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Research Article

Antimalarial and analgesic activities of root extract of *Panicum maximum*

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Background: *Panicum maximum* is used as malarial remedy traditionally and the leaf extract has been found to possess antimalarial, analgesic and anticancer properties.

Objective: The ethanol root extract of *Panicum maximum* were evaluated for antiplasmodial and analgesic activities in rodents.

Methods: The crude root extract (137 – 547mg/kg) of *Panicum maximum* were investigated for antiplasmodial activity against chloroquine sensitive *Plasmodium berghei* infections in mice. The antiplasmodial activity during early and established infections as well as prophylactic were investigated. Artesunate 5mg/kg and pyrimethamine 1.2mg/kg were used as positive controls. Analgesic activity of the crude extract/fractions was also evaluated against acetic acid, formalin and heat-induced pains.

Results: The extract 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 13 to 28 days relative to control ($p < 0.001$). The activity of extract was weak compared to that of the standard drugs used (artesunate and pyrimethamine). On chemically and thermally- induced pains, the extract inhibited acetic acid and formalin-induced inflammation as well as hot plate-induced pain in mice. These inhibitions were statistically significant ($p < 0.001$) and in a dose-dependent fashion.

Conclusion: *Panicum maximum* root extract has antiplasmodial and analgesic activities which may in part be mediated through the chemical constituents of the plant.

Key words: *Panicum maximum*, analgesic, antimalarial

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1. Introduction

Panicum maximum Jacq (Poaceae) is a perennial, tuft grass with a short, creeping rhizome regarded as the most valuable fodder plant and extensively used to make hay. The stem of this robust grass can reach a height of up to 2m, the leaf sheath are found at the bases of the stems and are covered in fine hairs. It is widely distributed in Africa where it originates and almost all tropical parts of the world (Van Oudtshoorn, 1999). The plant (leaf) is use traditionally by the Ibibios of Akwa

Ibom State, Nigeria in the treatment of various ailments such as malaria, microbial infections, rheumatism pain, inflammation and diabetes. Antidiabetic (Antia et al, 2010), antimalarial and analgesic (Okokon et al, 2012), antibacterial (Gothandam et al, 2010; Doss et al, 2011a; Doss et al, 2011b), anti-inflammatory and antipyretic (Okokon et al, 2011) and antifungal (Kanife, 2012), anticancer, antioxidative burst, antileishmanial activities of the leaf extract have been reported (Okokon et al, 2014). Phytochemical components such as phytol, pentadecanoic acid, Hexadecanoic acid, dodecanoic acid,

8,11,14-eicosatrienoic acid (Z,Z,Z), mono and sesquiterpenes such as terpinen-4-ol, borneol and germanicol have been reported on the leaf extract (Okokon et al, 2014). Ethnopharmacological and scientific reports on the root of this plant is scarce. In this study, we investigated the antimalarial and phytochemical components of ethanol root extract of *Panicum maximum*.

2. Materials and Methods

2.1 Plants collection

The fresh roots of *Panicum maximum* were collected in August, 2015 at Farmland in Uyo, Uyo LGA, Akwa Ibom State, Nigeria. The roots were identified and authenticated as *Panicum maximum* a taxonomist in the Department of Botany and Ecological studies, University of Uyo, Uyo, Nigeria. Herbarium Specimen was deposited at the Faculty of Pharmacy Herbarium (FPH 76c), University of Uyo, Nigeria.

2.2 Extraction

The plant parts (roots) were washed and air-dried on laboratory table for 2 weeks. The dried roots were pulverized using a pestle and mortar. The powdered root was macerated in 95% ethanol for 72 hours. The liquid ethanol extract obtained by filtration was evaporated to dryness in a water bath at 60°C. The yield of the extract was stored in a refrigerator at -4°C until it was used for the experiment reported in this study.

