

Isolation, Antimicrobial and Anticancer Activity of Compounds from *Ficus Platyphylla Del.* Stem Bark

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Abstract

Ficus platyphylla Del commonly known as Gamji in Hausa is a medicinal plant used in Northern Nigeria. This research aimed at investigating the medicinal efficacy of stem bark of *F. platyphylla* in an attempt to isolate the possible bioactive compound(s). Thus, the powdered stem bark was dissolved in methanol for three days with constant stirring to afford a crude extract. This was fractionated by increasing the polarity of solvents. Preliminary phytochemical screening of the fractions using standard procedures was carried out. This was followed by the determination of zones of inhibition of growth of some bacterial and fungal species using agar diffusion method. The most active fractions were evaluated for minimum bactericidal concentration, antifungal activity. Anticancer activity of the fractions was tested against Huh7it cell. Gravity column chromatography was performed on ethyl acetate fraction using gradient solvent elution methods. The results of the investigation showed that there were some secondary metabolites in the fractions. Among microorganisms tested, aqueous-methanol fractions exhibited the highest zone of inhibition against *S. aureus*. The lower MIC value (125 mg/mL) was exhibited by methanol fractions. The fractions show no zone of inhibition against any fungal species. The anticancer activity showed that aqueous methanol fraction gave negative values implies proliferations of the cancer cells. Column chromatography led to the isolation of 13,27-cycloursan-3-ol, acetate, which was characterize using FTIR, ¹HNMR and ¹³CNMR .

Keywords: Anticancer activity, antimicrobial screening, characterization, fractions, stem bark

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INTRODUCTION

Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Revathilakshmi, 2019). Herbal medicines are one type of complementary and alternative medicine whose use has dramatically increased over the past decade, growing from 2.5 - 12% in community survey (Carlson *et al.*, 2023). The most recent figure from a 2002 community survey is 14% (Siegel *et al.*, 2019). The phenolic compound is soluble in water and includes phenols, phenolic acids, phenyl coumarins, phenyl propenes, flavonoid pigments, anthocyanins, flavanols, flavones and tannins (Divya, 2017).

It has been discovered that the plant *Ficus platyphylla* which is employed in Togo and Nigerian traditional medicines possess antibacterial and wound healing qualities. The plant's stem bark is rich in phytochemicals that support its antioxidant qualities, including saponins, flavonoids, tannins, and alkaloids. In previous work it showed that the fractions of *Ficus platyphylla* exhibited negative suppression of cell growth, according to a study on the subject. Compared to miconazole, the n-butanol fraction showed 90% reduction of antifungal activity against *Fusarium lini*. Nevertheless, none of the three fractions examined showed any signs of cytotoxic activity. Further chemical separation is required to fully comprehend the potential advantages of the plant by (Shina *et al.*, 2024).

A number of microorganisms, including, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pyogene*, and *Candida albican*, have been reported. Paper disc and agar well diffusion were the techniques employed (Mittal *et al.*, 2020). At the concentrations used (100, 200, and 400 mg/mL), *K. pneumoniae*, *P. aeruginosa*, and *B. subtilis* growth were all significantly inhibited by the ethanol extract., whereas the aqueous leaf extracts in both methods showed no antibacterial and antifungal activity against the tested organisms (Mittal *et al.*, 2020). In the paper disc method, the ethanol extract's minimum inhibitory concentration (MIC) ranges from 125 to 100 mg/mL for *P. aeruginosa*, *B. subtilis*, and *K. pneumoniae*, respectively. This demonstrated that ethanol was a more effective extractive solvent for the antimicrobial active components of the plant's leaves, indicating that this could be a great tool for the development of medications that could be used to treat a variety of illnesses (Mohammed *et al.*, 2015). When the phytochemical and antimicrobial properties of *F. platyphylla* extracts in ethanol and water were examined for efficacy against *Salmonella spp.*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* according to Evbuomwan *et al.*, (2018). There were found the presence of tannins and saponins while the pathogens were found to be susceptible to the ethanol extract. The only bacteria that developed resistance to the water extract were *Staphylococcus aureus*. These bacterial species are known to cause infections of the skin, respiratory tract, urinary tract, and gastrointestinal tract (Mittal *et al.*, 2020: Paul *et al.*, 2020).

