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Full Length Research Article

Isolation and Characterization of Lupeol and Betulinic Acid from the Aerial Parts of *Euphorbia convolvuloides*

***[1]H. A. Kwazo, [2]U. Z. Faruq, [3]L. G. Hassan, [4]M. E. Sadiq, [5]A. J. Yusuf, [1]M. Salihu**
[1] Department of Chemistry, Shehu Shagari College of Education Sokoto, Nigeria
[2]Department of Energy and Applied Chemistry, Usmanu Danfodiyo University, Sokoto, Nigeria
[3]Department of Pure and Environmental Chemistry, Usmanu Danfodiyo University, Sokoto, Nigeria
[4]Department of Biochemistry and Molecular Biology, Usmanu Danfodiyo University, Sokoto, Nigeria
[5]Department of Pharmaceutical and Medicinal Chemistry, Usmanu Danfodiyo University, Sokoto, Nigeria
[*Corresponding Author: Email: khadijahkwazo@gmail.com; ☎:+2347065634929]

ABSTRACT
Euphorbia convolvuloides is used in traditional medicine to treat bacterial infection, peptic ulcers and asthma among others. The aim of this research is to isolate and characterize the antibacterial constituent(s) from the aerial parts of *E. convolvuloides.* The ethyl acetate fraction of *E. convolvuloides* was subjected to column chromatography using a combination of silica gel and sephadex LH-20, which led to the isolation of colorless and yellowish crystalline substance coded EAF_1 and $EAF_{5A,}$ respectively. Spectral analysis (1D-NMR) of the isolated compounds and comparison with the literature data indicated EAF_1 to be lupeol and $EAF_{5A,}$ to be betulinic acid. In conclusion, the two triterpenoidal compounds isolated from the ethyl acetate fraction of *E. convolvuloides* aerial parts are known for their range of biological effects which include anti-bacterial activity.
Keyword: *E. convolvuloides,* Isolation, Characterization, Anti-bacterial.

INTRODUCTION
Antimicrobial resistance (AMR) poses a significant global public health threat undermining formerly successful treatments, resulting in prolonged illnesses, heightened mortality rates, and elevated healthcare expenses (Alex-Asaolu *et al.,* 2023). The indiscriminate use of antibiotics, particularly with inappropriate prescription practices in Africa, has contributed to the predominance of antibiotic resistance as a major form of antimicrobial resistance. Alarmingly; certain bacteria such as *Escherichia coli, Klebsiella pneumonia, Salmonella spp., Acinetobacter baumannii*, and *Staphylococcus aureus* have exhibited high rates of antibiotic resistance (Walusansa *et al.,* 2022). Hence, it is imperative to explore alternative treatments from natural sources that are more effective, relatively cheap, easily accessible, and reduced side effects (Mailafiya *et al.,* 2018).

E. convolvuloides (Figure 1) belonging to the *Euphobiaceae* family, commonly known as "Dove milk" in English and locally "nononkurciya" in Hausa, and Egele in Igbo (Ijioma *et al.,* 2017; Muftau and Musa 2020). Most of the species in this family are mostly found in the tropics including tropical Africa and tropical America, (Adewumi *et al.,* 2019; GBIF, 2024). *E. convolvuloides* is an erect plant with drooping tops on its twigs, typically growing 10 to 40 cm tall with a simple or minimally branched axis. *E. convolvuloides* is commonly used in Northern Nigeria for treating diarrhea and dysentery (Jain *et al.,* 2017); respiratory tract infections and insect bite (Ijioma *et al.,* 2021).

