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Isolation and Characterization of Triterpene from n-hexane Stem Bark Extract of *Vitellaria paradoxa* (Shea Butter Tree)

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Vitellaria paradoxa is used in traditional medicine for treatment of fungal infections. The research was carried out to isolate and characterize a compound from n-hexane stem bark extract of *V. paradoxa* and to test the isolated compound for antifungal activity. One hundred grams of powdered stem bark (sample) was extracted by maceration using 500 cm^3 n-hexane (1:5 w/v). Chromatographic studies of the extract led to the isolation of a light-yellow oily compound which was readily soluble in chloroform and was characterized using Proton and Carbon-13 Nuclear Magnetic Resonance (NMR), Fourier Transformed Infrared (FTIR) spectroscopy, phytochemical test and was compared with the literature as a triterpene [3α-hydroxy-lup-20(29)-en-28-oic acid 7β-benzoate]. The compound was subjected to antifungal activity test against *Trichophyton*, *Aspergillus niger* and *Aspergillus flavus* and was active against all the three test organisms with the diameter of the zones of inhibition ranging from 8.5 mm to 14.5 mm which compares well with that of the control drug (ketoconazole). The results suggest that the isolated compound can be used to treat diseases associated with the tested clinical isolates and also can be used as a potential precursor for the synthesis of new antifungal drug.

Keywords: *Vitellaria paradoxa*, chromatography, Nuclear Magnetic Resonance, infrared, antifungal activity.

1. Introduction

Vitellaria paradoxa (formerly *Butyrospermum parkii*), is a tree of the Sapotaceae family. It is the only specie in the genus *Vitellaria* (Byakagaba, 2011) and is indigenous to Africa. Common names as Shea-butter tree in English, 'kadanya' or 'kade' in Hausa speaking language, 'okwuma' in Igbo and 'ori' in Yoruba languages. It can be found in 19 countries across the African continent, namely Benin, Burkina Faso, Cameroon, Central African Republic, Chad, Ethiopia, Ghana, Guinea Bissau, Ivory Coast, Mali, Niger, Nigeria, Senegal, Sierra Leone, South Sudan, Sudan, Togo, Uganda, Democratic Republic of Congo, and Guinea (Neumann *et al.*, 1998). The shea tree is a traditional African food plant. It has been claimed to have potential to improve nutrition, boost food supply in the "annual hungry season" (Masters *et al.,* 2010) foster rural development, and support sustainable land care. It was reported that *V. paradoxa* is the second most important oil crop in Africa after oil palm by Hall *et al*., (1996) and Tsafe *et al.,* (2019). Across the distribution area, the estimated actual number of productive trees and the estimated potential number of shea trees ranges from several hundred million (Lovett, 2004) to a couple of billion, making it one of the largest economic tree species in the region. Its medicinal properties are known to relieve rheumatic and joint pains and to quicken healing times and prevent infection of open wounds. It is also widely used to treat skin problems such as dryness, sunburn, burns, ulcers and dermatitis (Marchand, 1988 cited in Moore, 2008). Secondary shea products include honey and edible caterpillars, while shea processing yields abundant quantities of shea husks used as compost and shea cakes as a source of fuel. The wood is used for charcoal, construction, for furniture and as pounding mortars (Abbiw, 1990). The bark is used for traditional medicines and the latex is used for making glue. Shea trees provided fodder for 70% of surveyed households in Nyankpala, northern Ghana (Poudyal, 2011).

Researches carried out on *V. paradoxa* indicated that the following compounds were isolated from the plant: 2β,3β,19α-trihydroxyurs-12-en-28-oic acid, betulinic acid, 1α,2β,3β,19α-tretrahydroxyurs-12-en-28-oic acid, *β*-sitosterol,

stigmasterol, (-)-epicatechin, (+)-catechin and quercetin (Kenneth, *et al.,* 2018).

