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Full Length Research Paper

Larvicidal, antipyretic and antiplasmodial activity of some Zulu medicinal plants

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***Gardenia thunbergia, Siphonochilus aethiopicus, Schotia brachypetala, Acorus calamus, Withania somnifera, Elaeodendron transvalense, Hypoxis hemerocallidea, Vernonia adoensis* and *Acanthosperum australe* are medicinal plants commonly used by traditional healers in South Africa to treat malaria. Aqueous, dichloromethane and methanol extracts of these plants were screened for larvicidal, antioxidant, *in vivo* antipyretic and *in vitro* antiplasmodial activities. The plant extracts either killed or reduced spontaneous movement in *Culex quinquefascitus* larvae after 24 h following treatment. Methanol extracts exhibited antioxidant (2,2-diphenyl-1-picrylhydrazyl free radical (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) scavenging, Fe^{2+} chelating) activity, albeit to varying degree of efficiency. The dichloromethane and methanol extracts significantly (*$p \leq 0.05$*) reduced pyrexia with activity increasing in a concentration dependent manner. The antiplasmodial activity against chloroquine sensitive strain of *Plasmodium falciparum* (D10) showed that the methanol extracts of *G. thunbergia, V. adoensis* and the dichloromethane extracts of *E. transvalense, A. australe* and *W. somnifera* were active (IC_{50} of 1.04 to 5.07 µg/ml). The results support the use of these plants in folk medicine and suggest that these plants contained constituents that could be developed as potent antimalarial drugs (mosquito larvicide, anti-fever and anti-plasmodial).**

Key words: Antiplasmodial, larvicidal, antipyretic activity, medicinal plants.

INTRODUCTION

Malaria is one of the most live threatening and widespread infectious diseases of our time. It has been estimated that about 40% of the world's population live in countries where the disease is prevalent and about 250 million people suffer from the disease every year (WHO, 2008). Malaria is caused by parasites of the genus *Plasmodium* (*Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale* and *Plasmodium malariae*). The currently used methods of prevention and treatment of malaria include the use of insecticides and larvicide spraying and the use of bed nets and antimalarial drugs. Drugs are also available for *falciparum* malaria which is the most dangerous of the human malaria parasite. However, the malaria parasite has over the years developed resistance to available drugs, including artermisinin-based combination therapies (Afonso et al., 2006) which are otherwise the most effective antimalarial agents in current use. This is the reason why the search for alternative antimalarial therapies is an on-going exercise. In addition, the disease is well associated with poverty and underdeveloped communities. Poor people cannot afford mosquito nets and insecticides as a method of prevention of mosquito bites.

Historically, the majority of antimalarial drugs have been derived from plants (Newman et al., 2003). Phytochemicals are known to be used by plants to prevent attack from phytophagous (plant eating) insects.

Curtis et al. (1986) showed that this repellent property of plants to pest insects and to mosquitoes has been of

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interest before the rising of synthetic chemicals. Many medicinal plants with antimalarial activity have recently been reported (Xu et al., 2011; Bero and Quetin-Leclercq, 2011). Ethnobotanical survey of traditional healers around Kwa-Zulu Natal (South Africa) revealed that, in addition to the plants already studied (Clarckson et al., 2004; Pillay et al., 2008), *Gardenia thunbergia, Siphonochilus aethiopicus, Schotia brachypetala, Acorus calamus, Withania somnifera, Elaeodendron transvalense, Hypoxis hemerocallidea,Vernonia adoensis* and *Acanthospermum australe* were among the many plants used by South African traditional healers to treat malaria. *G. thunbergia* is an evergreen shrub with a smooth, whitish, usually straight main stem found in forest, occurring in the Eastern Cape, Natal and Transkei in South Africa (Hutchings et al., 1996). Traditional healers use the roots of *G. thunbergia* as a treatment for numerous ailments, including skin diseases, skin lesions caused by leprosy and as an emetic against fever.

The root bark is used as an emetic for biliousness and to treat gall bladder problems. The roots and leaves are used in various parts of Africa by traditional healers to treat syphilis and the latex is used as a purgative. *S. brachypetala* is a medium to large tree with a wide-spreading, densely branched, rounded crown. Flowers are rich deep red and are produced in masses.

