Isolation and characterization of bergapten from the root bark of *Ficus exasperata* (Vahl)

ABSTRACT

Despite the wide ethnomedicinal applications of *Ficus exasperata*, little is known about the active principles responsible for the observed biological effects, thus limiting opportunities for further therapeutic applications. In this study we isolated a furocoumarin (**D-1**) shown to be partly responsible for the acclaimed anti-diabetic effect of the plant

1. Introduction

Ficus exasperata (Moraceae) also known as the sandpaper tree is found in different parts of tropical Africa and Asia, and has been widely used for the treatment of various ailments in these regions. In Nigeria, decoctions of its roots and leaves are traditionally used as remedies for hypertension, cough, ulcer and microbial infections[1,2,3]. Attempts to provide scientific rationalization for these uses have unraveled several pharmacological activities of the plant including antiulcer, anti-inflammatory, antidiabetic, antihypertensive, antioxidant and hypolipidemic properties[3-7].

Phytochemical investigation of extracts from the genus *Ficus* revealed the presence of several bioactive secondary metabolites including flavonoids, alkaloids, phenolic acids, steroids, saponins, tannins, terpenoids and coumarins[8-13]. In 2016, Nnamonu *et al*[14] reported the isolation of α-amyrin from the ethyl acetate fraction of the stem bark extract of *Ficus exasperata* harvested in North Central Nigeria.

Whilst the ethnomedicinal value of *Ficus exasperata* continues to increase, relatively little achievement has been recorded in isolating and identifying its active principles. Against this backdrop, we aimed to investigate the plant for bioactive contents. We herein report the isolation and characterization of bergapten, a furocoumarin, as a major constituent in the root bark *of Ficus exasperata* as well as its hypoglycemic activity.

2. Materials and Methods

2.1 Chemicals

Analytical grade solvents and chemicals, thin layer chromatography (TLC) silica gel 60 F254 plates, and silica gel 60 (70-230 mesh) used for column chromatography were purchased from Merck (Germany), Sigma Aldrich (USA), and AK Scientific (USA).

2.2 Plant material

The roots of *Ficus exasperata* were collected in a plantation in Ondo, Ondo State, Nigeria. The samples were identified at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Nigeria where voucher specimens have been deposited in the herbarium (CSPH2614).

2.3 Extraction and Isolation

The root bark of *Ficus exasperata* was sun dried for 48 hours followed by oven drying at 40 ℃ for further 48 hours. The dried material (0.5 Kg) was coarsely milled and .subjected to soxhlet extraction using chloroform for 72 hr. Obtained extract (0.0025 Kg, 0.5% yield) was concentrated under reduced pressure by means of a rotary evaporator (R-114, Buchi, Switzerland), and fractionated using 100% pet ether, 80% pet ether in EtOAc, 60% pet ether in EtOAc, 100% EtOAc, and 80% EtOAc in MeOH.

The 100% EtOAc fraction termed CLE4 was dissolved in chloroform and loaded onto a pipette previously packed with silica gel (70-230 mesh ASTM). It was then successively eluted with a mixture of EtOAc and MeOH in increasing concentration. 12 fractions of 10 ml each were collected; fractions containing the same compounds as determined by their TLC profiles were combined and concentrated to dryness under reduced pressure. Five fractions were obtained (CLE4A-E). Fraction CLE4B (0.7 g) was reconstituted in chloroform and chromatographed using Silica gel as described above. Three bulked fractions were obtained, termed 4B1 – 4B3.

Fraction 4B2 was further column chromatographed over silica gel (70-230 mesh) using a column of diameter 2.5 cm and length 40 cm and isocratically eluting with pet-ether: ethyl acetate 85:15. 40 fractions of 2 ml each were collected. Isolate D-1 (250 mg, >99% by HPLC) was obtained from the fractions 1-10 as off-white needle-like crystals with a characteristic odour.

2.4 Structural Elucidation

Proton (1H, 500 MHz), carbon-13 (13C, 101 MHz), and two-dimensional NMR were acquired on Varian 400 spectrometer (Varian, California, United States of America). **D-1** was measured in deuterated chloroform ($CDCl_3$), with 1H and 13C chemical shifts of 7.26 and 77.16 ppm respectively. High resolution mass spectra of the isolate were obtained using a Brucker microTOF-Q spectrometer (Brucker, Bremen, Germany) with an electrospray ionization (ESI) source.

