ISOLATION, CHARACTERIZATION AND ANTIMYCOBACTERIAL POTENCY OF A FURAN DERIVATIVE FROM THE CHLOROFORM CRUDE EXTRACT OF *Icacina trichantha* oliv TUBER.

O. U. Akoh*[1], B. C. Anyanwu [1], O. M. Mac-kalunta [1]

[1]Department of Chemistry, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria.
*Corresponding Author: onyilac@gmail.com
anyanwubenedict5@gmail.com
marygemkal@gmail.com

ABSTRACT

Spectroscopic analysis of a pure isolate (OAU 2, a furan derivative) by column chromatography from the chloroform crude extract of *Icacina trichantha* tuber was done using FT-IR, ^{1}HNMR, ^{13}CNMR, COSY and was established by literature as well as the investigation of antimycobacterial potency. This anitmycobacterial activity revealed that this isolate actually has activity against DR-TB Strain and H37RV Strain which are American type culture collection strain and multi-drug resistant strains of *M. tuberculosis* being the test organisms. Hence, confirming the folk believe of the use of *I. trichantha* tuber in the treatment of "serious cough"

Keywords: *Icacina trichantha*, biological activity, spectroscopy, antimycobacterial activity, anti-tuberculosis assay, minimum inhibitory concentration, functionality.

INTRODUCTION

Icacina trichantha Oliv. (*Icacinaceae*), characterized as being a drought-resistant plant originates from Central and West Africa, is a medicinal shrub utilized by the people particularly the ethnic societies in Nigeria. It is called "Urumbia" or "Eriagbo" (denoting its emetic effect) among the Igbo tribe of Nigeria, or "Gbegbe" (connotating to purify) by the Yoruba tribe of western Nigeria.

Icacina trichantha Oliv. (*Icacinaceae*) is described as a perennial shrub which usually occurs in forest regeneration, field crops, and waste regions in many parts of Nigeria. The *Icacinaceae* belong to a family of flowering plants, [6], the leaves of *Icacina trichantha Oliv* have alternate, simple and broadly-elliptical arrangement. The stem is irregular, partially wooden, curved in cross-section, characterized by soft brown hairs and ascends from an underground tuber which also possesses soft brown hairs [1]. Traditional medical healers make use of the tubers to treat several medical conditions including rheumatism, malaria, constipation, poisoning, and toothache as well as to induce abortion and emesis [2]. The juice extracted from the tuber can be used for curing mumps [10]. The Yoruba tribe of Nigeria utilize the leaves for chieftaincy coronation [5]. The

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Igbos use the leaves for enfolding processed oil bean seeds traditionally called 'ugba' The plant is frequently used as drug in rural communities in Nigeria. They believe that the plant is an aphrodisiac [9]. *Icacina trichantha* tuber has been found to be rich in starch and contains alkaloids, saponins, steroids, tannins, and cardiac glycosides. 19-nor-pimarane-type diterpenes which includes *Icacinal, Humirianthol, Hydroxyicacino, methoxyhumirianthol, Icacilactone, Icacenone, Humiriantholide C, Icacenone and Icacinlactone A-J* among others has been isolated from this tuber

1.1: Botanical classification of *Icacinaceae*

Kingdom:	Plantae
SubKingdom:	Tracheophytes
Subdivision:	Angiosperms
Class:	Eudicts
Subclass:	*Asterids*
Order	*Icacinales*
Family:	*Icacinaceae*

Mycobacterium tuberculosis is a unique effective bacterial parasitic micro-organism of humans, infecting over 33% of the world population. This amazing accomplishment is because pathogenic mycobacteria can thrive in the unfriendly environment of a macrophage, even in the presence of a particular T cell immune response. Consequently, an insignificant amount of viable mycobacteria may persevere at the place of infection. At the expiration of dormancy, this micro-organism may start to reproduce, and this leads to the recurrence of infection and medical disease. Notwithstanding numerous years of research, the effector mechanisms by which *M. tuberculosis* is exterminated, when the immune response assumes its most effective form of defense, remain argumentative [4].

MATERIALS AND METHODS

Sample collection and identification

The plant was harvested at Umunakwukwu Chokoneze Mbaise, Imo state and was identified by Mr. Ibeh Ndukwe of plant taxonomy, Department of Forestry, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. A herbarium number ICA DALZ 1094 was assigned to the plant.

