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Shahin Aziz
Senior Scientific Officer,
Chemical Research Division,
BCSIR Laboratories, Dhaka,
Bangladesh

Ruma Khatun
Department of Applied
Chemistry and Chemical
Engineering, Islamic University,
Kushtia, Bangladesh

Sharif M Al-Reza
Department of Applied
Chemistry and Chemical
Engineering, Islamic University,
Kushtia, Bangladesh

Analysis of fixed oil from the seed of *Cassia obtusifolia* by GC-MS

Shahin Aziz, Ruma Khatun and Sharif M Al-Reza

Abstract

Cassia obtusifolia is a medicinally important plants belonging to the family of Fabaceae. It has been used extensively by ayurvedic practitioner and also reported to contain anthraquinones and flavonoids, these constituents are well documented to possess anti-inflammatory activity. The fatty acid compositions of the petroleum ether extract of seeds of this plant were determined by gas chromatography-mass spectrophotometer. 4 compounds were identified from the extract of seeds (99.98%) and they are palmitic acid, stearic acid, oleic acid and linoleic acid of methyl ester. Among all fatty acids of linoleic acid showed the highest concentration (50.65%).

Keywords: *Cassia obtusifolia*, GC-MS, fatty acid compositions

1. Introduction

Medicinal plant products have been part of phytomedicine since time immemorial. These can be derived from any part of the plant like leaves, flowers, bark roots, fruits and seeds etc. (Cragg, 2001) [1]. Any part of the plant may contain active components. Herbal medicines have become more popular in the treatment of any diseases due to popular belief that green medicine is safe, easily available and with less side effects. Many plants are cheaper and more accessible to most people especially in the developing countries than orthodox medicine, and there is lower incidence of adverse effects after use. These reasons might account for their worldwide attention and use (Safowara, 1993) [2]. The medicinal properties of some plants have been documented by some researches (Smith *et al.* 1977; Gill, 1992; Banso *et al.* 2007) [3, 4, 5]. Medicinal plant constitutes are the main source of new pharmaceuticals and healthcare products (Ivanova *et al.* 2005) [6]. Extraction and characterization of several phytochemicals of these green factories have given birth to some high activity profile drugs (Mandal *et al.* 2007) [7]. Indeed, the market and public demand has been so great that there is a great risk that many medicinal plants today, face either extinction or loss of genetic diversity (Misra *et al.* 2009) [8]. Knowledge of the chemical constituents of the plant is desirable because such information will be valuable for the synthesis of complex chemical substances.

Cassia obtusifolia is one of the medicinally important plants of *Cassia* genus belongs to family Leguminosae. Various species of the genus are well documented to possess anti-inflammatory activities and the genus is also reported to contain anthraquinones and flavonoids, these constituents are well documented to possess anti-inflammatory activity. The leaves of this plant are being used traditionally to get relief from vomiting, stomach-ache and head-ache (Zhang *et al.* 2005) [9].

The seeds of *Cassia obtusifolia* (Leguminosae), a plant widespread across North, Central, and South America; Asia; Africa; and Oceania, have been used in traditional Korean, Japanese, and Chinese medicine to treat eye inflammation, photophobia, and lacrimation (Kim *et al.* 2007) [10], in addition to dysentery, headache, and dizziness (Prohp *et al.* 2006) [11]. Furthermore, *Cassia obtusifolia* extract (COE) has been reported to have an anti-*Helicobacter pylori* effect, inhibitory actions on the growth of *Clostridium perfringens* and *Escherichia coli*, estrogenic effects, and inhibitory effects on histamine release from mast cells and platelet aggregation (Adefagha *et al.* 2011; AOAC, 2005, AOAC, 1995; Safowara, 2008) [12, 13, 15]. A recent study also reported that *Cassia obtusifolia* can attenuate memory impairments in mice induced by scopolamine administration or transient bilateral common carotid artery occlusion and that these effects were mediated via acetyl cholinesterase inhibition (Onwuka *et al.* 2005) [16].

However many researches have been carried out on *Cassia obtusifolia*, is but no systematic research on fatty acid composition of seeds of the plant by GC-MS analysis has been reported. Therefore, the present study was undertaken with an objective to carry out a complete

Correspondence

Shahin Aziz
Senior Scientific Officer,
Chemical Research Division,
BCSIR Laboratories, Dhaka,
Bangladesh

investigation of the compositions of fatty acids of seeds from petroleum ether extract of *Cassia obtusifolia* with GC-MS analysis.

2. Materials and Methods

2.1 Collection of plant material

Fully matured fresh seeds of *Cassia obtusifolia* were collected from local area of Rajshahi district, Bangladesh in the month of April 2016 and identified by the taxonomist of Bangladesh National Herbarium, Dhaka, where a voucher specimen (No. =43201) has been deposited.

2.2 Preparation of sample

The matured seeds were washed to remove dirt. Then it was oven-dried at reduced temperature less than 45 °C to make it suitable for grinding purpose. The screened (20 mesh) powder was then stored in air-tight container with marking for future experiment.

2.3 Solvents

Petroleum ether (b.p 40-60 °C, Merck, Germany) of AR grade, under normal atmospheric pressure was employed for extraction of plant material. Solvent from extract were recovered under distillation and the dried extracts were preserved in a refrigerator.

