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# Oleanolic Acid Isolated from Ethyl Acetate Leaf Extract of *Cissus populnea* and the Antimicrobial Activities

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## ABSTRACT

The leaves of *Cissus populnea* were harvested, washed, air-dried under shade, ground into powder and were extracted using ethyl acetate. Column chromatography was used to fractionate the ethyl extract, and thin layer chromatography (TLC) was used to monitor the fractions. All isolates of *Cissus populnea* leaf inhibited or exhibited antibacterial and antifungal activities against tested microbes, with the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Cissus populnea* fractions against tested microbes or organisms ranging from 6.25 to 100g/ml. Using Proton Nuclear Magnetic Resonance (1H-NMR) spectroscopy, the fraction was studied. Oleanolic acid was discovered using nuclear magnetic resonance analysis. The plant's component is used locally for therapeutic purposes, and the extracts and separated compounds' bioactive components, antibacterial activities confirm *Cissus populnea* status as a medicinal plant.

**Keywords:** *Cissus populnea*, ethyl acetate, Proton Nuclear Magnetic Resonance, Oleanolic acid, antibacterial and antifungal activities.

## INTRODUCTION

Most plant parts contain important active compounds. The main source of pharmaceuticals and healthcare products are mostly medicinal plants [1] and the active components of this plant lead to the development of high activity profile drugs [2]. The treatment of ailments and diseases in villages and local communities has been possible with the help of medicinal plants such as leaves, stems and roots of plants. These medicinal plants are used by mostly traditional medicine practitioners, and livestock owners in many African countries to cure ailments in man and animals [3]. The traditional knowledge of medicinal plants however needs extensive improvement as these plants have potentials of replacing conventional drugs and being used in modern drug development [4]. Medicinal plants can serve multiple purposes in the treatment of

ailments. For example, a plant can be used to treat cancer and in the same vein can be used to treat ulcer. The same plant may also find useful application in herbal preparations for a synergistic effect [5].

The medicinal ability of the plant is due to the presence of phytochemicals and diversities of bioactivities in medicinal plants [6]. The plant kingdom still holds many species of plants containing substances of medicinal value which are yet to be discovered. *Cissus populnea* which is a savannah shrub, climber and three meters tall or more depending on the supporting tree is one of the plants used in traditional medicine over decades.

The present work is to isolate Oleanolic acid from the ethyl acetate leaf extract of *Cissus populnea*, to determine the antimicrobial activity of the isolate against some microorganisms including bacteria and fungi and is designed to enrich the available scientific data on efficacy of *Cissus populnea*. Overall, the study aims to investigate the potential of Oleanolic acid as a natural antimicrobial agent and to contribute to the development of new antimicrobial drugs.

## MATERIALS AND METHODS

### Collection of Sample and Preparation

The leaves of *Cissus populnea* were collected from its natural habitat in Takum Local Government Area of Taraba State, Nigeria. The samples were collected and washed thoroughly in running tap water and then with distilled water. The leaves were cut into small pieces and dried for two weeks under shade. After drying the plant material was pounded using mortar and pestle into fine powder. Then the powders were stored in airtight container with proper labeling and were kept in the laboratory for use.

### Extraction

The method of extraction of bioactive compounds was carried out as described by Ipav *et al* [7] with slight modifications. The leaves (234 g) were macerated in n-hexane See title (2.5 L) using a Winchester bottle for 48 h. The extract was filtered into a glass jar using a Whatman number 1 filter paper. The extract was concentrated *in vacuo* on a rotary evaporator. The concentrate was air-dried in a fume hood until all trace of solvent was removed. The hexane extract (3.5 g) was, however, rather oily and was therefore discontinued. The marc was aired to remove solvent traces prior to extraction with ethyl acetate. The extract was designated CSL (*Cissus populnea*

leaf) and set aside for column chromatography. The marc was aired to remove solvent traces prior to extraction with methanol.

