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Isolation of Digeranyl From the Hexane Extract of *Moringa Peregrina* Leaves

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ABSTRACT: The plant kingdom provides many precious gifts to mankind. One of these gifts is its ability to produce an endless stream of natural products. *Moringa peregrina* (In Oman called Al Shua) provides a wide range of benefits to traditional medicine in many cultures, as well many nutritional values. This paper discusses the isolation, and purification of digeranyl from the hexane extract of Moringa peregrine's leaves, for the first time. Recent studies show that digeranyl phosphate derivative, digeranyl bisphosphonate, inhibits breast cancer cell migration by inhibiting geranylgeranyl diphosphate synthase (GGDPS). The structure of the isolated compound has been confirmed using different spectroscopic methods.

Keywords: *Moringa peregrina*; Omani medicinal plants; Diterpene; Digeranyl.

إستخلاص مادة كيميائية من نوع تربيني ثنائي (Diterpene) من مستخلص الهكسان لأوراق نبتة الشوع (*Moringa peregrina*)

صالح البوصافي، صفية الجابري و شريف الهاشمي

الملخص: ماتزال النباتات تقدم المزيد من الفوائد للإنسان ومن هذه الفوائد انتاجها العديد و المتنوع من المواد الطبيعية. ومن هذه النباتات شجرة الشوع (*Moringa peregrina*) التي اثبتت أهميتها من خلال خصائصها الطبية المتنوعة واستخدامها في الكثير من الأدوية الشعبية. في هذا المبحث نسجل, ولأول مرة, استخلاص مادة الدايجيرانيل وهي من نوع التربينات الثنائية. تأتي أهمية هذا البحث بعد نتائج الأبحاث المنشوره والتي تؤكد أهمية مركبات الدايجيرانيل كمادة ثنائي فوسفونات دايجيرانيل في علاج مرض سرطان الثدي من خلال تثبيط إنزيم جيرانيل جيرانيل ثنائي فوسفات سينثيس. لقد تم استخدام أنظمة الطيف الضوئي المختلفة للحصول على التركيب الجزيئي للمادة المعزولة.

الكلمات المفتاحية: مورينجا بيريجرينا، نباتات طبية عمانية، تربين ثنائي.

1. Introduction

Sultanate of Oman is a land of many medicinal plants. The diverse landscape in Oman represented by rocky hills and mountains, cultivated plains and wadis to desert and long beach's represent suitable environments for around 1204 native plants, many of them used in traditional medicine [1]. Throughout history, Omani traditional healers practiced numerous techniques to treat different diseases such as using lime, honey, and garlic to treat throat infection, diabetes, and obesity. The aqueous decoctions of frankincense and ginger are used to treat stomach ache and respiratory infection. Boiled leaves and stems of *Jada* (*Teucrium mascatense*) are used to reduce fever and rose water is used for eye disorders. The *yas* plant (*Myrtus communis*) is used to treat ulcer and scorpion stings and the leaves of *Alalaan* tree (*Juniper*) are used to relax muscles [2]. With the advantage of laboratory chemical isolation techniques, many researches started to study medicinal plants to isolate active natural products, elucidate their chemical structure and study their medical properties. One of the earliest efforts to isolate an active natural product from Omani medicinal plant was the isolation of aristolochic acid-A and aristolochic acid-D from *Aristolochia bracteolate* plant in 2004 [3]. Boswellic acid and its derivative have been isolated from Luban tree (Frankincense) with good yields and show good anti-cancer properties [4]. From the bark of *Commiphora wightii* (Mukul myrrh tree) an anti-microbial activity Muscanone-1 was isolated and elucidated [5].

Secondary metabolites isolated from plants continue to offer good opportunities to discover new drugs [6]. Part of our ongoing research activity is to investigate new biologically active secondary metabolites from Omani medicinal plants. We studied the metabolites of a plant, *Moringa peregrina*, collected from the Quriyat mountain in Muscat Region of Oman. This plant which belongs to the Moringaceae family, found across the NE Africa, throughout Arabia and as far north as Syria and range in size from tiny shoots to enormous trees with height up to about ten meters [7]. In Oman, it is locally called Al Shua, grows wildly and is found mainly in rocky wadis and on cliffs in the drier areas [8]. *Moringa peregrina* exhibited various benefits in Omani traditional medicine such as treatment of malaria, abdominal pain, hypertension, and diabetes [9]. The pod's extract was used to treat skin ailments, mange, itch, nosebleeds, diseased teeth, as well as removing freckles, and encouraging head hair to grow long and strong [10]. The leaf's extract is used to treat paralysis, wounds and skin rashes [11]. The oil extracted from the husks of this plant is used for cooking, moisturizing the skin, preventing sun burn, treatment of infantile paralysis and abdominal pain [12]. *M. peregrina* leaves are used in Saudi Arabia for treating wounds, while the bark juice is used for treating fever, headache, constipation, back and muscle pains, and burns [13]. In Egypt, the seeds of *M. peregrina* are used by pregnant woman for strengthen the muscles to facilitate birth [14]. Furthermore, Indian traditional healers used the green roots of *M. peregrina* to treat paralysis, epilepsy, hysteria and as a cream to treat chronic rheumatism (Figure 1) [10].