Sample preparation and isolation

The tubers were washed to remove sand, after which they were peeled and grated. The grated tuber was air dried for four weeks and weighed. The weight was found to be 1.2 kg. The extraction was done by maceration with chloroform for 72 hours, thereafter, it was decanted, filtered using

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Whatman No.1 filter paper and concentrated under reduced pressure using rotary evaporator to afford 9 g of the crude extract. The column used was 280 mm high and 35 mm in diameter. The slurry was formed with silica gel, crude mixtures (crude extract with 15 ml of chloroform) and celite in the ratio of 1:30:3. The slurry was left to evaporate for 48 hours in the laboratory. After which it was used in the column chromatography. The column chromatography was ran according to the standard procedure using solvents of different polarities, from hexane to chloroform to ethyl acetate and then methanol. 49 eluents were collected and each of the eluent spotted in aluminum precoated TLC plate using capillary tube. TLC on the chloroform column fractions 45 and 46 (butter colour eluted from 10:90 (ethyl acetate: methanol) both gave the same RF value of 0.51 in solvent mixture of hexane: ethyl acetate: methanol in the ratio of 2:1:1 (8ml) and so pulled together and named **OAU 2**. It was packaged in a sample bottle and sent to (ACEPRD) Jos for spectra analysis.

Spectroscopic analysis

Spectroscopic studies was carried out to elucidate the structure of the isolated compound. The corresponding spectra of FT-IR, HMNR, 13CNMR, COSY were recorded with $CDCL_3$ as solvent.

Methodology for anti-tuberculosis assay

- Plant Materials:
- Extraction of Plant Materials
- Mycobacterial Strain Inoculum Preparation

Mycobacterial strain Inoculum preparation was done as described by [1]. *Mycobacterium tuberculosis* H37Rv, an American Type Culture Collection strain (ATCC 27294) and Multi-drug resistant strains of *M. tuberculosis* were used to investigate the antimycobacterial activity of the plant extracts. The Mycobacterial strains were first subcultured on Lowenstein-Jensen medium for 14 days, and pure colonies were then inoculated in sterile liquid medium (Middlebrook 7H9) supplemented with glycerol, Tween 80 and OADC (mixture of Oleic acid, Albumin, Dextrose and Catalase). Inoculum was incubated in a shaking incubator at 37^0C with5% CO_2 for 14 5 days before adjusting the turbidity of the suspension to 0.5 MacFarland (1.5 x 108 CFU/ml). The adjusted suspension was used as inoculum to determine the inhibitory activity of the extracts.

Determination of Minimum Inhibitory Concentration using Microplate Alamar Blue Assay

The antimycobacterial activity of the plant extracts was determined using the microplate Alamar Blue Assay (MABA) as previously described by [8 and 7]. Briefly, the plant extracts were dissolved in 5% dimethyl Sulphoxide (DMSO) and then sonicated for 30 minutes to ensure total solubility concentration of 100 mg

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was used as working solutions. Dilutions of the test extracts and an approved anti-tuberculosis drug (Isoniazid and Moxifloxacin were used as standard control drugs) were made with varying concentrations. Hundred (100) micrograms of each concentration of the plant extracts and standard drugs were dispensed into corresponding sterile 96 wells microtitre plates with the exception of the wells used for growth control (contain microorganisms and culture media only) and negative control (containing only culture media). *M.tuberculosis* H37Rv amd *M.tuberculosis* resistant strains were also added to the corresponding 96 wells plates with the exception of the wells used for negative control, final volume in each well were 200 µl.

Plates were covered and sealed with parafilm and incubated at 37^{o}C for 7dyas. At day 7, 32.5 µl. of Alamar blue dye was added to all the 96 wells then incubated for 19 hours at 37^{o}C in the dark. After 19 hours, plates were read, and validation of the test was conditioned by oxidation-reduction reaction of Alamar blue dye and bacterial cells indicated by control wells (growth control, media control, and standard drug control wells). For the tested samples, the blue colour is synonymous to lack of bacterial growth and therefore indicating the anti-tuberculosis activity. A turn to pink means bacterial growth. The MIC was defined as the lowest concentration, which prevented a colour change from blue to pink.

RESULTS AND DISCUSSION

Spectroscopic results.

