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In-vivo antiplasmodial and antipyretic activities of *Smilax krausiana*

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Abstract

Antiplasmodial and antipyretic activities of whole plant extract and fractions of *Smilax krausiana* were evaluated to ascertain the folkloric claim of its antimalarial and antipyretic activities. The crude extract (24–72 mg/kg) and fractions (48 mg/kg) of *Smilax krausiana* were investigated for antiplasmodial activity against chloroquine-sensitive *Plasmodium berghei* infections in mice and for antipyretic activity against dinitrophenol and yeast-induced pyrexia. The antiplasmodial activity during early and established infections as well as prophylactic were investigated. Artesunate (5 mg/kg) and pyrimethamine (1.2 mg/kg) were used as positive controls. Thin films made from tail blood of each mouse were used to assess the level of parasitaemia of the mice. Antipyretic activity of the crude extract was also evaluated against dinitrophenol and yeast-induced pyrexia. The extract and its fractions dose-dependently reduced parasitaemia induced by chloroquine-sensitive *Plasmodium berghei* infection in prophylactic, suppressive and curative models in mice. These reductions were statistically significant ($p<0.001$). They also improved the mean survival time (MST) from 10 to 21 days relative to control ($p<0.01$–0.001). The activities of extract/fractions were comparable to that of the standard drugs used (artesunate and pyrimethamine). On pyrexia induce by dinitrophenol and yeast, the extract exerted considerable inhibitions especially in yeast-induced pyrexia. These inhibitions in yeast-induced pyrexia were statistically significant ($p< 0.05$-0.001) and in a dose-dependent fashion. The antiplasmodial and antipyretic effects may in part be mediated through the chemical constituents of the plant.

Keywords: *Smilax krausiana,* antiplasmodial, antipyretic, *Plasmodium berghei*

Introduction

Smilax krausiana (Smilaceae) is a tropical weed that is distributed from West Africa to South Africa especially in the rain forest zone. It is an evergreen shrub or semi-shrub with climbing branches and stapler tendrils (Inyang, 2000; Inyang 2003). The leaf is widely use in East Africa for the treatment of infertility especially in human and veterinary medicine, while the Ibibios of Niger Delta of Nigeria use the leaf in the treatment of inflammatory diseases

such as haemorrhoids as well as joint and stomach pains and the root as antidote (Inyang, 2003). The Yorubas of Western Nigeria use the root as febrifuge and malaria remedy (Odugbemi and Akinsurie, 2007). Reports of acute toxicity potential (Nwafor *et al.,* 2006) and anti-inflammatory and analgesic activities (Nwafor et al., 2010) have been published. We report in this study the antiplasmodial and antipyretic activities of the root extract and fractions of the plant to confirm the tradomedical use of the plant as malarial remedy.

Materials and methods

Plant material

Roots of *Smilax krausiana* were collected in a farmland in Uruan, Akwa Ibom State, Nigeria. The plant was identified and authenticated by Dr. Margaret Bassey, a taxonomist in the Department of Botany, University of Uyo, Uyo. Nigeria. A specimen voucher (UULHER, No. 44c) was made and deposited at the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, University of Uyo.

Extraction

The roots of the plant were washed and dried on laboratory table for 2 weeks. The dried roots were cut into small pieces and further reduced to powder. The powdered root (2 kg) was divided into two parts, one part (1kg) was macerated in 97% ethanol (3L) for 72 hours to give the crude ethanolic extract while the other part (1kg) was successively and gradiently macerated for 72 hours in 3L of each of these solvents; n-hexane, chloroform, ethyl acetate and methanol to give the corresponding gradient fractions of these solvents. The liquid filtrates were concentrated and evaporated to dryness in vacuo 40C using rotary evaporator. The yield of each extract was calculated. The dry extracts were stored in a refrigerator at -4°C until used for experiment reported in this study.

Animals

Albino Swiss mice (21-24g) of either sex were obtained from the University of Uyo animal house. They were maintained on standard animal pellets and water ad libitum.

Drug administration

The drugs (chloroquine and pyrimethamine), extract and fractions used in the antiplasmodial study were orally administered with the aid of a stainless metallic feeding cannula.

Microorganism

A chloroquine sensitive strain of *P. berghei berghei* (ANKA) was obtained from the National Institute of Medical Research (NIMER), Lagos and was maintained by subpassage in mice.