**Isolation of Compounds**

The method of isolation of compounds was carried out as described by Ipav *et al* [7] and Bako *et al* [8] with slight modifications. Dried extract of the sample was extracted with ethyl acetate. The extracts were combined (based on similarity on TLC) and subjected to column chromatography using silica gel in a glass column. The column was packed wet in a hexane: ethyl acetate (90:10) mixture and eluted with ethyl acetate in hexane gradient starting with 10% ethyl acetate in hexane and increasing the amount of ethyl acetate by 10% until 100% ethyl acetate yielding 10 ml vials. Further elution with ethanol in ethyl acetate starting with ethyl acetate: ethanol (90:10) mixture. A slurry of silica gel was prepared thus: Silica gel (50 g) was dispersed into hexane (200 mL) and stirred with a glass rod. The slurry was introduced into a glass column (4.5 cm by 47 cm). The column was gradiently ran [Hex (95%) in Ethyl-acetate →Hex (50%)]. (200 mL) was used for the column. Fractions (95 of 20 mL each) were collected. The fractions were designated CPL1- CPL-96 and subjected to Thin Layer Chromatography (TLC) and similar fractions were pooled together. Fraction CPL-38 (CPL-38.10 fid) gave a crystal orange This was stored at room temperature for spectroscopic analysis. The fraction CPL-38.10.fid was characterized using proton Nuclear Magnetic Resonance ($^1$H-NMR). Nuclear magnetic resonance (NMR) analyses were carried out on a Bruker AVIII (500 MHz) spectrophotometer using $CDCl_3$ as the solvent and TMS as the internal standard. MestReNova® and Chemsketch® (ACDLABs) software were used in elucidating signal properties [2D NMR correlations were illustrated using the ACD labs ChemSketch (2015) software].

**Antimicrobial Activities of the Isolates**

The bioassay was carried out by methods described by Ushie *et al* [9]. The antimicrobial activities of CPL 2 compound was determined using some pathogenic microbes, the microbes were obtained from the Department of Medical Microbiology, ABU Teaching Hospital, Zaria. The test organisms were collected from the Department of Medical Microbiology, ABU Teaching Hospital Zaria. *Cissus populnea* leaves and stem extracts was tested against eleven (11) test organisms such as *Methicillin resist Staph aureus, Vancomycin resistant enterococci,*

*Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Helicobacter pylori, Campylobacter fetus, Proteus mirabilis, Pseudomonas aeruginosa, Candida tropicalis, and Candida krusei.*

Given amount of 0.001 mg of the compound was weighed and dissolved in 10 ml of DMSO to obtain a concentration of 100 µg/ml. This was the initial concentration of the compound used to determine its antimicrobial activities. Diffusion method was the method used for screening the compound. Mueller Hinton agar was the medium used as the growth medium for the microbes.

The medium was prepared according to the manufacturer instructions sterilized at 121 ºC for 15 min, poured into the sterile petri dishes and was allowed to cool and solidify. The sterilized medium was seeded with 0.1 ml of the standard inoculum of the test microbe, the inoculums was spread evenly over the surface of the medium by the used of sterile swab. By the use of a standard cork borer of 6mm in diameter a well was cut at the center of each inoculated medium. 0.1 ml of solution of the compound of the concentration of 100 µg/ml was then introduced into the well on the inoculated medium. Incubation was made at 37 $^0$C for 24 h, after which the plates of the medium were observed for the zone of inhibition of growth, the zone was measured with a transparent ruler and the result recorded in millimeter.

**Minimum Inhibitory Concentration (MIC) Assay**

The minimum inhibition concentration of the compound was determined using the broth dilution method as described by Adamu *et al* [10] and Ushie *et al* [11]. Mueller Hinton broth was prepared, 10 ml was dispensed into test tubes and was sterilized at 121 $^0$C for 15 min. The broths were allowed to cool. MC-farland's turbidity standard scale number 0.5 was prepared to give solution. Normal saline was prepared, 10 ml was dispensed into sterile test tube and the test microbe was inoculated and incubated at 37 $^0$C for 6 h. Dilution of the microbe was done in the normal saline until the turbidity marched that of the MC-farland's scale by visual comparison at this point the test microbe has a concentration of about $1.5X10^8$c/ml.

Two-fold serial dilution of the compound was done in the sterile broth to obtain the concentrations of 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml. The initial concentration was obtained by dissolving 0.001 mg of the compound in 10 ml of the sterile broth. Having obtained the different concentrations of the compound in the sterile broth, 0.1 ml

of the test microbe in the normal saline was then inoculated into the different concentrations, incubation was made at 37 $^0$C for 24 h, after which the test tubes of the broth were observed for turbidity (growth) the lowest concentration of the compound in the sterile broth which shows no turbidity was recorded as the minimum inhibition concentration.

**Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)**

MBC/MFC was carried out to determine whether the test microbes were killed or only their growth was inhibited as described by Adamu *et al* [10] and Ushie *et al* [11]. Mueller Hinton agar was prepared sterilized at 121 $^o$C for 15 min, poured into sterile petri dishes and was allowed to cool and solidly. The contents of the MIC in the serial dilutions were then sub cultured onto the prepared medium, incubation was made at 37 $^0$C for 24 h, after which the plates of the medium were observed for colony growth. MBC/MFC were the plates with lowest concentration of the compound without colony growth.

## RESULTS AND DISCUSSION

**$^1$HNMR Spectra Data for Compound B**

1H NMR (400 MHz, Chloroform-*d*) δ 5.27 (m, 1H), 3.22 (dd, *J* = 11.2, 4.7 Hz, 1H), 2.82 (dd, *J* = 14.0, 4.6 Hz, 1H), 1.13 (s, 2H),0.98 (s, 3H), 0.93 (s, 2H), 0.90 (d, *J* = 1.2 Hz, 3H), 0.77 (s, 2H), 0.76 (s, 2H).

Table 1: $^1$HNMR for CPSF2 (Compound B) experimental, in comparison with data from previous literature.

| Position of Proton (H) | Experimental 1H Chemical shift δ (ppm), J (Hz) | |
|---|---|---|
| | | [12] |
| 1 | - | - |
| 2 | - | - |
| 3 | 3.22, dd | 4.49,m |
| 4 | - | - |
| 5 | - | - |
| 6 | - | - |
| 7 | - | - |

| | | |
|---|---|---|
| 8 | - | - |
| 9 | - | - |
| 11 | - | - |
| 12 | 5.27, t | 5.28,t |
| 13 | - | - |
| 14 | - | - |
| 15 | - | - |
| 16 | - | - |
| 17 | - | - |
| 18 | 2.82,dd | 2.82,dd |
| 19 | - | - |
| 20 | - | - |
| 21 | - | - |
| 22 | - | - |
| 23 | - | - |
| 24 | 0.77,s | 0.87,s |
| 25 | 0.98,s | 0.94,s |
| 26 | 0.76,s | 0.76,s |
| 27 | 1.13,s | 1.13,s |
| 28 | - | - |
| 29 | 0.93, s | 0.93,s |
| 30 | 0.90,s | 0.91,s |

Table 2: The Antimicrobial Activities of CPLF2 Compounds

| Test Organism | CPLF2 | Ciprofloxacin | Fluconazole |
|---|---|---|---|
| *Methicillin resist Staph aureus* | S | R | R |
| *Vancomycin resistant enterococci* | S | R | R |
| *Staphylococcus aureus* | S | S | R |
| *Escherichia coli* | R | S | R |
| *Klebsiella pneumonia* | S | R | R |
| *Helicobacter pylori* | R | S | R |

| | | | |
|---|---|---|---|
| *Campylobacter fetus* | S | R | R |
| *Proteus mirabilis* | R | S | R |
| *Pseudomonas aeruginosa* | S | R | R |
| *Candida tropicalis* | R | R | S |
| *Candida krusei* | S | R | S |

KEY: S = Sensitive R = Resistance

Table 3: Zone of Inhibition of the CPLF2 Compound against the Test Micro Organisms

| Test Organism | CPLF2 | Ciprofloxacin | Fluconazole |
|---|---|---|---|
| *Methicillin resist Staph aureus* | 24 | 0 | 0 |
| *Vancomycin resistant enterococci* | 27 | 0 | 0 |
| *Staphylococcus aureus* | 25 | 35 | 0 |
| *Escherichia coli* | 0 | 37 | 0 |
| *Klebsiell pneumonia* | 27 | 0 | 0 |
| *Helicobacter pylori* | 0 | 31 | 0 |
| *Campylobacter fetus* | 28 | 0 | 0 |
| *Proteus mirabilis* | 0 | 30 | 0 |
| *Pseudomonas aeruginosa* | 22 | 0 | 0 |
| *Candida tropicalis* | 0 | 0 | 32 |
| *Candida krusei* | 25 | 0 | 30 |

