Journal of Applied and Advanced Research, 2019: 4(3) 85–88
http://dx.doi.org/10.21839/jaar.2019.v4i3.294
ISSN 2519-9412 / © 2019 Phoenix Research Publishers

Research Article – Phytochemistry

Isolation and characterization of Stigmasta-7, 22-dien-3-ol (α-Spinasterol) from *Entada africana* stem bark crude extract

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(Received: 19-05-2019; Accepted 01-06-2019; Published Online 06-06-2019)
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Abstract

The phytochemical investigation of the stem bark extracts of *Entada africana* led to the isolation and characterization of Stigmasta-7,22-dien-3-ol from the dichloromethane soluble portion of acetone/methanol (1:1v/v) crude extract. The powdered stem bark sample of *Entada africana* was defatted with hexane and extracted with acetone/methanol (1:1v/v) mixture. The dichloromethane soluble fraction was purified on a low pressure column containing silica gel 60 (60-200 mesh). The purification afforded an isolate coded Enac3 (85 mg) with Rf value of 0.404 in hexane/ethyl acetate (4:1). The isolate was characterized using IR, NMR data and in comparison with literature. Analysis of spectroscopic data and literature comparison suggests Enac3 as stigmasta-7, 22-dien-3-ol. The isolation of stigmasta-7, 22-dien-3-ol from the stem bark of *Entada africana* suggests the presence of useful bioactive principles which could be exploited for medicinal purposes.

Keywords: *Entada africana,*Isolation, Stigmasta-7, 22-dien-3-ol, Characterization

Introduction

Entada africana is a medicinal plant of high value throughout the West African sub-region due to its usefulness in the management and treatment of several ailments and diseases such as diabetes, fever, hypertension, respiratory tract infections, gonorrhea, typhoid fever, stomach upset and as an arrow poison antidote among others in traditional medicine practice (Bako *et al*., 2005, Tibiri *et al*., 2010, Mbatchou *et al*., 2011). Various studies had substantiated the usefulness of *Entada africana* in traditional medicine practice such as the existence of phytochemical substances with significant antioxidant properties (Tibiri*et al*., 2010) and antibacterial activity (Aboaba *et al*., 2006; Marthe *et al*., 2014; Ifemeje *et al.,* 2014). Ahua *et al.* (2007) and Njayou *et al.* (2013) reported the antileishmanial, anti-inflammatory, wound healing and antibacterial properties of *Entada africana*.

Additionally, the root extract was found to demonstrate significant hepatoprotective, anti-inflammatory and antioxidant activity (Njayou *et al*., 2013 and Owona *et al*., *al.* (2011), Ezenyi*et al.* (2014) and Germanò *et al*. (2014). In another study, the anti-hepatitis C virus activity was also reported by Tietcheu *et al.* (2014). Despite the medicinal potentials of *Entada africana,* there exist only few studies on the isolation and characterization of its phytoconstituents. These studies include the isolation of acidic wound healing polysaccharides (Diallo *et al.*, 2001), myricetin and derivatives (Montoro *et al*., 2005), antiproliferative triterpene saponins (Cioffi *et al*., 2006) and betulin (Kwaji *et al.*, 2018) based on available literature. In furtherance of our effort to make more information available on the chemical constituents of *Entada africana,* we report the isolation and characterization ofStigmasta-7, 22-dien-3-ol from *Entada africana* stem bark crude extract for the first time.

Materials and methods

Apparatus/Equipments

Glass column (75 cm x 35 mm), Silica gel 60 (60 – 200 mesh), 100 mL and 50 mL conical flasks, Mini-spatula, Pencil and Transparent Meter Rule, Commercially prepared thin layer chromatography, TLC, plate (silica gel 60 F_{254} , Merck Germany, 20 x 20 cm, 1 mm thick), Chromatogram and Iodine tanks. Recirculating Cooler (Stuart-SRC4). Digital Water Bath Stuart RE300DB, Vacuum Pump CAT. RE3022C SN-000100188, Rotatory Evaporator (Stuart RE300/MS), Digital Electronic balance ae-*ADAM* PW254 (Max 250 g, Sensitivity = 0.0001 g).

IR spectrum was recorded on PerkinElmer Universal ATR (100 FT-IR spectrometer) while the ^{1}H and ^{13}C NMR spectra were recorded using NMR 400 MHz Bruker Avance. Deuterated chloroform ($CDCl_3$) was used as a solvent for the isolate. Sample analysis was carried out at the Department of , South Africa.

