

## Fatty Acid Methyl Ester Profile Analysis of *In-Vitro* Grown Accessions of *Plumbago zeylanica*

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### Abstract

*Plumbago zeylanica* is a pharmaceutically important plant which belongs to *Plumbaginaceae* family. It possesses wide range of pharmacological activities which includes antibacterial central nervous system stimulatory activity, anti-fungal, anti-inflammatory, anti-hyperglycemic, anti-cancer and anti-atherosclerotic activity. Fatty acid methyl ester analysis reveals the presence of various fatty acids in the plant. The present study deals with Fatty acid methyl ester profile of five different accession of *Plumbago zeylanica*. Results reveal that these accessions are rich in Octadecadienoic acid (8-22%), Octadecatrienoic acid (7-24%) and Pentadecanoic acid (11-22%) fatty acids. Accession number 421418 is contains the highest amount of Pentadecanoic acid and 9,12,15 Octadecatrienoic acid. Accession number 439212 contains highest amount of Hexadecanoic acid. Highest amount of 9,12 Octadecadienoic acid was present in accession number 524441.

**Keywords:** *Plumbago zeylanica*; Accessions; Fatty acid methyl ester; MS medium

### Introduction

*Plumbago zeylanica* is an important medicinal plant which belongs to the *Plumbaginaceae* family. It is commonly known as 'Chitra' and grows in the Bengal and Southern India [1]. It's a perennial herb and widely used in the treatment of diseases such as diarrhoea, dyspepsia, skin diseases, piles, rheumatism and leprosy [2]. It contains a variety of secondary metabolites like glycosides, steroids, saponins, flavonoids, alkaloids, tri-terpenoides, tannins, coumarins, phenolic compounds, carbohydrates, fixed oils, fats, proteins and naphthoquinones [3,4]. It possesses anti-bacterial, anti-fungal [5] anti-cancer [6], anti-plasmodial, anti-tumour, hepatoprotective, central nervous system stimulatory activity, anti-inflammatory [7], anti-hyperglycemic, anti-atherosclerotic activity [8]. An accession means collection of plant material of single species from a specific geographical location. Each accession is an attempt to capture the diversity present in a given plant population. Accession number is given a unique identifier, and it is used to maintain associated information in the database. It exhibits significant variations in morphological parameters like growth of leaf, flowering, stomatal frequency, etc. The purpose of using different accession was to choose the best accession for phytochemicals production [9]. Fatty acids are identified as active ingredients in herbal medicine. FAME analysis provides an extensive knowledge of volatile compounds present in the plants. Fatty acid profiling known as FAME determines presence of fatty acids in the sample. This process hydrolyzes fatty acids from triacylglycerols, phospholipids, sterols, and various other lipid structures and then adds a methyl group to carboxyl group of fatty acids which forms a methyl ester that acts as a label for the Gas Chromatography, which then reads and identifies those fatty acids. The system was termed FAME analysis since it utilizes fatty acid methyl esters. Resulting composition profile provides the critical assistance in valuable oil present in the sample. Unsaturated fatty acids which includes monounsaturated fatty acids (MUFA) and

polyunsaturated fatty acids (PUFA) are health-promoting and have significant metabolic and cardiovascular benefits [10]. Fatty acids play important role in the human diet, it is estimated that human brain contains nearly 60% fat [11]. Usually essential fatty acids are found in plants and they play vital role in brain functions [12]. They also regulate lymphocyte metabolism and proliferation induces T cell death and enhance auto-immunity [13]. Fatty acids also possess anti-cancer activity [14]. There is no reported study about the FAME analysis in the leaves of *P. zeylanica*. Thus, an attempt has been made to study the FAME analysis in this plant.

### Materials and Methods

#### Plant material

Five different accessions of *P. zeylanica* i.e., 398891, 524441, 421418, 439212, and 539866 were collected from NBPGR, New Delhi, India. They were maintained in MS basal media in the Plant Biotechnology Laboratory, Delhi Technological University.

#### Fatty acid methyl ester (FAME) analysis of accessions

For FAME analysis, 100 mg leaves of each accession of *Plumbago zeylanica* were slightly crushed in mortar pestle and then transferred to a screw-cap glass tubes. 1 ml of 2% methanolic HCl was added to the crushed leaves and samples were then incubated at 90°C for an hour. After that 0.9% of NaCl solution in water was added followed by 2 ml of hexane and mixed. Samples were then centrifuged for phase separation at 2000 rpm for 2 minutes. After centrifugation, the upper (hexane) layer of the sample was transferred into a fresh glass tube and dried under nitrogen flow. Dried samples were then diluted with the 100 µl of hexane. One by one 1 µl sample were then injected into GC-MS analysis.

