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Bioactivity guided isolation of analgesic and anti-inflammatory constituents of *Cnestis ferruginea* Vahl ex DC (Connaraceae) root

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ABSTRACT

Ethnopharmacological relevance: *Cnestis ferruginea* (CF) Vahl ex DC (Connaraceae) is a shrub widely used in Traditional African Medicine (TAM) for the treatment of various painful and inflammatory conditions.

Aim of the study: To isolate the active pharmacological constituents responsible for the anti-inflammatory and antinociceptive properties of the methanolic root extract of *C. ferruginea.*

Materials and methods: The crude methanolic root extract of CF was sequentially fractionated into four sub extracts (chloroform, ethylacetate, *n*-butanol and the remaining aqueous fraction). The aqueous–butanol fractions, having showed significant inhibition of inflammation and pain, were subjected to fractionation through successive column chromatography on silica gel 60–120 mesh, eluted with a gradient of $CHCl_3$–MeOH.

Sixty five fractions were collected; fractions with similar TLC profiles were grouped into seven major fractions (1–7). Fraction 4 being the most active in bioassay was rechromatographed to obtain CF-2. Analgesic activity was evaluated using the acetic acid-induced writhing and hot plate tests in mice while carrageenan induced paw oedema test was used to investigate the anti-inflammatory actions of the fractions obtained.

Result: Amentoflavone (CF-2) was isolated from the aqueous/*n*-butanol fraction. CF-2 (12.5, 25 and 100 mg/kg; p.o) produced significant ($P < 0.05$) dose dependent inhibition of pain response elicited by acetic acid and increased nociceptive reaction latency in hot plate test. In addition it produced significant ($P < 0.05$) dose-dependent inhibition of oedema in the carrageenan-induced inflammation.

Conclusion: This study showed that amentoflavone is responsible for the analgesic and anti-inflammatory activity of *Cnestis ferruginea*.

1. Introduction

Cnestis ferruginea Vahl ex DC (Connaraceae) is a short ornamental shrub, sometimes a climber, which is about 2.5 m high and is usually covered by dense, brown velvety hairs (Hutchinson and Dalziel, 1958). The plant is widely employed in the treatment of various ailments in traditional medicine throughout West Africa. In previous studies (Ishola et al., 2011), it has been demonstrated that the root methanolic extract of *C. ferruginea* produced significant dose-dependent inhibition of pain response elicited by acetic acid and formalin, while also increasing the nociceptive reaction latency in the tail clip and hot plate tests. In respect of its anti-inflammatory activity, *C. ferruginea* caused significant ($P < 0.05$) dose-dependent inhibition of oedema in the carrageenan, egg albumin, formaldehyde, and xylene-induced inflammation tests. The antistress property of CF has also been reported (Ishola and Ashorobi, 2007).

Tropical rain forests continue to support a vast reservoir of potential drug species by providing natural product chemists with valuable compounds as starting points for the development of new drugs; thus, the potential for developing newer compounds is enormous.

Olugbade et al. (1982) reported in their preliminary screening that the leaves of CF were positive for glycosidic anthraquinones, sterols, tannins and flavonoids as well as four major compounds squalene, β-sitosterol, myricyl alcohol and a higher homologue of methyl linolenate. Similarly, in our preliminary screening, the

Abbreviations: HMBC, Heteronuclear multiple bond correlation; NMR, Nuclear magnetic resonance; ESMS, Electrospray ionization mass spectrometry; TLC, Thin layer chromatography; UV, Ultraviolet; NSAID, Non-steroidal anti-inflammatory drugs; COX, Cyclooxygenase; p.o., Per oral; i.p., Intraperitoneal; DMSO, Dimethyl sulfoxide; H_2SO_4, Sulphuric acid; MeOH, Methanol

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roots of CF were shown to be positive for glycosidic anthraquinones, sterols, phenols, tannins, alkaloids, saponins and flavonoids (Ishola et al., 2011).

Therefore, the objective of this study was to isolate the putative the anti-inflammatory and antinociceptive bioactive constituents of *Cnestis ferruginea* through bioassay-guided fractionation in standard laboratory models.

2. Materials and methods

2.1. Plant material

The dried roots of *C. ferruginea* were purchased from a traditional herbal practitioner in Mushin, Lagos State, Nigeria. The botanical identification and authentication of the plant was done by Prof. J.D. Olowokudejo of the Department of Botany and Microbiology, Faculty of Science, University of Lagos, Lagos, Nigeria and Mr. Joseph Ariwaodo of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The voucher specimen of the plant deposited at the herbarium of the Institute (FHI 108219).

2.2. Preparation of extract

Powdered root of *C. ferruginea* (5.2 kg) was loaded into a glass percolator containing methanol (20 l). It was allowed to stand at room temperature (28 °C) for about 16 h (overnight). The percolate was collected and the process of extraction was repeated five times. The combined extract was filtered and concentrated on Buchi Rotavapor at 40 °C and was further dried under vacuum pump. The weight of the extract obtained was 560 g (brownish extract).