Reagents and Solvents

Solvents (n-Hexane, Dichloromethane (DCM), Ethyl acetate, Methanol, Diethyl ether, and Acetone) LOBA Chemie Analytical grade Reagents.

Collection, identification and preparation of Sample

The sample collection was carried out by a herbalist on 26/11/2014 and subsequently identified by a botanist Dr K. P. Yoriyo of Biological Sciences Department Gombe State University and assigned Voucher No. F. H. J – 227. The stem bark was dried under shade for twenty-one days at normal atmospheric temperature on a clean surface. After achieving substantial moisture reduction, sample was

powdered with a milling machine. The powdered plant sample was kept safe until use.

Extraction was carried out on the plant material using solvents of varying polarity. A portion of powdered *Entada africana* stem bark sample approximately 2.70 Kg was soaked in 10 liters of hexane for five days and defatted three times to obtain hexane filtrate. The combined filtrate was concentrated on a rotary evaporator at 45°C to obtain 46.25 gram hexane extract. The marc was shade dried and re-extracted with 10 Liters of acetone/methanol (1:1v/v) mixture three times. The combined volume of extracts was also concentrated over rotary evaporator to obtain 134 grams of Extract. The maceration technique(Tiwari *et al*., 2011) was adopted for the extraction of plant active principles from their matrix.

The isolation of stigmasta-7, 22-dien-3-ol was achieved by adopting a slightly modified procedure of Teke *et al*. (2011). About 9.40 grams of dichloromethane soluble fraction of acetone/methanol extract of the *Entada africana* crude extract was pre-adsorbed onto silica gel 60 and then loaded to a column packed with silica gel 60 (60-200 mesh). Gradient elution was performed with hexane/ethyl acetate at 5% increase in volume of eluting solvent; (100:00), (95:05), (90:10), (85:15), (80:20), (75:25), etc. to yield several fractions of 100 mL each. On the basis of their thin layer chromatography (TLC) profiles, fractions 43-47 were combined and concentrated on a rotary evaporator at 45°C. After washing and recrystallization from methanol, 85 mg of a pure Isolate coded Enac3 was obtained with Rf value of 0.404 in hexane/ethyl acetate (4:1).

Characterization of Enac3

On subjection of Enac3 to IR spectroscopy, several absorption bands were observed (Table 1), notably 3379.0 cm^{-1} corresponding to alcoholic O-H stretch, 2935.19 cm^{-1} is C-H stretch of alkanes, 2868.19 cm^{-1} is another C-H stretch band of alkanes, 1640.38 cm^{-1} is a C=C stretch of alkenes that appears as a weak band, 1446.61 cm^{-1} is the $(CH_2)_n$ bending stretch of cycloalkanes, 1382.03 cm^{-1} corresponds to O-H deformations of alcohols and 1038 cm^{-1} is C-H stretch of alkenes. The above frequencies are diagnostic of those of steroids (Billah *et al.*, 2013).

Table 1.FT-IR Spectrum of Enac3

S/No	Frequency (cm^{-1})	Type of Vibration
1	3379.00	O-H stretch of alcohols
2	2935.19	C-H stretching of alkanes
3	2868.19	C-H aliphatic symmetric stretching
4	1640.38	C=C stretching of alkenes
5	1446.61	C-H stretch of cycloalkanes
6	1382.03	O-H deformation of alcohols
7	1038.60	C-H stretch of alkenes

Table 2. Chemical Shifts (δ) of ^{13}C- and ^{1}H-NMR for Enac3 &Literature data (Meneses-Sagrero *et al.*, 2017*)

