



## Isolation and Characterization of 4',7-dihydroxy isoflavone from *Indoneesiella echioides* (L.) Nees plant leaves

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### ABSTRACT

The present study was carried out to isolation and characterization of flavones compounds present in the *Indoneesiella echioides* (L) Nees leaves. *Indoneesiella echioides* (L) Nees is an important herb widely distributed in south India. This is commonly known as False Water willow. *Indoneesiella echioides* (L) Nees is a traditional Indian medicine; the whole plant is highly medicinal value such as the leaf juice of this plant is used to cure fever. Different pharmacological properties of *Indoneesiella echioides* have already been reported. The plant was extracted for various solvents in increasing order of polarity from using n-hexane, chloroform, ethyl acetate, acetone, ethanol, butanol and methanol. Thus, the present study was performed to investigate the preliminary phytochemical screening, isolation and characterization of flavones compounds present in the *Indoneesiella echioides* leaves using FT-IR, GC-MS, NMR and MASS spectral techniques.

**Keywords:** *Indoneesiella echioides* (L) Nees; Phytochemical screening; Isolation; Characterization; FT-IR; GC-MS; NMR; MASS Spectra

## 1. INTRODUCTION

*Indoneesiella echioides* (L) Nees (Acanthaceae), also known as *Andrographis echioides* (L) Nees. This is commonly known as False Water Willow, is an abundantly growing in south India. *Indoneesiella echioides* (L) Nees is highly medicinal important. The genus of *Indoneesiella* is used in goiter, liver diseases [1], fertility problems, bacterial [2], malarial and fungal disorders.

The leaf juice of this plant is used to treating fever [3]. Several *Indoneesiella* species (about 40 species) has been used in treatment of influenza, malaria, dyspepsia and respiratory diseases. The *Indoneesiella* species also used to antidote for poisonous stings of some insects [4,5]. The leaf juice is mixed and boiled with coconut oil used to control falling and greying of hair [6]. Phytochemistry of *Indoneesiella echioides* has been investigated and reported to contain several flavonoids [7,8] and labdane diterpinoids [9-14].

In previous literatures are reported to only flavonoids as a major component in *Indoneesiella echioides* (L) Nees extracts [15-18]. It has been reported that variety of phytoconstituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotenoids, glycosides, flavonoids, organic acids, lipids, alkaloids and xanthene's [19].

The main focus of this study was Isolation and Characterization of Flavone compounds present in the *Indoneesiella echioides* (L) Nees leaves using FT-IR, GC-MS, NMR and MASS Spectral techniques.

## 2. MATERIALS AND METHODS

### 2. 1. Collection of plant materials

The leaves of *Indoneesiella echioides* (L) Nees was collected from Poondi village, Thanjavur District, Tamil Nadu. The botanical identity (Voucher No: A.A.R 001 on 04-02-2013) of the plant was confirmed by Dr. S. John Britto, Rapinat Herbarium, St. Joseph's College, Tiruchirappalli.

### 2. 2. Preparation of Extracts

The fine powder (5 kg) was extracted with 95% ethanol at room temperature for ten days. The extract were filtered and concentrated under reduced pressure in a rotary evaporator and extracted for various solvents in increasing order of polarity from using n-hexane, chloroform, ethyl acetate, acetone, ethanol, butanol and methanol.

After that the extract was taken in a beaker and kept in a water bath and heated at 30-40 °C till all the solvents got evaporated. All the extracts were tested for the presence bioactive compounds by using standard methods. The dried extract was subjected to preliminary phytochemicals, isolation and characterization of flavones compounds present in the *Indoneesiella echioides* (L) Nees leaves.

### 2. 3. Phytochemical screening

The preliminary phytochemical analysis of *Indoneesiella echioides* (L) Nees was carried out as per standard methods (Table.1).

**Table 1.** Preliminary phytochemical constituents of *Indoneesiella echioides* (L) Nees leaves.

S.N	Phytochemicals	Hexane Extract	Chloroform Extract	Ethyl acetate Extract	Acetone Extract	Ethanol Extract	Butanol Extract	Methanol Extract
1.	Alkaloids	-	-	-	Present	Present	-	-
2.	Flavonoids	-	Present	Present	Present	Present	Present	Present
3.	Terpenes	Present	Present	-	-	-	-	-
4.	Triterpenoid saponins	-	Present	Present	Present	-	-	-
5.	Saponins	-	Present	Present	Present	Present	Present	Present
6.	Glycosides	-	-	-	-	-	-	-
7.	Steroids	Present	Present	Present	Present	-	-	-
8.	Carbohydrates	-	-	-	-	-	-	-
9.	Phenolic compounds	Present	Present	Present	Present	-	Present	Present
10.	Tannins	-	-	-	-	-	-	-
11.	Amino acids	-	-	Present	Present	Present	-	Present

### 3. ANALYSIS OF PHYTOCOMPONENTS

The 95% ethanolic extract was subjected to column chromatographic separation eluted with chloroform/methanol system (4.75:0.25, 4.85:0.15, and 4.95:0.05). The fraction was crystallized with methanol to obtain white colour solid crystal. The isolate compounds were identified by spectral studies.

### 4. CHARACTERIZATION OF 4',7-DIHYDROXY ISOFLAVONE

#### 4. 1. GC-MS Analysis

The GC- MS analysis of ethyl acetate extract of *Indoneesiella echioides* (L) Nees leaves shows the RT value is 16.3 and Peak area is 50.4% composition. The data was compared with the spectrum of known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained. The results were clearly indicating the 4',7-dihydroxy isoflavone (Fig. 1).

