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# African Journal of Pure and Applied Chemistry

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## Full Length Research Paper

# Amino acid content, fatty acid content and anti nutritional factor of seeds of new hybrid varieties of *Echinochloa frumentacea* (Sanwa) minor millets

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Cereals are the staple diet of most of the world's population. The millets are very important staple food in the rural parts of India. Millets can secure India's food and farming in future because it is amazing in their nutrition contents. *Echinochloa frumentacea* (Sanwa) millet is good source of energy and provide protein, fatty acid, minerals, vitamins, dietary fibre and polyphenols. Proteins present in various foods differ in their nutritive value on account of the difference in the amino acid contents. The amino acid content, fatty acid content (TSFA and TUFA) and anti nutritional factor ranged from 0.0008 to 0.522%, 24.2 to 26.0%, 73.5 to 75.4% and 0.301 to 0.302, 0.0202 to 0.0204 g/100 g and 31.95 mg/100 g respectively. No cyanide content and haemagglutinin activity were found. Nutritionally the seeds of *E. frumentacea* variety DFM-1 and HR-374 are rich in aspartic acid (essential amino acid) content and total unsaturated fatty acid content.

**Key words:** Amino acid, fatty acid content, anti nutritional factor, minor millets variety of *Echinochloa frumentacea*.

## INTRODUCTION

Millets are a group of cereal species crops or grain like food that has been used by large group of people in rural, tribal and hilly areas in Asia and Africa (Ravindra et al., 2008; Anonymous, 2006; Rao et al., 2011; Odoemalam and Osu, 2009). Millet is a cereal crop plant belonging to different genera but all within the grass family, Poaceae and subfamily Panicoideae (FAO, 1972; 1991). As the minor millets are consumed by the poor, they guard them against food and nutritional insecurity imposed by various agronomic, socio economic and political factors. Minor millets can thus act as a shield against nutritional

deficiency disorders and provide nutritional security. These grains will be used for traditional as well as novel foods (Vanithasri et al., 2012). All of them are small seeded grasses having high capability of resistance to extreme environmental conditions in which major cereals fail to give substantial yields (Amadou et al., 2013; McDonoug et al., 2000; Black et al., 2006; Ahmed et al., 2013). *Echinochloa frumentacea* minor millets are high energy, nutritious foods comparable to other major cereals and some of them are even better with regard to protein and mineral content (Fe, Ca, Mn, Mg). Fat is one

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of the major nutrients which provide energy, promote body growth, maintain and repair body tissue, promote reproduction and lactation and regulate body process. Fats are carriers of fat soluble vitamins. Dietary fat must also provide essential fatty acids (EFA) which are the functional components of membrane lipids and have other important metabolic function. Fats are made up of fatty acids which include saturated fatty acids like palmitic and stearic, monounsaturated fatty acids (MUFA) like oleic and polyunsaturated fatty acid (PUFA) likes linoleic acid and linolenic acid (Singhai and Shrivastava, 2002; Nagraj, 1995). Lipids are relatively minor constituents in cereal grains; however, they contribute significantly to diet as a source of invisible fat and essential fatty acid (Achaya, 1986, 1987). Nevertheless *E. frumentacea* represents a good source of essential amino acid and essential fatty acid (linoleic acid and linolenic acid). However, it must be pointed out that, *E. frumentacea* also contains some anti-nutritional factors which inhibits proteolytic and amylolytic enzymes, limits mineral, protein and starch digestibility and makes poor human bioavailability of proteins.

This study was therefore conducted to assess the levels of amino acid content, fatty acid content and antinutritional factors in the seeds of BMVL-29 and BMVL-172 variety of *E. frumentacea* for awareness and exploitation.

## MATERIALS AND METHODS

In the present study two new hybrid, authentic, healthy and matured seeds of minor millets viz., *E. frumentacea* (variety BMVL-29 and BMVL-172), under investigation were procured from Agriculture Research Station of Jawaharlal Nehru krishi Vishwavidyalaya, Dindori (M.P.) and were studied for their amino acid, fatty acid and antinutritional factors.

### Amino acid analysis

The amino acid composition of seeds of hybrid variety BMVL-29 and BMVL-172 of *E. frumentacea* was analyzed by using liquid chromatography mass spectroscopy (LC-MS).

### Solvent extraction and sample preparation

Solvent extraction was done by Soxhlet apparatus and stock solution was prepared by dissolving 10 mg of each amino acid in 100 ml of diluents (acetonitrile/formic acid) and it was properly shaken. Working standard solution of 1 mg/L was prepared by this stock solution.

### LC-MS analysis

LC-MS analysis of sample was done by using C18 column (Brava Amino 5  $\mu$ , 4.6  $\times$  250 mm). Column temperature was maintained at 40°C. 10  $\mu$ l of sample was injected for 10 min, 0.1% Formic acid in water and 0.1% Formic acid in acetonitrile (95+5) were used as mobile phase and its flow rate was 0.8 ml/min. Ionization of sample component were performed on electron spin resonance (ESR) mode (70 eV).

