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Pharmacognostic Studies and Antiinflammatory Activities of *Clerodendrum Volubile* P Beauv Leaf

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Abstract

Clerodendrum volubile P Beauv (Verbenaceae) is a climbing shrub used traditionally in the treatment of swelling, gout and venereal diseases. In this study, the microscopic, macroscopic, phytochemical analysis and anti-inflammatory activities were investigated using standard procedures.
The results revealed the presence of alkaloids, flavonoids, saponins, anthraquinone and cardiac glycoside while the methanol extract, petroleum ether and ethyl acetate fractions exhibited significant ($p<0.05$) anti-inflammatory activities when compared to the effect of the reference drug Diclofenac sodium.

Keywords:anti-inflammatory, *Clerodendrum volubile,* macroscopy, microscopy, phytochemical screening

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Introduction

Clerodendrum volubile P. Beauv (Family; Verbenaceae) also known as the white Butterfly leaf is a climbing shrub grown in deciduous forests from Senegal to Fernando Po. [1] In folklore medicine, the plant is used to treat swellings, oedema, gout, arthritis, rheumatism and venereal diseases. [1]
Previous studies have reported the presence of manganese, copper, iron, phenols and flavonoids in the leaf of C. volubile [2] while, Fleischer et al., 2011 in their study reported the antimicrobial and anti-inflammatory activities of the petroleum ether, ethyl acetate and ethanol extracts of a species of Clerodendrum, Clerodendrum splendens leaves. [3]
In order to justify the use of Clerodendrum volubile in the folklore treatment of inflammation, this study investigates the pharmacognostic properties and anti-inflammatory activities of Clerodendrum volubile leaf methanol extract and fractions.

Material and Methods

Plant Material

The leaves of Clerodendrum volubile were collected from Agbele Community High School, Sagamu, and Ogun State, Nigeria. The plant was authenticated at the Department of Pharmacognosy, Olabisi Onabanjo University, and Sagamu where a voucher specimen was deposited.

Extraction

The leaves were air dried and ground into fine powder. The powdered plant were macerated in 80% methanol for three days, filtered and evaporated to dryness under vacuum. The residue was reconstituted in water and successively partitioned with petroleum ether and ethyl acetate.

Chemicals and Instruments

Digital camera, Compound microscope, glass and cover slips. All chemicals and reagents used were of analytical grade.

Phytochemical Studies

The Phytochemical screening of the leaf of *C. volubile* was screened for the presence of alkaloids, saponins, flavonoids, Tannins and cyanogenetic glycosides using established procedures. [4-5]

Pharmacognostic Studies

Macroscopical Features

The macroscopical features of the leaves of *C. volubile* such as the odour, colour, size and shape were studied. [6]

Microscopy

The sections for microscopy were prepared by free hand section of the leaf which was cleared with chloral hydrate and mounted in glycerine. [7-8] The leaf was viewed by using a compound

microscope and pictures of the sections made using a digital camera.

Anti-inflammatory Studies

Rats of both sexes weighing between 100 – 130gm obtained from the animal house of the Department of Veterinary Medicine, University of Ibadan were used for this study. The rats were housed and allowed to acclimatize at the animal house of the Department of Clinical Pharmacy and Biopharmacy, Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu. The animals were maintained at 25^{o}C, fasted for 12h and allowed free access to water before the study. Diclofenac sodium (Novartis) was used as the reference drug while egg albumin was used to induce inflammation by injecting 0.1ml of fresh egg-albumin into the subplantar surface of the right hind paw. [9-11]

The extracts were re-constituted in water and administered orally an hour before inducing the inflammation. The linear circumference of the injected paw was measured immediately after injection at 0, 30, 60, 90, 120, 150 and 180 minutes. Increase in the linear diameter indicated oedema in the paw.

% Inhibition

The percentage inhibition for each treatment was determined by using the equation

$$\% \text{ Inhibition of oedema} = \frac{\text{Mean Control} - \text{Mean treatment}}{\text{Control}}$$

Statistical Analysis

The results are presented as mean ± standard deviation and percentage decrease (%). The level of significance of the activities of the extract and fractions were determined by the one way Analysis of Variance (ANOVA).

Results and Discussion

The macroscopic study showed that *C. volubile* has a simple leaf with an entire blade, a reticulate venation, acuminate apex, and a cuneate base. The leaves are green with a bland odour. Table 1 while the phytochemical analysis of the leaf of *C volubile* showed the presence of flavonoids, tannins, saponins, anthraquinone, cardiac glycosides and alkaloids. Table 2

The microscopic study of the leaf of *C volubile* revealed the presence of a mixture of unicellular non-glandular and awn-shaped uniseriate glandular trichomes with one cell stock and multicellular head and an anomocytic type of stomata because the stomata

Table 1: Macroscopic characteristics of fresh *C. volubile* leaf.

