonies indicating bacteriostatic; a reduced number of colonies indicating a partial or slow bactericidal activity and no growth if the whole inoculum has been killed. The test must include a second set of the same dilutions inoculated with an organism of known sensitivity. 2000 µg/mL concentration as a stock solution was used to dilute the synthesized compounds. 500, 250 and 200 µg/mL concentrations of the synthesized compounds were taken in the primary screening. The most active compounds in this primary screening were further tested in a secondary screening against all microorganisms. The active compounds from primary screening were uniformly diluted to get 100, 62.5, 50, 25, 12.5 and 6.5 µg/mL concentrations. The highest dilution showing at least 99% inhibition is taken as MIC.

Furthermore, the antifungal activity of all the extracts were performed on three fungal strains (*Aspergillus clavatus* MTCC 1323, *Candida albicans* MTCC 227 and *Aspergillus niger* MTCC 282) using the agar dilution method. Ampicillin, Ciprofloxacin, and Chloramphenicol were used as standard control drugs for antibacterial activity, whereas Nystatin and Greseofulvin were used as standard drugs for antifungal activity.

In vitro anti-malarial activity

In vitro antimalarial activity was carried out by using modifications of the methods of Microassay protocol of Rieckmann *et al*¹⁶. *Plasmodium falciparum* strain was supplemented with 1% Dglucose, 25 mM HEPES, 0.23% bicarbonate and inactivated human serum. The culture was stored at 37°C temperature for 24 h. After the 5% D-sorbitol treatment, the asynchronous *P. falciparum* was synchronized. Primary stage of parasitaemia of 0.9 to 1.4% at 4% hematocrit in total volume of 200 µL of 25 mM HEPES. The slides were observed of ring stage plasmodium into Schizonts and Trophozoites in the presence of various concentrations of ring stage agents. The results of mean inhibitory concentration (IC₅₀) recorded with the Quinine and Chloroquine have been used as the standard drugs.

RESULTS AND DISCUSSION

In vitro anti-bacterial activity

All the plant extracts were evaluated for their in vitro antibacterial activity against *Staphylococcus aureus* MTCC 96, *Streptococcus pyogenes* MTCC 442 (Gram-positive), *Escherichia coli* MTCC 443 and *Pseudomonas aeruginosa* MTCC 1688 (Gram-negative) by conventional broth microdilution method using Chloramphenicol as a control drug for antibacterial activity (Hannan, 2000). All the extracts showed moderate antibacterial activity. The results of the antimicrobial activity are expressed in Table 1 and Graph 1. From the result table, Acetone bark extract of *Bauhinia foveolata* Dalzell (CB1) emerged as the most effective antibacterial extract with MIC (25 µg/mL) against *Streptococcus pyogenes* as compared to the standard drugs.

Acetone bark extract of *Bridelia spinosa* (Roxb.) Willd. (GB1) was found effective against *Staphylococcus aureus* as compared to the standard drug. Petroleum ether bark extract of *Cordia macleodii* Hook.f. & Thomson (AB4), Acetone leaf extract of *Bauhinia foveolata* Dalzell (CL1), acetone bark extract of *Bauhinia malabarica* Roxb. (HB1) and ethyl acetate leaf extract of *Acmella paniculata* (Wall. ex DC.) R.K.Jansen (EL2) exhibited comparable antibacterial activity with the standard drug. The remaining results of the tested compounds against the bacterial strains were comparatively poor. The lower effectiveness of the plant extracts against strains not being adapted to laboratory environments and different media, may affect their apparent susceptibility to certain inhibitors and they exhibit poor effect against strains resulting in the poor activity.

