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SPECTROSCOPIC CHARACTERIZATION AND IDENTIFICATION OF FRIEDELAN-3-OL FROM MANGIFERA INDICA ROOT AND ITS ANTI-MICROBIOLOGICAL ACTIVITIES AGAINST SOME CLINICALPATHOGENS

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Abstract
Plants used in ethnomedicine have remained a foundation of therapeutic agents in nature till date. Recently, there has been wider interest in research for new agents for treatment of both emerging and re-emerging infectious diseases as a result of anti-microbial resistance to drugs. The application of some common drugs which results in the development of their side effects, resistance and other deficiencies created the need for use of alternative natural remedies with better therapeutic properties. This research sought to contribute to this quest by extracting and identifying possible therapeutic compounds from the roots of *Mangifera indica,* as well as evaluating their efficacy against a variety of clinical microbiological infections. Using a Soxhlet device, the powdered root was extracted with ethyl acetate and methanol. The extracts were separated on a silica gel column and eluted in ethyl acetate with increasing quantities of methanol**.** Fractions obtained were examined by some spectroscopic methods such as TLC, UV, IR and based on their profiles 40 fractions obtained were pooled to four combined fractions which were analysed using NMR (1D and 2D) to confirm the structures of their constituents. The result showed that MI-03 is a triterpenoid (Friedelan-3-ol). The antimicrobial results showed high sensitivity of *Vancomycin resist Enterococci* (30mm), *Staphylococcus aureus* (28mm), *Escherichia coli* (31mm) and *Pseudomona aeruginosa* (30mm) to the isolated triterpenoid (Friedelan-3-ol), but *Methicillin resist Staphylococcus aureus* and *Salmonella typhi* was resistant to the compound. The fungi *Aspergillus fumigates* (30mm*), Aspergillus nigre* (31mm), *Fibrophoria vaillentii* (29mm), *Fomitopsis pinicola* (31mm), *Rhizopus SP* (20mm) and *Serpula lacrymans* (30mm) were sensitive to the isolated compound, while *Aspergillus flavus, Coniophora puteana, Fusarium oxysporum, Fusarium proliforatum* and *Sclerotium rofsii* were resistant to the compound. The sensitivity of these clinical pathogens indicates that the isolated natural organic triterpenoid (Friedelan-3-ol) canbe harnessed, prepared and used for their treatment.

Keywords: Spectroscopic, Characterization, Identification, Mangifera Indica Root and Its, Clinicalpathogens

Introduction

Medicinal plants have been recognised as a lead way to therapeutic agents for both man and animals for centuries now. This may be traced back to their usage in the manufacture of medicines, food, oxygen for living creatures (including humans and animals),and industrial raw materials. Plants have also shown to be the most effective in the treatment of illnesses, and chemical compounds contained in them play a significant part in the world's medicines (Nwokonkwo, 2014). Terpenes, steroids, alkaloids, flavonoids, saponins, tannins, anthraquinones, carotenoids, and glycosides are some of the chemical compounds found in plants. Phytochemicals or secondary metabolites are the terms used to describe them (Ajibesin, 2011, Ichiko,et al, 2016). Plant phytochemicals protect plants against pathogens, pollution, drought, and UV exposure, and they