Plate 1. *Vitellaria paradoxa* tree (Satoshi, 2009)

Extensive literature review has shown that there are no researches carried out on isolation and characterization of compounds from n-hexane stem bark extract of *V. paradoxa.* The research is aimed at isolating and characterizing a compound from n-hexane stem bark extract of *V. paradoxa* using ^{13}C-NMR, ^{1}H-NMR, IR respectively, so as to provide a basis for the potential use of the extract for synthesis of antifungal drugs.

2. Materials and Methods

2.1 Sample Collection and Treatment

The stem bark of *Vitellaria paradoxa* was collected by first removing the hard surface and then inner reddish part of the bark was taken as the sample. The leaves and fruits were also collected. Sample collection was done at Maiyama town, Maiyama Local Government Area of Kebbi State, Nigeria in November 2016. The plant sample was authenticated at the Herbarium Section of the Department of Biological Sciences, Usmanu Danfodiyo University Sokoto, with voucher number (UDUH/ANS/0114). The fresh samples were air-dried for two weeks, grounded into a fine powder and stored in a plastic container until further use.

2.2 Extraction

The extraction was carried out using the method of maceration in the ratio 1:5 (w/v). One hundred grams (100 g) of the powdered sample (stem bark) was placed into a clean air-tight jar. To the jar, 500 cm^3 of n-hexane was added. The jar was kept shaking for 24 hours using laboratory shaking machine. The mixture was then decanted, filtered with muslin cloth and then with Whatman no. 1 filter paper. Further extraction of the residue was carried out with fresh n-hexane until the solvent turn colorless. The filtrate was evaporated to dryness using rotary evaporator. This was then packed in a separate clean dry bottle and stored at room temperature until required for use (Tsafe *et al.,* 2019).

2.3 Chromatographic Analyses.

Thin Layer Chromatographic (TLC) Analysis.

Precoated silica gel TLC plates was used to determine the separation profile of the extracts using one-way ascending technique. Capillary tube was used to manually apply spots on the TLC plate and the chromatogram was developed in an air tight chromatographic tank at room temperature employing different solvent systems. The spots were visualized under day light, UV (254-366nm) and by use of spray reagent (10% Sulphuric acid) followed by heating in an oven at 105^{o}C for 5 minutes (Ogbiko *et al.,* (2018).

Retention Factor (R_f) values were calculated using Equation 1.

$$R_f = \frac{\text{Distance moved by sample}}{\text{Distance moved by solvent}} \quad \text{....................................(1)}$$

Column Chromatography of the extract

A 75 cm by 3.5 cm glass column and a stationary phase silica gel of 60-120 mesh size was used. Wet packing and wet sample loading (sample + silica gel + hexane) was used for silica gel column chromatography. The (3 g) of the extract was subjected to column chromatography using gradient elution; starting with 100% n-hexane (200 ml) followed by n-hexane: ethyl acetate (98:2), (96:4) and then (94:6). The column chromatography was monitored with co-TLC. Collections were pooled together on the basis of their TLC profiles and coded NH1-NH4 (Ogbiko *et al.,* (2018).

. 2.4 Characterization of the Isolated Compound

Solubility Test

The solubility of the isolated compound was checked in n-hexane, ethyl acetate, methanol and chloroform.

2.5 Characterization of the Isolated Compound

Characterization of the Isolate Compound was carried out using ^{1}H and ^{13}C NMR Spectroscopy at the Central Laboratory, University of Pretoria, South Africa and FTIR at the Central Laboratory, Sokoto Energy Research Center (FTIR) following the method of spectral analyses reported by Ogbiko *et al.,* (2018)

2.6 Phytochemical Test on the Isolated compound

This was carried out following the Salkowski's test and Liberman Burchard's test as reported by Evans (2002) and Sofowora (2008) respectively.

