IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
April 2018 Vol.:12, Issue:1

Isolation and Characterization of α-Amyrin and β-Sitosterol from Ethyl Acetate Extract of the Leaves of *Monechma ciliatum* (Jacquin)

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
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ISSN 2349-7203
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Submission: 27 March 2018
Accepted: 03 April 2018
Published: 30 April 2018

Keywords: *Monechma ciliatum*, Jacquin, Acanthaceae, HNMR, CNMR

ABSTRACT

M. ciliatum is extensively used in Nigeria as remedy for general body pain, liver, cold, diarrhea and sterility. The study aims to isolate and characterize compounds from the ethyl acetate leaves extract of the plant. Healthy leaves of the plant were collected, washed under running water to remove earthy impurities, shade dried, soxhlet extracted using ethyl acetate and concentrated to obtain the ethyl acetate extract. While column and thin layer chromatography were used for the isolation and purification of the ethyl acetate extract respectively, ^{1}HNMR and ^{13}CNMR were employed for the structural elucidation of the isolates. The result reveals colorless crystalline solids U and M whose ^{1}HNMR, ^{13}CNMR and DEPT spectra data agrees reasonably with literature for the compounds α-amyrin benzoate and β-sitosterol. From these results and to the best of our knowledge this is the first report of the isolation of α-amyrin benzoate and β-sitosterol from the ethyl acetate leaves extract of M. *ciliatum*.

INTRODUCTION

Monechma ciliatum (Jacquin) belong to the family Acanthaceae. Although it is cultivated in the Savannah, it grows wide in tropical Africa. The plant (Figure 1) is a small herb that grows a few inches above the ground. Its leaves are simple, measuring about 4–7 x 1–2 cm (Murtada and Abdelkarim, 2013). In Northern Nigeria, it is locally known as "Damfarkami" in the Hausa language. The leaves are linear or narrowly linear-lanceolate growing up to 10 cm long and 1.25 cm broad (Taha and Mustapha, 2015). The leaves of *M. ciliatum* has been reported to be used as remedy for general body pain, diarrhea, sterility in women and has been demonstrated to possess oxytocic property *in vivo* and *in vitro* (Mariod *et al*., 2010).

Natural products provide unlimited opportunities for new drug discoveries because of the unmatched availability of its chemical diversity (Cosa *et al*., 2006). The medicinal relevance of these plants lies in the presence of secondary metabolites present in them (Anyasor *et al*., 2011; Edeoga and Gomina, 2000). They serve as the source of structurally novel compounds that might serve as the lead for the development of novel drugs not yet attributed to known compounds (Hostettmann, 1987; Hamburger and Hostettmann, 1991). Pentacyclic triterpenes are ubiquitously distributed throughout the plant kingdom, in a free form as aglycones or in combined forms, and have long been known to have a number of biological effects. The compounds α-amyrin and β-sitosterol are commonly found in medicinal plants (Carretero *et al*., 2008). It is against this backdrop of the enormous ethnobotanic value and lack of commensurate isolations of active metabolites thereof that we investigate this plant. We hereby report the isolation and characterization of a triterpenoid and steroid from the ethyl acetate leaf extract of *M. ciliatum*.

Figure 1: *M. ciliatum* in its natural habitat

MATERIALS AND METHODS

Collection, Identification and Preparation of Plant Samples

Fresh leaves of *M. ciliatum* were collected from the Sokoto State University, Sokoto State Nigeria in June 2017. They were washed under running water to remove earthy impurities, identified and authenticated at the Botany Unit, Department of Biological Science, Usmanu Danfodiyo University Sokoto where a herbarium specimen was deposited and a voucher number UDOH/ANS/0191 issued. The plants were air dried for 3 weeks with occasional turning to prevent rot before they were reduced to fine powder with the aid of an electric milling machine. The powdered sample was stored in clean, air-tight glass container until required for use.

Extraction and Purification

Preparation of extract

Powdered plant material (1.00 kg) was extracted with ethyl acetate using soxhlet apparatus. The extract was concentrated to dryness using a rotary evaporator at 40ºC under reduced pressure. The concentrated extract was air dried, weighed and stored in an air-tight container.