The novelty in this current research was to make an attempt to isolate the potential compounds present in the stem bark of the plant and determine their antimicrobial and anticancer activity respectively.

In 2012, there were 14 067 894 new cases of cancer and 8 201 575 deaths from cancer worldwide (Bray *et al.*, 2018). Of all the cases that occur, 75% of them are in men with an average age of 63 to 69 years (Hamilton *et al.*, 2013). About 52.3% of patients with liver cancer have had chronic HBV infection and 20% have had HCV infection (Fong and Tanabe, 2014). Hepatocarcinogenesis can develop into HCC (Fong and Tanabe, 2014). Other options

include percutaneous ethanol injection, radiofrequency ablation, and transarterial chemoembolization (Fong and Tanabe, 2014). Recent trials have been made to create effective chemotherapy drugs, but there are still problems in terms of selectivity and toxicity by Hamilton *et al.*, . (2013). Henceforth the study aimed was to evaluate the antimicrobial and anticancer efficacy of *Ficus platyphylla* stem bark.

MATERIAL AND METHOD

Plant Materials

Collection, Identification and preparation.

The material was collected from the University farm (Federal University Dutse, Jigawa State, Nigeria, on 15th March, 2021). The plant was authenticated and identified by Botany department, Faculty of Life Sciences, Federal University Dutse, Jigawa State, Nigeria. A voucher number FUD/BOT/15/2021 was deposited at the Herbarium for future use. The *F. platyphylla* was collected, washed, then air dried in the laboratory twenty one days. Then the pulverised stem bark was mechanically grinded into coarse powder with the aid of pestle and mortal. One (1.0 kg) of the coarse powder sample was weighed and transferred into a container. 2 L of Methanol (95%) was transfer to the powdered sample, later dissolve for three days at room temperature with constant stirring and extract was then filtered using a clean fine cloth. The filtrate was evaporated using rotary evaporator (Gee and Bauder, 2018) and the weight of the crude extract was recorded.

Fractionation

Fractionation of crude methanol extract was done using modified 'Kupchan method of solvent-solvent partitioning as described by Qureshi *et al.*, (2019). The extract (500 g) of the stem-bark was redissolved with water. Then was fractionated with the aid of separating funnel using solvents of different polarity. All three fractions were evaporated with help of a rotary evaporator at reduced pressure the remaining aqueous fraction of methanol was then evaporated to yield residue. The aqueous methanol and butanol residue obtained was used for the qualitative analyses, antimicrobial and anticancer activity respectively.

Qualitative Phytochemical Analysis

The phytochemical constituents of *Ficus platyphylla* stem bark (FPSB) fractions were screened qualitatively using methods described by Sofowora, 1993.

Isolation of Compounds

Gravity Column Chromatography

A wet packed was performed in a glass column (30 x35 cm) using n-hexane. Then ethyl fraction (3.0 g) was mixed with silica gel (60 – 230 mesh) then loaded onto column. The column was further packed with n-hexane and allowed to stabilize for four hours. Different fractions (10 cm³ each) were collected and combined based on the TLC profile (n-hexane/ ethyl acetate in 7:3). Fractions with similar colours were pooled together and concentrated in vacuo to give one fraction. Microcolumn was used to purify the isolated compound coded FSP1a (35mg).

General and Spectroscopic Analysis

The isolated compound was subjected to spectroscopic analyses.

Nuclear Magnetic Resonance (NMR)

The isolated compound (10 mg) was dissolved in 0.5 ml and then subjected to 1D and 2D NMR analyses. All NMR spectra were obtained using pulsed Fourier transform methods on an Avance III HD Bruker 16.4 Tesla 700 MHz spectrometer (BioSpin GmbH). Deuterium lock signals were obtained by using deuterated solvent (CDCl_3) (Cambridge Isotope Laboratories, Inc). Measurements were made at ambient probe temperature 296 K in 5 mm spinning tubes (Wilmad-541-PP-7). The coupling constants (J values) and chemical shift (δ) was measured in parts per million, (ppm) and measured relative to 85% tetramethylsilane (TMS) external standard. ^1H NMR at 700.13 MHz spectra were obtained using a 8.00 μs pulse, ^{13}C NMR at 176.07 MHz spectra were obtained using a 12.00 s pulse.

