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Original Article

Evaluation of Non-polar Composition in *Plumbago Zeylanica* Leaves by Gas Chromatography and Mass Spectrometry

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Abstract

Background: *Plumbago zeylanica* plant belongs to *Plumbaginaceae*. The plant is reported for many pharmacological activities.

Aim: The objective of the study was to identify fatty acids and non-polar chemical compounds in *Plumbago zeylanica* leaves.

Materials and methods: Petroleum ether extract was prepared using soxhlet apparatus. Saponifiable and unsaponifiable matter was separated with saponification process. To identify fatty acids in saponifiable matter further esterification was performed. Gas chromatography and mass spectrometry analysis was performed of both saponifiable and unsaponifiable fractions. All the fatty acid methyl esters and non-polar chemical compounds were identified using NIST library data.

Results: A total of 14 compounds were identified with comparison of NIST data. From that, 8 fatty acid methyl esters and 6 non-polar chemical compounds were identified. Here we have analyzed fatty acids and non-polar chemical compounds by the same GC-MS method.

Conclusions: The present analysis showed that *Plumbago Zeylanica* leaves contain 8 fatty acids and 6 non-polar chemical compounds. Principal determination of the research was development of efficient method to identify non-polar compound from plant by single injection using chromatographic technique.

Keywords

fatty acid, GC-MS, plumbaginaceae, plumbagin, sterol

INTRODUCTION

Plumbago zeylanica is an important medicinal plant. It is found in tropical Africa and Asia, especially in the west zones.[1,2] The plant have antimicrobial and antiulcer activity, anti-obesity, anti-inflammatory, and hypocholesterolemic activity, hepatoprotective, wound healing activity, and cytotoxicity.[3] *Plumbago zeylanica* plant is mainly known for its bioactive compound – plumbagin. It is reported to have anti-carcinogenic activity, antioxidant, cardio-protective, antimalarial, antifungal, anti-atherosclerotic, central nervous system stimulatory, anti-hyperglycemic, and anti-inflammatory activities.[3-7] The *Plumbago zeylanica* plant contains many chemical compounds.[8] Phytochemical screening of the plant shows presence of fatty acids, saponins, carbohydrate, steroid, tannins, flavonoids, and alkaloids.[9-10] Chemical compounds can be categorized into polar and non-polar compounds. Non-polar profile of plant leaves was still not reported. Here we are reporting for the first time non-polar compounds presence in plant leaves of *Plumbago zeylanica*.

MATERIALS AND METHODS

Petroleum ether, methanol, KOH, n-hexane, ethyl acetate, diethyl ether, and chloroform of Merck Ltd. were used. *Plumbago zeylanica* plant (leaves) was obtained from RK University garden in Rajkot and it was identified by Dr. Anjisha Maharshi, School of Science, RK University.

Preparation of extracts [11]

Plumbago zeylanica plant was dried for one to two weeks at room temperature. The dried material was finely powdered. 126.50 gm of powder was extracted with petroleum ether for at 60-80°C using soxhlet apparatus and the obtained extract was 3.40 gm.

Saponification[12-14]

The petroleum ether extract obtained by soxhlet extraction was then suspended in 20% alcoholic KOH in round bottom flask. This solution was then refluxed for 8 hours with constant stirring. After it was refluxed, the solution was collected in a separating funnel and 30 ml distilled water was added. 100 ml of diethyl ether was added to the separating funnel stirred, shaken and then left to stand for separation of layers. Two layers were formed, an aqueous layer containing saponifiable fraction (A) and an organic layer containing unsaponifiable fraction (B). Multiple times extracted with diethyl ether and separated saponifiable fraction (A) and unsaponifiable fraction (B).

Saponifiable fraction (A)[15,16]

Saponifiable fraction (A) was acidified with 5N H_2SO_4 and the aqueous layer was extracted 3-4 times with diethyl ether. Ethereal layer was collected and washed with distilled water, dried over anhydrous Na_2SO_4 and evaporated to obtain fatty acid portion (100 mg). An accurately (90 mg) weighed fatty acid portion was taken with 10 ml methanol in presence of catalytic amount conc. H_2SO_4 and refluxed on water bath for 10 hrs. Cooled the same and diluted with (40 ml) water and extracted using hexane. The extract was dried over Na2SO4 and volume was made up to 10 ml with hexane and gas chromatography, and mass spectrometry analysis was performed.

