

Research Article

Isolation and Characterization of Dammara-20,24-Dien-3-ol from Ethyl Acetate Extract of *Diospyros Mespiliformis* (HOCHST ex. A.DC) Root Bark

*M.D. Mustapha¹, L.G. Hassan², K. J. Umar², K. Abubakar³, H.E. Mshelia⁴, A.J. Yusuf⁵
T. B. Taiwo⁶, S. Abubakar⁷ and M. Salihu⁸

¹Department of Chemistry, Borno State University, Maiduguri, Nigeria

²Department of Pure and Environmental, Usmanu Danfodiyo University, Sokoto, Nigeria

³Department of Pharmacology, Usmanu Danfodiyo University, Sokoto, Nigeria

⁴Department of Pharmacognosy and Ethnopharmacy, Usmanu Danfodiyo University, Sokoto, Nigeria

⁵Department of Pharmaceutical Chemistry, Usmanu Danfodiyo University, Sokoto, Nigeria

⁶Department of Pharmaceutical Chemistry, OAU, Ife, Nigeria

⁷Department of Chemistry, Zamfara state College of Education, Maru 2, Zamfara, Nigeria

⁸Department of Chemistry, Shehu Shagari College of Education, Sokoto, Nigeria

*Corresponding author: mohdmustapha812@gmail.com, doi.org/10.55639/607.2939

ARTICLE INFO:

Keywords:

Diospyros mespiliformis,
Ebenaceae,
Dammara-20,24-dien-3-ol,
Spectroscopic

ABSTRACT

Natural products research has been on an increase with the isolation and characterization of bioactive constituents targeted for pharmaceutical utilization and development. *Diospyros mespiliformis*, which belongs to the family *Ebeneceae*, is used locally or traditionally as an anti-bacterial, anti-fungal, and anti-inflammatory agent. This work aimed at isolating bioactive constituent(s) from the root bark of *Diospyros mespiliformis*. The pulverized root was extracted with solvents of increasing polarity: n-hexane, ethyl acetate, ethanol, and water, with 1.41, 1.44, 8.14, and 4.33 percentage yields obtained, respectively. Dammara-20,24-dien-3-ol was isolated from the ethyl acetate extract and characterized by spectroscopic technique (¹³C-NMR and ¹H-NMR) and by comparison with the literature data. The compound has been reported to be isolated from other plant

Corresponding author: M.D. Mustapha, Email: mohdmustapha812@gmail.com
Department of Chemistry, Borno State University, Maiduguri, Nigeria

INTRODUCTION

D. mespiliformis belongs to the family *Ebenaceae*, locally known as Kanya or Kaywa in Hausa. Mature trees have dark gray bark and can grow to be 4-6 meters tall. The tree produces flowers during the rainy season and is creamy in colour. The female tree bears oval-shaped fruits in the dry season; these fruits appear yellow or purple when ripe and are about 20–30 mm in diameter. The tree is used extensively for its medicinal and nutritional value in the majority of states in northern Nigeria. Available literature and ethnobotanical records suggest that the plant has been used to treat different ailments such as malaria, pneumonia, and syphilis (Orwa *et al.*, 2009). Different portions are also utilized as psychopharmacological medications, to treat dermatomycoses and helminthiases, and to facilitate childbirth (Orwa *et al.*, 2009).

Diospyros mespiliformis is the largest genus in *Ebenaceae* that has more than 500 species with remarkable economical values, especially *Diospyros kaki* Thunb which has traditionally been used as an important food resource in China, Korea, and Japan (Guo, and Luo, 2011). The tree is a native to Africa, Asia and some part of Europe. Leaves are usually simple, alternate, and coriaceous. The inflorescence is cymes, flowers are actinomorphic, and calyx is gamosepalous and persistent. *Diospyros* species are deciduous and evergreen shrubs and trees. The verified biological effects of members of *Diospyros* genus include antioxidant, hepatoprotective, anti-inflammatory, analgesic, antipyretic, antihypertensive, antidiabetic, neuroprotective, antimicrobial, anti-protozoal, fungicidal, anthelmintic, insecticidal, molluscicidal, cytotoxicity, anti-tumor, multidrug resistance (MDR) reversal, and sedative (Abdur Raufa *et al.*, 2017). The crude n-hexane fraction of the dry stem of *D. variegata* Kruz demonstrated significant antinociceptive effect in acetic acid and tail

immersion models (Aminu *et al.*, 2021). This review the current knowledge on ecological, ethnobotanical, and phytochemicals present in these plants and their uses in the treatment of different diseases and disorders. In the current study, we express the isolation and structure elucidation of Dammara-20,24-dien-3-ol from the ethyl acetate extract of *D. mespiliformis*. by means of chromatographic techniques, and its structure was established using ¹H-NMR and ¹³C-NMR spectroscopy.