Antibacterial and Antifungal Assays

All media used include nutrient agar, Sabouraud dextrose agar, nutrient broth and Sabouraud dextrose broth.

Bacterial and Fungal species

The following organisms used include *Pseudomonas aeruginosa*, *Escherichia coli* and *Citrobacter*, *Staphylococcus aureus*, *Salmonella typhi*, while fungal species used are *Candida albicans*, *Aspergillus niger*, and *Aspergillus flavus* were clinical isolates obtained from patients with different ailments. These bacterial species and the fungal species were screened using standard procedures.

Preparation of Stock Solution

About 4 mg of each fraction was weighed and dissolved in 1 mL dimethylsulfoxide (DMSO) in a sterilized bijou bottles and the mixture was shaken thoroughly to dissolve the solution giving a concentration of 4000 mg/mL. The concentrations were prepared by serial doubling dilution. 0.5 mL of each of the various stock solutions was weighed and mixed with 0.5 mL of DMSO to produce various concentrations 1000 mg/mL, 500 mg/mL, 125 mg/mL and 125 mg/mL respectively. The standard control (Ciprofloxacin and Nystatin) for bacteria and fungi were also prepared by dissolving 0.5 mL of each drug in 0.5 mL distilled water (Garba *et al.*, 2019).

Determination of Zone of Inhibition of Bacterial and Fungal Growth

The turbidity of the suspension was matched with that of 0.5 McFarland (Paul *et al.*, 2020). Standard inoculation of each isolate was swabbed onto the surface of nutrient agar in separate petri dishes of the extract and standard antibiotic placed. Five wells were bored in each plate by using 6 mm in diameter sterile cork borer, an aliquot of 100 mg/mL of the various concentrations of the extracts was added into the wells using dropper. The plates were inverted and allowed to stand for 30 mins for the fractions to diffuse into the agar after which the plates were incubated aerobically at 35°C for 18 hours. This was followed by measurement of zone of inhibition of extract and the standard antibiotic against the test organisms in mm by the use of transparent ruler.

Determination of Minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations (MIC) were investigated for the fractions using nutrient broth dilution method in accordance with (Clinical and Laboratory Standard Institute, 2021). Nutrient broth was prepared. The McFarland turbidity scale was prepared to give turbid solution. Later Normal saline was prepared and dispensed into test tubes and the microorganisms were inoculated and incubated at 37°C. Dilution of the test microorganisms in the normal saline was performed until the turbidity matched that of the McFarland's scale by visual comparison. Two-fold serial dilutions of the fractions / extracts in the broth were

performed to obtain the concentration of 1000mg/mL, 500mg/mL, 250mg/mL and 125 mg/mL for the fractions. 0.1 mL of the standard inoculum of the test microorganism in the normal saline was then inoculated into the different concentrations of the fractions. Incubation was made for 24 hours at 37°C after which each broth was observed for turbidity. The lowest concentration of the fractions in the broth which showed no turbidity was recorded as the minimum inhibitory concentration (MIC) (Garba *et al.*, 2019).

Determination of Minimum bactericidal concentration(MBC).

The minimum bactericidal concentration / minimum fungicidal concentration (MBC) was determined according to Naji *et al.* (2018). The solution of the MIC in the serial dilution was sub- cultured onto the prepared medium and incubation done at 37°C for 24 hours. Each plate of the medium was observed for colony growth. The values obtained in the plate with lowest concentration of the extracts without colony growth was recorded as the MBC (Garba *et al.*, 2019).

Anticancer Activity

Huh7it cells were cultured in petri dishes on DMEM (Invitrogen, Carlsbad, CA, USA) with 10% addition of FBS (Biowest, Nuaille, France), kanamycin 150 µg/mL (Sigma-Aldrich), and nonessential amino acids (Invitrogen).