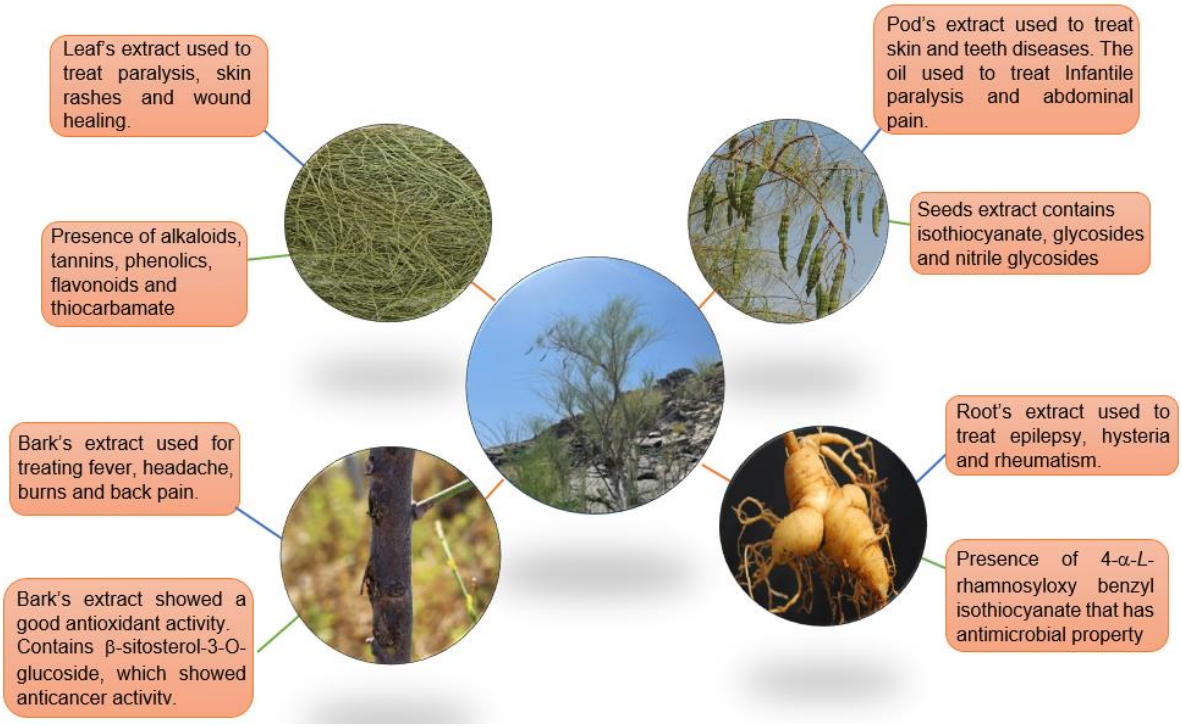

Figure 1. Traditional medicine use and phytochemicals of different parts of M. peregrina.

Pharmacological studies of extracts prepared from different parts of *M. peregrina* revealed that it possess antioxidant, antimicrobial, anti-inflammatory, anticancer, anti-obesity, hypoglycemic, anti-hyperlipidemic, and hepatoprotective properties [15-23]. These biological activities can be linked to the presence of phenolic compounds such as flavonoids and other phenolic natural products which are known to play an important role in preventing the progression of many diseases [8]. Numerous natural products belong to different classes of secondary metabolites have been isolated from *M. peregrina* including quercetin (Flavonoid), moringin (Isothiocyanate), neochlorogenic acid (Phenylpropanoid), β-cymene (Monoterpene), α-amyrin (Triterpene), Niaziminin (Thiocarbamate), campesterol (Steroid), oleic acid (Fatty acid), and 4-(4′-O-methyl-α-*L*-rhamnosyloxy)benzylnitrile) (Figure 2) [24-30].

Moreover, the essential oil extracted from the seeds of *M. peregrina* comprises 33 compounds, among them geijerene (33%), linalool (23.4%), caryophyllene oxide (19.3%), carvacrol (1.9%) and other compounds [31]. The oil isolated from the seed kernel contains isobutyl isothiocyanate (94.0%), isopropyl isothiocyanate (4.9%), sec-butyl isothiocyanate (0.5%) and benzyl isothiocyanate (0.5%) [32]. The oil exhibited good antibiotic activity against wide-range microorganisms [33].

We report herein the isolation and elucidation of the diterpene digeranyl (**1**) (Figure 3) from the hexane extract of *M. peregrina* leaves. This open-chain C20 diterpene, which is biosynthesized *via* head-to-head binding of two geranyl pyrophosphate molecules, has been isolated from bergamot oil [34], but this is the first time to be isolated from *M. peregrina*. Digeranyl derivatives, such as digeranyl bisphosphonate, exhibit good inhibition activity towards geranylgeranyl diphosphate synthase (GGDPS), the enzyme responsible of the biosynthesis of geranylgeranyl diphosphate (GGPP). Such inhibition induces depletion of intracellular GGPP level and hence inhibits cancer cell growth [35,36].