The aerial parts of the crude extract of *E. convolvuloides* exhibits a range of phytoconstituents including alkaloids, flavonoids, phlobatannins, saponins, and anthraquinones with antioxidant and antibacterial activities (Kwazo *et al.* (2022). When tested on isolated uterine tissue, *E. convolvuloides* demonstrated tocolytic effects; tocolytic agents have proven valuable in managing preterm labor and preventing premature birth (Ijioma *et al.,* 2020). The ethyl acetate fraction has demonstrated good antibacterial activity against *E. coli*, and *S. typhi* (Kwazo, 2024). In this paper, we report the isolation and characterization of Lupeol and Betulinic acid from the aerial parts of *E. convolvuloides.*

Figure 1: *E. convolvuloides* plant in its natural habitat (GBIF, 2024)

MATERIALS AND METHODS
Collection and Identification of Plant Materials
The aerial parts of *E. convolvuloides* were collected in December, 2022 from its native habitat in Dukai village, located in the Silame Local Government Area of Sokoto State, Nigeria. It was identified at the herbarium unit, Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto, Nigeria and a specimen was deposited with voucher number PCG/UDUS/EUPH/0013.

Preparation and Extraction of Plant Materials

The aerial parts were air-dried under shade and pulverized to powder, labeled, and stored at room temperature for use. The powdered sample of the plant was extracted with methanol to obtain crude extract, which was then fractionated with hexane, chloroform, ethyl acetate and n-butanol. The extract and its fractions were evaporated *in-vacuo* using a rotary evaporator at 40 ^{o}C. The antibacterial activity of the ethyl acetate fraction was previously demonstrated which is the basis for selection for chromatographic separation.

Chromatographic Separation of Ethyl Acetate Fraction

Column Chromatography using Silica Gel

The procedure described by Hassan *et al.* (2018) was adopted for column chromatography. In this method, 4.0 g of the EAF was pre-adsorbed with silica gel (1.0 g) and a glass column (50 cm x 5cm, 500 cm^3 capacity) was used. The column was wet-packed with silica gel which was made into a slurry with 100% DCM (250g; mesh sizes 60-120) and the pre-adsorbed EAF was loaded onto the column and allowed to stabilize for one hour before the elution commenced. The column was eluted using a gradient technique starting with 100 % DCM, mixtures of DCM and ethyl acetate to 100 % ethyl acetate. A total of 331 fractions were collected from the column and pooled together based on their TLC profile to give 19 fractions coded EAF_1 to EAF_{19}. Fraction EAF_1 was found to have a single and homogenous spot on TLC. EAF_5 fraction also gave a single spot with little impurities and thus was subjected to further purification with Sephadex LH-20 using isocratic elution with acetone resulting in the isolation of a compound coded EAF_{5A} with a single homogenous spot.

Gel Filtration Chromatography of Fraction EAF_5

The method outlined by Yusuf *et al.* (2020) was utilized to purify fraction EAF_5. Slurry was made from 10 g of Sephadex LH-20 and 50 cm^3 of acetone, allowing it to swell for 24 hours. The swollen Sephadex LH-20 was then loaded onto a 50 cm^3 column and EAF_5 (1.5 cm^3) was added to the column and eluted isocratically with acetone, resulting in EAF_{5A}. The purity of EAF_{5A} was further assessed using TLC with DCM: HE (4:1) solvent system and subjected to NMR spectroscopy analysis.

Characterization of EAF and EAF_{5A}

Physical appearance

The color and nature of EAF and EAF_{5A} were noted.

Solubility test

The solubility of EAF and EAF_{5A} were checked using DCM: EA (7:1) and DCH: HE (4:1) respectively.

Spectroscopic studies

The two compounds were subjected to 1D-NMR (400 MHz $CDCl_3$) spectroscopic analysis for structural elucidation.

RESULTS

Physical properties of EAF and EAF_{5A}

The physical properties of the isolated compounds EAF (Figure 2) and EAF_{5A} (Figure 2) such as weight, retention factors, colors and solubility are presented in Table 1.

Compound EAF

Compound EAF was isolated as a colorless compound with a mass of 25 mg from ethyl acetate fraction obtained from silica gel column chromatography of the ethyl acetate fraction, and it was found to be soluble in DCM and acetone. The identity of the compound was confirmed using ^{1}H-NMR and ^{13}C-NMR (400 MHz $CDCl_3$) by comparing its NMR data with the literature as summarized in Table 2.