Cancer Cell Proliferation Analysis

The test was carried out using an MTT assay (Sigma-Aldrich). A Huh7it cell was cultured on 96-well plates with a density of 2.4×10^4 per well and incubated at 37°C for 24 hours. After incubation, 100 µL of *F.platyphylla* was added in each well and diluted with DMSO with concentrations of 2000, 1000, 800, 400, 200, 100, 50, 25, 12, 5, 6, and 3 µg/mL. later, incubated at temperature of 37°C for two hours. While a positive control group was given doxorubicin (10 µg/mL) and IC₅₀ value was adopted. Medium was discarded after incubation and 150 µL of new medium containing 0.5 mg/mL MTT solution concentration in DMEM were added to each well. Then, the plate was incubated at 37°C for 4 hours until a formazan crystal formed. Then the cells was observed with an inverted microscope, if formazan was clearly formed, the medium was discarded and 100 µL DMSO was added to dissolve the precipitate formed from the MTT reaction. Absorbance was measured at a wavelength of 560 nm and 750 nm using GloMax-Multi Microplate Multimode Reader (Promega) by Purnamasari *et al.* (2019).

Apoptosis and Necrosis Analysis

Ficus platyphylla stem bark was added at concentrations of 400, 600, 800, 1000 µg/mL were placed on 6-well plates and incubated at 37°C overnight. . The plate was then incubated for two days at 37°C. Cells were collected by adding trypsin EDTA. All aspirates were placed on the microtube and centrifuged 13 000 rpm for 5 minutes at 4°C and washed with cold PBS. The cells were harvested and then centrifuged at speeds of 2500 rpm, for 10°C for 5 minutes. The supernatant was removed and the pellet was washed with PBS 1 mL and centrifuged at a speed of 2500 rpm, 10°C for 5 minutes. Pellets were added with Annexin-PI in PBS (50 µL) and incubated for 30 minutes (Purnamasari *et al.*, 2019).

Results

The preliminary phytochemical constituent analyses of the *F.platyphylla* fractions of stem bark are presented in Table 1.

Table 1: Phytochemical constituents of *F. platyphylla* stem bark fractions

Phytochemicals	Aqueous methanol Fraction	Ethylacetate Fraction	n-butanol Fraction	n-hexane
Alkaloids	-	-	-	-
Tannins	+	-	-	-
Saponins	+	+	+	-
Volatile oils	-	+	-	+
Terpenoids	+	+	+	+
Glycoside	+	+	+	-
Anthraquinones	-	-	-	-
Flavonoids	+	-	-	-
Resins	+	+	+	-

- = absent + = present

Table 2: Antimicrobial activity of *F. platyphylla* Stem bark against selected Bacteria and fungi

Microorganism	Fraction/Conc (mg/mL) n-hexane				Zone of Inhibition (mm) A.ME				ETA				BUT				CP	NTS
	1000	500	250	125	1000	500	250	125	1000	500	250	125	1000	500	250	125	74	NA
<i>Citrob</i>	0	0	0	0	22	20	17	0	0	0	0	0	0	0	0	0	44	NA
<i>P. A</i>	0	0	0	0	33	30	29	23	0	0	0	0	0	0	0	0	44	NA
<i>E. coli</i>	0	0	0	0	20	18	12	0	20	18	16	14	20	16	10	8	44	NA
<i>S. typhi</i>	0	0	0	0	20	18	17	0	20	18	16	14	20	16	10	8	44	NA
<i>S. a.</i>	0	0	0	0	40	32	24	22	35	30	26	23	36	31	21	1	44	NA
<i>A.niger</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
<i>A.Flav</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
<i>Candida bica</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	34

Key: CP= Ciprofloxacin, NTS=Nystatin, N H = N. hexane, A.ME= aqueous methanol, ETA=ethylacetate, BUT=butanol NA= no activity. P. A= *P.aeruginosa*, E= *Escherichia coli*, *Citrob.*=*Citrobater*, *S,a* = *Staphylococcus aureus*, *A.n*=*Aspergillus niger*, *A.F* = *Aspergillus flavus*, *candida albican*,

Table 3: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the stem bark of *F. platyphylla* del. fraction.

