



## 1-HEXADECENE FROM AERIAL PARTS OF *AGERATINA ADENOPHORA* (SPRENG)

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### ABSTRACT

*Ageratina* genus grows wildly in many parts of the world. A number of its species are toxic to grazing animals and some promising secondary metabolites for preparation of drugs and value-added products. From different solvent extractions and chromatographic techniques a compound was isolated from aerial parts of *Ageratina adenophora*. Mass spectroscopy, nuclear magnetic resonance ( $^1\text{H}$ ,  $^{13}\text{C}$ , DEPT,  $^1\text{H}$ - $^1\text{H}$ - COSY, HMQC and HMBC) studies showed that the isolated compound is 1-hexadecene, a known compound but being reported in this plant for the first time.

**Keywords:** *Ageratina adenophora*, chromatographic techniques, aerial parts, isolated, 1-hexadecene

### INTRODUCTION

The genus *Ageratina* belongs to the tribe of Eupatorieae, species, one of the thirteen tribes of the Asteraceae (Woerdenbag, 1993; Sharma et al., 1998). Over 44 species are known (Hooker and Jackson, 1960). Comparison of American, Asian and African samples of *E. odoratum* using descriptive taxonomy showed little differentiation. However, chemotaxonomy studies using enzymatic markers showed that plants from America were different from those of Asian origin (Lanaud et al., 1991; Sharma et al., 1998)

*Ageratina adenophora* (AA) is native to Mexico but has now spread to Hawaii, the Philippines, China, Thailand, Australia, New Zealand, India, Nepal, and California (Chen and Zhao, 1984). In the 1920s, the plant was suspected of causing chronic respiratory disease in horses in Hawaii. By 1940, outbreaks of the same syndrome were recorded in New South Wales and southern Queensland in Australia where the disease

was called Numinbah Horse Sickness or Tallebudyera Horse Disease (Oelrichs et al. 1995). *Ageratina adenophora* has encroached upon a vast expanse of pastures and forest areas in the northeastern and northwestern sub-Himalayan region in India (Sharma and Dawra, 1994). In northeastern India, it colonises rapidly after **jhum** (shifting) cultivation. The species is unpalatable to grazing cattle (Verma et al. 1987). In the Kangra Valley of Himachal Pradesh in India, grazing sheep, goats and cattle browse *Ageratina adenophora*. No epidemiological data are available on the toxicosis in these species of ruminants but farmers consider the plant as undesirable and noxious (Sharma et al. 1998).

*Eupatorium* species are characterized by the presence of flavonoids (Metwally and Ekejiuba, 1981; Ferraro et al., 1988), pyrrolizidine alkaloids (Perdersen, 1975), sesquiterpene lactones (Haruna, et al. 1986) and some of them are of interest because of

their use in popular medicine and because of their toxicological and pharmaceutical properties. Some *Eupatorium* species have been described to possess antioxidant (Delas et al. 1998), an antigonorrhoeal (Caseres et al. 1995), cytostatic (Woerdenbag et al. 1987; Woerdenbag, 1989) and also toxicity against some species of soil pathogens (Kumar and Tripathi, 1991). *E. cannabinum* L. is an herbaceous, perennial plant which has been extensively investigated (Aquiuno et al. 1985). In the extract of this plant, sesquiterpene lactones with biological activity (Woerdenbag, 1989) and polysaccharides with immunological activity (Vollmar, 1986) were reported. No toxic effects were seen in goats when *A. adenophora* comprised up to 67 % of their food ration but at higher concentrations voluntary intake was reduced due to low palatability (Neopane et al. 1992).

**In continuation of the work to identify bioactive secondary metabolites from plants, herein, 1-hexadecene has been isolated for the first time from *A. adenophora* and characterized on the basis of mass and NMR spectroscopy techniques.**

## MATERIALS AND METHODS

### Plant Material

The fresh aerial parts of *A. adenophora* Spreng used for the studies were collected from the CSIR- IHB T Campus, Palampur, Himachal Pradesh (HP) (32°06'05"N, 76°34'10"E) located in mid-hills of the western Himalaya. The specimen of the target plant was deposited in the herbarium of CSIR-IHB T (voucher number PLP 16569) and got authenticated by the Institute. The plants were collected in the month of December 2013.