#### 4. 2. FT-IR Analysis

IR (KBr) $\nu$  - The absorption appeared at 3433  $\text{cm}^{-1}$  indicating the  $\nu\text{OH}$  vibration of hydrogen bonds, the absorption 2925 and 2852  $\text{cm}^{-1}$  indicating the  $\nu\text{C-H}$  vibration of  $\text{CH}_2$  protons, the absorption 1625  $\text{cm}^{-1}$  indicating the  $\nu\text{C=C}$  vibration of  $\text{C=C}$  group and the absorption 1284  $\text{cm}^{-1}$  indicating  $\nu\text{OH}$  vibration of OH group present in the molecule.

**Table 2.**  $^1\text{H}$  and  $^{13}\text{C}$ - NMR data of compound in acetone - *d*6

S.N	Position	$\delta_{\text{H}}$ (J, Hz)	$\delta_{\text{C}}$
1.	2		158.31
2.	3	6.831, s	105.94
3.	5	8.037, d, (2.5)	102.45
4.	6	7.027, d, (2), 6.981, d, (2)	126.04
5.	7		163.76
6.	8	6.986, d, (2.5)	126.07
7.	9		160.63
8.	10		105.93
9.	1'		1
10.	2' and 6'	7.581, d, (6.5)	128.84
11.	3' and 5'	8.009, d, (7.5)	131.48
12.	4'		164.01
13.	C=O		178.94

#### 4. 3. $^1\text{H}$ -NMR Analysis

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , TMS) 500 MHz - The peaks appeared at  $\delta$ ppm: 6.831 (1H, s, H-3), 8.037 (1H, d, H-5), 7.027 (1H, d, H-6), 6.986 (1H, d, H-8), 7.581 (2H, d, H-2', H-6') and 8.009 (2H, d, H-3', H-5').

The peaks at  $\delta$  7.027, H-6 ( $J = 2$  Hz) and 6.981, H-6, ( $J = 2$  Hz) of two aromatic protons present in the phenol hydroxyl group showing *ortho/meta* coupled doublet of doublets, peak at  $\delta$  6.986, H-8, ( $J = 2.5$  Hz) also showing *ortho* coupled doublet, peak  $\delta$  8.037, H-5, ( $J = 2.5$ ) shows *meta* coupled doublet and the peak  $\delta$  6.831, H-3 shows one singlet in the ring A.

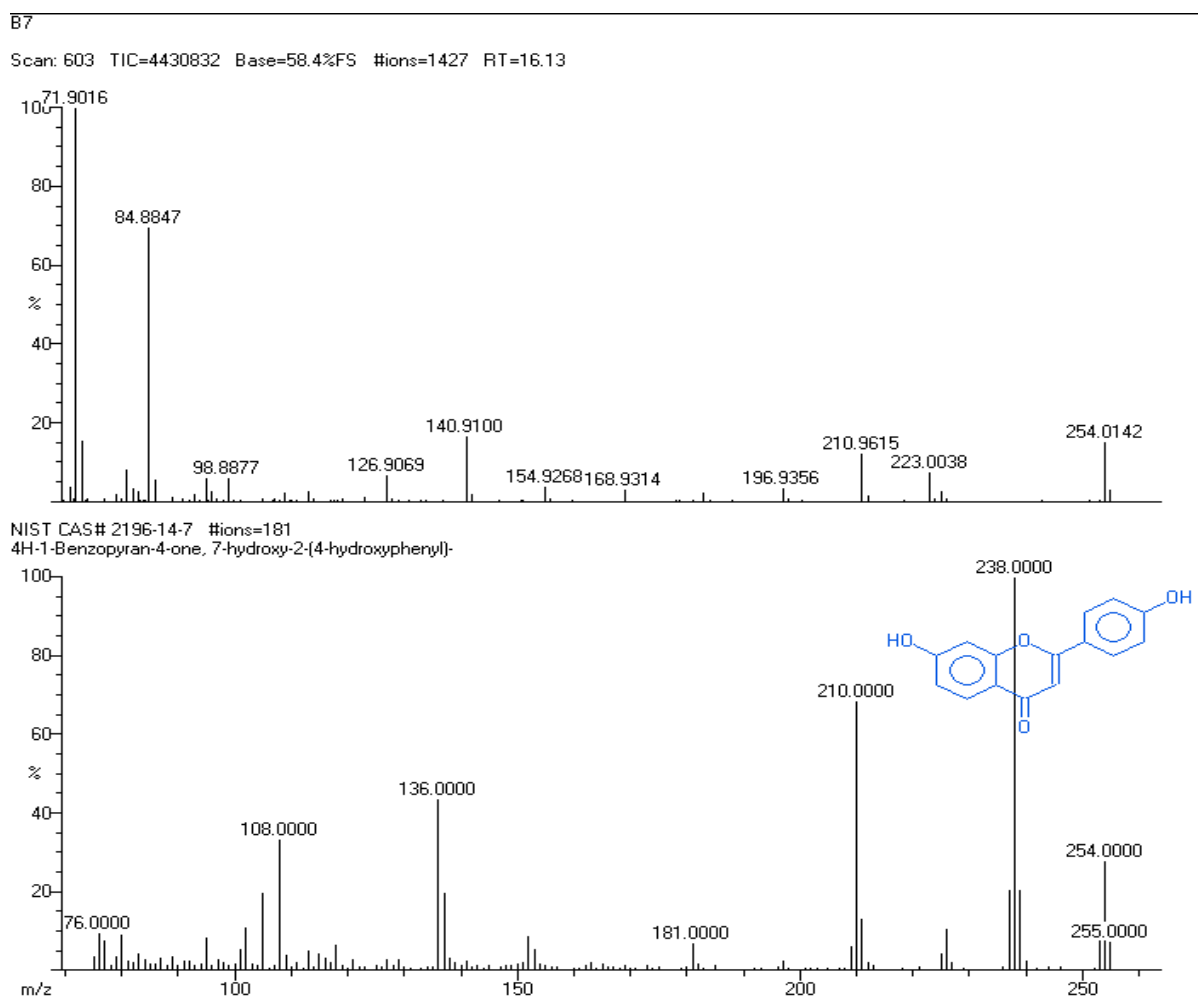
The peaks at  $\delta$  7.581, H-2' & H-6', ( $J = 6.5$ ) and  $\delta$  8.009, H-3' & H-5', ( $J = 7.5$ ) showing two *ortho/meta* coupled doublets appeared in the ring B.