2.5 Oral Glucose Tolerance Test

A total number of 20 Wistar male rats weighing between 120-180 g were purchased from the Animal House of the Federal University of Technology, Akure, Nigeria. Animal grooming and collection and testing of blood samples were conducted as described by Akhtar *et al*.,2008[15]. In summary, the animals were divided into four groups of five and kept in standard rat cages where they were adequately fed. All five groups were fasted for 16 hours, after which groups II-IV were induced with diabetes intragastric administration of glucose (3g/Kg body weight) The baseline glucose level was then measured by glucometer (Accu-Check glucose test meter). Group I represented the negative control group. Group II was treated with metformin (100 mg/kg

body weight). While groups III and IV received 10 and 30 mg/Kg body weight of **D-1** respectively, administered by intraperitoneal injection. Serum glucose was determined for all groups at 0, 30, 60, 90 and 150 min. Data obtained was statistically compared using two-way ANOVA (multiple comparison), observed difference was termed significant at $P < 0.05$. Statistical analysis was conducted using GraphPad Prism 7.03.

3. Results and Discussion

Structural determination of D-1

The structure of **D-1** was elucidated using ESI-MS and NMR, and was found to be spectroscopically similar to previously reported bergapten structure by Chunyan *et al.*(ref). As shown in table 1 and figure 1, the compound contains a total of twelve carbon atoms, eleven of which are sp^2 hybridized resonating at δ 93.8 –161.3 and the remaining one being a methoxy carbon resonated at δ 60.1. The ^{1}H NMR spectrum revealed the presence of five sp^2 protons (δ 6.27 – 8.16), and three highly deshielded methyl protons (δ 4.27). The full assignment of the proton and carbon signals was conducted with the aid of 2D NMR experiments. Heteronuclear single quantum coherence (HSQC) was used to assign individual protons to their corresponding carbon atoms as given in table 1, and also to confirm the absence of methylene protons. Homonuclear correlation spectroscopy (^{1}H – ^{1}H COSY) revealed protons that are coupled in an AB system, specifically δ 6.27 (1H, *d*, *J* = 9.8 Hz, H-3) and 8.16 (1H, *d*, *J* = 9.8 Hz, H-4); 7.59 (1H, *d*, *J* = 2.4 Hz, H-2') and 7.02 (1H, *d*, *J* = 2.4 Hz, H-3'). ESI-MS showed the sodiated mass ion $[M + Na]^+$ peak at 239.028, consistent with the 216.042 molecular weight of **D1**. Thus allowing the structural confirmation of **D1** as bergapten.

Table 1. ^{13}C and ^{1}H-NMR chemical shifts (ppm) of **D-1**.

Position	^{1}H, δ (multi, Hz)	^{13}C, δ
C-2	-	161.3
C-3	6.27 (1H, *d*, *J* = 9.8)	112.5
C-4	8.16 (1H, *d*, *J* = 9.8)	139.2
C-5	-	149.3
C-6	-	112.3
C-7	-	158.3
C-8	7.14 (1H, *s*)	93.8
C-9	-	152.6
C-4a	-	106.4
C-2'	7.59 (1H, *d*, *J* = 2.4)	144.8

C-3'	7.02 (1H, *d*, *J* = 2.4)	105.0
C-2''	4.27 (3H, *s*)	60.1

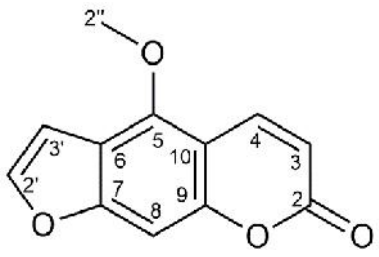

Figure 1. Structure of Compound **D-1** (Bergapten).

