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Research Article

Identification of Phytol and Cis-1,4-Polyisoprene from the Leaf Extracts of *Morinda lucida* (Rubiaceae)

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Abstract

Morinda lucida is a medicinal plant popular for its traditional uses in treating several illnesses such as malaria, inflammation, diabetes, jaundice, hypertension, and dysentery. Recently, the leaf which is the most commonly used part of the plant in ethnomedicine was reported to possess anti-malarial, anti-microbial, anti-inflammatory, and anti-oxidant activities. In this work the bioactive methanol-soluble fraction of the crude leaf extract was investigated for its chemical constituents. The fraction was subjected to column chromatography to give two components, which on FTIR and NMR spectral characterization were identified as phytol and 1,4-polyisoprene. While phytol may occur free in green plants or as a product of alkaline hydrolysis of the chlorophylls, the presence of isoprene or its polymeric derivative in a plant is a rare occurrence. To the best of our knowledge, this is the first report of these two compounds from a species of the genus *Morinda*, and in particular from *Morinda lucida*. These two identified compounds and the other phytochemicals present in the crude methanol extract have been associated with several biological activities and may account for the ethnomedicinal properties of the leaf of *Morinda lucida.* Thus, the leaf of *Morinda lucida* is a potential lead for the development of new drugs for the management of many human ailments. The results of this work may have pharmacological and taxonomic implications.

Keywords

Morinda Lucida, Leaf Extracts, Chromatography, Spectral Characterization, Phytol and 1,4-Polyisoprene

1. Introduction

Medicinal plants have long been a cornerstone of traditional healthcare systems across the globe, offering a diverse reservoir of bioactive compounds with therapeutic potential. *Morinda lucida* (Benth.) belongs to the family Rubiaceae. It is an evergreen shrub that can grow up to 18 m high [1]. The leaves are about 7-15 cm long by 3.5-7.5 cm broad and form a shining foliage [1, 2]. The leaf, stem bark, root bark, and fruit seed of *M. lucida* are widely used in rural areas in West and Central Africa to treat many diseases. In Central and West Africa, infusions and decoctions of root, bark, and leaves are used as treatment against various ailments, including fevers, trypanosomiasis, diabetes, insomnia, dysentery, cerebral congestion, stomach-ache, ulcers, wounds, microbial infections and worm infestations [1, 3]. The leaf extract of *M. lucida* has also been found to have reversible anti-spermatogenic properties [4]. In Nigeria and other parts of West Africa, the leaf of *M. lucida* is particularly valued, being the most frequently utilized part of the plant in herbal prepa-

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rations. The biological activities of the plant have continued to attract attention, leading to some interesting phytochemical and pharmacological findings as shown in Table 1.

Table 1. *Recent phytochemical and bioactivity studies on Morinda lucida (2022–2025).*

Activity	Extract / Fraction	Bioactives Identified	Key Findings	Literature Reference
Anti-inflammatory	Methanol stem bark fraction	Flavonoids, tannins, anthraquinone glycosides	Significant inhibition of carrageenan-induced paw edema; comparable to diclofenac and indomethacin	[5]
Immunostimulatory	Ethyl acetate leaf fraction (methanol extract base)	Molucidine, desmethyl-molucidine, luteolin, quercetin, hyperoside, cyclopenol	Marked increase in total and differential leukocyte counts in immunosuppressed mice	[6]
Antifungal	Leaf extracts: hexane, ethyl acetate, metha nol	Squalene, hexadecanoic acid methyl ester, eugenol, phytosterols	Strong antifungal activity against Candida albicans, Aspergillus fumigatus, Trichophyton mentagrophytes	[7]
Enzyme inhibItion	Hydroethanolic stem bark extract	Phenolics, flavonoids, alkaloids, saponins	Moderate α-amylase and α-glucosidase inhibition, with antioxidant potential	[8]
Antiplasmodial / antioxidant	Methanolic leaf extract	GC-MS detected anthraquinones, flavonoids; minerals	~67% reduction in parasitemia at 800 mg/kg; improved oxidative and hematological markers	[9]
Safety profile	Methanol stem bark extract (oral dose up to 5000 mg/kg)	Anthraquinones (digitoluteIn, rubiadin 1-methyl ether), fatty acids	No mortality, histological or biochemical abnor malities; safe at high doses	[10]
Antibacterial & in silico	Root extracts (chloroform and methanol)	Anthraquinones, phenolics, flavonoids	In vitro inhibition of MDR S. aureus And P. aeruginosa; Docking Studies showed high binding affinity	[11]

Previously, we reported on the phytochemical and biological screening of the leaf extracts, including anti-malarial, antimicrobial, anti-oxidant, anti-inflammatory and toxicity [12, 13]. Despite its pharmacological importance, detailed phytochemical profiling of *M. lucida* leaves remains limited. In particular, the isolation and structural characterization of individual compounds have not been comprehensively explored. To address this gap, the current study employs chromatographic separation and spectral analyses to identify key bioactive compounds in the methanol-soluble extract of *M. lucida* leaves. We report the isolation of phytol, a diterpenoid alcohol with well-documented biological activities, including antioxidant, anti-inflammatory, antimicrobial, and cytotoxic effects [14, 15] and cis-1,4-polyisoprene, a polymer derived from isoprene units. The presence of an isoprene polymer such as cis-1,4-polyisoprene in a plant extract is rare and represents, to the best of our knowledge, the first report of its occurrence in the genus *Morinda*. Before this work anthraquinones have been the most common constituents of the plant, though there have been some reports on tannins and sterols [16-22].

2. Materials and Methods

2.1. General

The methanol-soluble fraction for this work was obtained from previous work on the crude extract of the leaf [12, 13]. All the solvents used for chromatography were redistilled before use. Thin layer chromatography (TLC) was carried out on pre-coated Merck Millipore silica gel 60 F_{245} aluminum plates with a thickness of 0.25mm. The spots were visualized by exposure to iodine vapor and Hewlett Packard UV/Visible light at 254/366nm. Column chromatography was carried out over silica gel (200-400 mesh, Qingdao Marine Chemical Inc., China). The infrared (IR) spectra were recorded on a Perkin Elmer Spectrum 100 Fourier Transform Infrared Spectrophotometer (FT-IR) with a universal attenuated total reflectance (ATR) sampling accessory. NMR experiments were conducted on a 400MHz Brucker AVANCE III NMR spectrometer at the Department of Chemistry, Faculty of Engineering and Physical Science (FEPS), University of Surrey,

UK. The spectra were ecorded in deuterated chloroform ($CDCl_3$). The $CDCl_3$ was referenced according to the central line at δ 7.26 in the ^1H NMR spectrum and at δ 77.23 in the ^{13}C NMR spectrum. The spectra were processed using Brucker NMR academic Topspin software (TopSpin 4.0.6).