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also help them to be more helpful in tradomedicine (Sofowora, 1993). Medicinal plants are sources of many important drugs thus, the precise interaction of drugs with biomolecules such as nucleic acids and proteins in the body improves functional effects of drugs in the body. Thus, different drugs are required or useful for different purposes making plants valuable precursors for drug development (Ajibesin, 2011). Plants thus, play vital roles in traditional or herbal medicine, homeopathy and aromatherapy. For instance, flavors and foods gotten from medicinal plants are used in the meals of humans to enhance their immune system (Akinpela & Onukoya, 2006). A widespread of knowledge have been developed vis-à-vis the utility of plants as food and medicine for man as an outcome of some pharmacological and biological activities such as diuretics, anti-inflammatory, anti-plasmodics, laxative, antimicrobial and antihypertensive potencies of these plants (Adesokan *et al.,* 2007). As a result, it's not uncommon to see animals and snakes consume plants when they're ill. It's possible that early man found herbal medicines as a result of intuition or exposure, as many herbalists say. On the third day, God created plants. Plants can manufacture a broad range of organic molecules of virtually every structural class thanks to the unique ability bestowed upon them by God. As a consequence, plants serve as a valuable source of raw material for the chemical industry. God Almighty has decreed herbal healing. 'Now Isaiah said, let them take a chunk of fig and put it on the boil like a plaster, and he will recover" is one of the divine sanctions on herbal healing (Isaiah 38:21). 'Their fruits will be used as food, and their leaves will be used as medicine.' Ezekiel 47:12 is a prophecy from the prophet Ezekiel. God has given His permission and holy seal to herbal therapy, as shown by the following scriptural injunctions. Herbal healing forms a key characteristic of traditional medicinal practice in Nigeria (Douglas & Joseph, 2002, Okwu & Morah, 2007). The effectiveness of traditional medicinal practice is a function of the chemical ingredients of the plants.

Materials and Methods

Sample Collection and Preparation: The root of *Mangifera indica* was obtained from Barayira Tai, in Tai Local Government Area, Rivers State, Nigeria in August 2019, identified and authenticated at the Department of Plant Science and Biotechnology, Rivers State University, Port Harcourt by a Botanist and was allotted Voucher specimen RSU/2019/MI-103 and kept in the herbarium. The sample was air-dried for three weeks and crumpled to powder with the aid of a mortar and pistle. Thereafter, it was stored in a glass container and taken to Strathclyde Institute of Pharmacy and Biomedical Sciences laboratory, University of Strathclyde, Glasgow, United Kingdom for extraction and further analysis.

Extraction of Sample: About 400g of ground root of *Mangifera indica* was placed in soxhlet apparatus and extracted sequentially for two days each continuously with ethyl acetate and methanol. The extracts were evaporated to dryness in a rotary evaporator at 40^0C. All dried extracts were pulled together and kept in different sample bottles and labelled MI/EA-01, and MI/ ME-01 for ethyl acetate and methanol extracts respectively. About 20g of each crude extract was dissolved in silica gel and allowed to dry for column chromatography.

Isolation and Characterisation: 15 g of dried extract of Mangifera indica was dissolved in dichloromethane and transferred into a small beaker. 10 g of silica gel was added to the extract and stirred then allowed to dry in a fume cupboard. The silica gel was added to adsorb the extract. About 500 mL of hexane was added to 100 g of silica gel and stirred continuously until slurry was formed. The column was hung on a retort stand and rinsed three times with ethyl acetate to avoid contamination. Little quantity of hexane was poured unto the column and two minutes later, the slurry was transferred unto the column glass with column tap opened slowly and allowed to settle as to dril out the solvent. At about 10 cm of solvent above silica packing, tap was closed and adsorbed extract slurry was transferred unto the column and then the tap was opened for excess solvent above loaded extract to run out of the column and allowed to settle on the silica. 5 % ethylacetate mixed with hexane was poured unto the column and the tap was allowed to run at about 10-15 drops per minute. About 25-30 mL fractions were uniformly collected using serially numbered vials. The eluates were allowed to dry and kept in a dust free fume hood (Nande & Igoli, 2017). Similar column fractions were combined after TLC analysis (Hostettmann et al.,1998). Fractions 001,002 and 003 elute with hexane: ethyl acetate gave similar Tlc profile single creamy spot when charred with concentrated sulphuric acid, Rf value found to be 0.68. The combined fraction 001-003 were recrystallized in ethyl

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acetate to yield a compound labelled MI-001. The compound was subjected to spectroscopic analysis (NMR-Spectroscopy), its melting point was determined (Table 1).