A decoction of the bark is taken to treat heartburn and hangovers. Bark and root mixtures are used to strengthen the body and purify the blood, to treat nervous heart conditions and diarrhoea, as well as for facial saunas (Hutchings et al., 1996). Ramalivhana et al. (2010) reported that *S. branchypetala* possess antimicrobial constituents against extended spectrum beta-lactamase producing *Escherichia coli*, *Enterobacter cloace* and *Pseudomonas aeroginosa*.

The seeds are edible after roasting and although low in fat and protein they have high carbohydrate content. *W. somnifera* is commonly known as Winter cherry and *Ubuvimbha* in isiZulu. *W. somnifera* is claimed to have potent aphrodisiac, rejuvenative and life prolonging properties. It is used to treat insomnia, skin problems and coughing.

The roots are used for rheumatism, constipation nervous exhaustion and spermatorrhoea (Hutchings et al., 1996). Literature (Christian et al., 2004; Prakash et al., 2001; Davis and Kuttan, 2001) indicates that extracts of *W. somnifera* exhibit antitumor activity. Its antiangiogenic (Mathur et al., 2006; Mohan, 2004), and immunomodulatory (Davis and Kuttan, 2000) activity have also been studied. *A. australe* is known as *Unsukumbili* in Isizulu. The herb is mostly used to treat infertility, wound healing and coughs. *E. transvalense* is commonly known as Transvaal Safronwood and *Ingwavuma* in isiZulu. It is found in deciduous woodland, along streams and on rocky hillsides (Hutchings et al., 1996). *E. transvalense* is prescribed by traditional healers to people who suffer from human immunodeficiency virus/ acquired immune deficiency (HIV/Aids) and rash (Van Wyk and Gericke, 2000). The rootstocks of *H. hemerocallidea* are traditionally used to treat a wide variety of ailments such as epilepsy, urinary tract infection and prostate cancer. Weak infusions and decoctions of the corm are used as a strengthening tonic during convalescence and against tuberculosis and cancer.

Phytosterols have been isolated from *Hypoxis* (Nair and Kanfer, 2008). *V. adoensis* is a shrub that grows up to 2 m high. It is found mainly in the savanna, from Senegal to Nigeria and extending across Africa to Ethiopia. It is known as *Inyathelo* in isiZulu. The leaves are crushed in cold water and applied to cattle sores caused by ticks (Hutchings et al., 1996). The root is prepared into a bitter medicine and is used as a digestive and appetizer. Root-infusion is taken for stomach-pains and for tuberculosis and for gonorrhea. The anticellular and immunosuppressive activity of *A. calamus* has been reported (Mehrotra et al., 2003). In this study, we screened extracts of the nine plants for larvicidal, antipyretic, antioxidant and antiplasmodial activity.

MATERIALS AND METHODS

Plant material

G. thunbergia T.A Sprague, *S. aethiopicus* (Schweif.) B.L Burt, *S. brachypetala* Sond., *Acorus calamus* L., *W. somnifera* (L) Dunal in DC., *E. transvalense* (Burtt Davy) R.H. Archer, *H. hemerocallidea* Fisch., C.A. Mey and Ave-Lall, *Vernonia adoensis* Sch. Bip. Ex Walp and *A. australe* (Loefl.) Kuntze) were collected from the *muthi* (Zulu word for medicine) market at Empangeni, KwaZulu-Natal, South Africa. The plants were identified by Mrs NR Ntuli of the Botany Department, University of Zululand and voucher specimen were deposited at the University herbarium.

Extraction

The plants materials were dried at 50±2℃ for 24 h. The dried materials were ground into powder (2 mm mesh) and stored in brown bottles at 4℃ until used for extraction. Each powder was separately incubated (1:5 w/v) with methanol, distilled water and dichloromethane (24 h on orbital shaker, 150 rpm, room temperature). The extracts obtained after filtrations were concentrated under reduced pressure; the aqueous extracts were freeze-dried. The extracts were stored at 4℃.

Antioxidant activity

Methanolic extracts were screened for antioxidant activity: The DPPH, ABTS HO: hydroxyl radicals; SO: super oxide radicals; NO: nitric oxide radicals) scavenging activity, Fe^{2+} chelating activity, total antioxidant capacity and the SH (sulphur hydryl) content of the plant extracts were determined by the methods previously outlined (Opoku et al., 2002, 2007; Simelane et al., 2010).