2.2. Chromatographic Purification of Methanol-Soluble Fraction and Spectral Characterization of Isolates

The bioactive methanol-soluble fraction [12, 13] was investigated for chemical constituents by Flash column chromatographic purification, using a glass column of about 40 cm and 3 cm diameter, 150 g adsorbent silica gel of mesh size 200 – 400 mm packed in hexane to a height of about 24 cm. The methanol-soluble fraction (20.0 g) was pre-absorbed with silica gel which was allowed to dry and then loaded into the column and eluted with mixtures of hexane and ethyl acetate, totaling 700ml. The eluates were successively collected in 5 mL test tubes. The TLC of each collection was carried out to determine the degree of purity. Similar eluates were combined, based on R_f values of the components. From the TLC profiling, the following seven (7) groups of column fractions were obtained: A (hexane: ethyl acetate, 100 ml, 95:5); B (hexane: ethyl acetate, 150, 90:10); C (hexane: ethylacetate, 100 ml, 80:20); D (hexane: ethyl acetate, 150, 70:30); E (hexane: ethylacetate, 100 ml, 65:35); F (hexane: ethyl acetate, 100 ml, 1:1) and G (100% ethyl acetate, 50 ml). Among these, fractions D, coded MLD, and F, coded MLF were found to be fairly pure. The column chromatographic fractions MLD and MLF were subjected to FTIR and NMR spectral analysis.

3. Results and Discussion

Columnn chromatographic purification of the bioactive methanol-soluble fraction gave two compounds, MLD and MLF, which were subjected to FTIR and NMR spectral analysis to determine their structures. Compound MLD was obtained as a light yellow oil boiling at 202 – 205°C (Rf=0.65, hexane/ethylacetate 9:1).

The FTIR (KBr) spectrum (Figure 1) exhibited a characteristic OH absorption band at 3372.65 cm^{-1}. Absorptions at 2969.89 and 2726.37 cm^{-1} are due to aliphatic C-H stretching. The absorptions at 1664.53 and 1605.30 cm^{-1} are due to the C=C bond. The absorptions at 1454.89 and 1375.55 cm^{-1} are due to CH_3 bending. The absorptions between 1309 and 700 cm^{-1} are due to skeletal C-C vibrations.

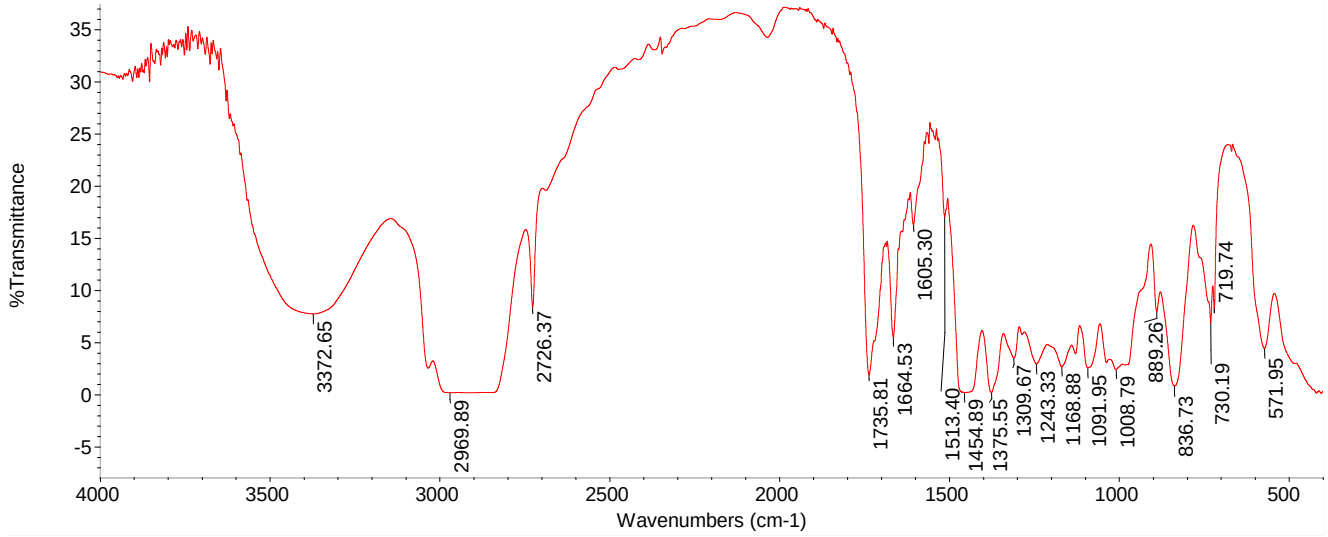

Figure 1. *FTIR spectrum of compound MLD.*

The ^1H-NMR spectrum (Figure 2) showed the presence of methyl protons at carbon-20 (δ_H 1.97 ppm, 3H, *singlet*), methyl protons at carbons-18 and 19 (δ_H 0.98 ppm, 3H, *doublet*), methyl protons at carbon-16 and 17 (δ_H 0.91, ppm, 3H, *doublet*), methine proton at carbon-15 (δ_H 1.63 ppm, 1H, *multiplet*), methine protons at carbons-11 and 7 (δ_H 1.67 ppm, 1H, *multiplet*), methylene protons at carbons-6,8,9,10,12,13 and 14 (δ_H 1.25 ppm, 2H, *quintet, quartet, quintet, quartet, multiplet, quintet*, respectively), methylene proton at carbon-5 (δ_H 1.33 ppm, 2H, *quintet*), methylene protons at carbon-4 (δ_H 1.97, 2H, *triplet*), methine proton at carbon-2 down field (δ_H 5.40, 1H, *triplet*) and methylene group attached to an sp^2 carbon to the left and a hydroxyl group to the right at carbon-1 (δ_H 4.16, 2H, *doublet*).

The ^{13}C-NMR spectrum (Figure 3) displayed several carbon signals including five methyl groups at δ: 16.4 ppm (C-20), 19.9 ppm (C-18,19), and 22.8 ppm (C-16,17). Four methine groups were also present with resonance frequencies at δ: 28.2 ppm (C-15), 32.9 ppm (C-7,11) and 123.3 ppm (C-2). The methylene group resonance frequency signals were

ten with chemical shift values of δ: 24.7 ppm (C-9), 24.9 ppm (C-13), 25.3 ppm (C-5), 36.9 ppm (C-6), 37.6 ppm (C-8,10,12), 39.6 ppm (C-14). 40.0 ppm (C-4) and 59.6 ppm (C-1).