In vitro anti-fungal activity

The in vitro antifungal activity of plant extracts were determined against *Candida albicans* MTCC 227, *Aspergillus niger* MTCC 282 and *Aspergillus clavatus* MTCC 1323 by Conventional broth microdilution method. The results of antifungal activity are presented in table 2 and Graph 2. The results indicated that acetone bark extract of *Bridelia spinosa* (Roxb.) Willd. (GB1) was found to be the most promising agent against *Candida albicans* with MIC (100 µg/mL) in comparison with control drug Greseofulvin. Acetone bark extract of *Bauhinia malabarica* Roxb. (HB1), hexane bark extract of *Miliusa tomentosa* (Roxb.) J.Sinclair (DB3), Acetone leaf extract of *Bauhinia foveolata* Dalzell (CL1), chloroform whole plant extract of *Rotala serpyllifolia* (Roth) Bremek. (RW5), petroleum ether bark extract of *Cordia macleodii* Hook.f. & Thomson (AB4), acetone bark extract of *Bauhinia foveolata* Dalzell (CB1), chloroform leaf extract of *Cordia macleodii* Hook.f. & Thomson (AL5) and acetone leaf extract of *Clematis hedysarifolia* DC. (BL1) displayed comparable antifungal activity as compared to the standard drug. However, all other extracts showed less inhibition against the tested microorganisms as compared to the standard drug.

In vitro anti-malarial Activity

All the plant extracts were screened for In Vitro antimalarial activity against *Plasmodium falciparum*. The results were compared with the standard drugs Quinine and Chloroquine. Most of the plant extracts exhibited good anti-malarial activity and the results are presented in Table 3 and Graph 3. We have observed that ethyl acetate leaf extract of *Bridelia spinosa* (Roxb.) Willd.(GL2) and ethyl acetate bark extract of *Bauhinia foveolata* Dalzell (CB2) showed significant antimalarial activity with IC₅₀ 0.15 and 0.10 µg/mL, respectively which is superior to the standard drug Quinine. Moreover, ethyl acetate leaf extract of *Cordia macleodii* Hook.f. & Thomson (AL2), chloroform leaf extract of *Cordia macleodii* Hook.f. & Thomson (AL5), acetone leaf extract of *Bauhinia foveolata* Dalzell (CL1), acetone bark extract of *Bridelia spinosa* (Roxb.) Willd. (GB1) and chloroform whole plant extract of *Rotala serpyllifolia* (Roth) Bremek. (RW5) show better inhibitory effect against pathogens. However, the remaining extract exhibited comparatively moderate antimalarial activity against tested malarial strains in comparison to the standard drugs Quinine and Chloroquine.

Table 1: Antibacterial activity (MIC µg/ml)

Sr. No.	Plant Name/STD. Drug	Herbarium Voucher Specimen Number	Plant Material	Solvent	Code	<i>Ec</i>	<i>Pa</i>	<i>Sa</i>	<i>Sp</i>
1	<i>Cordia macleodii</i> Hook.f. & Thomson	TP-54	Leaves	Ethyl acetate	AL2	250	100	250	100
2	<i>Cordia macleodii</i> Hook.f. & Thomson	TP-54	Leaves	Chloroform	AL5	125	62.5	125	500
3	<i>Clematis hedysarifolia</i> DC.	TP-177	Leaves	Acetone	BL1	100	100	125	500
4	<i>Bauhinia foveolata</i> Dalzell	TP-23	Bark	Acetone	CB1	62.5	100	100	25
5	<i>Bauhinia foveolata</i> Dalzell	TP-23	Leaves	Acetone	CL1	100	100	250	125
6	<i>Cordia macleodii</i> Hook.f. & Thomson	TP-54	Bark	Petroleum ether	AB4	50	250	125	125
7	<i>Bridelia spinosa</i> (Roxb.) Willd.	TP-178	Bark	Acetone	GB1	125	250	25	250
8	<i>Acmella paniculata</i> (Wall. ex DC.) R.K.Jansen	TP-179	Leaves	Ethyl acetate	EL2	100	62.5	125	100
9	<i>Bauhinia malabarica</i> Roxb.	TP-24	Bark	Chloroform	HB5.1	62.5	250	250	250
10	<i>Rotala serpyllifolia</i> (Roth) Bremek.	TP-144	Whole plant	Chloroform	RW5	100	250	100	500
11	<i>Bauhinia malabarica</i> Roxb.	TP-24	Leaves	Chloroform	HL5	125	250	100	125
12	<i>Bridelia spinosa</i> (Roxb.) Willd.	TP-178	Bark	Acetone	DB1	100	125	250	250
13	<i>Bridelia spinosa</i> (Roxb.) Willd.	TP-178	Leaves	Ethyl acetate	GL2	100	500	100	100
14	<i>Bauhinia foveolata</i> Dalzell	TP-23	Leaves	Acetone	CL1	62.5	125	250	250
15	<i>Bauhinia malabarica</i> Roxb.	TP-24	Leaves	Acetone	HL1	100	125	100	62.5
16	<i>Bauhinia malabarica</i> Roxb.	TP-24	Bark	Chloroform	HB5	250	250	100	100
17	<i>Bauhinia malabarica</i> Roxb.	TP-24	Bark	Acetone	HB1	62.5	500	125	100
18	<i>Miliusa tomentosa</i> (Roxb.) J.Sinclair	TP-180	Bark	Hexane	DB3	100	250	125	250
19	<i>Bauhinia foveolata</i> Dalzell	TP-23	Bark	Ethyl acetate	CB2	250	500	250	250
20	Gentamycin					0.05	1	0.25	0.5
21	Ampicillin					100	--	250	100
22	Chloramphenicol					50	50	50	50
23	Ciprofloxacin					25	25	50	50
24	Norfloxacin					10	10	10	10