#### 4. 4. $^{13}\text{C}$ -NMR Analysis

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , TMS) 500 MHz - The peaks appeared at  $\delta$ ppm: 158.31 (C-2), 105.94 (C-3), 178.94 (C=O), 102.45 (C-5), 126.04 (C-6), 163.76 (C-7), 126.07 (C-8), 160.63 (C-9), 105.93 (C-10), 115.43 (C-1'), 128.84 (C2' & C6'), 131.43 (C-3' & 5') and 164.01 (C-4').

The  $^{13}\text{C}$ -NMR spectrum showed the presence of 13 carbon skeleton including the overlapping peaks of C-3' & C-5' and C-2' & C-6' thus indicating the presence of 15-carbon skeleton. The  $^1\text{H}$  and  $^{13}\text{C}$ -NMR values for all the carbons were assigned on the basis of HSQC and HMBC correlations. The carbonyl carbon is bonded at C-4 position ( $\delta$  C 178.94 (C=O)) when the carbonyl is not hydrogen bonded. Carbon bonded to the hydroxyl group C-7 and C-4' appeared at  $\delta$  163.76 and  $\delta$  164.01. The  $^{13}\text{C}$  NMR resonance at  $\delta$  C 126 which showed HMBC correlations with  $^1\text{H}$  NMR resonance at H-6 & H-8 was attributed to C-7. In the  $^1\text{H}$  NMR spectrum of the compound shows the aromatic proton signals of two *m*-coupled doublets of  $\delta$  8.037, d, and  $\delta$  6.986, d, (each  $J = 2.5$  Hz) showing HSQC correlations to the carbon resonances at  $\delta$  C-5 102.45 and  $\delta$  C-8 126.07 was attributed to C-6 and C-7.

