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Analgesic activity of leaf extracts of *Culcasia scandens* P. Beauv

C O Okoli*, P A Akah & O N Egbuniwe

Department of Pharmacology & Toxicology, Faculty of
Pharmaceutical Sciences, University of Nigeria, Nsukka
Enugu State, Nigeria

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Analgesic activity of methanol leaf extract of *C. scandens* obtained by column chromatography and its graded solvent fractions, was evaluated in mice using acetic acid-induced abdominal writhing and formalin-induced paw licking. The extract and fractions significantly inhibited abdominal writhing and two phases of formalin-induced paw licking in mice, indicating that antinociceptive activity may involve inhibition of pain by peripheral and central mechanisms.

Keywords: Analgesic activity, *Culcasia scandens*

Inadequacies of available analgesic compounds make it imperative to develop more potent agents from available vast array of medicinal plants. *Culcasia scandens* P.Beauv (Araceae) is a tall climbing epiphyte¹⁻³ having anti-inflammatory activity⁴⁻⁶ and effective in toothache⁷. In the present study, an effort was made to elucidate the analgesic activity and evaluate the effect of the leaf extract and its fractions on central and peripheral pain mechanisms.

Fresh mature leaves of *C. scandens* were collected from plants growing on the trunks of palm trees, in Nanka, Orumba North L.G.A., Anambra State, Nigeria, and authenticated by Mr. A. O. Ozioko of Bioresources Development and Conservation Program (BDPC) Center, Nsukka, Enugu State, Nigeria. A voucher specimen (PC 97028) is preserved in the Pharmacy Herbarium, University of Nigeria, Nsukka.

The leaves were cleaned, sliced, dried in the open for 2 days and pulverized to coarse powder using a hand blender. The dry leaf powder (5 kg) was extracted by cold maceration in methanol for 48 h. Concentration of the methanol extract in a rotary evaporator under reduced pressure and subsequent freeze-drying afforded 584.85 g (12.13%) of the crude extract. The crude extract (500 g) was separated in a

dry-packed silica gel 60, 70-230 Mesh ASTM (EM Science) column successively eluted with ethylacetate (100 %) and methanol (100 %). The methanol range (ME; 120 g; 22.9%) was subjected to graded solvent separation using ethylacetate, chloroform, butanol, methanol, and water. The butanol and methanol fractions were concentrated in a rotary evaporator to afford 4.17 g (3.48%) of the butanol fraction (BF) and 45.8 g (38.17%) of the methanol fraction (MF). The extract and fractions were analyzed for phytochemical constituents using standard methods⁸ and subjected to antinociceptive tests in experimental animals.

Adult albino mice (15-25g) of either sex obtained from the laboratory animal facility of the Department of Pharmacology & Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka were used. They were housed in plastic cages within the facility and maintained on standard feeds (Bendel Feeds and Flourmills Ltd., Benin City, Nigeria) and provided drinking water *ad libitum*. The animals were acclimatized for two weeks and used according to the protocol approved by the University for care and handling of experimental animals.

Formalin-induced paw licking in mice—One hour after oral or 30 min after administration (ip) of test substances (ME, BF, MF: 200 or 400 mg/kg; n = 40 mice at 5 per dose level), 20 µl of 2.5 % formalin was injected into the paw of each animal. Duration of paw licking (s) was monitored 0-5 min (first phase) and 20-25 min (second phase) after formalin injection⁹. Control animals received either pentazocine (10 mg/kg; i.p) or equivalent volume of vehicle (3% v/v Tween 85) orally.

Acetic acid-induced abdominal writhing in mice—One hour after oral administration of test drugs (ME, BF, MF: 200 or 400 mg/kg orally; n = 40 mice at 5 per dose level) the pain stimulus was induced in mice by injection (ip) of 0.6% solution of acetic acid (10 ml/kg). The number of writhing by each mouse was counted for 20 min starting 10 min after injection of acetic acid¹⁰. Control animals received either acetylsalicylic acid (100 mg/kg) or equivalent volume of vehicle (3% v/v Tween 85) orally. Inhibition (%) was calculated as—Inhibition (%) = $\frac{[N_c - N_t]}{N_c} \times 100$; where, N_c = number of writhes by control animals; N_t = number of writhes by treated animals.

*Correspondent author
E-mail: charlesokoli@hotmail.com

Statistical analysis—Data obtained were analyzed using ANOVA and subjected to LSD post hoc test and the results expressed as mean \pm SEM. Differences between means were found to be significant at $P < 0.05$.

Phytochemical analysis—Extract and fractions of the leaves of *C. scandens* gave positive reaction for alkaloids, saponins, sterols, terpenoids, flavonoids, and carbohydrates.

Effect on formalin-induced paw licking—The methanol extract and fractions of the leaves significantly ($P < 0.05$) reduced the duration of paw licking induced by formalin in mice. The extract and fractions reduced paw licking in the first phase, while the fractions provoked 100% inhibition of the second phase response (Table 1). The fractions exhibited inhibitory effect greater than that of the extract and pentazocine (Table 1).

