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Quantification of methyl esters of fatty acids in the oil of *Physalis minima* by GC-MS

Muhammad Nasimullah Qureshi[1*], Fazal Wajid[1], Inayat-ur-Rahman[2]

[1]*Department of Chemistry, Abdul Wali Khan University Mardan, Pakistan*

[2]*Medicinal Botanic Centre, PCSIR Laboratories Complex Peshawar, Pakistan*

PEER REVIEW

Peer reviewer

Prof. Murad Ali Khan, Department of Chemistry, Kohat University of Science & Technology, 26000 Kohat KPK, Pakistan.
Tel: +92-922-52914449
E-mail: drmalikhan@yahoo.com

Comments

It is an important research. *P. minima* is an important medicinal plant. The oil of *P. minima*is is an important constituent which has been analyzed and quantified for the fatty acids for the first time.
Details on Page 160

ABSTRACT

Objective: To investigate quantification of methyl esters of fatty acids in the oil extracted from *Physalis minima* (*P. minima*) using gas chromatography-mass spectrometer.

Methods: Oil was extracted from the shade dried plant with *n*-hexane through Soxhlet extraction. Fatty acids that present in the oil were derivatized to fatty acid methyl esters and analysed through gas chromatography-mass spectrometer.

Results: A total of nine fatty acids were detected in quantifiable amount in the oil. Both the saturated fatty acids and unsaturated fatty acids were identified. Palmitic acid was found in the highest concentration as 46.83%. Linoleic acid (ω-6) and linolenic acid (ω-3) were obtained in appreciable amount as 16.98% and 14.80% respectively among the unsaturated fatty acids in the oil under study. From the literature review, it appeared that fatty acids were determined for the first time in the oil of *P. minima*.

Conclusions: Presence of these important fatty acids in high amount makes *P. minima* oil beneficial for health, which can be used in the preparation of phytopharmaceutical or pharmaceutical preparations. Moreover, the results of this study are useful for the phytopharmaceutical industries to establish their quality control profile.

KEYWORDS

Physalis minima, Fixed oil, Quality control, Fatty acids, FAMEs, GC-MS

1. Introduction

Qualitative and quantitative evaluation of the chemical constituents is vital for establishing the quality control profile and standardization of herbal extracts[1-6]. This makes a base for the investigation of metabolites which have various biological and pharmacological importance[1,4,7,8]. Fatty acids, the basic structural units of lipid, are among the important quality markers which have biological and pharmacological importance, such as, for the evaluation of nutritional value of food, enhancing immunity and for lowering the risks of heart related diseases[7-18]. Various analytical approaches like enzymatic, spectrophotometric and chromatographic based procedures, such as high performance liquid chromatography (HPLC) and gas chromatography (GC), have been employed for the analysis of fatty acids[7,8,19-22]. GC coupled to mass spectrometer (MS) is the mostly employed technique for the evaluation of fatty acids in samples[7-8,23,24]. In order to develop fatty acid profile through GC, it is required that the fatty acids must be gaseous phase. Therefore, fatty acids have to be derivatized before the GC-MS analysis. Methylation is the method generally applied for obtaining volatile fatty acids methyl esters (FAMEs) from non-volatile fatty acids[7,8].

Physalis minima (*P. minima*) is a medicinal herb of the family Solanaceae. It is commonly known as sun berry or ground cherry.

*Corresponding author: Dr. Muhammad Nasimullah Qureshi, Associate Professor, Department of Chemistry, Abdul Wali Khan University Mardan, Pakistan.
Tel: +92 937 929122
E-mail: mnasimuq@yahoo.com

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It is a small, delicate, erect, annual and pubescent herb, about 1.5 m tall. Leaves are 9.7 cm long and 8.1 cm broad having a dark green dorsal surface and light green ventral surface. They are petiolate (4.1 cm long), ovate to cordate, pubescent, delicate, exstipulate, acuminate and have reticulate palmate venation and undulate margins. Flowers are pedicellate having 1.2 cm long pedicel, hermaphrodite, complete, solitary, small campanulate, 1.2 to 1.4 cm in diameter having a 5-toothed green calyx and yellow corolla with 1.1 to 1.3 cm long five petals. A berry fruit enclosed within the enlarged calyx. Fully matured fruit is prime yellow rose colour with a juice inside and it is a good source of vitamin C[25]. A number of medicinal uses have been attributed to *P. minima* like diuretic, purgative, analgesic, anthelmintic, febrifuge, vermifuge, abortifacient, *etc*. Various pharmacological activities including anti-inflammatory, hypoglycemic, anti-bacterial, antifertility, analgesic, anti-malarial, anti-pyretic, *etc.* have been shown for this species[25-27].