Parasite inoculation

Each mouse used in the experiment was inoculated intraperitoneally with 0.2ml of infected blood containing about 1 x 10^7 *P. berghei berghei* parasitized erythrocytes. The ino-

culum consisted of 5 x 10^7 *P. berghei berghei* erythrocytes per ml. This was prepared by determining both the percentage parasitaemia and the erythrocytes count of the donor mouse and diluting the blood with isotonic saline in proportions indicated by both determinations (Odetola and Basir, 1980).

Evaluation of antiplasmodial activity of the extract/fractions

Evaluation of suppressive activity of the extract and fractions (4-day test)

This test was used to evaluate the schizontocidal activity of the extract, fractions and chloroquine against early *P. berghei berghei* infection in mice. This was done as described by Knight and Peters (1980). Forty-eight mice were randomly divided into seven groups of six mice each. On the first day (D_O), the forty-eight mice were infected with the parasite and randomly divided into various groups. These animals were administered with the extract, fractions and artesunate. The mice group 1 were administered with the 24 mg/kg, the group 2, 48 mg/kg and group 3, 72 mg/kg of crude extract, groups 4, 5 and 6 were administered with the 48 mg/kg of the chloroform, ethyl acetate and methanol fractions respectively, while group 7 was administered with 5mg/kg of artesunate (positive control), and 10ml/kg of distilled water to group 8 (negative control) for four consecutive days ($D_0 - D_3$) between 8am and 9am. On the fifth day (D_4), thin blood film was made from tail blood. The film was then stained with leishman stain to reveal parasitized erythocytes out of 500 in a random field of the microscope. The average percentage suppression of parasitaemia was calculated in comparison with the controls as follows:

$$\frac{\text{Average \% parasitaemia in negative control - Average \% parasitaemia in positive group}}{\text{Average \% parasitaemia in negative control.}}$$

Evaluation of curative activities of extract and fractions (Rane's test)

This was used to evaluate the schizontocidal activity of the extract, fractions and artesunate in established infection. This was done as described by Ryley and Peters (1970). *P. berghei berghei* was injected intraperitoneally into another 48 mice on the first day (D_O). Seventy–two hours later (D_3), the mice was divided randomly into eight groups of six mice each. Different doses of the extract, 24 mg/kg, 48 mg/kg and 72 mg/kg were orally administered respectively to mice in groups 1-3. 48 mg/kg of the chloroform, ethyl acetate and methanol fractions were administered to groups 4, 5, and 6 respectively, 5 mg/kg/day of artesunate to the group 7 (positive control) and group 8 was given 10 ml/kg of distilled water (negative control). The extract, fractions and drugs were administered once daily for 5 days. Leishman's stained thin smears were prepared from tail blood samples collected on each day of treatment to monitor parasitaemia level. The mean survival time (MST) of the mice in each treatment group was determined over a period of 29 days ($D_0 - D_{28}$).

$$\text{No of days survived} \times 100 / \text{Total No. of days (29)} = \text{MST}$$

Evaluation of prophylactic or repository activities of extract and fractions

The repository activity of the extract, fractions and pyrimethamine (daraprim) was assessed by using the method described by Peters (1965). The mice were randomly divided

into eight groups of six mice each. Groups 1 - 3 were administered with 24, 48 and 72 mg /kg/day of the extract respectively, while group 4 – 8 were respectively given 48 mg/kg/day of the chloroform, ethyl acetate and methanol fractions, 1.2 mg/kg/day of pyrimethamine (positive control) and 10 ml/kg of distilled water (negative control). Administration of the extract/fraction/drug continued for three consecutive days ($D_0 – D_2$). On the fourth day (D_3) the mice were inoculated with *P. berghei berghei.* The parasitaemia level was assessed by blood smears seventy-two hours later.

Evaluation of antipyretic activity of the extract

2,4–Dinitrophenol (DNP) induced pyrexia

Adult albino rats (150 – 170 g) of both sexes fasted for 24 hours but allowed water *ad libitum* were used for the experiment. They were randomized into groups of 6 rats each. DNP (10 mg/kg, i.p) was administered to the rats after obtaining the basal rectal temperatures. Hyperthermia developed within 30 min of DNP administration. Different doses of extract (24,48 and 72 mg/kg i.p), aspirin (100 mg/kg) and distilled water (10 ml/kg, orally) were administered respectively to the treatment and control groups of animals. Rectal temperatures of the animals were obtained at an hour interval for 5 hrs (Backhouse *et al*., 1994; Winter *et al*., 1962; Mbagwu *et al*., 2007).