MATERIALS AND METHODS

Collection and Identification of plant material

Fresh root bark of *D. mespiliformis* was collected in August 2018 at Airport Road in Bodinga Local Government Area, Sokoto State, Nigeria (Long. 5°12'26"E, Lat. 12°05'59"N). The root bark was washed with running water to remove earthy impurities, identified, and authenticated at the Herbarium unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, where the herbarium specimen was deposited with a voucher number (UDUSH/ANS/0244). The sample was shade-dried, pulverized into a fine powder, and packed into a polythene bag at room temperature until use.

Extraction of plant material

The dried powdered plant material (500.0 g) was extracted sequentially with 1.5 L of solvents of increasing polarity (n-hexane, ethyl acetate, ethanol, and water) using the Soxhlet extraction method. The heating mantle on the extractor was switched on and allowed to reflux the mixture for 2 h at 70°C, in order of their polarities. The extracts were concentrated under reduced pressure at 45°C in a rotary evaporator and dried at room temperature to a constant weight. The percentage yield of the extracts n-hexane extract (NHE), ethyl acetate extract (EAE), ethanol extract (ETE), and water extract (WE) was determined based on the amount of pulverized

material extracted in accordance to the procedures outlined by Cyril *et al.*, 2017 and Ogbiko *et al.*, 2018.

Chromatographic Separation and Purification

Thin layer chromatography

Pre-coated plates (Silica gel Kiesel gel 60G, MERCK Darmstadt Germany) were cut into appropriate sizes and n-hexane 100%, n-hexane: ethyl acetate (9:1, 8:2, 7:3), ethyl acetate: ethanol (9:1, 8:2), ethyl acetate: chloroform: water (6:2:2) and butanol: ethyl acetate: water (6:2:2) solvent combinations was employed as the mobile phase for the NHE, EAE, ETE and WE respectively. Spots were observed in daylight and UV light (254 nm), after which 10% sulphuric acid was sprayed before being heated in an oven for five minutes at 105°C. Ethyl acetate extract offered the best separation hence was subjected to further purification using column chromatography procedures as outlined by Hassan *et al.*, 2018.

Purification using column chromatography

The ethyl acetate fraction (3.0 g) was gradiently eluted on a silica gel column using a

combination of solvent systems, starting with 100% n-hexane and mixtures of n-hexane and ethyl acetate (99:1 to 88:12). A total of 30 eluent (15 cm³ each) were collected and merged based on their TLC profiles with the retardation factor (R_f) value determined for the major fraction.

Spectroscopic Analysis

1D and 2D NMR (¹H-NMR, ¹³C-NMR, COSY, HMBC, HSQC and DEPT) spectroscopic techniques were employed for the complete characterization of the isolated pure compound. While Tetramethylsilane (TMS) was used as an internal reference at 25°C, deuterated chloroform (CDCl₃) was employed as the dissolving solvent. The NMR spectrum was recorded using a varian-vnmrs 400MHz spectrometer at the Multi-User Laboratory Ahmadu Bello University Zaria, Kaduna State Nigeria

RESULTS AND DISCUSSION

Extraction yield

The percentage yield of the powdered root bark extracts of *D. mespiliformis* is as outlined in Table 1

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of the sample}} \times 100$$

Table 1: Percentage yield of the root bark extracts of *D. mespiliformis*

Extract	Percentage yield
n-hexane	1.41
Ethyl acetate	1.44
Ethanol	8.14
Water	4.33

Spectral analysis of EAE-A isolate

A whitish/ashy compound with R_f value of 0.74 coded EAE-A was isolated according to the

protocol established by Hassan *et al.*, 2018. The 1D and 2D spectra of EAE-A are presented in Figures 1 - 6.