^{1}H- NMR and ^{13}C-NMR Data of Compound EAF_{5A}

Compound EAF_{5A} was obtained as a yellowish solid substance with a total mass of 14 mg from the purification of EAF_5 fraction obtained from silica gel column chromatography of the ethyl acetate fraction and the compound was found to be soluble in DCM and acetone. The structure of the compound was confirmed by comparing its literature, as summarized in Table 3.

DISCUSSION

Compound EAF

Compound EAF was isolated as a colorless solid compound with a mass of 25 mg from the ethyl acetate fraction obtained from silica gel column chromatography of the ethyl acetate fraction. It was found to be soluble in DCM and acetone. The identity of the compound was confirmed using $^1H_{NMR}$ and $^{13}C_{NMR}$ (400 MHz $CDCl_3$) by comparing its NMR data with the literature, as summarized in Table 2. The $^1H_{NMR}$ spectrum of EAF indicated the presence of angular methyl protons at signal δ_H 0.76 (s), 0.80 (s), 0.83 (s), 0.95 (s), 1.02(s), and 1.64 (s) at position C-24, C-28, C-25, C-27, C-23, C-26, and C-30 respectively, which is indicative of methyl groups (Shwe *et al.,* 2019). The signal at δ_H 3.16 (H-3) was observed as doublet and olefinic protons δ_H 4.61 and δ_H 4.62 (H-29) were assigned to the exocyclic double bond these chemical shift values were in close agreement with those reported for lupeol (Shwe *et al.,* 2019). The $^{13}C_{NMR}$ on EAF indicated the presence of 30 carbon atoms which are inconsistent with the proton NMR, major resonances observed include δ_C 18.02(C-28), δ_C 109(C-29) and δ_C 151.0 (C-20).

Table 1: Physical properties of EAF and EAF_{5A}

COMPOUND	MASS (mg)	MELTING POINT	COLOR	SOLUBILITY
EAF	25	215°C	Colorless	DCM/Acetone
EAF_{5A}	14	316°C	Yellowish	DCM/Acetone

Key: EAF = Ethyl acetate fraction, and DCM = Dichloromethane.

Table 2: ^{1}H-NMR and ^{13}C-NMR data of EAF compared with Literature values

POSITION	^{1}H-NMR (ppm)	Shwe *et al.* (2019) δ_H (ppm)	^{13}C-NMR (ppm)	Shwe *et al.* (2019) δ_C (ppm)	(CH)n
1	0.90, 1.64	0.90, 1.65	38.87	38.85	CH_2
2	1.52, 1.56	1.52, 1.67	27.46	27.44	CH_2
3	3.16	3.2	79.03	78.99	CH
4	-	-	38.72	38.70	C
5	0.67	0.67	55.31	55.29	CH
6	1.35, 1.52	1.37, 1.52	18.33	18.31	CH_2
7	1.40	1.39	34.29	34.27	CH_2
8	-	-	40.85	40.83	C
9	1.26	1.25	50.45	50.43	CH
10	-	-	37.18	37.16	C
11	1.19, 1.40	1.20, 1.40	20.94	20.92	CH_2
12	1.07, 1.61	1.06, 1.62	25.15	25.13	CH_2
13	1.82	1.66	38.06	38.05	CH
14	-	-	42.85	42.82	C
15	1.07, 1.57	1.05, 1.60	27.43	27.41	CH_2
16	1.35, 145	1.35, 1.45	35.60	35.58	CH_2
17	-	-	42.85	42.99	C
18	1.32, 1.41	1.36, 1.37	48.32	48.30	CH
19	2.34, 1.45	2.40, 1.45	48.00	47.98	CH
20	-	-	151.00	150.97	C
21	1.32, 1.90	1.3, 1.91	29.86	29.84	CH_2
22	1.19, 1.35	1.18, 1.37	40.02	39.99	CH_2
23	0.90	0.90	28.00	27.98	CH_3
24	0.76	0.79	15.38	15.36	CH_3
25	0.83	0.83	16.11	16.11	CH_3
26	1.02	1.03	15.99	15.97	CH_3
27	0.93	0.94	14.60	14.60	CH_3
28	0.80	0.79	18.02	17.99	CH_3
29	4.61, 4.62	4.57, 4.69	109.34	109.31	CH_2
30	1.64	1.67	19.32	19.30	CH_3