2. Materials and methods

2.1. Chemicals and reagents

Sodium chloride (analytical grade), dichloromethane (analytical grade) and methanolic NaOH solution (0.5 mol/L) was obtained from Merck (Darmstadt, Germany). While boron trifluoride solution (methanolic, 10%) was purchased from Fluka Chemie (Buchs, Switzerland). Normal hexane (HPLC grade) and methanol (HPLC grade) were purchased from Fischer Scientific (Leicestershire, UK). Helium gas (99.9999%) was obtained from Pak gas (United Arab Emirates). Standard mixture of FAMEs[8] was procured from AccuStandard (Newhaven, Connecticut, USA). Water used for samples preparation was double distilled.

2.2. Collection of plant material

Aerial part of the *P. minima* was collected from Swat valley of Pakistan during the month of June and identified by a group of taxonomists of Pakistan Council of Scientific and Industrial Research, Laboratories Complex Peshawar, Pakistan. Voucher specimen (No. PLC-201) was deposited in the herbarium of the labs.

2.3. Standard preparation

A total of 10 mg FAMEs mix (37 components) standard was dissolved in 10 mL dichloromethane. Working standard solutions in the concentration range 0.01 mg/mL to 1 mg/mL were prepared by diluting stock solution with dichloromethane. Standard solutions were preserved at appropriate temperature until use.

2.4. Sample preparation for GC

Shade dried plant materials were powdered and passed through a sieve of 2 mm mesh size. Fatty acids were extracted from 100 g powdered seeds with 250 mL *n*-hexane for 6 h through Soxhlet extraction. The solvent was recovered yielding a concentrated extract.

FAMEs were produced from free fatty acids present in seeds oil using BF_3-methanol as derivatizing reagent. Extraction and derivatization were performed according to the published procedures[7,8]. About 1.5 mL of methanolic solution of sodium hydroxide (0.5 mol/L) was added to the seed oil (equivalent to 25 mg fat) in a reaction tube. The tube was sealed and heated in boiling water bath for 5 min. About 2.5 mL of methanolic solution of boron trifluoride (10%) was added to the cooled hydrolyzed sample. The sealed solution was heated again in boiling water bath for 30 min and then cooled. About 5 mL saturated sodium chloride solution was added to the cooled esterified solution and the FAMEs produced were extracted twice with 1 mL hexane. The hexane extract was filtered through 0.45 μm membrane filter and injected 1 μL into the GC column using auto injection system.

2.5. Chromatographic separation of FAMEs

GC-MS (QP 2010 Plus, Tokyo, Japan) from Shimadzu was used. A capillary column (TRB-FFAP, Technokroma) of 30 m length, 0.35 mm internal diameter and 0.25 μm thickness, which treated with polyethylene glycol, was employed for separation of analytes and helium was used as the carrier gas. Ion source temperature (electron impact) and interface temperature were kept at 250 ˚C and 240 ˚C respectively. Pressure was maintained at 100 kPa, and solvent cut time used was 1.8 min. About 1 μL of the prepared standard and sample were injected into the GC column using auto injection system. Mode of injector was split with a 1:50 ratio and injections were made at 240 ˚C. The temperature gradient commenced at 84 ˚C and kept at this temperature for 4 min. The temperature was increased at 15 ˚C/min to 175 ˚C. The temperature was kept constant at 175 ˚C for 15 min and then rose to 220 ˚C at the speed of 2.5 ˚C/min and hold for 5 min. The temperature was maintained for 25 min. Single analysis completed in 68.07 min. MS scanning was in the range of 85 to 380 m/z. The system was controlled and the data which were acquired using the GC-MS solutions software provided by the supplier. Compounds were identified by comparing the mass spectra obtained with those of standard from the NIST library (NIST 05).