2.7 Antifungal activity test on the isolated compound

The isolated compound was subjected to antifungal activity test against *Trichophyton*, *Aspergillus niger* and *Aspergillus flavus* using the method of Tsafe *et al.,* (2019) with some modifications in which three concentrations of the isolated compound were prepared (25, 50 and 100 mg/mL) and 6 mm diameter cork borer was used to bore holes.

3. Results and Discussion

3.1 Extraction Yield

The mass of the extract obtained from the extraction and percentage yield of 100 g of sample, is shown in Table 1.

Table 1. Extraction and percentage yield

Solvent	Yield (g)	Yield (%)	Color
n-hexane	4.10	4.10	Orange yellow

3.2 Thin-Layer Chromatography

Plate 2 and Table 1 give details of the TLC profile of the extract, with n-hexane: Ethyl acetate (8:2) as the best system for the separation.

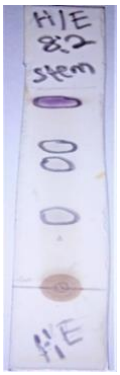

Plate 2. The chromatographic profile of the extract.

Table 2. TLC Profiles of the extract.

Sample	No. of spots	R_f values
n-hexane	4	0.34, 0.62, 0.70, 0.94

3.3 Column Chromatography of the Extract.

The result of column chromatography of n-hexane extract, number of collections pooled together, solvent ratios used and the number of spots on co-TLC are presented in Table 3.

Table 3. Column Chromatography of the extract.

No. of Collections	Solvent Ratio	No. of Spots on Co-TLC
1-25	n-hex 100%	0
26-44	n-hex:EA 98:2	0
45-60	96:4	1
61-70	94:6	0

Key: n-hex= n-hexane, EA= ethyl acetate

3.4 TLC Profile of the Extract from Column Chromatography

The Column Chromatography of n-hexane extract as monitored using TLC is as presented in Plate 3.

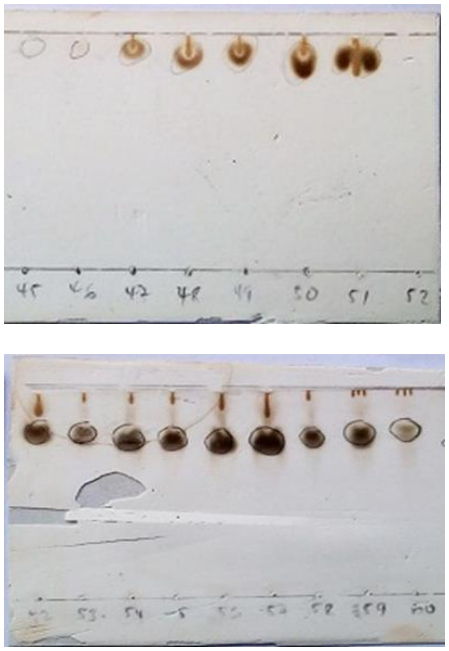

Plate 3. TLC Profile of column chromatography of the extract (collections 45-60).

3.5 TLC profile of the Isolated Compound

Collections 45-60 were pooled together to give one spot. TLC of the isolated compound gave a single, dark brown spot after heating with 10% sulphuric acid at 105^{o}C for five minutes as shown on Plate 4, Its R_f Value is 0.94.

TLC of the Isolated compound
in hexane:ethyl acetate (8:2)

Plate 4. co-TLC of collections 45-60 (the isolated compound).

3.6 Characterization of the Isolated Compound

The results of the spectral analyses of the compound isolated from the stem bark of *V. paradoxa* are presented in Appendix I (^{13}C-NMR), II (^{1}H-NMR) and III (FTIR). From Appendix I shows prominent peaks at 150.88 ppm and 109.44 ppm. Appendix II, diagnostic peak observed at 1.66 ppm, while in Appendix III, broad absorption at 3418 cm^{-1} is observed.