Table 1 presents FT-IR chart for isolate OAU 2 while Table 2 indicates ^{1}H NMR and ^{13}C NMR chemical shift for isolate OAU 2 which was recorded in $CDCl_3$

Table 1: Infrared spectral data of OAU2

The frequencies of the functional groups present in OAU2 is represented thus;

Functional group	**Absorption frequency (CM^{-1})**
OH/NH_2	3353.39
CH stretching	2924.57/2857.60
C = C	1661.54/1589.42
C = O	1743.96
CH bending	1450.22
CH_3 bending	1351.43
C – N	1244.93
C – O	1068.14

^{1}H NMR and ^{13}C NMR of OAU2 is represented in Table 2

ISSN 0795 - 22066

Table 2: ^{1}H NMR and ^{13}C NMR of OAU2

Position	Signal (PPM)	Type of proton	13C Signal (PPM)	Type of carbon
1	-	-	170.20	C = o
3	2.62 s	2H	99.58	CH_2
3a	-	-	143.71	=CH
4	7.67 s	1H	113.6	=CH
5	2.21 m	1 H	138.39	= CH
6	2.48 m	1 H	42.46	CH
7	2.54 m	2 H	2 60.13	CH_2
8	3.34 m	1 H	67.66 111.25	CH/CN
8a	-	-	140.10	= CH
9	7.71 s	1 H	124.92	= CH
9a	-	-	148.35	= CH
1^{1}	10.44 m	1H	194.78	^{19}CH
2^{1}	2.57 m	2H	63.68	CH_2
3^{1}	5.30 m	1H	88.50	$CH\text{-}_{OH}$
4^{1}	5.09 dd	1H	108.59	= CH
5^{1}	3.74	1H	103.20	= CH
1^{11}	1.28	3H	18.59	CH_3
2^{11}	1.28 m	2H	26.62	CH_2
8	1.65 s	NH_2	-	-
	3.80s	OH	-	-

Infrared spectrum of OAU2 in Table 1 revealed the presence of an alcohol functional group overlapping with absorption of amine at 3353. 39 cm^{-1}, carbonyl stretching of lactone was seen at 1743. 96 cm^{-1}, alkane CH stretching at 2924. 57 cm^{-1} and 2857.60 cm^{-1}. The absorption recorded at 1661.54 and 1589. 42 cm^{-1} is characteristic of strong in plane NH_2 scissoring absorption, the region of this absorption also indicates the presence of unsaturated functional group of alkene and aromatic compounds. Absorption band at 1244. 93 cm^{-1} is indicative of C-N amine group, bands at 1450.02 and 1351. 41 cm^{-1} is assigned CH_2 and CH_3 bending, while the 1068. 14 cm^{-1} accounts for the C-O of lactone and alcohol.

In the ^{1}HNMR spectrum of compound OAU2 in Table 2, the 1H signal at 10. 44 ppm has been assigned to aldehydic proton at position 1^{1}, the α hydrogen that is one bond away from the

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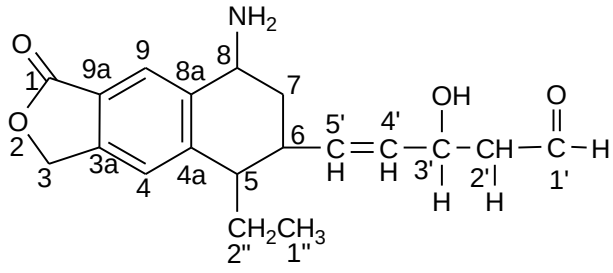
aldehyde proton split the aldehydic proton giving rise to a triplet, recorded as a multiplet in the spectrum. Position H-8 (methine proton) that is directly attached to the amine group resonated at 3.38 ppm (m), this hydrogen is deshielded by the electron withdrawing effect of the nitrogen. Aromatic protons resonated down field at 7.67ppm (H-4) and 7. 71 ppm (H-9). Methine protons at position 4^1 and 5^1 resonated at 5.09 ppm and 3. 74 ppm respectively. The H-4^1 chemical shift down field is as a result of the electron withdrawing effect of the hydroxyl group that is one bond away from 4^1. The ethyl attachment at position 5 was observed as 5H multiplet down field, this phenomenal indicates an over lapping signal arising from the splitting of CH_2/CH_3 protons. Amine proton gave characteristic singlet peak at 1.65 ppm

^{13}CNMR spectrum showed signal for carbonyl of aldehyde at 194. 78 ppm, carbonyl of lactone at 168. 50 and aromatic compound at 121 ppm. Following the critical analysis of OAU 2 spectra result, the structure of the isolated compound is proposed as.