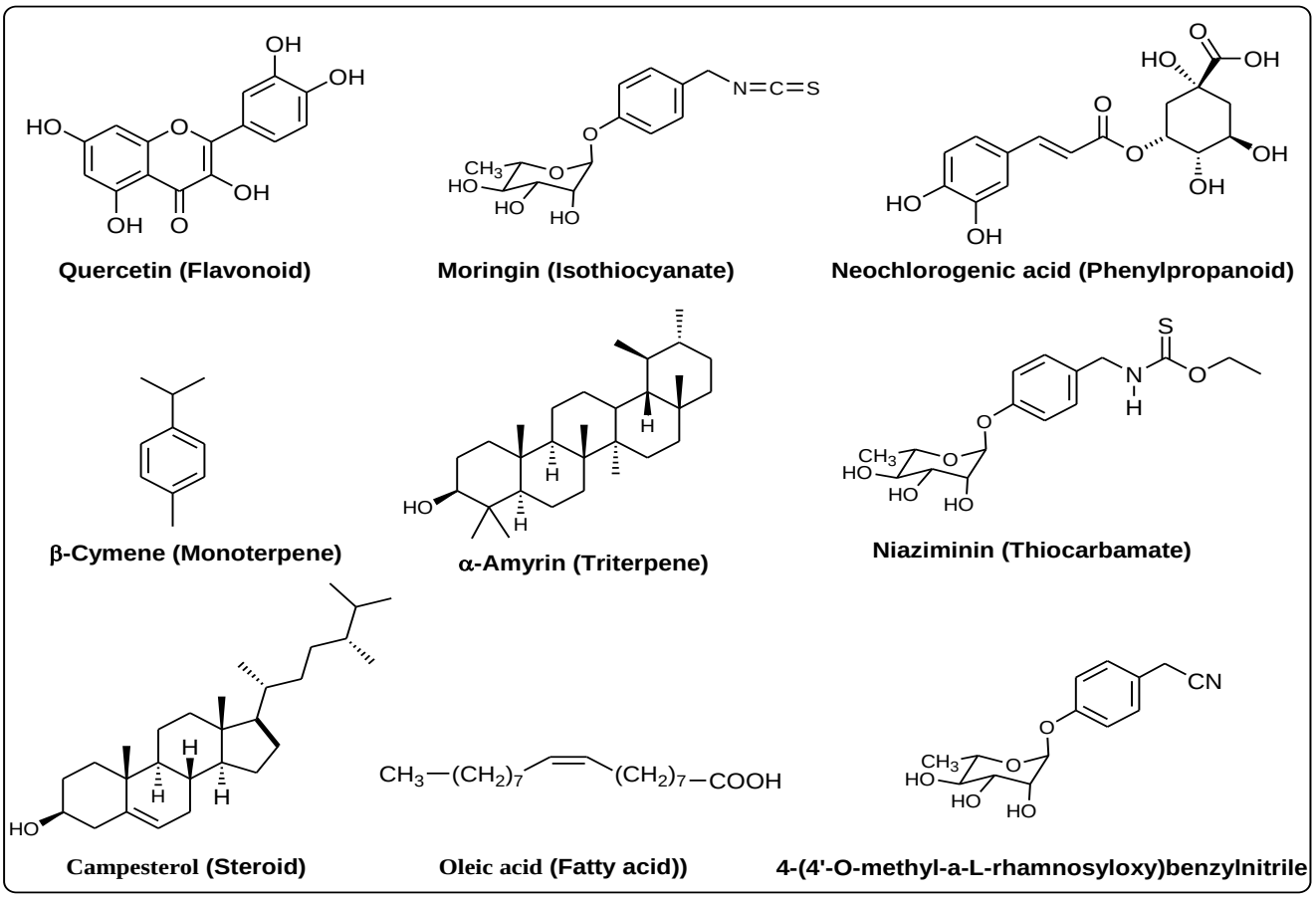

Figure 2. Selected natural products isolated from *M. peregrina*.

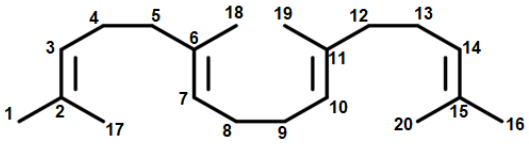

Figure 3. Chemical structure of digeranyl (**1**).

2. Materials and Methods

2.1 General

All reagents are commercially available and used as received without further purification. The infrared spectrum (IR) was measured by FTIR (Alpha II) spectrometer (Bruker, USA). ^{1}H NMR and ^{13}C NMR spectra were carried out in JEOL 400 MHz spectrometer (Japan) with $CDCl_3$ as the NMR solvent. Chemical shifts are expressed as δ values (ppm) downfield from TMS. ESI-MS spectra were recorded with Agilent, 6460 Triple quad LC/MS, 1200 Infinity series (Germany) equipped with electrospray ionization (ESI) interface and operated by Mass Hunter software. Thin-layer chromatography (TLC) was carried out on Merck F254 silica gel plates ().2 mm thickness) with mobile phase hexane-ethyl acetate (90:10) and the spots were detected under UV 254 nm and by using $KMnO_4$ dip solution to give permanent yellow spots.

2.2 Plant Material

Moringa peregrina was collected from a mountain in wilayat Quriyat, Sultanate of Oman in 2021 and was authenticated by a specialist in the Department of Biology, Sultan Qaboos University.