2.4 Extraction of fatty acids and preparation of methyl ester (FAMES)

The matured seeds of *Cassia obtusifolia* was collected and washed individually from running tap water to remove soil particles and other dust. Then they were dried at room temperature and powdered by Fritsch mortar grinder, Germany. The natural fatty acids were extracted separately from the seed powder (100 gm) of the plant with petroleum ether (b.p 40 °C-60 °C) in a Soxhlet apparatus for 72h. The extracts were concentrated under reduced pressure in a rotary evaporator. The extracts were filtered using What man No.1 filter paper and then vacuum distilled to remove solvent completely. The extracts from the seeds of *Cassia obtusifolia* was 4.46 gm (4.46% w/w) Petroleum ether extracts for seeds of *Cassia obtusifolia* were kept in a nitrogen atmosphere in a refrigerator. The fatty acids present in the extracts were converted to fatty acid methyl esters (FAMES) first and analyzed according to the method reported by Griffin (Griffin, 1960) [17] for GC-MS analysis.

The fatty acid composition was determined by analysis of their methyl esters. The fatty acid methyl esters (FAMES) were prepared by esterification reaction by using BF₃-MeOH complex according to AOAC method (AOAC, 1984) [18]. 10 mg of extract of seeds was taken in a screw capped glass tube. 1 ml of BF₃-MeOH complex were added and then heated at 100 °C for 1 hour in a water bath. After that it was cooled at room temperature and 1ml of deionized water & 2ml of hexane were added. The glass tube was vortexed and centrifuged at low RPM for two minutes. The upper layer was collected by means of syringe and kept in closely tight glass vial in refrigerator. Then the prepared FAMES were ready to analyze.

2.5 Gas Chromatograph-mass spectrum analysis

GC-MS analysis of seeds of *Cassia obtusifolia* from petroleum ether extracts were carried out on a Agilent 7890A system equipped with mass Spectrophotometer detector and split less injection system. The GC was fitted with a HP-5MS capillary column (30 m X 0.25mm: film thickness: 0.25µm).

The temperature program was as follows: injector temperature 260 °C, initial oven temperature at 70 °C, then increased at 10 °C/min to 150 °C for 5 min. then 12 °C/min to 200 °C for 15 min. and then 12 °C/min to 220 °C for 15 min. Helium was used as the carrier gas at 17.69 psi pressure with flow 0.6 ml/min. Samples were dissolved in methanol and 1µl aliquot was injected automatically. MS was set in scan mode. The ionization was electron ionization. The mass range was set in the range of 50-550 m/z. MS spectra of separated components were identified on NIST libraries for fatty acid compositions.

3. Result and Discussion

GC-MS analysis of fatty acids of seeds of *Cassia obtusifolia* petroleum ether extract showed the presence of 4 compounds for seeds of *Cassia obtusifolia*. GC analyzed results which include the active principles with their retention time, molecular formula, molecular weight and composition of the fatty acids of seeds of *Cassia obtusifolia* from petroleum ether extract are presented in table-1 & Figure-1 respectively.

Table 1: GC-MS analysis of fatty acids from petroleum ether extract of seeds of *Cassia obtusifolia*

S. No.	Retention time (min)	Name of the compound	Molecular weight	Molecular Formula	Conc. (%)
1.	13.60	Methyl Palmitate	270.45	C ₁₇ H ₃₄ O ₂	33.18
2.	16.44	Methyl Stearate	298.50	C ₁₉ H ₃₈ O ₂	4.80
3.	16.12	Methyl Oleate	296.49	C ₁₉ H ₃₆ O ₂	11.35
4.	16.05	Methyl Linoleate	294.47	C ₁₉ H ₃₄ O ₂	50.65

Total 4 fatty acids were identified as their methyl esters in the case of seeds of *Cassia obtusifolia*. The major constituent was Methyl Linoleate (50.65%) with retention time 16.05.

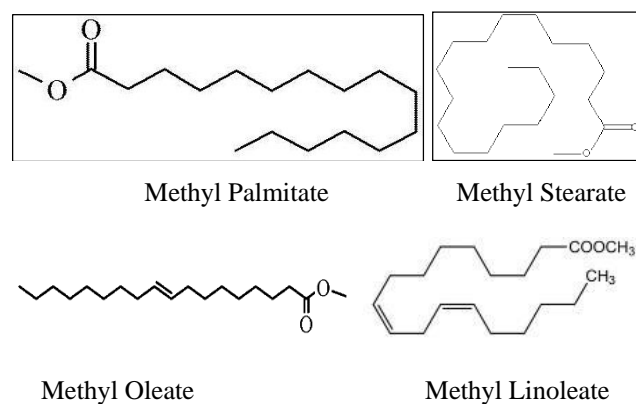


Fig 1: Structure of the identified fatty acid esters from GC-MS analysis of petroleum ether extract of seeds of *Cassia obtusifolia*

4. Conclusion

The present study found 4 constituents from seeds of petroleum ether extract of *Cassia obtusifolia* by Gas Chromatography-Mass spectroscopy (GC-MS) analysis. The presence of these chemical compounds justified the extensive uses of seeds of the plant by traditional practitioner to treat various ailments. It could be concluded that *Cassia obtusifolia* contains various chemical constituents that can be bioactive compounds of medical importance. However, further studies are needed to evaluate its bioactivity and toxicity profile.

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