2.3. General experimental procedures

^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX 300 MHz NMR spectrometer. Molecular weight was determined using ESMS on an advantage Max LCQ Thermo-Finnigan mass spectrometer. Column chromatography was performed using silica gel (60–120 mesh). TLC was carried out on precoated silica gel plates $60F_{254}$ (Merck). Spots were visualized by UV light or by spraying with H_2SO_4–MeOH or anisaldehyde-H_2SO_4 and vanillin-H_2SO_4 reagents.

2.4. Fractionation and isolation of active component(s)

The crude methanolic extract (560 g) was partitioned, which gave chloroform (114 g), ethylacetate (146 g), *n*-butanol (160 g) and an aqueous fraction (140 g).

Each fraction was subjected to the bioactivity assays using the carrageenan induced paw oedema, hot plate test and mouse writhing reflex. There were mixed activity between aqueous and *n*-butanol fraction. Hence, both fractions were mixed together; it was subjected to column chromatography (silica gel 60–120 mesh) using step gradient of $CHCl_3$–MeOH as eluents.

It was eluted with a gradient of $CHCl_3$–MeOH (100:00) to $CHCl_3$–MeOH (00: 100), 60 fractions were collected (1000 ml each) and their composition was monitored by TLC, those fractions having similar R_f were pooled together to give 7 subfractions (F1–F7) with the following yield: 2.2 g, 13.62, 17.4, 75, 9.44, 4.37 and 0.81 g, respectively.

These fractions were subjected to pharmacological evaluation to determine their anti-inflammatory and antinociceptive activities. Fr.4 (75 g) was found to be the most active one. It was rechromatographed using silica gel 60–120 mesh and eluted with a gradient of $CHCl_3$–MeOH (5%H_2O) (100:00) to MeOH-H_2O (95:05) to give 86 eluents (500 ml each).

Compound CF-2 was obtained at $CHCl_3$–MeOH. H_2O (90:10) as yellow amorphous powder (500 mg). Other eluents were checked with TLC, those fractions having similar R_f were pooled together to afford three fractions (Table 1 and Fig. 1).

Table 1
NMR data of amentoflavone (CF-2) (Pyridine-D5 at 400 MHz).

C	δc	δH, multiplicity, J (Hz)	HMBC correlations
2	164.4	–	H-3;H-2′; H-6′
3	103.3	6.92,s	–
4	182.5	–	H-3
5	162.8	–	H-6
6	99.6	6.19,s	–
7	165.6	–	H-6;H-8
8	94.6	6.33,s	H-6
9	158.3	–	–
10	104.7	–	H-6
1′	117.9	–	H-2′; H-5′;H-6′
2′	132.3	8.51, s	H-6′
3′	121.4	–	H-2′; H-5′
4′	164.3	–	H-2′; H-5′;H-6′
5′	117.0	7.45,d (8.0)	H-6′
6′	128.1	7.92, dd (8.0,1.8)	H-2′; H-5′
2″	164.5	–	H-3″;H-2‴; H-6‴
3″	103.9	7.01,s	–
4″	182.8	–	H-3‴
5″	161.0	–	H-6‴
6″	99.8	6.06,s	–
7″	164.4	–	H-6‴
8″	106.7	–	H-2′; H-6″
9″	155.6	–	–
10″	101.7	–	H-6″
1‴	121.7	–	H-2‴,H-6‴, H-3‴, H-5‴
2‴, 6‴	128.6	7.88, d(8.0)	H-3‴, H-5‴
3‴, 5‴	116.6	7.16, d (8.0)	H-2‴, H-6‴
4‴	160.6		H-2‴, H-6‴, H-3‴, H-5‴′
Chelated hydroxyl		13.7 and 13.8	

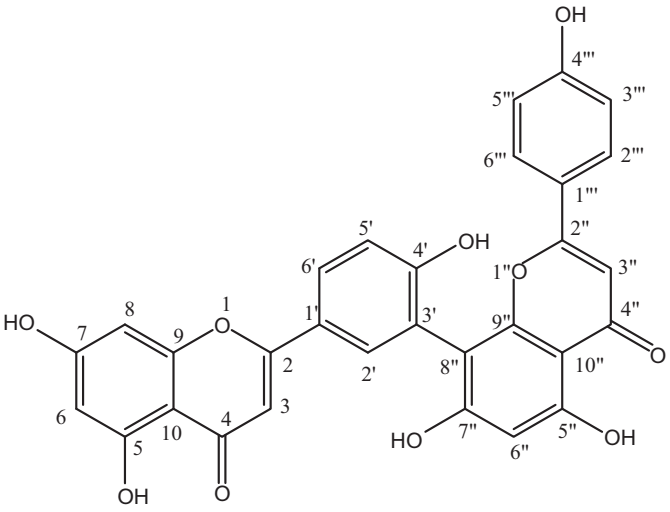

Fig. 1. Chemical structure of amentoflavone isolated from the methanolic root extract of *C. ferruginea*. NMR data of amentoflavone (Pyridine-D5 at 300 MHz).

2.5. Laboratory animals

Male Sprague Dawley rat (140–170 g) and 8 weeks old Swiss albino mice (20–30 g) were obtained from the Laboratory Animal Services Division of Central Drug Research Institute, Lucknow, UP, India. The animals were kept in polyacrylic cages containing six animals per cage and maintained under standard housing