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Chemical constituents and analgesic activity of *Telfaria occidentalis*

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Abstract

The seed of *Telfairia occidentalis* is use commonly for nutritional purposes and medicinally in the treatment of malaria and inflammatory diseases. The ethanolic seed extract of *Telfairia occidentalis* (450 -1350 mg/kg) was evaluated for analgesic activity using acetic acid-induced writhing, formalin-induced hind paw licking and thermal –induced pain models. The hexane and dichloromethane fractions were also analyzed using Gas chromatography-mass spectrometry (GCMS). The seed extract exhibited a dose-dependent inhibition of pains in the three experimental models. The GCMS analysis revealed the presence of pharmacological active compounds which are responsible for the analgesic activity.

Keywords: *Telfairia occidentalis;* analgesic; vegetable; GCMS; Chemical constituents

Introduction

Telfairia occidentalis (Hook. F) Vahl. popularly known as fluted pumpkin is a member of Cucurbitaceae family. The plant is cultivated in Southern Nigeria mainly for the leaves and seeds which are eaten because of their high content of protein, vitamins and minerals (Johnson and Johnson, 1996). *T. occidentalis* leaf are often used as vegetable in the preparation of soups, while the seeds are eaten raw or roasted and also ground into powder and used as soup thickening. Reports of hypoglycaemic and antidiabetic activities (Aderibigbe et al., 1999; Alada, 2000; Eseyin et al., 2000; Eseyin et al., 2005; Nwozo et al., 2004), antioxidant and antimicrobial activities (Oboh et al., 2006) of the leaf have been published. Several workers have reported on the nutritional composition, chemical characterization and functional properties of fluted pumpkin seed (Asiegbu, 1987; Badifu et al., 1995; Agatemor, 2006; Ezugwu and Nwodo, 2000; Fagbemi et al., 2005). The seed has been reported to possess antiplasmodial (Okokon et al., 2008). Most researches have focused on the leaf and information on the medicinal properties of the seed is scanty. We report in this study the analgesic activity as well as GC-MS analysis of hexane and dichloromethane fractions of the seed extract of *Telfairia occidentalis* from Nigeria.

Materials and methods

Plants collection

The plant material *Telfairia occidentalis* (seeds) were bought from local markets in Uruan area, *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. A voucher specimen was deposited in the faculty of Pharmacy Herbarium, University of Uyo, Uyo with voucher no. FPHUU 110.

Extraction

The seeds were washed and shade-dried for two weeks. The dried plants' materials were further chopped into small pieces and 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 ethanolic 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 activity

Acetic acid induced writhing in mice

The abdominal constrictions resulting from intraperitoneal (i.p) injection of 3% acetic acid consisting of the contraction of abdominal muscles together with the stretching of hindlimbs, was carried out 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 10ml/kg of normal saline, while groups 2, 3 and 4 were pre-treated with 450, 900 and 1350 mg/kg doses of *Telfairia occidentalis* seed 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.

Formalin–induced hind paw licking in mice

The procedure was essentially 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 used to analyze the first phase of formalin-induced licking and 20μL of 2.5% formalin solution (0.9% formaldehyde) made up in phosphate buffer solution (PBS concentration: NaCl 137mM, KCl 2.7mM and phosphate buffer, 10 mM) was injected 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 of the nociceptive response normally peaks 5 minutes after injection and the second phase 15 - 30 minutes after formalin injection, representing the neurogenic and inflammatory pain responses, respectively (Hunskaar and Hole, 1987). Adult albino mice (23 – 27 g) of either sex randomised into five groups of 6 mi-

ce 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 10ml/kg of normal saline, groups 2 - 4 received 450, 900 and 1350 mg/kg doses of the seed extract, while group 5 received 100mg/kg of acetyl salicylic acid 30 minutes before being challenged with buffered formalin. The responses were measured for 5mins after formalin injection (first phase) and 15–30 mins after formalin injection (second phase).

