ORIGINAL ARTICLE

Isolation, Quantitative Phytochemical Profiling, and NMR Spectroscopic Characterization of Lupeol Acetate from the Stem Bark of *Cissus populnea*

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ABSTRACT

Medicinal plants are one of the major plant sources for bioactive compounds which has a great therapeutic value. *Cissus populnea* is a medicinal plant, traditionally used in West Africa, but there is little information on phytochemicals and structural characterization of its bioactive metabolites. The aim of this study was to evaluate the quantitative phytochemical composition of stem bark of *Cissus populnea* in different solvents and isolate and characterize lupeol acetate by Nuclear Magnetic Resonance (NMR) spectroscopy. The powdered stem bark was sequentially extracted using hexane, ethyl acetate and methanol. The quantitative phytochemical analysis for phenolics, tannins, flavonoids, steroids, glycosides and terpenoids were performed using the standard spectrophotometric method. The bioactive compound was isolated by silica gel column chromatography and structural elucidation was accomplished by ^{1}H NMR, ^{13}C NMR, HSQC and HMBC spectroscopic methods. The results of the experiments were presented as mean ± standard deviation of three times determination and analyzed by one-way ANOVA at $P<0.05$. The methanol extract contained the highest amount of phenolics (0.513 ± 0.003), tannins (0.664 ± 0.003), flavonoids (0.822 ± 0.002) and terpenoids (0.165 ± 0.002); glycosides and steroids were the highest in the hexane and ethyl acetate extracts, respectively. The isolated compound was identified as lupeol acetate based on its diagnostic signals, which showed the presence of a lupane-type pentacyclic triterpenoid skeleton with an acetyl group.

Keywords: *Cissus populnea*, Lupeol acetate, Phytochemical profiling, Pentacyclic triterpenoids, NMR spectroscopy, Bioactive compounds.

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1.0 INTRODUCTION

Medicinal plants remain an important source of therapeutic agents and bioactive compounds in drug discovery and development (1). With the rise in chronic diseases, antimicrobial resistance, disorders associated with oxidative stress and inflammatory diseases, there has been increased interest in finding new phytochemicals with potent pharmacological activity from natural sources. Plant secondary metabolites like flavonoids, tannins, terpenoids, glycosides, alkaloids, steroids and phenolics have shown various biological activities such as antioxidant, anti-inflammatory, antimicrobial, antidiabetic, hepatoprotective and anticancer activities (1,2). As a result, phytochemical studies and characterization of bioactive compounds from medicinal plants continue to be the main objective of phytochemistry and natural product research.

Cissus L. (family *Vitaceae*) is an important genus of medicinal plants that is common in tropical and subtropical parts of Africa and Asia. Plants of this genus have long been used in ethnomedicine for treating various diseases such as inflammation, microbial infection, diabetes, gastrointestinal disorders, pain and bone related diseases (3). *Cissus populnea* is one of the least studied species in the genus and is traditionally employed in West African regions to treat various ailments; only a few studies have been conducted on them and their phytochemical composition and bioactive constituents are not well-known. Previous researches on related species has identified the presence of triterpenoids, flavonoids, stilbenes and phenolic compounds which have been reported to be linked to significant pharmacological properties (4,5). However, there is a lack of extensive phytochemical screening and spectroscopic identification of compounds of *Cissus populnea* stem bark.

A major problem in medicinal plant research is the poor level of characterization and validation of the bioactive principles that the medicinal claims of many indigenous plants attribute to. Even though some of the plants utilized in traditional medicine showed interesting pharmacological properties, it is impossible to standardize them, monitor their quality, and use them in preparation of drugs, due to insufficient phytochemical and structural elucidation studies (6). The literature available on *Cissus populnea* is inadequate in terms of isolation and spectroscopic identification of the secondary metabolites especially the triterpenoid components. This lack of knowledge will come in the way of scientific validation of the plant and also limit the chance of discovery of any new or important compounds of the plant that will aid in the development of new drugs.

Triterpenoids are significant class of naturally occurring compounds, well known for their therapeutic importance. Among these, lupeol and its derivatives, including lupeol acetate, have been the subject of significant scientific interest due to its wide range of biological activities including anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, anticancer and antidiabetic properties (7,8). Lupeol acetate is a pentacyclic triterpenoid compound identified in medicinal plants and shown to have good pharmacological potentials with relatively low toxicity. The effective spectroscopic characterization and structural elucidation of such compounds is critical for establishing their chemical identity and is necessary for further biological studies with the use of the advanced spectroscopic techniques such as one dimensional and two-dimensional nuclear magnetic resonance (1D and 2D NMR) spectroscopy.

In recent years, the use of modern spectroscopic techniques, in particular ^{1}H NMR, ^{13}C NMR, HSQC and HMBC spectroscopy has been a tremendous advancement in the elucidation of structure of the naturally occurring products. These analytical methods offer information regarding proton–carbon connectivity, functional group identification and molecular architecture, which will aid in the accurate characterization of complex phytochemicals (9,10) Thus, combined application of quantitative phytochemical profiling and spectroscopic characterization of the products offers valuable insights into the chemical composition and therapeutic potential of medicinal plants.

Due to the scarcity of phytochemical data of *Cissus populnea*, it is important to conduct a detailed study on the chemical composition of the plant. Hence the aim of this study was to assess the phytochemical composition of various solvent extracts of the stem bark of *Cissus pulponea* and isolate and characterize Lupeol acetate by NMR spectroscopic techniques. The results of this study will add another set of phytochemical data in the phytochemical database of the species and scientific evidence for its traditional medicine and also serve as a foundation for future pharmacological and drug discovery studies for bioactive triterpenoids from medicinal plants.

2.0 MATERIALS AND METHODS

2.1 Materials

Instruments used are rotary evaporator, suction pump, glass column (2.5cm x 100cm), reflux condenser, retort stand, Pasteur pipette, stirring rod, conical flask, beakers, glass funnels, 20mL vials, measuring cylinder (10mL, 100mL, 200mL, 500mL), cotton wool, latex gloves, spatula,

Whatman No 1 filter paper, round bottom flask, heating mantle, sample bottles, wood mortar and pestle, capillary tubes, precoated TLC plate (Aluminum baked).