Total phenolic, flavonoid, proanthocyanidin content

The plant materials were extracted into diethyl ether and the total

phenol (Kähkönen et al., 1999), flavonoids (Ordon et al., 2006) and Proanthocyanidin (Sun and Chen, 1998) of the various extracts determined and expressed as garlic acid equivalent, quercetin equivalent and catechin equivalent, respectively.

Anti-pyretic activity

Approval for experimental procedures was obtained from the Research Animal Ethic Committee, University of Zululand. Pyrexia was induced in Sprague-Dawley rats (both sexes, 150 to 180 g) by subcutaneous injection of 12% brewer's yeast (1 ml/kg bw). 24 h later the anal temperatures of the animals were taken and those that showed a minimum rise of 1.5℃ were selected as pyretic. Pyretic rats were divided into five groups of five animals each. Group one was the control and received carboxy-methylcellulose (CMC), the drug vehicle. Group two, three and four received the plant extract dissolved in CMC (100, 500 and 1000 mg/kg body weight, respectively). Group five was administered with paracetamol (panado) as the standard drug (100 mg/kg). The drugs were orally administered. The rats had free access to food and water. The anal temperatures were taken (three times each period) at 30 min, 1, 2 and 4 h after the administration of the drugs.

In vitro antiplasmodial activity

The plant extracts were tested in triplicate against chloroquine sensitive (CQS) strain of *P. falciparum* (D10). Continuous *in vitro* cultures of asexual erythrocytes stages of *P. falciparum* were maintained using a modified method of Trager and Jensen (1971). Quantitative assessment of antiplasmodial activity *in vitro* was determined via the parasite lactate dehydrogenase assay using a modified method described by Makler et al. (1993). The samples were prepared to a 2 mg/ml stock solution in 10% Dimethyl sulfoxide (DMSO) or 10% methanol and sonicated to enhance solubility. Stock solutions were stored at -20℃. Fur ther dilutions were prepared on the day of the experiment. Chloroquine (CQ) was used as the reference drug in all experiments. Test samples were tested at three concentrations which were 20 µg/ml. 10 and 5 µg/ml. CQ was tested at three concentrations (30, 14 and 7.5 ng/ml). A full dose was performed on active samples to determine the concentration inhibiting 50% of parasite growth (IC_{50}-value). Test samples were tested at a starting concentration of 100 µg/ml, which was then serially diluted 2-fold in complete medium to give 10 concentrations; with the lowest concentration being 0.2 µg/ml. The same dilution technique was used for all samples. CQ was tested at a starting concentration of 100 ng/ml. The highest concentration of solvent to which the parasites were exposed to had no measurable effect on the parasite viability (data not shown). The IC_{50}- values were obtained using a non-linear dose-response curve fitting analysis via Graph Pad Prism v.4.0 software.

Cytotoxicity

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] cell proliferation assay (Mosman, 1983) was used to determine the cytotoxicity of the extracts against human embryonic kidney (HEK293) and human hepatocellular carcinoma (HepG2) cells. The cells were seeded in a 48-well plate at a density of 2.5 x 10^4 cells per well. Following an overnight incubation at 37℃, the cells were incubated with the compound at different concentrations (50, 100, 150, 200, 250, 300 and 350 µg/200 µl) in medium (MEM + Gutamax + antibiotics + 10% fetal bovine serum) for 48 h. Thereafter, the medium was removed from the cells and 200 µl MTT solutions (5 mg/ml phosphate buffer saline) as well as 200 µ l of cell culture medium was added to the corresponding wells. The cells were incubated at 37℃ for 4 h and the reaction was termi nated by addition of DMSO (100, 200 and 400 µl). The cells viability was determined spectrophotometrically (Biomate spectrophotometer) at 570 nm. The experiment was done in triplicate- and the results were expressed as mean±SD. Lethal concentration of the compound that results in 50% cell death (LC_{50}) was determined by regression analysis using Quantum electrodynamics (QED) statistics programmer.

