ISSN: 0975-833X

Availableonlineathttp://www.journalcra.com

International Journal of Current Research
Vol. 11, Issue, 09, pp.7023-7028, September, 2019

DOI: https://doi.org/10.24941/ijcr.36258.09.2019

INTERNATIONAL JOURNAL OFCURRENTRESEARCH

RESEARCH ARTICLE

ISOLATION AND CHARACTERIZATION OF LEAVES EXTRACT OF *VERNONIA AURICULIFERA*

***Temesgen Orebo**

Department of Chemistry, College of Natural and Computational Science, Mizan Tepi University, Ethiopia

ARTICLEINFO

Article History:
Received 19th June, 2019
Received in revised form
20th July, 2019
Accepted 24th August, 2019
Published online 30st September, 2019

Key Words:

V. Auriculifera, Extraction, Isolation and Characterization.

ABSTRACT

The present study was carried out on the Isolation and characterization of the leaves extract of V. auriculifera because; the plant is used for traditional medicine especially its leaf juice is locally known by blood clotting and healing wounds. In the present study, the powdered leaf of plant was sequentially extracted with organic solvents: petroleum ether, chloroform, chloroform/methanol (1:1), methanol and water respectively. Repeated use of solvent extraction followed by Column Chromatography and Thin layer Chromatography, leaf extract of V. auriculifera yielded one compound which was characterized as (E)-methyl 11-hydroxy-1-(4-hydroxyphenyl) tetradec-7-en-14-onenamide using UV, FT-IR and NMR spectral data and by comparing with literature reports.

Citation: Temesgen Orebo. **2019.** "Isolation and Characterization of Leaves Extract of Vernonia auriculifera", *International Journal of Current Research*, 11, (09), 7023-7028.

INTRODUCTION

Organic chemistry as it stands today has developed largely from the chemistry of natural products. The study of natural products has been and continues to be a major deriving force in the development of the fields of organic and medicinal chemistry. Natural products are expected to play an important role as one of the major sources of new drugs in the years to come because of their incomparable structural diversity, the relatively small dimensions of many of them, and their "drug like'' properties, *i.e*. their ability to be absorbed and metabolized (Sarker *et al*., 2006). They are once served man as the source of all drugs, and higher plants provided most of therapeutic agents. Today, 50% of the small pharmaceutically important molecules discovered during the period from 2000 to 2010 have been connected to the field of natural products (Newman and Cragg, 2012). Obviously natural products will continue to be extremely important as sources of medicinal agents. The genus *Vernonia* is one of the largest genera of flowering plants and contributed that the highest number of medicinal plants in the *Asteraceae* family (Mirutse *et al*., 2009), which includes more than 1000 species distributed widely in the tropical and sub-tropical region of Africa, Asia and America. It has two major centers of origin, South America and tropical Africa, with approximately five hundred species found in Africa and Asia, 300 in Mexico, Central and South America and 16 in the USA (Oketch-Rabah *et al*., 1997). Of the 500 species from Africa, 49 species are endemic to Ethiopia (Mesfin, 2004). It has been difficult to establish relationships with in the *Vernonia* species due to their overlapping characteristics. This difficulty in classifying the species gave rise to the nickname, the 'evil tribe'. As such, there has been no phylogeny proposed for the tribe *Vernoniea* and only a few relationships had been suggested even among the best known species (Jones, 1977). Generally, *Vernonia* species are used in the treatment of infectious and parasitic diseases. The majority of these plants are used as vegetables. The vegetables have a bitter taste, hence the name "the bitter genus *Vernonia"* (Aliyu *et al*., 2011). Hydroperoxides of unsaturated fatty acid methyl esters previously isolated from *Vernonia auriculifera* Hiern were found to have lethal toxicity (Keriko *et al*., 1995a). Plant growth stimulators have also been identified from this plant. *Vernonia auriculifera* has a wide variety of applications in traditional medicine. A drop of the juice squeezed from the crushed stem bark, inserted into the nostrils, is known to relieve headache (Kusamba, 2001). The Kikuyu people of central Kenya use the leaves of this plant as a wrap for pounded material used as a poultice (Kokwaro, 2009). Heated crushed leaves of *Aspilia mossambicensis,* are tied in the leaf of *V. auriculifera,* and then applied over the eyes to treat conjunctivitis (Muthaura *et al*., 2007). A cold water infusion of *V. auriculifera* is administered orally in Uganda and Kenya to treat fever associated with viral and bacterial infections (Muthaura *et al*., 2007). In Congo, the dried and pounded leaves are applied on wounds (Keriko *et al*.,

****Corresponding author:*** Temesgen Orebo,
Department of Chemistry, College of Natural and Computational Science, Mizan Tepi University, Ethiopia.