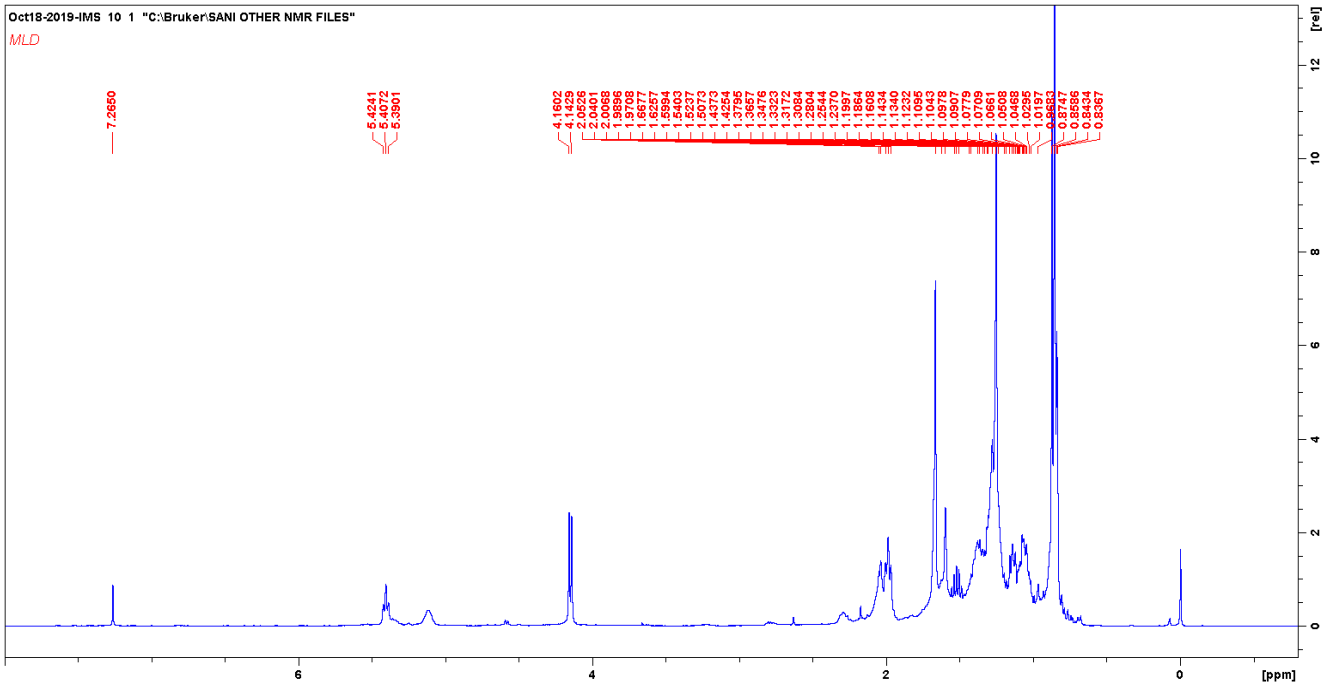

Figure 2. *^{1}H NMR Spectrum of compound MLD in $CDCl_3$.*

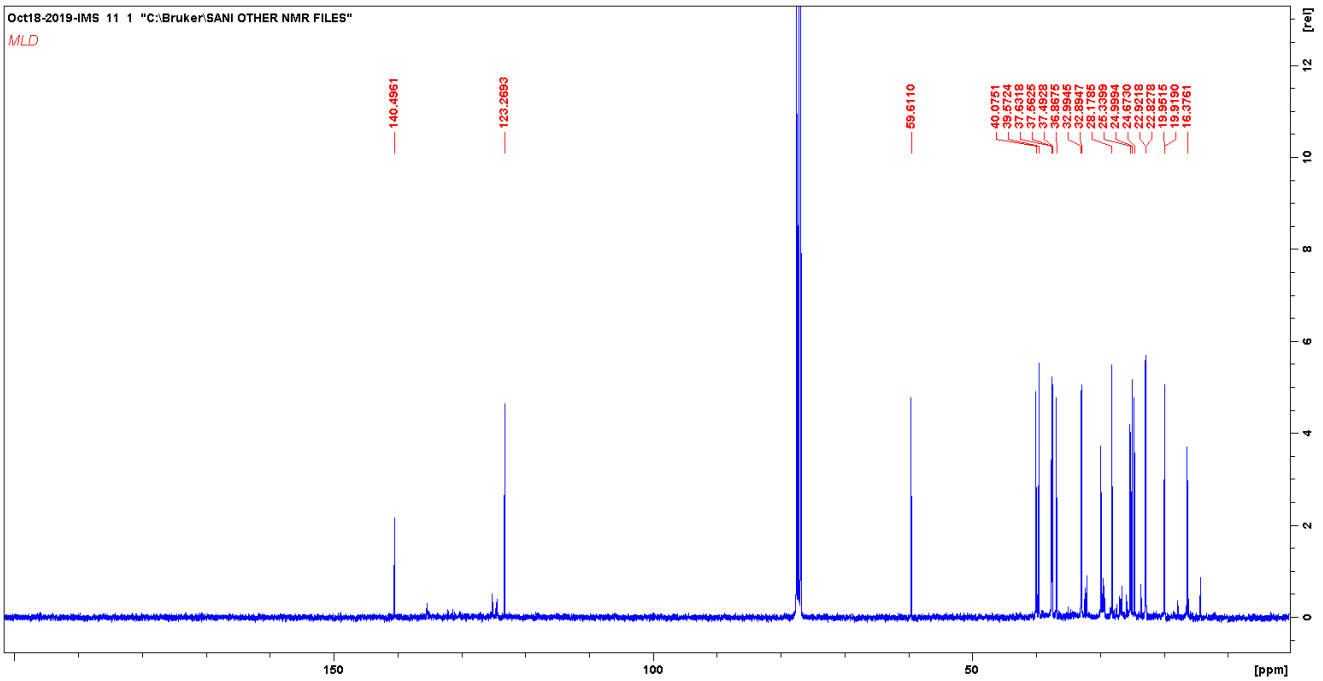

Figure 3. *^{13}C NMR spectrum of compound MLD in $CDCl_3$.*

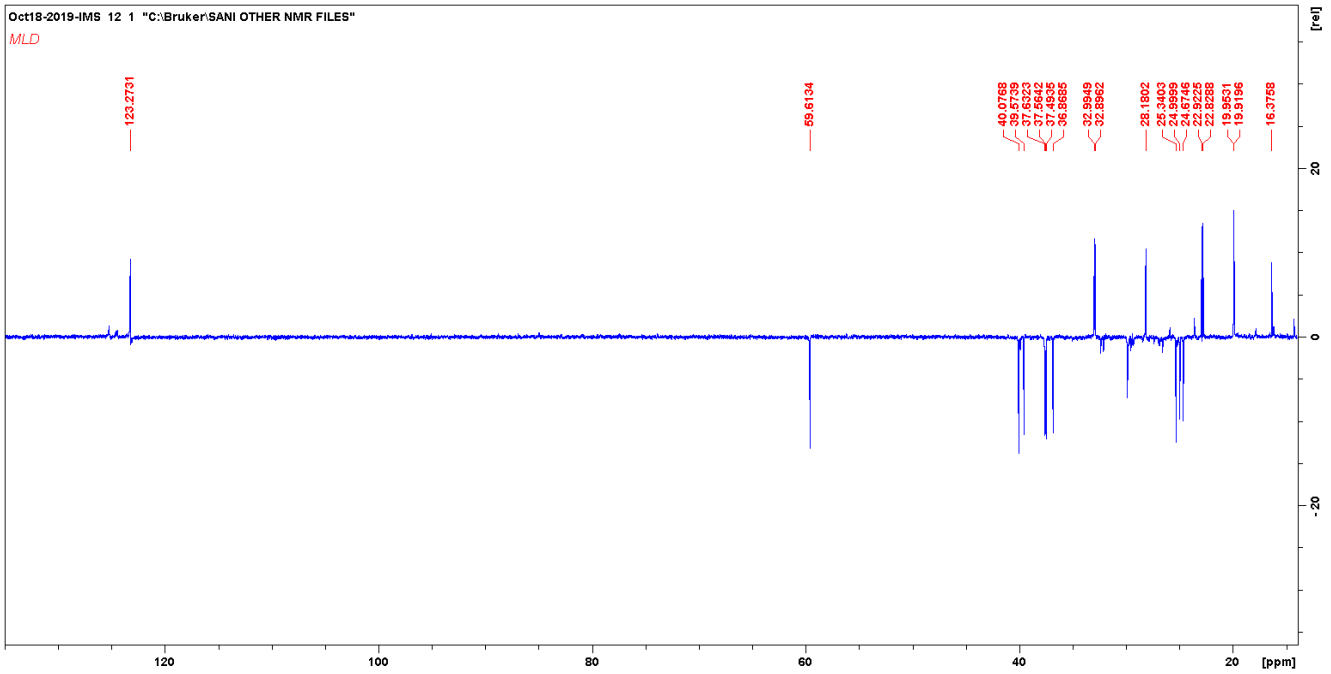

Figure 4. *DEPT spectrum of compound MLD in $CDCl_3$.*

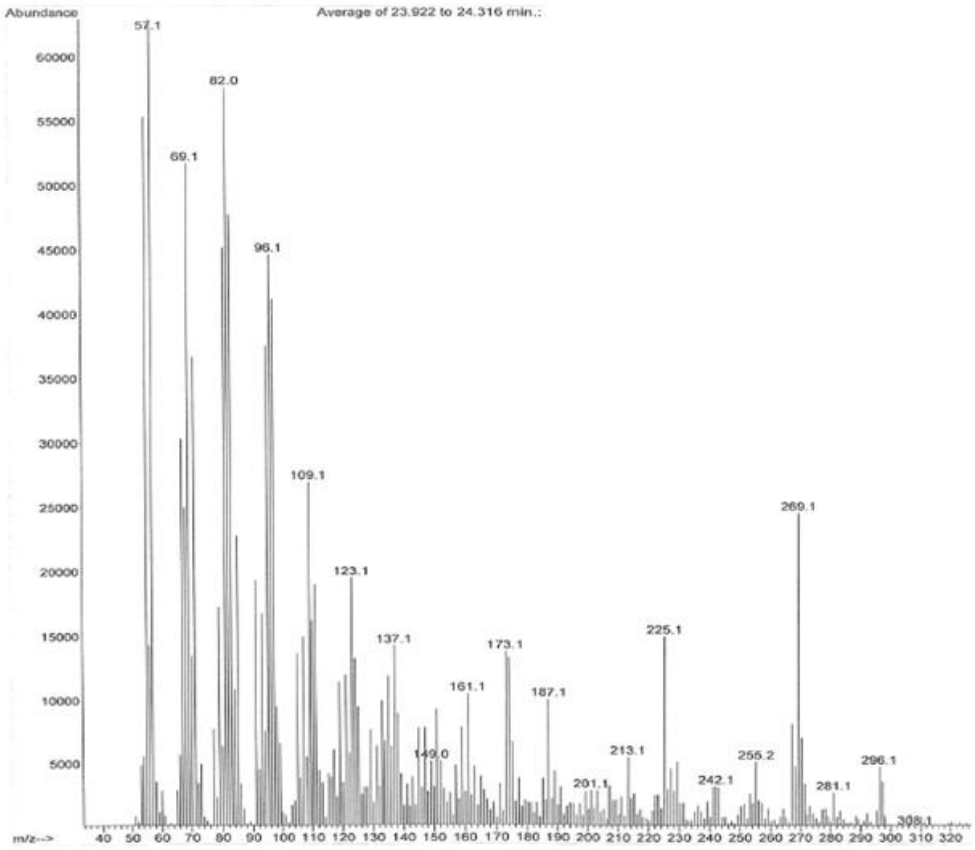

Figure 5. *Mass spectrum of compound MLD.*

DEPT-135 experiment (Figure 4) confirmed the presence of nine positive peaks representing five methyl and four methine groups; and ten negative peaks representing the methylene group. Carbon-3 is tetra-substituted and therefore does not produce signals in the DEPT-135 experiment. The comparative NMR spectral data for compound MLD and phytol

are summarized in Table 2.

In addition, the mass spectrum (Figure 5) of compound MLD showed a molecular ion peak at m/z 296.0, corresponding to a molecular formula of $C_{20}H_{40}O$. The above spectral characteristics and the boiling point are in agreement with those previously reported for the diterpenoid, phytol, (Figure 6) [23-28].

Table 2. *1-H NMR and 13-Carbon NMR spectral analysis of compound MLD.*

Carbon	δ^1H (MLD)	δ^1H *	δ^{13}C (MLD)	δ^{13}C*[23]
1	4.16 (2H, *doublet*)	4.18	59.6	59.1
2	5.40 (1H, *triplet*)	5.39	123.3	125.3
3	No proton	No proton	140.4	140.4
4	1.97 (2H, *triplet*)	1.96	40.0	39.9
5	1.33 (2H, *quintet*)	1.33	25.3	25.2
6	1.25 (2H, *quintet*)	1.25	36.9	36.9
7	1.67 (1H, *multiplet*)	1.65	32.9	32.6
8	1.25 (2H, *quartet*)	1.25	37.6	37.1
9	1.25 (2H, *quintet*)	1.25	24.7	24.2
10	1.25 (2H, *quartet*)	1.25	37.6	37.1
11	1.67 (1H, *multiplet*)	1.65	32.9	32.6
12	1.25 (2H, *quartet*)	1.25	37.6	37.1
13	1.25 (2H, *quintet*)	1.25	24.9	24.5