2.3 Extraction and Isolation

500 g of shade dried powdered leafs of *M. peregrina* was repeatedly extracted with methanol at room temperature. The combined extract was concentrated under reduced pressure to get 40 g of greenish viscous material which was partitioned between hexane/H_2O, then EtOAc/H_2O to give a hexane extract and an ethyl acetate extract. The concentrated hexane extract (21 g) was chromatographed over a silica gel column and eluted with chloroform to yield six different fractions. Fraction 3 was further purified by another column chromatography using hexane: ethyl acetate mixture (9:1) to give pure digeranyl (**1**) as an orange oil (1.03 g, 0.21%).

3. Results and Discussion

3.1 Isolation of digeranyl (1)

The methanol extract of *M. peregrina* leafs was first screened by TLC method. The developed TLC plate showed many close compounds (Figure 4). Our focus went to the upper spot at R_f value of 0.83 since it appeared the largest one. The target natural product was isolated by a standard column chromatography with silica gel (0.13-0.25 mm, 60-120 mesh), first eluted with hexane to get rid of the first low-polarity compounds, followed by a mixture of hexane: ethyl acetate (90%:10%) to obtain compound-1 with a yield equal to 1.33 g. Further purification of the desired product was achieved by applying a second smaller column chromatography using hexane: ethyl acetate (90%:10%) to get pure compound-1 (1.03 g) as a yellow oil.

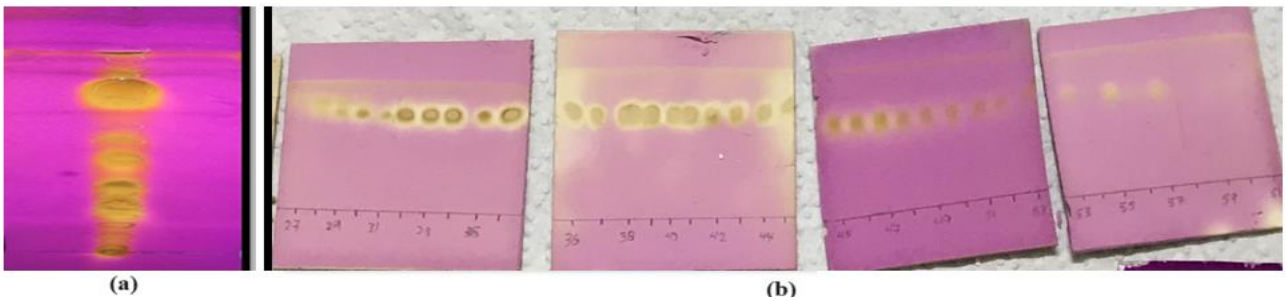

Figure 4. TLC plates of the methanol extract of *M. peregrina* (a) and digeranyl (1) after the second column (b).

3.2 Structural Elucidation of digeranyl (1)

The mass spectrum of compound-**1** exhibited a mass of 274 for its molecular ion peak $[M]^+$ establishing the molecular formula as $C_{20}H_{34}$ with 4 degrees of unsaturation (Figure 5). The base peak appears at 69.1 for 2-methyl-2-butene cation fragment which is produced *via* allylic fragmentation of 2-methyl substituted alkenes. This fragment is well known in similar polyene natural compounds like squalene (Figure 6). Other fragments like 81, 121, 137, and 205 overlap with that of squalene [37] and reports MS of digeranyl [38] (Figure 7).