Larvicidal

Larvae of *Culex quinquefascitus* were collected from the Hatchery unit of the Department of Zoology, University of Zululand, KwaDlangezwa. They were maintained at ambient rearing conditions in the environmental room. All bioassays were conducted at 28±1℃, 60.0±5 R H and 12 h dark photop eriod. A 10% yeast suspension was used as food source. Test for mosquito larval activity was conducted with some modifications using the method of Cheng et al. (2004) and Rafikali et al. (2001). Ten fourth-instar mosquito larvae were collected with a Pasteur pipette, placed on a filter paper to remove excess water and transferred to the Petri-dishes (100 ml) each containing 29.0 ml of degassed distilled water and 1000 µl of different concentrations of plant extract (10 to 250 µg/ml) in 1% DMSO with a tiny brush. Each Petri-dish was shaken lightly to ensure a homogenous test solution and was left at room temperature. Each test was performed in triplicate. The control was prepared with 29.0 ml of degassed distilled water and 1000 µL of DMSO solution without the plant extract to which larvae were added. Observation on larvae mortality was recorded after 24 h exposure, during which no food was given to the larvae. Larvae were considered dead, when they did not react to touching with a needle. The percentage of mortality and lethal concentrations (LC_{50}) values were determined using Abbots formula and probit analysis program, version 1.5, respectively.

Statistical analysis

Data were analysed using one-way analysis of variance (ANOVA) followed by Turkey-Kramer multiple comparison test. The results were presented as mean ± SEM. Statistical difference was accepted at p<0.05. Student's t-test was used to analyse statistical difference between control and treated groups.

RESULTS AND DISCUSSION

Antioxidant activity

In severe malaria, the red blood cell membrane is exposed to a local increase in oxidative stress (Griffiths et al., 2001). It is thus suggested (Griffiths et al., 2001; Ngouela et al., 2006) that compounds exhibiting both anti-plasmodial and antioxidant activities could be leads for new antimalarial drugs. Table 1 shows the antioxidant (free radicals scavenging and Fe^{2+} chelating) activity of the plants extracts. The extracts exhibited a concentration dependent antioxidant activity. However, except the *E. transvalense* and the *V. adoensis* (leaves) extracts (IC_{50} of 0.7 µg/ml and 04 µg/ml, respectively) all the other extracts were poorer scavengers of DPPH and ABTS than the standards (Antioxidant activity (AA) and butylated hydroxy toluene BHT). On the other hand, most of the extracts showed relatively better ability to

Table 1. Antioxidant (free radical scavenging and Fe $^{2+}$ chelating) activity (IC_{50}) of plants' methanol extracts.

Plant name	Activity IC_{50} µg/ml					
	DPPH	ABTS	Fe^{2+} chelating	SO	OH	N O
H. hemerocallidea	1.13	5	5	2.6	0.8	2.1
A. australe	3.2	5	5	0.84	0.82	2.05
V. adoensis (roots)	2.3	5.2	5	0.70	0.78	3.4
V. adoensis (leaves)	4	1.04	4	2.68	0.88	1.57
A. calamus	2.8	>5	>5	1.8	3.0	4.2
E. transvalense	0.7	4.1	3.9	1.6	3.6	3.6
W. somnifera	2.2	4.6	4.3	0.8	1.2	4.7
G. thunbergia	6.39	5.58	7.48	16.47	4.35	20.2
S. aethiopicus	3.62	13.72	>5	8.58	>5	15.26
S. brachypetala	8.12	11.00	>5	0.82	7.21	4.45
BHT	0.7	3	NA	2.46	1.37	2.74
Ascorbic acid	0.5	5	NA	0.84	3.7	3.76
EDTA	NA	NA	0.6	NA	NA	NA
Citric acid	NA	NA	2.9	NA	NA	NA

Table 2. Total phenol, Proanthocyanidin, flavonoids (mg/g) and SH (µg/g) content of the plants.

Plant	SH Content (µg/g)	Total phenol (mg/g)	Proanthocyanidin (mg/g)	Flavonoid (mg/g)
H. hemerocallidea	1.2	0.12	0.32	0.07
A. australe	0.8	0.13	0.17	0.09
V. adoensis (roots)	1.58	0.16	0.05	0.07
V. adoensis (leaves)	0.43	0.05	0.21	0.02
A. calamus	2.57	0.051	0.11	0.107
E. transvalense	0.36	0.035	0.25	0.098
W. somnifera	2.74	0.051	0.21	0.050
G. thunbergia	1.35	0.17	0.16	0.07
S. aethiopicus	$1.25x10^{-03}$	0.22	0.11	0 .15
S. branchypetala	7.25	0.36	2.74	0.12

scavenge the biological free radicals (SO, OH and NO) than the standards. Most of the plant extracts did not show any potential to inhibit Fe^{2+} chelating activity. However, the relatively high content of SH, phenol, proanthocyanidin and flavonoids (Table 2) suggests that the plants in this study have the potential of combating oxidative stress (Moure et al., 2001). Two inhibitors of NO production have been isolated from plants that are used for their insecticide activity (Hossay et al., 2011).

