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A comparative study of fatty acids and sterols profiles in seed oil of five *Cassia* species

[1]Lalita Ledwani* and [2]Shelley Oberoi

[1]*Pandit Deen Dayal Petroleum University, Raison, Gandhinagar, Gujarat, India*
[2]*Inderprastha Engineering College, Ghaziabad, India*

Abstract

The fatty acids and sterols composition of seed oil were determined from five Cassia species namely: *Cassia absus* Linn., *Cassia alata* Linn., *Cassia javanica* Linn., *Cassia laevigata* Linn., *Cassia roxburghii* DC. The oil content was ranged from 3.5% to 6.2 %. The fatty acid and sterols composition of seed oil of five Cassia species were analyzed by Gas Chromatography. Four fatty acids: C16:0 palmitic acid, C18:0 stearic acid, C18:1 oleic acid and C18:2 linoleic acid (along with minor percentage of C18:3 linolenic acid in *C. roxburghii & C. absus*) were identified in all five species. The tested seed oil contained mainly unsaturated fatty acids i.e. C18:2 linoleic acid in the range from 45.96% to 60.25% and C18:1 oleic acid from 26.29% to 34.91%**.** C16:0 Palmitic acid was the most abundant saturated acid. Three sterols cholesterol, stigma sterol and β-sitosterol were found in all species. β-sitosterol was found to be major sterol in *C. javanica* (67.50 %), *C. alata* (64.79 %) and *C. roxburghii* (63.29 %) and *C. absus* (53.42%) while sigmasterol was major in *C. laevigata* (45.34 %). Linoleic acid, oleic acid, stigmasterol and β-sitosterol were identified as the major constituent of the oil.

Keywords: linoleic acid, oleic acid, β-sitosterol, stigma sterol, palmitic acid, cholesterol stearic acid.

Introduction

Cassia is a genus of Fabaceae in the subfamily Caesalpinioideae. It is large and predominantly tropical genus of about 580 species of herbs, shrubs and trees with about 20 representatives in India. Many of them are medicinal: a few provide tanning materials of economic value. Cassia plants are considered very potent in Ayurvedic Medicine. It contains elevated quantities of anthraquinones [1, 2] and consequently is mainly useful against gastrointestinal conditions and still bleeding. Its fruit pulp is considered a mild remedy, the roots are said to be so potent. There

exists some culinary use for Cassia. The fruit pulp of some is eaten as a refreshing treat; similar to the related tamarind. Species of Cassia [3-5] are rich source of flavonoids, anthraquinone and polysaccharides.

Five Cassia species *Cassia absus* Linn. *Cassia alata* Linn., *Cassia javanica* Linn., *Cassia laevigata* Linn., *Cassia roxburghii* DC. were selected for present investigation. *Cassia absus* Linn. (Local name: Chaksu) is an erect annual plant 1-2 ft. height, distributed throughout India. The leaves are bitter and astringent and find use as a cough remedy. The seeds are flat oblong. *Cassia alata* Linn.(Local name: Guajava) is an erect tropical, annual herb with leathery compounded leaves. It grows up to 6' tall. It is a medicinal plant. The root, leaves, wood, flowers and seeds of this plant are decocted for a remedy against intestinal parasites and hepatitis. The leaves of this plant are used in the treatment of ringworm, the seeds as an anthelmintic while the roots can be used against uterus disorders. The *Cassia javanica* Linn. [6] (Local Name: Java Cassia) is an evergreen tree that reaches a height of up to 15 meters. Brightly colored pink, orange, yellow, red or white flowers cover the tree in summer. The tree is also called rainbow shower tree, pink shower tree and apple blossom shower tree because of the resemblance of the flower to apple flowers. The fruits are long and accommodate several seeds within them. *Cassia laevigata* Wild [7] (Local Name: Irwin) is a shrub of about 3 m height. Tender fruits are cooked as vegetable. A paste of seeds is applied to treat skin disease. Roasted seeds are chewed in case of cough. *Cassia roxiburghii* DC [8] (Local Name: Red Cassia) is a large shrub like tree grows to height of five meters and produces flowers of red, pink colour. They are planted as avenue trees along side roads and paths for shades and coolness. The flowers appear in clusters. The long fruit bears several seeds.