### Preparation and extraction of the plant material

Fresh aerial parts of *A. adenophora* Spreng was dried under mild sunlight and pulverized into powder using a grinding

machine. Cold extraction was performed using methanol for 24 h with intermittent shaking at interval. Removal of solvent with rotary evaporator at 45 °C afforded 0.06% w/w yield.

### Fractionation of the crude extract and isolation

100g of crude methanol extract of AA was fractionated using silica gel GLR stationary phase using different solvent combination on the basis of polarity such as; hexane:ethylacetate (50:50), ethylacetate (100%), ethylacetate:methanol (50:50) and methanol (100%). Methanolic fraction was subjected to column chromatography with Silica gel (230-400 mesh) as the stationary phase. 100% n-hexane was used as mobile phase (eluent) and it resulted in the isolation of Eupa 4 (1-Hexadecene) as a single compound.

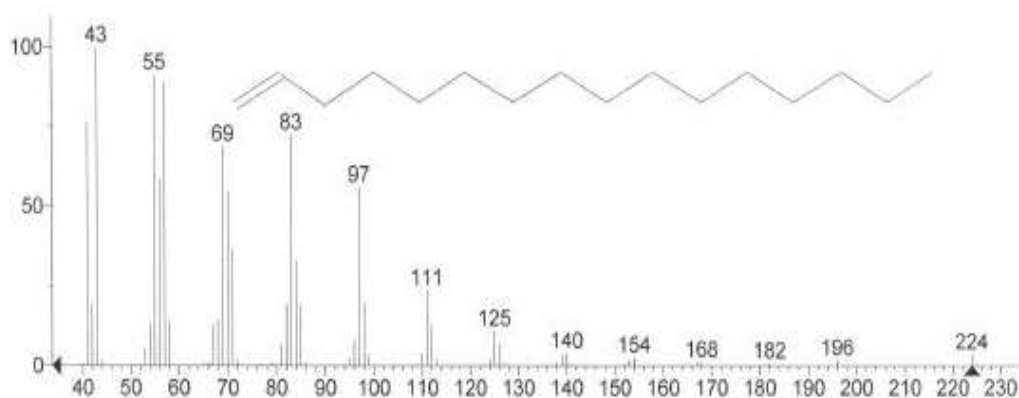
### Characterization of Isolated compounds

The structure of the isolated compound was characterized at the Institute of Himalayan Bioresource Technology, Palampur India by <sup>1</sup>H NMR, <sup>13</sup>C NMR and mass spectrometry. NMR spectra were recorded on a Bruker Avance-600 MHz (<sup>1</sup>H and <sup>13</sup>C), in CDCl<sub>3</sub> solution with TMS as internal reference. Mass spectra were recorded on QTOF-Micro of Waters Micromass and Maxis- Bruker

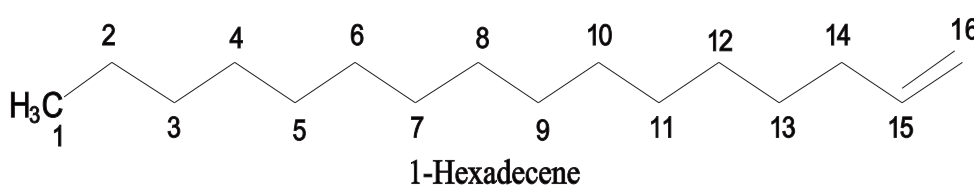
## RESULTS AND DISCUSSION

For homogeneity of the compound, in all cases of thin layer chromatographic experiments using two different solvent systems, single spot was obtained. Thus, it was a single compound. 1-Hexadecene (fig.2) was obtained as a colourless oily substance. Its molecular weight was assigned for C<sub>16</sub>H<sub>32</sub> by MS showing an [M]<sup>+</sup> at m/z 224.43 with fragmentation pattern; 224 [M]<sup>+</sup> 196, 125, 111, 97, 83, 69, 55, and 43 as shown in figure 1. Table 1 shows the complete <sup>1</sup>H and <sup>13</sup>C NMR assignment. The spectrum of <sup>1</sup>H, <sup>13</sup>C NMR and DEPT are as shown in figure 3, 4 and 5.