2.2 Chemicals/Reagents

All the chemicals and reagents used during this analysis were of analytical grade and properly distilled. The chemical used were: hexane, ethyl acetate, methanol, chloroform, acetone, sulfuric acid, Liebermann Burchard spray, silica gel (60-120 mesh) as stationary phase, dimethyl sulfoxide (DMSO) and distilled water.

2.3 Collection and authentication of Plant Material

Cissus populnea stem was collected from Wannune market in Tarka Local Government Area of Benue State. The plant material was authenticated at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka with a herbarium voucher number (UNH No. 902) assigned to it.

After identification, the plant material was then taken to the Directorate of Specialized Equipment Centre of Joseph Sarwuan Tarka University, Makurdi, Nigeria for further laboratory processing. The collected stem bark samples were washed thoroughly with distilled water to get rid of other earthy materials and contaminants, air dried under shade at room temperature for 14 day and then ground into coarse powder by mechanical grader.

2.4 Preparation of Plant Extracts

The stem bark was powdered and sequentially extracted with hexane, ethyl acetate and methanol. About 1.5 kg of the powdered plant material was macerated in hexane for 72 hours, with intermittent shaking at room temperature. The extract was concentrated under reduced pressure using rotary evaporator at 40 °C and filtered through Whatman No.1 filter paper to yield hexane extract.

After the first extraction with ethyl acetate, the plant residue (marc) was then extracted with ethyl acetate, followed by methanol extraction. The obtained extracts have been concentrated separately, under reduced pressure and conserved in airtight containers at 4 °C for further analysis.

2.5 Quantitative Phytochemical Analysis

Quantitative phytochemical analysis of the hexane, ethyl acetate and methanol extracts of *Cissus populnea* stem bark were carried out to determine the contents of phenolics, tannins, flavonoids, steroids, glycosides and terpenoids.

2.5.1 Quantification of the Total Phenolic Content.

The total phenolics were quantified according to (11) by the spectrophotometric Folin–Ciocalteu method. In brief, a part of each extract was reacted with Folin–Ciocalteu reagent and sodium carbonate solution. This reaction mixture was incubated at room temperature for 30 min and then the absorbance was taken at 765 nm with UV–Visible spectrophotometer. Gallic acid was used as calibration standard.

2.5.2 Quantification of tannin content using the Folin-Ferricyanide method.

The tannin content was determined as per (12). The extract solution was mixed with ferric chloride reagent and the absorbance of the complex was spectrophotometrically measured at the spectral band of 760nm. The reference standard used was tannic acid.

2.5.3 Quantification of total flavonoid

The total flavonoid content was determined by the aluminum chloride colorimetric method of (13). In brief, the extracts were incubated with aluminum chloride solution and potassium acetate at the room temperature for 30 min and absorbance was measured at 415 nm in UV–Visible spectrophotometer. Quercetin was used as the calibration standard.

2.5.4 Determination of the steroids:

The estimation of steroid content was carried out spectrophotometrically as described by (14). Cholesterol was used as the reference standard.

2.5.5 Determination of the glycosides

The gravimetric and spectrophotometric standard methods described by **(15)** were used to determine the glycoside content. The extracts were subjected to controlled hydrolysis and quantitatively analyzed.

2.5.6 Determination of the terpenoids

The total terpenoid content was estimated according to the method of **(16)**. Solvent partitioning of the extract solutions followed by spectrophotometric analysis was used to determine the concentration of terpenoids.

2.6 Isolation of Bioactive Compound

The methanol extract was found to contain significant amounts of phytochemical constituents and was separated using chromatography for isolation of compounds. The crude methanol extract was passed through a silica gel column and open column chromatography was performed using silica gel (60–120 mesh size).

The elution was performed with hexane, increasing polarity mixtures of hexane and ethyl acetate, and methanol. Fractions were collected with the aid of thin layer chromatography (TLC) on pre-coated silica gel. The spots were visualized by ultraviolet light (254 and 365 nm) and then developed by spraying with the reagents and heating.

Fractions with the same TLC mobility were collected and were repeatedly chromatographed on the same columns to obtain a pure compound named KAE-2**.**

2.7 Nuclear Magnetic Resonance (NMR) Spectroscopic Analysis

The isolated compound (KAE-2) was characterized by spectroscopic studies such as one-dimensional and two-dimensional nuclear magnetic resonance (NMR).

About 10 mg of the purified compound was dissolved in deuterated chloroform ($CDCl_3$) and placed in an NMR tube for analysis. Bruker NMR spectrometer was used to obtain 1H NMR and 13C NMR spectrum at appropriate frequencies under standard experimental conditions. The internal standard was tetramethylsilane (TMS) and chemical shifts (δ) were recorded in parts per million (ppm).

To establish proton–carbon connectivity and long-range correlation within the molecule, a series of 2-D NMR experiments were also conducted, such as Heteronuclear Single Quantum Coherence (HSQC) and Heteronuclear Multiple Bond Correlation (HMBC) spectroscopy.

The spectra are interpreted by comparing the chemical shift values and coupling constants, with the reported literature data of pentacyclic triterpenoids, in particular lupeol acetate.

2.8 Identification of Isolated Compound (KAE-2)

^{1}H NMR, ^{13}C NMR, HSQC, and HMBC spectra were used to gather comprehensive data for structural elucidation of the isolated compound (KAE-2). The spectroscopic characteristics like the signals assignable to oxygenated methine proton, exomethylene functionality, presence of multiple methyl substituents and presence of acetyl carbonyl group were compared with the published literature values.

2.9 Statistical Analysis

Experimental data obtained from quantitative phytochemical analyses were expressed as mean ± standard deviation (SD) of triplicate determinations. Statistical differences among extract groups were analyzed using one-way analysis of variance (ANOVA) followed by appropriate post hoc tests. Differences were considered statistically significant at $p < 0.05$.