14	1.25 (2H, *quartet*)	1.25	39.6	39.5
15	1.63 (1H, *multiplet*)	1.62	28.2	27.9
16	0.91 (3H, *doublet*)	0.91	22.8	22.6
17	0.91 (3H, *doublet*)	0.91	22.8	22.6
18	0.98 (3H, *doublet*)	0.96	19.9	19.6
19	0.98 (3H, *doublet*)	0.96	19.9	19.6
20	1.97 (3H, *singlet*)	1.82	16.4	17.2

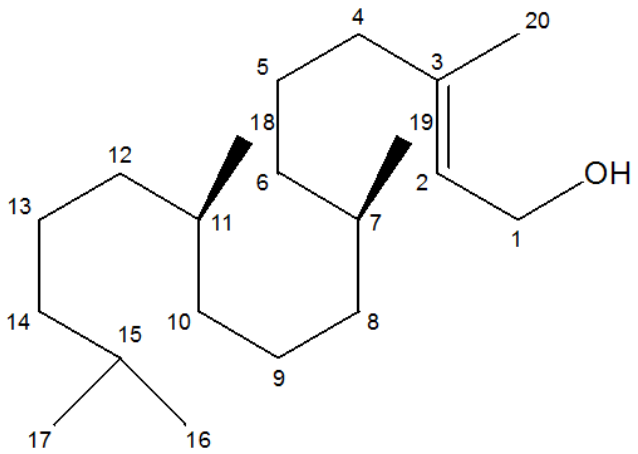

Figure 6. *The structure of phytol.*

Phytol, a diterpenoid, is an oxygenated acyclic diterpene that is an important building block for the chlorophyll molecule. The arrangement of isoprene units in phytol is identical to that in vitamin A, a monocyclic diterpene derivative. Though phytol is a hydrolysis product of chlorophyll, it has been isolated free from extracts of some plants or identified using GC-MS as volatile constituents of such plants. Also, it has been associated with important biological activities such as antimicrobial, anti-oxidant, anti-cancer, anti-inflammatory, and anti-proliferative [24-28]. More recently, potentially important biological studies have been reported on phytol including its ant-ulcer/gastro-protective [29], anti-inflammatory [30], anti-diarrheal [31], antimicrobial [32], and antibacterial activities [33].

The compound MLF was isolated as a colourless liquid

with a boiling point, 122-142 ℃. It has an R_f value of 0.66 (Hex/EtOAc, 7:3). The IR (KBr) spectrum (Figure 7) exhibited characteristic absorptions at 2924.24 and 2726.30 cm^{-1} which are due to aliphatic C-H stretching. The absorption at 1664.06 cm^{-1} is attributable to C=C bond. The absorptions at 1450.15 and 1375.86 cm^{-} are due to CH_3 bending. The absorptions between 1309.25 – 738.65 cm^{-1} are due to skeletal C-C vibrations.