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**Fig. 1** Fragmentation pattern of 1-Hexadecene.



**Fig 2.** Name and structure of isolated compound

Position	$\delta\text{H}$	$\delta\text{C}$
1	0.79-0.81, m	14.2
2	1.05-1.34 <sup>a</sup> , m	22.7
3	1.05-1.34 <sup>a</sup> , m	32.3
4	1.05-1.34 <sup>a</sup> , m	29.3-30.0 <sup>a</sup>
5	1.05-1.34 <sup>a</sup> , m	29.3-30.0 <sup>a</sup>
6	1.05-1.34 <sup>a</sup> , m	29.3-30.0 <sup>a</sup>
7	1.05-1.34 <sup>a</sup> , m	29.3-30.0 <sup>a</sup>
8	1.05-1.34 <sup>a</sup> , m	29.3-30.0 <sup>a</sup>
9	1.05-1.34 <sup>a</sup> , m	29.3-30.0 <sup>a</sup>
10	1.05-1.34 <sup>a</sup> , m	29.3-30.0 <sup>a</sup>
11	1.05-1.34 <sup>a</sup> , m	29.3-30.0 <sup>a</sup>
12	1.05-1.34 <sup>a</sup> , m	29.3-30.0 <sup>a</sup>
13	1.05-1.34 <sup>a</sup> , m	29.3-30.0 <sup>a</sup>
14	1.92-1.95, m	34.1
15	4.82, d ( $J = 10.0$ Hz), 4.88, d ( $J = 12.0$ Hz)	139.5
16	5.70-5.71, m	114.4

<sup>a</sup> Overlapped signals

**Table 1.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of 1-Hexadecene

The  $^{13}\text{C}$  NMR spectrum gave a total of 16 resonances and the DEPT spectral data showed the presence of 14 methylenes, 1 methines and 1 methyl. The presence of one downfield methylene (C 114.4) and one methine group (C 139.5) corresponds to the presence of terminal alkene bond. In the HMBC spectrum a correlation peak was observed between the methine proton (H 4.82, 4.88) and downfield methylene (C 114.4) carbon, and vice-versa. The methine carbon (C 139.5) also shows correlation with C-14 protons (H 34.1) in HMBC spectrum. Complete  $^1\text{H}$  and  $^{13}\text{C}$  assignments of **Eupa4** are given in Table 1. From the above spectral data, the structure of compound **Eupa4** was established as 1-hexadecene earlier, this compound has been isolated from *Bacillus subtilis* and was found to be active against bacterial

pathogen (Hameedha et al., 2014). In addition, the 1-hexadecene was found to be an enhancer of metabolite production in microorganisms. Recent studies reported that the metabolite production was enhanced by 1-hexadecene in microbial culture. 1-Hexadecene, belonging to the alkenes, probably either acts as the oxygen-vector or plays a role in the redox system to increase palmarumycin C2 and C3 production in *Berkleasium* sp. (Yau, et al., 2013). Palmarumycins C2 and C3 were considered as the intermediates in the biosynthetic pathway of spiro bisnaphthalenes (Bode and Zeeck, 2000). The HMQC, HMBC, AND COSY spectral which further confirms the structure of 1-hexadecene are as shown in figure 6, 7, and 8, respectively.