3.0 RESULTS

3.1 Quantitative Phytochemical Analysis

The phytochemical analysis of the extracts of *Cissus populnea* obtained by different solvents showed significant differences as displayed in Table 3.1, suggesting that the different solvents used varied in their extraction efficacy for bioactive constituents as a result of their varying polarities. The phenolics, tannins, flavonoids and terpenoids were detected at the highest concentration in the methanol extract, indicating that the highly polar methanol solvent extracted these predominantly polar phytochemicals most effectively. This observation suggests that the methanol extract might have better antioxidant and therapeutic potentials as phenolics, flavonoids, and tannins have been widely recognized for their free radical scavenging, anti-inflammatory, and antimicrobial effects. This is because moderately polar and non-polar solvents such as hexane and ethyl acetate have a higher affinity to the lipophilic phytoconstituents, hence glycoside content was found to be maximum in hexane extract (1.328 ± 0.006) and steroid content was found to be maximum in ethyl acetate extract (1.020 ± 0.004). The concentration of terpenoids in all the extracts was comparatively small indicating that they are present in small amounts in the plant material. Moreover, the extracts differed significantly ($p < 0.05$) for each phytochemical parameter as shown by the superscripts (a–c) which signifies the importance of choosing the appropriate solvent for the extraction of qualitative and quantitative phytochemical yield of *Cissus populnea.*

3.2 Characterization of KAE-2 as Lupeol acetate

The NMR spectroscopic data shown in Table 3.2 strongly support the identification of isolated compound as Lupeol

acetate as the result of the close agreement between the experimental values and those reported **(17)**. The ^{1}H NMR spectrum is typical for a lupane-type pentacyclic triterpenoid, and has several methyl singlets between δ_H 0.85–1.66 ppm indicating tertiary methyl groups attached to quaternary carbons in a rigid hydrophobic framework. The proton signal at δ_H 4.47 is assigned to H-3 and together with the carbon resonance at δ_C 81.1 ppm, the presence of an oxygenated methine carbon, which is also esterified by an acetyl group, is strongly indicated, thus showing the substitution at C-3. This is corroborated by the presence of the acetyl methyl resonance at δ_H 2.02 and of the carbonyl carbon of the ester at δ_C 170.9 ppm typical of acetate esters. Two olefinic proton signals centred at δ_H 4.66 and 4.54, and the carbon signals at δ_C 150.7 and 109.4 ppm, are consistent with the presence of an exocyclic methylene group typical of the lup-20(29)-ene skeleton. Furthermore, the protonated carbons were unambiguously assigned by the HSQC correlations (H-3/C-3 and H-29/C-29) and the HMBC cross-peak of H-3 with the ester carbonyl carbon confirmed the location of the acetate group at C-3. There are hardly any deviations from the chemical shifts in the literature, which shows that the isolated compound is pure and that there are no significant modification of its structure or any impurities. The spectroscopic results clearly confirm the compound as lupeol acetate and corroborate previous reports that mentioned this triterpenoid as a biologically active metabolite.

3.3 ^{1}H NMR Spectrum and Proton Signal Assignments of Lupeol Acetate in $CDCl_3$

The ^{1}H NMR spectrum of Lupeol acetate in $CDCl_3$ is shown in Figure 3.1 as the spectrum shows proton resonances which are quite characteristic of a lupane-type pentacyclic triterpenoid with an acetate group. The upfield methyl resonances are very strong between δ_H 0.79–1.03 ppm, and are singlets due to the presence of the methyl groups on quaternary carbons in the rigid pentacyclic framework. In particular, the signals at δ_H 1.03, 0.85, 0.79, 0.87, 0.94, and 0.82 are assigned to the tertiary methyl protons of C-23, C-24, C-25, C-26, C-27, and C-29, respectively, confirming the highly substituted aliphatic skeleton characteristic of lupeol derivatives. The allylic methyl group attached to the olefinic carbon system was also observed as a distinctive singlet at δ_H 1.69, which also confirmed the presence of the lup-20(29)-ene nucleus. The spectrum also shows two exomethylene proton signals at δ_H 4.69 and 4.57, corresponding to H-29a and H-29b, respectively, and diagnostic for a terminal methylene (=CH_2) functionality, thus indicating the presence of an exocyclic double bond at C-20/C-29. A resonance of the oxygenated methine proton at C-3 is deshielded at δ_H 4.47 as a doublet of doublets demonstrating the presence of an ester oxygen, which is present at C-3. The acetyl methyl protons give rise to another signal at δ_H 2.02, which further establishes the presence of an acetate ester group. From the multiplicity pattern and the distribution of chemical shifts, the structure of the triterpene backbone is deduced as being predominantly saturated pentacyclic, with the unsaturation being only present in the exomethylene group. Moreover, the coincidences of these resonances with published spectral data for lupeol acetate confirm the structure and provide further evidence to the successful isolation of a chemically pure triterpenoid compound with characteristic structural components with pharmacological relevance.

3.4: ^{13}C NMR Spectrum and Carbon Signal Assignments of Lupeol Acetate in $CDCl_3$

The ^{13}C NMR spectrum of Lupeol acetate in $CDCl_3$ is shown in Figure 3.2 and the carbon resonances are fully in accordance with a lupane-type pentacyclic triterpenoid with an acetyl group. Thirty-two carbon signals are observed in the spectrum, which is indicative of the presence of the thirty carbons from the triterpene skeleton and of two other carbons from the acetate moiety. The presence of a prominent downfield resonance at δ_C 170.91 is a hallmark of an ester carbonyl carbon, and is strong evidence for the presence of acetylation in the molecule. The oxygenated methine carbon signal at δ_C 81.08 is characteristic of lupeol acetate, and is assigned to C-3. Two diagnostic olefinic carbon resonances at δ_C 150.77 and 109.44 can also be seen in the spectrum, corresponding to the C-20 and C-29 atoms, respectively, indicating the presence of an exocyclic double bond typical of the lup-20(29)-ene system. The remaining carbon signals are mostly in the aliphatic region (δ_C 14–55 ppm) corresponding to methyl, methylene, methine and quaternary carbons which are common for a highly substituted pentacyclic triterpene skeleton.