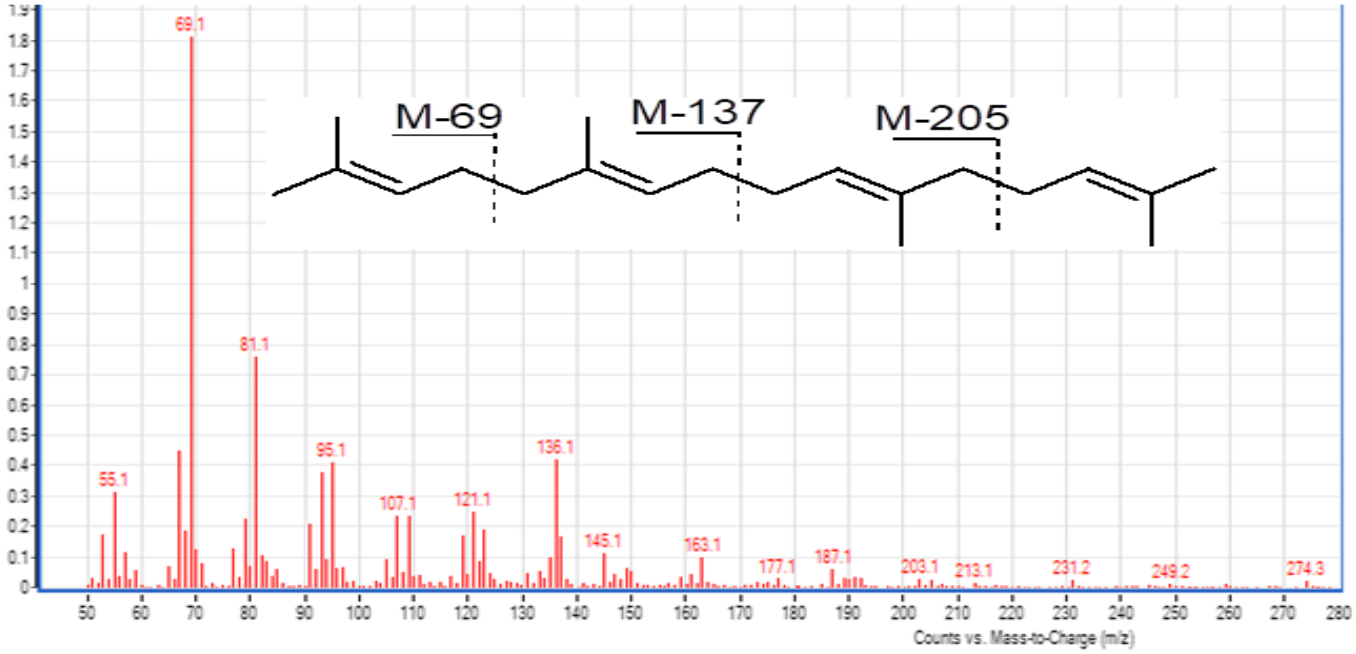

Figure 5. Mass spectrum of isolated digeranyl (1) and its mode of fragmentations.

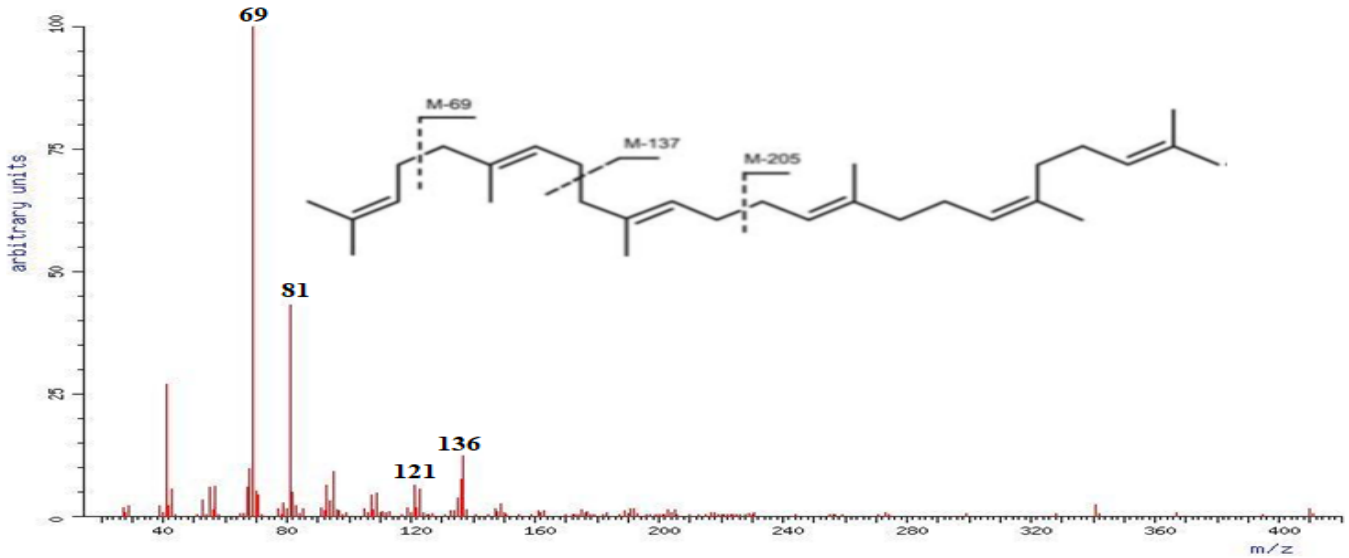

Figure 6. MS spectrum and mode of fragmentation of squalene.

