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EVALUATION OF ACETONE EXTRACT OF THREE INDIAN MEDICINAL PLANTS FOR SCHIZONTICIDAL PROPERTIES IN *PLASMODIUM FALCIPARUM*

Bharat Gami[1*], Farzin Parabia[2],
[1]Ipcowala Santram Institute of Biotechnology & Emerging Sciences,
The H.M.Patel School's complex, Dharmaj – 388430, Gujarat (India).
[2]Ashok & Rita Patel Institute of Integrated Study & Research in Biotechnology and Allied Sciences (ARIBAS)
New Vallabh Vidyanagar - 388 121.
Email: bharat11_gami@yahoo.com

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Abstract:

Increased resistance against *Plasmodium falciparum* in the present drugs is an impetus for dynamic study filed for new antimalarial agent from plants & chemically synthesized molecule. In present attempt, acetone extract of *Alstonia scholaris*, *Moringa oleifera*, and *Tinospora cordifolia,* was tested for schizonticidal activity of *Plasmodium falciparum*. The 50 % inhibitory concentration (IC_{50}) value range from 0.08 µM to 0.26µM. Components of acetone fraction were separated by high performance thin layer chromatography (HPTLC) and scanned at 366 nm. All plants showed strong schizonticidal activity. All the three plant extracts by further purification could be use as an effective, alternative source of herbal anti-malarial drugs.

Key words: *Plasmodium falciparum*, acetone extract, schizonticidal activity, antimalarial agent.

Introduction:

Plasmodium falciparum is the causative agent of most severe form of human malaria[1], and is a leading cause of morbidity and mortality in developing countries, infecting hundreds of millions of individuals[2-5]. *P. falciparum* infection is more frequent and severe in children than in adults[6]. This death toll will rise as drug-resistant parasites spread[7] and so it is difficult to select appropriate drugs for both prophylaxis and treatment[1].

Due to drug-resistant parasites, new treatments for malaria are urgently needed[8], as well as the promise of broadly effective malaria vaccine remains unfulfilled despite important technological advances[9]. The long established use of

quinine and the more recent introduction of artemisinin and its derivatives as highly effective antimalarials demonstrate that plant species are an important resource for the discovery of new antimalarial agents. Furthermore, many plant species continue to be used in traditional medicines for the treatment of malaria and many people depend on such remedies as they cannot afford and/or do not have access to effective antimalarial drugs.

In present investigation acetone extract of *Tinospora cordifolia*, *Alstonia scholaris* Linn. R.Br, and *Moringa Moringa oleifera* Lam were evaluate for *in vitro* schizonticidal activity of *Plasmodium falciparum*.

Materials and Methods:

Plant material:

Authentic sample (powder) of *Alstonia scholaris* Linn. R.Br. (Stem bark, Apocynaceae), *Moringa oleifera* Lam. (Leaves, Morinaceae) and *Tinospora cordifolia* (Stem, Menispermaceae) were collected from Bapalal Botanical Vaidya Research Center Surat (Gujarat).

Extraction:

Coarsely powdered air-dried material 4 g was placed in a glass stoppered conical flask and macerated with 100 ml of analytic grade solvents (acetone) shaking frequently and then allowing it to stand for 18 hours. Filter it rapidly through Whatman No. 1 filter paper, taking care not to lose any solvent. Transfer filtrate to flat- bottom dish and evaporate solvent on a water bath. Dry at 105°C for 6 hours, cool in a desiccator for 30 minutes and weigh without delay, calculate the content of extractable matter in mg/g of air-dried material[10].

Sample preparation:

Clear acetone extract (10 ml) treated with 200µl of 50 % lead acetate (Hi-media, Mumbai), mix it properly and precipitated by centrifugation at 10000 rpm (REMI, India). Care fully remove the supernatant in watch glass, evaporate at room temperature, dry at 105 ^{o}C for 6 hours, cool in a desiccator for 30 minute. Dry acetone extract was used in study of HPTLC finger printing and schizonticidal activity.