3.6 HMBC spectrum of Lupeol acetate in $CDCl_3$ is presented as an illustration of the long-range proton–carbon interactions.

The HMBC (Heteronuclear Multiple Bond Correlation) spectrum of Lupeol acetate in $CDCl_3$ is displayed in Figure 3.4 and is used to obtain the important long-range proton–carbon correlation information, which is necessary to confirm the structural framework and substitution pattern of this

compound. There is a strong correlation between the acetyl methyl proton signal at δ_H 2.01 and the carbonyl carbon signal at δ_C 170.91, which is the signature of the presence of an acetate functional group. Importantly, the oxygenated methine proton at δ_H 4.47 also shows long range correlation with the same carbonyl carbon, thereby confirming that the acetyl group is attached to C-3 of the triterpenoid skeleton. The HMBC correlations for the exocyclic double bond in the lup-20(29)-ene moiety were confirmed by correlations of the resonances of the exomethylene protons at δ_H 4.68 and 4.55 with the olefinic quaternary carbon at δ_C 150.77. The other long-range interactions between the methyl proton signals in the vicinity of δ_H 0.79-1.01 and other quaternary or methine carbon signals further corroborates the fused pentacyclic rings and the substitution pattern of the molecule. The HMBC cross-peaks which are observed in conjunction with the assignments made in the ^{1}H, ^{13}C and HSQC spectra, give a clear idea about the structure and hence the identification of the compound isolated in the present study as lupeol acetate.

4.0 DISCUSSION

The present study revealed that secondary metabolites and structurally important bioactive constituents such as pentacyclic triterpenoid lupeol acetate are abundant in the stem bark of *Cissus populnea*. The quantitative phytochemical analysis results presented in Table 3.1 showed that the solvent extracts varied significantly in their phytochemical composition, highlighting the significance of the polarity of the solvents on the efficiency of extraction and distribution of the phytochemicals. The methanol extract had the highest phenolics, flavonoids, tannins and terpenoids content while the hexane extract had the highest content of glycoside and the ethyl acetate extract had the highest content of steroid. The results obtained are similar to those previously reported, showing that the polar solvents, such as methanol, have better extraction potential for polyphenolic compounds than the nonpolar solvents because of their ability to form strong hydrogen bonds, which penetrates the plant cellular matrices better **(18–20)**. There were also other medicinal plants that were studied with methanol extract that gave significantly more amount of antioxidant phytochemicals than lesser polar solvents **(21)**.

It can be seen that the phytochemicals distribution of this species (*Cissus populnea*) (Table 3.1) indicates that it has great pharmacological potential. The methanol extract recorded high phenolic and flavonoid contents and thus are recognized for their capacity to scavenge ROS and donate hydrogen atoms to the stabilization of free radicals **(22)** and thus have possible antioxidant and anti-inflammatory properties. Similarly, the significant tannin content found in the extracts could be responsible for antimicrobial and wound healing properties, via their protein-precipitating and microbial enzyme-inhibitory properties **(23)**. The predominance of glycosides in hexane extract and steroids in ethyl acetate extract is also indicative of the differential solubility of solvents for the phytochemicals of different polarities and complexity of their molecules. The significant differences observed between the extracts highlight the significance of solvent selection in phytochemical investigations and could account for the differences often reported on the biological activities of medicinal plants extracted with different solvent systems.

Lupeol acetate was the most important phytochemical discovery made in the present study. The chromatographic purification and spectroscopic analysis presented in Figures 3.1 – 3.4 gave strong evidence in favour of the identity of the isolated compound as lupeol acetate. The NMR spectrum shown in Figure 3.1 showed several upfield methyl singlets (δ_H 0.79–1.69) which are characteristic of highly substituted pentacyclic triterpenoids having shielded tertiary methyl groups on quaternary carbons. Proton resonance pattern has been reported earlier for lupeol acetate extracted from medicinal plant Ficus polita **(24,25)**. The many proton signals in the methyl region seen in the present work further substantiates the high level of substitution and the highly saturated character of the pentacyclic carbon skeleton.

One particularly significant signal in the ^{1}H NMR spectrum (Figure 3.1) was the deshielded proton resonance at δ_H 4.47, which was a doublet of doublets and was assigned to H-3. This signal was a strong indication of the presence of an oxygenated methine proton attached to an esterified carbon. Further, the acetyl methyl resonance at δ_H 2.02 was particularly consistent with acetyl substitution at C-3 and was consistent with the structure of lupeol acetate. The proton resonance pattern observed here in protonated lupeol acetate was consistent with what was reported in previous studies with other pentacyclic triterpenoids such as lupeol acetate **(25)**. In addition, the exo-methylene proton signals at δ_H 4.69 and 4.57 established the location of a terminal methylene group on the olefinic carbon skeleton of the lup-20(29)-ene nucleus. The spectral values of these resonances were found to be very similar to the published values of genuine lupeol acetate compounds **(26)**, thus establishing the purity and integrity of the isolated compound.

The assignment of the isolated compound was further confirmed by the ^{13}C NMR spectrum (Figure 3.2). Thirty-two

signals for the thirty carbon atoms of the triterpenoid skeleton and two others for the two carbon atoms of the acetate group were observed on the spectrum. The carbonyl resonance of the ester carbonyl group (δ_C 170.91) was a positive indication of the presence of an acetyl functional group, and the oxygenated methine carbon signal (δ_C 81.08) confirmed substitution at the C-3 position. These observations are quite similar to the previous reports in which the ester carbonyl groups showed typical signals around 170–171 ppm and oxygenated methine groups showed typical signals around 80–81 ppm **(27)**. The exocyclic double bond of the lup-20(29)-ene skeleton was confirmed by the olefinic carbon resonances at δ_C 150.77 and 109.44. The majority of the remaining carbon resonances were found in the aliphatic region (between 14 and 55 ppm), characteristic of saturated methylene, methine, methyl and quaternary carbons of pentacyclic triterpenoids.