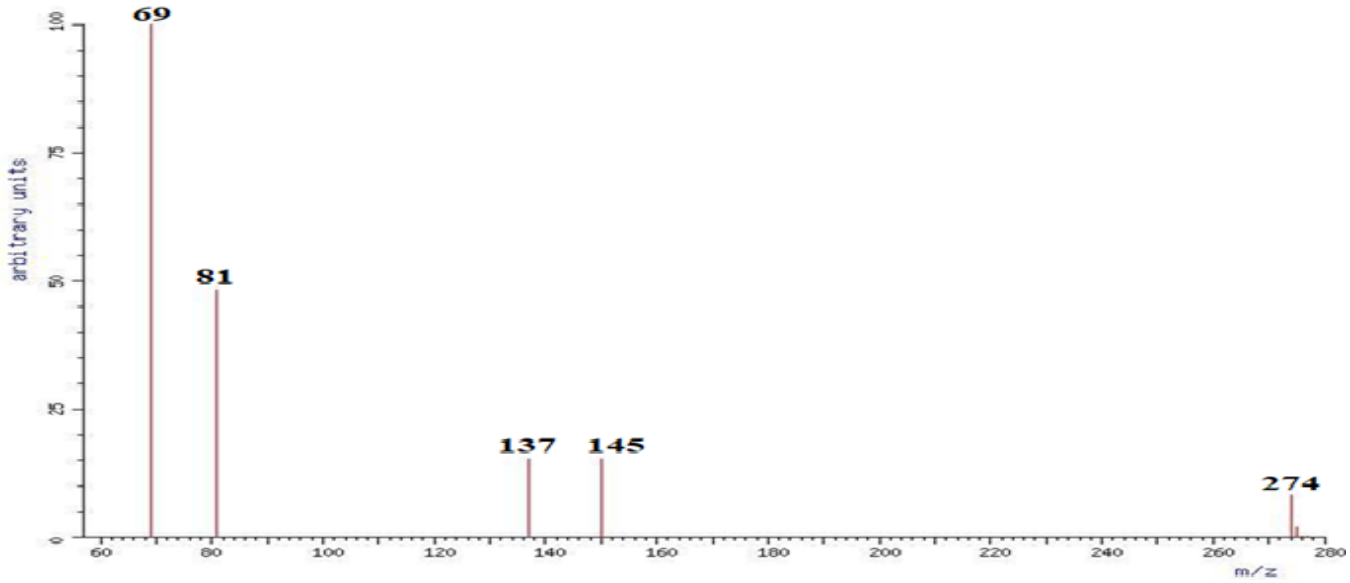

Figure 7. Mass spectrum of reported digeranyl (1).

The structure of digeranyl (1) consists of various chemical bonds such as aliphatic C-H, olefinic C-H and olefinic C=C bonds and all of them appear in the IR spectrum of the compound. Therefore, five characteristic absorption bands were revealed by the IR spectrum of compound-1 (**Figure-8**). The alkene C-H bond showed a weak stretching absorption at 3050 cm^{-1} and the alkane C-H stretching appeared as a strong band around 2960 cm^{-1}. The weak band at 1664 cm^{-1} is for unconjugated C=C stretching frequency and the two frequencies at 1445 and 1377 cm^{-1} are for the CH_2 and CH_3 groups, respectively.

The ^{1}H nuclear magnetic resonance (^{1}H-NMR) spectrum of compound **1** showed 7 different signals ranging between 1.59 and 5.19 ppm that correspond to 7 different proton environments (Figure 9 (a) and Table 2). The signal at δ 1.59 ppm (6H, s) belongs to the two symmetrical methyl groups **18** and **19**, while the four terminal CH_3 groups, **1**, **16**, **17**, and **20**, revealed a singlet at δ 1.68 ppm. Multiple overlapping signals appeared at 1.92-2.09 ppm range accounts for 12 hydrogens in six CH_2 groups **4**, **5**, **8**, **9**, **12** and **13**. The two most deshielded (up field) signals that appeared at δ 5.09-5.19 ppm are for the four alkene hydrogens **3**, **7**, **10** and **14**. These ^{1}H-NMR signals are comparable to the reported ones (Table 2) [39].

Table 1: Infrared (IR) data of digeranyl (1)

ν (cm^{-1})	Functional group	Intensity
3050	=C-H stretching	Weak
2960	C-H stretching	strong
1664	C=C starching	Weak
1445	C-H bending	medium
1377	C-H bending	medium

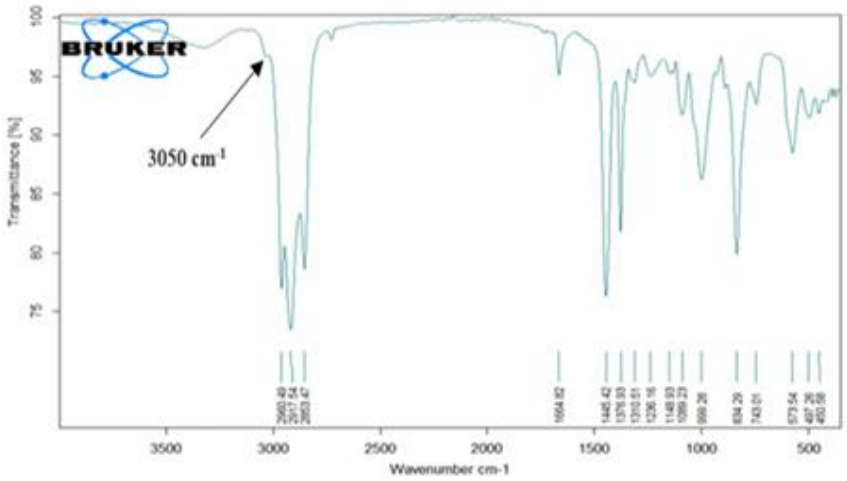

Figure 8. IR spectrum and table of digeranyl (1).