Larvicidal, cytotoxicity and *in vitro* antiplasmodial activity

The control of mosquitoes and thus malaria and other related diseases, is a major concern of both governmental and non-governmental organizations. Despite all the different control measures malaria still remains a threat to the human race. It is envisioned that the only successful method of reducing mosquito densities to an appreciable level for which a malaria epidemic can be controlled is by attacking the larval breeding places through the use of larvicides (WHO, 1996). Plants generally offer an alternative source of insect control agents, because they are available locally, cheap and contain a wide range of bioactive chemicals, which have little or no harmful effect on non-target organisms and the environment. The results of the bioactivity of the extracts are presented in Table 3. The efficacies of the extracts were observed to be dose dependent. No larvae emergence into pupae or adult was observed within 24 h exposure with the treated groups, but the larvae developed into pupae and then adult within 24 to 48 h in the control experiment. The plant extracts either killed or reduced spontaneous movement in *C. quinquefascitus* larvae after 24 h following treatment.

The results of the percentage mortality of the fourth-instar larvae of *C. quinquefascitus* indicate that the aqueous extracts of the plants had little or no larvicidal activity. The dichloromethane extracts were the most

Table 3. The Larvicidal, *in vitro* antiplasmodial activity, and cytotoxicity of plants extracts.

Plant extract	Larvicidal activity (µg/ml)		Antiplasmodial activity (µg/ml)		MTT assay LC_{50} (µg/ml)	
	% mortality	IC_{50}	Activity	IC_{50}	Human embryonic kidney cells	Human hepatocellular carcinoma cells
A. calamus						
Dichloromethane	64	10.98	NA		ND	ND
Methanol	58	11.6	NA		ND	ND
Aqueous	37	23.67	NA		ND	ND
E. transvalense						
Dichloromethane	60	18.18	5 µg/ml	5.07	512	394
Methanol	47	9.78	NA		ND	ND
Aqueous	35		NA		ND	ND
W. somnifera						
Dichloromethane	77	14.78	5 µg/ml	4.94	635	911
Methanol	80	22.88	NA		ND	ND
Aqueous	33	47.66	NA		ND	ND
H. hemerocallidea						
Dichloromethane	40		NA		ND	ND
Methanol	57		NA		ND	ND
A. australe						
Dichloromethane	65		5 µg/ml	1.04	534	512
Methanol	60		NA		ND	ND
***V. adoensis*(roots)**						
Dichloromethane	40		10-20 µg/ml	NA	ND	ND
Methanol	43		NA		ND	ND
V. adoensis (leaves)						
Dichloromethane	80		NA		ND	ND
Methanol	73		5 µg/ml	2.90	361	421
S. brachypetala						
Dichloromethane	74		10-20 µg/ml	NA	ND	ND
Methanol	60		NA		ND	ND
Aqueous	30	84.56	NA		ND	ND
G. thunbergia						
Dichloromethane	52		10-20 µg/ml	NA	ND	ND
Methanol	77		<10 µg/ml	4.36	363	338

Table 3. Contd.

Aqueous	0	0	NA		ND	ND
S. aethiopicus						
Dichloromethane	66		10-20 µg/ml	NA	ND	ND
Aqueous	30		NA		ND	ND

(ND=Not determined and NA=not active).

Table 4. The antipyretic activity of (100 mg/kg) plants extracts.