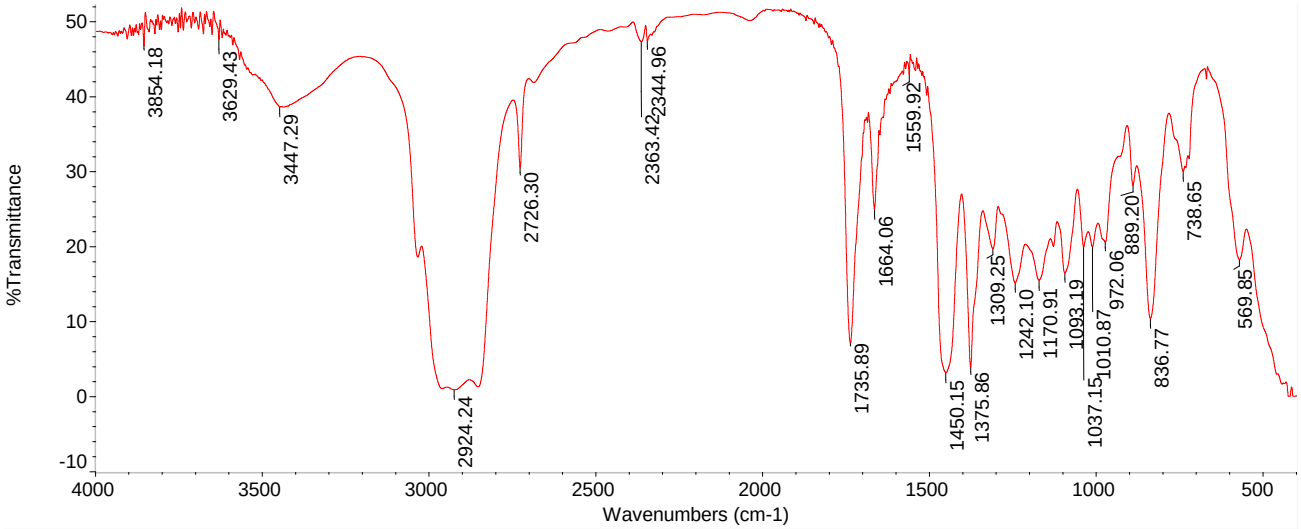

Figure 7. *FTIR spectrum of compound MLF.*

The ^{1}H-NMR spectrum (Figure 8) showed the presence of methyl protons at carbon-5 (δ_H 1.31 ppm, 3H, *singlet*), methylene protons at carbons-1 (δ_H 1.69 ppm, 2H, *singlet*), methyl protons at carbon-4 (δ_H 2.04, ppm, 2H, *doublet*), and methine proton at carbon-3 (δ_H 5.12 ppm, 1H, *triplet*). The ^{13}C NMR spectrum (Figure 9) displayed several carbon signals including methyl groups at δ23.65 ppm (C-5). a methylene carbon with resonance frequency at δ26.61 ppm (C-4) and another methylene group with chemical shift of 32.42 ppm (C-1). The only methine group present resonated at the resonance frequency of δ125.24 ppm (C-3). A tetra-substituted carbon was observed at δ135.42 ppm (carbon-2). The DEPT-135 experiment (Figure 10) confirmed the presence of two positive peaks representing one methyl and one methine groups and two negative peaks representing the methylene groups. Carbon-2 which is tetra-substituted and did not produce signal in the DEPT-135 experiment.

