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Research Article

ISOLATION AND CHARACTERIZATION OF STIGMA-5-EN-O-B-GLUCOSIDE FROM ETHYLACETATE LEAF EXTRACT OF *BYRSOCARPUS COCCINEUS* SHUM &THONN

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ABSTRACT

Objective: The aim of this study is to isolate and characterized the active compound from ethylacetate leaf extract of *Byrsocarpus coccineous* Schum and Thonn.

Methodology: For isolation of the compound, 10gm of ethylacetate extract was subjected to fractionation using column chromatography using silica gel 60-120 mesh. Off-white crystals were observed in fractions 191-210 using solvent system chloroform / methanol 9:1. Crystals were washed with acetone at which Thin Layer Chromatography was performed.

Result: Mass spectral data and acquired Nanospray (NSI) technique was used for characterization of the compound which turned out to be Stigma-5-en-O-β-glucoside.

Conclusion: The isolated compound may be responsible for the uterotonic activity of ethylacetate leaf extract and this justify the traditional use of this plant in augmentation of labour.

Keyword: *Byrsocarpus coccineous*, Ethylacetate, Characterization, Stigma-5-en-O-β-glucoside

INTRODUCTION

The use of plants and their preparations to treat various diseases is an age-long practice [1]. However, the systemic study of plants for the treatment of different ailments is of recent origin that has been triggered by the emergence and spread of many diseases [2]. Hence plant kingdom is being screened for newer and effective drugs. Today in many countries modern medicine has replaced plant medicine with many synthetic products but it must be emphasized that almost 30% of pharmaceutical preparations are still obtained directly from plants [3]. A large percentage of studies have been carried out on herbal traditional medicines and significant amount of laboratory data have been generated and published on their efficiency [4]. *Byrsocarpus coccineus* schum and thonn (family connaraceae) is one of such plant that has been known and used in traditional medicine in several parts of West Africa [5]. *Byrsocarpus coccineus* is popularly known in Ghana by the Twi and Gar people as "awenda" or "awende." In Northern Nigeria, it is referred to by the Hausas as "Tsamiyar kasa or kimbar maharba." The Fulani people call it "wangarabubi or yangara-bubihi", while the Bassange people call it "Kogi." In the southern part of Nigeria the Yoruba people call it "Oke abolo" or "Mybo-apepea" [5]. Kilba people in Adamawa State call it ''mblakiki''. *Byrsocarpus coccineus* have been shown to be useful in oro-pharngeal, dermatological, urogenital tract and haematological problems [6-10]. In central Nigeria the leaf decoction of *Byrsocarpus coccineus* have been used traditionally to augment labour. *Byrsocarpus coccineus* leaves have been found to contain a lot of bioactive phytochemical compounds which may be responsible for the observed uterotonic effects of the ethyl acetate leaf extract on pregnant uterus of albino rat [11, 12]. The purpose of this study is to identify and characterize the bioactive principle from the leaves of *Byrsocarpus coccineus*.

MATERIALS AND METHOD

Identification, collection and authentication of plant materials

Samples of the plant material *Byrsocarpus coccineus* were collected from Idu, Abuja during the month of April 2009 under the guide of a professional plant collector Mr.Yakubu Habi of the Department of Medicinal Plant Research and Traditional Medicine of the National Institute of Pharmaceutical Research and Development Abuja where a voucher specimen number (3452) was assigned and deposited at the herbarium for future reference.