Molecular Formular: $C_{19}H_{24}NO_4$

Molecular Mass = 341 $gmol^{-1}$

5-(8-amino-5-ethyl-1-oxo-1,3,5,6,7,8-hexahydronaphtho[2,3-*c*]furan-6-yl) 3-hydroxyl-pent-4-enal

Fig 1: Proposed structure of OAU 2.

The compound was eluted from the column using at solvent system ethyl acetate/methanol (10:90), the TLC gave R_f of 0.51, using a solvent system of hexane/ethyl acetate/methanol (2:1:1)

ISSN 0795 - 22066

Antimycobacterial assay result.

Table 3: Anti-tuberculosis activity of OAU 2 - Minimum inhibitory concentration result

Plant isolate	MIC (mg/ml) DR-TB Strain	MIC (mg/ml) H37RV Strain
OAU 2	10.34±3.53[b]	6.28±0.06[a]
Control 1 – Moxifloxacin (for MDR-TB Strain)	4.25±1.95[a]	
Control 2- Isoniazid (for H37RV Strain)		1.31±0.46[a]

Values are presented as mean ± standard deviation (n = 3); and values with a different letter superscript are significantly ($p < 0.05$) different from paired mean with the column.

Mycobacterium tuberculosis infections are transmitted through breathing of infective bacilli. Bacteria are inhabited by alveolar macrophages and develop infection centers in the lung tissue. These centers increase through bacterial development and production of macrophages and lymphocytes that make up the granuloma that describes this infection. OAU 2 showed activity against MDR-TB strain as compared to the control (Moxifloxacin). OAU 2 had low mean MIC value against H37RV strain as compared to the control drug (Isoniazid). Hence OAU 2 had appreciable antimybobacterium activity against the test organisms.

CONCLUSION

Plants have been employed for ages for the treatment of various diseases by the traditional medical practitioners. The tubers of *I.trichantha* have been reported to contain flavonoids, tannins, alkaloids, saponnins, phenols and cyanide, with flavonoid being the most abundant. Theses phytochemicals no doubt are responsible for its use in the treatments of various illments. The pure isolate (OAU 2) from the chloroform extract went through the in-vitro anti-mycobacterium anaylsis to acertain its potency for the folk stories, however, it came out successful proving its ability for the treatment of tuberculosis and related upper respiratory illments.

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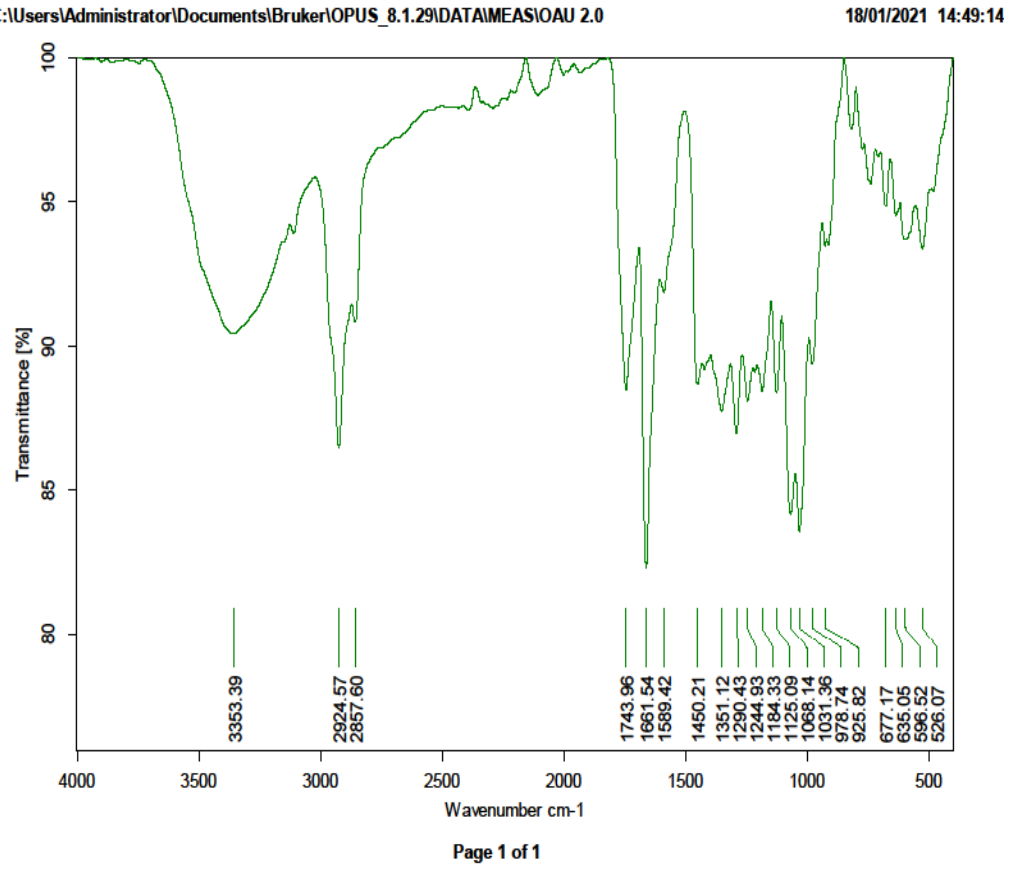