## Results and Discussion

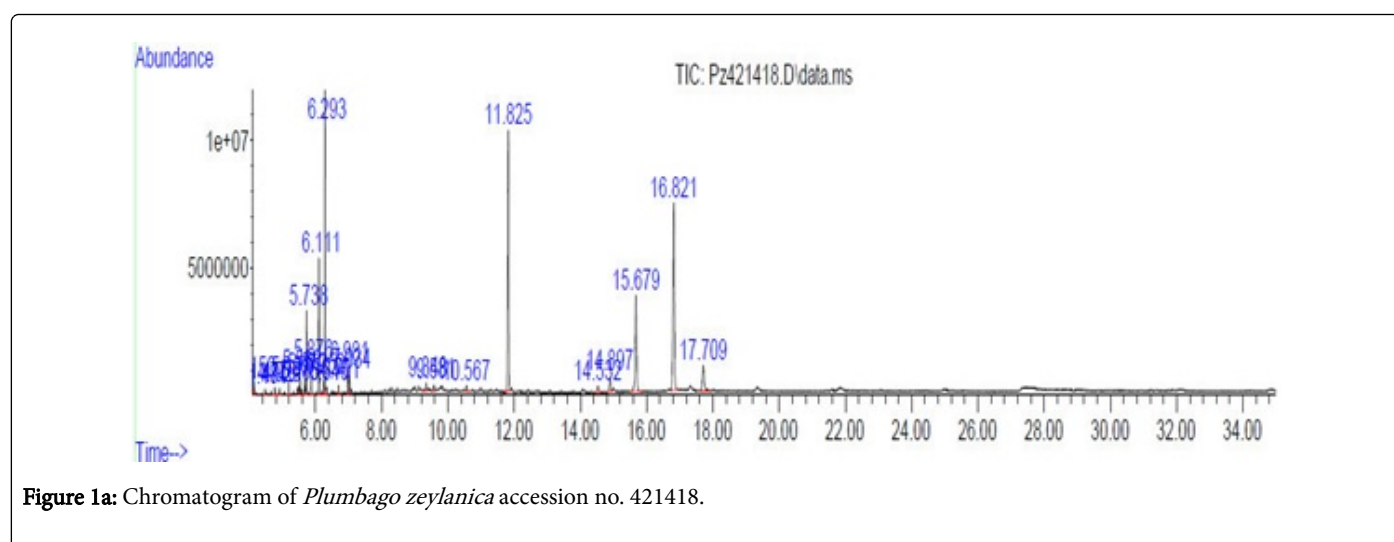
### Fatty acid methyl ester (FAME) production and analysis

Fatty acid as well as their relative percentage composition obtained from the GC-MS analysis of the n-hexane extracts of five different accessions of *Plumbago zeylanica* (Table 1 and Figures 1a-1e). The plant sample contain various fatty acids, among which Octadecadienoic acid (8-22%), Octadecatrienoic acid (7-24%) and Pentadecanoic acid (11-22%) being present in maximum amounts as per the chromatograms obtained. Tetradecanoic acid (0-2%), Hexadecanoic acid (1-2%) and Octadecanoic acid (1-7%) were also obtained but comparatively low amount in all the five accessions of *P. zeylanica*. Accession number 524441 contains maximum number of fatty acids compared to the other accessions. Unsaturated fatty acids, Octadecadienoic and Octadecatrienoic acid are the most important essential fatty acids as our body cannot synthesize these fatty acids.

Accession no. 539866 contain highest percent of Octadecadienoic acid (approximately 23%) followed by 524441 (18.45%), 398891 (14.57%), 421418 (12.38%) and 439212 (8.87%). When we consider the presence of Octadecatrienoic acids, it is recorded that accession no. 421418 contain the highest percent approx. 24% followed by 43921 (9.05%), 524441 (7.85%), 539866 (7.53%) and 398891 (7.004%). Linoleic acid (Octadecadienoic acid) is essential for maintenance of growth and shown to be potent cyclooxygenase-2 (COX-2) catalysed prostaglandin biosynthesis inhibitors. Palmitic acid (Hexadecanoic acid) reduces the risk of cardiovascular disease. Stearic acid used in baked food items. Only three accessions of *P. zeylanica* contain approximately 2 percent of Hexadecanoic acid (stearic acid) (Table 1). Thus it can be concluded that this plant is a good source of fatty acids and can be used in cholesterol-lowering diets. Similar study has been conducted by Roy et al. [15] in case of *Centella asiatica* where they have reported the presence of Pentadecanoic, Hexadecanoic and Octadecanoic acid, Octadecadienoic and Octadecatrienoic.