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 $45 \pm 1^{o}C$, 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 10ml/kg of normal saline. Groups 2, 3 and 4 were pre-treated intraperitoneally with 450, 900 and 1350 mg/kg doses of *Telfairia occidentalis* seed extract respectively, while group 5 animals received 100 mg/kg of acetyl salicylic acid intraperitoneally, 30 min-utes prior to the placement on the hot plate.

GC-MS analysis of hexane and dichloromethane fraction

Quantitative and qualitative data were determined by GC and GC-MS, respectively. Each 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 (H_2/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 various active fractions of the plants' extracts 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

Data obtained from this work were analyzed statistically using Students' t-test and 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 $P < 0.01$ and 0.05.

Results

Effect of on acetic acid-induced writhing in mice

The extract (450–1350 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, 1350 mg/kg. (Table 1).

Effect on formalin-induced hind paw licking in mice

The extract 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, 1350 mg/kg. (Table 2).

Effect 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).

Table 1. Effect of *Telfairia occidentalis* on acetic acid induced writhing in mice.

Dose (mg/kg)	Time interval (hr)						
	5	10	15	20	25	30	Total
Control	5.00 1.06	8.83±1.42	19.16±2.13	18.16±1.49	15.7± 0.71	12.33±1.20	79.14±3.03
Extract 450	0.00c	9.60±0.88	11.16±2.27[a]	12.50±1.36[a]	11.5±0 2.3	10.66±1.82	55.42±3.26[c]
Extract 900	0.00[c]	9.16±1.40	11.0±0.96[a]	11.16±0.94[a]	8.50±1.23[a]	6.80±1.37[a]	46.62±2.23[c]
Extract 1350	0.00 [c]	9.50±1.54	11.08±0.67[a]	9.50±1.28[b]	7.83±1.53[a]	3.83±0.87[b]	41.74±2.33[c]
ASA 100	0.00 [c]	2.00±0.00[b]	6.40±1.93[c]	9.50±1.73[b]	9.50±1.28[b]	9.30±1.85[b]	36.70±2.56[c]

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

Table 2. Effect of *Telfairia occidentalis* on formalin –induced hind paw licking in mice.

Dose (mg/kg)	Time interval (hr)						
	5	10	15	20	25	30	Total
Control	35.46±0.19	15.27±0.60	16.10±0.24	12.44±0.54	8.35±0.38	5.88±0.11	87.94±3.25
Extract 450	29.50±88	6.00±0.25[b]	5.00±0.36[c]	4.33±0.42[c]	4.00±0.36[c]	3.66±0.21[c]	52.49±2.71[c]
Extract 900	24.16±0.28[c]	5.33±0.21[c]	4.83±0.33[c]	4.16±0.16[c]	3.33±0.21[c]	3.00±0.36[c]	44.76±1.61[c]
Extract 1350	18.5±2.74[c]	0.00[c]	0.50±0.49[c]	3.00±0.516[c]	2.50±0.22[c]	2.50±0.22[c]	27.00±4.16[c]
ASA 100	8.83±0.43[c]	1.66±0.21[c]	2.80±0.16[c]	2.16±0.16[c]	1.16±0.16[c]	0.00[c]	16.61±0.43[c]

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

Table 3. Effect of *Telfairia occidentalis* on hot-plate test in mice.

Group	Dose (Mg/kg)	Reaction time (sec)(Mean±SEM)	% inhibition
Control	-	5.10±0.29	-
T. occidentalis	450	6.95±0.81[a]	36.27
T. occidentalis	900	10.77±0.75[a]	111.17
T. occidentalis	1350	16.38±0.40[b]	221.17
ASA	100	18.04±0.25[b]	253.72

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

GC-MS analysis

The GCMS analysis of the hexane and dichloromethane fractions of *Telfairia occide-ntalis* seed revealed the presence of 11 bioactive compounds each as represented in Tables 4 and 5.

Discussion

The seed of *Telfairia occidentalis* use by the Ibibios of Niger Delta of Nigeria for nut-ritive purposes in the making of soup was evaluated for antinociceptive activity in rodent. The seed extract in this study, 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 (Am-

Table 4. GC-MS analysis of dichloromethane fraction of *Telfairia occidentalis* seeds.