1995a). In Ethiopia, the roots are used to treat toothache (Mirutse *et al*., 2009) and snake poison (Mesfin *et al*., 2009). Although several drugs have been developed based on natural product chemistry, to the best of our knowledge the current study was undertaken primarily isolates and characterizes compounds from the leaves of plant since leaves were found to be the most frequently used plant part in Ethiopian traditional medicine (Kalayu *et al.,* 2013). The plant was selected for this study, because it is used as traditional medicine in Kambata and Hadya Zones. The people in these area recommend it, especially its leaf juice, *''for healing wounds and blood clotting''*. Although, the people use this plant as a traditional medicine for blood clotting and healing wounds, still the chemical component of the plant was not studied. However, up to now many people do not avoid using unidentified crude drug. For this reason the researchers are intended to perform isolation and characterization of the leaves of plant. Finally, this study looks into the fundamental scientific bases for the use of traditional medicinal *V. auriculifera* plant by determining the phytochemical constituents present in the crude extract of this plant. Hence, an extensive research and development work should be under taken on the leaves of *V. auriculifera* and its product for their better economic and therapeutic utilization. Therefore, the study will help people in the area to upgrade the indigenous knowledge of this plant as well as to make the traditional knowledge of the plant scientific and confirm the role of study for Pharmaceutical Companies. This will also lead to the domestication and preservation of this plant in the world as the medicinal plant.

MATERIALS AND METHODS

General Experimental Procedures: The solvents used in extraction and column chromatography including: *n*-hexane, petroleum ether, chloroform, ethyl acetate and methanol were pure HPLC grades. TLC analyses were carried out on TLC plates 0.2 mm thick layer of Merck silica gel 60 (mesh) coated on aluminum foil and pre-coated Silica gel plates (Merck, Kiesel gel 60 F_{254}, 0.25mm). Compounds on TLC were detected using UV light (254 and 365 nm); while column chromatography was performed using silica gel (230-400 mesh, Merck) in HU Organic Chemistry laboratory. The FT-IR samples were prepared using spectral grade KBr and made into pellets and measured on Spectrum 65-FT-IR (Perkin Elmer) in the range between 4000cm^{-1} and 400 cm^{-1} in Addis Ababa University. ^{1}H-NMR ^{13}C-NMR and DEPT-135 spectra were recorded on a bruker advance spectrometer in the Addis Ababa University, NMR laboratory, Ethiopia at 400 MHz (^{1}H) and 100 MHz(^{13}C) at room temperature using $CDCl_3$ as solvent and TMS as the internal standard. The chemical shifts reported in δ (ppm) units relative to TMS signal.

Collection and preparation of plant leaves for extraction: The leaves of *V. auriculifera* plant were collected in August 2014 from Angacha district, which is located at 260 km south of Addis Ababa in Sothern Nation Nationalities and Peoples Regional State (SNNPR), Ethiopia. A voucher of specimen was identified as *Vernonia auriculifera* in the herbarium of Haramaya University. The leaves were dried in an open air protected from direct exposure to sun light. The dried plant leaves were finely grounded and ready for extraction.

Solvent Extraction at room temperature (Cold Extraction): 2 kg of air dried leaves powder of *V. auriculifera* was first soaked with 5 L petroleum ether for 72 h at room temperature. The mixtures were filtered and the filtrate was concentrated under reduced pressure and temperature of 40^{o}C using rotary evaporator and afforded 268 g deep yellow crude extract. The marc, remaining in the extraction flask after exhaustive extraction of petroleum ether, was dried and 1 kg of powder was extracted with 3 L chloroform after soaking for 72 h at room temperature. Then the extract was concentrated in rotary evaporator and afforded (146.4 g) deep blue black crude. The marc remaining was then extracted using $CHCl_3$: MeOH (1:1) following similar procedure and yielded 123.5 g of dark green crude extract. Then, 3L of methanol was added to the dried marc in the flask and afforded 117.8 g of deep green extract. Lastly, 2 L of distilled water was added to the marc in the flask and gave 158.7 g of dark violet crude.

