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ANTINOCICEPTIVE AND ANTIPYRETIC ACTIVITY OF LEAF EXTRACT OF LASIANTHERA AFRICANA

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ABSTRACT

The leaf of *Lasianthera africana* is used commonly for nutritional purposes and medicinally in the treatment of fever, malaria and inflammatory diseases. The ethanol leaf extract of *Lasianthera africana* (500 - 1500 mg/kg) was evaluated for analgesic and antipyretic activities against acetic acid-induced writhing, formalin-induced hind paw licking, thermal – induced pain, amphetamine and yeast - induced pyrexia models. The dichloromethane fraction was also analyzed using Gas chromatography-mass spectrometry (GCMS). The leaf extract exhibited a dose-dependent inhibition of pains in the three experimental models and significant ($p<0.01$) inhibition of pyrexia induced by amphetamine and yeast. The GCMS analysis revealed the presence of pharmacologically active compounds which are likely responsible for the observed activities suggesting that the leaf extract possess analgesic and antipyretic properties.

KEYWORDS: *Lasianthera africana,* analgesic, antipyretic, vegetable, GCMS

INTRODUCTION

Lasianthera africana (P.Beav.) is a perennial glabrous shrub of the family Icacinaceae whose height may reach from 61 to 136 cm and is widely distributed in the tropical rain forest (Hutchinson and Dalziel,1973). There are four ethno varieties distinguished by their taste, leaf colour and ecological distribution. The leaves are majorly consumed as vegetable in southern Nigeria. Ethnobotanically, L. africana is used as antacid, analgesic, antispasmodic, laxative, antipyretic, antiulcerogenic, antidiabetic and antimalarial (Okokon et al., 2007). Lasianthera africana has been reported to possess bacteriostatic (Itah, 1997), fungicidal (Itah, 1996), antidiabetic (Ekanem, 2006), antiplasmodial (Okokon et al., 2007), antimicrobial (Andy et al., 2008), antiulcer (Okokon et al., 2009), immunomodulatory and antileishmanial activities (Okokon et al., 2013). The leaf whose LD50 has been reported to be 5000 mg/kg contain alkaloids, terpenes, saponins, tannins, flavonoids, anthraquinones and cardiac glycosides (Okokon et al., 2007). The present study was aimed at evaluating the analgesic and antipyretic activities of the crude ethanol extract of the dark green variety and chemical composition of the dichloromethane fraction.

MATERIALS AND METHODS

Plants collection

The plant material, Lasianthera africana (leaf), was procured from a market in Uyo metropolis, Akwa Ibom State, Nigeria in April, 2011. The plant was identified and authenticated by Dr. Magaret Bassey of Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria.

Extraction

The leaves were washed and shade-dried for two weeks. The dried plants' materials were reduced to powder. The powdered material was macerated in 70% ethanol. The liquid filtrates were concentrated and evaporated to dryness in vacuo 40□C using rotary evaporator. The crude ethanol extract (100 g) was further partitioned successively into 1L each of n-hexane, dichloromethane, ethyl acetate and butanol to give the corresponding fractions of these solvents.

Evaluation of analgesic potential of the extract

Acetic acid induced writhing in mice

The abdominal constrictions consisting of the contraction of abdominal muscles together with the stretching of hindlimbs was induced by intraperitoneal (i.p) injection of 2% acetic acid 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 500, 1000 and 1500 mg/kg doses of Lasianthera africana leaf extract intraperitoneally, and group 5 received 100 mg/kg of acetyl salicylic acid (ASA). 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.