Processing and extraction of the powdered plant material

The leaves of *Byrsocarpus coccineus* were carefully separated from the other morphological parts of the plant and washed clean with water, air dried under shade for seven days pounded with pestle and mortar into fine particles. Six kilograms of the powdered leaves of *Byrsocarpus coccineus* was extracted by marceration with 6 L of N-hexane to cover the powder. The set up was closed tightly for 72 hours with occasional agitation and stirring. Afterwards the mark was filtered, squeezed for remaining N-hexane and were air dried under shade until it was completely free from n-hexane. The mark was then macerated with absolute ethanol. The same procedure as indicated for n-hexane was repeated. Hexane was recovered by rotary evaporator. Ethanol extract was then subjected to evaporation using rotary evaporator. Two hundred grams of dried and crude ethanol extract of *Byrscocarpus coccineus* leaf was suspended in 150 ml of distilled water and successively extracted with 500 ml x 3 ethylacetate and 500 ml x 3 N-butanol. At every stage of the partitioning and before switching over to the next organic phase, it was ensured that the organic phase exhaust the aqueous phase of the needed ingredients and this was indicated by colour change in the organic phase. In addition it was also ensured that the next partitioning organic solvent does not interfere with the previous one; the two organic phases are used though successively but strictly exclusively. The ethyl acetate and N-butanol pooled fractions were filtered and evaporated to dryness separately at reduced pressure under rotary evaporator and the dried fractions stored in the desiccator until constant weight was obtained. The percentage yields for ethanol, ethylacetate, N-butanol and aqueous leaf extracts of *Byrsocarpus coccineus* were 16.7%, 3.9%, 1.2% and 1.0% respectively.

Fractionation of the Ethyl Acetate ($CH_3100CH_2CH_3$) Extract

Column Chromatography:- The ethylacetate extract was subjected to column chromatography:

The weight of extract used was 35 g, weight of silica gel mesh 60-120 for sample preparation was 50 g, while the weight of silica gel for packing column was 150 g. Fractions were pooled together and labeled F1 to F13 based on thin layer chromatography spotting. Fractions 5 and 6 were found to be the same so were combined and evaporated. Another smaller column was mounted for further fractionation of fractions 5 and 6. The weight of sample was 10 g,

weight of silica gel mesh 60-120 for sample preparation was 15 g, and weight of silica gel for packing column was 46 g, while Hexane was used for washing the column. Twenty milliliters were collected at a time. The fractions were left open for three days under room temperature for the solvent to evaporate. Thin layer chromatography was carried out and similar fractions were combined. Crystals were observed in fractions 191 to 201. They were observed to be similar on thin layer chromatography so the fractions were combined, dissolved in acetone and then filtered. The crystals were dried and weighed. Thin layer chromatography of the crystal was performed. The solvent system used was chloroform/ methanol 9:1. The plate was sprayed with 10% sulphuric acid and then heated on hot plate.

Rf value of the spot = Distance moved by solute/Distance moved by solvent

Rf value = 2.7cm/6.7cm = 0.402

Weight of pure sample + bottle = 12.290g

Weight of empty bottle = 12.120g

Therefore, weight of pure sample = 12.290 g – 12.120 g = 0.170 g (170mg)

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RESULT ANALYSIS

Structure and Molecular Weight Elucidation

Mass spectral data, acquired using Nanospray Ionisation (NSI) technique, gave a pseudo molecular ion $[M+H]^+$ at m/z 577 corresponding to $C_{35}H_{60}O_6$. The peak at m/z 413.30, corresponding to $[C_{29}H_{49}O]^+$ is characteristic of β-sitosterol. The difference in mass between this fragment and the molecular ion (i.e. 163) can be ascribed to glucose moiety. Hence the data strongly indicates that compound W1 is β-sitosterol glucoside. Further confirmation of the β-sitosterol nucleus was obtained from MS data. The cyclohexene ring of β-sitosterol is expected to undergo Retro Diels-Alder (RDA) fragmentation to yield two fragments – a diene at m/z 300 $[C_{15}H_{24}O_6]^+$ and a dienophile at m/z 276 $[C_{20}H_{36}]^+$. These peaks were located in the mass spectrum of W1. The ^1H and ^{13}C NMR data of W1 (including H-H COSY, a 2D NMR experiment) are summarised in Table 1.