Fig 2: FT-IR spectrum of compound OAU 2 from the tuber of *I. trichantha*

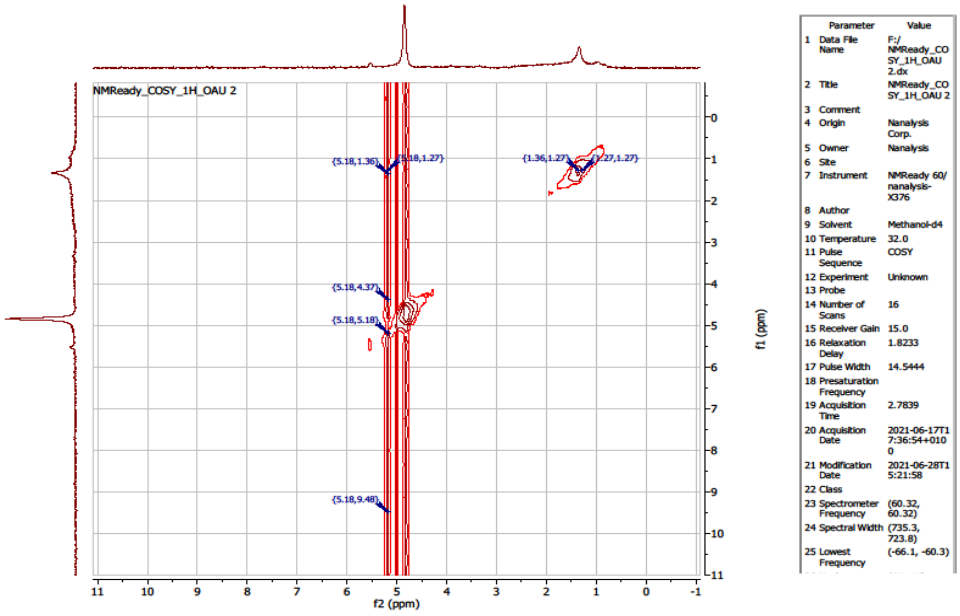

Fig 3: COSY NMR spectrum of compound OAU 2 from the tuber of *I. trichantha*

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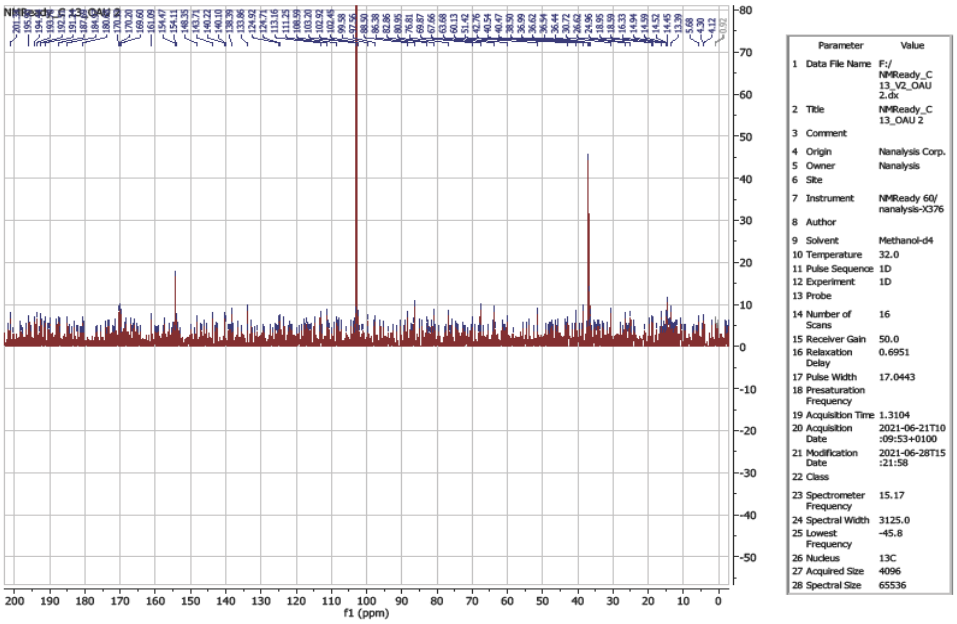
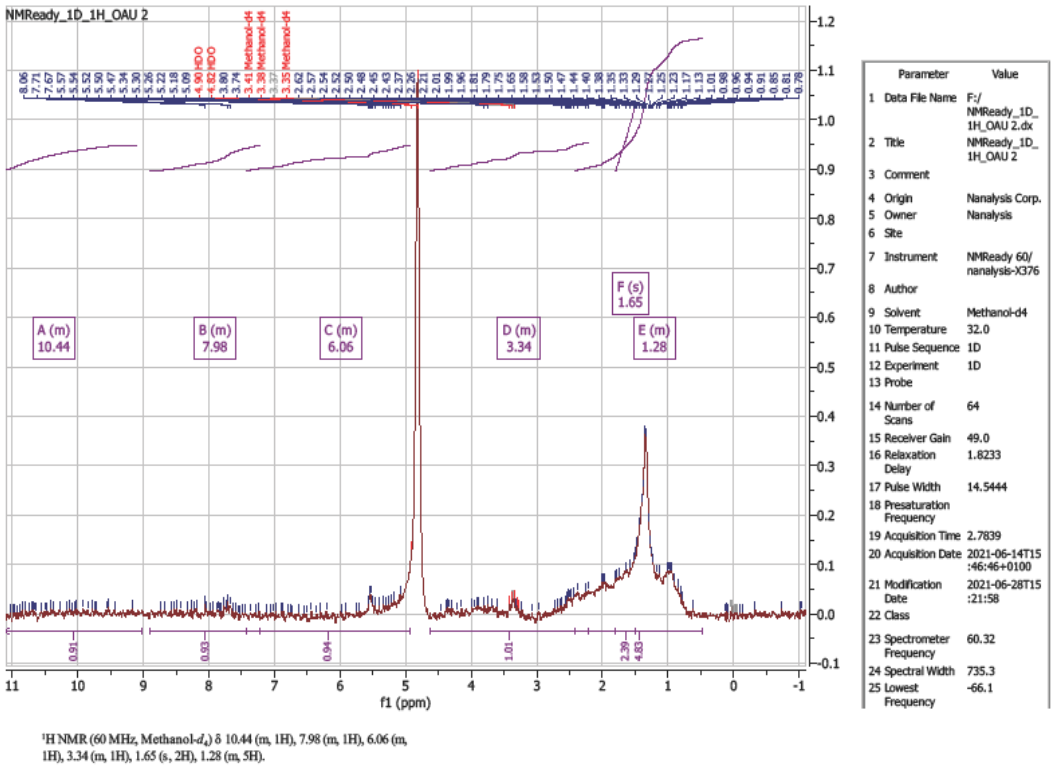


Fig 4: ^{13}C-NMR spectrum of compound OAU 2 from the tuber of *I. trichantha*



^{1}H NMR (60 MHz, Methanol-d_4) δ 10.44 (m, 1H), 7.98 (m, 1H), 6.06 (m, 1H), 3.34 (m, 1H), 1.65 (s, 2H), 1.28 (m, 5H).

Fig 5: ^{1}H-NMR spectrum of compound OAU 2 from the tuber of *I. trichantha*

ISSN 0795 - 22066

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