Fats and oil are chief source of energy in human diet .The extract from Cassia seed oil have been used in folk medicine for many years as analgesic, anti-inflammatory and anti-contractive medicaments . Both unsaturated fatty acids and sterols revel distinct pharmacological activity. Essential fatty acids (EFAS) are unsaturated fatty acids. Linoleic and gama-linolenic acids are important unsaturated fatty acids which can be used in the synthesis of tissue hormones, which regulate blood pressure and take part in immunological response. Sterol show anti inflammatory and immunostimulatory activity. The aim of the study was to compare [9, 10] the composition of sterols and fatty acids in the seeds of the five Cassia species.

Materials and Methods

The object of the study was the seeds [11, 12] of five Cassia plants namely *Cassia absus* Linn., *Cassia alata* Linn., *Cassia javanica* Linn., *Cassia laevigata* Linn., *Cassia roxburghii* DC. Seeds were purchased from the local market of Ahmedabad. The Phytochemical analysis was done in the Department, Petroleum University, Gandhinagar. The result is the mean value from one year.

Cleaned and dried seeds (500 g) of all five species were crushed and extracted separately into five different reservoirs. Extraction was done with light petroleum ether (60-80 ^{0}C) until colourless extract obtained. Evaporation of solvent under reduced pressure afforded a yellowish oil which was saponified by refluxing with 10% methanolic KOH for 2 hrs in the atmosphere of nitrogen. The solvent was removed under reduced pressure and residue was diluted with water. The unsaponified matter was used for the identification of sterols.

A) Physico-chemical values: The physcio-chemical values like iodine value, saponification value, ester value, free fatty acids were determined according to the procedure of British Standard Specification 684. Iodine value indicated the composition of unsaturated methyl esters. Refractive index was determined with Abbe's refractometer.

B) Fatty acid analysis: The mixed fatty acids were obtained by acidification of the aqueous layer left after extraction of unsaponifiable matter with diethyl ether. The aqueous layer was acidified with dil. HCl to pH 2-3. This was then extracted with diethyl ether. The ethereal layer was washed with water and dried over anhydrous sodium sulphate. The five samples of dried extracts were concentrated in presence of nitrogen to afford an oily viscous mass separately. All the samples were esterified separately with anhydrous methanol in presence of conc. H_2SO_4 for 2-3 hrs. The esters so formed were extracted with water and dried over anhydrous Na_2SO_4. The solvent was concentrated under the stream of nitrogen. The methyl esters thus formed were used for GLC.

The methyl esters of standard and unknown fatty acids were dissolved in petroleum ether and injected to GLC column. GLC[13] of the fatty acids were carried out on model, 4890 II series on GLC gas chromatogram equipped with flame ionization detector using stainless steel column (3 mm x 2m) packed with 20% DEGS on chromosorb and using nitrogen as a carrier gas. The block was maintained at 210^{o}C, 170^{o}C and 220^{o}C, respectively.

The identification of individual fatty acid was carried out by comparison of retention time of unknown methyl esters of fatty acids with standard fatty acid methyl esters. Presence of four to five peaks, confirmed the presence of four to five fatty acids. The characterization of individual mixture of the seed oil of all five cassia plants were carried out by comparing the retention time of unknown fatty acids with that of methyl esters of authentic fatty acid[14-16] esters (Fig.1).

C) Sterol analysis**:** The unsaponifiable material was extracted with ether and dried over anhydrous Na_2SO_4. The ether layer was dried and evaporated to afford residue of unsaponifiable matter. The unsaponifiable matter was column chromatographed over silica gel (40 g) and eluted with increasing concentration of diethyl ether in petroleum ether (5, 10, 15, and 20 %) to afford a solid from. The last elute was crystallized from methanol - diethyl ether. This gave the positive Liebermann – Buchard colour test for sterols. The sterol mixture was dissolved in 2 ml of acetic anhydride – pyridine mixture (2:1) and kept for two nights. The solvent was evaporated in vacuo and residue was crystallized from methanol. The crystalline acetate derivative was obtained.

Sterol content and composition in all five seed samples were assessed by gas chromatography. The GC of the acetate derivative mixture showed three peaks in the gas chromatograms. It indicates the presence of three sterols. The identification of individual sterol was carried out by comparison of the retention time of the unknown sterol acetates with that of standard sterol acetates (Fig.-2).

GLC of sterols (standard and unknown) was performed on OV-17 and DB-17 fused silica column (length 30m, i.d. 0.3nm) on Hewlett-Packerd (HP5 890A) gas chromatograph equipped with flame ionization detector. Operating conditions were; carrier gas,

(He); flow rate, 30min/min; injector and detector temperature, 290°C; column temp. 270°C isothermal.