2.3 Phytochemical Screening

Phytochemical screening of the crude root extract was carried out employing standard procedures and tests (Trease and Evans, 1996, Sofowora, 1993), to reveal the presence of chemical constituents such as alkaloids, flavonoids, tannins, terpenes, saponins, anthraquinones, reducing sugars, and cardiac glycosides and others.

2.4 Animals

The animals (Swiss albino mice) of either sex were used for these experiments. The animals were housed in standard cages and were maintained on a standard pelleted feed (Guinea feed) and water ad libitum. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo.

2.5 Microorganisms

A chloroquine sensitive strain of *Plasmodium berghei* (ANKA) was obtained from the National Institute of Medical Research (NIMR), Yaba Lagos, Nigeria and was maintained by sub-passage in mice.

2.6 Determination of Median Lethal Dose (LD₅₀)

The median lethal dose (LD₅₀) of the extract was estimated using albino mice by intraperitoneal (i.p) route using the method described by Lorke (1983). This involved intraperitoneal administration of different doses of the extract (1000, 2000, 2500, 3000, 4000 and 5000 mg/kg) to groups of five mice each. The animals were observed for manifestation of physical

signs of toxicity such as writhing, decreased motor activity, decreased body/limb tone, decreased respiration and death. The number of deaths in each group within 24 hours was recorded. The LD₅₀ was calculated as geometrical means of the maximum dose producing 0% (a) and the minimum dose producing 100% mortality (b).

$$LD_{50} = \sqrt{ab}$$

2.7 Parasite Inoculation

Each mouse used in the experiment was inoculated intraperitoneally with 0.2ml of infected blood containing about 1×10^7 *P. berghei berghei* parasitized erythrocytes. The inoculum consisted of 5×10^7 *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).

2.8 Drug Administration

The drug (artesunate), extract used in the antiplasmodial study were orally administered with the aid of a stainless metallic feeding cannula.

2.9 Evaluation of anti-plasmodial activity of ethanol crude root extract of *Panicum maximum*

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

This test was used to evaluate the schizontocidal activity of the extract and artesunate against early *P. berghei berghei* infection in mice. This was done as described by Knight and Peters (1980). Thirty mice were randomly divided into five groups of six mice each. On the first day (D₀), the 30 mice were infected with the parasite and randomly divided into various groups. These were administered with the extract and artesunate. The mice in group 1 were administered with the 137 mg/kg, the group 2, 273 mg/kg and group 3, 547 mg/kg of crude extract, while group 4 was administered with 5 mg/kg of artesunate (positive control), and 10ml/kg of distilled water to group 5 (negative control) for four consecutive days (D₀ - D₃) between 8am and 9am. On the fifth day (D₄), thin blood film was made from tail blood. The film was then stained with leishman stain to reveal parasitized erythrocytes 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:

$$\frac{\text{Average \% parasitaemia in negative control} - \text{Average \% parasitaemia in Positive groups}}{\text{Average \% parasitaemia in negative control}}$$

Evaluation of prophylactic or repository activities of extract

The repository activity of the extract and artesunate was assessed by using the method described by Peters (1965). The mice were randomly divided into seven

groups of six mice each. Groups 1 - 3 were administered with 137, 273 and 547 mg/kg/day of the extract respectively, Groups 4 and 5 were respectively administered with 1.2 mg/kg/day of pyrimethamine (positive control) and 10 ml/kg of distilled water (negative control). Administration of the extract/drug continued for three consecutive days (D₀ - D₂). On the fourth day (D₃) the mice were inoculated with *P. berghei berghei*. The parasitaemia level was assessed by blood smears seventy-two hours later.

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

This was used to evaluate the schizontocidal activity of the extract, and artesunate in established infection. This was done as described by Ryley and Peters (1970). *P. berghei berghei* was injected intraperitoneally into another 30 mice on the first day (D₀). Seventy-two hours later (D₃), the mice was divided randomly into five groups of six mice each. Different doses of the extract, 137 mg/kg, 273 mg/kg and 547 mg/kg were orally administered respectively to mice in groups 1-3. 5 mg/kg/day of artesunate was administered to the group 4 (positive control) and group 5 was given 10 ml/kg of distilled water (negative control). The extract 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 (D₀ - D₂₈).