Test organisms: Using animal and plant diseases, the biological activities of the isolated chemical from the understudied plant were identified. The animal and plant pathogens were acquired from the ABU Teaching Hospital Zaria's Department of Medical Microbiology. The antimicrobial assay used the following animal pathogens (bacteria): *Methicillin Resist Staph Aureus, Vancomycin Resist Enterococci, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella tryphi; and plant pathogens* (fungi): *Aspergillus fumigatus, Aspergillus flavus, Coniophora* All microorganisms were tested for purity and kept in agar slants (Tor-Anyiin et al., 2016).

Cultivation and standardization of test organisms: The test organisms were taken from the agar slant and subcultured in test tubes containing sterile nutritional agar for bacteria and sabouraud dextrose medium (20 mL) for fungus, respectively. The test tubes were incubated for 48 hours at 37°C. Broth cultures were standardised using sterile normal saline to reach a density of 106 cfu/mL for bacteria. A sporulated test fungal spore was collected in sterile normal with 0.05 percent Tween80 and standardised to 106 spores/mL. (Nna et al,2019)..

Antimicrobial profile (Sensitivity test): To achieve a concentration of 20 g/mL, 0.002 mg of a chemical was weighed and dissolved in 10 mL of DMSO. For screening, the diffusion technique was employed. Mueller Hinton agar and Sabouraud dextrose agar were employed as the microorganisms' growth medium. The media were made according to the manufacturer's instructions, sterilised for 15 minutes at 121°C, put onto sterile petri dishes, covered, and allowed to cool and harden. Mueller Hinton agar received 0.1 mL of standard test bacteria inoculum, whereas subouraud dextrose agar received 0.1 mL of test fungus. A sterile swab was used to distribute the inoculum uniformly over the surface of the medium. A well was cut in the centre of each infected medium using a standard sterile cork borer with a diameter of 6 mm. After that, around 0.1 mL of a 20 g/mL solution of the chemical was added to the well on the infected medium and allowed to diffuse into the agar. After incubation at 37 °C for 24 hours for bacteria and 30 °C for 1-7 days for fungi, the plates containing the medium were examined for zones of growth inhibition. With a clear ruler, the observed zones were measured and the results were recorded in millimeters (Nwisah*et al*., 2020).

Determination of Minimum Inhibitory Concentration (MIC): A broth dilution technique was used to determine a compound's minimum inhibitory concentration. Mueller Hinton broth and Sabouraud dextrose broth were made; 10 mL of each broth was poured into test tubes and sterilised at 121 °C for 15 minutes, after which the broth was allowed to cool. A turbid solution was produced using Mc-turbidity Farland's standard scale number 0.5. The test microbe was injected and cultured at 37 °C for 6 hours after 10 mL of normal saline was poured into sterile test tubes. The test microorganism was diluted in normal saline until the turbidity matched Mc-scale farland's when compared visually. The test microorganism has a concentration of 1.5 108cfu/mL at this time. The chemical was serially diluted twice in sterile broth to achieve concentrations of 20 g/mL, 10 g/mL, 5 g/mL, 2.5 g/mL, and 1.25 g/mL. 0.002 mg of a chemical was dissolved in 10 mL of sterile broth to achieve the starting concentration. After obtaining various concentrations of a chemical in sterile broth, 0.1 mL of a test microbe in normal saline was inoculated into the various concentrations and incubated at 37 °C for 24 hours for bacteria and 30 °C for 1-7 days for fungus. Following that, the turbidity of the broth test tubes was examined (growth). The minimal inhibitory concentration was determined by taking the lowest concentration of a chemical in sterile broth that produced no turbidity (MIC).