Column Chromatography (CC)

120.0 g of silica gel (60-120 mesh sizes) was made into the slurry with 100% n-hexane and then packed into a 2.5 cm × 63 cm glass column. It was allowed to stand for 24 hours to attain stability. 3.0 g of the ethyl acetate extract pre-adsorbed on 3 g of silica gel was loaded into the column. The loaded sample was eluted gradiently starting with n-hexane (100 %), n-hexane: ethyl acetate (99:1), n-hexane: ethyl acetate (98:2), n-hexane: ethyl acetate (97:3) and n-hexane: ethyl acetate (96:4). Increase in polarity of the solvent system of n-hexane: ethyl acetate results in rapid elution of the entire colored compounds, so the solvent system of n-hexane: ethyl acetate (96:4) was adopted.

Thin Layer Chromatography

Concentrated fractions were subjected to Thin Layer Chromatography (TLC) and similar fractions were pooled together on the basis of TLC similarity. Fractions U and M obtained were stored at room temperature for further analysis. Both fractions were characterized using

NMR analyses (^{1}H, ^{13}C, and DEPT-135). Isolates U and M were tested for the presence of steroidal nucleus using the Liebermann Burchard and Salkowski tests (Nnamonu *et al*., 2016; Luhata and Munkombwe, 2015; Arjun *et al*., 2010).

RESULTS

The soxhlet extraction of the plant material gave a percentage yield of 4.9%. Isolates M and U (Figures 2 and 3) were colorless and whitish needle-like crystalline solids respectively soluble in n-hexane, ethyl acetate, and chloroform. They both tested positive for the presence of steroidal nucleus.

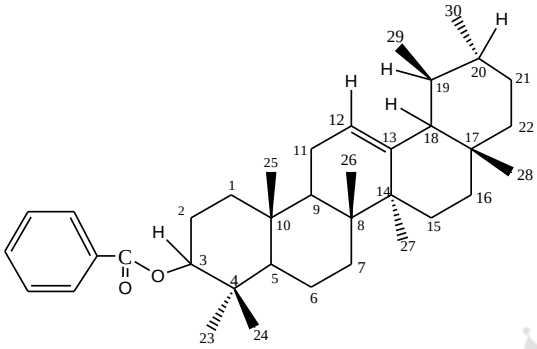

Figure 2: α-Amyrin benzoate (Isolate M)

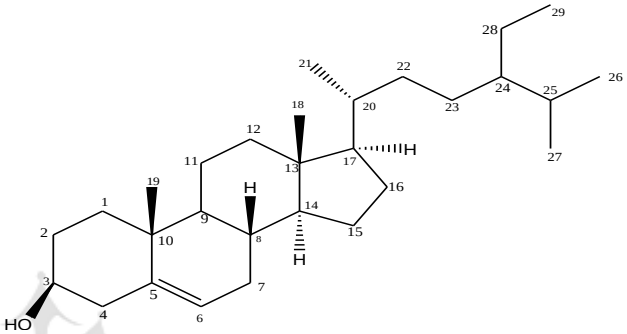

Figure 3: β-sitosterol (Isolate U)

^{1}H-NMR and ^{13}C-NMR spectra data of isolates M and U were consistent with those published for α-amyrin and β-sitosterol respectively (Tables 1 and 2) (Okoye *et al*., 2014; Habib *et al*., 2007; Patra *et al*., 2010).

Table 1: ^{13}C, ^{1}H, and DEPT NMR Data of Isolate M Compared with Literature Values (300 MHz, $CDCl_3$)