Table 4: Minimum Inhibitory Concentration of the Compound of *Cissus populnea* Leaf Isolates against the Test Microorganisms

| Test Organism | CPLF2 | | | | |
|---|---|---|---|---|---|
| | 100µg/ml | 50µg/ml | 25µg/ml | 12.5µg/ml | 6.25µg/ml |
| *Methicillin Resist Staph aureus* | - | - | 0* | + | ++ |
| *Vancomycin Resistant enterococci* | - | - | - | 0* | + |
| *Staphylococcus aureus* | - | - | 0* | + | ++ |
| *Escherichia coli* | | | | | |
| *Klebsiella pneumonia* | - | - | - | 0* | + |
| *Helicobacter pylori* | | | | | |
| *Campylobacter fetus* | - | - | - | 0* | + |
| *Proteus mirabilis* | | | | | |
| *Pseudomonas aeruginosa* | - | - | 0* | + | ++ |
| *Candida tropicalis* | | | | | |
| *Candida krusei* | - | - | 0* | + | ++ |

KEY: =>No turbidity (No growth), 0*=>MIC, +=>Turbid (light growth), ++=>Moderate turbidity.

Table 5: Minimum Bactericidal/Fungicidal Concentration of the Compound *Cissus populnea* Stem against the Test Microorganisms

| **Test Organism** | **CPLF2** | | | | |
|---|---|---|---|---|---|
| | 100µg/ml | 50µg/ml | 25µg/ml | 12.5µg/ml | 6.25µg/ml |
| *Methicillin Resist Staph aureus* | - | 0* | + | ++ | ++ |
| *Vancomycin Resistant enterococci* | - | 0* | + | ++ | ++ |
| *Staphylococcus aureus* | - | 0* | + | ++ | ++ |
| *Escherichia coli* | | | | | |
| *Klebsiella pneumonia* | - | 0* | + | ++ | ++ |
| *Helicobacter pylori* | | | | | |
| *Campylobacter fetus* | - | - | 0* | + | ++ |
| *Proteus mirabilis* | | | | | |
| *Pseudomonas aeruginosa* | 0* | + | + | + | ++ |
| *Candida tropicalis* | | | | | |
| *Candida Krusei* | - | 0* | + | ++ | ++ |

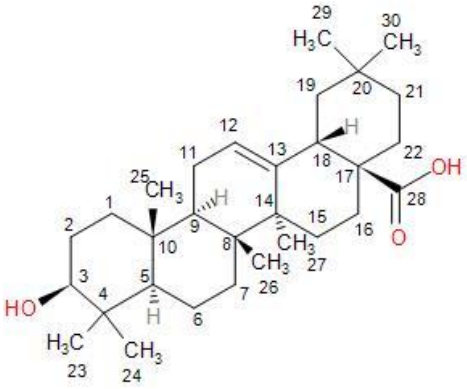
**KEY:** - => No Colony Growth, o*=>MBC/MFC, + =>Scanty colonies growth,++ => Moderate colonies growth, +++ =>Heavy colonies growth.

**Structural Elucidation**

Evidence from the 1H NMR (Table 1), there are seven six characteristic angular (tertiary) methyl groups but only six were detected, appearing as 3H singlets, at δ 0.77, 0.98, 0.76, 1.13, 0.93, and 0.90 (H-24, H-25, H-26, H-27, H-29 and H-30 respectively. The characteristic vinyl proton (H-12) is seen at δ 5.27 ppm while another characteristic signal, H-18 methine proton, appears at δ 2.82 ppm. These point to the presence of olean-12-ene skeleton [13]. The methine proton at H-3,

deshielded due to attached OH, has its signal moved downfield, as expected, to δ 3.22 ppm. These signals are consistent with those cited in literature for oleanolic acid [12]. Hence, Compound B is oleanolic acid [13].

Oleanolic acid, also known as oleanic acid with a molecular formula, $C_{30}H_{48}O_3$, is a pentacyclic triterpenoid that occurs naturally and is related to betulinic acid. It occurs as a free acid, as a conjugate acid of an oleanolate and as an aglycone of triterpenoid saponins in a variety of plant and food sources. [14]. This acid is a pentacyclic triterpenoid and plays a major role as a plant metabolite [15]. It has properties of both hydroxy monocarboxylic acid and pentacyclic triterpenoid. This compound reportedly demonstrated antiviral, anti-HIV, antidiabetic, antibacterial, antifungal, anticarcinogenic, anti-inflammatory, hepatoprotective effects [16]. Others include α-glucosidase inhibitory activity [17] and immunomodulatory activity [18]. Oleanolic acid has been used as a hepatic drug for over 20 years in China because of its hepatoprotective effect [19]. The investigation of the additional biological functions of OA and its synthetic derivatives may result in the creation of powerful pharmaceuticals for the management or treatment of human disorders.