3.7 Phytochemical Test of the Isolate Compound.

Both Salkowski's and Liebermann-Burchard's tests showed the presence of triterpenes with pure red color in the upper layer (Salkowski's and Liebermann-Burchard's tests). **R**eddish brown color at the interface (Salkowski's test), deep red coloration at the junction of the two layers (Liebermann-Burchard's Test).

3.8 Antifungal Activity of the Isolated Compound

The antifungal activity of the isolated compound is presented in Table 4.

Table 4. Antifungal Activity of the Isolated Compound.

	Diameter	of zones of	inhibition	(mm)
Test organism	25 mm/mL	50 mm/mL	100 mm/mL	Ketoconazole (20 mm/mL)
Trichophyton	0	9.0	14.5	8.5
Aspergillus niger	0	8.5	11.5	11.0
Aspergillus flavus	8.5	10.5	12.5	15.5

Column Chromatography of the extract led to the isolation of the compound which was insoluble in hexane, ethyl acetate and methanol but completely and readily soluble in chloroform. The isolated compound gave a single, dark brown spot on TLC plate using the solvent systems 8:2 hexane: ethyl acetate and 2:8 hexane: ethyl acetate and after heating the developed plate at 105^{o}C for 5 minutes with spray (10 % sulfuric acid). The isolated compound was light yellow viscous oil which is soluble in chloroform and insoluble in n-hexane, dichloromethane, ethyl acetate and methanol.

The result of ^{13}C NMR of the compound (Appendix I) confirmed the nature of the skeleton of the compound as lupane benzoate with diagnostic signals of the terminal olefinic bond at 150.98 ppm (C-20) and 109.44 ppm (C-29). Key (prominent) signals indicated other functional groups of the lupane skeleton: the signal at 170.79 ppm corresponding to a carbonyl carbon of a carboxylic acid function; carbonyl carbon of conjugated ester function (166.72 ppm); and aromatic signals (between 125-150 ppm) corresponding to the benzoic ester ring (Appendix I and V). On comparing the spectra of the triterpene benzoate isolated by Tatiana *et al.,* (2001) and that of the isolated compound (Appendix I), other signals observed are 130.51 ppm (C-1'), 128.63 ppm (C-2' and 6' in Appendix V), 128.05 ppm (C-3' and C-5' in Appendix V) and 134.57 ppm (C-4' in Appendix V) for the benzene ring, all which correspond to the 131.00 ppm, 129.60 ppm, 128.40 ppm and 132.70 ppm signals of the reference compound for carbons 1', 2' & 6', 3' & 5' and 4' respectively.

^{1}H NMR signals at 7.62 ppm [7.6 ppm of the compound isolated by Tatiana *et al.,* (2001)], 7.41 ppm (7.44 ppm of the reference compound) and 7.55 ppm (7.55 ppm of the reference compound as isolated by Tatiana *et al.,* (2001) are for the five protons (2' & 6', 3' & 5' and 4') of the benzoate respectively. The spectrum also displayed six signals for the methyl groups at 0.79, 0.90, 0.96, 1.11, 1.24 and 1.66 ppm (Appendix II and V). This last value suggested the presence of one methyl group attached to an unsaturated carbon (Tatiana *et al.,* (2001). Furthermore, vinylic proton signals appeared at 4.75 and 4.62 ppm which exactly corresponded to the vinylic protons signals of the reference compound. Signals at 7.46-8.10 showed the typical pattern of the benzoate group (Appendix II). All these signals compare well with the report of Tatiana *et al.,* (2001).

FTIR Spectrum of the isolated compound (Appendix III) gave a strong absorption at 1722 cm^{-1} which is a characteristic of a carbonyl carbon of an ester (C=O). Absorption at 1475 cm^{-1} was also observed for C=C in aromatics. Similarly, broad absorption at 3418 cm^{-1} indicates the presence of OH group corresponding to the absorption at 3420 cm^{-1} of the compound as reported by Tatiana *et al.,* (2001). Strong absorption at 1270 cm^{-1} [as

reported by Tatiana *et al.,* (2001)] confirmed the presence of carbon to oxygen single bond (C-O). Additionally, an absorption at 713 cm^{-1}, which corresponds to aromatic ring, was also observed as reported by Tatiana *et al.,* (2001).