#### 4. 5. MASS Spectral Analysis



**Fig. 1.** GC-MS spectrum of 4',7-dihydroxy isoflavone.

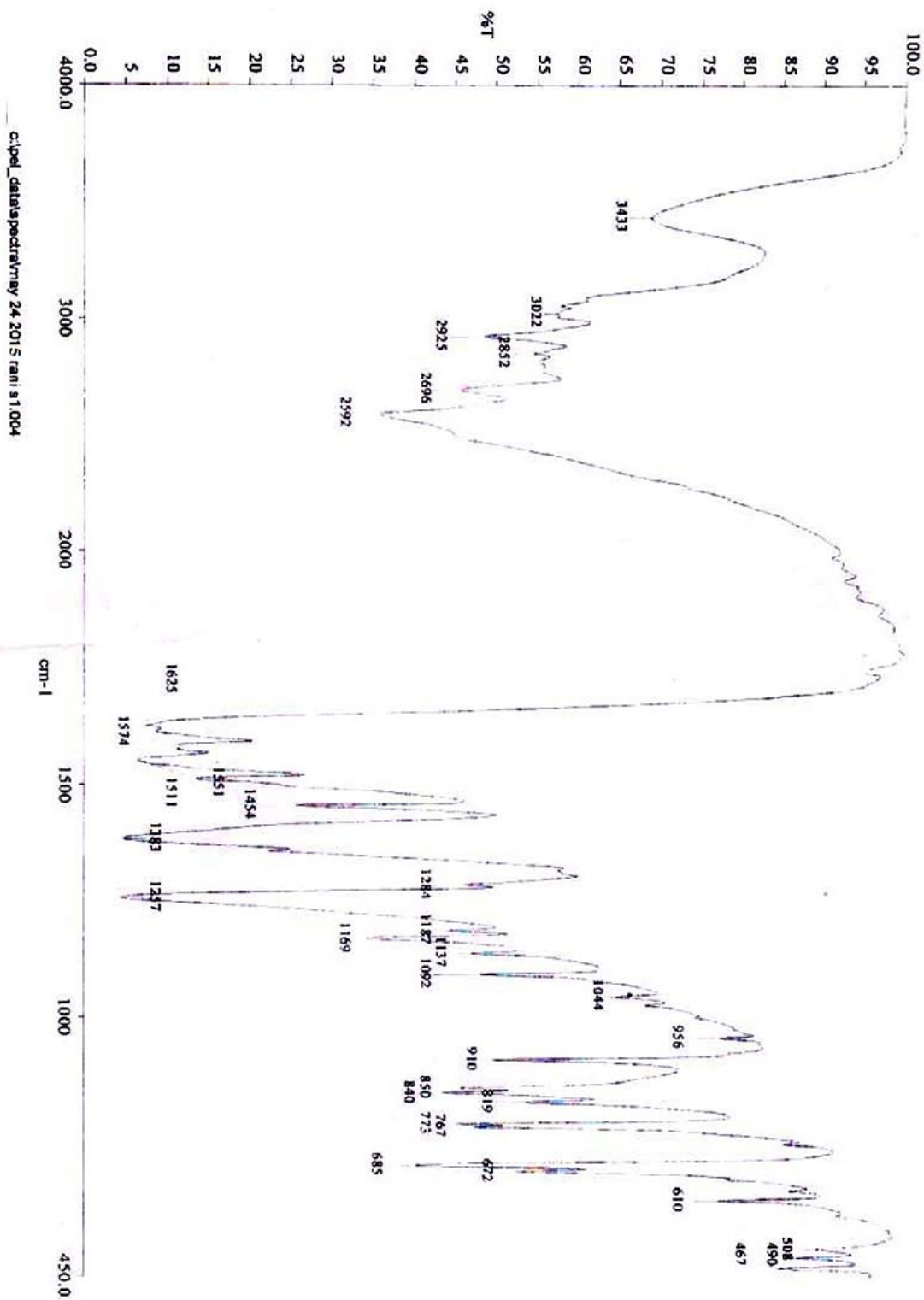
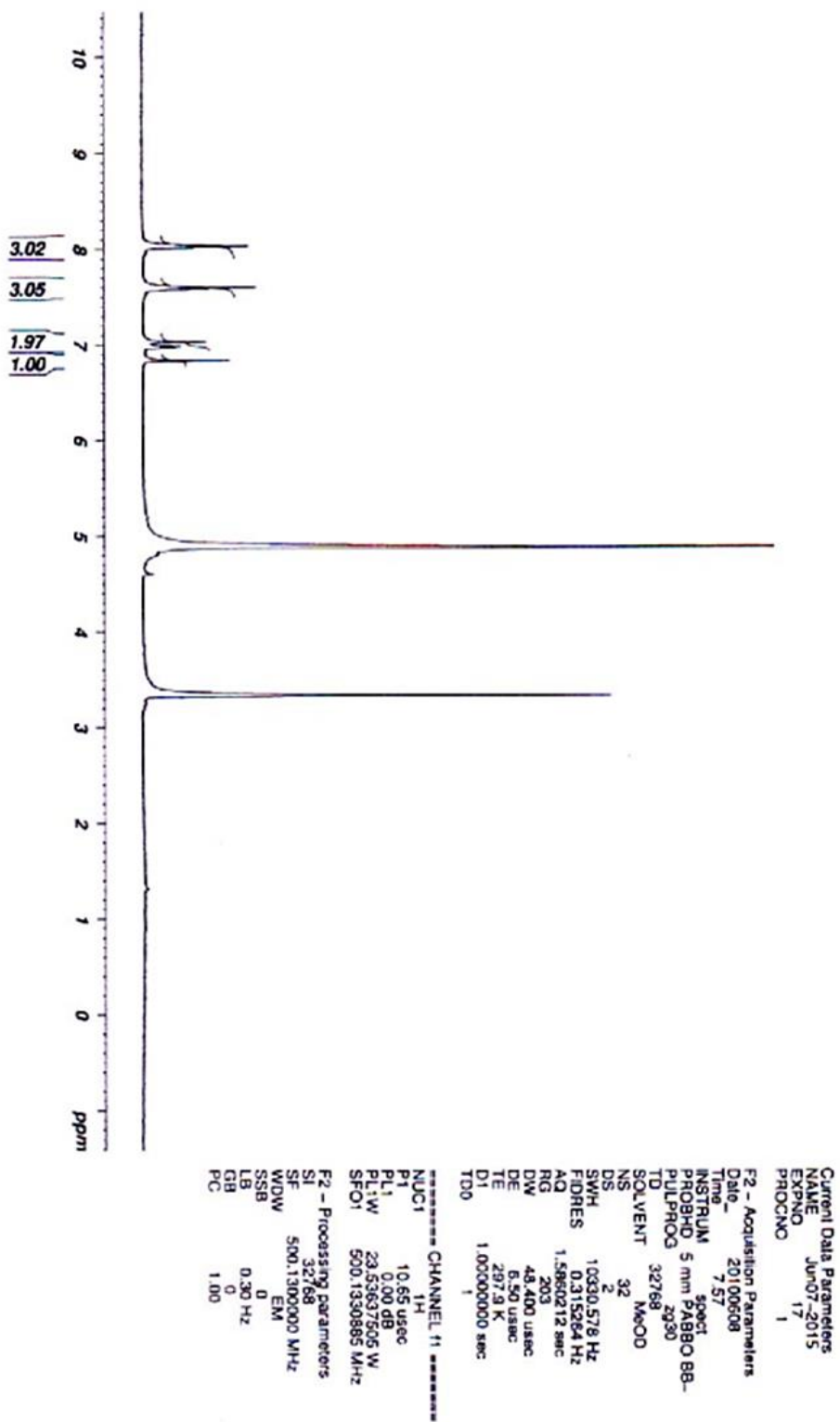