Unsaponifiable fraction (B)

The unsaponifiable fraction (B) was washed with water till it became neutral and then dried with sodium sulphate.[17] It was filtered and dried. The obtained dried unsaponifiable matter was 0.812 gm. The unsaponifiable extract was further dissolved in di-ethyl ether and gas chromatography and mass spectrometry analysis was performed.

GC-MS Protocol

The GC-MS analysis was carried out using Agilent Technologies Model 5977B MS occupied to 7820A GC equipped with HP-5 capillary column (30 m×0.32 mm i.d.; 0.25 µm film thickness). The column temperature was initially held at 60°C for 5 min, then increased to 100°C with a rate of 10°C/min, held for 1 min further, raise the temperature to 150°C at a rate of 10°C/min, held for 10 min after that to increase temperature up to 200°C at a rate of 10°C/min, hold time 10 min, continue to 250°C with rate of 10°C/min, hold time 10 min. Raise the temperature to 300°C at a rate of 10°C/min and hold time 5 min. Helium flow rate was 1.3 ml/min. The ion source of the MS was operated at 230°C and the transfer line at 260°C. Electron impact (EI) ionization was carried out at 1195.2 EMV, and quantitative determination was based on the total ion current corrected for the detector response of each compound. For analysis, compounds were dissolved in diethyl ether (2 ml) and 1 µl aliquots by auto-injector. The mass range from 50-850 Amu was scanned at a rate of 1562 [N= 2] unit/second. Compounds were identified by direct comparison of mass and their patterns were matched with data from the NIST 14 Library having more than 2,50,000 mass patterns of reported compounds.

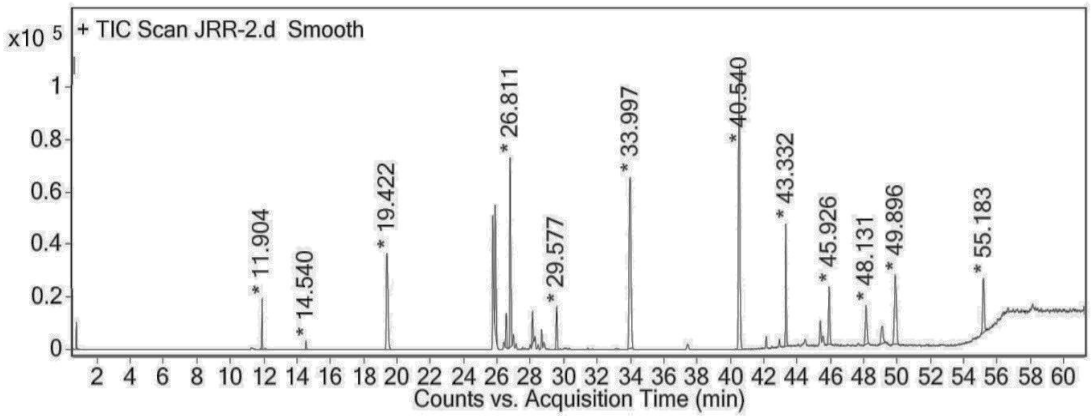

Figure 1. GC graph of saponifiable fraction.

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Table 1. Fatty acids identified from *Plumbago zeylanica*

Peak	RT	Name of the compound	Molecular formula	M.W.	Peak area %
1	14.54	Pimelic acid, isobutyl 4-octyl ester	$C_{19}H_{36}O_4$	328.5	0.38
2	26.557	Methyl stearate	$C_{19}H_{38}O_2$	298.50	1.64
3	29.577	Adipic acid,3-oxobut-2-yl undecy ester	$C_{21}H_{38}O_5$	370.52	2.21
4	33.997	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	354.61	16.76
5	40.54	Tetracosanoic acid, methyl ester	$C_{24}H_{50}O_2$	382.66	21.23
6	43.332	Hexacosanoic acid, methyl ester	$C_{27}H_{54}O_2$	410.72	6.17
7	45.926	Octacosanoic acid, methyl ester	$C_{29}H_{58}O_2$	438.77	4.09
8	49.896	Triacontanoic acid, methyl ester	$C_{31}H_{62}O_2$	466.82	7.77