The 2D NMR spectra shown in Figure 3.3 and Figure 3.4 further confirmed the molecular structure of the isolated compound. The one-bond proton-carbon correlation was confirmed from the HSQC spectrum (Figure 3.3) and the assignments made from the one-dimensional spectra were confirmed. The proton at H-1 (δ_H 4.47) was found to be coupled to the carbon at C-3 (δ_C 80.87) as part of the study, which confirmed the presence of an oxygenated methine carbon. Likewise, the cross-peaks of the exomethylene protons at δ_H 4.67 and 4.55 with δ_C 109.24 confirmed that the protons were attached to the terminal methylene carbon. These are the similar HSQC correlations reported earlier for lupeol acetate obtained from several medicinal plants which have been regarded as diagnostic features of lupane skeleton **(28)**.

A HMBC-spectrum as illustrated in figure 3.4 proved to be of great value in assigning long-range proton-carbon connectivity, and in locating functional groups in the molecule. The methyl proton signal at δ_H 2.01 was correlated with the ester carbonyl carbon at δ_C 170.91 to definitely establish the acetate functionality. More importantly, the long-range interaction of H-3 and the carbonyl carbon of the ester provided evidence that the acetyl group is specifically attached to C-3 of the triterpenoid framework. Further confirmation of the position of the terminal double bond in the lup-20(29)-ene skeleton was provided by the exomethylene proton correlations with the olefinic carbon at δ_C 150.77. These HMBC interactions are in close agreement with other reported for lupeol acetate, and it is clear that this isolated compound is lupeol acetate **(28)**.The agreement found between one and two dimensional NMR data thus confirms the reliability and accuracy of the spectroscopic characterisation carried out in this study.

The presence of lupeol acetate in *Cissus populnea* is of pharmacological importance since pentacyclic triterpenoids have been widely reported with various biological properties. Lupeol acetate and lupeol derivatives have been shown to have anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, antidiabetic and anticancer properties **(29)**. Pharmacological activity of lupeol derivatives has been shown to be anti-inflammatory, which is associated with the inhibition of inflammatory mediators like interleukins, NF-κB, COX-2 and TNF-α, thereby decreasing the oxidative stress and inflammatory responses **(30)**. Furthermore, the antioxidant activity of lupeol acetate might be responsible for membrane stabilization and prevention of oxidative damage of cells and lipoperoxidation. This finding of lupeol acetate in the *Cissus populnea* thus offers a scientific basis for its traditional uses, and indicates that the therapeutic properties that have been attributed to the plant may be due to the combined activity of lupeol acetate and other phytochemicals identified in the extracts, such as flavonoids, phenolics and tannins.

5.0 Conclusion

The results of the present work showed that the stem bark of *Cissus populnea* is a good source of pharmacologically important secondary metabolites and validated the efficient extraction and characterization of lupeol acetate from the plant by using comprehensive spectroscopic data. The phytochemical findings from the present study (Table 3.1) along with the spectroscopic data (Figures 3.1-3.4) collectively justify the medicinal importance of the species and also highlight the significance of isolation and structural elucidation of bioactive compounds through bioassay guided approach to validate the traditional medicinal plants. The study also extends the knowledge base of phytochemical constituents of the genus Cissus and the medical importance of *Cissus populnea* as a source of bioactive natural products for pharmaceutical preparation. However, more studies with pharmacological evaluation, molecular mechanism research, toxicity studies, and quantitative analysis of lupeol acetate are still required to be able to completely understand the therapeutic efficacy, safety profile and its pharmaceutical application of both the isolated compound and the crude plant extracts.

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TABLES

Table 3.1: Quantification of phytochemical content of *Cissus populnea* extract

Phytochemical	Hexane Extract	Ethyl Acetate Extract	Methanol Extract
Phenolics	0.393 ± 0.002^{b}	0.244 ± 0.002^{c}	0.513 ± 0.003^{a}
Tannins	0.230 ± 0.003^{b}	0.153 ± 0.003^{c}	0.664 ± 0.003^{a}
Flavonoids	0.477 ± 0.002^{c}	0.605 ± 0.002^{b}	0.822 ± 0.002^{a}
Steroids	1.005 ± 0.004^{b}	1.020 ± 0.004^{a}	0.677 ± 0.000^{c}
Glycosides	1.328 ± 0.006^{a}	1.036 ± 0.008^{b}	0.635 ± 0.008^{c}
Terpenoids	0.070 ± 0.003^{c}	0.110 ± 0.002^{b}	0.165 ± 0.002^{a}

Table 3.2: ^{1}H and ^{13}C NMR data for Lupeol acetate in $CDCl_3$

Position	Experimental Data ($DCCI_3$)		Literature Data δ ^{1}H chemical shift (δ, ppm), J(Hz) (17)	
	δ^{13}C (ppm)	δ ^{1}H (δ, ppm), J(Hz)	δ^{13}C (ppm)	
1	38.5	-	-	38.6
2	21.6	-	-	21.7
3	81.1	4.47	4.47 (1H, dd, 7.4, 12.0)	81.2
4	38.0	-	-	38.0
5	55.2	-	-	55.6
6	18.3	-	-	18.4
7	34.3	-	-	34.4
8	40.8	-	-	41.0
9	50.3	-	-	50.5
10	37.1	-	-	37.3
11	20.9	-	-	21.1
12	24.2	-	-	24.0
13	36.2	-	-	36.2
14	43.0	-	-	43.0
15	25.2	-	-	25.3
16	35.6	-	-	35.8
17	42.0	-	-	43.2
18	48.4	-	-	48.5
19	48.3	-	-	48.2
20	40.0	-	-	40.2
21	150.7	-	-	151.2
22	29.8	-	-	30.0
23	16.5	1.01	1.03 (3H, s)	16.4
24	27.6	0.85	0.85 (3H, s)	27.6
25	16.2	0.85	0.85 (3H, s)	16.2
26	14.2	0.88	0.79 (3H, s)	14.7
27	18.2	0.94	0.94 (3H, s)	18.2
28	109.4	4.66 and 4.54	4.69 (1H, s), 4.57 (1H, s)	109.6
29	16.7	0.87	0.87 (3H, s)	16.7
30	19.3	1.66	1.69 (3H, s)	19.5
a	170.9	-	-	171.3
b	28.1	2.02	2.05 (3H, s)	28.2

