

Short Communication

Phytochemical investigation and anti-inflammatory property of ethanol-water extract of the roots of *Anthocleista djalonensis* A. Chev. (Gentianiaceae)

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***Anthocleista djalonensis* A.Chev. (Gentianiaceae)** a plant of West African origin is used for the treatment of inflammatory disorders traditionally. Phytochemical screening of the plant root material gave positive result for tannins, saponins and carbohydrate; but negative for alkaloids and flavonoids. The ethanol-water (1:1) extract of the root material was subjected to anti-inflammatory activity using experimental animal model. From the results obtained, the crude extract showed significant activity ($p < 0.05$) comparable to the reference drug used. Chromatographic separation of the crude extract furnished a yellow viscous liquid, identified spectroscopically as sweroside. The pure compound was also subjected to anti-inflammatory activity, this showed significant activity ($p < 0.05$) at 100 mg/kg. The results of the study showed the justification of the use of the plant in the treatment of inflammatory disorders in ethnomedicine.

Key words: Anti-inflammatory activity, *Anthocleista djalonensis*, ethanol-water extract, sweroside.

INTRODUCTION

The immense structural diversity of compounds produced by plants and empirical knowledge about their activity has been essential inspiration for developing novel medicines used throughout the world. At the same time, this knowledge is the basis for the local preparation of plants remedies, which are still an indispensable resource for everyday health care, particularly in developing countries (Johanna et al., 2005). The renewed interest in medicinal plants has focused on herbal cures among many communities around the world. This is especially true among indigenous peoples in the tropical rain forest (Kong et al., 2003). Tropical rain forest is a vital source of medicines. Today, less than 1% of the world's tropical forest plants have been tested for pharmaceutical properties, yet at least 25% of all modern drugs originally come from rain forests where most were first discovered

and used by indigenous people.

Anthocleista djalonensis (A. Chev.) is widely used for various ailments in Nigeria. It is used as an antipyretic, laxative and remedy for various stomach disorders. Aqueous extracts of the leaves mixed with lemon juice is used by the Abros of Ghana to cure epilepsy (Irvine, 1961; Watt and Breyer-Brandwijk, 1967), while in Casamane, Senegal, it is used as a diuretic (Keharo and Adam, 1974). Aqueous extract of *A. djalonensis* has also been reported to have hypertensive effect on cats as well as increase the tone and amplitude of rabbit duodenal movement (Okoli and Iroegbu, 2004).

The present study was motivated by the traditional use of the roots of *A. djalonensis* in the treatment of inflammatory disorders.

MATERIALS AND METHODS

Collection and identification of plant material

The roots were collected from the outskirts of Benin, in July and identified by Dr. B. A. Ayinde, Department of Pharmacognosy,

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Abbreviations: TLC, Thin layer chromatographic; NMR, nuclear magnetic resonance.

Table 1. Anti-inflammatory property of *A. djalonenis* root extract and sweroside in rats.

Drugs	Doses (mg/kg, p. o)	Change in paw oedema; mean (mm)	Oedema inhibition relative to control at the 4 th h (%)
Control (normal saline, 0.9%)	0.3 ml	1.88 ± 0.17	-
Indomethacin	10	0.48 ± 0.15**	74.5
Extract	100	1.2 ± 0.23*	36.2
	200	1.18 ± 0.3	37.2
	400	0.74 ± 0.25*	60.6
Pure compound(sweroside)	100	0.5 ± 0.44**	73.4

Values are mean ± S.E.M. * P < 0.05, ** P < 0.001, significantly different from control paired t- test (n = 5), p.o = oral

Table 2. Phytochemical composition of the powdered root material of *A. djalonenis*.

Phytochemical composition	Detection level
Alkaloids	—
Flavonoids	—
Tannins	++
Saponins	+++
Anthraquinones	—
Glycosides	++

(—), Not detected; (+), low concentration detected; (++) , moderate concentration detected; (+++), high concentration detected.

Faculty of Pharmacy, University of Benin. It was confirmed at the Forestry Research Institute, Ibadan, Nigeria, with herbarium number: FH: 107625, where a voucher specimen is deposited. Immediately after collection, the roots were washed, cut into pieces and left to dry on the laboratory bench.

Preparation and extraction of the root material

The dried root material was pulverized using mechanical grinder. A weighed portion of the powdered root material (450 g) was extracted by cold maceration in 1 L mixture of absolute ethanol-water (1:1) and shaken intermittently for 72 h. The extract was filtered through a Whatman no.1 filter paper and the filtrate was concentrated on the rotary evaporator connected to a vacuum pump at 40°C, giving a yield of 5.72%.

Phytochemical screening

Phytochemical screening was carried out to test for the presence of alkaloids, saponins, flavonoids, anthraquinones and carbohydrate (Trease and Evans 1989).