Schizonticidal activity

The strains of *P. falciparum*, was collected from the Malaria Parasite bank, maintained by the National Institute of Malaria Research, New Delhi, India. The cultures have been maintained in the laboratory using method of Trager &

Jensen[11] in human red blood cells (blood type O+) at a 5% haematocrit in RPMI 1640 medium with 25 mM HEPES[12], 0.2% sodium bicarbonate and 15% human AB+ serum.

Determination of *in vitro* schizonticidal activity of *Plasmodium falciparum*

The parasite cultures, prior to experimentation, were synchronized by treatment with 5% D-sorbitol[13]. Synchronized cultures containing ring-staged parasites were suspended in equal volume of human serum. Stock solutions were prepared separately from 10 mg of dried acetone extract of these three plants in 10 µl of dimethyl sulfoxide (DMSO) and finally diluted with serum free medium to a concentration of 1 mg/ml. Serial double dilutions of each set of plant extracts were made in triplicate in 96 well - plates (Axygen, Germany) with concentration ranging from 0.4 - 0.0125 µg/ml against a control containing the incomplete medium with same concentration of DMSO. In each well 100 µl of the diluted extract, 10 µl parasitized blood in 100 µl incomplete medium and 5% haematocrit were added. Four wells of the last row were set as general controls to check the normal growth of the parasite. Schizont maturation time was calculated from the growth of the parasites cultured in general control wells. Accordingly thin smears were drawn (approximately 24–28 hrs of incubation) from each of the experimental and control wells on properly labeled slides. The blood smears were air dried and fixed in methanol. Dried slides were JSB stained[14] and observed in 100 × with oil immersion under microscope (Zeiss) for the study of parasitaemia, particularly the inhibition of schizont maturation. Numbers of schizonts were counted per 200 asexual stage parasites. The values were compared between test and control wells. The percentage of inhibition was calculated as (1-Number of schizonts in test well/Number of schizonts per control well) × 100. The 50% inhibitory concentration (IC_{50}) of these plant extracts were estimated from the graph drawn on the inhibition (%) data.

HPTLC finger printing

Activation of aluminum precoated 250 µm thick Silica Gel 60_{254} TLC Plates (Merck) was done by placing in oven at 110°-120° C for 30 min prior to sample spotting[15]. The samples were applied on TLC plates by CAMAG Linomate-VI automated sample applicator. For each extract two spot were loaded, first spot with 10µl extract while second spot with 5 µl. Plates were developed with solvent system of Toulene: Methanol (8:2) Rf values of the

separate constitute of extracts were detected by densitometric scanner. The compounds were visualized under visible and UV light (245 and 366 nm).

Phytochemical Screening

Acetone extracts of *A. scholaris, M. oleifera* and *T.cordifolia* were teststed for alkaloids, flavonoids, sterols, triterpen, and glycosides according to the method of Harbone[16].

Results:

In vitro schizonticidal activity

Schizonticidal activity of *P. falciparum* against acetone fraction of *M.olefera* (Table 1), *A. scholaris* (Table 2) and *T.cordifolia* (Table 3) were carried out *in vitro* and stages like ring stage, trophozoit stage, and schizont stages were identified and reduction in schizont was recorded against applied concentration.

Table-1: Schizonticidal activity of acetone fraction of *Moringa oleifera*. Data are expressed as mean value of four replicate.

	Drug Concentration	No. of infected cells	Ring stage	Trophozoit stage	Schizont stage	Schizonts reduction percentage
A	-	-	-	-	-	-
B	-	200	55	73	82	-
C	0.4 µl.	200	36	131	33	59.8%
D	0.2 µl.	200	55	101	44	46.4%
E	0.1 µl.	200	40	103	57	30.5%
F	0.05 µl.	200	25	107	68	17.1%
G	0.025 µl.	200	30	100	70	14.7%
H	0.0125 µl	200	27	101	72	12.2%

Table-2: Schizonticidal activity of Acetone fraction of *Alstonia scholaris*. Data are expressed as mean value of four replicate.