Fig. 2. IR-Spectrum of 4',7-dihydroxy isoflavone



Bruker AVIII 500 MHz NMR Facility  
 SAIF, IIT Madras Chennai 600 036

Fig. 3. <sup>1</sup>H-NMR - Spectrum of 4',7-dihydroxy isoflavone

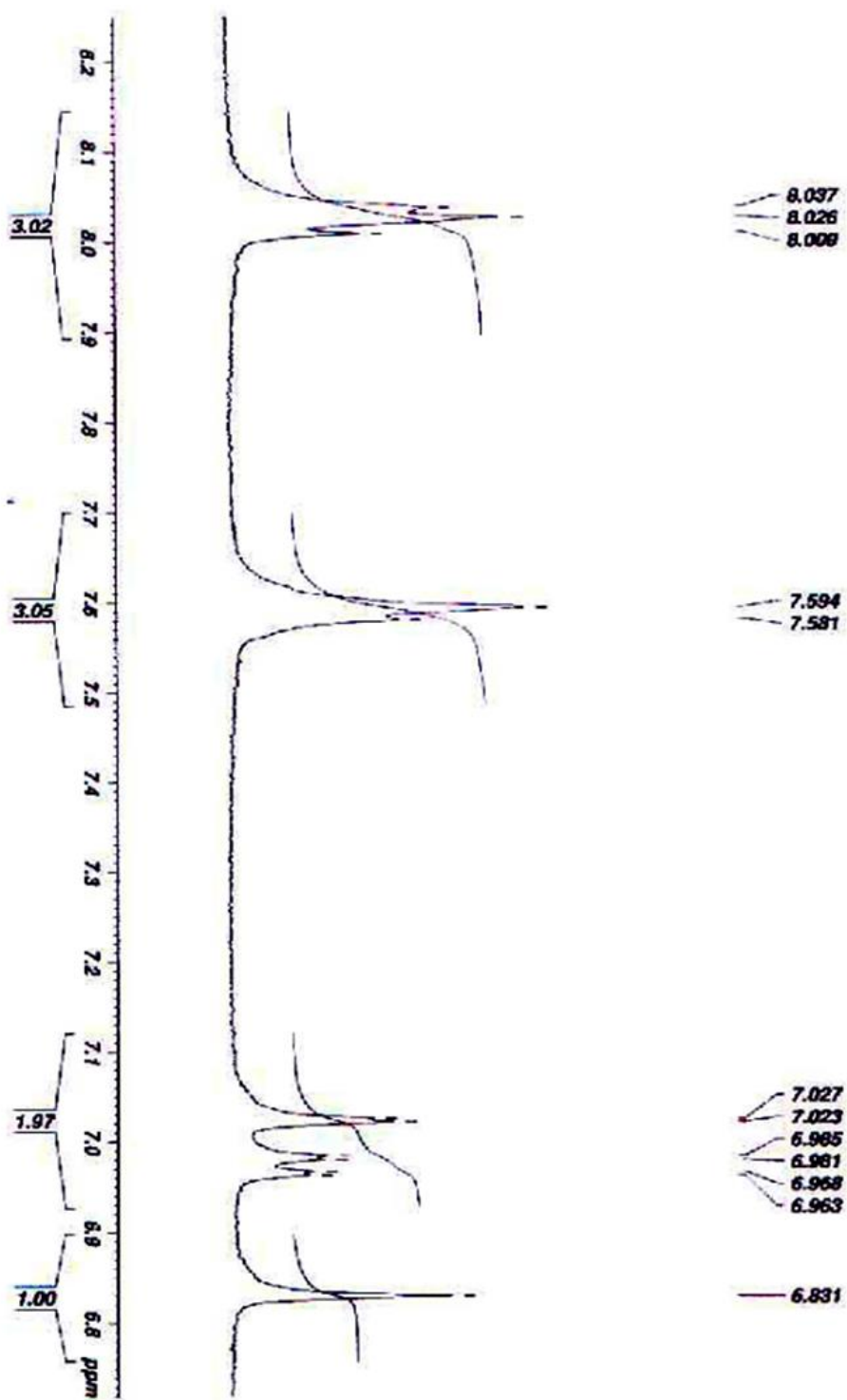