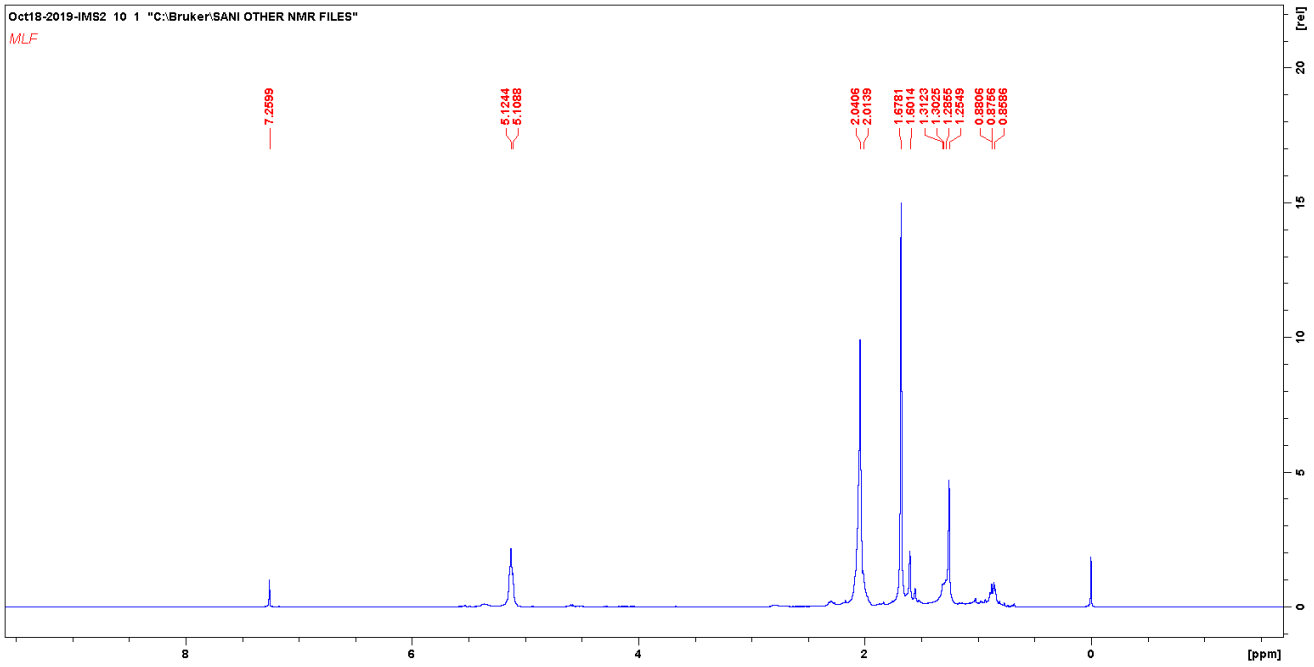

Figure 8. *^{1}H NMR spectrum of compound MLF in $CDCl_3$.*

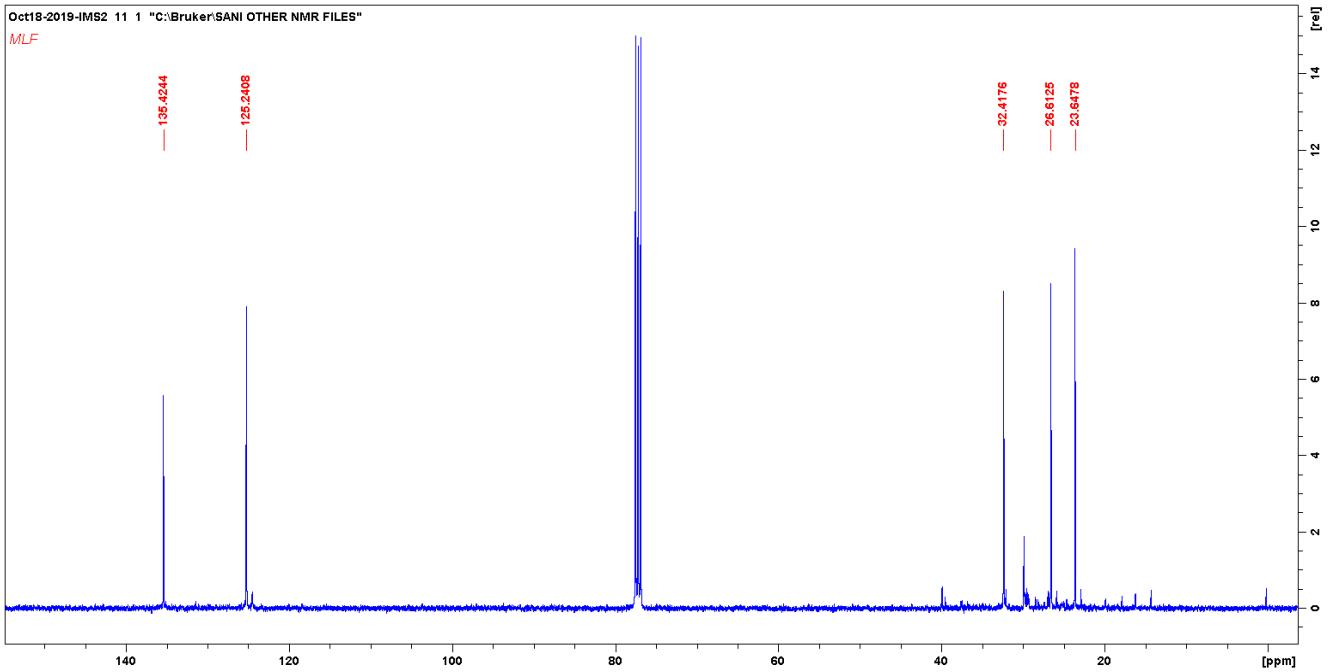

Figure 9. *^{13}C NMR spectrum of compound MLF in $CDCl_3$.*

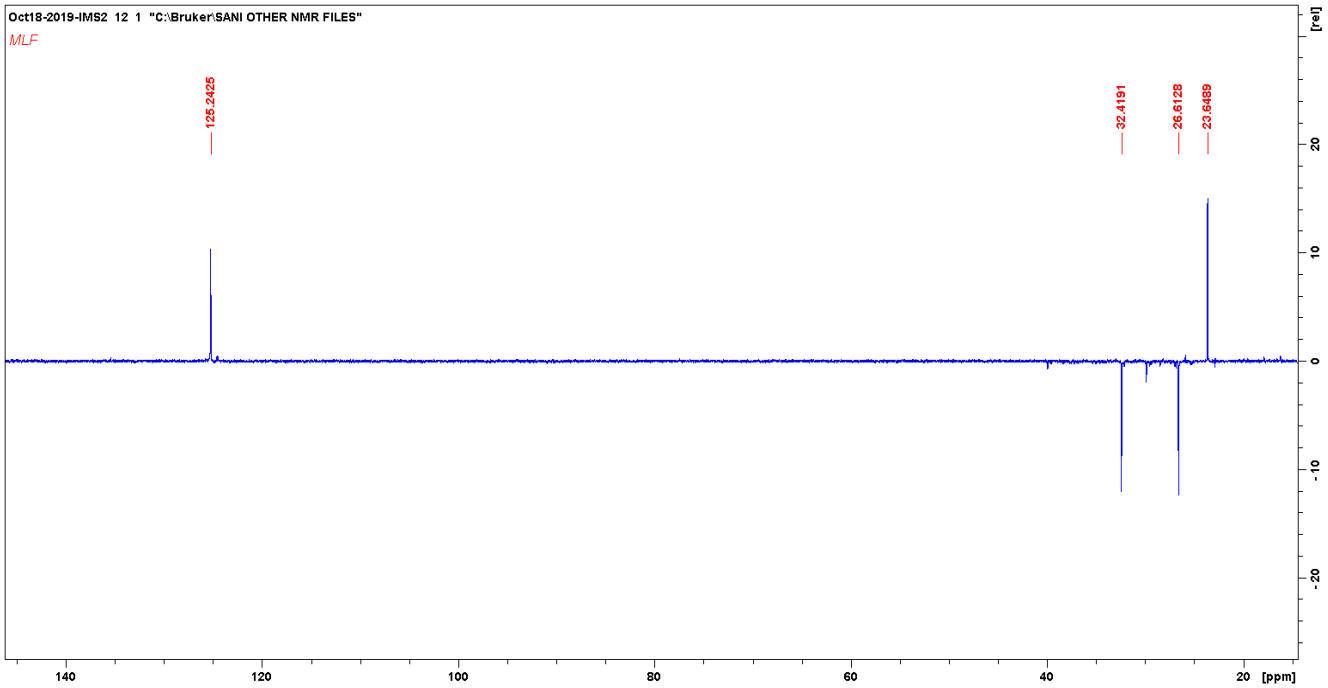

Figure 10. *DEPT spectrum of compound MLF in $CDCl_3$.*

The proton-carbon correlation (HSQC) (Figure 11), the correlation between protons and carbons separated by multiple bonds (HMBC) (Figure 12), the correlation between protons which are coupled to each other in the ^{1}H NMR spectrum (^{1}H-^{1}HCOSY) (Figure 13) and the cross-relaxation and measure of cross-relation rates of the nuclear spins (NOESY) (Figure 14) support the ^{1}H and ^{13}C NMR data for compound MLD. All the spectral data for the NMR analysis of compound MLF when compared with that of established and known compound from literature [23] suggest that compound MLF has the polymeric cis-2-methylbut-2-ene units (Figure 15).

The above spectral characteristics for compound MLF, as well as spectral characteristics recorded for synthetic and natural poly-isoprenes by some researchers [23, 34-38] (Table 3) suggest that compound MLF may be cis-1,4-polyisoprene (Figure 15).