Formalin – induced hind paw licking in mice

The procedure adopted was similar to that described by Hunskaar and Hole (1987), Correa and Calixto (1993), Gorki et al., (1993) and Okokon and Nwafor,(2010). The animals were injected with 20 µL of 2.5% formalin solution (0.9% formaldehyde) made up in phosphate buffer solution (PBS concentration: NaCl 137 mM, KCl 2.7 mM and phosphate buffer, 10 mM) subcutaneously under the surface of the right hind paw. The amount of time spent licking the injected paw was timed and considered as indication of pain. The first phase of the nociceptive response normally peaks 5 min after injection and the second phase 15 - 30 min after formalin injection, representing the neurogenic and inflammatory pain responses, respectively (Hunskaar and Hole, 1987). Adult albino mice (25 – 29 g) of either sex randomised into five groups of 6 mice each were used for the experiment. The mice were fasted for 24 hours before used but allowed access to water. The animals in group 1 (negative control) received 10 ml/kg of normal saline, groups 2 - 4 received 500, 1000 and 1500 mg/kg doses of the leaf extract, while group 5 received 100 mg/kg of acetyl salicylic acid 30 min before being challenged with buffered formalin. The responses were measured for 30 min after formalin injection.

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^{o}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 500, 1000 and 1500 mg/kg doses of Lasianthera africana leaf extract respectively, while group 5 animals received 100 mg/kg of acetyl salicylic acid intraperitoneally, 30 min prior to the placement on the hot plate.

Evaluation *of antipyretic activity of the extract*

D-amphetamine induced pyrexia

Adult albino rats (122 – 160 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. Amphetamine (5 mg/kg, i.p) was administered to the animals after obtaining basal temperatures. Hyperthermia developed 0.5 h following amphetamine administration. Different doses of extract (500, 1000 and 1500 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 h (Backhouse et al., 1994; Mbagwu et al., 2007).

Yeast-induced pyrexia

Adult albino rats (125 – 150 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, Okokon and Nwafor, 2010). 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 doses of 500, 1000 and 1500 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 1h interval for 5 h.

GC-MS analysis of dichloromethane fraction

Quantitative and qualitative data were determined by GC and GC-MS, respectively.

The fraction was injected onto a Shimadzu GC-17A system, equipped with an AOC-20i autosampler and a split/ splitless injector. The column used was an DB-5 (Optima-5), 30 m, 0.25 mm i.d., 0.25 µm df, coated with 5% diphenyl-95 % polydimethylsiloxane, operated with the following oven temperature programme: 50°C, held for 1 min, rising at 3°C/min to 250°C, held for 5 min, rising at 2°C/min to 280˚C, held for 3 min; injection temperature and volume, 250°C and 1.0 µl, respectively; injection mode, split; split ratio, 30:1; carrier gas, nitrogen at 30 cm/s linear velocity and inlet pressure 99.8 KPa; detector temperature, 280°C; hydrogen, flow rate, 50 ml/min; air flow rate, 400 ml/min; make-up (H2/air), flow rate, 50 ml/min; sampling rate, 40 ms. Data were acquired by means of GC solution software (Shimadzu).

Agilent 6890N GC was interfaced with a VG Analytical 70-250 s double -focusing mass spectrometer. Helium was used as the carrier gas. The MS operating conditions were: ionization voltage 70 eV, ion source 250°C. The GC was fitted with a 30 m x 0.32 mm fused capillary silica column coated with DB-5. The GC operating parameters were identical with those of GC analysis described above.

The identification of components present in the dichloromethane fraction of the plants' extract was based on direct comparison of the retention times and mass spectral data with those for standard compounds, and by computer matching with the Wiley 229 and Nist 21 Library, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literatures (Adams, 2001; Setzer et al., 2007).

Statistical analysis and data evaluation

Data obtained from this work were analyzed statistically using ANOVA (One- or Two- 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 of ethanol extract of leaf of Lasianthera africana on acetic acid-induced writhing in mice

The leaf extract of Lasianthera africana (500 – 1500 mg/kg) demonstrated a dose-dependent reduction in acetic acid-induced writhing in mice. The reductions were statistically significant ($p<0.001$) relative to control and comparable to that of the standard drug, ASA, at the highest dose, 1500 mg/kg. (Table 1).

Table 1: Effect of *Lasianthera africana* leaf extract on acetic acid -induced writhing in mice.