Position	^{13}C NMR of M	^{1}H NMR of M	DEPT	^{13}C NMR (Reference)	^{1}H NMR (Reference)
1	37.89	1.55	CH_2	38.46	….
2	23.44	1.61	CH_2	23.45	1.61
3	81	4.44	CH	81.03	4.48
4	37.84	….	C	37.78	….
5	….	0.80	CH	55.33	0.81
6	18.23	1.34	CH_2	18.32	1.34
7	32.8	1.26	CH_2	32.94	….
8	39.87	….	C	39.72	….
9	48	1.53	CH	48.08	1.54
10	36.64	….	C	36.86	….
11	23.44	1.8	CH_2	23.45	1.89
12	124.27	5.05	CH	124.39	5.1
13	135.20	….	C	139.72	….
14	41.76	….	C	42.14	….
15	27.94	1.18	CH_2	28.15	….
16	26.92	0.91	CH_2	26.68	….
17	32.64	….	C	33.82	….
18	62.22	1.30	CH	59.13	1.29
19	39.56	1.36	CH	39.72	1.38
20	39.87	1.96	CH	40.8	1.98
21	51.48	1.31	CH_2	31.33	….
22	41	1.23	CH_2	41.6	….
23	29.18	0.86	CH_3	28.15	0.85
24	16.23	0.82	CH_3	16.06	0.84
25	16.53	0.96	CH_3	16.26	0.96
26	16.9	0.96	CH_3	16.83	0.98
27	22.86	0.99	CH_3	23.21	1.04
28	27.60	1.02	CH_3	28.83	0.78
29	17.51	0.78	CH_3	17.59	0.77
30	21.03	0.76	CH_3	21.49	0.83
1^0	173.35		C	171.07	
1^{00}	129.91				
2^{00}	124.20				
3^{00}	125.02				

Table 2: ^{13}C, ^{1}H, and DEPT NMR Data of Isolate U Compared with Literature Values (300 MHz, $CDCl_3$)

Position	^{13}C-NMR of U	^{1}H-NMR	DEPT	^{13}NMR (Reference)	$^{1}HNMR$ (Reference)
1	37.32	1.47	CH2	37.2	1.47
2	31.94	1.55	CH2	31.69	1.56
3	68.20	3.5	CH	71.82	3.52
4	39.88	2.14	CH2	42.33	2.28
5	139.33	….	C	140.7	….
6	124.20	5.2	CH2	121.72	5.36
7	30.36	2.3	CH	31.69	2.03
8	32.91	1.67	CH	31.93	1.67
9	50.8	1.49	CH	50.13	1.48
10	37.03	….	C	36.52	….
11	22.88	1.53	CH2	21.1	1.52
12	39.57	1.47	CH2	39.8	1.49
13	42.39	….	C	42.33	….
14	56.87	1.5	CH	56.79	1.5
15	25.71	1.61	CH2	24.37	1.6
16	28.23	1.88	CH2	28.25	1.84
17	56.26	1.47	CH	56.09	1.49
18	14.06	0.67	CH3	11.86	0.68
19	17.51	1.02	CH3	19.4	1.02
20	37.02	1.61	CH	36.32	1.64
21	16.24	0.94	CH3	18.32	0.94
22	33.84	0.88	CH2	33.98	0.88
23	26.52	1.07	CH3	26.14	1.04
24	45.83	1.5	CH	45.88	1.5
25	29.72	1.72	CH	28.91	1.65
26	19.73	0.83	CH3	19.8	0.83
27	18.79	0.85	CH3	18.79	0.85
28	22.87	1.03	CH2	23.1	1.04
29	11.5	0.89	CH3	11.99	0.88

DISCUSSION

The ^{1}H-NMR spectrum of compound M revealed the presence of several signals between 0.77 and 1.02 ppm (Table 1) which are attributed to overlapping methyl, methylene and methine protons typical of triterpenes. The triplet observed at 5.05 ppm is typical of an olefinic proton (H-12); that at δ 4.45ppm corresponds to the oxymethine proton typical of hydrogen at C-3 of triterpenes. This relatively deshielded signal is indicative of substitution of the hydroxyl group with benzoate group at C-3. The deshielding of this methyl proton was as a result of its proximity to a carbonyl functional group.

The ^{13}C-NMR spectrum showed recognizable signals at δ 124.27ppm and 138.27 ppm. The peak at 124.27 ppm was assigned to C- 12 while that at 138.27 ppm was assigned to C-13. These are indicative of olefinic carbons at C-12 and C-13. Also, there was an observed carbon signal at δ 62.11 ppm which corresponds to the methane carbon, C-18 of the alpha-amyrin moiety. The ^{13}C-NMR signal at 172.23 ppm is attributed to the carbonyl carbon of a benzoate group attached to C-3 of the alpha amyrin, while the peak at 81.00 ppm is indicative of oxymethine carbon (C-3) which was slightly deshielded due to its attachment to the benzoate group. The compound consists of 30 carbon atoms and a benzoate group at 172.23 ppm (C1$^{\bullet}$) which is characteristics of amyrin skeleton (Okaye *et al*., 2014). Other signals observed are 129.72 ppm and 125.03 ppm for the benzene ring in the same chemical environment.