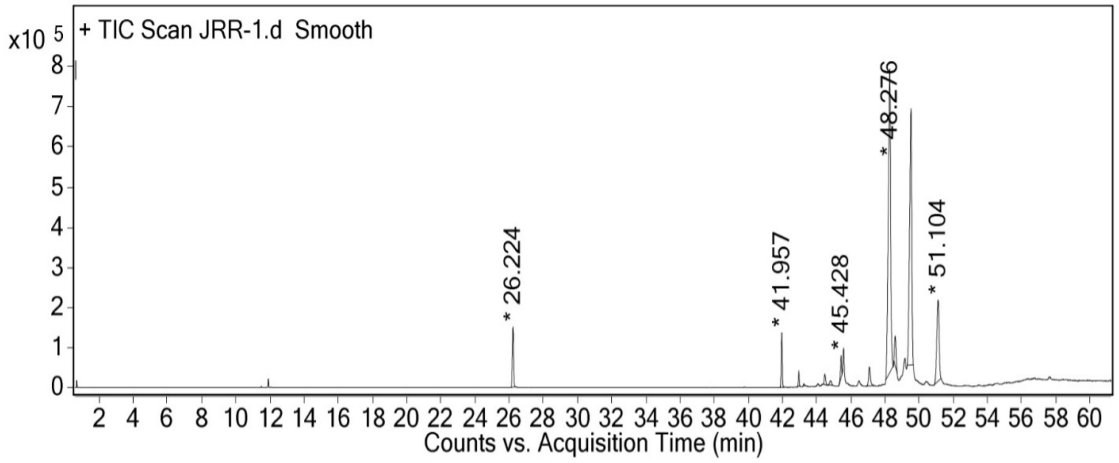

Figure 2. GC graph of unsaponifiable fraction.

Table 2. Non-polar compounds in *Plumbago zeylanica*

Peak	RT	Name of the compound	Molecular formula	M.W.	Peak area %
1	41.957	Longiverbenone	$C_{15}H_{22}O$	218.33	3.37
2	47.088	24-Norchol-22-ene-3,12-diol, diacetate, (3α., 5β., 12α)	$C_{27}H_{42}O_4$	430.63	1.96
3	48.276	Gamma-sitosterol	$C_{28}H_{50}O$	414.71	40.99
4	48.598	Stigmasta-5,24(28)-dien-3-ol, (3 β.,24z)	$C_{29}H_{48}O$	412.69	2.69
5	49.517	Taraxasterol	$C_{30}H_{50}O$	426.72	32.42
6	51.104	Lanosterol	$C_{30}H_{50}O$	426.72	10.96

RESULTS

Saponifiable fraction (A)

Plant contains non-polar compounds as lipid and other compounds. Here we have converted lipid into their esters by esterification and then identified using GC-MS. Direct injection of lipid was not possible into GC-MS. So, it was necessary to convert lipids into their esters. The result showed that by the esterification procedure eight fatty acids were identified by GC-MS. Single injection of esters mixture in GC-MS gives accurate results for identification of fatty acids with use of NIST library. Here we have identified eight fatty acids methyl esters from leaves of *Plumbago zeylanica* (**Fig. 1**, **Table 1**).

Unsaponifiable fraction (B)

Unsaponifiable fraction was injected into GC-MS and six non-polar compounds were identified confirmed by GC-MS. The comparison of the mass spectrums with the NIST database gave more as confirmatory compound structure match (**Fig. 2**, **Table 2**).

Compound-1 (JRR-1): Longiverbenone, M.F.- $C_{15}H_{22}O$, M.W. – 218.33, RT- 41.957. **GC-MS fragment**: The peak at 41.957 minutes had a mass [M^+] 218.33. The daughter ion spectra of compound revealed the characteristic fragments *m/z* 121.1, 163.2, 203.2, 218.33.

Compound-2 (JRR-1): 24-Norchol-22-ene-3,12-diol,-diacetate,(3.alpha.,5.beta.,12.alpha.),M.F.- $C_{27}H_{42}O_4$, M.W. – 430.63, RT-47.088. **GC-MS fragment**: The peak at 47.088 minutes had a mass [M^+] 430.63. The daughter ion spectra of the compound revealed the characteristic fragments *m/z* 133.1, 159.1, 191.1, 255.2, 271.2, 430.63.

Compound-3 (JRR-1): γ-sitosterol, M.F. - $C_{29}H_{50}O$, M.W. - 414.71, RT-53.983. **GC-MS fragment**: The peak at 48.276 minutes had a mass [M^+] 414.4. The daughter ion spectra of the compound revealed the characteristic fragments *m/z* 145.1, 213.2, 255.2, 329.3, 414.4.