3. Results

Table 1 shows the relative concentration of individual esterified fatty acids based on the external standard method in the *P. minima* oil. GC-MS chromatogram for the standard fatty acids mixture is shown in Figure 1. While the GC-MS chromatogram acquired from the quantification of fatty acids in seeds oil is shown in Figure 2. Saturated fatty acids and unsaturated fatty acids were detected and quantified. The saturated FAMEs of capric acid, myristic acid, pentadecanoic acid, palmitic acid, stearic acid and behenic acid were quantified. Palmitic acid was found in high amount as 46.83% among the saturated fatty acids. Linoleic acid (ω-6) and linolenic acid (ω-3) were obtained in appreciable quantities as 16.98% and 14.80% respectively.

Table 1
Amount of various fatty acids in *P. minima* oil.

Fatty acids	Chemical formula	Acronym	*Concentration (%)	**SD	Relative SD	LOD (%)	LOQ (%)
Capric acid	$C_{10}H_{20}O_2$	C10:0	1.11	0.021	0.019	0.062	0.208
Myristic acid	$C_{14}H_{28}O_2$	C14:0	6.03	0.010	0.002	0.030	0.100
Pentadecanoic acid	$C_{15}H_{30}O_2$	C15:0	0.94	0.031	0.032	0.092	0.306
Palmitic acid	$C_{16}H_{32}O_2$	C16:0	46.83	0.030	0.001	0.090	0.300
Stearic acid	$C_{18}H_{36}O_2$	C18:0	8.61	0.025	0.003	0.075	0.252
Oleic acid	$C_{18}H_{34}O_2$	C18:1c	2.81	0.015	0.005	0.046	0.153
Linoleic acid (ω-6)	$C_{18}H_{32}O_2$	C18:2c	16.98	0.010	0.001	0.030	0.100
Linolenic acid (ω-3)	$C_{18}H_{30}O_2$	C18:3c	14.80	0.021	0.001	0.062	0.208
Behenic acid	$C_{22}H_{44}O_2$	C22	1.87	0.015	0.008	0.046	0.153

SD: Standard deviation; LOD: Limit of detection; LOQ: Limit of quantification. *: Average of three measurement results; **: SD values for the three measurement results.

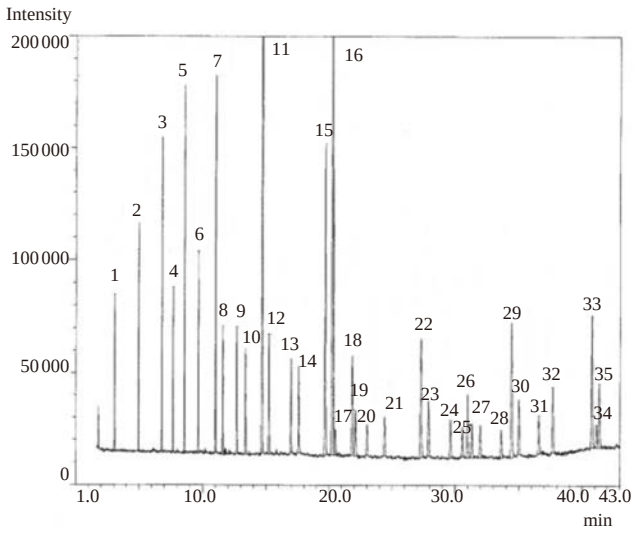

Figure 1. GC-MS chromatogram for the standard fatty acids mixture. Chromatographic conditions: injection volume: 1 μL; carrier gas: helium; column: TRB-FFAP capillary column (length 30 m, internal diameter 0.35 mm, thickness 0.25 μm, treated with polyethylene glycol); MS scanning: 85-380 m/z; 1: Hexanoic acid; 2: Caprylic acid; 3: Capric acid; 4: Undecanoic acid; 5: Lauric acid; 6: Tridecanoic acid; 7: Myristic acid; 8: Myristoleic acid; 9: Pentadecanoic acid; 10: Pentadecenoic acid; 11: Palmitic acid; 12: Palmitoleic acid; 13: Margaric acid; 14: Heptadecenoic acid; 15: Stearic acid; 16: Oleic acid; 17: Elaidic acid; 18: Linoleic acid; 19: Octadecadienoic acid; 20: g-Linolenic acid; 21: Linolenic acid; 22: Arachidic acid; 23: Eicosenoic acid; 24: Eicosadienoic acid; 25: 8,11,14-Eicosatrienoic acid; 26: Heneicosanoic acid; 27: Arachidonic acid; 28: Eicosapentaenoic acid; 29: Behenic acid; 30: Erucic acid; 31: Docosadienoic acid; 32: Tricosanoic acid; 33: Tetracosanoic acid; 34: Tetracosenoic acid; 35: Docosahexaenoic acid.