Yeast-induced pyrexia

Adult albino rats (140 – 180 g) of both sexes fasted for 24 hours but allowed water ad libitum were used for the experiment. They were randomized into groups of 6 rats each. At zero hour, the basal temperature of the rats was taken using digital clinical thermometer. Thereafter, each animal was administered subcutaneously with 20 % w/v aqueous suspension of yeast at a volume of 10 ml/kg (Gural *et al*., 1955,). At suitable intervals beginning one hour after yeast injection, rectal temperature of animals were taken, animals with increase of 1°C were selected and grouped for the study. The extract understudy was administered i.p. after the pyrogen at the dose of 24, 48 and 72 mg/kg to respective groups of rats. The control group received distilled water (10 ml/kg) and the reference group administered with ASA (100 mg/kg) both intraperitoneally. The rectal temperature of the groups was taken at 1hr interval for 5 hrs.

Statistical analysis

Data obtained from this work were analyzed statistically using ANOVA (One - way) followed by a post test (Tukey-Kramer multiple comparison test). Differences between means will be considered significant at 1% and 5% level of significance i.e $P \leq 0.01$ and 0.05.

Results

Effect on suppressive activity of ethanolic root extract and fractions of Smilax krausiana

The ethanolic root extract of *Smilax krausiana* produced a dose-dependent chemotherapeutic effect at the different doses employed in the study. The chemosuppressions were

10.44, 25.37 and 62.68 % for 24, 48 and 72 mg/kg/day doses respectively. The effects produced by the extract were statistically significant (P<0.001) relative to control though incomparable to that of standard drug (artesunate 5 mg/kg) with a chemosuppression of 77.61% (Table 1). The fractions exerted different levels of chemosuppression with methanol (48 mg/kg) exerting the highest effect with a chemosuppression of 68.65 %. This was followed by ethyl acetate and chloroform fractions (55.22 and 41.79 %) (Table 1).

Prophylactic activity of ethanolic root extract and fractions of Smilax krausiana

The ethanolic extract of *Smilax krausiana* exerted a dose-dependent prophylactic activity at the various doses employed resulting in significant (P< 0.05 - 0.001) reduction of parasitaemia in extract treated groups when compared to control. Chemotherapeutic effects of 5.37, 27.95 and 51.61 % were respectively recorded for the corresponding dose of extract (24, 48 and 72 mg/kg/day). The chemosuppressions exerted by the doses of the extract were incomparable to that of the standard drug, pyrimethamine with chemosuppression of 77.41 % (Table 2). The results of repository activities of the various *S. krausiana* root fractions are shown in table 2. Methanol fraction had the highest chemosuppression though incomparable to that of the standard drug, artesunate 5 mg/kg. This was followed by ethyl acetate and chloroform.

Curative activity of ethanolic root extract and fractions of Smilax krausiana

The extract and its fractions showed a dose-dependent schizonticidal effect on the parasitaemia similar to that of the artesunate-treated group. These effects were statistically sign-

Table 1. Suppressive activity of ethanolic root extract and fractions of *Smilax krausiana* on *Plasmodium berghei* infection in mice (4-day test).

Treatments	Dose (mg/kg)	Parasitaemia	% Chemosuppression
Normal saline	10ml/kg	67.0± 0.01	-
S. krausiana crude extract	24	60.0± 1.14[a]	10.44
	48	50.0±0.48[a]	25.37
	72	25.0±1.14 [a]	62.68
Chloroform fraction	48	39.0±2.27[a]	41.79
Ethyl acetate fraction	48	30.0±1.01 [a]	55.22
methanol fraction	48	21.0±0.48[a]	68.65
Artesunate	5.0	15.0± 0.76[a]	77.61

Values are expressed as mean ± SEM. Significance relative to control [a]p<0.01, [b]p<0.001, n = 6.