Hypoglycemic activity of D-1

As shown in figure 2, hyperglycemia was observed approximately 30 minutes after intragastric administration of glucose. Upon administration of the standard drug metformin, and **D-1**, the most significant blood glucose reduction was observed for metformin and 10mg/Kg **D-1** at 60 min and 90 min relative to the control. Metformin produced a reduction of 29% at 60 min and 22% at 90 min, while 10mg/Kg **D-1** gave a reduction of 23% and 24% at 60 and 90 min respectively. However, 30 mg/Kg **D-1** showed a significantly lower decrease in blood glucose both at 60 min (7%) and at 90 min (9%) compared to 10mg/Kg **D-1**; also at 150 min, a slight increase in blood sugar compared to what was observed at 90 min. The observed non-dose dependent hypoglycemic activity of **D-1** is most likely due to lack of target specificity.

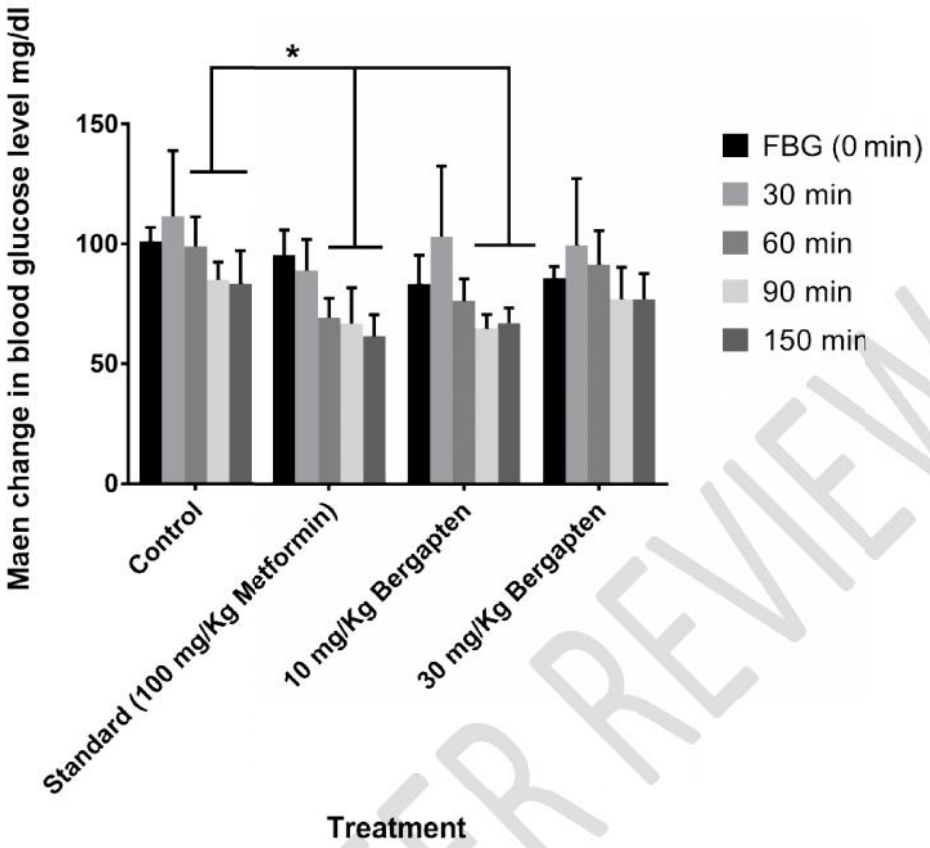

Figure 2. Effect of **D-1** on blood glucose levels of glucose-fed rats. Error bars indicate SEM obtained from experimental replicates (minimum of 5). *P < 0.05, significant difference in mean glucose levels at similar time intervals in comparison to the control. Intraperitoneal metformin as standard drug.

Conclusion

Overall, this study shows that bergaten has considerable hypoglycemic effect, which in part justifies the reported anti-diabetic activity of *Ficus exasperata*. The comparable hypoglycemic effect displayed by bergapten at 10 mg/Kg body weight, and the clinically used metformin, positions bergapten as a viable lead molecule for the development of novel anti-diabetic agents. It is thus research-worthy to unravel the precise mechanism of action of the molecule as a hypoglycemic agent, followed by rational structural modification to optimize it for potency and safety.

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