Fig. 3(a). Enlarged <sup>1</sup>H-NMR - Spectrum of 4',7-dihydroxy isoflavone



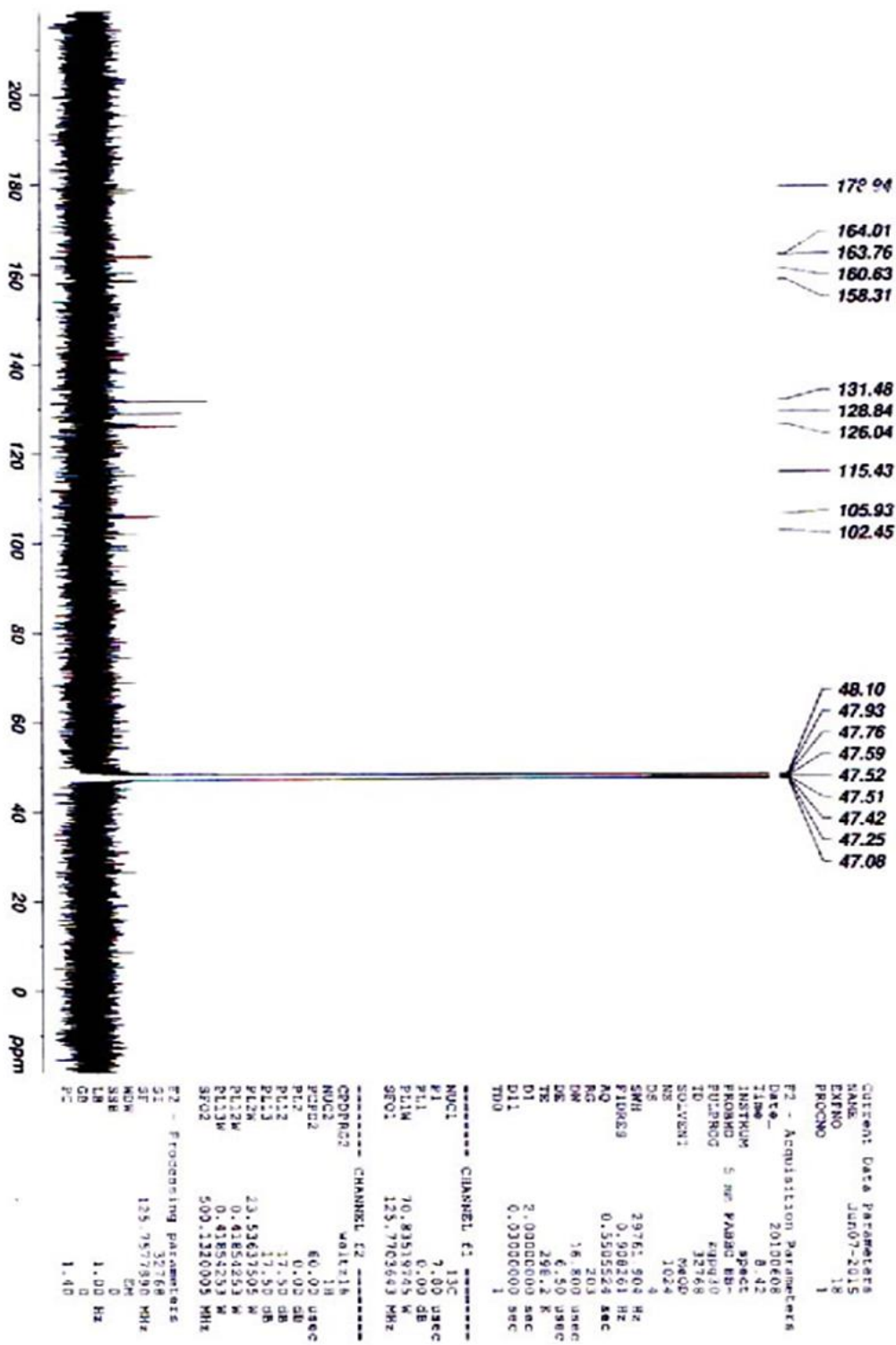


Fig. 4. <sup>13</sup>C-NMR - Spectrum of 4',7-dihydroxy isoflavone

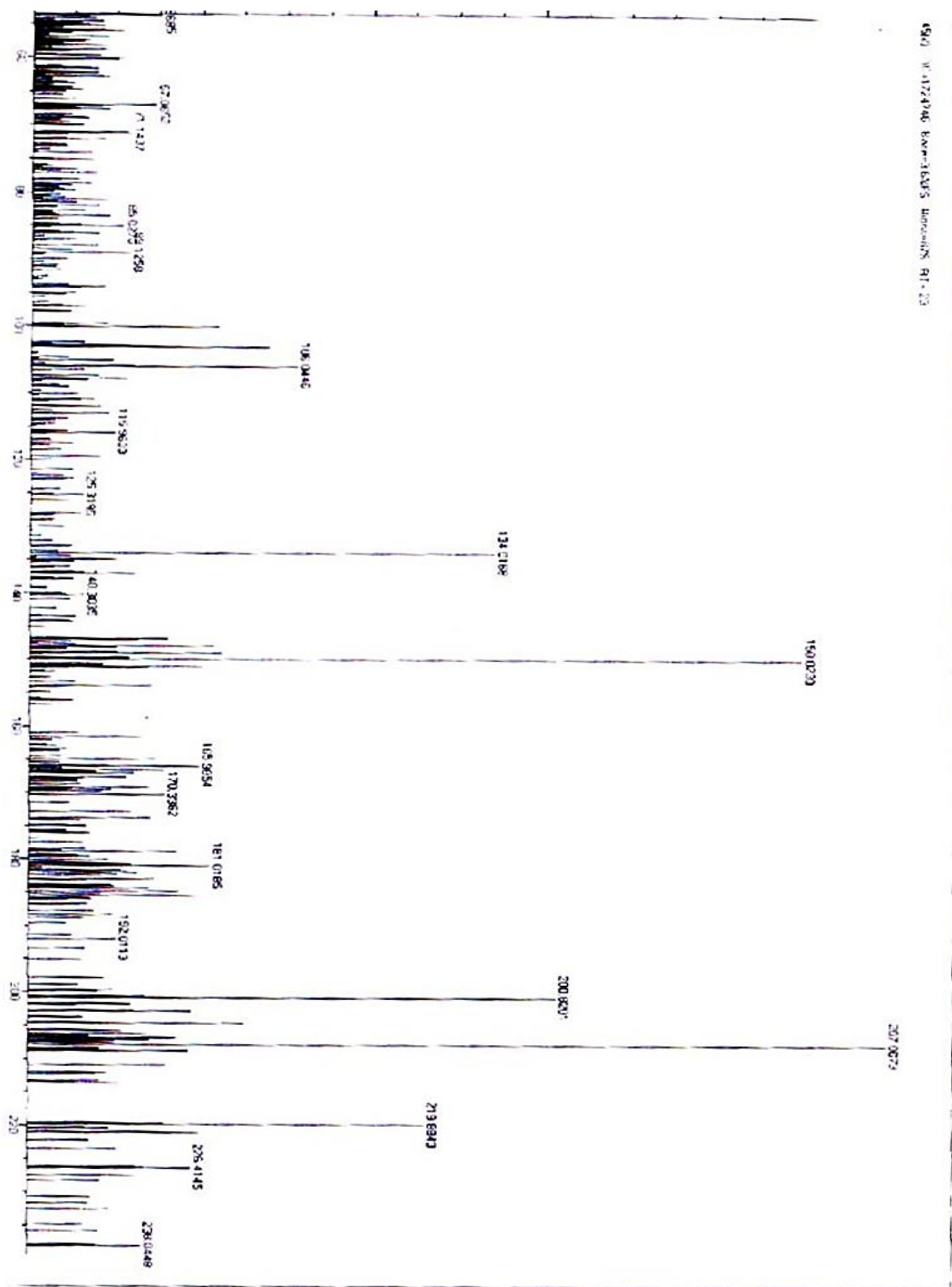
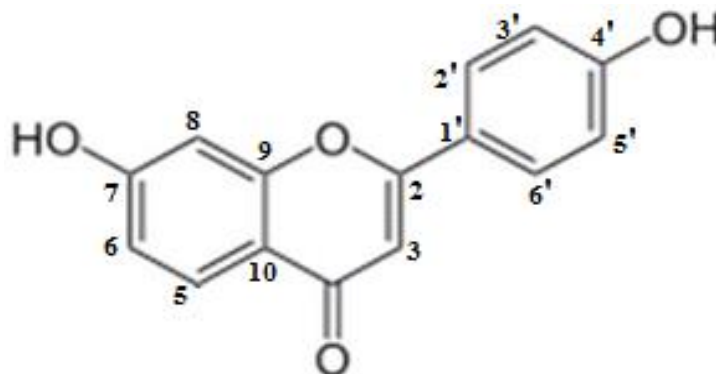