TREATMENT/ DOSE (mg/kg)	TIME INTERVALS (hr)						
	5	10	15	20	25	30	TOTAL
CONTROL	5.12 ± 0.96	9.32 ± 1.15	21.03 ± 1.12	19.33 ± 1.24	16.47 ± 0.43	12.14 ± 0.28	83.41 ± 5.18
EXTRACT							
500	0.00[c]	8.10 ± 0.32	12.23 ± 0.96[a]	13.35 ± 0.99[a]	11.12 ± 1.24	10.14 ± 0.31	55.02 ± 3.82[c]
1000	0.00[c]	8.20 ± 1.38	11.15 ± 0.34[a]	10.08 ± 0.36[a]	9.10 ± 0.42[a]	7.71 ± 1.35[a]	46.24 ± 3.85[c]
1500	0.00[c]	8.56 ± 1.22	11.10 ± 0.12[a]	10.45 ± 0.37[b]	8.13 ± 1.33[a]	5.62 ± 0.44[b]	43.86 ± 3.48[c]
ASA 100	0.00[c]	2.16 ± 0.16[b]	7.14 ± 0.93[c]	8.21 ± 0.54[b]	7.54 ± 0.96[b]	6.45 ± 0.52[b]	31.50 ± 3.11[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 2: Effect of *Lasianthera africana* leaf extract on formalin –induced hind paw licking in mice

TREATMENT/ DOSE (mg/kg)	TIME INTERVALS (MINS)						
	5	10	15	20	25	30	TOTAL
CONTROL	31.23 ± 0.24	14.17 ± 0.29	12.12 ± 0.46	10.35 ± 0.33	7.42 ± 0.24	5.04 ± 0.28	80.33 ± 1.84
EXTRACT							
500	27.56 ± 0.92	7.12 ± 0.36[c]	6.26 ± 0.89[c]	5.66 ± 0.84[c]	4.26 ± 0.16[c]	3.98 ± 0.32[c]	54.84 ± 3.49[c]
1000	22.21 ± 0.14[c]	5.16 ± 0.35[c]	4.22 ± 0.46[c]	4.32 ± 0.23[c]	3.12 ± 0.46[c]	2.24 ± 0.43[c]	41.27 ± 2.07[c]
1500	16.18 ± 1.36[c]	3.04 ± 0.68[c]	2.50 ± 0.18[c]	2.14 ± 0.51[c]	1.85 ± 0.22[c]	2.02 ± 0.56[c]	27.73 ± 3.51[c]
ASA 100	6.56 ± 0.18[c]	2.47 ± 0.71[c]	2.20 ± 0.16[c]	2.11 ± 0.26[c]	1.16 ± 0.16[c]	0.92 ± 0.10[c]	15.42 ± 1.57[c]

Data are expressed as mean ± SEM. Significant at [c]P < 0.001. when compared to control .n = 6.

Table 3: Effect of *Lasianthera africana* leaf extract on hot plate test

Group	Dose Mg/kg	Reaction time (sec) (mean ± SEM)	% inhibition
Control	-	5.22 ± 0.23	-
L. africana	450	7.21 ± 0.27[a]	38.12
	900	13.78 ± 0.87[a]	163.98
	1350	15.53 ± 0.68[b]	197.50
ASA	100	20.12 ± 0.93[b]	285.44

Data are expressed as mean ± SEM. Significant at [a]P < 0.05, [b] P < 0.01 when compared to control. n = 6.