The minimal bactericidal/fungicidal concentration (MBC/MFC) was determined in order to see whether the test microorganisms were killed or if their development was slowed. Mueller agar and sabouraud dextrose agar were made, sterilised at 121 °C for 15 minutes, and then put onto sterile petri dishes to cool and solidify. The contents of the MIC were then sub-cultured onto prepared medium in successive dilutions. Fungi were subcultured on Sabouraud dextrose agar, whereas bacteria were subcultured on Mueller agar. Bacteria were incubated at 37°C for 24 hours and fungi were

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incubated at 30°C for 1-7 days. The plates of the medium were then examined for colony growths. MBC/MFC were plates lacking colony development that had the lowest concentration of the chemical. After 24 hours, the results were recorded (Usman *et al.,* 2007).

Results and Discussion

Table 1: ^{1}HNMR and ^{13}C NMR Chemical Shifts for NJP-D2

Spectroscopic technique	Data
1R(cm^{-1})	3478, 1715
Rf [Hex:EAC(7:3)]	0.49
Mp	260-262 ^{0}C
^{1}HNMR($CDCl_3$)	δ 1.86, 1.81, 3.65, 1.19, 0.87, 0.86, 0.92, 0.94, 0.79, 1.17, 1.10, 0.92 ppm
^{13}C NMR($CDCl_3$)	δ 16.4, 35.4, 72.8, 53.2, 39.7, 41.7, 17.6, 49.2, 37.9, 64.4, 32.8, 30.7, 38.4, 37.1, 28.2, 36.0, 36.1, 39.8, 34.1, 29.7, 32.4, 39.3, 11.6, 15.8, 20.1, 18.7, 18.3, 31.8, 30.0, 32.1 ppm.

Characterization for NPJ-D2 as Friedelan-3-ol

Ethyl acetate extract of *Mangifera indica* on column root gave a fraction coded NPJ-D2, obtained from column chromatography on silica gel with hexane: ethyl acetate (70:30). Its infrared spectrum showed a strong absorption band at 3478 cm^{-1} and 1715 cm^{-1} indicative of a saturated alcohol (OH) group and olefenic methylene group respectively (Igoli &Gray,2008). The compound on TLC plate gave a R_f value of 0.49 and its melting point 248-250 ^{0}C.The ^{1}H spectrum showed a signal at δ 1.19 (1H, q) and another signal due to a methyl at δ 0.87 (d). Other signals appeared at δ 0.86 (s), 0.92 (s), 0.94 (s), 0.79 (s), 1.17 (s), 1.10 (s) and 0.92 (s) which are typical pentacyclic triterpenoid. ^{13}C-NMR showed signals for 30 carbon atoms 16.4, 35.4, 72.8, 53.2, 39.7, 41.7.,17.6, 49.2, 37.7, 64.4, 32.8, 30.7, 38.4, 37.1, 28.2, 36.0, 36.1, 39.8, 34.1, 29.7, 32.4, 39.3, 11.6, 15.8, 20.1, 18.7, 18.3, 31.8, 30.0 and 32.1 ppm (Appendix 7b). Its ^{13}C spectrum gave a hydroxyl signal at 72.8 ppm typical of a saturated ring OH; other peaks (Table 40) were in agreement with reported literature (Igoli & Gray, 2008). The spectra data of NPJ 40-42 shown in Table 40 were utilized together with literature reports (Igoli & Gray, 2008). From the COSY, the compound gave a correlation between the proton signals at δ 1.19 (H-4) and δ 0.87 (H-23) indicating a methyl substituent at position C-4 typical of a friedalanol moiety (Igoli & Gray, 2008). In HMBC, the methyl at 0.87 (H-23) gave correlations to the hydroxyl at C-3. The proton (H-4) also gave correlation to this hydroxyl, a confirmation for friedelanol ring A. Combination of NMR spectra data 1D (^{1}H and ^{13}C) and 2-D (COSY and HMBC) were consistent with literature values for a triterpenoid of friedelanol (Igoli & Gray, 2008). Thus NPJ 40-42 was identified as friedelanol with NMR spectra analysis (Table 2).