Table 2. Repository/Prophylactic activity of ethanolic root extract and fractions of *Smilax krausiana* on *Plasmodium berghei* infection in mice (4-day test)

Treatments	Dose (mg/kg)	Parasitaemia	% Chemosuppression
Normal saline	10ml/kg	93.0±0.79	-
S. krausiana crude extract	24	88.0± 1.45 [a]	5.37
	48	67.0±0.18 [b]	27.95
	72	45.0±1.62 [b]	51.61
Chloroform fraction	48	75.0±0.65 [b]	19.35
Ethyl acetate fraction	48	70.0±0.48 [b]	24.73
methanol fraction	48	38.0±1.43 [b]	59.13
Pyrimethamine	1.2	21.0±1.06 [b]	77.41

Values are expressed as mean ± SEM. Significance relative to control [a]p<0.05, [b]p<0.001, n = 6.

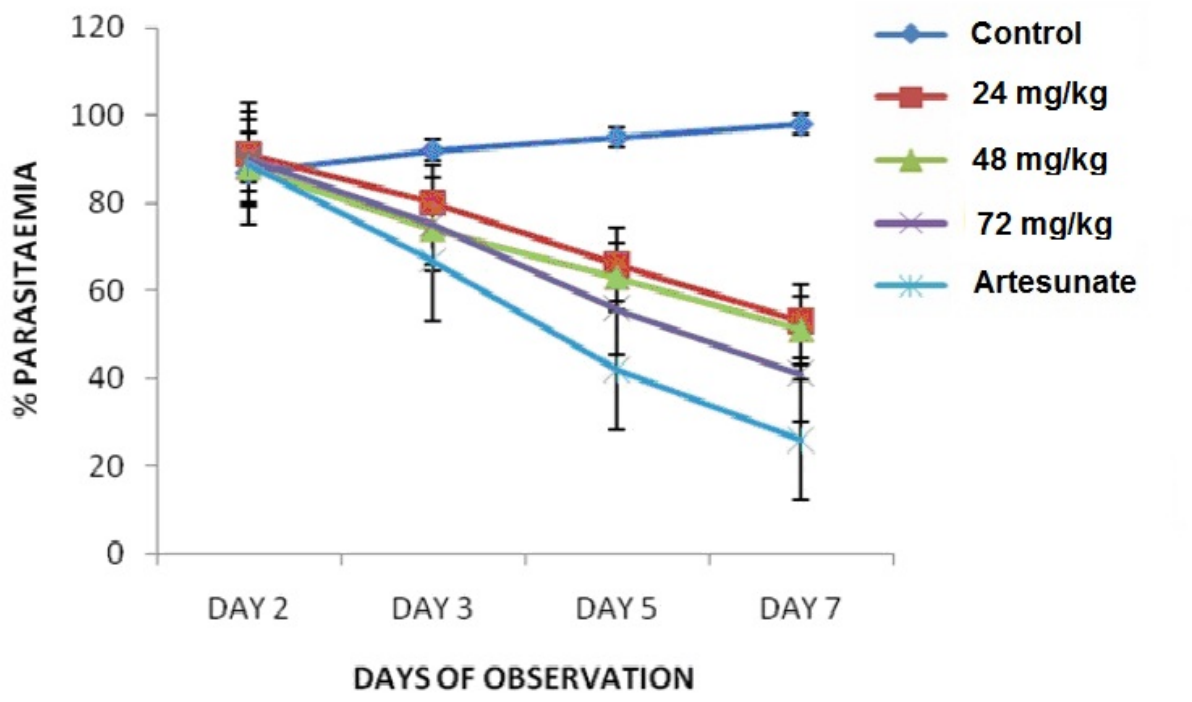

Figure 1: Curative activity of ethanolic crude root extract of *Smilax krausiana* on established infection.