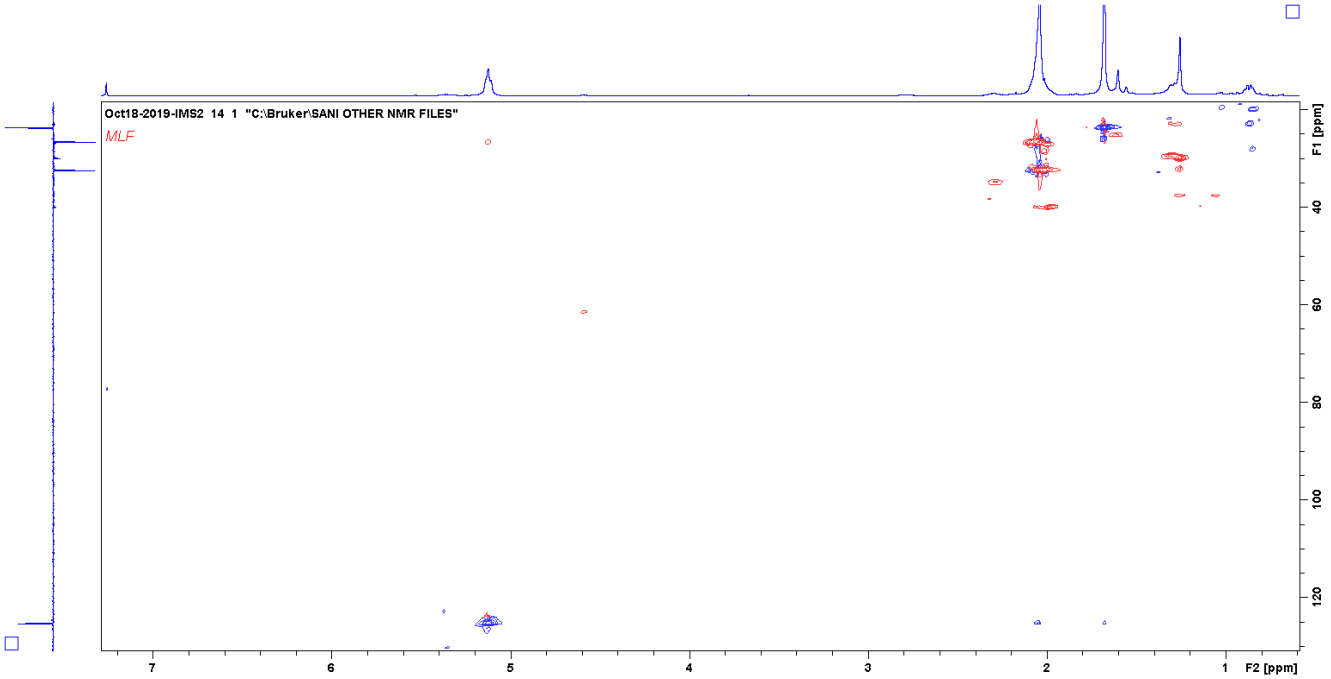

Figure 11. *HSQCDEPT spectrum of MLF in $CDCl_3$.*

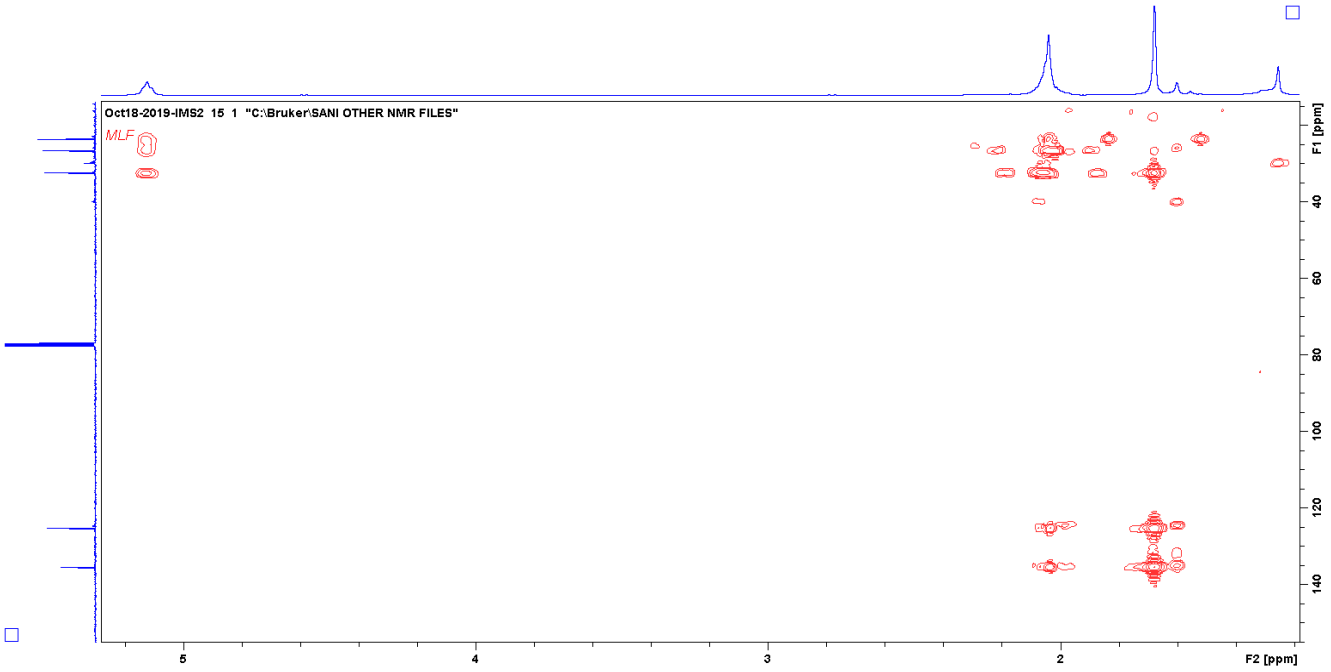

Figure 12. *HMBC spectrum of MLF in $CDCl_3$.*

Table 3. *NMR spectral data of compound MLF [23].*

Carbon	δ^1H (MLF)	δ^1H*	δ^{13}C (MLF)	δ^{13}C*
1	1.69 (2H, *singlet)*	1.69	32.42	32.29
2	No proton	No proton	135.42	135.08
3	5.12 (1H, *triplet)*	5.12	125.24	124.92

Carbon	$\delta^{1}H$ (MLF)	$\delta^{1}H$*	$\delta^{13}C$ (MLF)	$\delta^{13}C$*
4	2.04 (2H, *doublet*)	2.04	26.61	26.50
5	1.31 (3H, *singlet*)	1.31	23.65	23.53