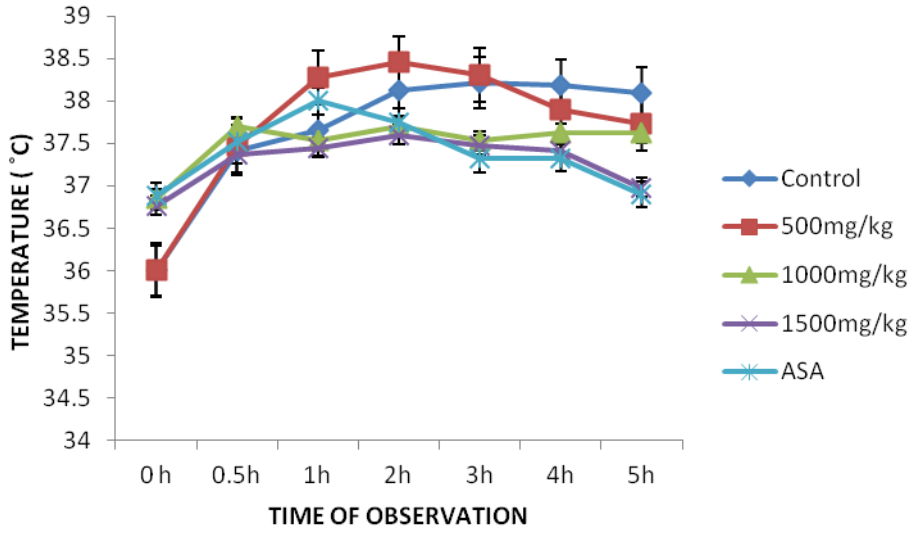

Figure 1: Effect of *Lasianthera africana* leaf extract on D-amphetamine induced pyrexia in rat

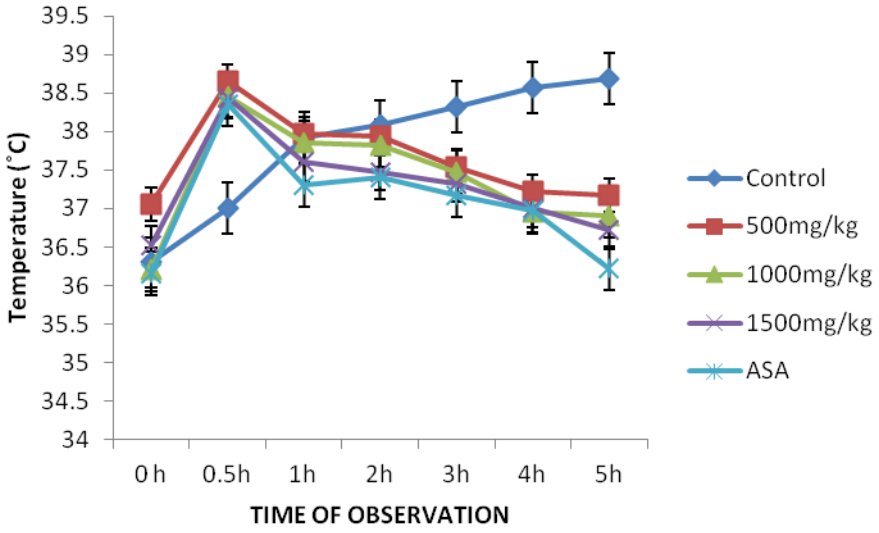

Figure 2: Effect of *Lasianthera africana* leaf extract on yeast-induced pyrexia in rat

TABLE 4: GC – MS analysis of dichloromethane fraction of *Lasianthera africana*

S/No.	NAME OF COMPOUND	MOL.WT	CHEMICAL FORMULA	RI
1.	4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	144	$C_6H_8O_4$	137
2.	3H-pyrazol-3-one,2,4-dihydro-4,4,5-trimethyl	126	$C_6H_{10}N_2O$	203
3.	Benzaldehyde, 4-hydroxy-,oxime	137	$C_7H_7NO_2$	576
4.	Desulphosinigrin	299	$C_{10}H_{17}NO_6S$	525
5.	Phenol, 4-(3-hydroxy-1-propenyl)2-methoxy	180	$C_{10}H_{12}O_3$	525
6.	9,10-anthracenedione, 2-methyl	222	$C_{15}H_{10}O_2$	654
7.	4-phenylisocoumarin	222	$C_{15}H_{10}O_2$	695
8	Camphene			949
9	Terpinene-4-ol	154	$C_{15}H_{16}O$	1176

Effect of ethanol leaf extract of Lasianthera africana on formalin-induced hind paw licking in mice

The leaf extract of Lasianthera africana exhibited a dose- dependent effect on formalin-induced hind paw licking in mice. This inhibition was significant relative to the control ($p<0.001$) and comparable to that of the standard drug, ASA, at the highest dose, 1500 mg/kg. (Table 2).

Effect of ethanol extract of leaf of Lasianthera africana on thermally-induced pain in mice

The extract exhibited a dose - dependent effect on thermally-induced pain in mice. This inhibition was statistically significant ($p<0.001$) relative to the control (Table 3).