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

2.10 Evaluation of analgesic potential of the extract

Acetic acid induced writhing in mice

Intraperitoneal (i.p) injection of 2% acetic acid was used to induce writhings (abdominal constrictions consisting of the contraction of abdominal muscles together with the stretching of hindlimbs) according to the procedure described by Santos et al. (1994), Correa et al. (1996) and Nwafor et al, (2010). The animals were divided into 5 groups of 6 mice per group. Group 1 served as negative control and received 10 ml/kg of normal saline, while groups 2, 3 and 4 were pre-treated with 137, 273 and 547 mg/kg doses of *P. maximum* root extract intraperitoneally, and group 5 received 100 mg/kg of acetyl salicylic acid. After 30 minutes, 0.2 ml of 2% acetic acid was administered intraperitoneally (i.p). The number of writhing movements was counted for 30 minutes. Antinociception (analgesia) was expressed as the reduction of the number of abdominal constrictions between control animals and mice pretreated with extracts.

Thermally induced pain in mice

The effect of extract on hot plate induced pain was investigated in adult mice. The hot plate was used to measure the response latencies according to the method of Vaz et al, (1996) and Okokon and Nwafor, (2010). In these experiments, the hot plate was

maintained at 45±1°C, each animal was placed into a glass beaker of 50 cm diameter on the heated surface, and the time(s) between placement and shaking or licking of the paws or jumping was recorded as the index of response latency. An automatic 30-second cut-off was used to prevent tissue damage. The animals were randomly divided into 5 groups of 6 mice each and fasted for 24 hours but allowed access to water. Group 1 animal served as negative control and received 10 ml/kg of normal saline. Groups 2, 3 and 4 were pre-treated intraperitoneally with 137, 273 and 547 mg/kg doses of *P. maximum* extract respectively, while group 5 animals received 100 mg/kg of acetyl salicylic acid intraperitoneally, 30 minutes prior to the placement on the hot plate.

2.11 Statistical analysis and data evaluation

Data obtained from this work were analyzed statistically using Students' t-test and ANOVA (One- or Two- way) followed by a post test (Turkey-Kramer multiple comparison test). Differences between means was considered significant at 1% and 5% level of significance, that is $P \leq 0.01$ and 0.05 .

3. Results

3.1 Phytochemical Screening

Phytochemical screening of the crude root extract was carried out employing standard procedures and tests revealed the presence of chemical constituents such as alkaloids, flavonoids, tannins, terpenes, saponins, cardiac glycosides among others.

3.2 Determination of Median Lethal Dose (LD₅₀)

The various doses employed in the study produced different degrees of mortality (Table 1). The median lethal dose (LD₅₀) was calculated to be 2738.1 mg/kg. The physical signs of toxicity included excitation, paw licking, increased respiratory rate, decreased motor activity, gasping and coma which was followed by death. These signs were dose dependent.

Table 1: Acute toxicity profile of ethanolic root extract of *Zea mays*

Doses (mg/kg)	Mortality
1000	0/3
2000	0/3
2500	0/3
3000	3/3
5000	3/3

3.3 Effect on suppressive activity of ethanol root extract of *P. maximum*

The extract showed a dose-dependent chemosuppressive effect on the parasitaemia. These effects were statistically significant relative to the control ($p < 0.05$ - 0.001). The chemoinhibitory percentages ranged from 38.71 to 75.80 (Table 2). However, the effect of the extract was still much lower

than that of artesunate, which exhibited a chemosuppression of 98.74% (Table 2).

3.4 Effect on repository activity of ethanol root extract of *P. maximum*

The ethanol root extract of *P. maximum* showed a dose-dependent chemosuppressive effect (35.47 – 54.04%) on the parasitaemia during prophylactic studies. These effects were statistically significant relative to the control ($p < 0.001$).