Sample Name **MOHAMMED_EAE** Pulse sequence **PROTON** Temperature **25** Study owner **vmr1**
 Date collected **2021-10-06** Solvent **cdcl3** Spectrometer **varian-vnmrs400** Operator **vmr1**

MOHAMMED_EAE

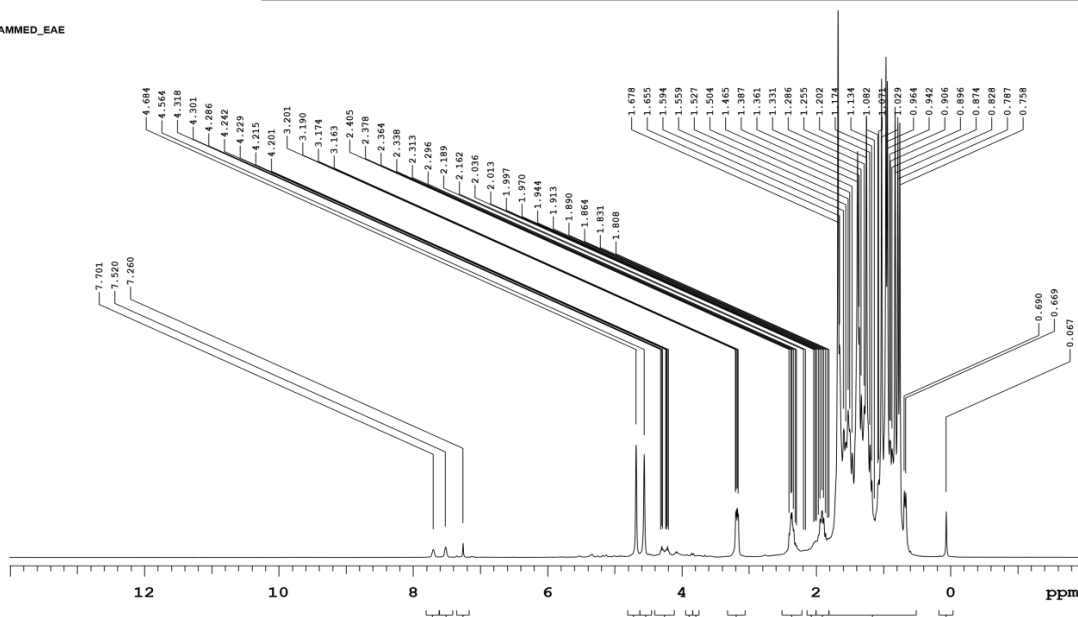


Figure 1: ¹H-NMR spectrum of EAE-A

Sample Name **MOHAMMED_EAE** Pulse sequence **CARBON** Temperature **25** Study owner **vmr1**
 Date collected **2021-10-06** Solvent **cdcl3** Spectrometer **varian-vnmrs400** Operator **vmr1**