	Drug Concentration	No. of infected cells	Ring stage	Trophozoit stage	Schizont stage	Schizonts reduction percentage
A	-	-	-	-	-	-
B	-	200	45	75	80	-
C	0.4 µl.	200	33	140	27	66.3
D	0.2 µl.	200	58	110	32	60.0
E	0.1 µl.	200	62	102	36	55.0
F	0.05 µl.	200	60	100	40	50.0
G	0.025 µl.	200	31	101	58	27.5
H	0.0125 µl	200	36	100	64	20.0

Table-3: **Schizonticidal activity of Acetone fraction of *Tinospora cordifolia*. Data are expressed as mean value of four replicate.**

	Drug Concentration	No. of infected cells	Ring stage	Trophozoit stage	Schizont stage	Schizonts reduction percentage
A	-	-	-	-	-	-
B	-	200	23	92	85	-
C	0.4 µl.	200	33	137	30	64.7
D	0.2 µl.	200	64	129	37	56.5
E	0.1 µl.	200	46	105	49	42.3
F	0.05 µl.	200	23	112	65	23.5
G	0.025 µl.	200	19	110	71	16.5
H	0.0125 µl	200	18	107	75	11.7

Acetone extract of *M.oleifera* showed highest percentage (59.8 %) of reduction at 0.4 µl/well, while least percentage of reduction (12.2 %) was observed at 0.0125 µl/well (Table 1). For acetone fraction of *M.oleifera* IC_{50} and IC_{90} values were 0.26 µl and 4.0µl respectively. *A.scholaris* showed highest reduction in schizont percentage; 66.25 % at 0.4µl, followed by 60.0 % at 0.2µl, 55 % at 0.1µl, 50.0 % at 0.05µl, 27.5 % at 0.025 µl, and 20.0% at 0.0125µl (Table 2). IC_{50} (0.08) and IC_{90} (0.55) values were found for the acetone extract of *A.scholarish*. In *T.cordifolia* highest reduction in schizont formation was found at 0.4 µl (64.7 %) and lowest value 11.76 % was

observed at 0.0125 µl extract concentration (Table 3). IC_{50} and IC_{90} values of *T.cordifolia* were found to be 0.16 and 2.4 respectively.

HPTLC finger printing

Separation of secondary metabolites was achieved by applied solvent system. Separated plates were observed under UV light, visible light and visual observation after staining with detection reagent (Fig. 1,2,3). Track loaded by 10 µl of acetone extract were scanned at 366nm after developing in respective solvent system. Six substances were recorded in acetone fraction of *M.oleifera* (Table 4). In *A.scholaris* also six substances were detected with Rf range of 0.12 to 0.62 (Table 4). Only four substances with Rf range 0.06 to 0.40 were detected in *T.cordifolia* (Table 4). In all extracts for 10 µl and 5 µl similar banding patterns was observed.

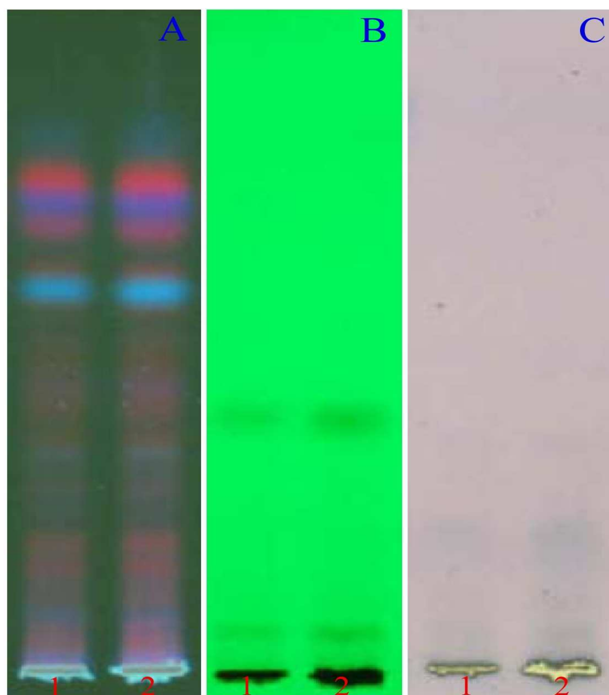

Fig. 1 HPTLC fingerprinting of Acetone extract of *Tinospora cordifolia.*
A. View at 366 nm
B. View at 254 nm
C. View after staining with spray reagents
Track 1- loaded with 10µl sample
Track 2- loaded with 05µl sample