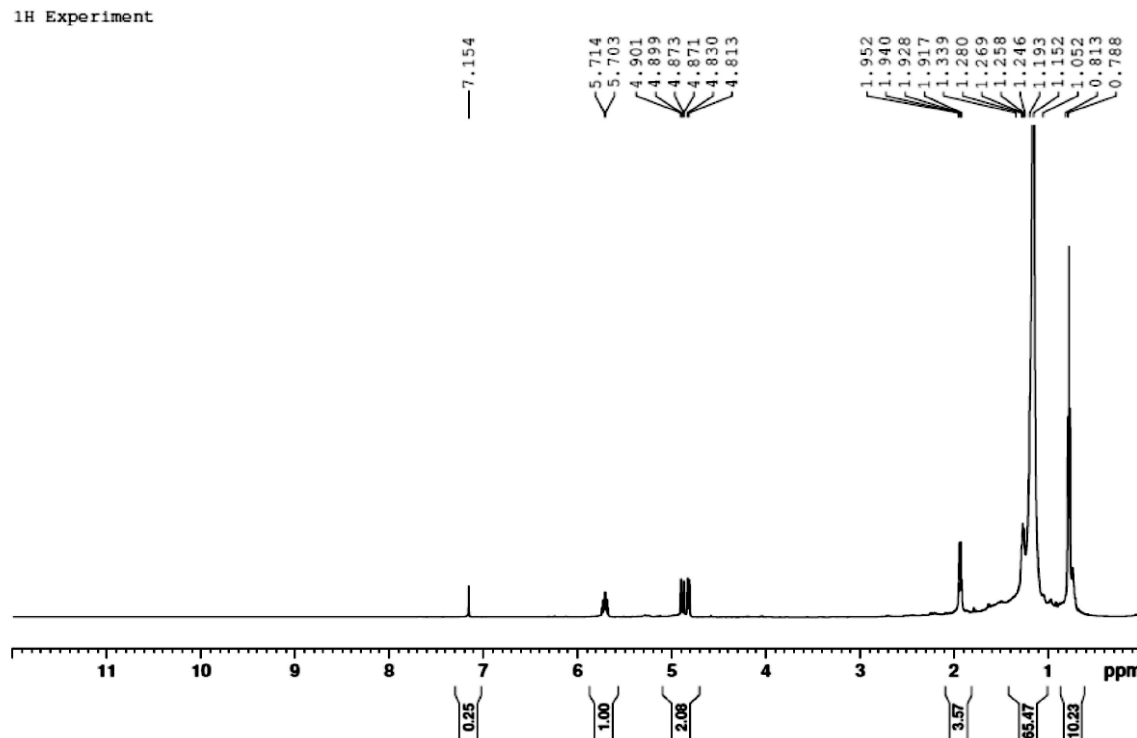
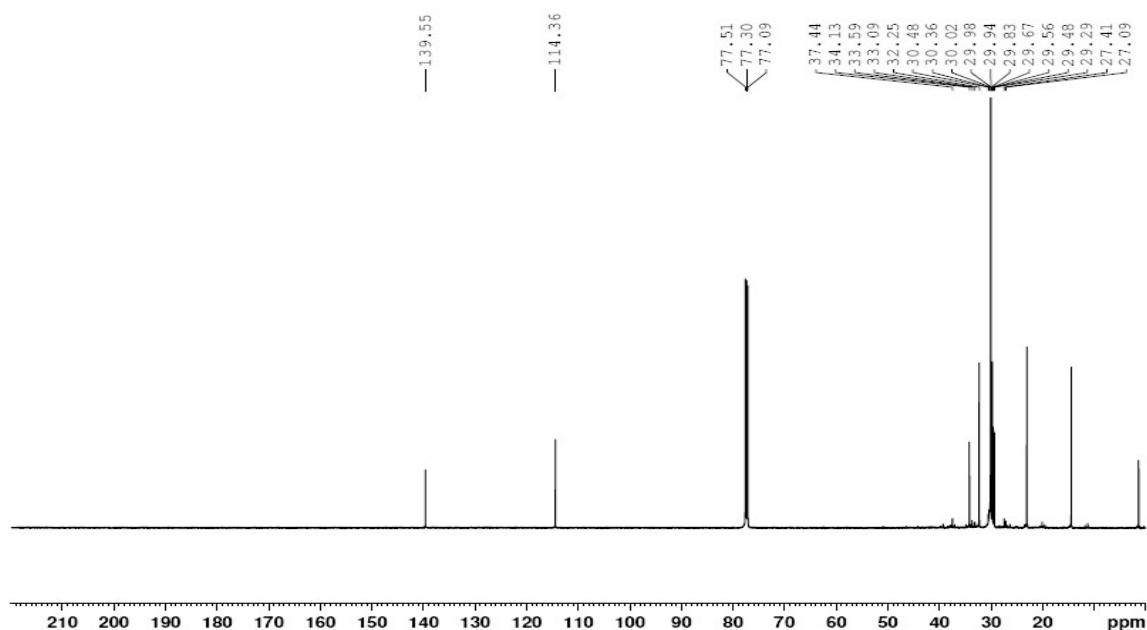