MOHAMMED_EAE

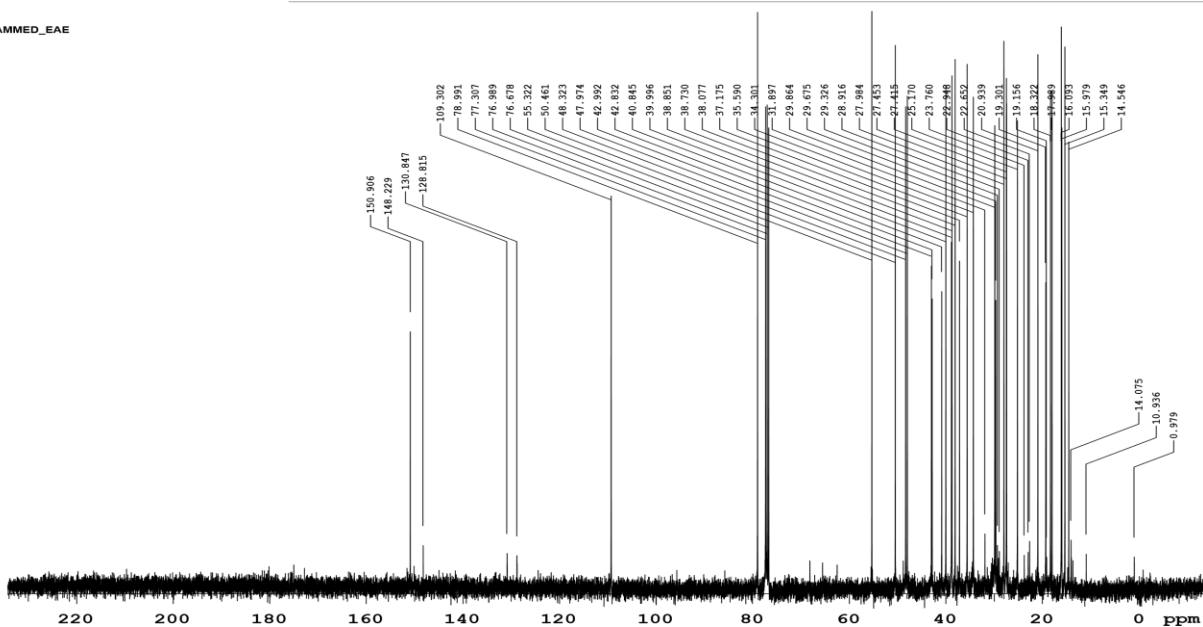


Figure 2: ¹³C-NMR spectrum of EAE-A

MOHAMMED_EAE

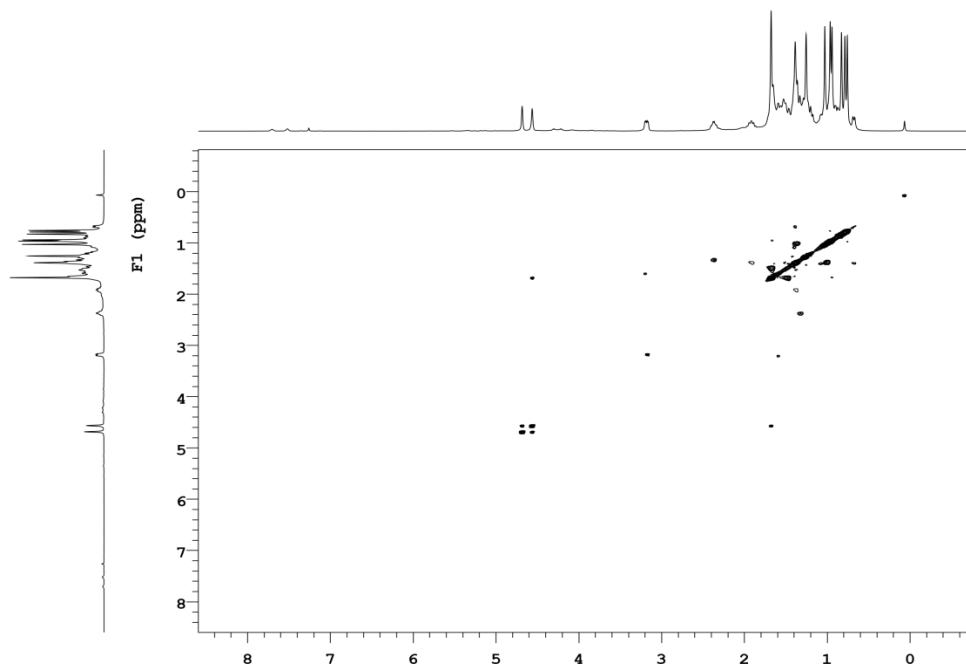


Figure 3: COSY spectrum of EAE-A

MOHAMMED_EAE

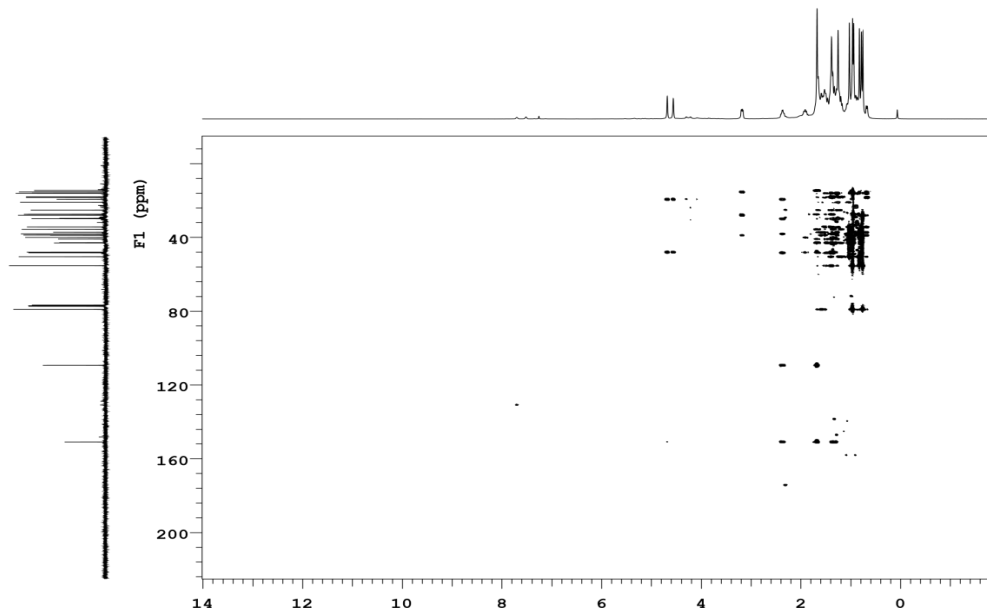


Figure 4: HMBC spectrum of EAE-A

MOHAMMED_EAE

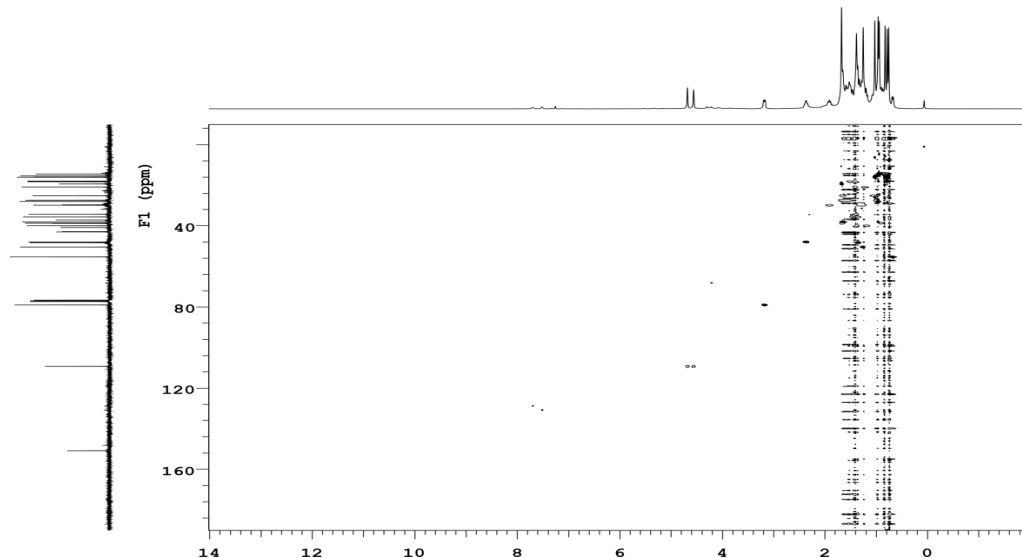


Figure 5: HSQC spectrum of EAE-A

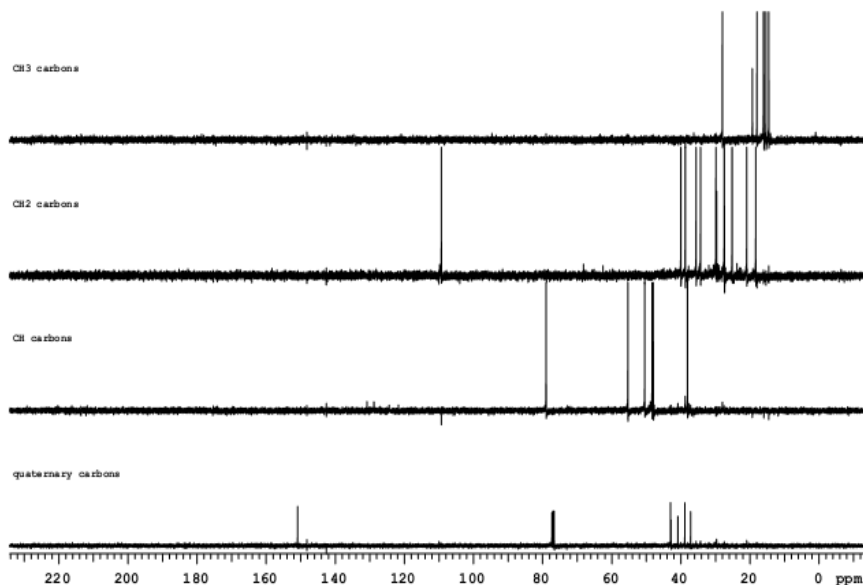


Figure 6: DEPT spectrum of EAE-A

The $^1\text{H-NMR}$ spectrum (Figure 1) showed signals of seven methyl groups at δ_H 0.83 (3H, s), 0.91 (3H, s), 1.08 (3H, s), 1.29 (3H, s), 1.39 (3H, s), 1.56 (3H, s) and 1.68 (3H, s), singly with no neighboring protons at their respective positions. One deshielded proton at δ_H 3.19 provided a distinctive signal of an oxymethine proton (*dd*, $J = 4.4, 10.8$ Hz) assigned as H-3. The coupling constant indicates that the H-2 protons (2H) are magnetically non-equivalent