### Fatty acid analysis

The hybrid variety (BMVL-29 and BMVL-172), of *E. frumentacea* seeds were studied for their fatty acid composition by gas chromatography. Powdered sample of experimental seeds were subjected to solvent extraction in Soxhlet apparatus for 20 h, using petroleum ether (40 to 60°C) as solvent. Lipids were then estimated gravimetrically by the method of Colowick and Kaplan (1957). Methyl esters of the lipids were prepared by the method of Chowdhary et al. (1984) and analysed by gas liquid chromatogram (GLC). Gas chromatograms were recorded using flame ionization detector (FID) with split ratio 1:50.

### Antinutritional factors

The seeds of *E. frumentacea* variety BMVL-29 and BMVL-172 were studied for their tannin content, oxalate content, trypsin inhibitor activity, cyanide content and haemagglutinin activity. Cyanide and tannin contents of seeds were determined by the method of AOAC (1970). The total oxalate content in the form of oxalic acid was determined by using the method of Talpatra et al. (1948). Trypsin inhibitor activity was determined according to the method as described by Kakade et al. (1969) with certain modifications by Gupta and Deodhar (1975). Haemagglutinin activity was determined by the method as given by Liener (1955).

## RESULTS AND DISCUSSION

The results of amino acid composition of seed protein of *E. frumentacea* variety BMVL-29 and BMVL-172 are given in the Table 1. The seeds of *E. frumentacea* variety BMVL-29 and BMVL-172 were found to have highest amount of Aspartic acid (0.522%), whereas Lysine content was reported 0.047 and 0.046% in *E. frumentacea* variety BMVL-29 and BMVL-172 respectively. In both the variety of *E. frumentacea* other amino acid in the decreasing order were glutamic, methionine, L-omithine HCl, alanine, arginine HCl, DL-Tryptophan, serine, glycine, proline = valine, threonine, tyrosine, phenylalanine, leucine = L-Hydroxyproline>isoleucine.

From the perusal of the data it appears that both the varieties of *E. frumentacea* minor millets seeds are lacking in Cystine, Histidine, 2-Aminobutanic and L-cysteine amino acids. It has been found that the amount of aspartic acid was maximum while the quantity of isoleucine was minimum but methionine levels of these variety of minor millets was more than the amount present in cereal grains. Methionine is of special importance to animals as a therapeutic and nutritional factor. It protects animals against liver injuries by chloroform, industrial halogenated fumes, and protein deficient diets and prevents the great loss of body nitrogen in the case of fractures, burns and surgical operations (Crocker and Barton, 1952). However, the amino acid composition of seed protein of both the variety (BMVL-29 and BMVL-172) of *E. frumentacea* under study was found to be in general accordance with reported values (FAO, 1970; Glew et al., 2008; Hui, 1996).

**Table 1.** Amino acid composition of *E. frumentacea* variety BMVL-29 and BMVL-172.

S/No	Amino acid	Amino acid analysis (content in %)	
		<i>Echinochloa frumentacea</i>	<i>Echinochloa frumentacea</i>
		BMVL-29	BMVL-172
1	Alanine	0.015	0.016
2	Arginine HCl	0.014	0.013
3	Aspartic	0.522	0.522
4	Cystine	ND	ND
5	Glutamic	0.027	0.034
6	Glycine	0.006	0.006
7	Histidine	ND	ND
8	Isoleucine	0.0009	0.0008
9	Leucine	0.001	0.002
10	Lysine	0.047	0.046
11	Methionine	0.027	0.025
12	Phenylalanine	0.002	0.002
13	Proliney	0.004	0.004
14	Serine	0.011	0.011
15	Threonine	0.003	0.002
16	Tyrosine	0.002	0.003
17	Valine	0.004	0.004
18	2-Aminobutaric	ND	ND
19	L-Omithine HCl	0.023	0.023
20	L-Cysteine HCl	ND	ND
21	DL-Tryptophan	0.012	0.012
22	LHydroxyproline	0.001	0.001

**Table 2.** Saturated fatty acid composition of *E. frumentacea* variety BMVL-29 and BMVL-172.

Name of variety	Saturated fatty acid %							Total saturated fatty acid (%)
	Caprylic acid	Lauric acid	Myristic acid	Palmitic acid	Stearic acid	Arachidic acid	Bahenic acid	
<i>E. frumentacea</i> BMVL-29	2.1	-	0.1	15.8	5.0	0.9	0.3	24.2
<i>E. frumentacea</i> BMVL-172	1.1	0.1	0.1	17.1	6.1	1.1	0.4	26.0