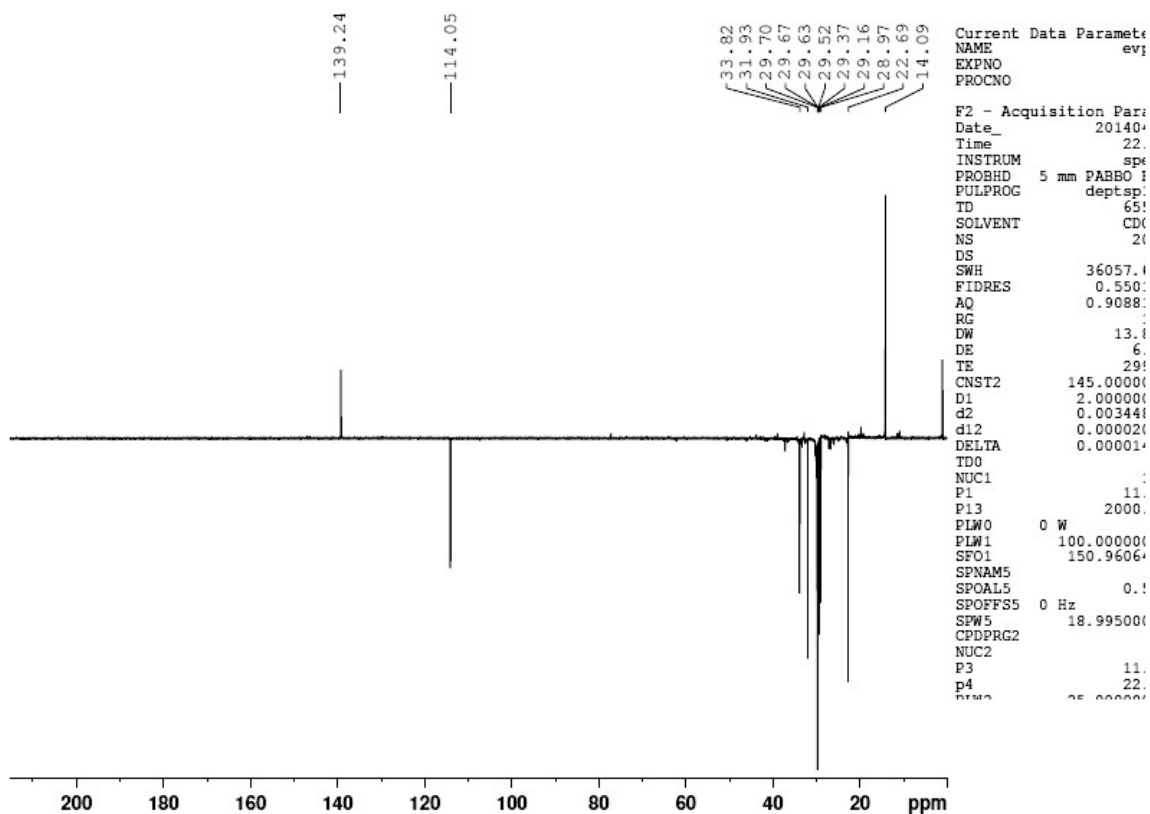