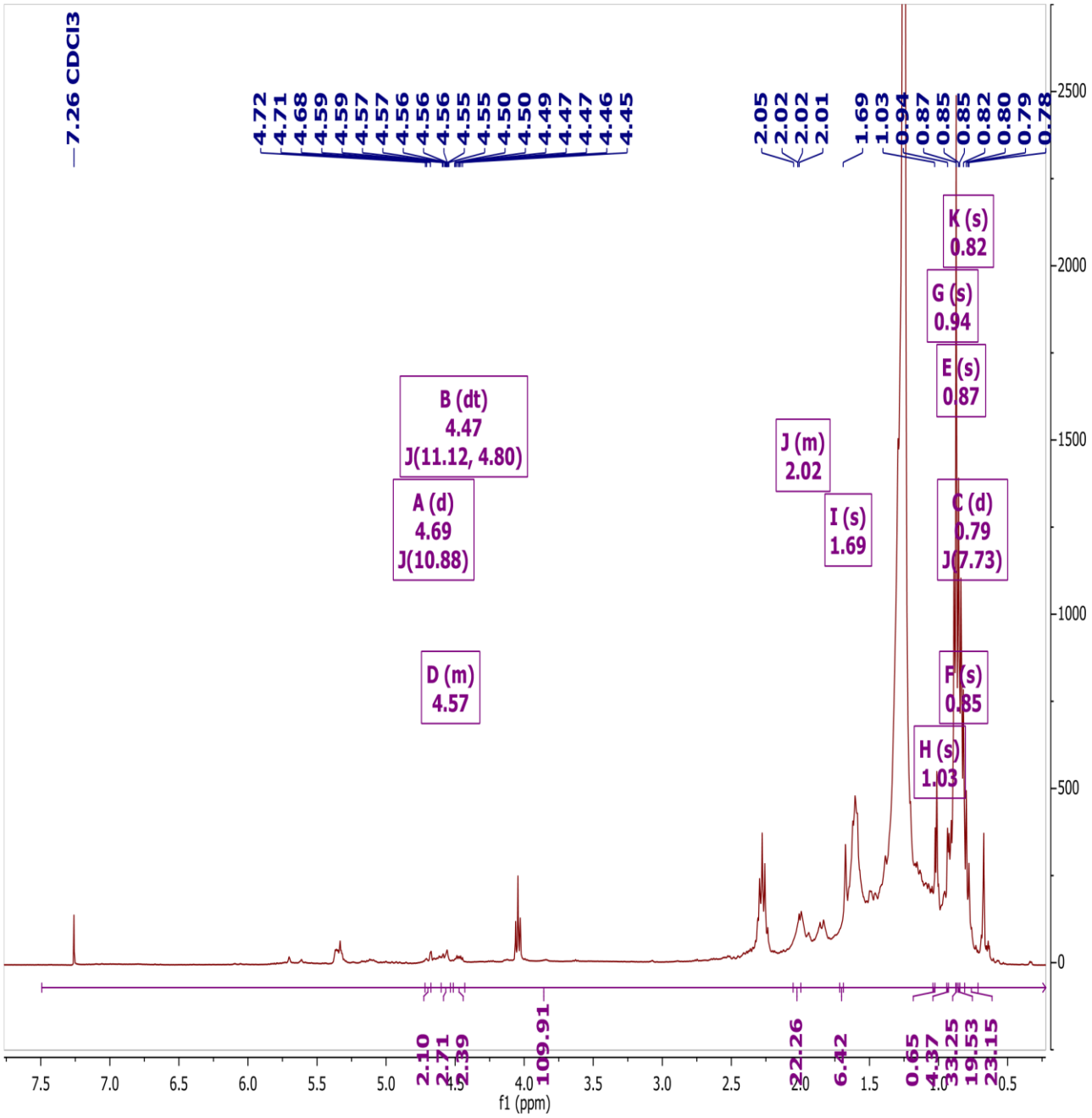


Figure 3.1: ^{1}H NMR Spectrum and Proton Signal Assignments of Lupeol Acetate in $CDCl_3$