Tables 2 and 3 showed the variation of fatty acid content of hybrid *E. frumentacea* variety BMVL-29 and BMVL-172. The saturated fatty acid, Caprylic acid was found to be highest (2.1%) in variety *E. frumentacea* BMVL-29 and lowest (1.1%) in variety *E. frumentacea* BMVL-172. The Palmitic acid content was reported higher (17.1%) in variety *Echinochloa frumentacea* BMVL-172 and lower in the variety *E. frumentacea* BMVL-29. The percentage of Stearic acid was found maximum (6.1%) in the variety *E. frumentacea* BMVL - 172 and minimum (5.0%) in the variety *E. frumentacea* BMVL-29. The Arachidic acid was found to be maximum (1.1%) in the variety *E. frumentacea* BMVL-172, while minimum in the variety *E. frumentacea* BMVL-29. The

variety *E. frumentacea* BMVL-172 has maximum (0.4%) Bahenic acid content while minimum (0.3%) in the variety of *E. frumentacea* BMVL-29. The total saturated fatty acid (TSFA) content was to be greater (26.0%) in the variety of *E. frumentacea* BMVL-172 than the variety *E. frumentacea* BMVL-29(24.2%).

The unsaturated fatty acid, *E. frumentacea* variety BMVL-172 contain maximum amount (29.5%) of Oleic acid (MUFA). Whereas the Linoleic acid was found to be highest (46.9%) in the variety *E. frumentacea* BMVL -29. The Linolenic acid and Ecosenoic acid content was found to be greater (1.0 and 0.5% respectively) in the variety *E. frumentacea* BMVL-29 than the variety *E. frumentacea* BMVL-172 (0.7 and 0.4% respectively). The variety *E.*



**Table 3.** Unsaturated fatty acid composition of *E. frumentacea* variety BMVL-29 and BMVL-172.

Name of variety	Unsaturated fatty acid (%)				Total unsaturated fatty acid
	Oleic acid	Linoleic acid	Linolenic acid	Ecosenoic acid	
<i>E. frumentacea</i> BMVL-29	27.0	46.9	1.0	0.5	75.4
<i>E. frumentacea</i> BMVL-172	29.5	42.9	0.7	0.4	73.5

**Table 4.** Anti nutrients of *E. frumentacea* variety BMVL-29 and BMVL-172.

Name of variety	Tannin content g/100 g	Oxalate content g/100 g	Trypsin inhibitor activity TIU/mg protein	Cyanide content	Haemagglutinin activity		
					Human blood	Goat blood	Hen blood
<i>Echinochloa frumentacea</i> BMVL-29	0.301	0.0202	31.95	ND	ND	ND	ND
<i>Echinochloa frumentacea</i> BMVL-172	0.302	0.0204	ND	ND	ND	ND	ND

\*ND- not detected, \* The values given in the tables are the mean of the triplicate values obtained.

*frumentacea* BMVL-29 contain minimum value of mono unsaturated fatty acid (MUFA) and maximum value of polyunsaturated fatty acid (PUFA). Total unsaturated fatty acid (TUFA) content was found to be highest (75.4%) in the variety *E. frumentacea* BMVL-29 and lowest (73.5%) in the variety *E. frumentacea* BMVL-172.

*E. frumentacea* variety BMVL -29 was found to be superior to the variety *E. frumentacea* BMVL-172 under investigation. It contains highest content of linoleic acid (46.9%). The amount of linoleic acid in millet oil is higher in comparison with most other types of vegetable oils (Ravindra et al., 2008). The linoleic acid is one of the most important polyunsaturated fatty acid in human food, because of its prevention of distinct heart vascular disease (Boelhouwer, 1983). This acid is most important essential fatty acid required for growth, physiological function and maintenance, which cannot be synthesized by the human body and one, has to depend on dietary source for their adequate supply. The body metabolizes linoleic and linolenic acid into arachidonic acid and docosahexaenoic acid (DHA) respectively which are essential to the normal development of central nervous system (Brich et al., 2007; Jacobson et al., 2008). Various developmental problems including attention-deficit/hyperactivity disorder (ADHD) in children have been linked to biological deficiencies in polyunsaturated fatty acids. Additionally, there is evidence that symptoms may be reduced with PUFA supplementation (Sinn and Bryan, 2007).

The result of anti nutritional factors of the *E. frumentacea* varieties are shown in Table 4. The tannin content of different varieties of *E. frumentacea* ranged from 0.301 to 0.302 g/100 g. These values are lower than the earlier findings of Pasala and Bjorn (1989) and are well below the fatal dose (Sarjekar and Shrivastava,

1994). The total oxalate content (in terms of oxalic acid) was found to be maximum (0.0204 g/100 g) in the variety of *E. frumentacea* BMVL-172 while it was minimum (0.0202 g/100 g) in the variety of *E. frumentacea* BMVL-29.