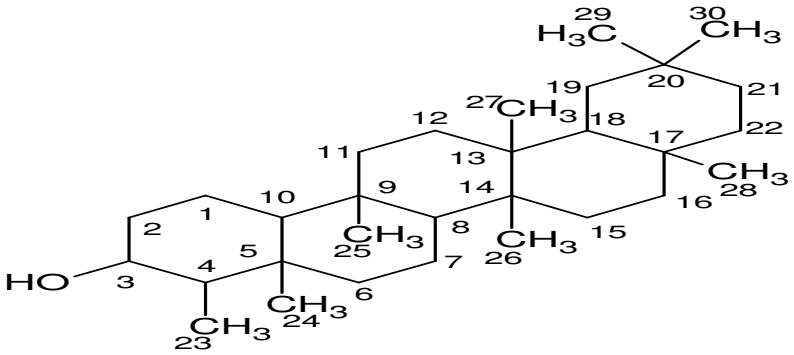

Figure 1: Compound D2 Friedelanol

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Table 2: NMR-Data for NPJ-D2

Position	Experimental		Literature	
	$^{1}H(\delta)$	$^{13}C(\delta)$	$^{1}H(\delta)$ Igoli and Gray (2008)	$^{13}C(\delta)$
1	1.86	16.4	1.88	18.66
2	1.90, 1.81	35.4	1.73	39.30
3	3.65	72.8	3.64	72.78
4	1.19	53.2	1.73	52.22
5		39.7		39.70
6		41.7		41.75
7		17.6		17.57
8		49.2		49.20
9		37.9		37.85
10		64.4		61.38
11		32.8		32.84
12		30.7		30.66
13		38.4		38.39
14		37.1		37.13
15		28.2		30.66
16		36.0		32.36
17		36.1		37.86
18		39.8		42.85
19		34.1		32.84
20		29.7		30.04
21		32.4		32.35
22		39.3		41.75
23	0.87	11.6	0.95	11.63
24	0.86	15.8	0.86	16.41
25	0.92	20.1	0.92	20.13
26	0.94	18.7	1.00	18.66
27	0.79	18.3	1.01	18.26
28	1.17	31.8	1.17	31.81
29	1.10	30.0	1.05	30.04
30	0.92	32.1	0.94	32.10

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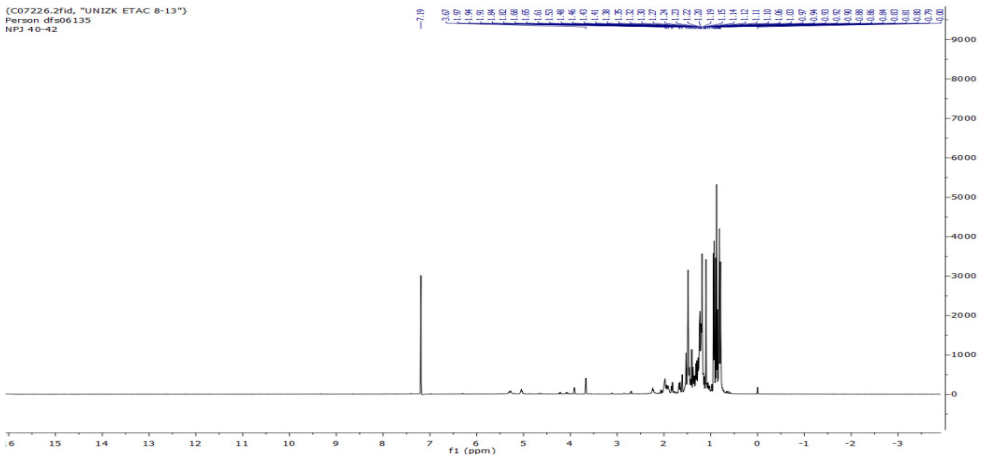

Figure 2: ^{1}H-NMR SPECTRUM OF NPJ-D2 (Friedelan-3-ol)