Compound-4 (JRR-1): Stigmasta-5,24(28)-dien-3-ol,(3β, 24z), M.F. - $C_{29}H_{48}O$, M.W. - 412.69, RT-48.598. **GC-MS fragment**: The peak at 48.598 minutes had a mass [M^+] 412.69. The daughter ion spectra of the compound revealed the characteristic fragments *m/z* 119.1, 145.1, 159.2, 213.2, 281.2, 314.3, 394.4

Compound-5 (JRR-1): Taraxasterol**,** M.F.- $C_{30}H_{50}O$, M.W. - 426.72, RT-49.517. **GC-MS fragment**: The peak at 49.517 minutes had a mass [M^+] 426.7, the daughter ion spectra of the compound revealed the characteristic fragments *m/z* 189.2, 207.2, 218.2, 411.4, 426.7.

Compound-6 (JRR-1): Lanosterol, M.F. - $C_{30}H_{50}O$, M.W. - 426.72, RT-51.104. **GC-MS fragment**: The peak at 51.104 minutes had a mass [M^+] 426.72. The daughter ion spectra of the compound revealed the characteristic fragments *m/z* 137.2, 159.2, 187.1, 229.2, 259.2, 393.4, 411.4, 426.7.

DISCUSSION

The saponifiable fraction which was separated through saponification from petroleum ether extract contains eight fatty acids i.e. pimelic acid isobutyl 4-octyl ester, methyl stearate, adipic acid, 3-oxobut-2-yl undecy ester, docosanoic acid methyl ester, tetracosanoic acid methyl ester, hexacosanoic acid methyl ester, octacosanoic acid methyl ester, triacontanoic acid methyl ester. All the fatty acid was identified as their fatty acid methyl ester. The unsaponifiable matter was separated through extraction with diethyl ether. From the unsaponifiable fraction longiverbenone, 24-Norchol-22-ene-3,12-diol, diacetate (3α., 5β., 12α), gamma-sitosterol, stigmasta-5, 24(28)-dien-3-ol, (3β,24z), taraxasterol, lanosterol were identified. Previously, Ajayi et al. reported GC-MS analysis of roots of *Plumbago zeylanica.* In the study, ethanol extract was used for GC-MS analysis. Here we used petroleum ether solvent to identify particularly non-polar compounds with respect to fatty acids and other non-polar chemical entity. On the other hand, our study used the leaves of *Plumbago zeylanica.* Here we are reporting for the first time the non-polar compound profile of *Plumbago zeylanica* leaves. So, the objective of study was completely achieved with the use of chromatographic techniques i.e. TLC and GC-MS.

CONCLUSION

An efficient and rapid method was developed for efficient separation of fatty acids and nonpolar chemical entity using GC-MS. The fatty acids and non-polar chemical compounds identified in this experiment may be responsible for the biological activity of the plant. The GC-MS method can be used in pharmaceutical and natural product industry. Here we have done a non-polar study of *Plumbago zeylanica* plant leaves. Researchers can take initiative to study the polar compound in leaves of this plant for further studies.

Acknowledgments

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Оценка неполярного состава листьев *Plumbago Zeylanica* методом газовой хроматографии и масс-спектрометрии

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Резюме

Введение: Растение Plumbago Zeylanica (читрак) относится к Plumbaginaceae. Растение обладает широким спектром фармакологического действия..

Цель: Целью данного исследования было выявление жирных кислот и неполярных химических веществ в листьях Plumbago Zeylanica.

Материалы и методы: Экстракт петролейного эфира изготовили с использованием аппарата Сокслета. Омыленная и неомыленная фракция была получена в процессе омыления. Была проведена дополнительная этерификация для установления наличия жирных кислот в омыленной массе. Газовую хроматографию и масс-спектрометрию проводили как на омыленных, так и на неомыленных фракциях. Все метиловые эфиры жирных кислот и неполярные химические составляющие были идентифицированы с использованием данных библиотеки NIST (Национальный институт стандартов и технологий).

Результаты: Было установлено общее количество в 14 ингредиентов при помощи сравнения данных NIST. Из них было выявлено 8 метиловых эфиров жирных кислот и 6 неполярных химических веществ. Здесь мы проанализировали жирные кислоты и неполярные химические вещества, используя тот же метод GC-MS.

Выводы: Настоящий анализ показал, что листья Plumbago Zeylanica содержат 8 жирных кислот и 6 неполярных химических веществ. Основная цель исследования заключалась в разработке эффективного метода идентификации неполярных компонентов растения путём однократной инъекции методом хроматографии.

Ключевые слова

жирные кислоты, GC-MS, plumbaginaceae, plumbagin, стерол