Name	Plant extract	Treatment (mg/kg)	Induced temp	Minutes after plant extract feeding			
				30	60	120	240
CMC	Control	100	37.5±0.06	37.8±0.1	37.6±0.1	37.4±0.1	37.5±0.1
Panado	Standard	100	37.6±0.2	36.5±0.1***	36.0±0.1***	35.6±0.1***	36.0±0.1***
A. calamus	Methanol	100	37.4±0.06	37.1±0.13*	37.2±0.2	37.1±0.3	37.0±0.2
A. calamus	DCM	100	37.8±0.3	37.5±0.4	37.8±0.7	37.6±0.6	37.8±0.1
E. transvalense	Methanol	100	38.3±0.2	38.4±0.3*	38.3±0.3*	38.1±0.19**	38.0±0.15**
E. transvalense	DCM	100	37.7±0.1	37.9±0.2*	37.6±0.2*	37.8±0.1*	37.5±0.3*
W. somnifera	DCM	100	37.3±0.3	36.2±0.2*	35.7±0.7*	35.5±0.2*	36.0±0.4*
W. somnifera	Aqueous	100	37.6±0.1	37±0.3*	36.7±0.4*	36.3±0.5*	37.0±0.2*
A. Australe	DCM	100	37.9±0.1	36.4±0.2*	37.6±0.0*	37.3±0.5	37.8±0.0
V. adoensis (leaves)	Methanol	100	38.5±0.2	38.2±0.2*	37.8±0.8*	37.5±0.2*	37±0.4*
V. adoensis (leaves)	Aqueous	100	39±0.1	38±0.2*	37.9±0.48*	37.7±0.2*	37±0.3*
V. adoensis (roots)	Aqueous	100	37.9±0.0	37.8±0.3	37.8±0.6	37.6±0.8	37.8±0.1
S. brachypetala	Aqueous	100	38.4±0.08	37.1±0.13*	37.2±0.2	37.1±0.3	37.0±0.3
G. thunbergia	DCM	100	38.4±0.2	37.5±0.4	37.8±0.7	37.6±0.6	37.8±0.6

(Data represents the mean ± SE of the experiment. *P< 0.05, **P< 0.001, ***p<0.0001).

efficient (≥ 50% mortality) of all the extracts. The larvicidal activity of some plants has been reported (Howard et al., 2011); however, such plants were not reported to exhibit any antiplasmodial activity.

The results of the antiplasmodial studies show that the methanol extracts of *A. australe* showed activity between 5 and 10 µg/ml. The dichloromethane extracts of *V. adoensis, S. aethiopicus, S. brachypetala,* and *G. thunbergia* and the methanol extracts of *A. calamus* showed activity between 10 to 20 µg/ml. The dichloromethane extracts of *A. australe, E. transvalense* and *W. somnifera* and methanolic extracts of *V. adoensis* (leaves) and *G. thunbergia* were the most active. The IC_{50}-values of 1.04 to 5.07 µg/ml were obtained for these extracts. These samples were therefore selected to determine their cytotoxicity levels against both human kidney (HEK293) and human hepatocellular carcinoma (HepG2) cell lines. It is worth noting that *A. australe* extracts exhibited the most active antioxidant and antiplasmodial properties.

Antipyretic activity

Most of the plant extracts exhibited the potential to reduce pyrexia in the induced rats (Tables 4, 5 and 6). The activity was time and concentration

Table 5. The antipyretic activity of (500 mg/kg) plants extracts.

Name	Plant extract	Treatment (mg/kg)	Induced temp	Minutes after plant extract feeding			
				30	60	120	240
Panado	Standard	1000	37.6±0.2	36.5±0.1***	36.0±0.1***	35.6±0.1***	36.0±0.1***
CMC	Control	1000	37.5±0.06	37.8±0.1	37.6±0.1	37.4±0.1	37.5±0.1
A. calamus	Methanol	500	37.5±0.06	37.8±0.1	37.6±0.1	37.4±0.1	37.5±0.1
A. calamus	DCM	500	38.6±0.1	37.9±0.02*	37.5±0.3*	37.5±0.5	37.7±0.2*
E. transvalense	Methanol	500	38.6±0.16	38.3±0.2**	38.2±0.18**	37.9±0.14**	38.1±0.2**
E. transvalense	DCM	500	38.0±0.2	37.9±0.2*	37.5±0.4*	37. ±0.6**	37.1±0.3*
W. somnifera	DCM	500	37.4±0.3	36.3±0.3*	36.6±0.5*	36.5±0.3*	35.3±0.3*
A. australe	DCM	500	38.7±0.3	36.8±0.2*	37.7±0.2*	37.3±0.3*	38.3±0.1*
W. somnifera	Aqueous	500	38.7±0.3	36.6±0.2*	36.3±0.2*	38.4±0.3*	38.3±0.3*
V. adoensis (leaves)	Methanol	500	38.6±0.5	38.5±0.3*	37.6±0.6 *	37±0.1*	36±0.2*
V. adoensis (leaves)	Aqueous	500	38.9±0.1	38.7±0.5*	38±0.6 *	37.5±0.1*	37.2±0.7*
V. adoensis (roots)	Aqueous	500	38±0.1	38±0.1*	37.9±0.3*	37.8±0.8	37.9±0.3*
G. Thunbergia	DCM	500	35.7±0.4	35±0.4*	34.4±0.1*	35.6±0.2	35±0.3*
S. brachypetala	Aqueous	500	38±0.2	37±0.1*	34.9±0.3*	38.8±0.3	35.9±0.3*