Isolation and Purification of Compound: 100 g of silica gel was mixed with 200 mL of *n*-hexane and the slurry was used to pack the column. Then 5 mL of *n*-hexane was added to the 20 g concentrated chloroform crude extract and mixed well and adsorbed. The concentrated adsorbed extract was then subjected to the top of packed silica gel column using a dropper. The column was then eluted with increasing polarity of solvent systems (Table 1).Then, after the column chromatography using solvent as the eluting system; 36 fractions were collected. Fraction C-1 was obtained from 100% hexane which shows single spot on TLC plate. Fractions C-2 and C-3 were combined by observing their TLC spots. Fraction C-4 kept separately and C-5 and C-6 were showed similar spot on TLC and kept for characterization. From chloroform crude extract after column chromatography five isolated pure compounds and different mixtures were obtained. From these the compound, VAH-C1 is characterized.

RESULTS AND DISCUSSION

Structural Elucidation of compound VAH-C1: The chloroform extract of the leaves of *V. auriculifera* was subjected to a series of chromatographic techniques, leading to the isolation of a compound VAH-C1 yield (93 mg). Compound VAH-C1 is amorphous, deep blue black and obtained from column chromatography of chloroform extract. For isolation the best mobile phase selected by TLC was chloroform: methanol (8:2) and its R_f value was determined as 0.63. UV-Vis spectrum of this compound showed absorption peak at 234 nm indicating the presence of aromatic conjugation whereas the maximum peak at 276 nm indicates the presence of carbonyl group.

The FT-IR spectrum of the compound displayed absorption bands indicating the presence of hydroxyl (3435 cm^{-1}), carbonyl (1711 cm^{-1}), the methyl and methylene C-H stretching a sharp peak at 2919 and 2850 cm^{-1} respectively. The absorption band around 1600 cm^{-1} and 1450 cm^{-1} and the weak band around 3050 cm^{-1} are characteristic of aromatic functionalities in the compound. The ^{1}H-NMR spectrum of VAH-C1 exhibited signals at δ 2.40 (3H, t, H-5 J = 15.09 Hz) due to a methine proton which is further confirmed by ^{13}C signal at δ 68.1. This carbon is attached to an OH group which was also supported by FT-IR absorption at 3445 cm^{-1}. Two signals were observed for four aromatic protons at δ 7.75 (2H, overlapped, H-3`, and 5`) δ 7.55 (2H, overlapped, H-2`, and 6`) which suggests the presence of a 4-hydroxy phenyl group.

Table 1. Solvent system used for chromatographic isolation of compounds

Fractions	Solvents	Ratio of solvents	Volume (ml)	Code of fractions
1	*n*-hexane:chloro form	10:0	20	C1
2	,,	9:1	20	C2
3	,,	8:2	20	C3
4	,,	7:3	20	C4
5	,,	6:4	20	C5
6-36	:	:	:	:

Table 2. Comparison of ^{1}H-NMR spectral data of compound VAH-C1 with literature reports (Lishuang *et al.,* 2013; Farid *et al.,* 2014)

Position of H	Chemical shift (δH)		
	VAH-C1	Literature value	Remark
15	0.9 (3H)	0.8 -0.9 (3H)	$-CH_3$
1, 2, 4, 6, 7, 10-14	1.25-2.39 (20H)	1.25-2.64 (20H)	$-CH_2CH_2-$
5	3.98 (1H)	3.98 (1H)	Hydroxymethine
7,8	5.35 (2H)	5.23-5.29 (2H)	Olefinic proton
2', 3', 5', 6'	7.55 δ-7.75 δ (3H)	7.13- 7.24 (3H)	Aromatic proton

Table 3. Comparison of ^{13}C NMR and DEPT-135 data of compound VAH-C1 with literature reports (Lishuang *et al.,* 2013; Farid *et al.,* 2014)

Carbon No.	^{13}C-NMR δ data	Reference data	Type of carbon	Remark
1	29.3	29.3	CH_2	
2	38.8	36.3	CH_2	
3	22.7	22.6	CH_2	
4	31.9	31.5	CH_2	
5	24.6	22.8	CH_2	
6	29.3	29.3	CH_2	
7	128.5	128.7	CH	Olefinic
8	131.0	130.9	CH	Olefinic
9	29.3	29.4	CH_2	
10	29.7	29.7	CH_2	
11	68.1	67.2	CH	Hydroxmethine
12	24.6	22.8	CH_2	
13	29.6	29.6	CH_2	
14	178.0	177.6	Quaternary	Carbonyl carbon
15	14.12	14.1	CH_3	
1'	129.0	129.0	Quaternary	
2'6'	128.7	128.7	CH	Aromatic
3'5'	138.0	139.0	CH	Aromatic
4'	145.0	144.0	Quaternary	