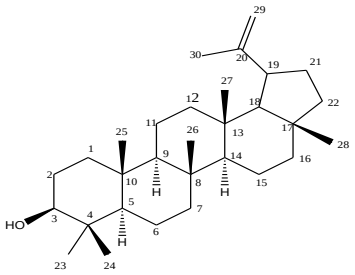

Figure 2: Structure of EAF (Lupeol)

Table 3: Comparison of 1D- NMR Data of EAF_{5A} with reported literature

POSITION	^{1}H-NMR (ppm)	Halilu *et al.* (2013) δ_H (ppm)	^{13}C-NMR (ppm)	Halilu *et al.* (2013) δ_C (ppm)	(CH)n
1	0.86 (2H, *m*)	0.98	40.01	38.37	CH_2
2	1.54 (2H, *m*)	1.83	27.42	27.38	CH_2
3	4.39 (1H, *t*)	3.31	79.01	78.98	CH
4	--	--	38.86	38.85	C
5	1.43 (1H, *m*)	0.82	55.30	55.33	CH
6	1.51 (2H, *m*)	1.56	16.12	16.07	CH_2
7	1.22 (2H, *m*)	1.43	34.29	34.31	CH_2
8	--	--	42.84	40.67	C
9	1.09 (1H, *s*)	1.37	50.44	50.49	CH
10	--	--	38.05	37.19	C
11	1.73 (2H, *m*)	1.43	20.93	20.83	CH_2
12	1.09 (2H, *m*)	1.21	28.00	25.48	CH_2
13	1.76 (1H, *s*)	1.90	38.71	38.71	CH
14	--	--	55.18	42.42	C
15	1.77 (2H, *m*)	1.26	28.00	27.97	CH_2
16	1.85 (2H, *m*)	1.85	40.01	29.68	CH_2
17	--	--	47.99	46.87	C
18	3.04 (1H, *t*)	3.07	48.31	49.25	CH
19	2.20 (1H, *m*)	2.11	77.22	42.42	CH
20	--	--	150.99	150.42	C
21	3.01 (2H, *m*)	3.03	124.42	123.92	CH_2
22	3.00 (2H, *t*)	3.01	35.59	34.31	CH_2
23	0.53 (3H, *s*)	1.00	18.01	20.83	CH_3
24	0.50 (3H, *s*)	0.99	18.01	20.83	CH_3
25	0.80 (3H, *s*)	0.83	15.98	16.06	CH_3
26	0.59 (3H, *s*)	0.60	18.32	18.27	CH_3
27	0.66 (3H, *s*)	0.86	15.38	15.32	CH_3
28	--	--	59.07	56.28	C
29	4.96, 4.52 (2H, *s*)	4.67	109.33	109.66	CH_2
30	1.76 (3H, *s*)	1.76	14.56	19.36	CH_3

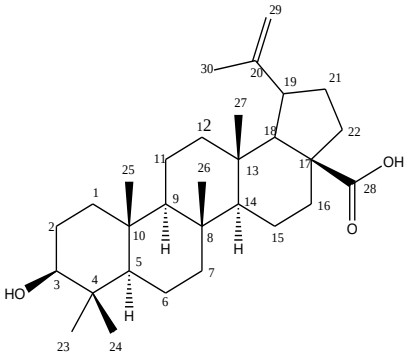

Figure 3: Structure of EAF_{5A} (Betulinic acid)