with each of them splitting H-3 signals. The splitting pattern (or the multiplicity) of H-3 indicated that carbon 4 is a quaternary. The last signal at δ_H 4.56 and 4.68 were detected as *exomethylene* signals at H-21a and H-21b, respectively. The two signals observed in the heteronuclear quantum correlation (HSQC) spectrum (Figure 5) correlate with the same carbon at δ_C 109.3 which is a terminal olefinic carbon signal.

The ^{13}C -NMR displayed a total of 30 separate resonances consisting of six methine signals; as indicated in DEPT- experiment (Figure 6) that included C-3 at δ_{C} 78.99, C-5 at δ_{C} 55.32, C-9 at δ_{C} 51.0, C-13 at δ_{C} 38.73, C-17 at δ_{C} 47.97 and C-24 at δ_{C} 128.8 respectively. Seven signals were of methyl groups observed as positive absorptions of C-18 at δ_{C} 15.97, C-19 at δ_{C} 15.34, C-26 at δ_{C} 23.76, C-27 at δ_{C} 17.98, C-28 at δ_{C} 27.98, C-29 at 14.54 and C-30 at δ_{C} 16.09, respectively. While eleven methylene signals absorption shown in DEPT spectrum were C-1,

C-2, C-6, C-7, C-11, C-12, C-15, C-16, C-21, C-22 and C-23. Six quaternary signals, two of which are olefinic group C-20 at δ_{C} 150.90 and C-24 at δ_{C} 128.8, the others were C-4, C-8, C-10, C-14, C-20 and C-24 respectively. All carbon bearing proton were seen in HSQC spectrum (Figure 5). Examination of both 1D and 2D-NMR spectra confirmed the compound to be Dammara-20,24-dien-3-ol correlating with that reported by Bianchini *et al.*, (1988) as presented in Table 2. The structure of compound is presented in Figure 7.

Table 2: ^{13}C -NMR data of compound EAE-A compared with Bianchini *et al.* (1988)

Position	EAE A		Bianchini <i>et al.</i> , 1988
	δ_{C} (ppm)	δ_{H} (ppm)	δ_{C}
1.	39.99	CH ₂	39.2
2.	27.45	CH ₂	27.5
3.	78.99	CH	78.9
4.	38.85	Q	39.0
5.	55.32	CH	55.9
6.	18.32	CH ₂	18.3
7.	35.59	CH ₂	35.5
8.	40.84	Q	40.5
9.	50.46	CH	51.0
10.	37.17	Q	37.3
11.	20.93	CH ₂	21.4
12.	25.17	CH ₂	25.0
13.	38.73	CH	45.3
14.	48.32	Q	49.5
15.	31.89	CH ₂	31.4
16.	27.41	CH ₂	27.1
17.	47.97	CH	47.8
18.	15.97	CH ₃	16.0
19.	15.24	CH ₃	15.7
20.	150.90	Q	152.7
21.	109.3	CH ₂	107.6
22.	34.30	CH ₂	34.1
23.	28.91	CH ₂	28.9
24.	128.8	CH	124.5
25.	130.84	Q	131.4
26.	23.76	CH ₃	25.7
27.	17.98	CH ₃	17.7
28.	27.98	CH ₃	28.1
29.	14.54	CH ₃	15.4
30.	16.09	CH ₃	16.3

Key: CH₃ = methyl; CH₂ = Methylene; CH = Methine; Q = quaternary; δ_{C} = ^{13}C (Carbon-13) chemical shift; δ_{H} = ^1H (proton) chemical shift