**Oleanolic Acid**

**Antimicrobial Activity of the Isolates**

The results of the antimicrobial activity of isolates from *Cissus populnea* leaf fractions (CPLF2) are shown in Tables 2-5. The findings revealed that all the isolates of the leaf inhibited or exhibited antibacterial activities against *Methicillin Resist Staph aureus, Vancomycin Resistant enterococci, Staphylococcus aureus, Klebsiella pneumonia, Campylobacter fetus, Pseudomonas aeruginosa* and *Candida Krusei.* The *Cissus populnea* leaf isolates or fractions (CPLF1) were

active against seven clinical isolates; *Methicillin Resist Staph aureus*and *Campylobacter fetus* (28 mm zone diameter inhibition), *Staphylococcus aureus*and *Klebsiella pneumonia* (27 mm zone diameter inhibition), *Candida Krusei* (25 mm zone diameter inhibition), *Vancomycin Resistant enterococci* (23 mm zone diameter inhibition), and *Pseudomonas aeruginosa* (22 mm zone diameter inhibition) while for CPLF2 isolates were active against seven of the clinical isolates; *Campylobacter fetus* (28 mm zone diameter inhibition), *Klebsiella pneumonia* and *Vancomycin Resistant enterococci* (27 mm zone diameter inhibition),*Staphylococcus aureus* and *Candida Krusei* (25 mm zone diameter inhibition), *Methicillin Resist Staph aureus* (24 mm zone diameter inhibition), and *Pseudomonas aeruginosa* (22 mm zone diameter inhibition). CPLF2 isolates do not show any bacteria and fungi activities against *E. coli*, *Helicobacter pylori*, *Pseudomonas aeruginosa* and *Candida tropicalis.*

The MIC and MBC of the isolates ranged from 6.25–100 µg/ml. CPLF 2 isolates demonstrating the lowest values (MIC 12.5 µg/ml: MBC 25 µg/ml each) against *Methicillin Resist Staph aureus*, *Klebsiella pneumonia*, *Campylobacter fetus*: *Campylobacter fetus.* The activity of CPLF2 showed highest values (MIC 25 µg/ml) against *Methicillin Resist Staph aureus, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Candida krusei* : MBC (50 & 100 µg/ml) against all the test organisms except *Campylobacter fetus* which is the lowest values (25 µg/ml). From the result obtained, MIC values were lower than the MBC values indicating that the isolates could be bactericidal in action. Low MIC and MBC values are also an indication of high efficacy. The activities against both gram-negative and gram- positive bacterial and fungi is an indication that the plant can be a source of bioactive substances that could be of broad spectrum of activity. The fact that the plant *Cissus populnea* leaf was active against clinical isolates is also an indication that it can be a source of very potent antimicrobial substances that can be used against drug resistant microorganisms prevalent in hospital environment.

## CONCLUSION

In conclusion, the study showed that the ethyl acetate leaf extract of *Cissus populnea* contained oleanolic acid, which exhibited antimicrobial activity against only seven (7) of the tested microorganisms. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the isolated oleanolic acid were also determined. Despite the limited antimicrobial activity of the plant isolates, *Cissus populnea* has been traditionally used as a

medicinal plant. The established compound, oleanolic acid, has been reported to possess various pharmacological properties, including antimicrobial, anti-inflammatory, and antidiabetic activities. The antimicrobial activity of the isolates in this study supports the traditional use of *Cissus populnea* as a medicinal plant for the treatment of infectious diseases. Further research is needed to investigate the potential of oleanolic acid as a natural antimicrobial agent and to develop new antimicrobial drugs based on this compound.

## REFERENCES

[1] Tungmunnithum, D., Thongboonyou, A., Pholboon, A., & Yangsabai, A. (2018). Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines*, *5*(3), 93-105.

[2] Thapa, N., Thapa, P., Bhandari, J., Niraula, P., Shrestha, N., & Shrestha, B. G. (2016). Study of phytochemical, antioxidant and antimicrobial activity of Artocarpus heterophyllus. *Nepal Journal of Biotechnology*, 4(1), 47-53.

[3] Ozioma, E. O. J. & Chinwe, O. A. N. (2019). Herbal medicines in African traditional medicine. *Herbal medicine*, 10, 191-214.