Table 1: Structure and Molecular Weight Elucidation (NMR data of W1)

Position	δ_C	DEPT	δ_H	H-H COSY
1	33.79	CH_2		
2	32.68	CH_2		
3	73.90	CH_2	3.39	
4	39.67	CH_2		
5	140.88	C		
6	121.65	CH	5.32	H-7
7	29.71	CH_2		
8	31.86	CH		
9	50.05	CH		
10	36.66	C		
11	20.17	CH_2		
12	37.28	CH_2		
13	42.30	C		
14	56.62	CH		
15	23.05	CH_2		
16	24.0	CH_2		
17	55.87	CH		
18	12.23	CH_3	0.64 br s	
19	21.0	CH_3	0.94 br s	
20	35.94	CH		
21	19.07	CH_3	0.89 d	H-20
22	31.82	CH_2		
23	25.87	CH_2		
24	45.58	CH		
25	29.14	CH		
26	19.38	CH_3	0.81 d	H-25
27	19.55	CH_3	0.81 d	H-25
28	28.86	CH_2		
29	12.12	CH_3	0.80 d	H-28
1’	101.23	CH	4.42	H-2
2’	77.20	CH	3.62	H-1
3’	70.53	CH	3.45	
4’	70.552	CH	3.11	
5’	77.34	CH	4.21	H-6
6’	61.53	CH_2	2.88	H-5

DEPT = Distortionless Enhancement by Polarization Transfer,

COSY = COrrelated SpectroscopY

The ^{13}C NMR of this compound showed 35 peaks comprising 6 methyl ($-CH_3$), 12 methylene ($-CH_2-$), 4 methine (=CH-) and 3 quaternary (=C=) carbon types, as revealed by DEPT technique. The C-5, C-6 olefinic C resonances were located at δ140.88 and 121.65 respectively The C-3 carbinol signal was found at δ73.90. The six methyl carbon signals of β-sitosterol at C-18, 19, 21, 26, 27, 29 were also found at δ12.23, 21.0, 19.07, 19.38, 19.55, 12.12 respectively. The anomeric carbon (C-1') of the glucose moiety appears at δ101.23. The remaining sugar C atoms (C2'-6') resonated within the range of δ61.53-77.34. The ^{1}H NMR spectrum showed signals for vinyl proton (H-6) at δ5.32. Six methyl signals comprising two broad singlets and four narrow doublets could be seen between δ0.64 and δ0.94. A broad signal centred at δ1.25, integrating 24 protons, was ascribed to methylene protons of β-sitosterol. The H-H COSY spectrum also revealed useful ^{3}J connectivities which confirm the assignment of the NMR signals. Thus considering the available NMR and MS data, W1 was determined to be β-sitosterol-5-O-glucoside (Table 1) (Fig 1-8).