The Trypsin inhibitor activity was found to be maximum (31.95 mg/100 g) in the variety *E. frumentacea* BMVL-29. However, no trypsin inhibitor activity was reported in the variety of *E. frumentacea* BMVL-172. No Cyanide content and haemagglutinin activity were found in the varieties of *E. frumentacea* under study. The value of anti nutritional factors reported in study was lies within the leather dose. These anti nutritional factors may be reduced by simple soaking, heating and germination or fermentation. It is now established that phytates, polyphenols and tannins can contribute to antioxidant activity of the millet foods, which is an important factor in health, aging and metabolic diseases (Bravo, 1998).

## Conclusion

Cereals and millets constitute a major component of diet consumed in developing countries like India. *E. frumentacea* (Sanwa) millets are the staple food for millions of poor people in the world. The seed of *E. frumentacea* (variety BMVL-29 and BMVL-172) millets contain significant quantities of essential amino acids particularly the sulphur containing amino acid (methionine), essential fatty acids (PUFA and MUFA) and leather amount of antinutritional factor. It will be a useful and economical source of protein provided that some legume or milk is consumed along with these minor millet, that is, suitable for good nutritional supplementation. Variations in the various constituents of the *E. frumentacea*

millet seeds have been attributed to variety, conditions, fertilizer treatments and climatic conditions. Most of the anti nutritional factors are heat-labile and since only humans consume millets after cooking, it would not constitute any major health hazard. It can be concluded that nutritional benefit of *E. frumentacea* minor millets can be enhanced when all type of processing treatments employed at domestic levels were effective in reducing the biological active factors and therefore could be used to enhance better quality in food materials. Compared to rice and wheat, minor millets contain little high amount of anti nutritional factors. But these anti nutritional factors are plant based phytochemicals that possess therapeutic qualities and hence are recommended by doctors for various diseases. Diabetics need to control their blood sugar, hypertension as well as cholesterol level and that is why doctors recommend minor millets for these problems.

### Conflict of Interest

The authors have not declared any conflict of interest.

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## Full Length Research Paper

# Isolation and characterization of a steroidal compound from the hexane extract of the leaves of *Artabotrys odoratissimus*

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*Artabotrys odoratissimus* has been investigated by many workers for its constituents. Previous phytochemical studies have revealed this genus to be rich in secondary metabolites including phenylcoumarins, xanthenes and triterpenoids. So far, not many studies have been carried out on this genus but there are some reports on this plant. Our recent study on the hexane extract of the leaves of *A. odoratissimus*, have led to the isolation of steroidal compound. The structure of the compound has been established by modern spectroscopic techniques such as IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR and mass-spectroscopy and identified as tetracontan-15-one.

**Key words:** *Artabotrys odoratissimus*, medicinal plant, tetracontan-15-one.

## INTRODUCTION

*Artabotrys odoratissimus*, commonly known as KantiliChampa, is an ornamental shrub. Leaves are oblong, lanceolate, glabrous, shining acute at the base, petioles are 6 to 10 mm long. The size of the leaves are up to 18 by 3.8 to 5.0 cm. It is available in Bangladesh and India (Chopra et al., 1956). Ayurvedic and Yunani doctors use the leaves, flowers as a remedy for cholera, vomiting, thirst and headache. Volatile oils from the leaves show antifungal and antimicrobial activity. The antifertility activity of *A. odoratissimus*, plant has been reported in albino rats (Chakarabarti et al., 1968). The fruit extracts showed cardiac stimulatory effects on some animals and cardiac depressant effects on others (Trivedi et al., 1971). So far, not many studies have been carried out on this genus but previous

phytochemical studies have revealed this genus to be rich in secondary metabolites including phenylcoumarins, xanthenes and triterpenoids (Connolly et al., 1994; Haider et al., 1991; Perold et al., 1978; Bheemasankara et al., 1984; Sharma et al., 2002; Singh et al., 2009; Chakabati et al., 1968). The compounds so far isolated from the leaves of *A. odoratissimus* are pentadecyl-7-hydroxy-dodecanote, pentadecyltritriacontanone, 4,5-epoxy-26-ol-dopentacontane,  $\beta$ -sitosterol, Aplysterol, Nonacosanylhexaconsanoate, Pentatetracont-19-ol, triacont-2-ol, Dotriacont-7-ene, Octacose-7-ene, 1-hydroxy-2,5-dimethyl-9, 10-antraquinone, 1,4,5-trihydroxy-9, 10-antraquinone, 13-hydroxynonacosane, Nonanoic acid, Methylphenylpropanoate, Decanoic acid,

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Diethylphthalate, Dibutylphthalate, 2-amino-3-ethylbiphenyl, 5-methyl-9-phenylnonane-3-ol, 1-phenylundecane, 2,5-dimethyl-1-phenylheptane-1-one, hexadeca-2,7,11-triene, 1-isopropyl-4,6-dimethylnaphthalene, 1-phenyldeca-1-one, 1-phenylundecan-1-one, 5-(2-butylphenyl) pent-3-en-2-ol, 2,5-dimethyltetradecanhydrophenanthrene. Our recent study on the leaf extracts have led to the isolation of steroidal compound. In this paper, isolation and structural elucidation of the isolated compound from the hexane extract of the leaves of *A. odoratissimus* is described. The isolated compound identified by spectral data, has not been reported before.