(Data represents the mean ± SE of the experiment. *P< 0.05, **P< 0.001, ***p<0.0001).

Table 6. The antipyretic activity of (1000 mg/kg) plants extracts.

Name	Plant extract	Treatment (mg/kg)	Induced temp	Minutes after plant extract feeding			
				30	60	120	240
CMC	Control	1000	37.5±0.06	37.8±0.1	37.6±0.1	37.4±0.1	37.5±0.1
Panado	Standard	1000	37.6±0.2	36.5±0.1***	36.0±0.1***	35.6±0.1***	36.0±0.1***
A. calamus	Methanol	1000	37.7±0.1	37.1±0.13*	37.1±0.06*	36.8±0.1**	36.7±0.1 ***
A. calamus	DCM	1000	36.9±0.4	37.6±0.1*	37.5±0.04*	37.6±0.3	37.8±0.3**
E. transvalense	Methanol	1000	38.7±0.12	37.8±0.3**	37.6±0.04**	37.2±0.3**	37.6±0.3**
E. transvalense	DCM	1000	38.3±0.3	37.8±0.2*	37.6±0.2*	36.5±0.2*	36.4±0.1*
W. somnifera	DCM	1000	36.5±0.4	36.1±0.1*	37.2±0.04*	36.1±0.2*	35.8±0.5*
W. somnifera	Aqueous	1000	37.2±0.1	38.8±0.5*	37.8±0.3	38.5±0.5*	38.4±0.4*
A. australe	DCM	1000	38.2±0.1	37.9±0.5*	37.7±0.3	38±0.5*	38±0.4*
V. adoensis (leaves)	Methanol	1000	38.7±0.7	38.3±0.1*	37.2±0.5*	35±0.0	35±0.6*
V. adoensis (leaves)	Aqueous	1000	38.2±0.5	37±0.3*	37.2±0.6*	36.6±0.6*	36.3±0.1*
V. adoensis (roots)	Aqueous	1000	37.9±0.3	37.7±0.1	37.5±0.4*	37.6±0.4	37.5±0.2*
S. branchypetala	Aqueous	1000	38.7±0.1	37.1±0.13*	37.1±0.06*	36.8±0.1**	36.7±0.1 ***
G. Thunbergia	DCM	1000	37.9±0.5	37.6±0.1*	37.5±0.04*	37.6±0.3	37.8±0.3**

(Data represents the mean ± SE of the experiment. *P< 0.05, **P< 0.001, ***p<0.0001).

dependent with most of the extracts showing activity as early as from 30 min even at the lowest concentration (100 mg/kg). *E. transvalense* methanol extract showed significant (P< 0.001) activity that was comparable to that of the reference drug (Paracetamol). Fever is the early symptom of malaria. Even though fever may be caused by other infections, the onset of fever is easily attributed to malaria infection in folk medicine. Most of the extracts in this study did not exhibit antiplasmodial activity but showed antipyretic properties. It is likely that traditional healers use these plants to treat the symptoms rather than the plasmodium infection.

In conclusion, we found that *A. australe, G. thumbergia, W. somnifera*, and *E. transvalense* possessed components that showed anti-plasmodial activity. With the relatively high anti pyretic, antioxidant and larvicidal activity, coupled to the weak levels of cytotoxicity, the plants could be candidates for the isolation and characterization of compounds that could be used in the management of malaria.

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