However these effects were still much lower than that of pyrimethamine which exhibited chemosuppression of 90.92 % (Table 3).

3.5 Antiplasmodial effect of ethanol root extract of *P. maximum* on established infection

The extract showed a dose- dependent schizonticidal effect on the parasitaemia. There were reductions in the

percentage parasitaemia of the extract/ artesunate-treated groups compared to that of the control in which prominent increases were recorded. These reductions were statistically significant relative to the control ($p < 0.05 - 0.001$) (Figure 1). Though the extract showed a significant ($p < 0.05 - 0.001$), dose -dependent mean survival time on established infection (12.66 – 15.66 days), the effect of the extract (137 – 547 mg/kg) was weak compared to that of the standard drug, artesunate (Table 4).

3.6 Effect of ethanol root extract of *P. maximum* on acetic acid-induced writhing in mice

The administration of *P. maximum* extract (137 , 273 and 547 mg/kg) demonstrated a dose-dependent reduction in acetic acid-induced writhing in mice. The reductions were statistically significant ($p < 0.05 - 0.001$) relative to control and the effect of the highest dose(547 mg/kg) at 30 minute was more than that of the standard drug, ASA (Table 5).

Table 2: Suppressive Activities of root extract of *Panicum maximum* (4-day test).

Drug/extract	Dose (mg/kg)	Parasitaemia	% Chemosuppression
Distilled water	10ml/kg	41.33 ± 6.67	-
Extract	137	25.33 ± 5.84 ^a	38.71
	273	21.33 ± 1.85 ^b	48.39
	547	10.00 ± 0.57 ^b	75.80
Artesunate	5	0.52 ± 0.01 ^b	98.74

Values are expressed as mean ± S.E.M. Significance relative to control: ^a P < 0.05; ^b P < 0.001; n=6

Table 3: Repository/Prophylactic activity of root extract of *Panicum maximum* on *Plasmodium berghei* infection in mice

Treatments	Dose(mg/kg)	Parasitaemia	% Chemosuppression
Normal saline	10ml/kg	14.66±0.66	-
Crude extract	137	9.46± 2.15 ^a	35.47
	273	7.40±5.68 ^a	49.52
	547	6.73 ± 3.11 ^a	54.09
Pyrimethamine	1.2	1.33±0.66 ^a	90.92

Values are expressed as mean ± SEM. Significance relative to control ^a P < 0.001. n = 6.

Table 4: Mean Survival Time (MST) of Mice receiving different doses of root extract of *Panicum maximum* during established infection

Drug/extract	Dose(mg/kg)	MST (days)
Distilled water	10ml/kg	11.66±0.88
Extract	137	12.66 ± 0.33
	273	14.00 ± 0.57 ^a
	547	15.66± 0.33 ^c
Artesunate	5	30.00 ± 0.00 ^c

Values are expressed as mean ± S.E.M. Significance relative to control: ^a P < 0.05; ^b P < 0.001, n=6.

3.7 Effect of ethanol root extract of *P. maximum* on thermally-induced pain in mice.

The extract (137, 273 and 547 mg/kg) exhibited a dose-dependent effect on thermally-induced pain in mice.

These inhibitions were statistically significant ($p < 0.05$) only at the highest dose of the extract (547 mg/kg) relative to the control but weak compared to that of the standard drug, ASA (100mg/kg) (Table 6).