According to available literature, this is the first report of the presence of 3α-hydroxy-lup-20(29)-en-28-oic acid 7β-benzoate isolated from the stem bark of *V. paradoxa.* Based on the spectral data (^{1}H NMR, ^{13}C NMR and FTIR), the proposed structure of the isolated compound is presented in Figure 1.

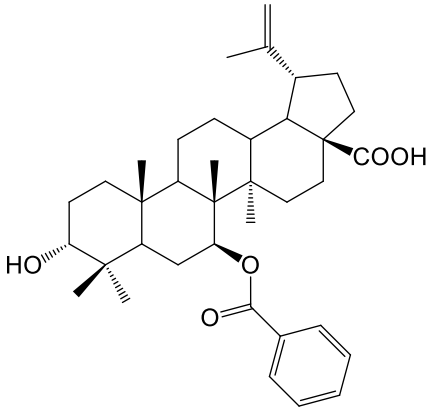

Figure 1. Structure of Isolated Compound: 3α-hydroxy-lup-20(29)-en-28-oic acid-7β-benzoate

The result of the phytochemical test carried out on the isolate confirmed that the isolated compound is a triterpene for the following reasons: i) formation of reddish brown color at the interface (Salkowski's test), ii) deep red coloration at the junction of the two layers (Liebermann-Burchard's Test) and iii) pure red color in the upper layer (Salkowski's and Liebermann-Burchard's tests).

The compound 3α-hydroxy-lup-20(29)-en-28-oic acid-7β-benzoate with molecular formula $C_{37}H_{52}O_5$ was isolated by Tatiana *et al.,* (2001) from the Bark of *Picramnia teapensis* (Simaroubaceae). It was also reported to be a viscous oil (Tatiana *et al.*, 2001).

The isolated compound showed a significant antifungal activity against *Trichophyton*, *Aspergillus niger* and *Aspergillus flavus* with the diameter of the zones of inhibition ranging from 8.5 mm to 14.5 mm and 8.5 mm to 15.5 mm for the control. This justifies the use of the plant's extracts in traditional medicine for treatment of diseases associated with the organisms. The result also showed that the activity of the isolated compound on the tested organisms is concentration dependent. Terpenoids have been proved to have antifungal activity (Ghoshal *et al.,* 2006 and Taddeo and Claude, 2011). This agrees with what was reported by Ghoshal *et al.* (2006) that alkaloids, terpenoids and lactones are responsible for antifungal activity of some plant extracts.

4. Conclusion

Chromatographic separation of the n-hexane extract of the stem bark of *Vitellaria paradoxa* afforded a triterpene compound 3α-hydroxy-lup-20(29)-en-28-oic acid-7β-benzoate. The isolated compound showed antifungal activity against some test fungi, rationalizing its ethnomedicinal use in the treatment of fungal diseases. Thus, the isolated compound can be used to treat diseases associated with the tested clinical isolates and can be used as a potential precursor for new antifungal drug synthesis.

Acknowledgement

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Conflict of interest

The authors declare no conflict of interest.