Results and Discussion

Table 1: Total oil content (%) in Cassia seeds

Cassia absus	Cassia alata	Cassia javanica	Cassia laevigata	Cassia roxburghii
3.5%	6.2%	4.2 %	4.9%	5.6 %

A) Oil content- The highest oil yield was found in the *Cassia alata* Linn. seeds (6.2%) and least was obtained from the seeds of *Cassia absus* Linn. (3.5%). The oil content of *Cassia javanica* Linn., *Cassia laevigata* Linn., *Cassia roxburghii* DC were 4.2 %, 4.9%, 5.6 % respectively [Table-1].

B) Physico-chemical properties- The seeds oil of five Cassia were determined. The oil extracted from the seeds of Cassia species was free from unwanted materials such as glucose, salt, urea, sucrose, etc. The following physico-chemical properties were investigated: moisture (3.91% to 5.87%), ash (2.68% to 4.40 %), refractive index(1.394 to 1.481), saponification value (160 to191), unsaturated fatty acids (78.50 to 95.16%), iodine value (109 to 144), unsaponifiable matter (2.6 to 4.2 %), in all cassia species. Physico- chemical properties are summarized in Table-2.

Table 2: Physico-chemical characterization of Cassia seeds

Values/Species	*Cassia absus*	*Cassia alata*	*Cassia javanica*	*Cassia laevigata*	*Cassia roxburghii*
Moisture (%)	3.91	4.67	3.82	4.87	5.87
Ash (%)	2.68	3.95	4.40	3.98	4.08
Fiber (%)	6.56	7.69	2.20	7.45	7.45
Refractive Index(40^{0}C)	1.435	1.481	1.394	1.467	1.457
Saponification Value	160	191	179	185	189
Unsaturated fatty acids (%)	78.50	79.26	93.87	95.16	93.32
Iodine value	109	110	143	144	141
Unsaponifiable matter (%)	3.8	2.6	3.3	2.9	4.2
Sterol content in Oil (%)	2.9	3.9	2.7	3.4	3.5

C) Fatty acid characterization- The GLC analysis of both Cassia species showed that they have uniform and usual fatty acid composition. It indicated the presence of four fatty acids [17, 18]

in three species and five fatty acids in two species (Fig.1). The extracted seed oil of Cassia species contained significant amount of linoleic acid (45.96% to60.25 %), which is the one of the most important unsaturated fatty acid. Oleic acid was second major unsaturated fatty acid (34.91% in *Cassia laevigata,* 34.80% in *Cassia javanica* and 30.11% in *Cassia alata* and 26.29 % in *Cassia absus*) except in *Cassia roxburghii* where the percentage of oleic acid and linoleic acid were 46.08% and 45.96 % respectively. Palmitic acid and stearic acid exhibited the third and fourth highest FA content and ranged between 16.41% to 2.61% and 1.62 % to 8.10% respectively. Minor percentage of linolenic acid was observed in *Cassia absus* (1.96%) and in *Cassia roxburghii* (1.28%). The content and composition of fatty acid are summarized in Table-3.

D) Sterol Characterization- The sterol fractions obtained from the unsaponifiable oil of the seed oils of the five cassia species were identified as acetates based on comparison of the GLC data with those of authentic samples. Three sterols namely stigmosterol, β-sitosterol and cholesterol were identified (Fig. : 2). β-sitosterol was found to be major sterol in *C. javanica* (67.50 %), *C. alata* (64.79 %) and *C. roxburghii* (63.29 %) and *C. absus* (53.42%) while sigmasterol was major in *C. laevigata* (45.34 %). Table-4 summarized the sterol compositions of the five Cassia species.

Table-3: Fatty acid composition (relative abundance %) in seed oil of *Cassia* species

Species/Fatty Acids	Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)	Linolenic acid (C18:3)
Cassia absus	11.28	8.10	26.29	50.25	1.96
Cassia alata	16.41	4.10	30.11	49.15	-
Cassia javanica	4.48	1.62	34.80	59.07	-
Cassia laevigata	2.61	2.21	34.91	60.25	-
Cassia roxburghii	4.27	2.27	46.08	45.96	1.28

Table-4: Sterol composition (relative abundance %) in seed oil of *Cassia* species

Species/Sterols	Stigma sterol	□-sitosterol	Cholesterol
Cassia absus	40.26	53.42	3.52
Cassia alata	27.45	64.79	6.30
Cassia javanica	17.60	67.50	8.71
Cassia laevigata	45.34	44.42	8.51
Cassia roxburghii	20.45	63.29	5.28