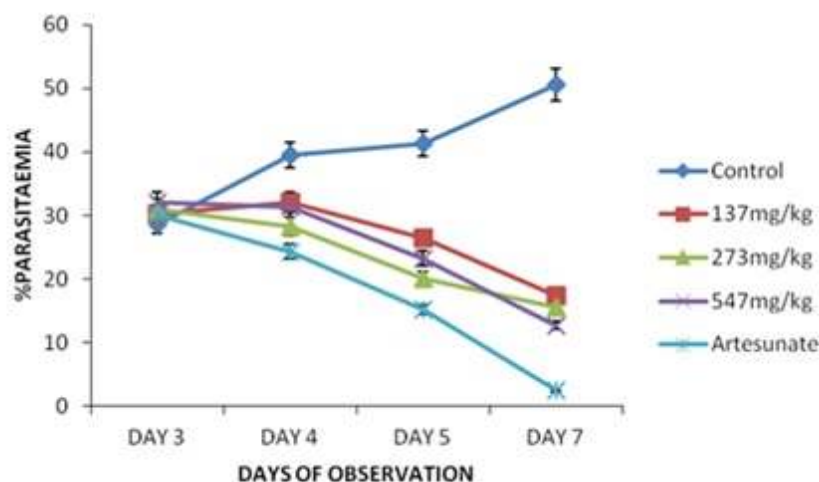


Figure 1: Antiplasmodial activity of root extract of *Panicum maximum* (Curative test).

Table 5: Effect of *P. maximum* root extract on acetic acid induced writhing in mice.

Treatment	Time intervals (hr)						Total
	5	10	15	20	25	30	
Control	8.32 ± 0.18	13.24 ± 1.11	22.66 ± 1.85	18.10 ± 0.38	15.45 ± 0.15	12.0 ± 1.10	89.77 ± 4.77
Extract							
137 mg/kg	4.33 ± 0.66 ^a	3.00 ± 1.12 ^a	10.53 ± 0.15 ^a	12.11 ± 1.20	10.38 ± 0.67 ^a	9.56 ± 0.35	49.91 ± 4.15 ^c
273 mg/kg	3.00 ± 0.57 ^c	2.33 ± 0.35 ^b	8.42 ± 1.65 ^b	10.24 ± 0.28 ^a	8.56 ± 1.58 ^b	7.15 ± 0.38 ^a	39.70 ± 4.81 ^c
547 mg/kg	2.00 ± 0.57 ^c	2.00 ± 0.57 ^b	8.12 ± 0.44 ^c	8.55 ± 0.46 ^b	5.00 ± 0.26 ^c	6.30 ± 0.76 ^b	31.97 ± 3.06 ^c
ASA 100 mg/kg	4.00 ± 0.51 ^a	3.00 ± 0.22 ^a	6.33 ± 0.18 ^c	7.89 ± 0.55 ^c	6.32 ± 0.16 ^c	4.00 ± 0.57 ^c	31.54 ± 2.19 ^c

Data are expressed as mean ± SEM. significant at ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$ when compared to control $n = 6$.

Table 6: Effect of *P. maximum* root extract on hot plate test

Group	Dose (mg/kg)	Reaction time (sec) (mean ± SEM)	% inhibition
Control	-	4.92 ± 0.23	
<i>P. maximum</i>	137	6.59 ± 0.27	33.94
	273	8.24 ± 0.11	67.47
	547	13.46 ± 0.21 ^a	173.57
	ASA	100	29.53 ± 3.48 ^c

Data are expressed as mean ± SEM. Significant at ^a $P < 0.05$, ^b $P < 0.001$ when compared to control. $n = 6$.

4. Discussion

In this work, median lethal dose (LD_{50}) was determined to be 2738.61 mg/kg, and the extract was found to be relatively safe with slight toxicity (Homburger, 1989).

The antiplasmodial activity of root extract of *P. maximum* was also investigated using standard models. It was found that the extract significantly reduced the parasitaemia in prophylactic, suppressive and curative models in a dose - dependent fashion.

These results corroborate our previously reported findings of significant antiplasmodial activity in the leaf extract of this plant (Okokon et al, 2011).

Some secondary metabolites of plants have been reported to have antiplasmodial activity. Among these metabolites are flavonoids, alkaloids and triterpenoids (Philipson and Wright, 1991; Christensen and Kharazmi, 2001; Kirby et al, 1989).