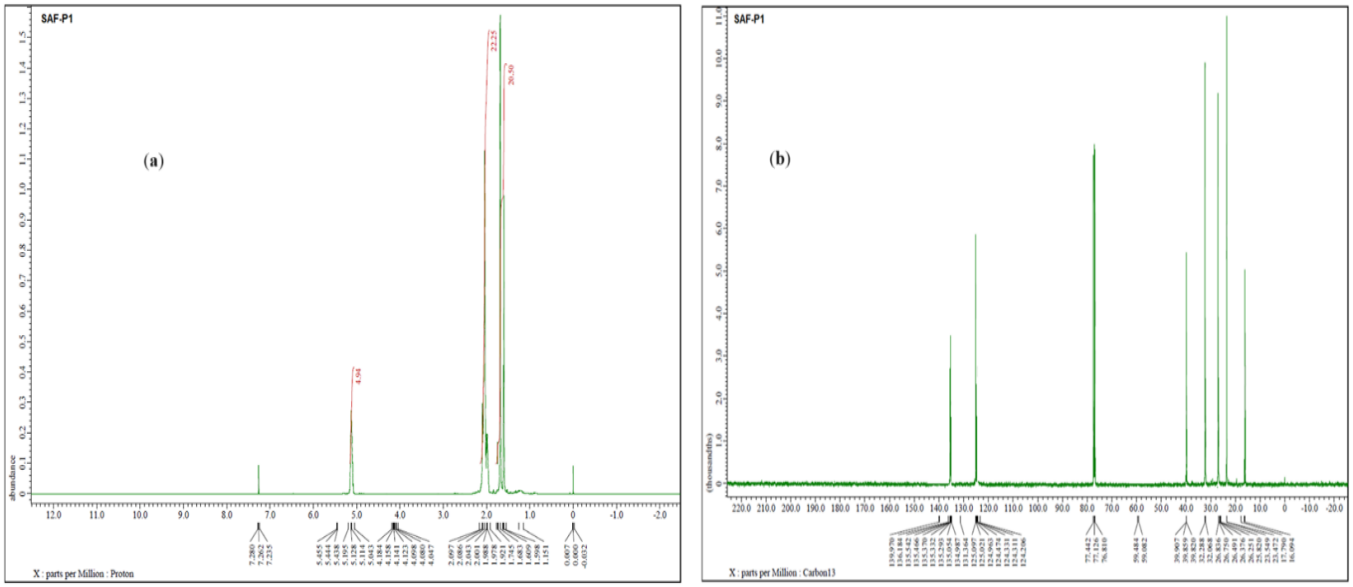

Figure 9. ^{1}H- NMR (a) and ^{13}C-NMR (b) spectra of digeranyl (1).

The ^{13}C-NMR spectrum of digeranyl (**1)** shows 9 signals related to 9 type of carbon atoms (Figure 9 (b) and Table 2). The most deshielded two peak appear at 135.37 and 135.47 ppm belong, because of symmetry, to the four quaternary alkene carbons (=C-) C2, C6, C11 and C15, respectively. The other two peaks at δ 125.02 and 125.1 ppm belong to the four tertiary alkene carbons (=CH-) C3, C14, C7 and C10, respectively. The aliphatic carbon atoms in digeranyl (**1**) are as follow: the six -CH_2 groups (C4, C5, C8, C9, C12 and C13) appear at 39.86, 32.29, and 26.49 ppm and the six –CH_3 (C1, C16, C17, C18, C19 and C20) appear at 23.55 ppm (4 methyl groups) and 16.09 ppm (2 methyl groups). The distinction between CH_3, CH_2, CH, and quaternary carbon atoms was achieved *via* distortion less enhancement by polarization transfer (DEPT) spectra (Figure **10**). In DEPT 90^0 (a) there is only one CH carbon in digeranyl which an alkene carbon, and in DEPT 135^0 the three invers signals at 39.86, 32.29, and 26.49 ppm represent aliphatic CH_2 groups while the two signals at 23.55 ppm and 16.09 ppm represent the CH_3 groups.

More detailed structural features of the isolated compound were attained using 2D-NMR techniques like ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMQC spectra (Figure 11). In H-H COSY (a) spectrum we can detect the coupling between the alkene hydrogens at δ 125.02-125.1 ppm with CH_2 hydrogens at 1.92-2.09 ppm. The H-C HMQC spectrum facilitated the assigning of the hydrogen and carbon atoms in digeranyl by connecting each hydrogen with its carbon.