It also revealed the presence of seven methyl, eleven methylene, six methine, and six quaternary carbons. Based on the 1D-NMR data of EAF, and comparison with related data in the existing literature (Shwe *et al.,* 2019) a tentative structure of EAF was proposed as Lupeol (Figure 2). Lupeol occurs naturally as pentacyclic triterpenoid and is present in a variety of plants (Lalthanpuii *et al.,* 2023) and has been identified as a promising antibiotic agent with susceptibility

against *E. coli, S. typhi,* and *S. saprophyticus*, providing evidence to support its traditional uses in folklore (Musa *et al.,* 2024). The antibacterial effect of lupeol reported in the literature further confirmed the antibacterial activities of fraction ethyl acetate from where it was isolated.

Compound EAF_{5A}

A total of 14 mg of compound EAF_{5A} was isolated as a yellowish solid substance from the ethyl acetate fraction of the aerial parts of *E. convolvuloides*. It was solubilized in DCM and acetone. The $^{1}H_{NMR}$ of EAF_{5A} showed the presence of six tertiary methyl protons at δ_H 0.50, 0.53, 0.59, 0.66, 0.80, and 1.76 assigned to C-23, C-24, C-25, C-26 C-27, and C-30 respectively, thus suggesting a characteristic of triterpenoidal nucleus (Halilu *et al.,* 2013). The spectrum showed two singlets at δ_H 3.00 and 3.08, characteristic of protons of an oxygenated carbon assigned to C-3 and C-28, respectively. The chemical shift value on C-28 indicates the carboxylic acid group (Batool *et al.,* 2024). The two exocyclic olefinic protons were observed at signals at δ_H 4.96 and 4.52 ppm (1H, *m*) assigned to C-29 at δ_C 109.33 suggesting the compound to be betulinic acid (Halilu *et al.,* 2013).

Furthermore, the $^{13}C_{NMR}$ of the compound clearly showed the presence of thirty carbon atoms (Table 3) of which seven are quaternary carbon at δ_c 38.86, 42.84, 38.05, 55.18, 47.99, 150.99, and 59.07 ppm assigned to C-4, C-8, C-10, C-14, C-17, C-20 and C-28, respectively. The spectrum also indicated the presence of eleven methylene groups at δ_C 40.01, 27.42, 16.12, 34.29, 20.93, 28.00, 28.00, 40.01, 124.42, 35.59 and 109.33 ppm assigned to C-1, C-2, C-6, C-7, C-11, C-12, C-15, C-16, C-21, C-22 and C-29 respectively. A comparison of these spectral data of compound EAF_{5A} with those reported by Halilu *et al.* (2013) (Table 2) confirmed the structure of EAF_{5A} to be betulinic acid (Figure 3).

Betulinic acid, a pentacyclic triterpene, is distributed in various plants, such as birch, eucalyptus, and other aerial parts of several plants (Lou *et al.,* 2021). It shows a wide spectrum of biological and pharmacological properties, such as anti-inflammatory, antibacterial, antiviral, antidiabetic, antimalarial, anti-HIV, and antitumor effects (Halilu *et al.,* 2013; Lou *et al.,* 2021). It also protects plants against pathogens and pests through its antifungal, antibacterial, and antiviral properties, and shields cells from oxidative stress caused by environmental factors. Additionally, it regulates plant growth, aids in stress recovery, and inhibits the growth of competing species through allelopathic interactions (Said *et al.,* 2018).

CONCLUSION

Separation via column chromatography and purification using Sephadex LH-20 monitored using thin layer chromatography of the ethyl acetate fraction derived from the aerial parts of *Euphorbia convolvuloides* resulted in the isolation and characterization of lupeol and betulinic acid. Both compounds are triterpenoids which belong to the terpenes family and have been studied for their medical properties, including anti-inflammatory, anti-viral and anti-cancer.

DECLARATION OF COMPETING INTEREST

The authors declared no conflict of interest.

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