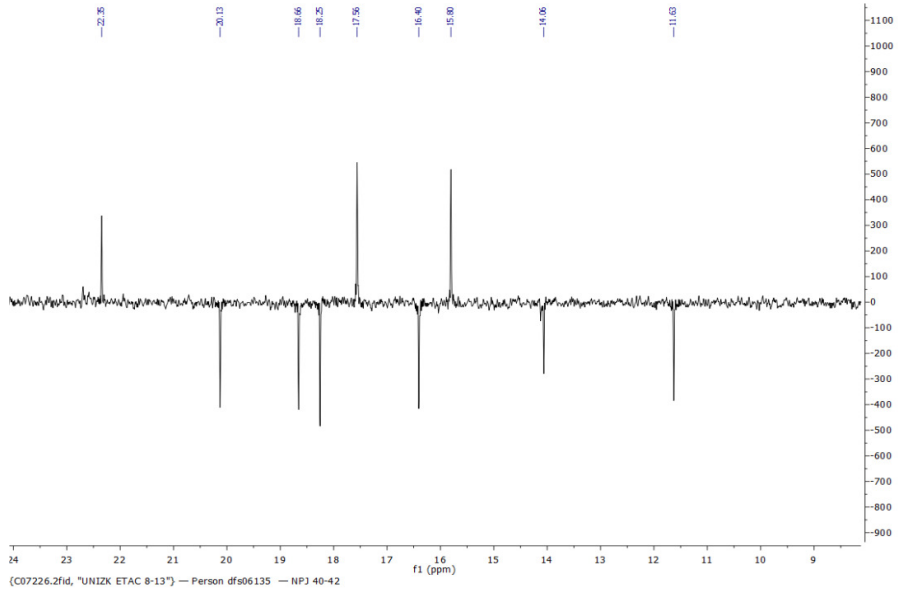

Figure 3: ^{13}C-NMR SPECTRUM OF NPJ-D2 (Friedelan-3-ol)

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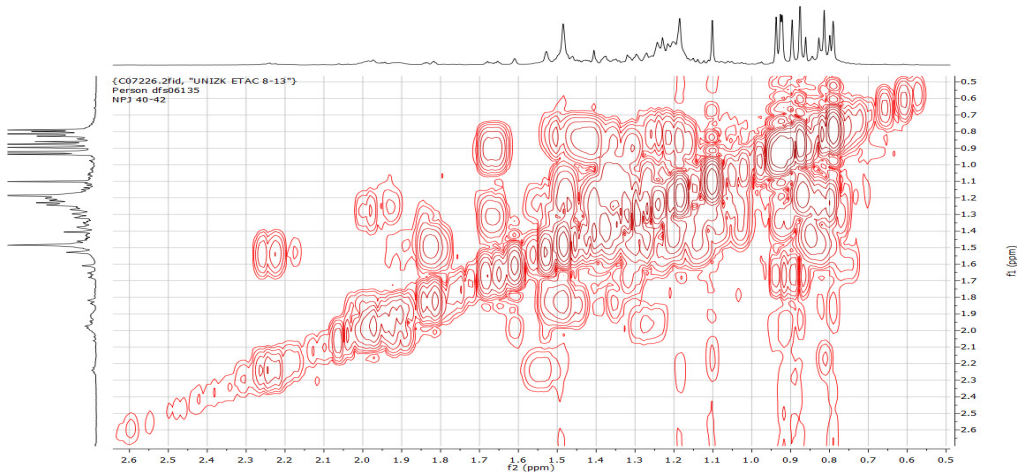

Figure 4: COSY SPECTRUM OF NPJ-D2 (Friedelan-3-ol)