Three spectra were obtained from the DEPT spectra. The first is a normal broad-band decouple spectrum, the second spectrum (DEPT 90 spectrum) is obtained under special condition in which only carbon bonded to a single hydrogen appear and the third spectrum (DEP-135 spectrum) is obtained under conditions in which CH's and CH_3's appear as normal signals, but CH_2's appear as negative absorptions and no peaks for quaternary carbons. The DEPT 135 of isolate M showed positive signals due to methane (CH) and methyl (CH_3). The negative signals were seen due to methylene (CH_2). The expected signal for 81.00 ppm (C3) was lost in the DEPT-135 spectrum because it is quaternary carbon. All these assignments were consistent with the data obtained from the literature for alpha-amyrin acetate (Okoye *et al*., 2014; Rasoanaivo *et al*., 2014 and Nnamonu *et al*., 2016). α-amyrin derivatives have been reported to have erectile dysfunction (Watcho *et al*., 2012), anti-inflammatory (Nnamonu *et al*., 2016), growth inhibitory effect on *Streptococcus* (Diaz-Ruiz and Hernandez-Vazquez, 2012), antihepatotoxic as well as antioxidant potentials (Donfack *et al.*, 2010).

The ^{1}HNMR spectrum of isolate U has revealed that the proton of H-3 appeared as a multiplet at δ 3.50 ppm and showed the existence of signals for olefinic proton at δ5.03 ppm. Angular methyl proton at 0.68 and 1.02 ppm corresponds to C18 and C19 proton respectively. This compound is having six overlapping methyl proton (0.7-1.08 ppm), eleven methylene and three quaternary carbons with a hydroxyl group. The above spectral features are in close agreement with those observed for β – Sitosterol (Manoharan *et al*., 2005; Bulama *et al*., 2015).

The ^{13}C-NMR has shown recognizable signals at 139.29 ppm and 124.28 ppm which are assigned to C5 and C6 double bonds respectively. The peak at 17.69ppm corresponds to an angular carbon atom (C19), C25 17.69 ppm and secondary hydroxyl-bearing carbon at 68.16 ppm for C3. The ^{13}C NMR also showed recognizable signals 179.21 ppm, which are assigned to C1. The structure was simulated using DEPT/NMR program to obtain the chemical shifts of both proton and carbon (Bulama *et al*., 2015; Luhata and Munkombwe, 2015 and Arjun *et al*., 2010).

CONCLUSION

α-amyrin benzoate and β-sitosterol are bioactive compounds commonly found in all parts of medicinal plants and their extensive pharmacological activities have been reported. The fact that both compounds were isolated from the leaves of *M. ciliatum* supports some of the ethnomedicinal applications of the plant.

DECLARATION OF INTEREST

The authors declare that they have no competing interests

AUTHOR CONTRIBUTIONS

LGH designed and supervised the work, UAU carried out the work with the help of HEM. KJU and CO provided some literature information as well as assisted in interpreting spectra data. All authors read and approved the final manuscript.