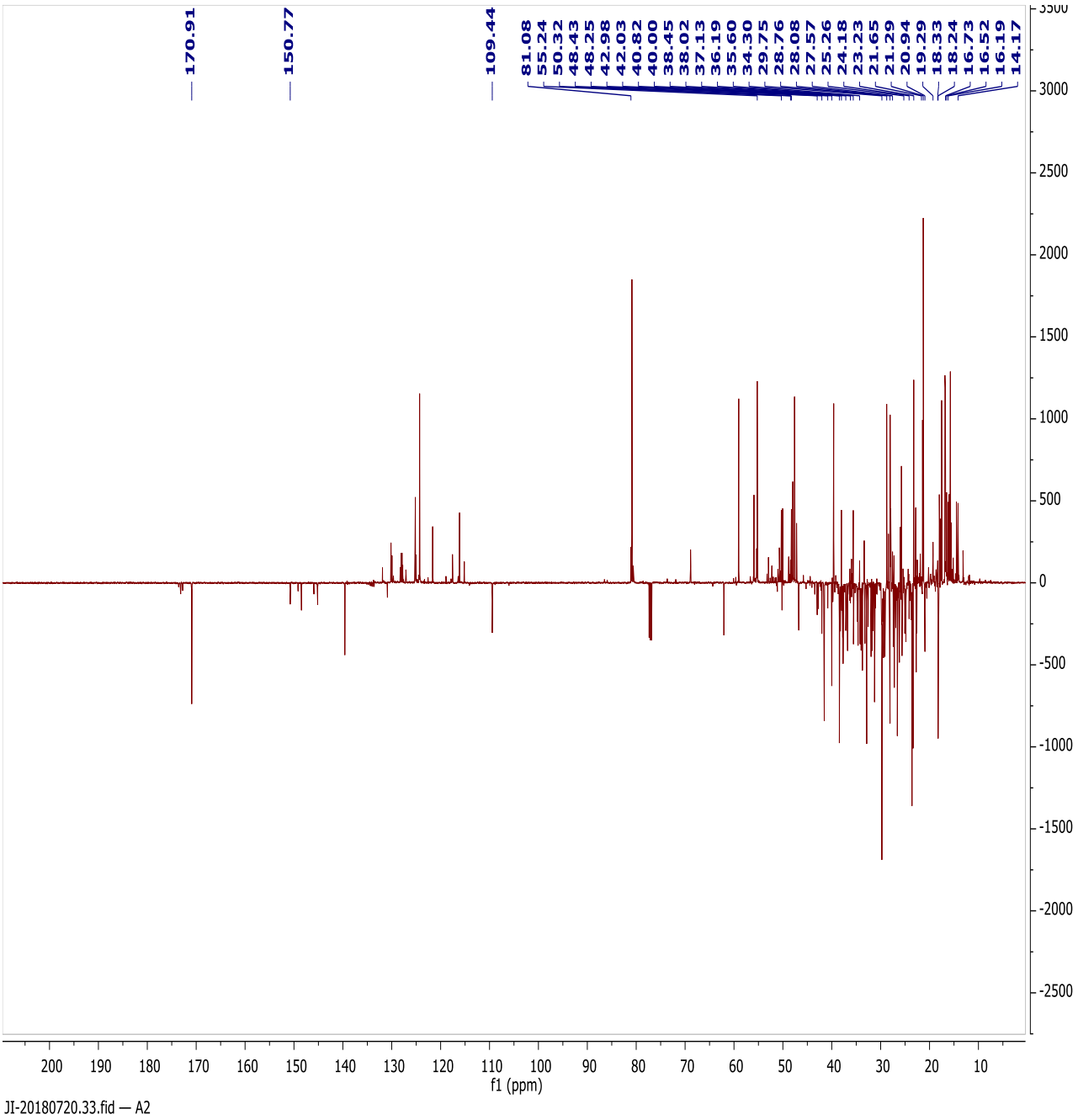


Figure 3.2: ^{13}C NMR Spectrum and Carbon Signal Assignments of Lupeol Acetate in $CDCl_3$

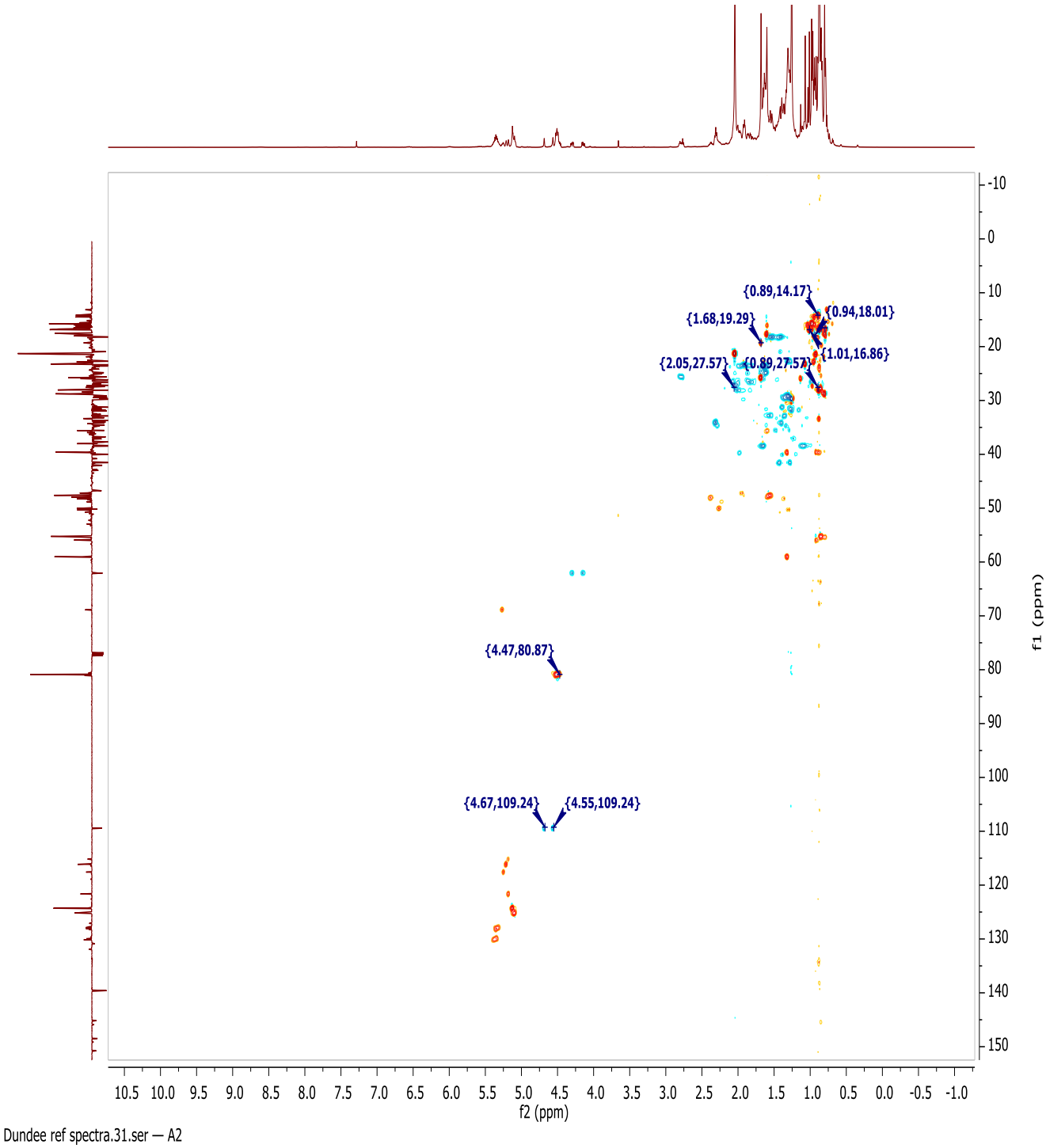


Figure 3.3: HSQC Spectrum Showing Proton–Carbon Correlations of Lupeol Acetate in $CDCl_3$