BACTERIAL/FUNGAL	ETA (mg/ml)		Aq. MT (mg/ml)		BUT (mg/ml)	
	MIC	MBC	MIC	MBC	MIC	MBC
<i>Citrobacter</i>	-	-	125	250	-	-
<i>P. aeruginosa</i>	-	-	125	250	-	-
<i>Staphylococcus aureus</i>	125	500	250	500	-	-
<i>E. coli</i>	125	500	500	1000	-	-
<i>Salmonella typhi</i>	-	-	250	500	-	-
<i>Apergillus niger</i>	-	-	-	-	-	-
<i>Apergillus flavus</i>	-	-	-	-	-	-
<i>Candida albican</i>	-	-	-	-	-	-

KEYS: N. H = N. Hexane, ETA= Ethylacetate fraction, Aq. MT= Aqueous Methanol fraction, BUT= Butanol Fraction, P= pseudomonas, E= *Escherichia*. MIC= Minimum inhibitory concentration
MBC= Minimum Bactericidal Concentration - = No Activity

Table 4. Anticancer Activity of stem bark *F.platyphylla*

Fractions	Concentration (ug/ml)	% Inhibition/Stimulation	IC ₅₀ +SD
Ethylacetate	30	-22.7	Inactive
Aqueous Methanol	30	-33.4	Inactive
N- Butanol	30	-7.6	Inactive
Doxarucin	30	101.21	0.9+-0.14

- = Negative values exhibit proliferation affects. SD= Standard deviation

Table 5 Infrared (IR) Spectra of compound 1

Functional group	Frequency (cm ⁻¹) Literature	Frequency (cm ⁻¹) Observed	Intensity
Alcohol (-OH)	3380-3650	3290	Strong
Alkane (sp ³ C-H)	2937	293.5	Strong
Alkanes CH ₂ CH ₃	2950-2975	2921	Strong
Alkanes CH ₂ CH ₃	2850-3000	2868	Strong
Alkenes CH=CH ₂	2200	2100.4	weak
Carbonyl(-C=O)	1636	1635.7	Strong
-C-O	1060	1057.7	Weak
Alkenes CH=CH	1660-1800	1682	Weak

Key; R- Range, IR- Infrared Spectroscopy

DISCUSSION

Phytochemicals Constituents

for the results revealed that aqueous methanol fraction contains saponins, volatile oils, terpenoids, glycosides, flavonoids, resins, steroids.

Meanwhile ethyl acetate fraction revealed the present of metabolite such as glycosides, tannins, saponins, terpenoids and steroids. While n-butanol fraction contains the presence of metabolite such as flavonoids, glycosides, saponins and terpenoids.

However, the n-hexane fraction revealed the presence of volatile oils, steroids and resins respectively. It is however reported that the presence of these secondary metabolite which include saponins, flavonoids, terpenoids and steroids could possess antimicrobial activities and anticancer properties (Hussein and El-Anssary (2019).

Antimicrobial activity of *F.platyphylla*

The antibacterial activity of *F.platyphylla* of aqueous methanol fraction showed the higher zone of inhibition of 40 mm against *Staphylococcus aureus* 1000 mg/mL, 500 mg/mL, 250 mg/mL and 125 g/mL. While it shows zone of inhibition against *E. coli* at 20mm, *Salmonella typhi* gave 20mm, while *Pseudomonas aeruginosa* revealed moderate inhibition and *citrobacter* revealed moderate inhibition while the control -the standard drug Ciproflaxin revealed 44 mm. this result implied that aqueous methanol fraction of stem bark of *F.platyphylla*.

Meanwhile, ethyl acetate fraction gave zone of inhibition against *Staphylococcus aureus* and *E. coli* revealed moderate inhibition. While butanol fraction revealed active zone of inhibition against *Staphylococcus aureus* and *E. coli* values ranges from 36 to 8mm-but n-hexane fraction revealed no zone of inhibition or inactive against the organism. This was compared with work reported by Truchan *et al.*, 2015 that the ethanol extract at different concentrations (25, 50 and 75 mg/ml) of roots showed moderate anti-bacterial activity against *S. aureus*, *P. aeruginosa*

and *K. pneumonia* respectively. While MIC and MBC of ethyl acetate fractions gave 125 and 500 mg/mL respectively against *Staphylococcus aureus* and *E. coli* respectively.

The MIC and MBC of aqueous methanol fraction gave 125 and 250 µg/mL against *Citrobacter*, and *P. aeruginosa*, while the MIC and MBC gave 250 and 500 µg/mL against *Salmonella typhi* and *Staphylococcus aureus* and while it gave 500 and 1000 µg/mL against *E. coli*. According to Roy *et al.* (2019), agar well diffusion method allows better diffusion of the extracts into the medium thus enhancing contact with the organisms.