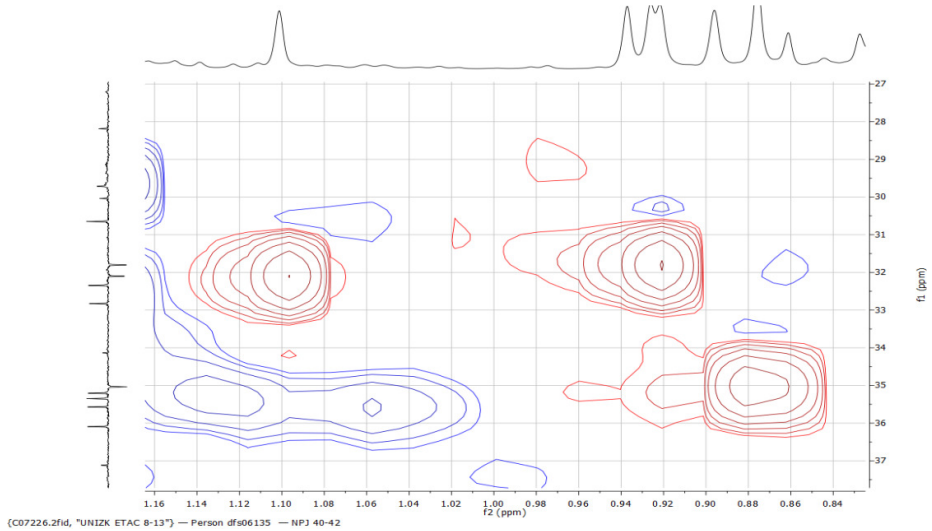

Figure 5: HSQC SPECTRUM OF NPJ-D2 (Friedelan-3-ol)

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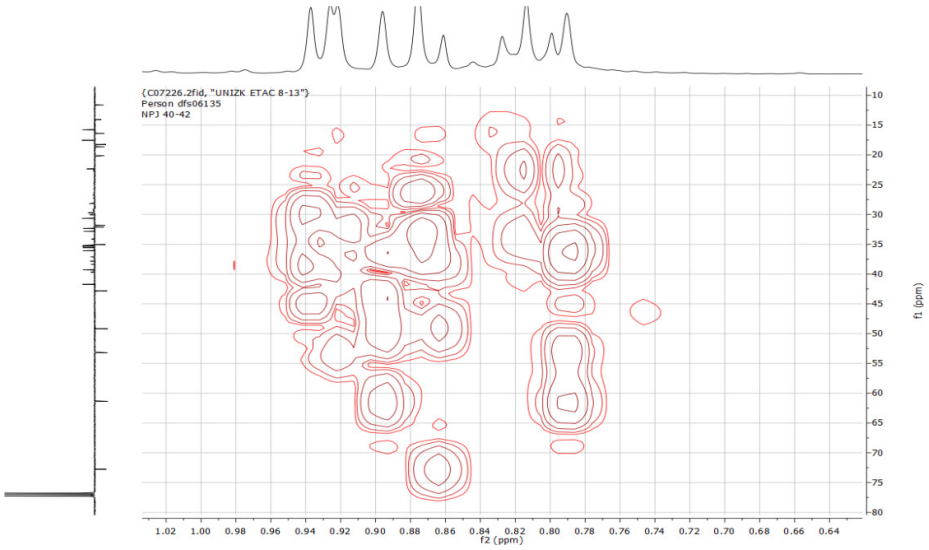

Figure 6: HMBC SPECTRUM OF NPJ-D2 (Friedelan-3-ol)

Table 3: Antibacterial Activities and zone of inhibition of isolated compound.

Test organism	Isolated compound	Sporfloxacin	Ciprofloxacin
Methicillinresiststaphaureus	R(0)	S(30)	R(0)
Vancomycinresistenterococci	S(30)	S(29)	S(30)
Staphylococcusaureus	S(28)	S(32)	S(26)
Escherichia coli	S(31)	S(27)	S(37)
Salmonella typhic	R(0)	R(O)	S(40)
Pseudomona aeruginosa	S(30)	R(0)	(25)

N/B: S=Sensitivity
R=Resistance

Table 4: The Antifungal Activities and Zone of inhibition of isolated compound.