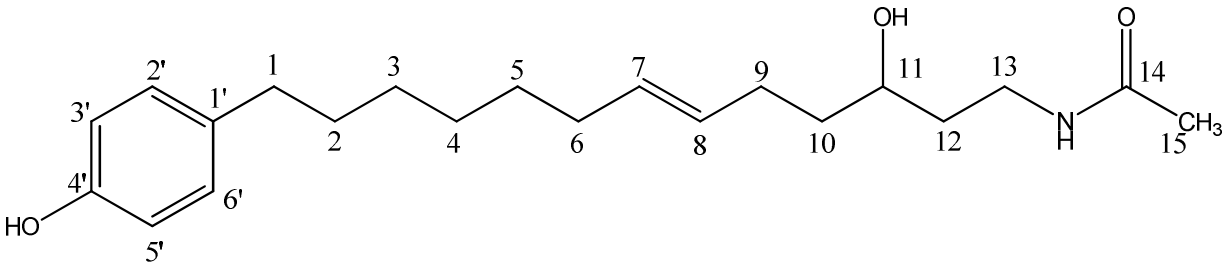

Figure 1. Proposed structure of compound VAH-C1

This was confirmed also by the presence of an oxygenated phenyl carbon at 145 C-4', 138.00, 128.70 and 129.0 (6 aromatic carbons) in the ^{13}C-NMR spectrum (Table 3). The ^{13}C-NMR and DEPT-135 spectra of compound VAH-C1 revealed that the presence of 13 signals for 21 carbons that included one methyl, 10 methylenes, two signals for four aromatic methines, two aromatic quaternary, three methine protons (one hydroxyl at δ 68.1 and two olefinic protons at δ 128.5 and δ 131 indicating carbon signals unsaturation). The presence of an extra double bond was also revealed from the ^{1}H-NMR spectrum at δ 5.4, the peak at δ 178 is due to carbonyl carbon. Furthermore, the presence of hydroxymethine carbon at (δ 68.1, C-11) and carbonyl carbon at (δ 178, C-14) indicated that compound VAH-C1 is in line with the ^{1}H-NMR spectrum and its NMR spectra data is close to literature report (Lishuang *et al*., 2013; Farid *et al*., 2014). Based on the above evidences and comparison with literature reports the *(E)*-methyl 11-hydroxy-1-(4-hydroxy- phenyl) tetradec-7-en-14-one amide structure was proposed.

Conclusions and Recommendation

After repeated successive solvent extraction and column chromatography, different compounds were isolated from the *V.auriculifera* leaf extracts. Among the isolated compounds, one compound coded as VAH-C1, was characterized (*E*)-methyl 11-hydroxy-1-(4-hydroxy phenyl) tetradec-7-en-14-one amide.

Other fractions contain chlorophyll as well as mixed compounds even after combining and attempts to purify. To the best of our knowledge three compounds are reported here for the first time. As the researchers' observed during analysis of chemical component, the plant is rich with different secondary metabolites. The TLC analysis of most fraction of methanol extract indicates flavonoid but due to lack of Preparative Tin layer Chromatography isolation of pure flavonoid compounds could not be achieved. Therefore, any researcher interested to study on this plant better to concentrate on the methanol and water extracts since based on TLC analysis of spots these extract have more compounds rather than nonpolar solvent extracts. Further studies are needed on this plant under study to characterize and elucidate structures of more bioactive compounds. Further studies should be carried on structure activity, relationships and mode of action may be a guide to a better understanding of the relationships between the structures and antimicrobial activities of these compounds. In addition to this the following recommendations were made.

- Further bioassay guided isolation and characterization work should be done to fully characterize more bioactive constituents of the plant leaf extracts to identify all active compounds against a variety of bacteria and fungi.
- It is recommended that further similar studies should be conducted on other parts of the plant such as root, stem, bark, etc.
- Toxicity studies of the plant should also be done to determine the safety indices of the extracts.

REFERENCES

Aliyu, A.B., Musa, A.M., Abdullah, M.S., Ibrahim, H. and Oyewale, O.A. 2011. Phytochemical screening and antibacterial activities of *V. ambigua, V. blumeoides, V. oocephala* (Asteraceae). *Acta Poloniae Pharmaceutical Drug Research*, 68: 67-73.

Chien-Chang Shen, Yuh-Chi Kuo, Ray-Ling Huang, Lie-Chwen Lin, Ming-Jaw Don, Tun Tschu Chang and Cheng-Jen Chou. 2003. New Ergostane and Lanostane from Antro dia Camphorata. *Journal of China Medicine*, 14(4): 247-258.