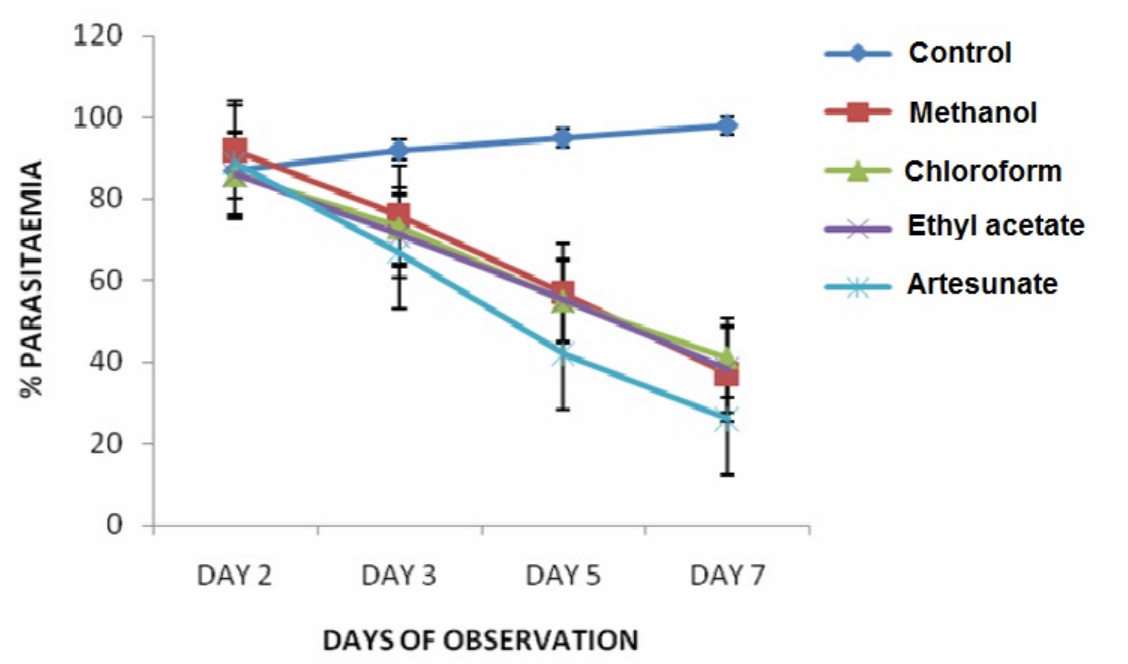

Figure 2: Curative activity of *Smilax krausiana* root fractions on established infection.

ificant relative to the control ($p<0.01$-0.001)(Figures 1 and 2). The control group showed daily increase in parasitaemia. The result of mean survival time (MST) is shown in Table 3.

Table 3. Mean survival time of mice receiving the various doses of ethanolic root extract and fractions of *Smilax krausiana* during established *P.berghei* infections in mice.

Treatments	Dose (mg/kg)	Parasitaemia
Normal saline	10ml/kg	10.75 0.42
S. krausiana crude extract	24	13.25 0.56a
	48	14.50 0.25 b
	72	16.25 0.67 b
Chloroform fraction	48	15.25 0.42b
Ethyl acetate fraction	48	17.25 0.42 b
methanol fraction	48	21.75 0.69 b
Pyrimethamine	5	25.25 0.20 b

Values are expressed as mean ± SEM. Significance relative to control [a]p<0.05, [b]p<0.001, n = 6.

Table 4. Effect of *Smilax krausiana* root extract on 2,4-dinitrophenol-induced pyrexia in rats.

Treatmen Dose (mg/kg))	Time interval (Hr)						
	Basal Temperature	0.5	1	2	3	4	5
Control	36.00± 0.18	37.42± 0.20	37.65 ± 0.44	38.12 ±0.33	38.22± 0.28	38.18 ± 0.24	38.10 ± 0.22
E. littorale 260	36.60± 0.55	37.48± 0.47	38.28 ± 0.23	38.45 ± 0.51	38.30 ± 0.33	37.90± 0.48	37.73 ± 0.32
E. littorale 520	36.85±0.14	37.70±0.28	37.53± 0.21	37.70 ± 0.30	37.53 ±0.24	37.62 ± 0.25	37.62 ± 0.15
E. littorale 780	36.77±0.24	37.37± 0.31	37.45± 0.40	37.60 ± 0.21	37.48 ± 0.23	37.42 ± 0.14	36.98 ±0.16[a]
E. littorale 100	36.88±0.28	37.52± 0.30	38.0 ± 0.27	37.75 ± 0.18	37.32 ± 0.18	37.23 ± 0.13	36.98 ± 0.12[a]

Values are expressed as mean ± SEM. Significance relative to control.[a]P<0.01, n =6

The MST of the extract- treated groups were significantly (P<0.01) longer than that of the control. Though both the extract and its fractions showed a significant dose-dependent mean survival time on established infection (p<0.001), the methanol fraction showed a greater protective effect followed by ethyl acetate fraction,crude extract and chloroform fraction. The mean survival time of the extract and fractions were incomparable to that of the standard drug, artesunate 5 mg/kg.