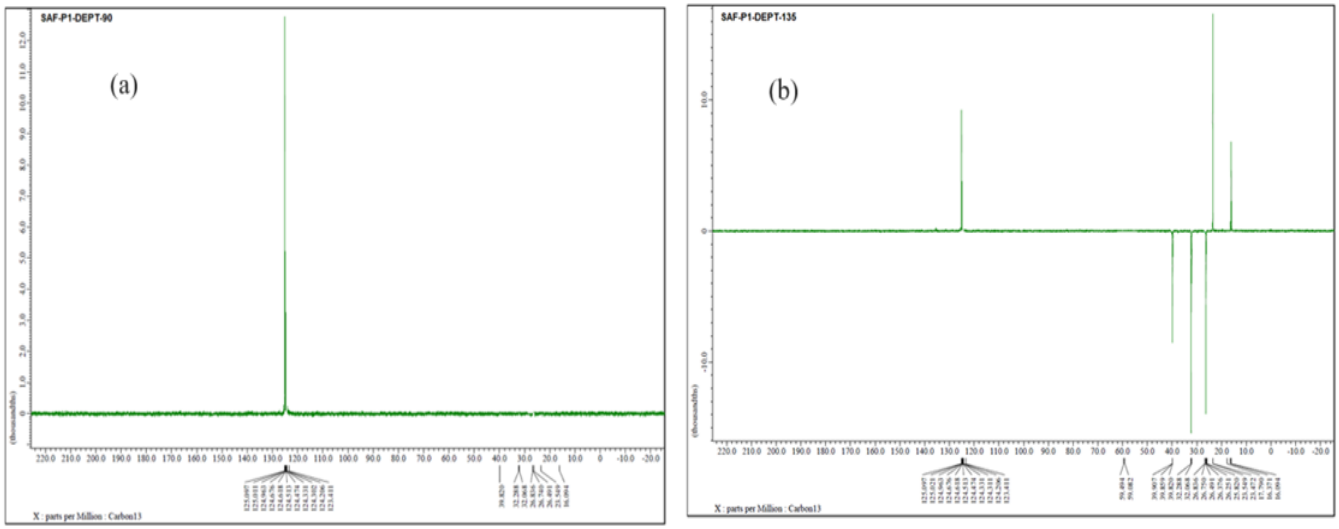

Figure 10. The 90^0 (a) & 135^0 (b) DEPT spectra of digeranyl (1).

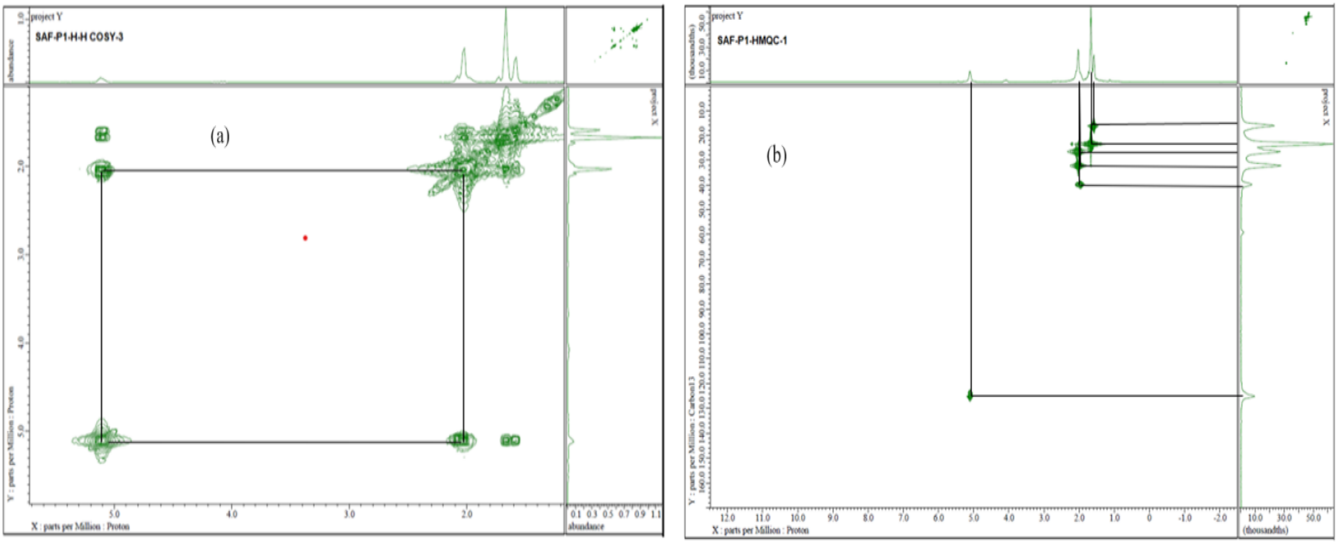

Figure 11. ^{1}H-^{1}H COSY (a) & ^{1}H-^{13}C HMQC (b) spectra.

Table 2. The ^{1}H and ^{13}C NMR data of digeranyl (1).

δ_H (ppm)	δ_H (ppm)[39] (Reported)	No. of H	splitting	H- number	δ_C (ppm)	δ_C (ppm)[39] (Reported)	C- number
1.59	1.60	6 H	s	CH_3-18 CH_3-19	16.09	15.9	C-18, C-19
1.68	1.68	12 H	s	CH_3-1 CH_3-16 CH_3-17 CH_3-20	23.55	25.6	C-1, C-16 C-17, C-20
1.92-2.09	1.92-2.13	12 H	m	CH_2-4 CH_2-5 CH_2-8 CH_2-9 CH_2-12 CH_2-13	26.49 32.29 39.86	26.7 28.1 39.7	C-4, C-5 C-8, C-9 C-12, C-13
5.09-5.19	5.05-5.19	4 H	m	H-3 H-7 H-10 H-14	125.02 125.1	124.2 124.3	C-3, C-7 C-10, C-14
					135.37 135.47	131.1 34.9	C-2, C-6 C-11, C-15

4. Conclusion

In this study, digeranyl, a polyunsaturated diterpene, was isolated, for the first time, from the hexane extract of *Moringa peregrina* leaves. The characterization of the isolated natural product was carried out using MS, IR, NMR, and 2D NMR techniques. The spectroscopic results were coincide with the reported ones. Additional research studies are required to reveal the potential therapeutic properties of digeranyl and other natural products isolated from *M. peregrina*.

Acknowledgment

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Conflict of interest

The authors declare no conflict of interest.

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