The MIC showed that low concentrations of 12.5mg/mL and 25mg/mL of the plant extract, in disc method, can inhibit the growth of *P. aeruginosa* and *B. subtilis* respectively as described by Evbuomwan *et al.* (2018), and at as high concentration as 100mg/mL, *K. pneumoniae* can be inhibited by the plant extract. The MIC showed that the concentration of 6.3mg/mL and 12.5mg/mL of the extract, in well diffusion method, can inhibit the growth of *P. aeruginosa* and *B. subtilis* respectively, but *K. pneumoniae* can be inhibited at concentrations of 50mg/mL. The MBC of concentrations of 12.5 mg/mL, 50 mg/mL, and 100mg/mL of the plant extract showed that the plant extract, were bactericidal against *K. pneumoniae*, *P. aeruginosa* and *B. subtilis* at the respective concentrations.

Anticancer Activity of various fractions of *F. platyphylla* Del stem bark.

The results of the anticancer activity revealed that aqueous methanol fraction gave -36.30 µg/mL, ethyl acetate fraction is -26.2 µg/mL and n-butanol fraction gave -12.6 µg/mL respectively. This implies the negative values exhibit proliferative effect by the fractions. Indicate LC50 were all inactive to the MTT Helas cell.

CONCLUSION

The antimicrobial analyses of *F. platyphylla* Del stem bark fractions, however, have revealed that it has antibacterial potential against *Staphylococcus aureus*, *Salmonella typhi* and *Citrobacter*. Moreover it has revealed the isolation of the proposed Compound 13, 27-cycloursan-3-ol, acetate. This further supported the claims made for some of the plant's traditional medicinal uses. These may be especially interesting for determining its undiscovered efficacy and may be a source of chemically and pharmacologically significant drug development candidates.

Acknowledgements

I want to thank the International Center for Chemical and Biological Sciences (ICCBS), the H.E.J. Research Institute of Chemistry, and the DR Panjwani Center for Molecular Medicine and Drug Research Karachi, Pakistan for hosting me and enable me to carried out this research in order to use Nuclear Magnetic Resonance (NMR) Facility, I want to express my gratitude to the Center's director, Prof. M. I. Choudhary and other Faculty Staff
Funding: None.

Conflict of Interest:

All the authors declared that there is no conflict of interest

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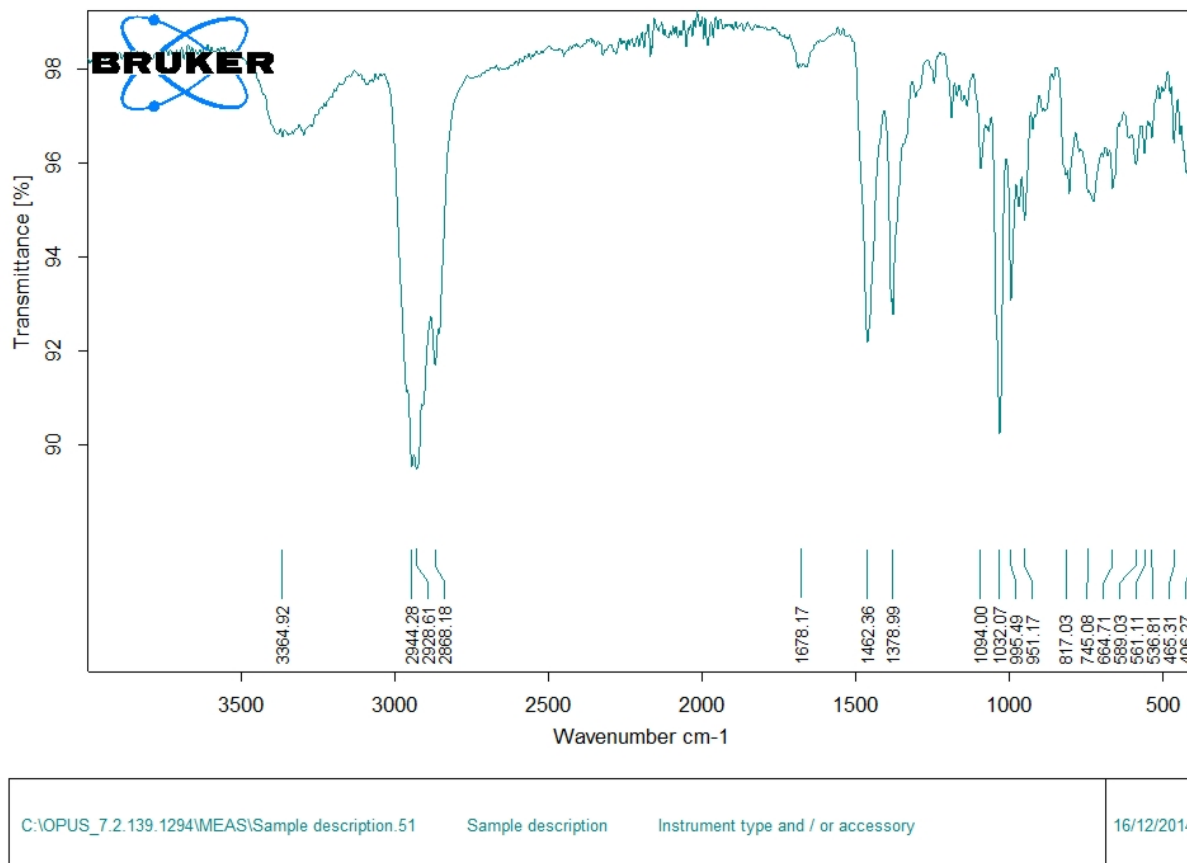