REFERENCES

1. Anyasor GN. Aina DA. Olushola M. Aniyikaye AF. 2011. Phytochemical constituent, proximate analysis, antioxidant, antibacterial and wound healing properties of leaf extracts of *Chromolaena Odorata*. Ann Biol Res. 2011; 2(2): 441-451.
2. Arjun P. Jha S. Murthy PN. Manik M. Sharone A. Isolation and Characterization of β – Sitosterol from the leaves of *Hygrophila spinosa*. Int J Pharma Sci. Res. 2010; 1(2): 95 - 100
3. Bulama JS. Dangoggo SM. Mathias SN. Isolation and Characterization of Beta-Sitosterol from ethyl acetate extract of root bark of *Terminalia glaucescens.* Int J Sci Res Pub. 2015; 5(3): 1-3
4. Carretero ME. López-Pérez JL. Abad MJ. Bermejo P. Tillet S. Israel A. Noguera PB. Preliminary study of the anti-inflammatory activity of hexane extract and fraction from *Bursera simaruba* (Linneo) Sarg. (Burseraceae) leaves. J Ethnopharmacol. 2008; 116: 11-15.
5. Cosa P. Vlietinck AJ. Berghe DV. Maes L. Anti-infective potential of natural products: How to develop a stronger *in vitro* 'proof-of-concept'. J Ethnopharmacol. 2006; 106:290–301.
6. Diaz-Ruiz G. Hernandez-Vazquez L. Growth inhibition of Streptococcus from the oral cavity by α-amyrin esters. Molecules. 2012; 17: 12603-12611.
7. Donfack JH. Simo CCF. Ngameni B. Tchana AN. Kerr PG. Finzi PV. Antihepatotoxic and antioxidant activities of methanol extract and isolated compounds from *Ficus chlamydocarpa*. Nat Prod Commun. 2010; 5(10): 1607–1612
8. Edeoga HO. Gomina A. Nutritional values of some non-conventional leafy vegetable in Nigeria. J Eco Tax Bot. 2000; 24:7-13.
9. Habib MR. Nikkon F. Rahman M. Haque ME. Karim MR. Isolation of stigmasterol and beta-sitosterol from methanolic extract of root bark of *Calotropis gigantea* (Linn). Pak J Biol Sci. 2007; 15;10(22): 4174-4176.
10. Hamburger M. Hostettmann K. Bioactivity in plants: the link between phytochemistry and medicine. J Phytochem. 1991; 30 (12):3864 – 3874.
11. Hostettmann K. Bull Soc Fib Sc Nat. 1987; 76: 51-63.
12. Luhata LP. Munkombwe NM. Isolation and Characterisation of Stigmasterol and β -Sitosterol from *Odontonema Strictum* (Acanthaceae). J Innov Pharma Biol Sci. 2015; 2(1): 88-95
13. Manoharan KP. Haut BTK. Yang D. Cycloartane types triterpenoids from the rhizomes of *Polygonum bistorta*. Phytochem. 2005; 66: 1168 – 1173
14. Mariod AA. AL-Naqeb G. Ismail M. *Monechma ciliatum* methanolic extract regulates low-density lipoprotein receptor and 3-hydroxy-3- methylglutaryl coenzyme A reductase genes expression in HepG2 cells. Afr. J Biotech. 2010; 9(36): 5813-5819
15. Murtada AO. Abdelkarim MA. Phytochemical screening and evaluation of *Monechma ciliatum* (black mahlab) seed extract as antimicrobial agents. Avi J Phytomed. 2013; 3(2):126-134.
16. Nnamonu LA. Tor-Anyiin TA. Ugbenyo NO. Anyam JV. Isolation and characterization of α-Amyrin from stem Back of *Ficus exasperata (Vahl)*. Biotech J Int. 2016; 16(4): 23-32.
17. Okoye NN. Ajaghaku DL. Okeke HN. Ilodigwe EE. Nworu CS. Okoye FBC. Beta-Amyrin and alpha-amyrin acetate isolated from the stem bark of Alstonia boonei display profound anti-inflammatory activity. Pharm Biol. 2014; 52(11): 1478–1486.
18. Patra A. Jha S. Murthy PN. Sharone A. Isolation and characterization of stigmast-5-en-3β-ol (β-sitosterol) from the leaves of *Hygrophila spinosa* T. Anders. Int J Pharma Sci. Res. 200; 1(2): 95-100
19. Rasoanaivo LH. Wadouachi A. Andriamampianina TT. Andriamalala SG. Razafindrakoto EJB. Raharisololalao A. Randimbivololona F. Triterpenes and steroids from the stem bark of *Gambeya boiviniana* Pierre. J Pharmacogn Phytochem. 2014; 3(1):68–72.
20. Taha MSM. Mustapha MAE. The effect of agricultural practices on growth and yield of Black Mahlab (*Monechma ciliatum).* Agri Biol Sci J. 2015; 1(2): 31-36.
21. Watcho P. Zelefack F. Ngouela S. Nguelefack TB. Kamtchouing P. Tsamo E. Enhancement of erectile function of sexually naïve rats by β–sitosterol and α–β–amyrin acetate isolated from the hexane extract of *Mondia whitei*. Asian Pac J Trop Biomed. 2012; 2(3): 1266–1269.