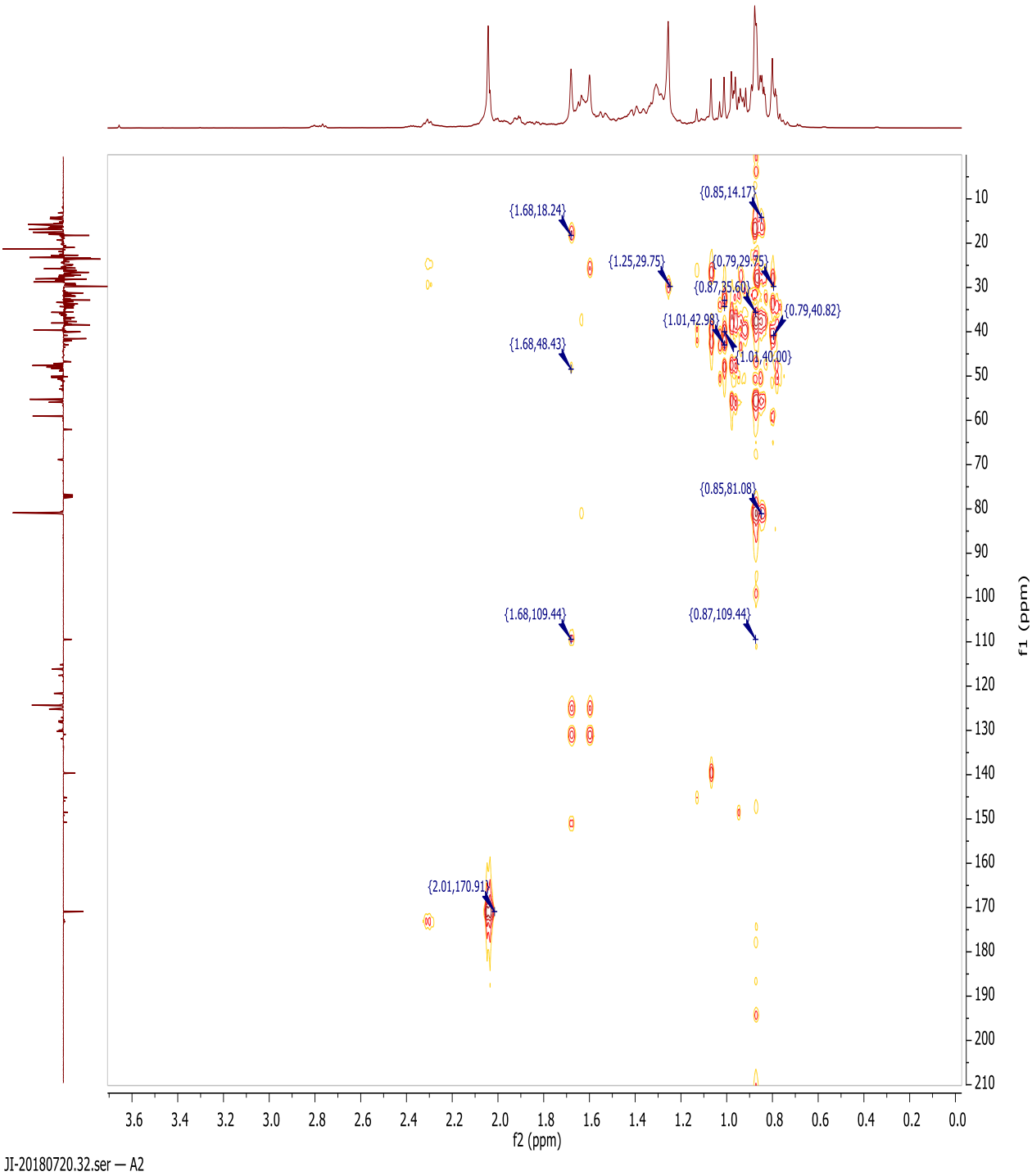
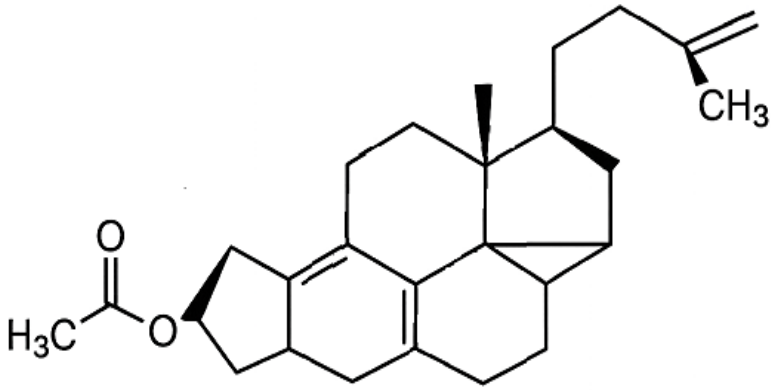


Figure 3.4: HMBC Spectrum Showing Long-Range Proton–Carbon Correlations of Lupeol Acetate in $CDCl_3$



Lupeol acetate

3β-Acetoxy-lup-20(29)-ene

Figure: 3.5. The structure displayed contains the compound Lupeol acetate which is also referred to as Lupeol-3-acetate or Lup-20(29)-en-3β-yl acetate. It is a pentacyclic triterpenoid with a lupane skeleton and is characterized by an exocyclic double bond at C-20/C-29 as well as a hydroxyl group which is acetylated at the C-3 position. (Molecular formula: $C_{32}H_{52}O_2$,)