Carbon	Enac3		Literature data*		DEPT
C-atom	δH	δC	δC	δH	
1	1.06	37.154	37.1	1.09,1.82	CH_2
2	1.32	31.49	31.5	1.39, 1.77	CH_2
3	3.59	71.07	71.1	3.6	CH
4	1.28	38.01	38.0	1.27, 1.70	CH_2
5	1.41	40.28	40.3	1.4	CH
6	1.75	29.65	29.4	1.22, 1.74	CH_2
7	5.14	117.46	117.5	5.18	CH
8		139.57	139.6		C
9	1.51	49.47	49.5	1.66	CH
10		34.23	34.2		C
11	1.52	21.55	21.6	1.48	CH_2
12	2.00	39.47	39.6	1.23, 2.0	CH_2
13		43.3	43.3		C
14	1.78	51.25	51.15	1.81	CH
15	1.41	23.02	23.0	1.40, 1.52	CH_2
16	1.27	28.49	28.5	1.25	CH_2
17	1.24	55.92	55.95	1.25	CH
18	0.57	12.05	12.05	0.55	CH_3
19	0.72	13.04	13.0	0.8	CH_3
20	2.03	40.81	40.8	2.05	CH
21	1.03	21.37	21.4	1.02	CH_3
22	5.17	138.16	138.15	5.17	CH
23	5.07	129.46	129.48	5.09	CH
24	1.60	51.25	51.26	1.55	CH
25	1.50	31.87	31.9	1.55	CH
26	0.85	21.08	21.2	0.85	CH_3
27	0.83	18.99	19.0	0.84	CH_3
28	1.14	25.39	25.4	1.18, 1.42	CH_2
29	0.80	12.23	12.2	0.81	CH_3

From Table 2, the proton, H-3 corresponds to a sterol moiety and appears as a triplet of doublet at δ_H 3.59 ppm. The angular methyl protons at δ_H 0.57 and 0.72 ppm corresponds to C_{18} and C_{19} protons respectively. The protons at δ 5.17 (1H, s) and 5.07 (1H, s) and the carbonyl protons at δ_H 3.59 ppm are typical of an olefinic steroid nucleus. The olefinic protons which appeared as characteristic downfield signals at 5.17 and 5.07 ppm are identical with the chemical shifts of H-22 and H-23 of steroids. Additionally, two doublets at δ 0.84 (3H, d) and 0.83 (3H, d) can be assigned

to H-26 and H-27. These assignments are consistent with reported values (Françaet *al*., 2016).

From the C-13 NMR spectrum values (Table 2), the signals at 139.57 and 117.46 ppm are characteristic and assignable to C-8 and C-7 double bond respectively while the signal at δ_C 71.07 ppm is due the C-3 hydroxyl group. The signals at 12.05 ppm and 13.04 ppm are due to methyl groups at C-18 and C-19 respectively. The DEPT spectrum indicates the presence of six methyl carbons (C-18, C-19, C-21, C-26, C-27 and C-29), nine methylene carbons (C-1, C-2, C-4, C-6, C-11, C-12, C-15, C-16 and C-28), eleven methine carbons (C-3, C-5, C-7, C-9, C-14, C-17, C-20, C-22, C-23, C-24 and C-25) and three quaternary carbons (C-8, C-10 and C-13). These observations are consistent with literature (Fufa *et al.*, 2018). On the strength of spectra evidence (Table 2 & appendix 1) and in comparison with literature reports (Jessica *et al.*, 2017), Enac3 is Stigmasta-7, 22-dien-3-ol (α-spinasterol) with the molecular structure below (Figure 1).

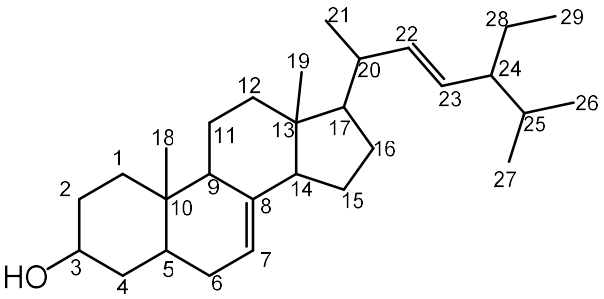

Figure 1: Structure of Stigmasta-7, 22-dien-3-ol

Alpha(α)-Spinasterol was reportedly found to exhibit interesting biological properties such as the demonstration of antiproliferative activity against the cancer cell lines HeLa and RAW 264.7 (Meneses-Sagrero *et al*., 2017). It was also found to cause reduction in cholesterol levels of plasma and liver and possesses anti-inflammatory properties (Jessica *et al.*, 2017).

Acknowledgement

I wish to thank Prof. H. M. Adamu and Prof. I. Y. Chindo for guidance during the course of the Research Work. My sincere thanks also go to Dr. Ismail Abubakar for assistance with the spectroscopic analysis of the isolate. Above all, I am grateful to Gombe State University Management who provided the opportunity for Research Work.

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