Fatty Acid	Relative Percentage of Fatty acid in different Accessions				
	421418	398891	539866	439212	524441
Tetradecanoic acid	0	1.16	0	0	0.47
Pentadecanoic acid	22.33	11.62	15.69	17.66	15.27
Hexadecanoic acid	0	0	1.45	2.18	1.12
Octadecanoic acid	1.57	4.17	5.87	4.16	6.65
9,12 Octadecadienoic acid	12.38	14.57	22.46	8.87	18.45
9,12,15 Octadecatrienoic acid	23.86	7.004	7.53	9.05	7.85
Total	60.14	38.52	53	41.92	49.81

**Table 1:** Percentage of methylated fatty acids of five different accession of *Plumbago zeylanica*.



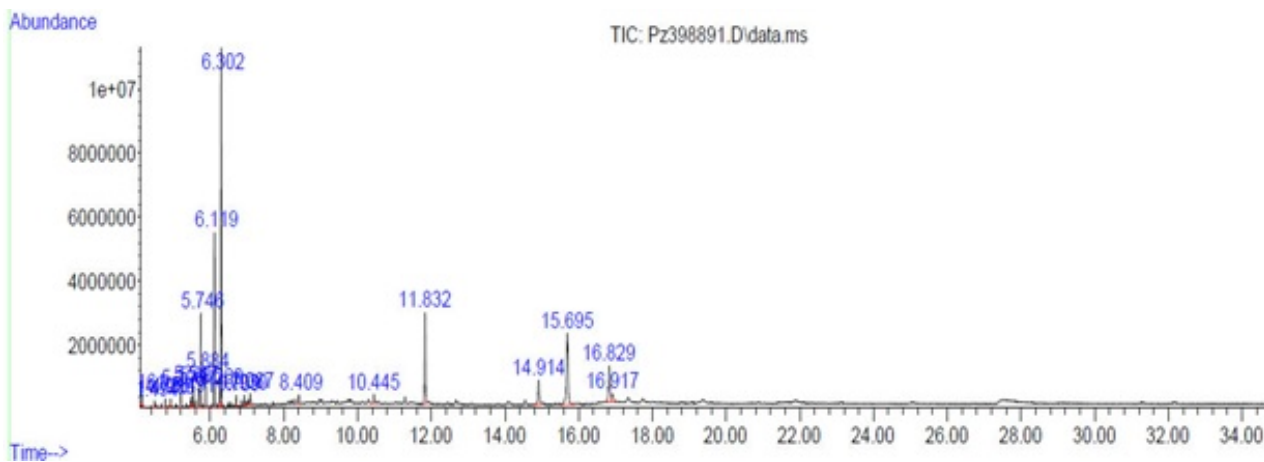


Figure 1b: Chromatogram of *Plumbago zeylanica* accession no. 398891.

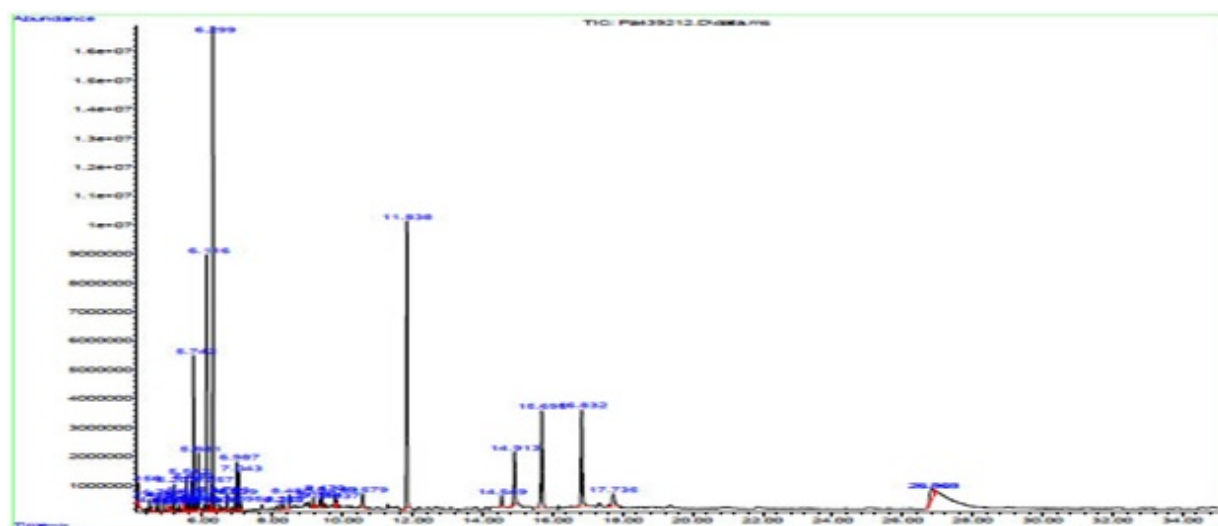


Figure 1c: Chromatogram of *Plumbago zeylanica* accession no. 539866.