Antipyretic test

D-amphetamine induced pyrexia

The antipyretic effect of the extract on amphetamine- induced pyrexia is shown in Figure 1. L. africana leaf extract (500, 1000 and 1500 mg/kg), in the presence of the pyrogen, caused a significant ($P<0.05$ – 0.001) reduction in the temperatures of the extract-treated rats when compared with the control. The antipyretic effect was dose-dependent and comparable to that of the standard drug, ASA (100 mg/kg).

Yeast-induced pyrexia

The results of the effect of leaf extract of Lasianthera africana (500, 1000 and 1500 mg/kg), on yeast-induced pyrexia in rats is shown in Figure 2. There was a dose-dependent reduction in the temperature of rats treated with the leaf extract. The reductions caused by the extract was significant ($P<0.05$ – 0.001) when compared to control and comparable to that of the standard drug, ASA (100 mg/kg).

GC-MS analysis

The GCMS analysis of the dichloromethane fraction of Lasianthera africana leaf revealed the presence of some bioactive compounds as represented in Tables 4.

DISCUSSION

The leaf of Lasianthera africana used by the Ibibios of Niger Delta of Nigeria mostly for nutritive purposes in the making of soup and treatment of various diseases traditionally; pain, fever, ulcer, constipation, diabetes and malaria as well as microbial infections, was evaluated for antinociceptive and antipyretic activities in rodent. The extract significantly reduced acetic acid-induced writhing, formalin-induced hind paw licking as well as 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 suggested 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.

Formalin- induced pains involves two different types of pains which are in phases; neurogenic and inflammatory pains (Vaz et al., 1996, 1997) and measures both centrally and peripherally mediated

activities that are characteristic of biphasic pain response. The first phase (0 to 5 min), named neurogenic phase resulted from chemical stimulation that provoked the release of bradykinin and substance P while the second and late phase initiated after 15 to 30 min of formalin injection resulted in the release of inflammatory mediators such as histamine and prostaglandin (Wibool et al., 2008; Yi-Yu et al., 2008). The injection of formalin has been reported to cause an immediate and intense increase in the spontaneous activity of C fiber afferent and evokes a distinct quantifiable behavior indicative of pain demonstrated in paw licking by the animals (Heapy et al., 1987). The first phase of formalin-induced hind paw licking is selective for centrally acting analgesics such as morphine (Berken et al.,1991), while the late phase of formalin-induced hind paw licking is peripherally mediated. Analgesic (nociceptive) receptors mediate both the neurogenic and non-neurogenic pains (Lembeck and Holzer, 1979). The extract ability to inhibit both phases of formalin-induced paw licking suggests its central and peripheral activities as well as its ability to inhibit bradykinins, substance P, histamine and prostaglandins which are mediators in these pains.

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 opiod receptors.

On antipyretic activity, the extract inhibited significantly amphetamine and yeast-induced pyrexia. Amphetamine acts on the brain causing the release of biogenic amines from their storage sites in nerve terminals resulting in increased level of cAMP and subsequent synthesis of prostaglandins from arachidonic acids produced in neurons by receptor-mediated hydrolysis of phospholipids (Westfall and Westfall, 2006). This leads to hyperthermia. 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 E2 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 GC-MS analysis of the dichloromethane fraction has revealed the presence of terpinen-4-ol which has been reported to suppress production of prostaglandin and in vitro of TNF-α, IL-1β, as well as IL-8, IL-10 and PGE2 by LPS-activated human blood monocytes (Hart et al., 2000; Miguel, 2010). These compounds may in part be responsible for the observed analgesic and antipyretic activities of the leaf extract.

The antinociceptive and antipyretic activities exerted by this extract may be attributed to the presence of phytochemical compounds as revealed by the GCMS analysis of the leaf extract. That the extract inhibited neurogenic and non-neurogenic pains as well as narcotic pains may in part explain the mechanisms of its action and these effects are due to the present of phytochemical components in the extract.

CONCLUSION

The results of this study demonstrated that Lasianthera africana possesses considerable analgesic and antipyretic activities. These confirm its use as analgesic and febrifuge in folkloric medicine. Therefore, it would be interesting if the active principle is isolated, identified and characterized.

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