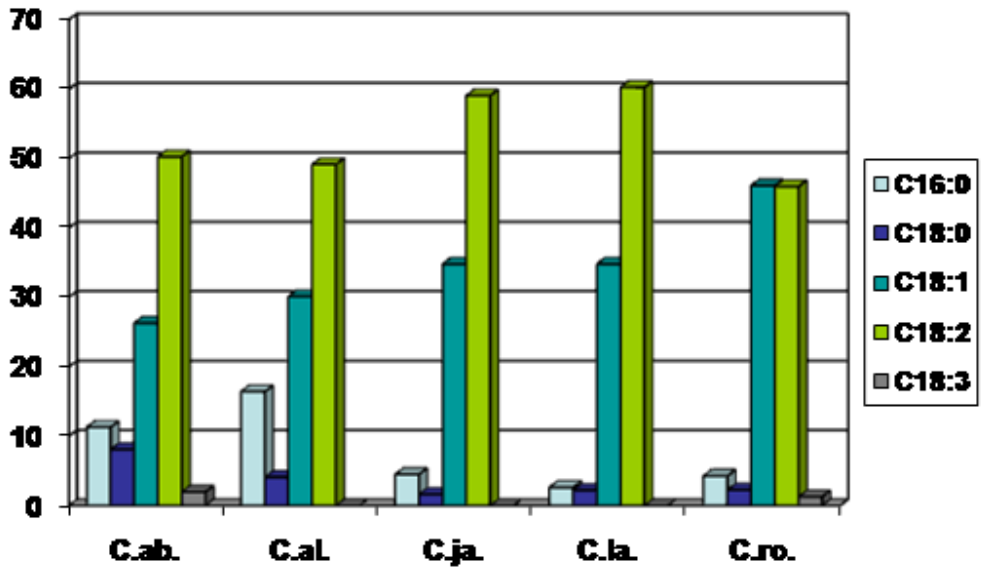

Fig. 1: The fatty acids composition (relative abundance %) in *C.absus (C.ab), C. lata(C.al.), C.javanica(C.ja.), C.laevigata(C.la.)* and *C. roxburghii(C.ro.)* Ca*: Cassia auriculata;* Cs: *Cassia siamea*

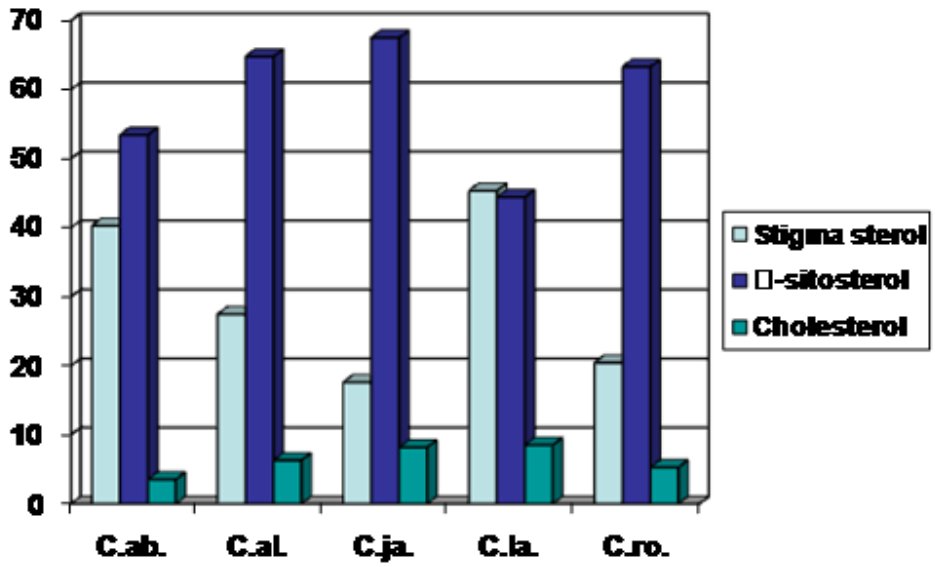

Fig. 2: The sterol composition (relative abundance %) in *C.absus (C.ab.), C. alata(C.al.), C. javanica(C.ja.), C .laevigata(C.la.)* and *C. roxburghii(C.ro.)* Ca*: Cassia auriculata;* Cs: *Cassia siamea*

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