Figure 1.0: IR Spectrum of comp I

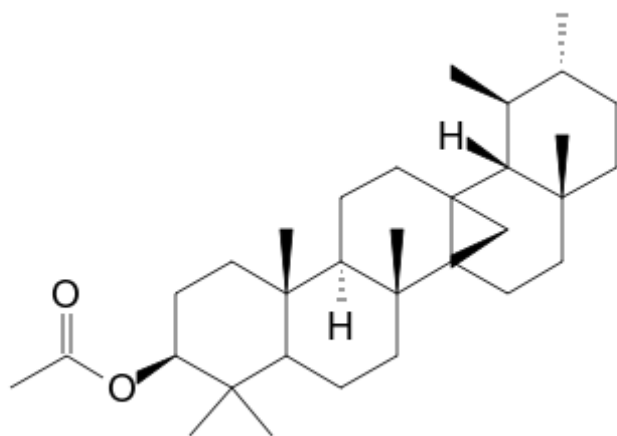
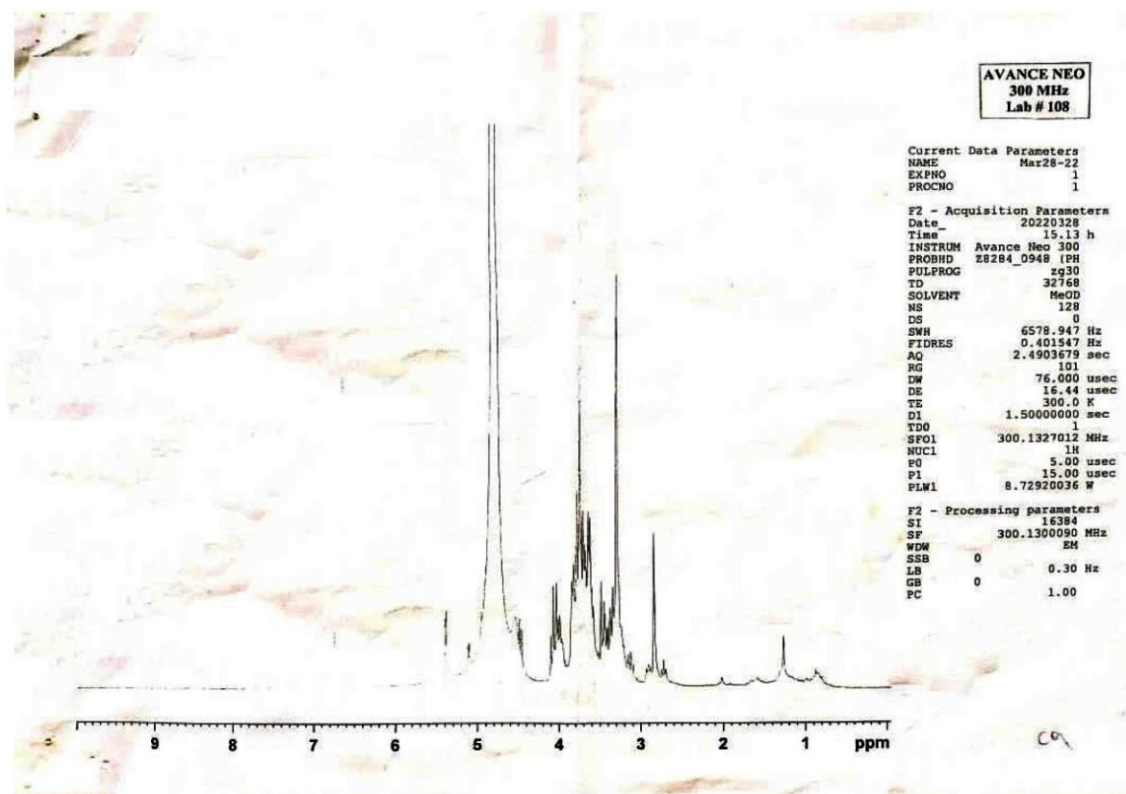