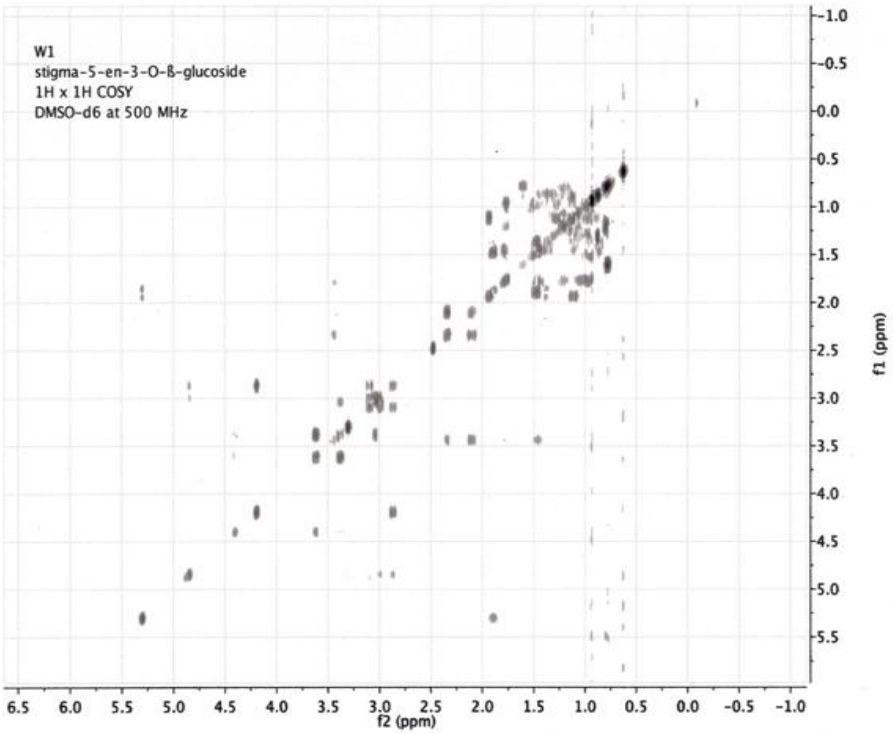

Fig. 1: Characterisation of the pure sample W1

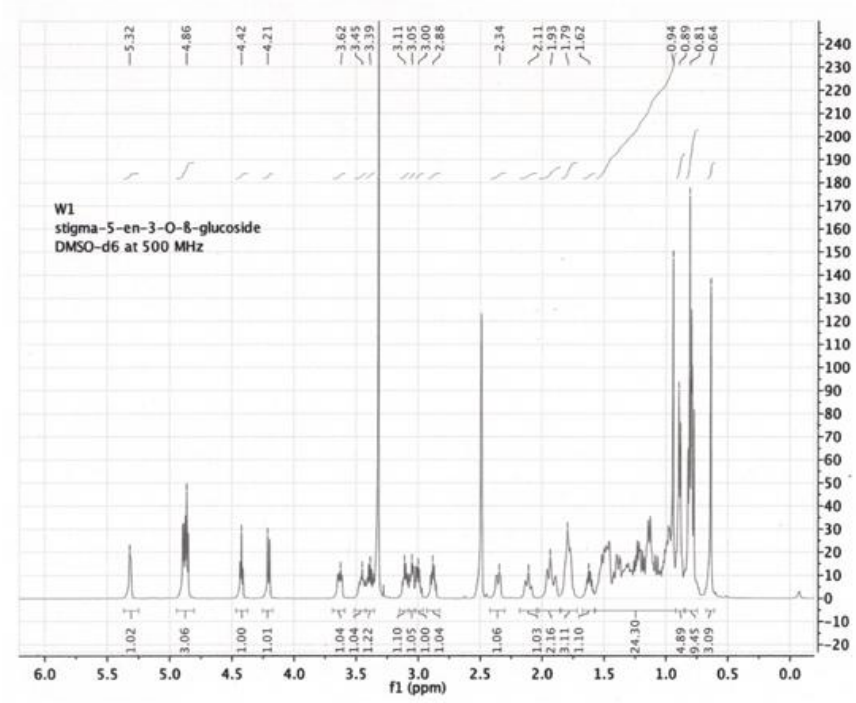

Fig. 2: Characterisation of the pure sample W1

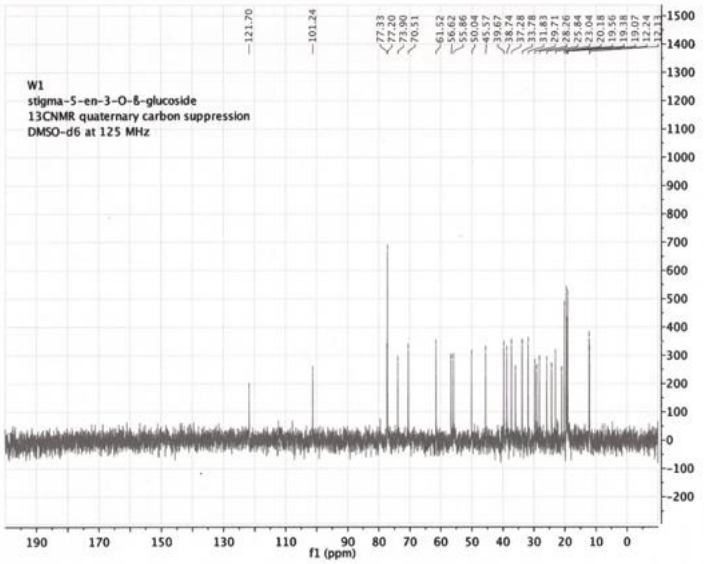

Fig. 3: Characterisation of the pure sample W1

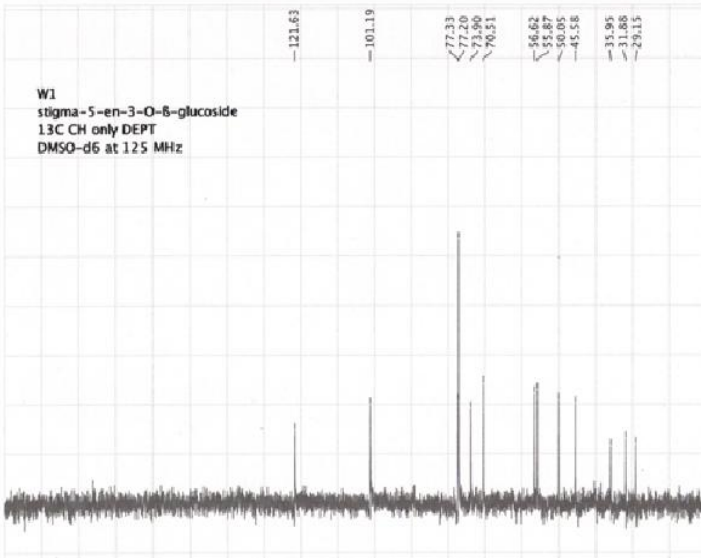

Fig. 4: Characterisation of the pure sample W1

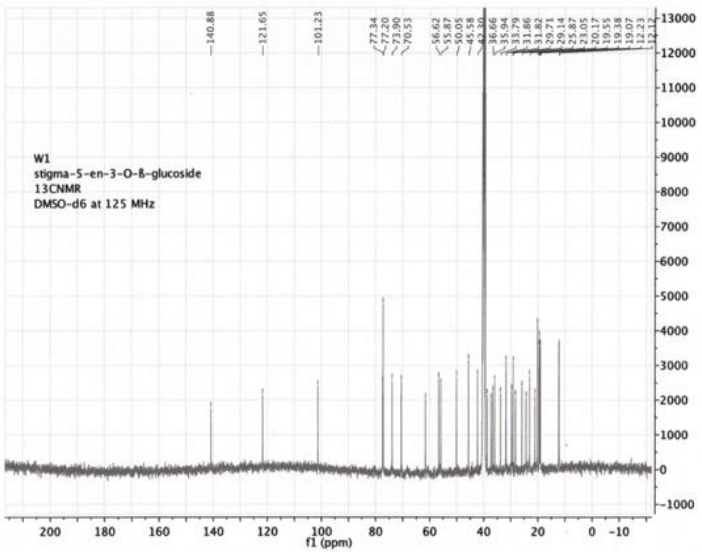

Fig. 5: Characterisation of the pure sample W1

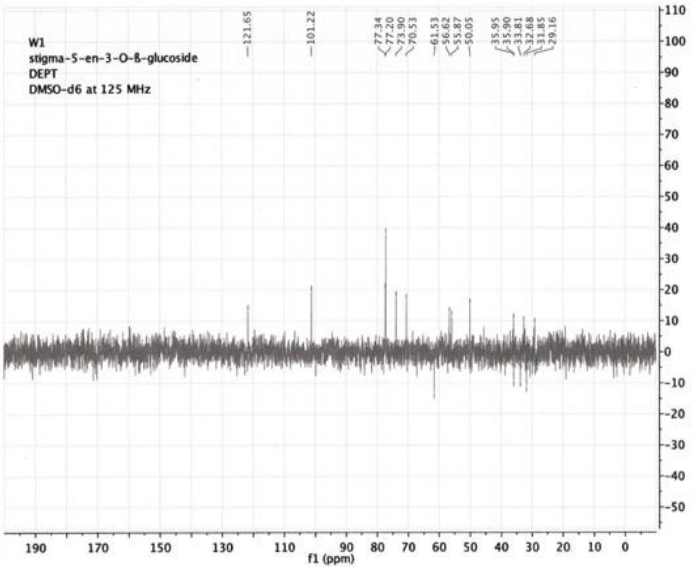

Fig. 6: Characterisation of the pure sample W1

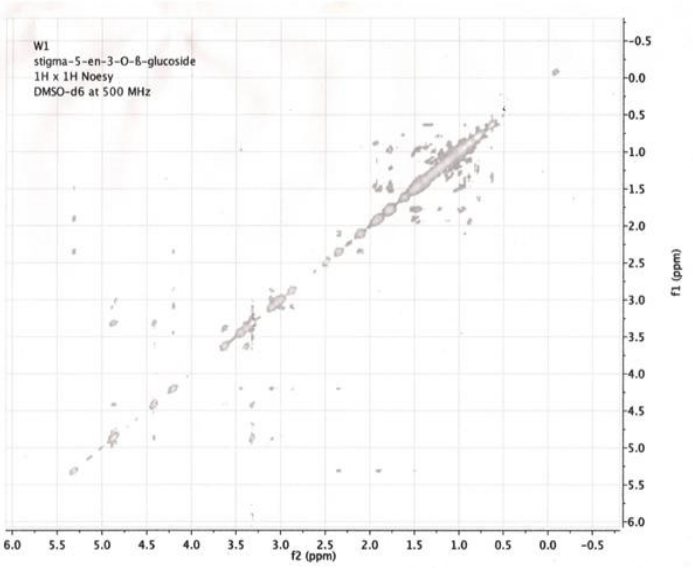

Fig. 7: Characterisation of the pure sample W1

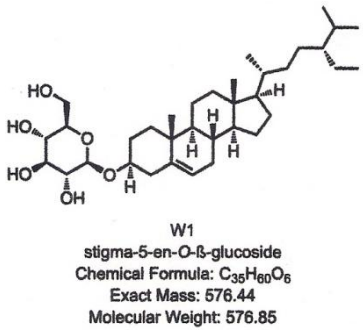

Fig. 8: The structure of the pure sample (W1)

DISCUSSION