Test Organism	Isolated compound	Fulcin	Kefeconazole
Aspergillusflavus	R(0)	S(26)	R(0)
Aspergillusfumigates	S(30)	S(28)	R(0)
Aspargillusnigre	S(31)	S(30)	S(25)
Coniophoraputeana	R(0)	R(0)	S(23)
Fibrophoriavaillentii	S(29)	S(28)	R(0)
Fomitopsispinicola	S(31)	S(30)	S(27)
Fusariumoxysporum	R(0)	S(32)	R(0)
Fusariumproliforatum	R(0)	S(27)	R(0)
Rhizopussp	S(20)	S(26)	S(28)
Sclerotium rofsii	R(0)	R(0)	S(25)
Serpula lacrymans	S(30)	S(20)	S(26)

N/B: S=Sensitivity,
R=Resistance

The antimicrobial results showed high sensitivity of *Vancomycin resist enterococci* (30mm), *Staphylococcus aureus* (28mm), *Escherichia coli* (31mm) and *Pseudomona aeruginosa* (30mm) to the isolated compound but *Methicillin resist staph aureus* and *Salmonella typhic* was resistant to the compound. The result also showed that the following fungi were sensitive to the isolated compound, *Aspergillus fumigates* (30mm*), Aspergillus*

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nigre (31mm), *Fibrophoria vaillentii* (29mm), *Fomitopsis pinicola* (31mm), *Rhizopus SP* (20mm) and *Serpula lacrymans* (30mm). Omeke et al., (2019) also reported the antibacterial activity of leaf extracts of *Chromolaena odorata* plant against *Pseudomonas aeruginosa* isolated from wound infections. The following fungi; *Aspergillus flavus, Coniophora puteana, Fusarium oxysporum, Fusarium proliforatum* and *Sclerotium rofsii* were resistant to the isolated compound. In a similar study, Okwelle and George (2008) noted the antifungal effectiveness of the use of bitter leaf (*Vernonia amygdalina)* extract against *Sclerotium rolfsi,* the fungus that causes soft rot disease in plants. The efficacy of the successful control of growth of *Fusarium oxysporium,* the fungus that causes wilt disease with cold and hot aqueous extracts of Cassava leaf was equally documented by Okwelle and George, (2010).

The result suggested that the compound deactivated various cellular enzymes, which play a vital role in various metabolic pathways of these screened microorganisms (Amise et al*.,* 2016). The high zones of inhibition are indications of strong zones of clearance by the compounds against the microbes. *Staphylococcus aureus* is known to cause several diseases such as blood stream infection and skin disease such as boil. *Pseudomonas aeruginosa* is a gram negative bacteria that is known to cause several diseases in humans like bacteremia, pneumonia, eye and ear infections (Lan & Sandip, 2012, Odeh et al., 2016). Escherichia coli is a gram-negative bacteria found in the intestines of warm-blooded mammals. Although certain strains of E. coli are safe to humans and other animals, they have been linked to pneumonia, urinary tract infections, and diarrhoea in humans (Allocate et al*.,* 2013). According to Ahmed et al., (2012), antimicrobials' mode of action may include interfering with the build-up of cellular walls, engendering damage which could lead to changed cell permeability features or remodelled lipoprotein arrangements, eventually ensuing in cell death, and inactivation of several cellular enzymes that play key roles in the cell cycle(Dzubak et al., 2006, Achika et al., 2020).

Conclusion

The structure of the compound was elucidated based on spectroscopic analysis and comparison with existing literature. Melting point determination was a viable physical method employed in the study. The molecule that was isolated, described, and identified is a terpene with antifeedant and anti-inflammatory properties. It has been discovered to have hepatoprotective properties. The isolated compound was subjected to bioassay to ascertain its usefulness in trado-medical claims. It was confirmed that the plant under investigation as well as the isolated compound could serve as a useful precursor or as candidate for drugs to combat bacterial and fungal caused diseases with special reference to the microorganisms used in this study.

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