Purification of the extract

A weighed portion of the extract (17.45 g) was dissolved in distilled water and partitioned with chloroform (4 × 25 ml), butanol (4 × 25 ml), in succession. The fractions were concentrated using rotary evaporator connected to the vacuum pump at 40°C. A portion of the butanol fraction (8.06 g) was eluted on gravity column (2 cm in diameter), packed with silica gel (mesh 70 to 230 µm), using hexane, chloroform and methanol gradient wise. The eluate was

monitored by the thin layer chromatographic (TLC) plates using UV lamp set at 254 and 366 nm as detector. A brown viscous liquid was eluted at the solvent combination of chloroform/methanol (9.5:0.5) and (9:1). This gave a single spot under the UV lamp (R_f 0.65, $CHCl_3$ -MeOH, 4:1). The fractions of the same spot were bulked together and concentrated with the rotary evaporator connected to the vacuum pump at 40°C. The concentrate was analyzed using ¹H and ¹³C nuclear magnetic resonance (NMR) spectroscopy. The spectroscopic data was compared with literature source (Angelica et al., 2005; Onocha et al., 1995) to establish the structure.

Anti-inflammatory activity

Wistar rats (110 to 140 g) of either sex kept at the laboratory animal house of the Faculty of Pharmacy, University of Benin, Benin City, Nigeria were used. The animals were maintained under standard environmental conditions and have free access to standard diet and water. Anti-inflammatory activity was measured using carageenan-induced rat paw oedema assay (Winter et al., 1962; Adeyemi et al., 2002). Groups of 5 rats of both sexes (pregnant females excluded) were given a dose of the extract. After 1 h, 0.1 ml 1% carageenan suspension in 0.9% NaCl solution was injected into the sub-planter tissue of the right hind paw. The swelling of the carageenan was measured before and 1, 2, 3 and 4 h after injection of carageenan. Saline solution was used for control group, while indomethacin served as reference drug.

$$\% \text{ Inhibition (I)} = ((\Delta Vc - \Delta T) / \Delta Vc) \times 100$$

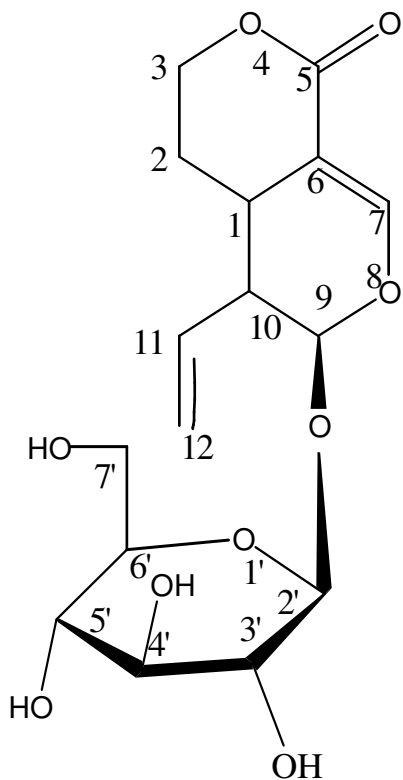
Where ΔVc , is the average difference in thickness of hind paw of control group and ΔT is that of drug treated group.

Statistical analysis

All data were expressed as mean ± SEM and the student's t-test was applied to determine the significance of the difference between the control group and drug treated groups.

RESULTS AND DISCUSSION

The phytochemical study revealed the presence of tannins, saponins and carbohydrate. Carageenan-induced rat paw oedema is used widely as a working model of inflammation in the search for new anti-inflammatory drug (Manueli et al., 1994) and appeared to be the basis of the discovery of indomethacin anti-



Sweroside

Figure 1. Isolated compound. IUPAC: 10-ethenyl-9-((2S, 3R, 4S, 5R, 6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl)oxy-4,8-dioxabicyclo(4.4.0)dec-6-en-5-one. Molecular weight, 358.340; Molecular formula: C₁₆H₂₂O₉

inflammatory drug (Winter et al., 1965). The anti-inflammatory activity of the ethanol/water extract of *A. djalensis* was evaluated by carageenan-induced rat paw oedema method (Winter et al., 1962; Adeyemi et al., 2002) and the result is shown in Table 1. The extract was tested at three different dose levels to know if they were dose dependent. From the result obtained, the crude ethanol/water extract showed significant activity ($p < 0.05$) comparable to the reference drug used. At the different dose range used (100, 200 and 400 mg/kg), there was no significant difference in the anti-inflammatory activity between 100 and 200 mg/kg, but as the dose was increased from 200 to 400 mg/kg, the activity almost doubled. The significant level of anti-inflammatory activity of the ethanol/water extract could be attributed to the presence of secoiridoids reported to be present in this genus (Jensen and Shripsema, 2002). The

secoiridoids, which sweroside is one are known for their anti-inflammatory activity (Farung et al., 2004). This work justifies the use of *A. djalensis* in the treatment of inflammatory disorders in ethno medicine.

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