Colour	Green
Odour	Bland
Taste	Slightly bitter
Type	Simple
Margin	Entire
Venation	Reticulate
Shape	Lanceolate
Apex	Acuminate
Base	Cuneate
Surface	Smooth
Texture	Smooth/soft

Blade length: Breadth 10cm; 3.2cm

lacked distinct subsidiary cells Fig1, Fig 2. A comparison of the stomata and hair types are in agreement with previous studies which have reported the presence of diacytic, anomocytic, anisocytic types of stomata in the members of the family verbenaceae. In addition, a comparison of the microscopic, macroscopic and phytochemical analysis of *C. volubile* with another *Clerodendrum species* (*Clerodendrum inerme*) has reported some similarities. [12-13]

The anti-inflammatory activity of the leaf extracts of *C. volubile* was established by using a non-steroidal (NSAID) model of fresh albumin- induced rat paw oedema. The model used is known to act in a biphasic manner where the initial phase of inflammation (0-2) is attributed to the release of histamine and kinnins while the second phase is sustained by the release of prostaglandins. [14-15]

When the activities of the test extracts were compared to the untreated control (water), the methanol extract, ethyl acetate and pet ether fractions exhibited an anti-inflammatory effect of 70%, 73.3% and 70% respectively. The study revealed that the activity exhibited by the methanol, ethyl acetate and pet. ether extracts were more pronounced than that exhibited by the reference drug (Diclofenac Sodium) at 180 min.

Though the extracts did not exhibit anti-inflammatory effect until after 60 mins. This therefore confirms that the constituents in the methanol, pet ether and ethyl acetate of *C volubile* acted like most clinically effective anti-inflammatory drugs. [16]

In all the groups except that administered water only (control), maximum inflammation was observed at 60 mins Table 3

At 150 mins, the anti-inflammatory effect of the Pet. ether and ethyl acetate extracts were similar (11.1%) Table 3The maximum anti-inflammatory effect of 15.6%, 20% and 15.6% were exhibited by the methanol, ethyl acetate and pet ether extracts respectively

Table 2 : Phytochemical Screening of Leaf of *C. volubile*.

Anthraquinone		Saponins	Tannins	Cardiac glycoside	alkaloids	Flavonoids
Free	Combined					
+	++	++	++	++	++	++

Table3 : Antiinflamatory Activities of the methanol extract, Pet ether, and Ethyl acetate fractions of *Clerodendrum volubile* leaf

	MEAN RAT PAW SIZE (cm)						
	0	30	60	90	120	150	180 (min)
Water	2.20 ± 0.21	2.35 ±0.07	2.40 ±0.14	2.75 ±0.21	2.80 ±0.35	2.90 ±0.56	3.00 ±0.14
Petroleum ether	2.40 ±0.14	2.55 ±0.14	2.90 ±0.07	2.60±0.14 **5.5%	2.10±0.14 **25%	2.00±0.00 **31.0%	1.90±0.14 **70%
Ethyl acetate	2.30± 0.14	2.40± 0.14	2.70 ±0.14	2.40± 0.14** 12.7%	2.35 ±0.25 **16.1%	2.00± 0.10 **31%	1.80 ±0.00** 73.3%
methanol	1.95± 0.07	2.25±0.07	3.00±0.14	2.35±0.21** 14.7%	2.30±0.14 **17.9%	2.15±0.21 **25.9%	1.90±0.14 **70%
Diclofenac Sodium	2.35±0.07	2.50±0.14	2.65±0.07	2.45± 0.07 **10.9%	2.40±0.00 **14.3%	2.25±0.07 **22.4%	2.25±0.07 **25%

P<0.05 when compared to the effect of Diclofenac sodium.
** % Decrease when compared to the untreated control.