S/No.	Compound	Mol.Wt	Chemical Formula	RI
1.	Pentadecanoic acid, 14-methyl-,methyl ester	270	$C_{17}H_{34}O_2$	619
2.	Hexadecanoic acid	256	$C_{16}H_{32}O_2$	648
3.	8,11-Octadecadienoic acid, methyl ester	294	$C_{19}H_{34}O_2$	703
4.	16-Octadecenoic acid,methyl ester	296	$C_{19}H_{36}O_2$	706
5.	Heptadecanoic acid,6-methyl, methyl ester	298	$C_{19}H_{38}O_2$	719
6.	9, 12-Octadecadienoyl chloride(Z,Z)-	298	$C_{18}H_{31}ClO$	735
7.	9-Octadecadienoic acid (Z)-,2,3-dihydroxypropyl ester	356	$C_{21}H_{40}O_4$	741
8.	Octadecanoic acid	284	$C_{18}H_{36}O_2$	749
9.	Hexadecanoic acid, 2,3-bis[(trimethylsilyl)oxy] propyl ester	474	$C_{25}H_{54}O_4Si_2$	876
10.	Cyclohexanespiro-5'-(4'-methyl-2'-phenyl-2'-oxazoline	229	$C_{15}H_{19}NO$	947
11.	9-Octadecenoic acid (Z)-,2-hydroxy-1-(hydroxymethyl) ethyl ester.	356	$C_{21}H_{40}O_4$	966

Table 4. GC-MS analysis of n-hexane fraction of *Telfairia occidentalis* seeds.

S/No.	Compound	Mol. Wt	Chemical Formula	RI
1.	2,4-Heptadien-6-ynal,(E,E)-	106	C_7H_6O	190
2.	Benzoic acid	122	$C_7H_{14}O$	195
3.	Dodecanoic acid	200	$C_{12}H_{24}O_2$	201
4.	Linoleic acid ethyl ester	308	$C_{20}H_{36}O_2$	729
5.	Hexadecanoic acid, methyl ester	284	$C_{18}H_{36}O_2$	756
6.	α-phellandrene	136	$C_{10}H_{16}$	1005
7.	α-campholene aldehyde	152	$C_{19}H_{16}O$	1123
8.	Terpinen-4-ol	154	$C_{10}H_{18}O$	1137
9.	Trans-β-ocimene	136	$C_{10}H_{16}$	1150
10.	Borneol	154	$C_{10}H_{18}O$	1164
11.	Stigmastan-3-ol, 5-chloro-,acetate,(3a',5a')-	492	$C_{13}H_{53}ClO_2$	1175

ico-Roxas et al.,1984; Nwafor et al., 2007), and in part through local peritoneal receptors from peritoneal fluid concentration of PGE_2 and $PGF_2\alpha$ (Deraedt *et al*.,1980; Bentley *et al*.,1983). This model is used to distinguish between central and peripheral pain.

Formalin exhibits 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 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 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.

The GCMS analysis of the hexane fraction has revealed the present of a-phellandrene, an acyclic monoterpene which has been reported for significant anti-inflammatory and antinociceptive activities against acetic acid-induced writhing and capsaicin test. It is also reported to inhibit both phases of formalin-induced paw licking and in addition decreased carragennan-induced hyperalgesia (Lima et al., 2012). Moreover, Terpinen-4-ol which has been found in the seed extract has been reported to suppress production of prostaglandin and *in vitro* of TNF-α, IL-1β, as well as IL-8, IL-10 and PGE_2 by LPS-activated human blood monocytes (Hart et al., 2000; Miguel, 2010). These compounds may in part be responsible for the observed analgesic activity of the seed extract.

The antinociceptive activities exerted by this extract may be attributed to the presence of phytochemical compounds as revealed by the GCMS analysis of the seed 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. In conclusion, the seed extract has analgesic property which is due to its phytochemical components. Hence, can be exploited in the management of pains.

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Conflict of interest

There is no conflict of interest associated with the authors of this paper.

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