[4] Dawurung, C.J., Jurbe, G.G., Usman, J.G., Elisha, I.L., Lombin, L.H. & Pyne, S.G. (2019). Antidiarrheal activity of some selected Nigerian plants used in traditional medicine. *Pharmacognosy Research*, 11(4), 371–7. https://doi.org/10.4103/pr.pr_43_19.

[5] Zhou, X., Seto, S. W., Chang, D., Kiat, H., Razmovski-Naumovski, V., & Chan, K. (2016). Synergistic effects of Chinese herbal medicine: A comprehensive review of methodology and current research. *Frontiers in Pharmacology*, 7, 201. doi:10.3389/fphar.2016.00201.

[6] Dawurung, C. J., Nguyen, M. T., Pengon, J., Dokladda, K., Bunyong, R., Rattanajak, R. & Pyne, S. G. (2021). Isolation of bioactive compounds from medicinal plants used in traditional medicine: Rautandiol B, a potential lead compound against Plasmodium falciparum. *BMC Complementary Medicine and Therapies*, *21*, 1-12.

[7] Ipav, S. S., Igoli, J. O., Tor-Anyiin, T. A., & Anyam, J. V. (2022). Isolation and Characterisation of Lupeol and Lupeol Acetate from Propolis Obtained from Benue State. *Journal of Chemical Society of Nigeria*, 47(1), 9-17.

[8] Bako, B., Ushie, O. A. & Malu, S. P. (2023). Lupeol and Lauric Acid Isolated from Ethyl Acetate Stem Extract of Justicia Secunda and their Antimicrobial Activity. *Journal of Chemical Society of Nigeria*, 48(1), 081 – 094.

[9] Ushie, O. A., Neji, P. A., Muktar, M., Ogah, E., Longbab, B. D., & Olumide, V. B. (2018). Estimation of some phytochemicals in Swietenia macrophylla leaves. *Journal of Pharmaceutical Research and Reviews*, 2(15), 1-7.

[10] Adamu, H.M., Ushie, O. A, D.S. Lawal & I. A. Oga (2013). Phytochemical Screening of the Fruit of the Azanza garckeana and the Root of Acacia Macrothyrsa. *International Journal of Traditional and Natural Medicines*, 3(1): 19-25.

[11] Ushie, O. A., Neji, P. A. Abeng, F. E., Okpashi, V. E. Baba, N. H. & T. I. Azuaga (2019). Phytochemical Screening and Antimicrobial Activities of Chloroform and Ethyl Acetate Extracts of Physalis angulata. *Journal of Chemical Society of Nigeria*, (44) 6, 1062 -1069.

[12] Endo, M., Shigetomi, K., Shinya, M., Makoto, I., & Makoto, U. (2019). Isolation, structure determination and structure–activity relationship of anti-toxoplasma triterpenoids from Quercus crispula Blume outer bark. *Journal of Wood Science*, 65(3), 189–196. https://doi.org/10.1007/s10086-018-1772-x

[13] Gohari, A. R., Saeidnia, S., Hadjiakhoondi, A., Abdoullahi, M., & Nezafati, M. (2009). Isolation and Quantificative Analysis of Oleanolic Acid from Satureja mutica Fisch.&C.A. Mey. *Journal of Medicinal Plants*, 8 (5), 65-69.

[14] Parikh, N., Chauhan, B., & Mashru, R. C. (2018). RP-HPLC method development and validation of simultaneous estimation of gallic acid and Oleanolic acid in antihyperlipidemic Polyherbal tablets. *Journal of Pharmacognosy and Phytochemistry*, *7*(5), 209-216.

[15] Cappiello, F., Loffredo, M. R., Del Plato, C., Cammarone, S., Casciaro, B., Quaglio, D., ... & Ghirga, F. (2020). The revaluation of plant-derived terpenes to fight antibiotic-resistant infections. *Antibiotics*, 9(6), 325-333.

[16] Castellano, J. M., Rames-Romero, S. R., & Peroma, J. S. (2022). Oleanolic acid: Extraction, Characterization and Biological activity. *Nutrients*, 14, 623-632.

[17] Eli, H. A., Nur, S. S. Z., Ali, A. & Syed, A. A. S. (2015): α-glucosidase activity of oleanolic acid and its oxidative metabolites: DFT and docking studies; *Mini Reviews in Medicinal Chemistry*, 15(4), 1148 – 1158.

[18] Harun, N. K., Septama, A. W., Ahmed, W. A. N. W. & Suppian, R. (2020): Immunomodulatory effects and structure-activity relationship of botanical pentacyclic triterpenes: A review, *Chinese Herbal Medicines*, 12(2), 118 – 124.