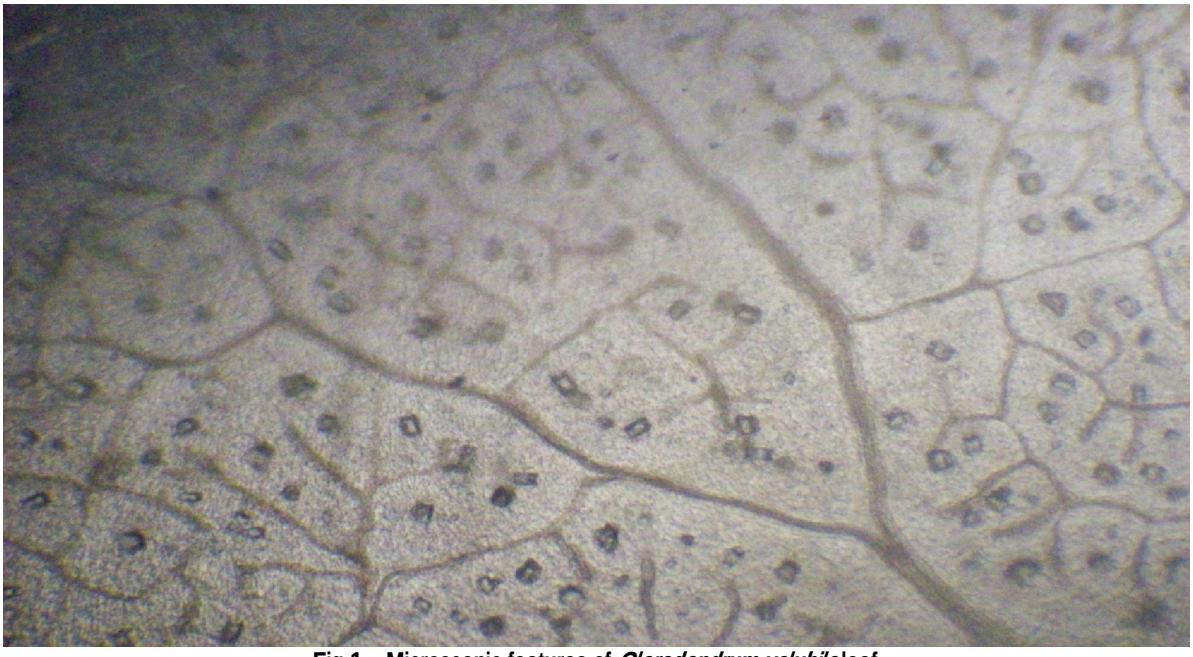

Fig 1. Microscopic features of *Clerodendrum volubile* leaf

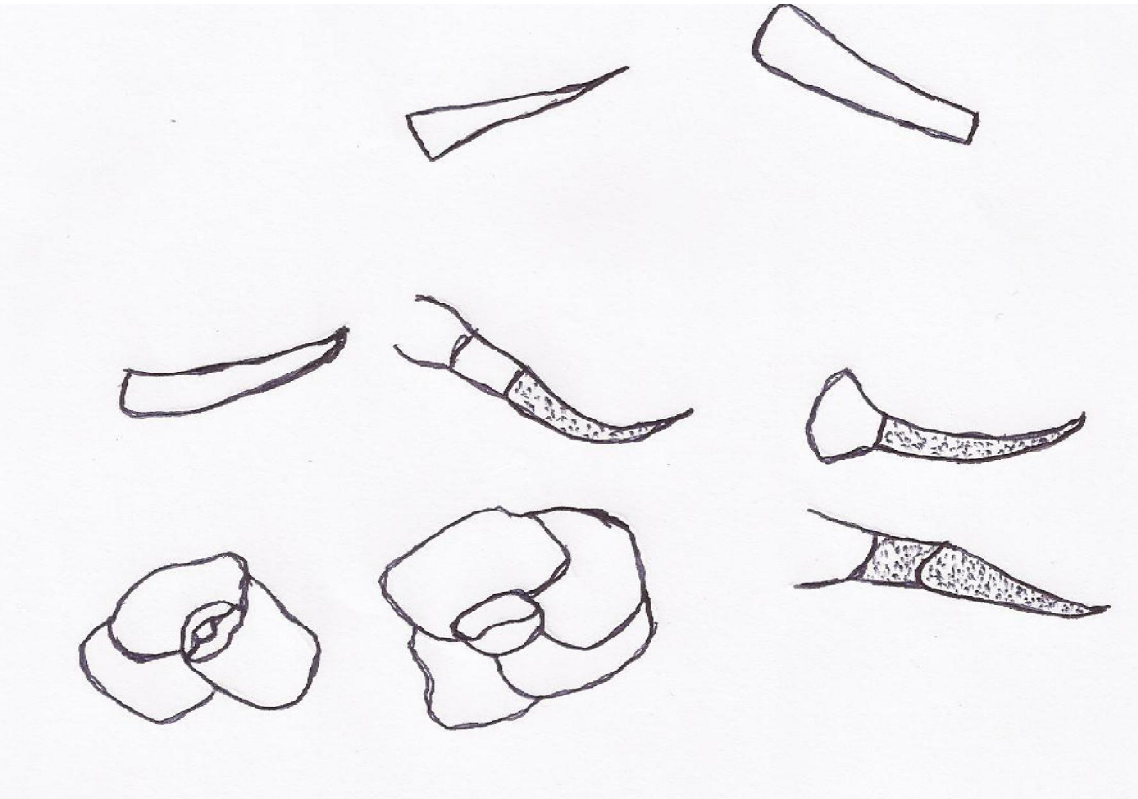

Figure 2: Trichomes and stomata types found in *Clerodendrum volubile* leaf.

when compared to the anti-inflammatory effect of the reference drug (Diclofenac Sodium) at 180 min.

Furthermore, this study revealed that the ethyl acetate fraction of *C volubile* was the most active on a time course curve. There was however no significant difference in the anti-inflammatory effects exhibited by the extract and fractions ($p<0.05$) when compared to the effect of the reference drug Diclofenac sodium.

It could therefore be suggested that the active extract and/or constituents of *C volubile* leaf may be exhibiting an inhibitory effect on chemical mediators such as prostaglandins.

This anti-inflammatory effect reported in this study and that reported in other *Clerodendrum species* [3] could suggest that *Clerodendrum* species may be a possible source of novel ant inflammatory drugs and this study therefore justifies the use of *C. volubile* as an antiiflammatory agent in folklore medicine.

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