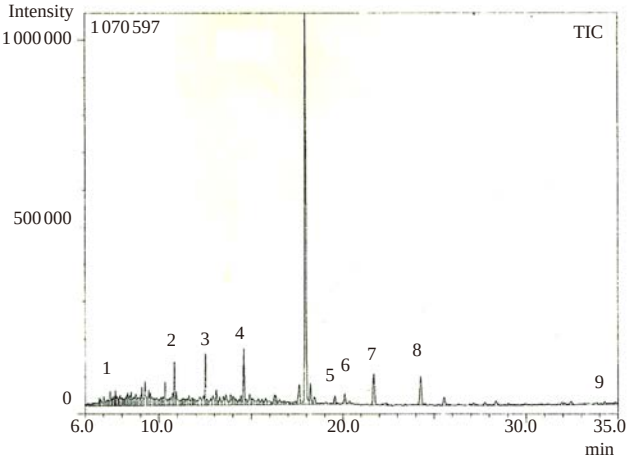

Figure 2. GC-MS chromatogram for quantification of fatty acids in the oil of *P. minima*. Chromatographic conditions: injection volume: 1 μL; carrier gas: helium; column: TRB-FFAP capillary column (length 30 m, internal diameter 0.35 mm, thickness 0.25 μm, treated with polyethylene glycol); MS scanning: 85-380 m/z; 1: Capric acid; 2: Myristic acid; 3: Pentadecanoic acid; 4: Palmitic acid; 5: Stearic acid; 6: Oleic acid; 7: Linoleic acid; 8: Linolenic acid; 9: Behenic acid.

4. Discussion

Quality control in phytopharmaceutical and pharmaceutical industries was based on the physical and chemical profile of the raw materials and the finished products. Determination of fatty acids was an important quality control parameter which made a base for the current study in *P. minima* oil. Analyses were repeated three times and the concentration (%) was the average of three measurement results. SD values among the three measurement results and their relative SD values were also tabulated in this study. LOD and LOQ were calculated using the Microsoft Office Excel 2003 program based on SD. Quantification of FAMEs was performed using three points calibration curve with R^2 value greater than 0.99 (R^2>0.99) in each case individually. The instrument was calibrated using known quantities of the components in the standard mixture of fatty acids.

The study evaluated the fatty acids profile quantitatively in the *P. minima* oil for the first time. Palmitic acid, linoleic acid and linolenic acid were obtained in appreciable amounts. Oleic acid and linoleic acid, which are responsible for the reduction of low density lipoprotein cholesterol and increase of high density lipoprotein cholesterol[8], have health benefits especially in heart patients. Presence of these important fatty acids in high amount makes *P. minima* oil beneficial for health, which can be used in the preparation of phytopharmaceutical or pharmaceutical preparations. Moreover, the results of this study are useful for the phytopharmaceutical industries to establish their quality control profile.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

P. minima is an important medicinal plant of the region and has various reported constituents. The oils and its components have an active role in the medicinal properties. So it is needed to be analyzed and identified.

Research frontiers

The present research work describes the fatty acids of the medicinal plant *P. minima*. The composition and quantity of the fatty acid in the oils of the plant has been analyzed for the first time.

Related reports

The physical and chemical profile of raw materials and finished products is the basis of quality control in pharmaceutical industry. The determination of fatty acids in oil is an important parameter in quality control.

Innovations and breakthroughs

The *P. minima* is an important medicinal plant. The authors have analyzed the oil of the plant, identified the fatty acids and determined the present quantitatively for the first time.

Applications

The work will have a fruitful application in herbal pharmaceutics and pharmaceutical industry.

Peer review

It is an important research. *P. minima* is an important medicinal plant. The oil of *P. minima*is is an important constituent which has been analyzed and quantified for the fatty acids for the first time.

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