Figure 2.0: Propose structure of Compound - 13,27-Cycloursan-3-ol,acetate.



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Figure 3.0: Structure for proton NMR

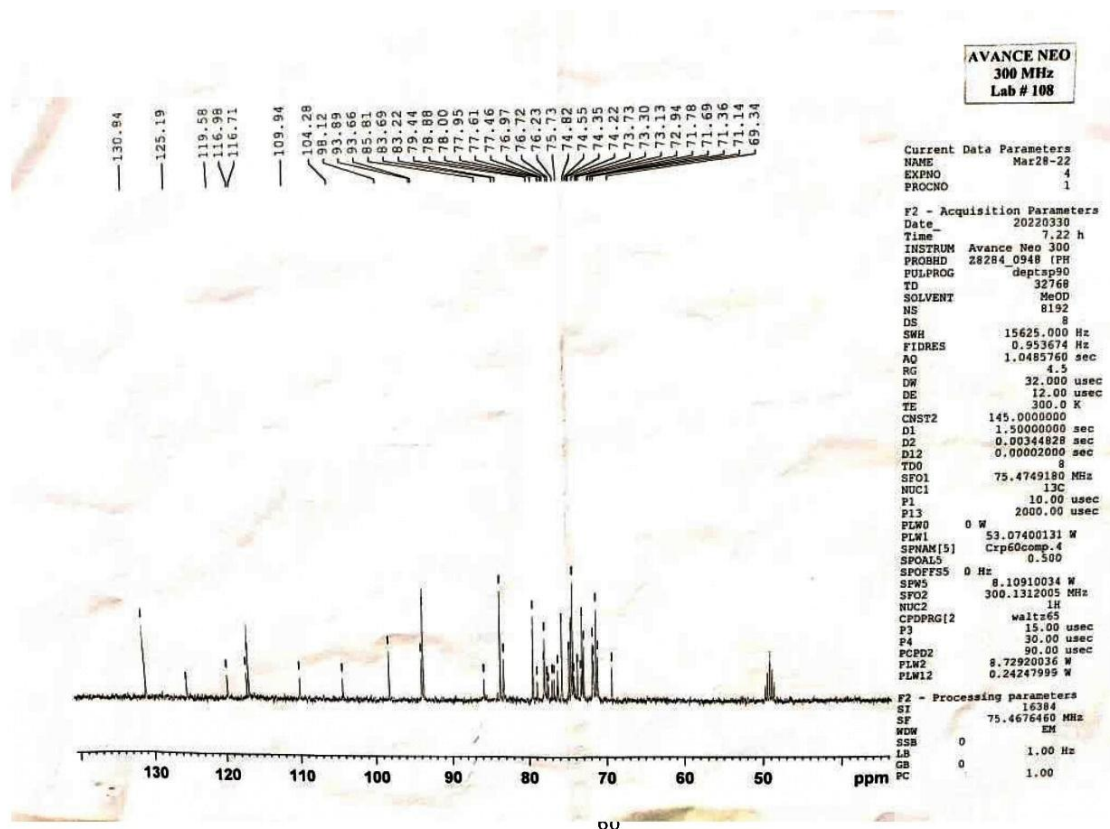


Figure 4.0: Carbon NMR