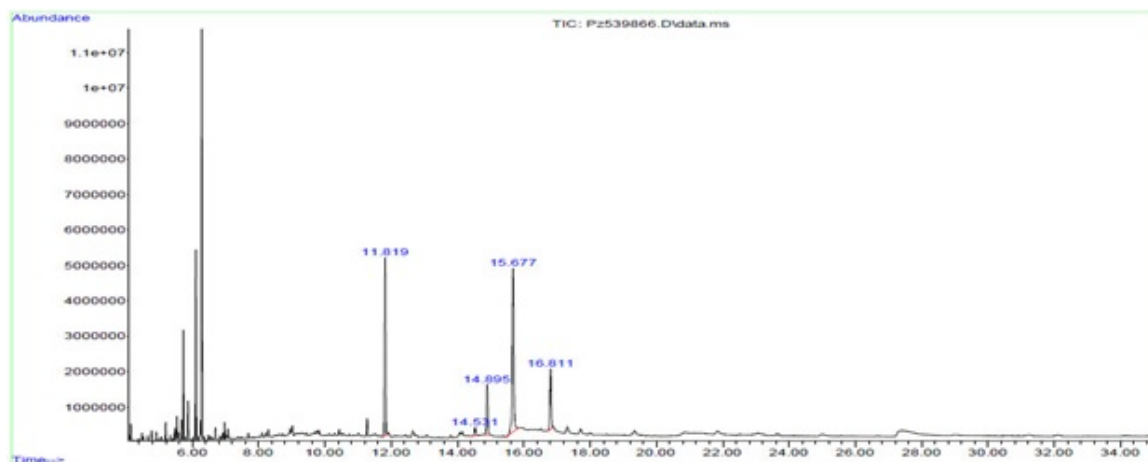


Figure 1d: Chromatogram of *Plumbago zeylanica* accession no. 439212.

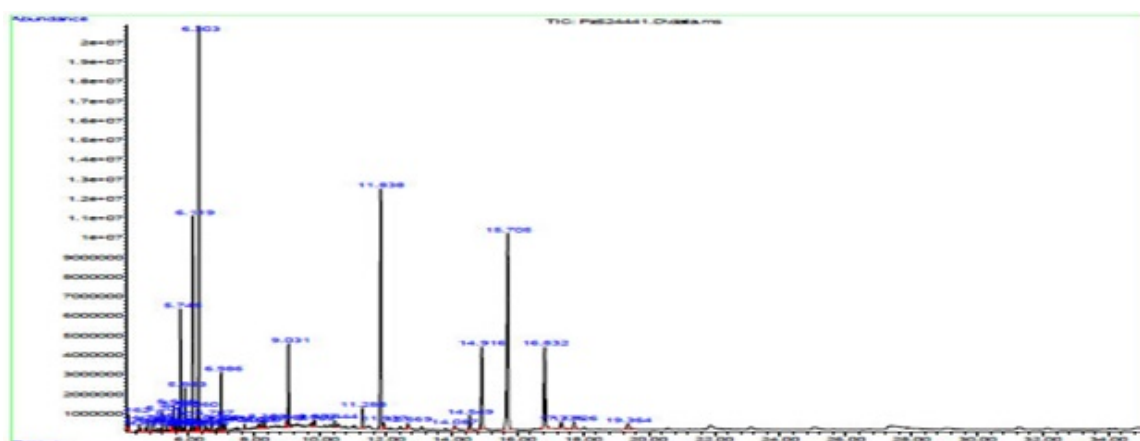


Figure 1e: Chromatogram of *Plumbago zeylanica* accession no. 524441.

## Conclusion

FAME analysis respectively showed that this plant is rich in Octadecadienoic acid (8-22%), Octadecatrienoic acid (7-24%), Pentadecanoic acid (11-22%) fatty acids. Pentadecanoic acid, Hexadecanoic acid, Octadecanoic acid are saturated fatty acids while other are unsaturated fatty acids. Accession number 421418 showed highest amount of Pentadecanoic acid and 9,12,15 Octadecatrienoic acid.

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