Fig. 3  $^1\text{H}$  NMR spectrum of 1-Hexadecene

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**Fig. 4 <sup>13</sup>C NMR spectrum of 1-Hexadecene**



**Fig. 5 The DEPT spectrum of 1-Hexadecene**



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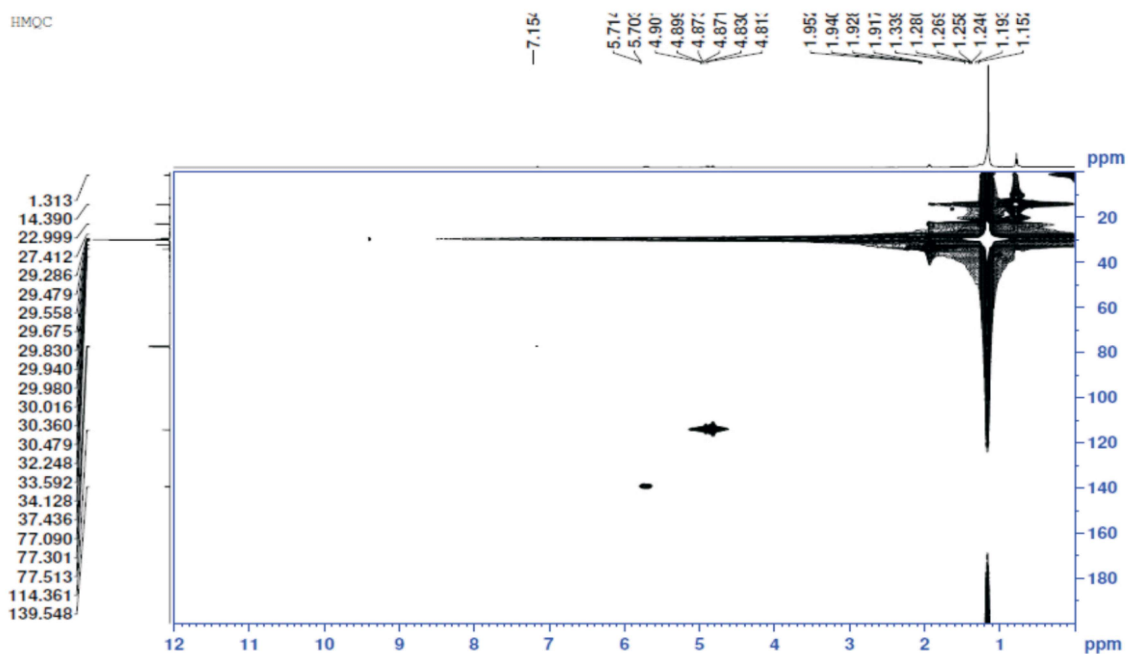