## EXPERIMENTAL: GENERAL

Freshly distilled solvents were used for extraction, isolation and purification. Evaporations were performed under reduced pressure in a Buchii rotary evaporator. IR spectra were recorded (KBr discs) on a Shimadzu UV-168A Spectrophotometer, validation ( $\nu_{\max}$  in  $\text{cm}^{-1}$ ).  $^1\text{H-NMR}$  were recorded on a Bruker R-32 (300 MHz) instrument in  $\text{CDCl}_3$  and  $\text{DMSO-d}_6$  with TMS as an internal standard (Chemical Shifts in  $\delta$ , ppm). All solvents used were of analytical grade. Thin-layer chromatography (TLC) was performed using Silica gel GF254.

### Plant materials

The leaves of *A. odoratissimus* were collected from the gardens of Ujjain city and university campus and were identified by the authorities of IEMPS, Vikram University Ujjain (M.P) India.

### Extraction of the Compound D1

Dried leaves of the plant (5 kg) were milled into powder and then extracted with hexane (8 L) in a Soxhlet extractor for 36 h. The extract was evaporated in a rotatory evaporator and dried by vacuum pump. The hexane extract (50 g) was suspended on water and extracted successively with hexane, hexane: Benzene, Benzene: Ethyl acetate and Ethyl acetate: Chloroform to yield hexane (15 g), Hexane: Benzene (5 g), Benzene: Ethyl acetate (12 g), Ethyl acetate: Chloroform (3.2 g), soluble fractions respectively. The hexane (15 g) soluble fraction was subjected to column chromatography was eluted initially with hexane, hexane: Benzene followed by the mixture of Benzene with increasing amount of ethyl acetate and finally with methanol. These elutes were collected in a series of test tubes with 15 ml in each fraction. Each fraction was examined by TLC. Based on the similar TLC behavior, these elutes were combined to yield D1, D2, D3, D4, D5, D6, D7, D8, D9 and D10. The fractions D1-D3 (that is, hexane fractions) were concentrated and allowed to stand at room temperature when a circular white amorphous crystal was obtained and this was marked as compound D1. The amorphous crystal was washed with methanol successively. The crystal dissolved in chloroform, applied in TLC micro-glass slide, and viewed in an iodine chamber were two different spots were detected. Therefore, the compound was needed further purification and dissolving in the mixture of  $\text{CHCl}_3$  and methanol (10:1) and kept at room temperature. After 24 h a white amorphous crystal was precipitated out at the bottom of the conical flask. The crystallization process was repeated three times and finally white crystal (80 mg) were obtained.

### Compound D1

Finally the Compound D1 was crystallized from Benzene: ETOAC (3:7v/v) to give white crystals (90 mg), M.P. 285.

IR.  $\lambda$  (KBr): 2919, 2850, 1735, 1636, 1541, 1463, 1261, 1074, 801-668  $\text{cm}^{-1}$ .

$^1\text{H-NMR}$  ( $\text{CDCl}_3$ , TMS): 2.35(t, 4H,  $\text{CH}_2\text{COCH}_2$ ,  $j=6.9\text{Hz}$ ), 1.56(s, 4H,  $2\beta\text{CH}_2$ ), 1.26(s, 66H,  $-33\text{CH}_2$ )

$^{13}\text{C-NMR}$  (75 MHz,  $\text{CDCl}_3$ , TMS): 15.5, 22.7-29.9, 31.9, 35.5, 173.8 ppm.

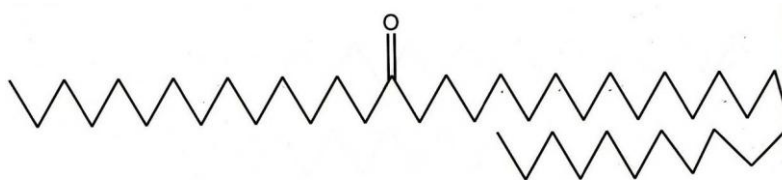
EIMS(m/z, rel.int): M+576(5.85), 533(4.05), 504(4.95), 476(9.9), 462(12.16), 435(11.26), 406(7.20), 378(14.86), 337(27.77), 308(19.81), 280(19.80), 253(24.54), 240(28.37), 238(247.77), 182(27.47), 141(34.68), 126(34.68), 84(34.68), 58(100), 43(27.92), 28(24.77).