Fig. 5. Mass Spectrum of 4',7-dihydroxy isoflavone



**Fig. 6.** Structure of 4',7-dihydroxy isoflavone (Daidzein).

Mass spectra of isolated compound show molecular ion  $m/z$  238  $[M^+]$  corresponding to the molecular formula  $C_{15}H_{10}O_4$ . Based on the above spectral and chemical studies it was suggested that compound having an isoflavonoid skeleton having two phenolic hydroxyl groups. The placement of the phenolic hydroxyl groups were identified at C-7 and C-4' positions. Thus, based on the above spectral data, structure of was assigned as 4',7-dihydroxyisoflavone (Daidzein) consistent to the reported literature values [20].

## 5. RESULT AND DISCUSSION

4',7-dihydroxyisoflavone (Daidzein) has been isolated successfully from the medicinal plant *Indoneesiella echioides* (L) Nees leaf under present study. Similarly De-Yang Shen et al [21], reported that the new compounds of androgechosome A (5,8,2'-trihydroxy-7-methoxyflavone-5-*O*- $\beta$ -D-glucopyranoside), androgechosome B (2*R*)-5,2'-dihydroxy-7-methoxyflavanone-5-*O*- $\beta$ -D-glucopyranoside), androechioside A (2-*O*- $\beta$ -D-glucopyranosyl-4-methoxy-2,4,6-trihydroxybenzoate), androechioside B (methyl 3-(2-hydroxyphenyl)-3-oxopropanoate 2-*O*- $\beta$ -D-glucopyranoside) are isolated and structurally elucidated by spectral analysis and chemical transformation and 37 known compounds were identified to be, 2',6'-dihydroxyacetophenone 2'-*O*- $\beta$ -D-glucopyranoside, echioidinin 5-*O*- $\beta$ -D-glucopyranoside, echioidinin, pinostrobin, andrographidine C, dihydroechioidinin, tectochrysin 5-glucoside, methyl salicylate glucoside, 7,8-dimethoxy-5-hydroxyflavone, 5,7,8-trimethoxyflavone, skullcapflavone I 2'-methyl ether, acetophenone-2-*O*- $\beta$ -D-glucopyranoside, androechin, skullcapflavone I 2'-*O*- $\beta$ -D-glucopyranoside, tectochrysin, 5,7,2'-trimethoxyflavone, echioidin, skullcapflavone I, 5,7-dimethoxyflavone, negletein 6-*O*- $\beta$ -D-glucopyranoside, andrographidine E, 4-hydroxy-3-methoxy-*trans*-cinnamic acid methyl ester, 4-hydroxybenzaldehyde, 4-hydroxy-*trans*-cinnamic acid methyl ester, *O*-coumaric acid, 2,6-dihydroxybenzoic acid, 132-hydroxy-(132-*R*)-phaeophytin, (*E*)-phytyl-epoxide, phytol, phytene 1,2-diol, (+)-dehydrovomifoliol, 3 $\beta$ -hydroxy-5 $\alpha$ , 6 $\alpha$ ,-epoxy-7-megastigmen-9-one,  $\beta$ -sitosterol,  $\beta$ -sitosteryl-3-*O*- $\beta$ -glucopyranoside, squalene, 1*H*-indole-3-carbaldehyde, and loliolide by comparison of their physical and spectral data with those reported in the literature.