Fig. 6 The HMQC spectra of 1-Hexadecene

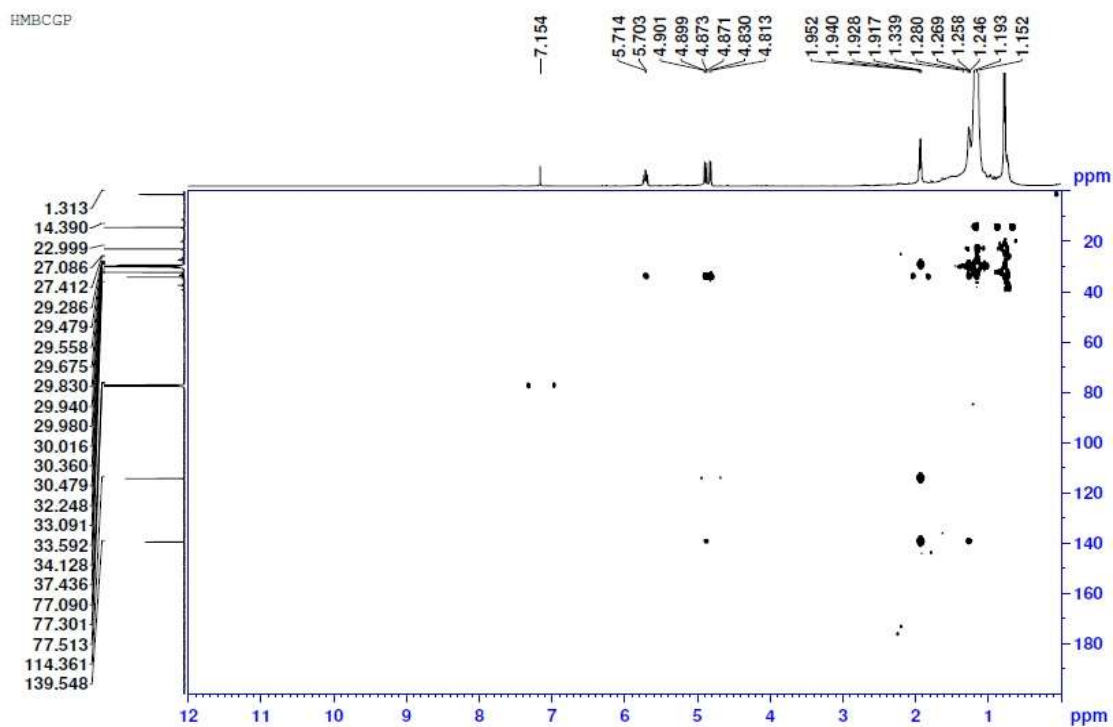


Fig. 7. The HMBC spectral of 1-Hexadecene

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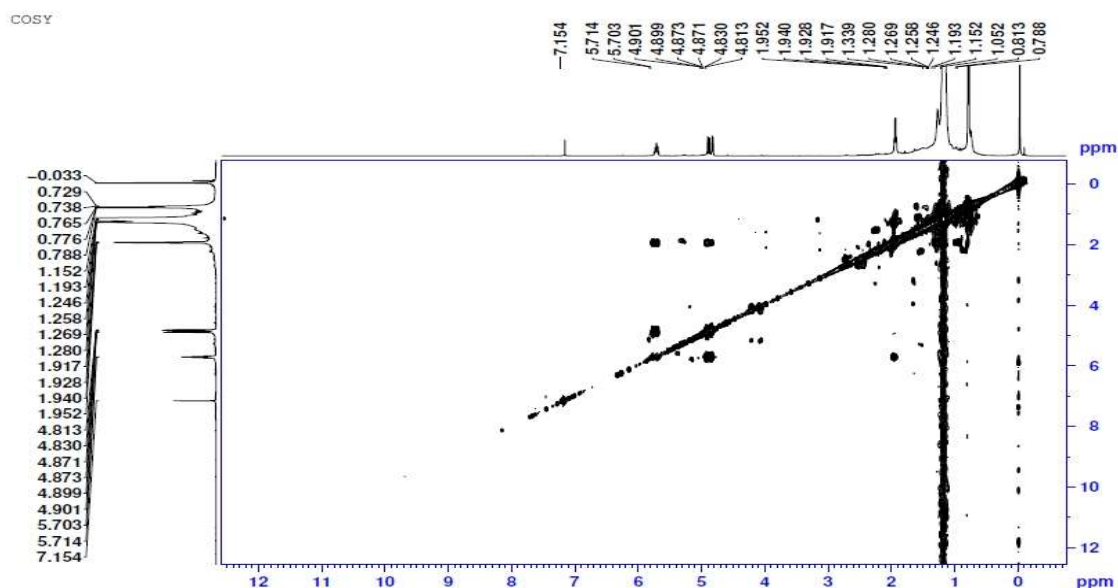


Fig. 8 The COSY spectral of 1-Hexadecene

### CONCLUSION

1-Hexadecene has been isolated for the first time from the aerial parts of *Ageratina adenophora* and characterized on the basis of its  $^1\text{H}$ ,  $^{13}\text{C}$  NMR and mass spectral analysis. Phytochemicals present in the *Ageratina adenophora*, 1-hexadecene was, thus included.

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