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Isolation and Characterization of Stigmasterol and B-Sitosterol from the leaves of *Emilia coccinea* (Sims) *G.Don*

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Abstract: *In view of its unique medicinal value and other applications, stigmasterol and* ***β-sitosterol were isolated from*** *Emilia coccinea leaves. Extraction of the compounds from the plant was carried out using Soxhlet apparatus, while column and thin layer chromatographic techniques were used for isolation and purification, which afforded white crystalline powder. Structural elucidation using* ^{1}*H-NMR, DEPT, COSY, HSQC and HMBC followed by comparison, with literature values confirmed the isolated compounds as stigmasterol and β-sitosterol. The isolated compounds may contribute to the medicinal properties of E. coccinea. This is the first isolation of stigmasterol and β-sitosterol from E. coccinea.*

Key Words: *Emilia coccinea, extraction, stigmasterol and β-sitosterol, characterization*

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1.0 Introduction

Nature is blessed with abundant bioresources which could be used in the treatment of various diseases and infections or as intermediates for pharmaceutical formulations. Medicinal and pharmaceutical roles of plant or plant materials have long been established and is currently receiving concentrated research attention in different fields such as alternative medicine and natural product chemistry (Igwe and Echeme, 2013). Scientific investigations have revealed that the medicinal value of any plant depends on its phytochemical constituents which may include glycosides, alkaloids, flavonoids and others (Friday *et al*.,2018) *Emilia coccinea* Yellow tassel (*Emilia coccinea*) belongs to the family Asteraceae and is native to Africa, tropical Asia and Oceanica. The specie *E. coccinea* is an annual herbaceous plant, weak-stemmed to 1 m high. It is dispersed from Guinea to Nigeria, Cameroon and occurring through eastern and western Africa and into tropical Asia. It is an attractive plant of easy culture, bearing small scarlet or sometimes golden-yellow heads. *Emilia coccinea* (SIMS) G.DON commonly known as Scarlet tassel flower is one medicinal plant that has been used traditionally for medicinal purposes to treat a variety of ailments such as tumor, inflammation, cough, rheumatism, fever, dysentery, wounds and in preventing miscarriage (Teke *et al.*, 2007, Ojiako *et al.*, 2015; Nwachukwu *et al.*, 2017). The juice of the edible leaves is reportedly used in treating eye inflammations, night blindness, and ear-aches. Several research works have been executed to study the phytochemical components of *Emilia coccinea* and also on the antimicrobial activity of the plant

(Kamboj and Sulaj 2011). The pharmacological properties such as antioxidant, antidiarrheal, anti-microbial, and neuroprotective activities have been reported (Teke *et al.,* 2002, Zhang *et al.,* 2013 and Foyet *et al.,* 2014). Shetonde *et al.,* (2015) isolated a total of 24 compounds from the leaf extracts (19 and 7 compounds from the Hexane and DCM extracts, respectively; 2 being common to the two extracts. The most abundant components were caryophyllene (22.07%), 1-octadecanol (19.34%), caryophyllene oxide (17.74%), 1-tridecene (7.70%), geranylgeraniol (7.46%), tetracosane (5.50%), and ethyl hexadecanoate (2.82%) from hexane and pentadecanal (40.03%), 1,E-11,Z-13-octadecatriene (11.35%), 1-octadecanol (7.31%), 1-tridecene (6.39%) and 4,8,12,16-tetramethylheptadecan-4-olide (2.59%) for the DCM extract. .

Application roles of plants that are related to their phytochemical constituents are as local anesthetics/ analgesics (Alqareer *et al*., 2006), antiseptic, astringents, which is mostly related to the presence of tannins and polyphenols (13), anti-inflammatory, etc. Stigmasterol is a plant sterol that contain an unsaturated bond between C22 and C23. Stigmasterol has been identified in some plants and their anti-inflammatory roles were established (Bone and Mill, 2013). Some of the reported plants were *Aesculus hippocastanum* (for the treatment of odedema and swelling caused by inflammation, *Cimicifuga racemosa* (for the treatment of arthritis, especially small joint osteoarthritis), *Rehmannia glutinosa* (for the treatment of adrenal tonic and anti-inflammatory in autoimmune diseases). Stigmasterol is a known food additive, a precursor for industrial manufacturing of vitamin D, semisynthetic progesterone (useful in rebuilding mechanisms related to estrogen effect) and cortisone. It has been reported that stigmasterol is a sterol compound in the diet that has the potential to reduce the risk of cardiovascular diseases (Kaur *et al*., 2011). It is also used as a phytosterol c on liver X-receptor-α activation, in the preparation of lipid monolayers and as internal standard for the quantification of yeast cell wall sterols using gas chromatography mass spectrometry. Literature has also revealed that stigmasterol have anti-hypercholestrolemic, antitumor and antioxidant functionality. Due to the wider application of stigmasterol, commercial production is ongoing but not cost effective. Besides, the product may also have some toxic content. Hence, a search for availability of stigmasterol in plant can provide information on green source of this vital steroid. Consequently, the aim of this study is to isolate and characterized stigmasterol and β-sitosterol from the leaves of Emilia coccinea.