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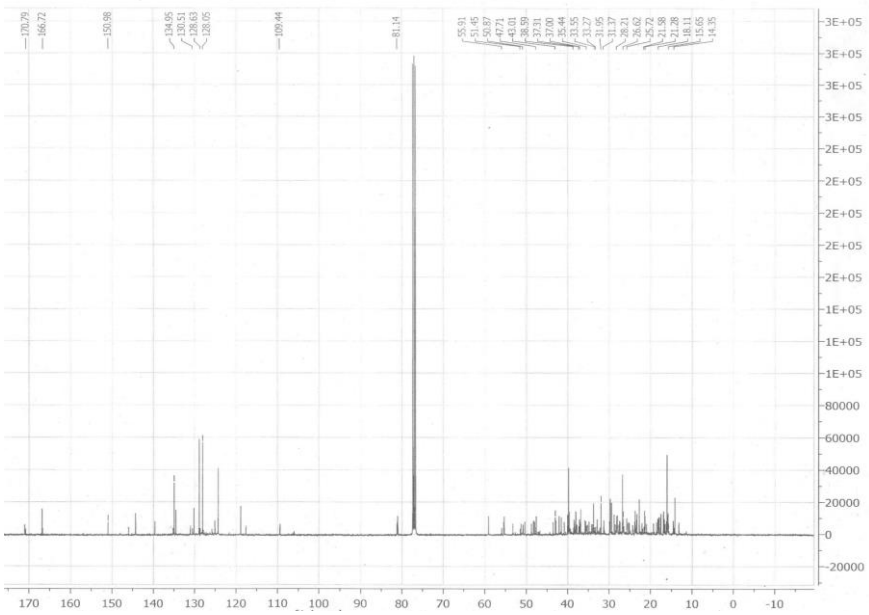
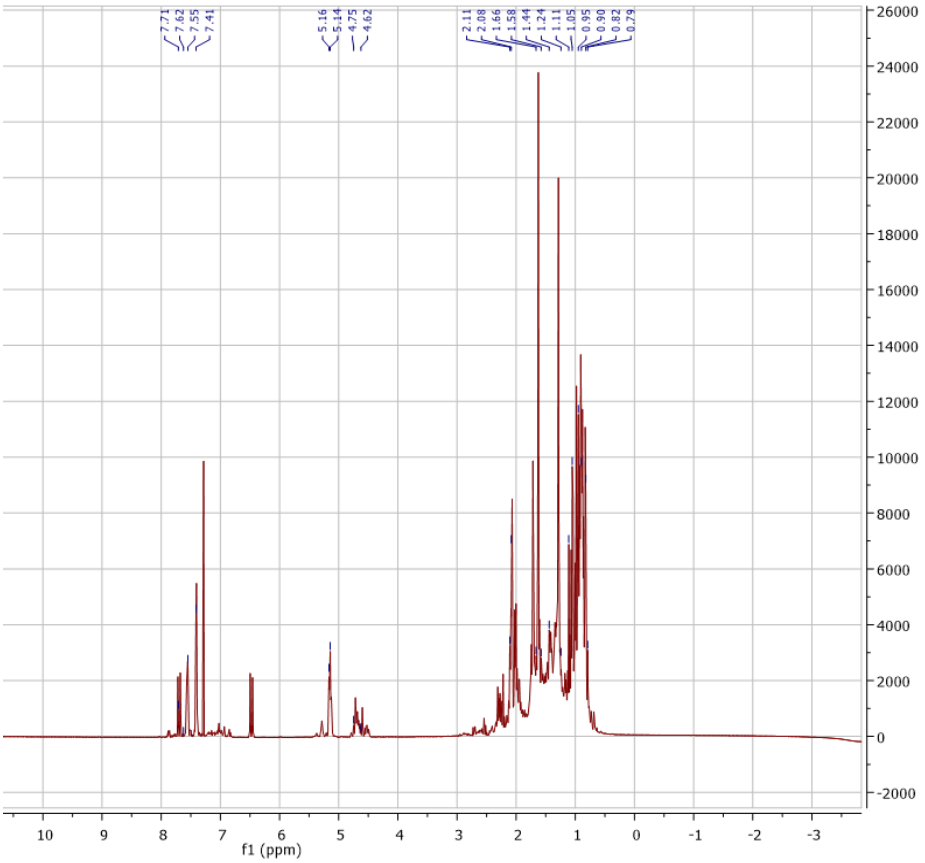
APPENDICES