[19] Zhao, H., Zhou, M., Duan, L., Wang, W., Zhang, J., Wang, D., & Liang, X. (2013). Efficient synthesis and anti-fungal activity of oleanolic acid oxime esters. *Molecules*, *18*(3), 3615-3629.

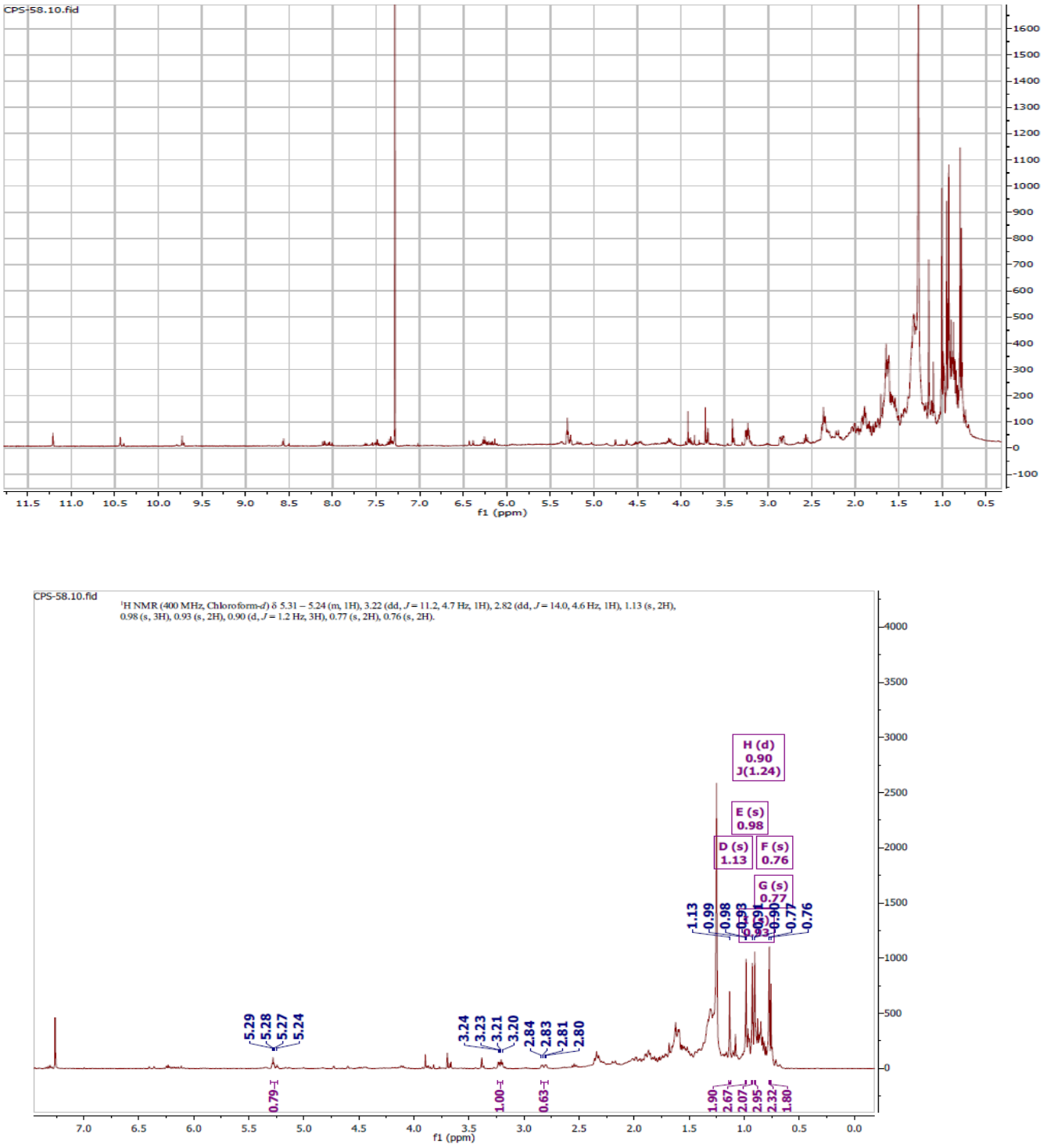



**Fig 1: $^{1}$H-NMR Spectra of CPS-58.10.fid**