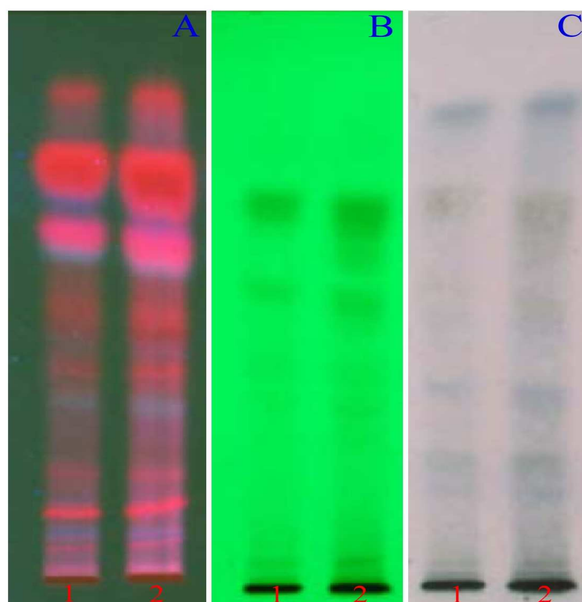

Fig. 2 HPTLC fingerprinting of Acetone extract of *Alstonia scholaris.*
A. View at 366 nm
B. View at 254 nm
C. View after staining with spray reagents
Track 1- loaded with 10µl sample
Track 2- loaded with 05µl sample

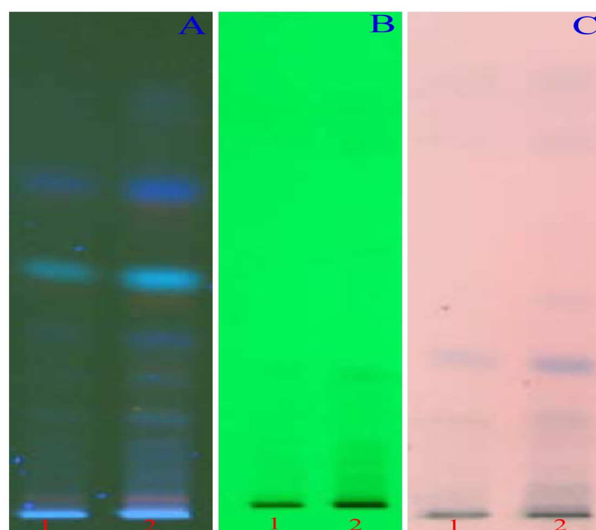

Fig. 3 HPTLC fingerprinting of Acetone extract of *Moringa oleifera.*
A. View at 366 nm
B. View at 254 nm
C. View after staining with spray reagents
Track 1- loaded with 10µl sample
Track 2- loaded with 05µl sample

Table-4: Rf value of substance in acetone extracts of three plants detected by HPTLC scanning at 366 nm.

No	*M. oleifera*	*A.scholaris*	*T.cordifolia*
1	0.10	0.12	0.06
2	0.13	0.20	0.14
3	0.15	0.26	0.34
4	0.23	0.35	0.40
5	0.28	0.52	-
6	0.51	0.62	-

Phytochemical Screening

Table 5 represent the respective group of secondary metabolites present in acetone extract of *A.scholaris, M. oleifera* and *T.cordifolia.*

Table-5: Priliminary phytochemical analysis of acetone extracts of *M.oleifera, A.scholaris*, and *T. cordifolia.*

Plant material	**Phytoconstituents present**
Moringa oleifera	Alkaloids, saponins, tannins, glycoside, terpenoids, flavanoids
Alstonia scholaris	Alkaloids, glycoside saponins, tannins, terpenoids, flavanoids
Tinospora cordifolia	Saponins, glycoside, terpenoids

Discussion:

Different extracts of known and unknown plant extracts in form of crude as well as purified are recently screened for antimalarial activity. Antimalarial activity of aqueous extract of *Uapaca nitida*[17], crude ethanol extract of *Bidens pilosa*[18], bioactivity guided fractionation of *Cajanus cajas*[19] and methanol & chloroform extract of *Guiera senegalensis*, *Feretia apodanthera*, *Combretum micranthum*, *Securidaca longepedunculata*[20] are reported as well. Acetone extract of *A. scholaris, M. oleifera* and *T.cordifolia* showed excellent results for scizonticidal activity against *P.falcifarum*.