Damtoft, S., Rosendal, W., Jensen and Nielsen, B.J. 1981.^{13}C and ^{1}H NMR Spectroscopy as a tool in the Configurational Analysis of Iridoid Glucosides. *Journal of Phytochemistry,* Farid, A.B., Mai, H., El-Naggar., Fatma, M., Abdel B. and Mohamed, M.A. 2014. New gingerol derivative and other related compounds from *Zingiber officinale*. *Journal of Drug Discovery and Therapeutics,* 2(13): 53-59. 20(12): 2717-2732.

Feng, W., Zhan-Lin, L., Hong, H.C., Hui-Ming, H., Yong-Kui J. and Sheng-Wang, L. 2011.Two new triterpenoids from the resin of Boswellia carterii. *Journal of Asian Natural Products Research*, 13(03): 193-197.

Haba, H., Lavaud, C., Magid, A.A. and Benkhaled, M. 2009. Diterpenoids and triterpenoids from *Euphorbia retusa*. *Journal of Natural Product*, 72(7): 1258-1264.

Jones, S.B. 1977. *Vernoniea*-Systematic Review. *In*: Heywood, V.H., Harborne, J.B. and Turner B.L. (ed). The Bio and Chem. of Compositae Academic Press, London. pp. 503-521.

Kalayu Mesfin, Gebru Tekle, and Teklemichael Tesfay. 2013. *Journal of Pharmacognosy and Phytochemistry,* 1(4): 37.

Keriko, J.M., Nakajima, S., Baba, N., Isozaki, Y., Iwasa, J. and Karanfa, P.N. 1995a. 8-Desay lvernodalol plant growth regulator from *V .auriculifera* Z. *Natureforsch,* 50(c): 455-458.

Kokwaro, J.O. 2009. Medicinal plants of east Africa. Nairobi: University Press.

Kusamba, C. 2001. Contribution to the inventory of medicinal plants from the Bushi area, South Kivu Province, Democratic Republic of Congo. *Fitoterapia*, 72: 351-368.

Lia, Chang-Jun Linb, Zhan-Xin Zhanga and Zhong-Jian Jia. 2007. Three New Triterpenoids from *Potentilla multicaulis*. *Chemistry and biodiversity*, 4.

Lishuang, L., Huadong, C.,Dominique, S., Xiaoxin, C.,Tin, C.L. andShengmin, S. 2013. 6 Gingerdiols as the Major Metabolites of 6-Gingerol in Cancer Cells and in Mice and Their Cytotoxic Effects on Human Cancer Cells. *Journal of Agriculture and Food Chemistry*, 60(45): 11372–11377.

Mesfin Tadesse. 2004. *Flora of Ethiopia and Eritrea*. Asteraceae Addis Ababa University, Addis Ababa, Ethiopia and Uppsala University, Uppsala Sweden, 4(2): 408.

Mesfin, F., Demissew, S. and Teklehaymanot, T. 2009. An Ethnobotanical study of medicinal plants in Wonago Woreda, SNNPR, Ethiopia. *Journal of Ethnobiology Ethnomed*, 5: 28.

Mirutse, G., Zemede, A. and Zerihun, W. 2009. Medicinal plants of the Meinit ethnic group of Ethiopia: An ethnobotanical study. *Journal of Ethnopharmacology,* 124: 513–521.

Muthaura, C.N., Rukunga, G.M., Chhabra, S.C., Mungai, G.M. and Njagi, E.N.M. 2007. Traditional phytotheraphy of some remedies used in treatment of malaria in Meru District of Kenya. *South African Journal of Botany,* 73: 402-411.

Newman, D.J. and Cragg, G.M. 2012. Natural products as sources of new drugs. *Journal of Natural Product*, 75: 311–335.

Oketch-Rabah, H.A., Lemmich, E., Dossaji, S.F., Theander, T.G., Olsen, C.E., Cornett, C., Arsalan, K. and Christensen, B.S. 1997.Two new antiprotozoal 5-methylcoumarins from *V. brachycalyx*. *Journal of Natural Product,* 60: 458-461.

Sarker, S.D., Latif, Z. and Gray, A.I. 2006. '*Natural Product Isolation'*, in *Natural Product Isolation* 2nded, Human Press, Totowa, New Jersey. pp. 1-25.