Effect on acetic acid-induced abdominal writhing—The methanol extract and fractions significantly ($P < 0.05$) reduced the number of abdominal writhing induced by acetic acid in mice (Table 2). The fractions caused greater inhibition than the extract and comparable to that of acetylsalicylic acid (Table 2).

Graded solvent fractionation of the methanol extract obtained from chromatographic separation of the leaf extract of *C. scandens* produced five solvent

fractions which were subjected to analgesic tests. The results of the study suggested that out of the five fractions, the butanol and methanol fractions exhibited high levels of analgesic activity by effectively inhibiting pain induced by acetic acid and formalin in mice. Abdominal constriction-induced by acetic acid is used to screen for peripheral analgesic effect¹¹ mediated by local peritoneal receptors¹². Effect of the extracts on acetic acid-induced abdominal writhing suggested that they might inhibit or modify responses to pain mediated by nociceptors peripherally. The formalin test distinguishes two (early and late) phases of pain, which can reveal mechanisms of pain and analgesia¹³. While centrally acting drugs such as narcotics could inhibit both phases equally¹⁴, the peripherally acting drugs like NSAIDs (e.g. aspirin, oxyphenbutazone, etc) only inhibit the late phase¹⁵⁻¹⁷ indicating a possible development of an inflammatory response and the release of algescic mediators¹⁵. The antinociceptive activity of the extracts in two phases of formalin-induced pain suggested that they might relieve pain by both peripheral and central mechanisms. The latter is consistent with our earlier findings, where the methanol extract increased reaction latency to thermal pain induced by the hot plate in mice⁷ that is a specific central antinociceptive test¹⁸. Although the second phase of the formalin-induced pain is attributed to activation of the inflammatory response, screening of these fractions for anti-inflammatory effect did not reveal any significant inhibition of inflammation (unpublished data) suggesting that these fractions may relieve pain through other antinociceptive mechanisms than suppression of the inflammatory response. Thus, there was a possibility

Table 1—Effect of extract and fractions on formalin-induced pain
[Values are mean \pm SE of 5 subjects]

Extract/fraction	Dose (mg/kg)	Duration of paw licking (s)	
		1 Phase (0-5 min)	2 Phase (20-25 min)
Control	-	180.0 \pm 11.5	120.0 \pm 11.5
ME	200	113.3 \pm 16.7* (37)	140.0 \pm 20.0 (NI)
	400	143.0 \pm 18.8 (21)	36.7 \pm 4.4* (69)
BF	200	80.0 \pm 10.0* (56)	00.0 \pm 0.0* (100)
	400	56.7 \pm 26.7* (69)	00.0 \pm 0.0* (100)
MF	200	61.7 \pm 6.0* (66)	00.0 \pm 0.0* (100)
	400	20.0 \pm 8.8* (89)	00.0 \pm 0.0* (100)
Pentazocine	10	96.7 \pm 18.6* (47)	56.7 \pm 8.8* (53)

NI = No inhibition; * $P < 0.05$ vs control (ANOVA; LSD post hoc test; n = 5). Values in parenthesis represent inhibition (%).

ME = Methanol extract; BF = Butanol fraction; MF = Methanol fraction.

Table 2—Effect of extract and fractions on acetic acid-induced pain in mice

Extract/fraction	Dose (mg/kg)	[Values are mean \pm SE of 5 subjects]	
		No. of writhing	Inhibition (%)
Control	-	37.3 \pm 1.8	-
ME	200	26.7 \pm 1.5*	40
	400	16.7 \pm 2.3*	55
BF	200	21.7 \pm 6.1*	42
	400	6.7 \pm 1.8*	82
MF	200	23.7 \pm 0.8*	37
	400	13.3 \pm 1.9*	64
Acetylsalicylic acid	100	13.3 \pm 0.3*	64

* $P < 0.05$ vs Control (ANOVA, LSD post hoc test; n = 5).

ME = Methanol extract; BF = Butanol fraction; MF = Methanol fraction.

that the fractions might interact with tachykinin pathways¹⁹ and inhibit other pain-producing mediators such as histamine or the sensitization of nociceptors. Inhibition of histamine or kinin pathway may reduce pain, but is not known to cause anti-inflammatory effect as neither antihistamines nor kinin antagonists are anti-inflammatory agents. The results of the present study also showed that butanol and methanol fractions exhibited comparable magnitude of antinociceptive activity in both models of pain which suggested that the phytochemical constituents responsible for the analgesic effect might concentrate mainly within these two fractions. Phytochemical analysis of the extract and fractions revealed the presence of a variety of plant principles and sitosterol has been isolated from the leaf extract⁶. The analgesic activity of some flavonoids²⁰ and terpenoids^{21,22} has been reported suggesting that these or similar constituents may be responsible for the analgesic effect of the extracts.

In conclusion, the results of the present study indicated that leaf extract of *C. scandens* might contain constituents capable of relieving or modifying responses to pain caused by either mechanical or chemical stimulation of the nociceptors mediated by both central and peripheral mechanisms. Effect of the extracts might not be due to inhibition of inflammatory response. Further studies to isolate the active component are underway.

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