The root extract of *P. maximum* have been found to contain alkaloids, saponins, tannins, phlobatannins, flavonoids and cardiac glycosides among others. Also the leaf extract of *P. maximum* has been found to contain mono and sesquiterpenes such as terpinen-4-ol, borneol and germanicol (Okokon et al, 2014) which are likely to be present in the root. Flavonoids are known to exert antiplasmodial activity by chelating with nucleic acid base pairing of the parasite (Lui et al, 1992), thereby producing plasmocidal effect 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.

Phytochemical compounds such as terpenes and their derivatives such as monoterpenes and sesquiterpenes have been implicated in antiplasmodial activity of many plants (Philipson and Wright, 1991; Christensen and Kharazmi, 2001). Monoterpenes such as limonene have been implicated in endoperoxidation leading to plasmocidal activity (Hatzakis et al, 2000). These could have also contributed to the antiplasmodial activity of this extract

The extract significantly reduced acetic acid-induced writhing and delayed the reaction time of animals (mice) to thermally induced pain. Acetic acid causes inflammatory pain by inducing capillary permeability (Amico-Roxas et al, 1984; Nwafor et al, 2007), and in part through local peritoneal receptors from peritoneal fluid concentration of PGE₂ and PGF₂α (Deraedt et al, 1980; Bentley et al, 1983). The acetic acid-induced abdominal writhing is a visceral pain model in which the processor releases arachidonic acid via cyclooxygenase, and prostaglandin biosynthesis plays a role in the nociceptive mechanism (Franzotti et al, 2002). It is used to distinguish between central and peripheral pain. These results suggest that the extract may be exerting its action partly through the lipoxygenase and/or cyclooxygenase system.

The organic acid has also been suggested to induce the release of endogenous mediators indirectly, which stimulates the nociceptive neurons that are sensitive to NSAIDs and narcotics (Adzu et al, 2003). The inhibition of acetic acid-induced writhing by the extract at all the doses suggests antinociceptive effect which might have resulted from the inhibition of the synthesis of arachidonic acid metabolites.

The study also shows that the extract significantly delayed the reaction time of thermally- induced (hot plate) test. This model is selective for centrally acting analgesics and indicates narcotic involvement (Turner, 1995) with opioid receptors.

The root extract of *P. maximum* have been reported above to contain alkaloids, saponins, tannins, phlobatannins, flavonoids and cardiac glycosides among others which might have contributed to the observed analgesic activity in this study.

Flavonoids are known anti-inflammatory compounds acting through inhibition of the cyclo-oxygenase pathway (Liang et al, 1999). Some flavonoids are reported to block both the cyclooxygenase and lipoxygenase pathways of the arachidonate cascade at relatively high concentrations, while at lower concentrations they only block lipoxygenase pathway (Carlo et al, 1999). Some flavonoids exert their antinociception via opioid receptor activation activity (Suh et al, 1996; Rajendran et al, 2000; Otuki et al, 2005). Flavonoids also exhibit inhibitory effects against phospholipase A2 and phospholipase C (Middleton et al, 2000), and cyclooxygenase and/or lipoxygenase pathways (Robak et al, 1998).

Triterpenes have been implicated in analgesic activities of plants (Liu, 1995; Krogh et al, 1999; Taponjoui et al, 2003; Maia et al, 2006). Other secondary metabolites of plants such as ursolic acid, a selective inhibitor of cyclooxygenase-2 (Ringbom et al, 1998) and oleanolic acid which acts through an opioid mechanism, and possibly, modulates vanilloid receptors (Maia et al, 2006) have been reported to possess analgesic activities.

5. Conclusion

The result obtained in this study indicated that the root of *P. maximum* plant possesses a significant antiplasmodial and analgesic activities which justify the usage of this plant in the treatment of malaria and associated symptoms.

Conflict of Interest Declaration

The authors declare no conflict of interest.

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