Siddiqui, B. S., Farhana Afshan., Sham-Sul-Arfeen and Tahsin Gulzar. 2006. A new tetracyclic triterpenoid from the leaves of Azadirachta indica. Natural Product Research: Formerly Natural Product Letters, 20(12): 1036–1040.

Toshihiro, A., Yoshiyuki, M., Motohiko, U., Manabu, O., Seizo, K., Yumiko, K., Takashi, S.and Takaaki T. 2004. Dehydrotrametenonic Acid and Dehydroeburiconic Acid from Poria cocos and Their Inhibitory Effects on Eukaryotic DNA Polymerase α and β, *Bioscience, Biotechnology, and Biochemistry*, 68(2): 448-450.

Yan, Z and Xiu-Wei Yang. 2008. Poriacosones A and B: two new lanostane triterpenoids from Poria cocos. *Journal of Asian Natural Products Research*, 10(7): 640-646.

Ying-Chi Du, Tung-Ying Wu, Fang-Rong Chang, Wan-Yu Lin, Yu-Ming Hsu, Fu-Ting Cheng, Chi-Yu Lu, Ming-Hong Yen, Ya-Ting Tsui, Hsuan-Lun Chen, Mei-Chin Lu,Yang-Chang Wu. 2012. *Journal of Pharmaceutical and Biomedical Analysis*, 58: 182–192.