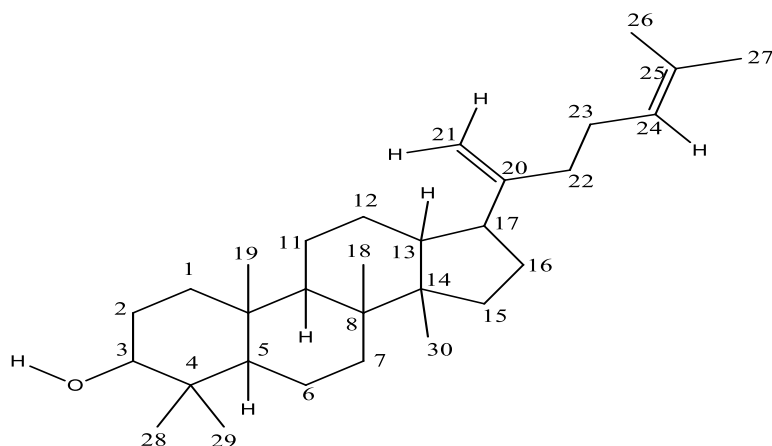


Figure 7: Dammara-20,24-dien-3-ol

CONCLUSION

Our present investigation of the ethyl acetate extract of *D. mespiliformis* root bark resulted in the isolation of the compound dammara-20,24-dien-3-ol confirmed by all its spectroscopic and structural characteristics. The abundance of dammarane derivatives in *D. mespiliformis* indicates that they are frequently used as an intermediate in the biosynthesis of lupane, ursane, and oleanane compounds. Given that the antitumor properties of compounds of

dammarane have been demonstrated, Itoh *et al.*, 1981; Bianchini *et al.*, (1988).

ACKNOWLEDGMENT

Our special gratitude goes to Mal Mohammed Mu'azu, of Multi-User Laboratory, Chemistry Department, ABU, Zaria Kaduna State, Nigeria for running the 1D and 2D NMR spectroscopy

CONFLICT OF INTEREST

The authors declare no conflict of interest. The authors alone are responsible for the content and writing of this article.

REFERENCES

- Abdur Raufa, Ghias, U., Seema, P., Ajmal. K., Sobia, A. Halime, F, Saud, B., Khalid, A., Naveed, M.I, and Mohammad, S. M. (2011). *Diospyros*, an underutilized, multi-purpose plant genus: A review. *Biomedicine and pharmacotherapy*. 91. 741-730.
- Aminu, S. A., Ibrahim, Y, Ismail, H.A and Ibrahim, I. O. 2021. Medicinal and Traditional Utilization of African Ebony (*Diospyros mespiliformis*): A Review. *Int.J.Curr.Microbiol.App.Sci* 10 (06):81817. doi.https://doi.org/10.20546/ijcmas.2021.1006.086.
- Bianchini, J. P., Gaydou, E. M., Rafaralahitsimba, G., Waegell, B. and Zahra, J. P. (1988). Dammarane derivatives in the fruit lipids of *Oleamada gascariensis*. *Phytochemistry*, 27(7), 2301-2304.
- Cyril, O., Jonathan, C.E., Ighodora, I. and Dabai, M.U. (2017). Anti-ulcer activity of methanol extract of *Plantago rugelii* Decne. (Plantagenaceae). *Tropical Journal of Natural Product*, 1(2), 84-88. doi: 10.26538/tjnpr/v1i2.7
- Guo, D. L and Luo. Z. R (2011). Genetic relationships of the Japanese persimmon *Diospyros*

- kaki* (Ebenaceae) and related species revealed by SSR analysis, *Genet. Mol. Res.* 10. 1060–1068
- Hassan, L.G., Halilu, E.M., Kabiru, J.U., Umar, A.U. and Cyril, O. (2018). Isolation and characterization of α -Amyrin and β -Sitosterol from ethyl acetate of the leaves of *Monechma ciliatum* (Jacquin). *International Journal of Pharmacy and Pharmaceutical Research*, 12(1), 289-306
- Itoh, T., Yoshida, K., Yatsu, T., Tamura, T., and Matsumoto, T. (1981). *J. Chromatograph.* 58: 545.
- Ogbiko, C., Eboka, C.J. and Tambuwal, A.D. (2018). Extraction and fractionation of whole black seed Plantain (*Plantago rugelii* Decne) for in-vitro antioxidant, antibacterial and phytochemical screening. *Journal of Applied Science and Environmental Management*, 22(5):613-618. doi: 10.4314/jasem.v22i5.1
- Orwa, C; Mutua, A; Kindt, R., Jamnadas, R. and Simons, A. (2009). Agro-forestry data base: A tree reference and selection guide. Version 4.0