Effect of ethanolic crude root extract of Smilax krausiana on 2,4, dinitronitrophenol (DNP)-induced pyrexia in rats

The extract (24–72 mg/kg) demonstrated an insignificant dose-dependent lowering of temperature in DNP-induced pyretic rats. The antipyretic effect was however pronounced (p<0.001) at the 5h with the highest dose of the extract (72 mg/kg). The effect was comparable to that of the standard drug, ASA, 100 mg/kg (Table 4).

Effect of ethanolic crude root extract of Smilax krausiana on yeast-induced pyrexia in rats

Ethanolic crude extract of *Smilax krausiana* (24 – 72 mg/kg) exhibited a significant (p<0.05-0.001) dose-dependent lowering of rats' body temperature elevated by the administration of yeast. These effects were pronounced at the 4h and 5h post-treatment with extract The antipyretic effects of the extract were comparable to that of the standard, ASA, 100 mg/kg (Table 5).

Discussion

The antiplasmodial properties of the extract and fractions of *Smilax krausiana* were investigated using standard models. It was found that both the extract and its fractions sign-

Table 5. Effect of *Smilax krausiana* root extract on yeast-induced pyrexia in rats.

Treatmen Dose (mg/kg))	Time interval (Hr)						
	Basal Temperature	0.5	1	2	3	4	5
Control	36.30± 0.12	37.00± 0.23	37.92 ± 0.18	38.08 ±0.22	38.32± 0.20	38.57 ± 0.24	38.68 ± 0.29
E. littorale 260	37.05± 0.20	38.65± 0.28	37.60 ± 0.21	37.40± 0.22	37.33± 0.28	37.25±0.24[b]	36.85 ± 0.18[c]
E. littorale 520	37.00±0.23	38.45±0.26	37.30± 0.20	37.48± 0.30	37.18±0.26[a]	36.95 ± 0.15[c]	36.72 ± 0.16[c]
E. littorale 780	37.00±0.25	38.43± 0.15	37.97± 0.12	37.92± 0.24	37.80 ± 0.25	37.47 ± 0.22[a]	37.18 ±0.28[c]
E. littorale 100	36.65±0.84	38.35± 0.18	37.87 ± 0.18	37.83± 0.28	37.55± 0.30[b]	37.38± 0.21[b]	37.13 ± 0.20[c]

Values are expressed as mean ± SEM. Significance relative to control.[a]P<0.01, n =6

ificantly reduced the parasitaemia in prophylactic, suppressive and curative models in a dose-dependent fashion. Some secondary metabolites of plants are said to have antiplasmodial activity. Among these metabolites are flavonoids and triterpenoids such as quassinoids (Philipson and Wright,1991; Christensen and Kharazmi,2001; Kirby et al., 1989). Flavonoids are reported to chelate with nucleic acid base pairing of the parasite (Lui et al.,1992) and triterpenes like quassinoids are potent protein inhibitors (Liao et al.,1976). These compounds (flavonoids and triterpenoids) present in this plant extract may in part have contributed to the plasmocidal activity of this extract and therefore explained the mechanism of antiplasmodial effect of the extract and its fractions.

On antipyretic activity, the extract inhibited significantly dinitrophenol, amphetamine and yeast-induced pyrexia. Dinitrophenol induces hyperthermia by uncoupling oxidative phosphorylation causing release of calcium from mitochondrial stores and also prevent calcium reuptake. This results in increased level of intracellular calcium, muscle contraction and hyperthermia (Kumar *et al.*, 2002). Yeast induces pyrexia by increasing the synthesis of prostaglandins (Al-Ghamdi, 2001). The extract may in part reduced pyrexia by reducing brain concentration of prostaglandin E_2 especially in the hypothalamus through its action on COX-2 or by enhancement of the production of the body's own antipyretic substances like vasopressin and arginine (Chandrasekharan, 2002). The hypothermic activity of the extract could have also been mediated by vasodilatation of superficial blood vessels leading to increased dissipation of heat following resetting of hypothalamic temperature control center (Rang *et al*., 2007). This action may be due to the phytochemical compounds in this plant. Therefore, the temperature lowering activity of the extract may not be unconnected with the inhibition of one or combination of the mechanisms mentioned above. The results of this study demonstrated that *Smilax krausiana* possesses considerable antiplasmodial and antipyretic activities. These confirm its use to treat malaria and fever in folkloric medicine. Therefore, it would be interesting if the active principle is isolated, identified and characterized.

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Conflict of interest

There is no conflict asscociated with authors of the paper.

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