2.0 Materials and Methods

2.1 Sample collection

Emilia coccinea leaves were collected from Osusu, Isiala-Ngwa North L.G.A., Abia State, Nigeria, between September and March, 2017. The plant material was identified and authenticated by Mr. I. K. Ndukwe, a specialist in Plant Taxonomy, Forestry Department, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

The leaf samples were washed thoroughly using tap water, followed by distilled water. The leaves were air-dried under shades to a constant weight before milling with an electric blender.

2.2 Extraction and isolation

Extraction of plant material was carried out by Soxhlet extraction method using hexane and ethylacetate as solvents respectively. The extract was concentrated using a rotary evaporator at room temperature and left on the laboratory bench for 2 days. The column was washed with acetone and rinsed with n-hexane. The column was prepared by packing a glass column (2.5 cm by 80 cm) with slurry of silica gel (60.2 g) in 200 ml of n-hexane. The slurry was introduced in one smooth flow and the solvent drained off to the top of the column bed. 100 ml of n-hexane was used to wash down sides of the column and also fill it up. Solvent mixture of n-hexane and ethylacetate (90:10 ml) was introduced and collection of fractions in well labeled vials began just before the plant material travelled to the column neck. This continued for the following solvent mixtures - 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100. Thereafter, a more polar solvent, methanol (100 ml) was used to elute the more polar components from the column. A total of 57 vials were collected. ach fraction was spotted using a capillary tube on a precoated TLC plate and developed in a solvent mixture of 3 ml: 7 ml (ethylacetate: hexane) and fraction A20 gave a single spot on TLC with R_f value of 0.51. The fraction A20 was packaged in a vial and sent to University of Strathclyde, Glasgow, Scotland, for spectral analysis.

2.3 Spectroscopic characterization

Spectroscopic methods were used to elucidate the structure of the isolated compound. The spectra (^{1}H-NMR, HSQC, DEPT, COSY and HMBC) were recorded using CDC as a solvent

3.0 Results and Discussion

Table 1 presents ^{1}H NMR chemical shift for fraction A 20 recorded in $CDCl_3$ while Table 2 presents ^{13}C (DEPT) chemical shifts of fraction A20.

Table 1: ^{1}H-NMR chemical shift for fraction A20 recorded in $CDCl_3$

Position of hydrogen	Chemical shift (ppm)	Assignment (type of proton)
1	-	$-CH_2$
2	-	$-CH_2$
3	3.54	-CH
4	-	$-CH_2$
5	-	-CH
6	5.38	=CH
7	-	$-CH_2$
8	-	-CH
9	-	-CH
10	-	-
11	-	$-CH_2$
12	-	$-CH_2$
13	-	-
14	-	-CH
15	-	$-CH_2$
16	-	$-CH_2$
17	-	-CH
18	1.53	$-CH_3$
19	0.71	$-CH_3$
20	-	-CH
21	1.86	$-CH_3$
22	5.04	-CH
23	5.18	=CH
24	-	-CH
25	-	-CH
26	0.73	$-CH_3$
27	0.95	$-CH_3$
28	-	$-CH_3$
29	1.04	$-CH_3$

Table 2: ^{13}C (DEPT) chemical shift of fraction A20

Position of carbon atom	Chemical shift (ppm)	Assignment
1	37.27	$-CH_2$
2	31.68	$-CH_2$
3	71.84	-CH
4	42.31	$-CH_2$
5	-	-
6	121.74	=CH
7	31.90	$-CH_2$
8	29.71	-CH

9	50.16	-CH
10	-	-
11	24.32	$-CH_2$
12	39.80	$-CH_2$
13	-	-
14	56.79	-CH
15	24.32	$-CH_2$
16	28.93	$-CH_2$
17	56.08	-CH
18	12.06	$-CH_3$
19	19.41	$-CH_3$
20	39.80	-CH
21	-	$-CH_3$
22	138.33	-CH
23	129.30	=CH
24	51.25	-CH
25	36.16	-CH
26	21.10	$-CH_3$
27	21.23	$-CH_3$
28	25.42	$-CH_2$
29	11.86	$-CH_3$

The ^1H – NMR for fraction A20 showed the presence of three olefinic ptotons at 5.04 ppm (H-22, dd) J (15.15Hz,8.57Hz), 5.18ppm (H-23, d) J (15.15Hz,8.65Hz) and 5.38 ppm (H-6, d) J (5.13) while the signals at 3.54 ppm (H-3, td) J (11.15, 5.42) are features of a sterol moiety. The signals at 0.71 ppm and 1.53 ppm are characteristic of H-19 and H-18 angular protons attached to the sterol moiety at position 10 and 13 respectively. The other signals at 0.73 ppm and 1.04 ppm corresponds to the methyl and ethyl protons of H-26 and H-29 attached at position 25 and 28 respectively. Other signals observed at 0.95 ppm J (6.34) corresponds to the H-27 protons.