Appendix Figures

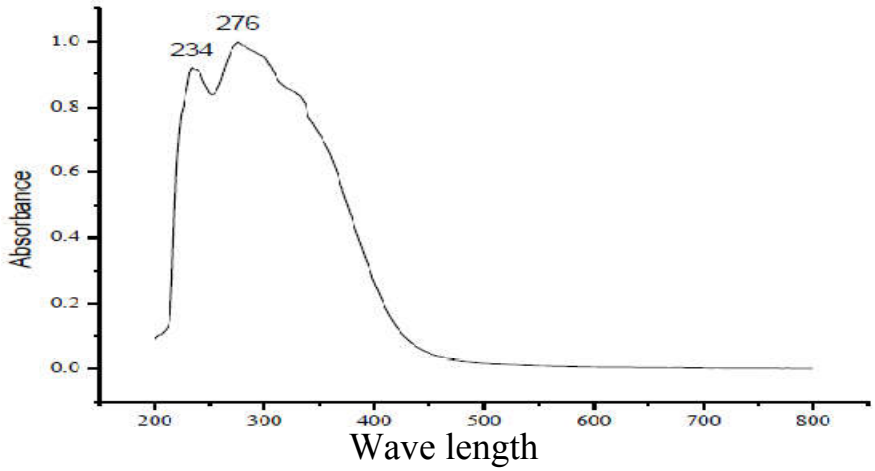

Figure 4. UV- Vis Spectrum of compound VAH-C1

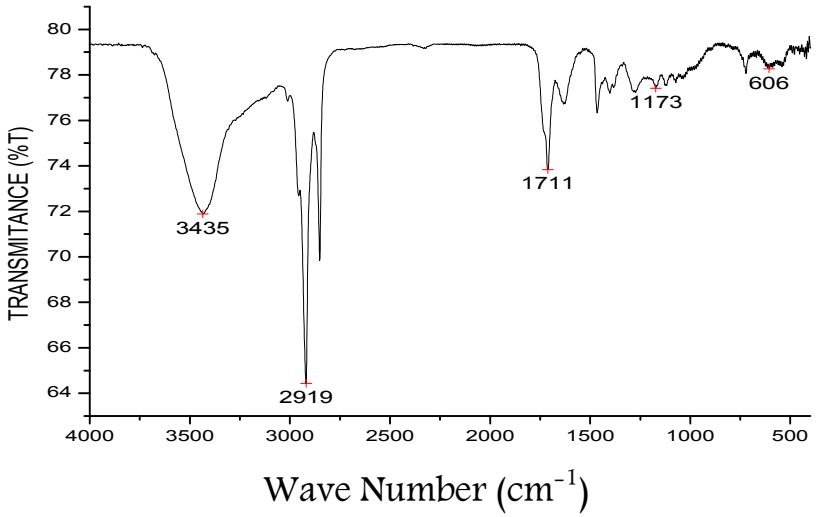

Figure 1. FT-IR Spectrum of Compound VAH-C1

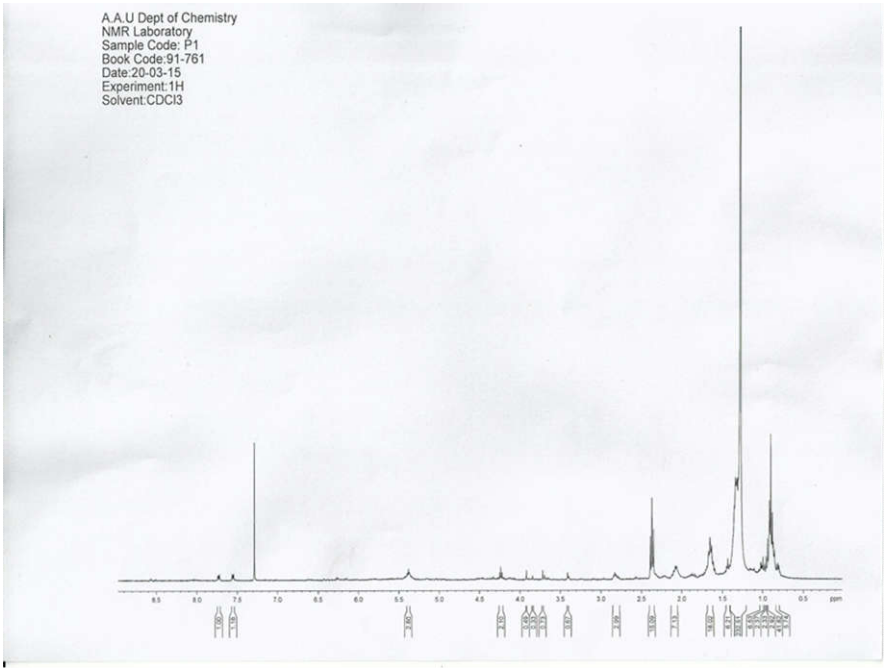