## RESULTS AND DISCUSSION

The TLC examination of the isolated compound from the hexane extract of the leaves of *Artabotrys odoratissimus* upon exposure to iodine vapour showed a bright single spot. The compound was obtained as white crystals (m.p.=285°C). HRMS of compound D1 exhibited molecular ion at m/z 576, which is consistent with the molecular formula  $\text{C}_{40}\text{H}_{80}\text{O}$ . It was readily soluble in  $\text{CHCl}_3$ . IR spectrum showed bands at 2919, 2850, 1735, 1541, 1463, 1074, 801, 730 to 720  $\text{cm}^{-1}$  suggesting aliphatic ketonic nature of the compound (Mehta et al., 1999; Bellamy, 1962).

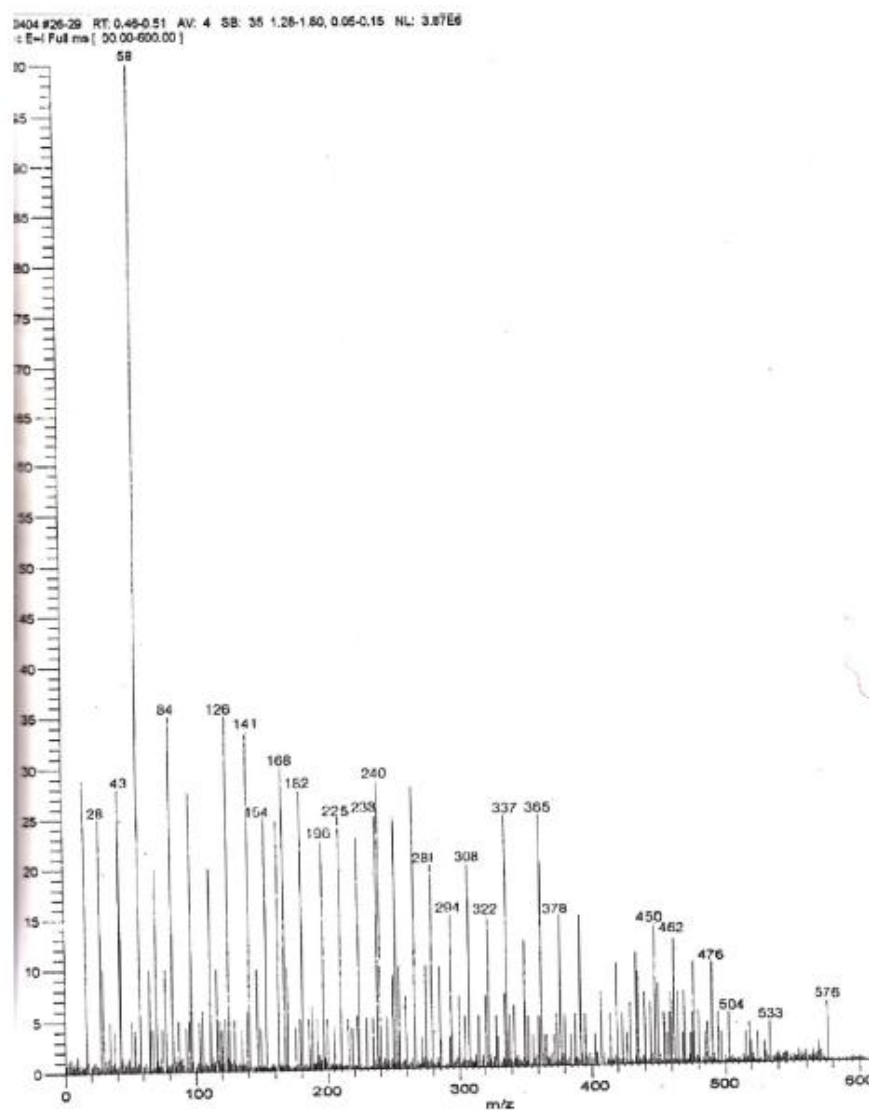
The  $^1\text{H}$  NMR spectrum showed triplet at  $\delta$  0.86 for six protons of terminal methyl groups. A triplet at  $\delta$  2.35 for alpha methylene ( $\text{CH}_2\text{OCH}_2$ ) protons to the carbonyl group. A broad singlet at  $\delta$  1.56 for beta methylene protons. A broad singlet at  $\delta$  1.26 assigned to rest of the methylene protons (Falch et al., 2004; Fauhl et al., 2000; Guillen and Ruiz, 2005; Guillen and Ruiz, 2003; Panico et al., 1994; Gunstone et al., 1975; Lie Ken Jie et al., 2003).  $\text{C}^{13}$  NMR spectrum value were consistent with the proposed structure, it showed a peak at  $\delta$  173.8 for carbonyl carbon. The peaks at  $\delta$  35.5 and 31.9 attributed to the methylene carbons attached at alpha and beta positions of the carbonyl group. A bunch of peaks at  $\delta$  29.9-22.7 corresponds to the remaining methylene carbons. Peak at  $\delta$  15.5 correspond to the methyl carbons.