Escherichia coli MTCC 443 (*Ec*), *Pseudomonas aeruginosa* MTCC 1688 (*Pa*).
Staphylococcus aureus MTCC 96 (*Sa*), *Streptococcus pyogenes* MTCC 442 (*Sp*).

Table 2: Antifungal activity (MIC µg/ml)

Sr. No.	Plant Name	Herbarium Voucher Specimen Number	Plant Material	Solvent	Code	<i>Ca</i>	<i>An</i>	<i>Ac</i>
1	<i>Cordia macleodii</i> Hook.f. & Thomson	TP-54	Leaves	Ethyl acetate	AL2	1000	>1000	>1000
2	<i>Cordia macleodii</i> Hook.f. & Thomson	TP-54	Leaves	Chloroform	AL5	500	>1000	>1000
3	<i>Clematis hedysarifolia</i> DC.	TP-177	Leaves	Acetone	BL1	500	>1000	>1000
4	<i>Bauhinia foveolata</i> Dalzell	TP-23	Bark	Acetone	CB1	500	500	500
5	<i>Bauhinia foveolata</i> Dalzell	TP-23	Leaves	Acetone	CL1	250	500	1000
6	<i>Cordia macleodii</i> Hook.f. & Thomson	TP-54	Bark	Petroleum ether	AB4	500	500	500
7	<i>Bridelia spinosa</i> (Roxb.) Willd.	TP-178	Bark	Acetone	GB1	100	1000	1000
8	<i>Acmella paniculata</i> (Wall. ex DC.) R.K.Jansen	TP-179	Leaves	Ethyl acetate	EL2	1000	>1000	>1000
9	<i>Bauhinia malabarica</i> Roxb.	TP-24	Bark	Chloroform	HB5.1	500	1000	1000
10	<i>Rotala serpyllifolia</i> (Roth) Bremek.	TP-144	Whole plant	Chloroform	RW5	250	500	500
11	<i>Bauhinia malabarica</i> Roxb.	TP-24	Leaves	Chloroform	HL5	250	>1000	>1000
12	<i>Bridelia spinosa</i> (Roxb.) Willd.	TP-178	Bark	Acetone	DB1	500	>1000	>1000
13	<i>Bridelia spinosa</i> (Roxb.) Willd.	TP-178	Leaves	Ethyl acetate	GL2	500	>1000	>1000
14	<i>Bauhinia foveolata</i> Dalzell	TP-23	Leaves	Acetone	CL1	250	>1000	>1000
15	<i>Bauhinia malabarica</i> Roxb.	TP-24	Leaves	Acetone	HL1	500	500	500
16	<i>Bauhinia malabarica</i> Roxb.	TP-24	Bark	Chloroform	HB5	1000	>1000	>1000
17	<i>Bauhinia malabarica</i> Roxb.	TP-24	Bark	Acetone	HB1	500	1000	1000
18	<i>Miliusa tomentosa</i> (Roxb.) J.Sinclair	TP-180	Bark	Hexane	DB3	500	1000	1000

19	<i>Bauhinia foveolata</i> Dalzell	TP-23	Bark	Ethyl acetate	CB2	1000	>1000	>1000
20	Nystatin					100	100	100
21	Griseofulvin					500	100	100

Candida albicans MTCC 227 (Ca), *Aspergillus niger* MTCC 282 (An), *Aspergillus clavatus* MTCC 1323 (Ac).