In this study, a substance (W1) with chemical formular $C_{35}H_{60}O_6$ (molecular weight of 576.85) have been isolated from the ethylacetate soluble part of ethanolic extract of *Byrsocarpus coccineus* leaf. To the best of our knowledge, there has been no documented evidence of the isolation of stigma-5-en-0-*B*-glucoside from this plant. However, three flavonoids identified as quercetin 3-0-*B*-D-glucoside has been isolated from the ethylacetate and n-butanol soluble parts of ethanolic extract of *Byrsocarpus coccineus* by Ahmadu and his colleaques [13]. Interestingly, no study that evaluated the effect of this compound on isolated pregnant rat uterus to the best of our knowledge has been reported. Faizi and colleagues [14] isolated a similar compound Stigma-5-en-3-O-β-glucoside and its acetyl derivative from oil cakes of *Brassica rapa* but was not tested on rat uterus. However, the effect of this compound

was tested and found useful for the treatment of human immunodeficiency virus (HIV) infection at which the compound has already been patented [15,16]. The data on W1 also agrees closely with those found in literature [1,17].

CONCLUSION

The isolated compound Stigma-5-en-O-β-glucoside may be responsible for the uterotonic activity of the crude leaf extract reported by traditional healers in some part of Nigeria and this justify the traditional use of this plant in augmentation of labour.

ACKNOWLEDGEMENT

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