## 6. CONCLUSION

In the present study preliminary phytochemical analysis of the *Indoneesiella echioides* (L) Nees revealed the presence of flavonoids, alkaloids, terpenoids, triterpenoids saponins, saponins, phenolic compound, sterols and amino acids are qualitatively analysed and the results are listed in Table 1. The GC- MS studies of ethyl acetate fraction of *Indoneesiella echioides* (L) Nees leaves was clearly indicate that the 4',7-dihydroxyisoflavone (Daidzein) and also further confirmed by IR, <sup>1</sup>H, <sup>13</sup>C-NMR and MASS spectral data's. The isolation of the characterized flavonoids would be useful to prepare plant based pharmaceutical preparation to treat various complications linked with human diseases.

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### Reference

- [1] Nadkarni AK, Nadkarni KM. Indian Materia Medica, Vol. 1. Bombay: Popular Prakashan, 1976.
- [2] Qadrie ZL, Beena Jacob, Anandan R, Raj Kapoor B, Rahamathulla M., Antibacterial activity of ethanol extracts of *Indoneesiella echioides* evaluated by the filter paper disc method. *Pak J Pharm Sci*, 2009, 22: 123-125.
- [3] Kirtikar KR, Basu BD. In Indian medicinal plants, 3. New Delhi: Periodical Experts. 1975.
- [4] Kirtikar K.R., Basu B.D., *Indian Medicinal Plants*; Periodical Experts Book Agency: New Delhi, India, 3 (1975) 1884-1886.
- [5] Chopra R.N., Nayer S.L., Chopra I.C., *Glossary of Indian Medicinal Plants*; Council of Scientific and Industrial Research: New Delhi, India, 1980; p. 18.
- [6] Pandi Kumar P., Ayyanar M, Ignacimuthu S., Medicinal plants used by Malasar tribes of Coimbatore district, Tamil Nadu, *Indian J Tredit Knowl*, 2007; 6: 579-582.
- [7] Harbone, J.B. *The Flavonoids: Advances in research science 1986*; Chapman and Hall: London, UK, 1994: pp. 280-290.
- [8] Inuma, M.; Mizuno, M. Natural occurrence and synthesis of 2' – oxygenated flavones, flavonols, flavonones and chalcones. *Phytochemistry* 1989, 28, 681-694.
- [9] Kleipool, R.J.C. Constituents of *Andrographis paniculata* Nees. *Nature* 1952, 169, 33-34.
- [10] Chan, W.R.; Taylor, C.R.; Willis, R.L.; Bodden, R.L. The structure and stereochemistry of neoandrographolide, a diterpene glucoside from *Andrographis paniculata* Nees. *Tetrahedron* 1971, 27, 5081-5091.

- [11] Balmain, A.; Connolly, J.D. Minor diterpenoid constituents of *Andrographis paniculata* Nees. *J. Chem. Soc., Perkin Trans. I* 1973, *I*, 1247-1251.
- [12] Fujita, T.; Fujitani, R.; Takeda, Y.; Takaishi, Y.; Yamada, T.; Kido, M.; Miura, I. On the diterpenoids of *Andrographis paniculata*: X – ray crystallographic analysis of andrographolide and structure determination of new minor diterpenoids. *Chem. Pharm. Bull.* 1994, *42*, 1216-1225.
- [13] Matsuda, T.; Kuroyanagi, M.; Sugiyama, S.; Umehara, K.; Ueno, A.; Nishi, K. Cell differentiation – inducing diterpenes from *Andrographis paniculata* Nees. *Chem. Pharm. Bull.* 1994, *42*, 1216-1225.
- [14] Reddy, M.K.; Reddy, M.V.; Gunasekar, D.; Murthy, M.M.; Caux, C.; Bodo, B.A flavone and an unusual 23 – carbon terpenoid from *Andrographis paniculata*. *Phytochemistry* 2003, *62*, 1271-1275.
- [15] Govindachari, T.R.; Parthasarathy P.C.; Pai, B.R.; Subramaniam, P.S. *Andrographis echioides*.I. Structure and synthesis of echioidinin. *Tetrahedron* 1956, *21*, 2633-2640.
- [16] Govindachari, T.R.; Parthasarathy P.C.; Pai, B.R.; Subramaniam, P.S. *Andrographis echioides*. II. Structure and synthesis of echioidinin. *Tetrahedron* 1956, *21*, 3715-3720.
- [17] Jayaprakasam, B.; Damu, A.G.; Gunasekar, D.; Blond, A.; Bodo, B. Dihydroechioidinin, a flavone from *Andrographis echioides*. *Phytochemistry* 1999, *52*, 935-937.
- [18] Jayaprakasam, B.; Gunasekar, D.; Rao, K.V.; Blond, A.; Bodo, B. Androechin, a new chalcone glucoside from *Andrographis echioides*. *J. Asian Nat. Prod. Res.* 2001, *3*, 43-48.
- [19] Sharma, SK, Ali M, Gupta J: Hepatoprotective activity of aqueous ethanolic extract of *Chamomile capitula* in paracetamol intoxicated albino rats. *Phytochem Pharmacol*, 2002; *2*, 253-270.
- [20] Kinjo, J.E.; Furusawa, J.I.; Baba, J.; Takeshita, T.; Yamasaki, T.; Nohara, T. Studies on the constituents of *Pueraria lobata*. III. Isoflavonoids and related compounds in the roots and the voluble Stems, *Chem. Pharm. Bull.*, 1987, *35*, 4846-4850.
- [21] De-Yang Shen, Shin-Hun Juang, Ping-Chung Kuo, Guan-Jhong Huang, Yu-Yi Chan, Amooru G. Damu and Tian-Shung Wu: Chemical Constituents from *Andrographis echioides* and Their Anti-Inflammatory activity, *Int. J. Mol. Sci.* 2013, *14*, 496-514.

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