Figure 2.^{1}H-NMR Spectrum of Compound VAH-C1 in $CDCl_3$

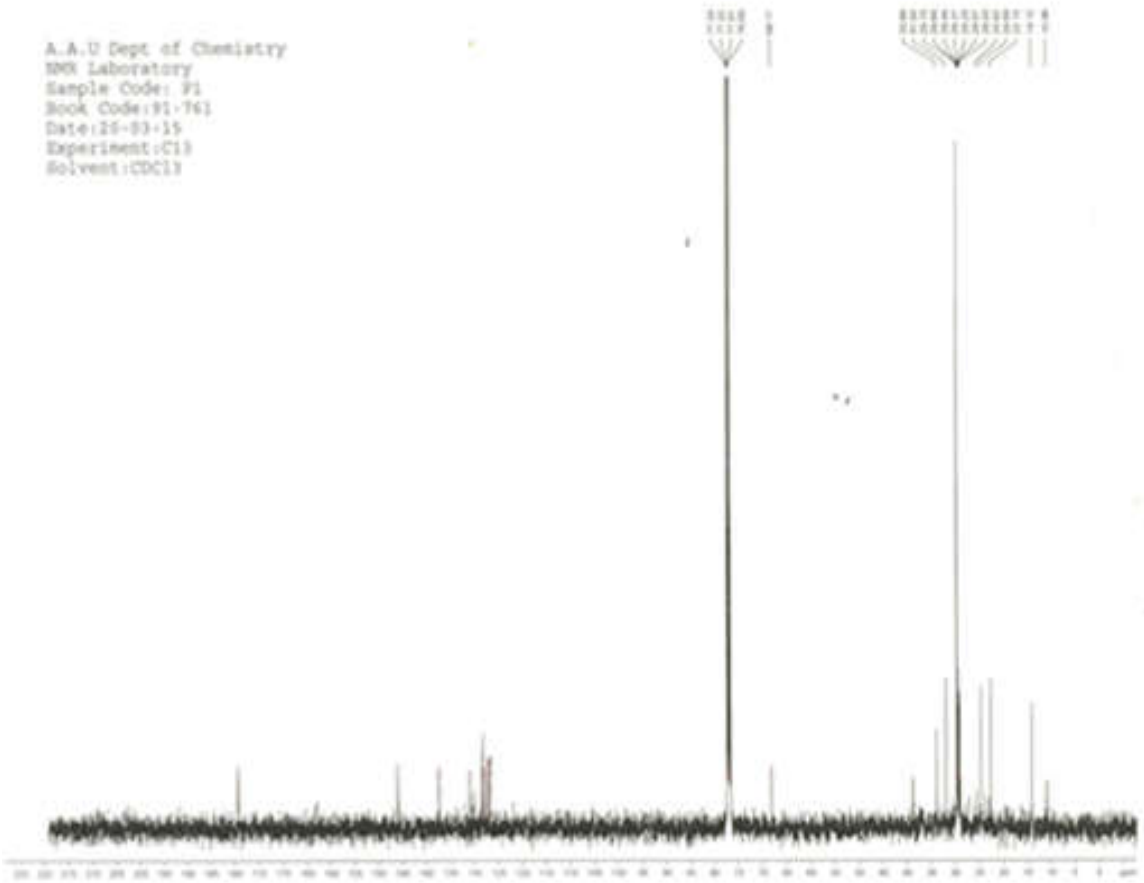

Figure 3.^{13}C NMR Spectrum of Compound VAH-C1 in $CDCl_3$

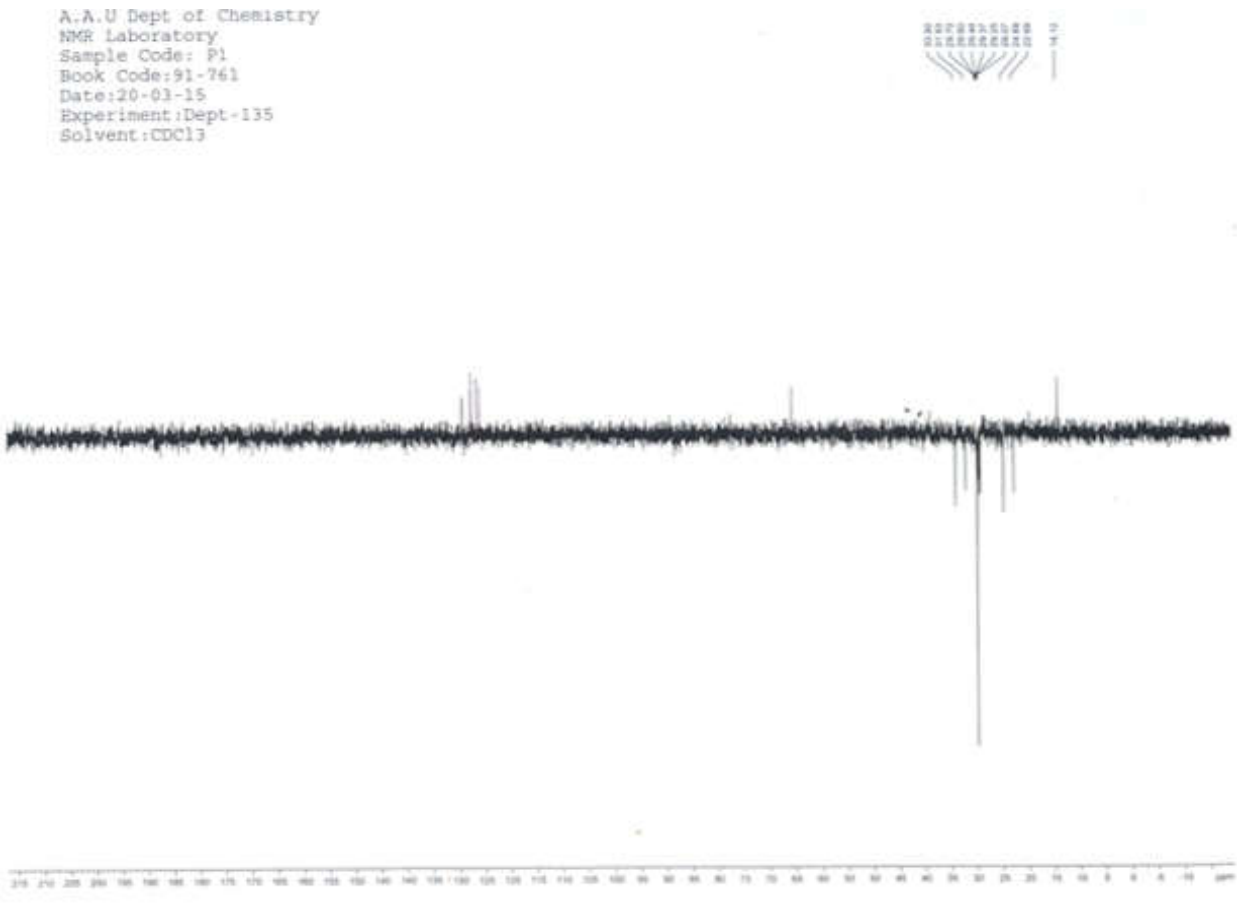

Figure 4. DEPT-135 Spectrum of Compound VAH-C1 in

****