The EIMS showed the molecular ion peak at m/z 576 suggesting its molecular formula as  $\text{C}_{40}\text{H}_{80}\text{O}$ . The mass fragmentation was characteristic of long chain hydrocarbon. The separation of most of the peaks by 14 and 28 mass units and appearance of  $\text{C}_n\text{H}_{2n+1}$  and  $\text{C}_n\text{H}_{2n-1}$  ion series confirmed its long chain aliphatic nature. The fragment at m/z 240 formed by the McLafferty rearrangement indicated the position of carbonyl group. Other abundant fragments at m/z 504, 476, 462, 450, 435, 378, 182, 141 and 58 were characteristic of the long chain aliphatic ketone (Mehta et al., 1999; Gowariker et al., 1997; Bellamy, 1962; Manorangani et al., 1999; Silverstein et al., 1984; Christie et al., 1993; Ali et al., 2001; Dobson and Sebedio, 1999; McLafferty, 1973).



Tetracontan-15-one

Figure 1. Tetracontan-15-one.



EI-MS spectrum of D1

Figure 2. EI-MS spectrum of D1.

Thus the IR, <sup>1</sup>HNMR, <sup>13</sup>CNMR and mass spectral analysis with physical properties established the identity of Compound D1 as tetracontan-15-One (Figures 1 to 5).

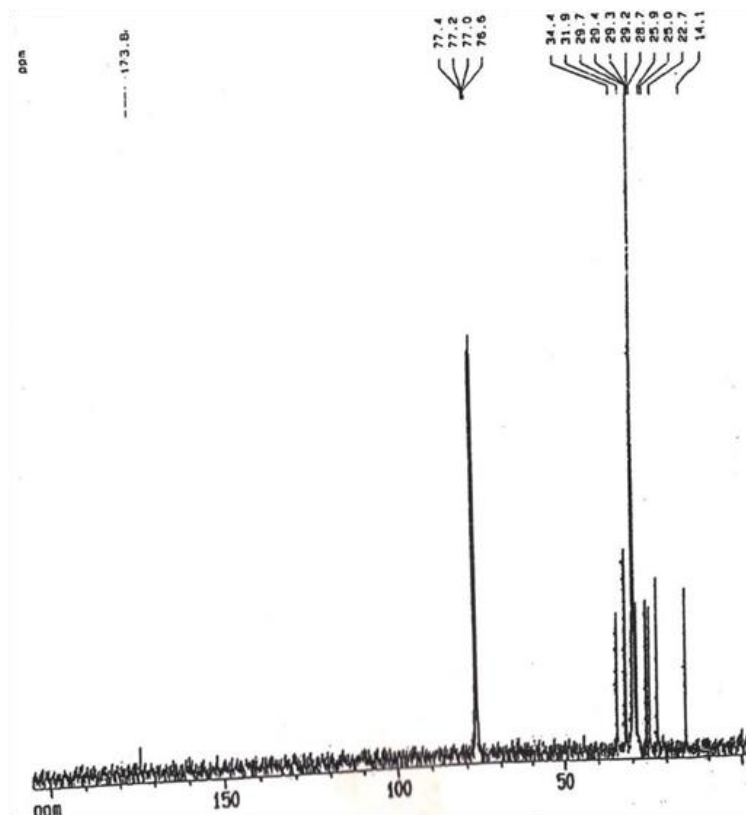


Figure 3. <sup>13</sup>C NMR spectrum of D1.