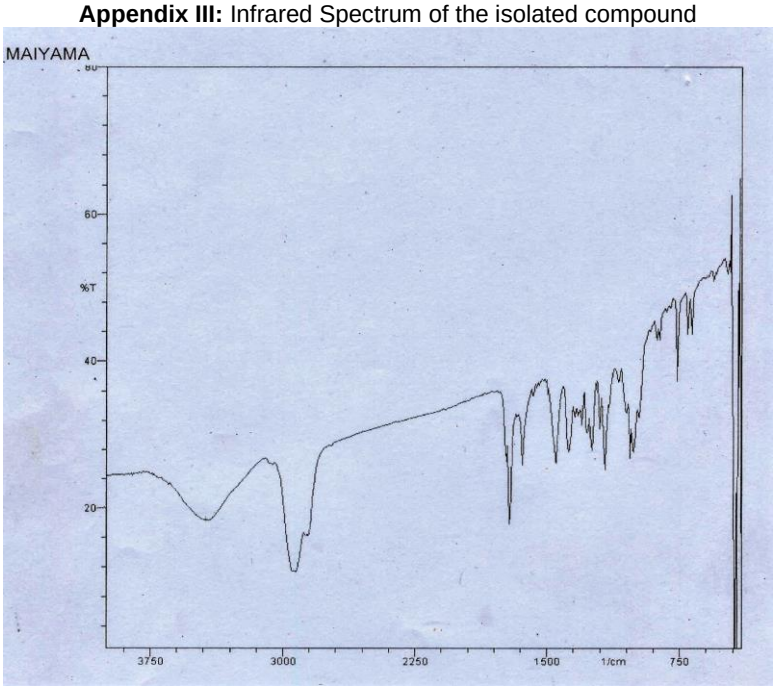
Appendix I: ^{13}C-NMR Spectrum of the isolated compound



Appendix II: ^{1}H-NMR Spectrum of the isolated compound



Appendix III: Infrared Spectrum of the isolated compound



Appendix IV: Prominent Signals Observed from isolated Compound Compared with Literature.

Frequency (cm^{-1}) of the isolated compound	Frequency (cm^{-1}) of literature*	Inference
3418	3420	OH
2862	2865	C-H
1722	1722	C=O
1270	1274	C-O
713	712	Aromatic ring

*= Tatiana *et al.,* (2001)

Appendix V: Prominent Signals Observed from the Isolated Compound Compared with Literature.

Position	*δC (ppm)**	*δH**	*δC (ppm)*	*δH*
1	33.30	1.05	33.55	1.05
2	25.40		25.72	
3	75.90	2.2		2.08
4	37.60		37.31	
5	46.20	0.81		0.82
6	25.40		25.72	
7	77.60	5.32		5.16
8	46.00			
9	50.70	1.45	50.87	1.44
10	37.10		37.00	
11	20.40		21.28	
12	25.50		26.62	
13	38.70	2.25	38.59	2.11
14	43.90		43.01	
15	33.20		33.27	
16	32.50		31.95	
17	55.90		55..91	
18	49.20		51.45	
19	47.20	5.20	47.71	5.14
20	150.40		150.98	
21	30.60		31.37	
22	37.10		37.00	
23	28.00	0.96	28.21	0.96
24	22.00	0.80	21.58	0.82
25	15.70	0.89	15.65	0.90
26	11.60	1.26		1.24
27	15.10	1.12	14.54	1.11
28	no[C]		170.79	
29	109.8	4.75 & 4.62	109.44	4.75 & 4.62
30	19.40	1.68	18.11	1.66
1’	131.00		130.51	
C=O (1’)	165.70		166.72	
2’ & 6’	129.60	7.60	128.63	7.62
3’ & 5’	128.40	7.70	128.05	7.71
4’	132.70	7.55	134.95	7.55

Key: no[c]= not observed
*= Tatiana *et al.,* (2001)