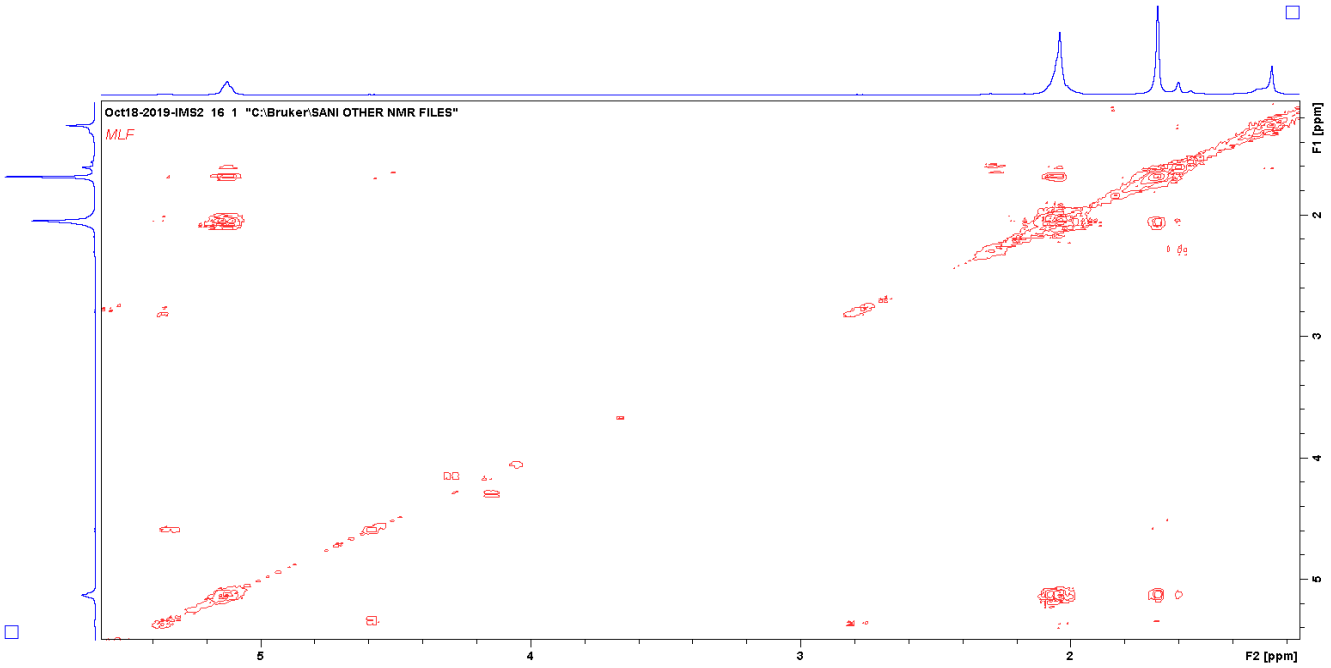

Figure 13. *COSY spectrum of MLF in $CDCl_3$.*

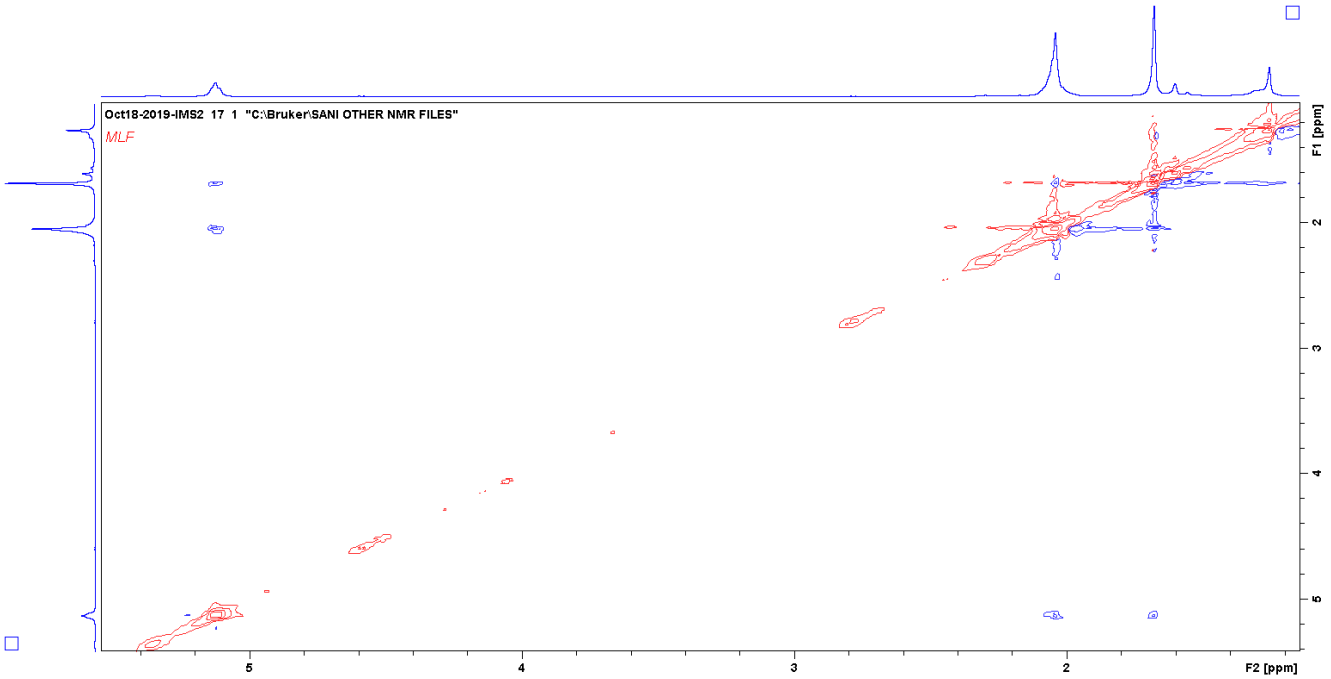

Figure 14. *NOESY spectrum of MLF in $CDCl_3$.*

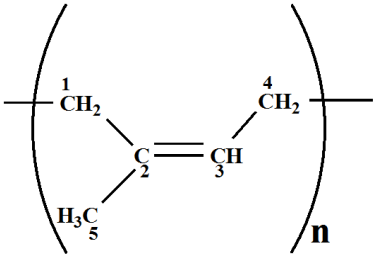

Figure 15. *The structure of 1,4-polyisoprene unit.*

The presence of isoprene molecule or its polymer in plants is rare. However, isoprene is emitted by some plants challenged by environmental pressures such as draught. Such plants are said to experience isoprene responses as a coping mechanism for those plants [39]. Isoprene has also been associated with anti-oxidant and other regulatory functions in plants [40, 41]. Based on spectral characteristics, including the 2D NMR spectra and comparison with literature [23] (Table 3), it is most probable that the compound MLF obtained in this work is cis-1,4- polyisoprene (Figure 15), This is a significant result as this is the first time a rubber-like polyisoprene has been identified in *Morinda lucida*. Poly-isoprenoids are known to be among the eight major classes of naturally-occuring biopolymers [42]. The structure of a polyisoprene normally consists of a methyl-branched carbon-carbon framework and is a derivative of isoprene (2-methyl-1,3-butadiene) [43]. Today, about 2,500 species of plants are known to generate the polyisoprene, natural rubber (NR). However, the rubber plant, *Hevea brasiliensis*, growing only in subtropical and tropical climates, is by far the most commercially important source [44]. Some recent studies have reported on cis-1,4-polyisoprene not only as a material but its bioactivity including, the herbivory reducing property [45], wound-healing activity in rats [46], antimicrobial activity of polyisoprene-based surfaces [47], elastomeric composites with ZnO [48], and genome sequencing for the purpose of understanding how to increase the production of natural rubber (NR) [49].

4. Conclusion

In this work, chemical investigation of the extracts of the leaves of *Morinda lucida* has led to the isolation and characterization of two constituents, the diterpenoid, phytol, and cis-1,4-polyisoprene. Phytol is a constituent of green plants, found in the structure of chlorophyll, but was found in this work as a free alcohol in the methanol-soluble fraction of the leaf extract of *Morinda lucida*. It is known to have important bioactivities such as antimicrobial, anti-cancer, anti-inflammatory, and antioxidant. Isoprene and polyisoprenes are important in plants for self-help and are of industrial importance as sealants and antioxidants. Very significantly, the natural occurrence of isoprene or its direct polymer is rare in plants. This is probably the first report of these two compounds from a plant belonging to the family, Rubiaceae. The results of this work may therefore have pharmacological and taxonomical implications.

Abbreviations

FTIR	Fourier Transform Infrared
NMR	Nuclear Magnetic Resonance
$CDCl_3$	Deuterated Chloroform
ML	*Morinda lucida*
HSQC	Heteronuclear Single Quantum Correlation
HMBC	Heteronuclear Multiple Bond Correlations
^1H-^1HCOSY	Hydrogen-Hydrogen Correlation Spectroscopy
NOESY	Nuclear Overhauser Effect Spectroscopy

Acknowledgments

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Author Contributions

Simon Koma Okwute: Conceptualization, Data curation, Formal Analysis, Investigation, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing

Isaacs Okwori Ochi: Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Resources, Writing – original draft

Additional Information

The preliminary work on this plant in terms of extraction of the leaf, phytochemical analysis of the extracts, and the bioassay studies has been published according to references [12] and [13]. Those details are therefore left out in this report.

Conflicts of Interest

The authors declare no conflicts of interest.

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Biography

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