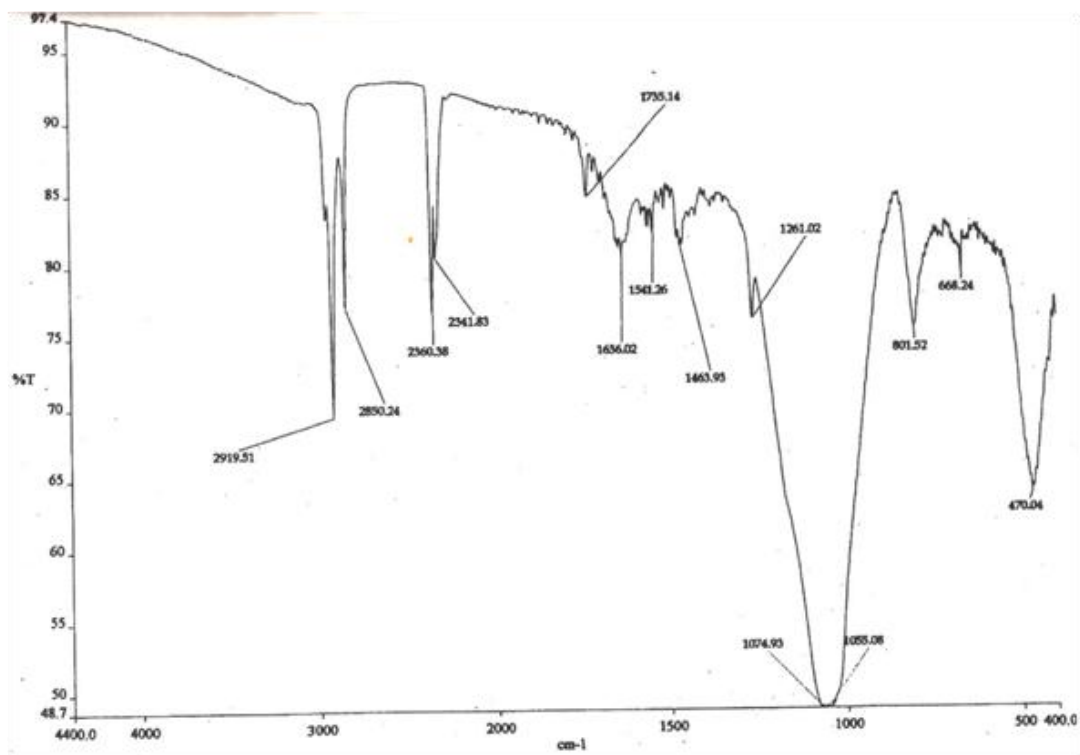


Figure 4. IR spectrum of D1.

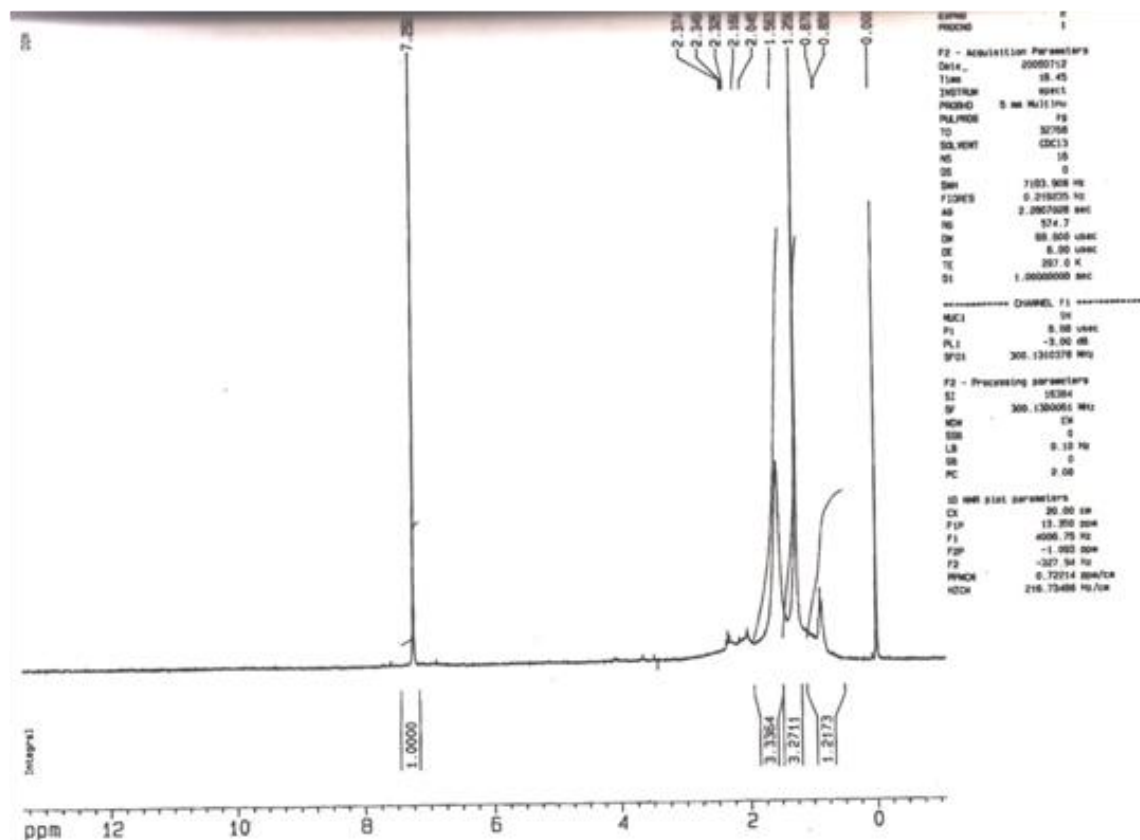


Figure 5.  $^1\text{H}$  NMR spectrum of D1.

## Conclusion

The results of the present investigation showed the occurrence of Tetracontan-15-one type compound in plant kingdom. The title Compound was isolated from this plant for the first time.

## Conflict of Interest

The authors have not declared any conflict of interest.

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