Table 3: Antimalarial activity (MIC µg/ml)

Sr. No.	Plant Name	Herbarium Voucher Specimen Number	Plant Material	Solvent	Code	<i>Pf</i> Mean IC ₅₀ (µg/ml)
1	<i>Cordia macleodii</i> Hook.f. & Thomson	TP-54	Leaves	Ethyl acetate	AL2	0.59
2	<i>Cordia macleodii</i> Hook.f. & Thomson	TP-54	Leaves	Chloroform	AL5	0.33
3	<i>Clematis hedysarifolia</i> DC.	TP-177	Leaves	Acetone	BL1	1.98
4	<i>Bauhinia foveolata</i> Dalzell	TP-23	Bark	Acetone	CB1	1.25
5	<i>Bauhinia foveolata</i> Dalzell	TP-23	Leaves	Acetone	CL1	0.41
6	<i>Cordia macleodii</i> Hook.f. & Thomson	TP-54	Bark	Petroleum ether	AB4	1.08
7	<i>Bridelia spinosa</i> (Roxb.) Willd.	TP-178	Bark	Acetone	GB1	0.40
8	<i>Acmella paniculata</i> (Wall. ex DC.) R.K.Jansen	TP-179	Leaves	Ethyl acetate	EL2	1.12
9	<i>Bauhinia malabarica</i> Roxb.	TP-24	Bark	Chloroform	HB5.1	0.45
10	<i>Rotala serpyllifolia</i> (Roth) Bremek.	TP-144	Whole plant	Chloroform	RW5	0.57
11	<i>Bauhinia malabarica</i> Roxb.	TP-24	Leaves	Chloroform	HL5	2.05
12	<i>Bridelia spinosa</i> (Roxb.) Willd.	TP-178	Bark	Acetone	DB1	1.89
13	<i>Bridelia spinosa</i> (Roxb.) Willd.	TP-178	Leaves	Ethyl acetate	GL2	0.15
14	<i>Bauhinia foveolata</i> Dalzell	TP-23	Leaves	Acetone	CL1	0.94
15	<i>Bauhinia malabarica</i> Roxb.	TP-24	Leaves	Acetone	HL1	1.52
16	<i>Bauhinia malabarica</i> Roxb.	TP-24	Bark	Chloroform	HB5	0.63
17	<i>Bauhinia malabarica</i> Roxb.	TP-24	Bark	Acetone	HB1	0.73
18	<i>Milusa tomentosa</i> (Roxb.) J.Sinclair	TP-180	Bark	Hexane	DB3	1.312
19	<i>Bauhinia foveolata</i> Dalzell	TP-23	Bark	Ethyl acetate	CB2	0.10
20	Quinine					0.26
21	Chloroquine					0.02

Antimalarial screening using *Plasmodium falciparum* strain (*Pf*), values expressed in IC₅₀ µg/mL.

Graph 1: Plot of antibacterial activity MIC µg/ml vs Plant extract.

Graph 2: Plot of antifungal activity MIC µg/ml vs Plant extract.

Graph 3: Plot of antimalarial activity MIC µg/ml vs Plant extract.

CONCLUSION

It is concluded that acetone bark extract of *Bauhinia foveolata* Dalzell showed most effective antibacterial action with MIC (25 µg/mL) against *Streptococcus pyogenes* as compared to the standard drugs. Acetone bark extract of *Bridelia spinosa* (Roxb.) Willd. was found most promising agent against *Candida albicans* with MIC (100 µg/mL) in comparison with control drug Griseofulvin. Ethyl acetate leaf extract of *Bridelia spinosa* (Roxb.) Willd. And ethyl acetate bark extract of *Bauhinia foveolata* Dalzell showed significant antimalarial activity with

IC₅₀ 0.15 and 0.10 µg/mL, respectively which is superior to the standard drug Quinine. These biological properties support the traditional uses of plants by various tribal people to cure some diseases. However, further research will be needed to isolate and characterize the active compounds involved.

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