Among three plants *A. scholaris* was found most effective in scizont reduction. Results indicate *A. scholaris* show higher scizont reduction compared to other two plants at the same concentration. Further purification and screening of acetone extract of *A. scholaris* would be developed as new antimalarial drug. Traditionally *A.scholarish* is also known for its malarial fever treatment [21-23]. IC_{50} value of acetone extract of *A.scholarish* indicates the strong scizonticidal activity in present investigation, while methanolic extract of same plants was reported ineffective as scizont reduction by Gandhi and Vinayak[24]. The conflict might be due to scizonticidal agent, having more affinity

towards acetone than methanol. Brandao[18] & Andayi[25] reported that non-polar solvents like chloroform, butanol and acetone are good for extraction of antimalarial agent. Results of phytochemical screening of acetone extract of *A. scholaris* shows presence of glycoside saponins, tannins, terpenoids, and flavanoids. These compounds are also well known for their antimicrobial activity [22, 26 -28].

Phytochemically *T.cordifolia* is well reported for their secondary metabolites and their bioactivity. Terpenoid was found major group in *T.cordifolia*, variety of terpenoids reported like; furanolactone[29], Lactones[30,31], Tinosporon[32], Tinosporides & its derivatives[33-35] and Tinocordifolin[36]. Terpenoids were also detected in acetone extract of *T.cordifolia.* IC_{50} value for acetone extract of *T.cordifolia* was 0.16 µM. This strong scizonticidal activity was may be due to the presence of terpenoids. Terpenoids are reported for their antimalarial activity & antiplasmodial activity[37]. In *T.cordifolia* apart from terpenoids, glycoside is also well documented [38] they also contribute for scizonticidal activity. Previous scizonticidal activity of *T. cordifolia* was dealt with crude extract[39-40]. In present study acetone extract was used and showed encouraging results. Further characterization is required to identify biologically active chemical constitute. The acetone extract of *M.oleifera* was positive for alkaloids, saponins, tannins, glycoside, terpenoids and flavanoids. Acetone extract of *M.oleifera* posses remarkable scizonticidal activity with IC_{50} value of 0.26 µM. Acetone fraction of *M.oleifera* contain flavanoids as major constitute. Flavanoids are well reported having antiplasmodial activity in *Erythrina sacleuxii*[25]. Three flavanoids namely acacetin, genkwanin and 7-methoxyaacacetin are reported for *in vitro* activity with IC_{50} values ranges from 4.3 mg/ml to 12.6 mg/ml in *Artimisia afara*[27]. In present investigation by acetone extract of *M.oleifera* showed good scizonticidal activity, may be due to results of cumulative effects of all phytoconstituents present in extract.

High Performance Thin layer chromatography (HPTLC) was chosen over other chromatography methods because it is a simple, quick and inexpensive procedure compare to other separation techniques that can be used for the analysis of mixtures [41]. Plates can be visualized, depending on the chemical structure of the compounds at visible light, UV-254 nm and 365 nm or by using spray reagents[42]. Acetone extracts of *T.cordifolia* (Fig. 1) *A. scholaris* (Fig. 2) *M. oleifera* (Fig. 3) and were successfully separated through HPTLC and Rf values determined

respectively. The effectiveness of the separation depends on the mixture to be separated, the choice of the mobile phase and the adsorption layer[43]. It has been reported for the first time that acetone extracts of selected plants showed encouraging results for scizonticidal activity against *P.falcifarum*.

Conclusion:

The acetone extract of *Alstonia scholaris*, *Moringa oleifera* and *Tinospora cordifolia* has strong scizonticidal activity. IC_{50} value of acetone extracts of all three plants against *Plasmodium falciparum* pose a claim for the new antimalarial compounds. Further pharmacological screenings with chemical fractions of acetone extracts would allow the isolation, and identification of active compounds.

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Corresponding Author:
R & D division, Abellon Cleanenergy Ltd,
Premchand Nagar Road, Bodakdev,
Ahmedabad – 380054, Tel- +91-79-66309332.
Email: *bharat11_gami@yahoo.com*