The ^1H-^1H- COSY showed correlations at 5.04 ppm (H-22, d), 5.18 ppm (H-23, d) and 5.38 ppm (H-6, d) characteristics of vinylic protons. ^1H-^1H coupling signals were also observed at 3.54 ppm (H-3.td, J=11.15, 5.42 Hz), 0.71 ppm (H-19 s,) and 1.53 ppm (H-18) all confirming the presence of a sterol moiety. The DEPT-135 spectrum showed the presence 6 $-CH_3$, 9 $-CH_2$ and 11-CH carbons. The signals at 138.33 ppm (C-22), 129.30 ppm (C-25) and 121.74 ppm (C-6) are characteristics of the three olefinic carbons of the sterol moiety. Also, the signals at 71.84 ppm corresponds to the methine carbon (C-3) of the sterol moiety.

However, all the signals were equally observed for the angular methyl carbons (C-18 and C-19) at 12.06 ppm and 19.41 ppm (attached to the sterol moiety at C-10 and C-13 respectively.

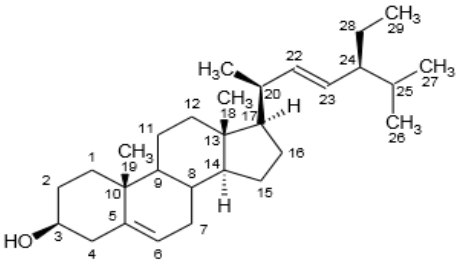
^1H - ^{13}C HSQC spectra showed correlations between the carbon atom at (C-22) at 138.33 ppm and the proton (H-22) at 5.04 ppm, C-23 at 129.30 ppm and (H-23) at 5.18 ppm and the carbon atom (C-6) at 121.74 ppm and the proton (H-6) at 5.38 ppm as well as between the carbon atom (C-3) at 71. 84 ppm and the proton (H-3) at 3.54 ppm.

The signals at 37.27 (C-1),39.80 (C-12), 24.32 ppm (C-15) and 28.93 ppm (C-16) were assigned to the methylene carbons at protons 1,2,15 and 16 respectively. Also, H - ^{13}C HSQC showed single bond coupling between carbon atoms (C-26) at 21.10 ppm and the proton (H-26) at 0.84 ppm as well as the carbon atom (C-27) at 21.23 ppm and the proton (H-27) at 0.95 ppm.

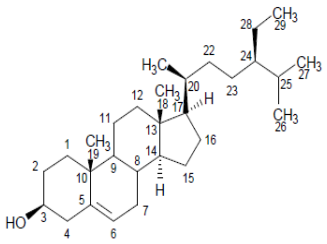
^1H - ^{13}C (HMBC) spectrum showed correlation between the Olefinic carbon (C-23) at 129.30 ppm with ^1H-22 at 5.04 ppm (^2J CH). There were also coupling between carbon (C-25) at 36.16 ppm with ^3H - 27 at 0.95 ppm (^2J CH). The methyl carbon (C-26) at 21.10 ppm coupled with ^3H- 27 at 0.95 ppm (^3J CH). Also, the methine carbon (C-28) at 25.42 ppm coupled with ^3H - 29 at 1.04ppm (^2J CH). The

analysis of the ^{1}H, ^{1}H-^{1}H COSY, DEPT, HSQC and HMBC were in agreement with reported literatures for stigmasterol (Habib *et al.*,2007; Jain and Bari,2010; Kamboj and Suleja, 2011; Chaturvedula and Indra, 2012; Pierre *et al.*, 2015; Okoro *et al., 2017*). This confirm that A20 isolated from *E. coccinea* leaves is stigmasterol.

It is important to note that stigmasterol as a sterol compound comes with β-sitosterol. they are difficult to obtain in their pure forms from the mixture. The only difference between the two compounds is the presence of C22=C23 double bond in stigmasterol and C22-C23 single bond in β-sitosterol. Stigmasterol is a ubiquitous phytosterol occurring naturally in various varieties of plants. However, this is the first time it is reported for *E. coccinea* leaves.



Stigmasterol $C_{29}H_{48}O$



β-sitosterol $C_{29}H_{50}O$

Fig 1. Structure of stigmasterol and β-sitosterol

4.0 Conclusion

This is a novel report on the isolation of stigmasterol and β-sitosterol from *E. coccinea* leaves. The isolated compounds (Stigmasterol and β-sitosterol) might contribute to the use of the plant in forklore medicine for the treatment of various ailmentssuch as tumor, inflammation, cough, rheumatism, fever, dysentery, wounds, eye inflammations, night blindness, ear-aches and in preventing miscarriages.

5.0 Acknowledgements

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Conflict of Interest

The authors declare no conflict of interest