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Analysis of fatty acid and determination of total protein and phytochemical content of *Cassia sophera* Linn leaf, stem, flower, and seed

Shahin Aziz* and Tahmina Khondkar Mitu

Abstract

Background: *Cassia sophera* Linn is a medicinally important plant belonging to the family of Caesalpiniaceae. The whole part of the plant is used as traditional folk medicine and is reported to possess analgesic, anticonvulsant, antioxidant, anti-inflammatory, hepatoprotective, and antiasthmatic activity. The present communication attempt is to evaluate fatty acids from different parts of the plant by GC-MS spectrophotometer and total protein content by the Kjeldahl method and to quantify some active constituents, i.e., alkaloid, saponin, and flavonoid.

Results: From fatty acid compositions of the petroleum ether extract of leaves, stems, flowers, and seeds of this plant grown in Bangladesh, 22 compounds from leaves, 8 compounds from stems, 9 compounds from flowers, and 12 compounds from seeds were identified. The main fatty acid was arachidic acid (38.66%) from leaves. Linoleic acid (40.12% and 42.40%) was found mainly from stems and seeds, whereas from flowers, it was docosadienoic acid (27.14%).

Conclusion: The findings from the present study showed that the protein content for seeds has higher value (19.20%) than other parts of the plant. Also the present investigation showed that different parts of the plant contain phytochemicals in appreciable quantities in the form of flavonoids, alkaloids, and saponins. The flavonoid and alkaloid content of leaves showed higher value. But the stem part showed higher saponin content than other parts of the plant.

Keywords: *Cassia sophera* Linn, Fatty acids, Gas chromatography coupled to mass spectrophotometer, Kjeldahl method

1 Background

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 [9]. Herbal medicines have become more popular in the treatment of any diseases due to the 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 a lower incidence of adverse effects after use. These reasons might account for their worldwide attention and use [30]. The medicinal properties of some plants have been documented by some researchers [4, 16, 34]. Medicinal plant constitutes the main source of new pharmaceuticals and healthcare products [17]. Extraction and characterization of several phytocompounds of these green factories have given birth to some high activity profile drugs [21]. 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 less of genetic diversity [22]. Knowledge of the chemical constituents of the plant is desirable because such information will be valuable for the synthesis of complex chemical substances.

* Correspondence: shaziz2408@yahoo.com
Chemical Research Division, BCSIR Laboratories, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka 1205, Bangladesh

Cassia sophera Linn belongs to the family of Caesalpiniaceae. It is one of the important medicinal plants in the tropical and subtropical region in Asia especially in India, Sri Lanka, Pakistan, Malaysia, Myanmar, and Bangladesh. In Bangladesh, the plant is locally known as "Kulkashunda." It grows abundantly in the plain land, hilly areas of Chittagong Hill Tracks, Sylhet, and patches throughout Bangladesh [2]. It has a number of traditional and medicinal uses in the traditional system of medicine such as Ayrveda and Unani [2, 11, 18]. The plant is described in Unani literature to be repulsive of morbid humors, resolvent, blood purifier, carminative, purgative, digestive, and diaphoretic and reported to be useful in epilepsy, ascites, dyscrasia of the liver, skin disorders, piles, jaundice, fever, articular pain, and palpitation. In ethnobotanical literature, this plant is mentioned to be effective for pityriasis, psoriasis, asthma, acute bronchitis, cough, diabetes, and convulsions of children [1]. Although in traditional medicine *Cassia sophera* L have been well known for their laxative and purgative properties and for the treatment of skin diseases [5], there is now an increasing body of scientific evidence demonstrating that the plants possess many other beneficial properties [26]. The different parts of the plant, *Cassia sophera* Linn, are used for medicinal purposes for thousands of years in Bangladesh, India, or subcontinent. The bark, leaves, and seeds are used as a cathartic, and the juice of the leaves is specific for ringworm, especially when made into plaster in combination with sandalwood. The root is administrated internally with black pepper for snake bite [18]. The essence of dried *Cassia sophera* Linn with the same amount of mint into water is used to cure apositia [19]. The flowers of this important medicinal plant is prescribed as a tonic, astringent, febrifuge, and strong purgative and useful in fever, heart diseases, joint pain, migraine, and blood dysentery [35]. The seeds of this important medicinal plant are used as a traditional medicine in Japan, Korea, and China for the treatment of eye in ammation, phytophobia, and lacrimation; dysentery; and headache as well as dizziness [10]. Seed oil is used to promote a healthy immune function. It is also a great oil to diffuse during cold months due to its warming properties and spicy scent [13]. The plant *Cassia sophera* Linn revealed the presence of ascorbic acid, dehydroascorbic acid, ß-sitosterol, glycosides, and a rich source of flavonoids and anthraquinones [1].

Several studies have been carried out on the isolation of pharmacologically active compounds on different parts of *Cassia sophera* Linn [1], but no systematic work has been reported about the analysis of fatty acid by gas chromatography and mass spectrum (GC-MS) and protein content by the Kjeldahl method and the quantification of some active secondary metabolities, i.e., alkaloid, saponin, and flavonoid content of different part (leaves, stems, flowers, and seeds) so far. Keeping in mind the wide application of different plant parts of *Cassis sophera* Linn in traditional medicine and ayurvedic system, the abovementioned analysis was carried out.

2 Materials and methods

2.1 Collection of plant material

Fully matured fresh leaves, stems, flowers, and seeds of *Cassia sophera* Linn were collected from Sylhet, Bangladesh, in the month of June 2015 and identified by the taxonomist of Bangladesh National Herbarium, Dhaka, where a voucher specimen (No. 43734) has been deposited. Leaves, stems, flowers, and seeds were separately air dried. These dried samples were powdered using a 20-mesh screen in Willey mill and then used for subsequent analysis.

2.2 Reagents and standards

All reagents used were from Merck (Darmstadt, Germany) or Sigma Aldrich (Buchs, Switzerland). For fatty acid analysis, petroleum ether (b.p 40–60 °C, Merck, Germany) of AR grade, under normal atmospheric pressure, was employed for extraction.

2.3 Determination of protein content (Kjeldahl method)

Protein content was determined through the micro-Kjeldahl method [25] using 2 g of the dried powder sample of leaves, stems, flowers, and seeds of the plant separately and according to the following equation.

$$Nitrogen\,(\%) = \frac{(\text{ml.standard acid-ml Blank}) \times \text{N of acid} \times 14 \times 100}{\text{Weight of sample taken in grams}}$$

$$\text{Protein content}\,(\%) = \text{Nitrogen content}\% \times 6.25$$

2.4 Preparation of fatty acid extract and analysis by GC-MS

2.4.1 Preparation of fatty acid extract

Fatty acids were extracted separately from the powder (50 g) of leaves, stems, flowers, and seeds of *Cassia sophera* Linn with petroleum ether (b.p 40–60 °C) in a Soxhlet apparatus for 72 h using the method adopted by Aziz et al. [3]. The extracts were filtered using Whatman No.1 filter paper and then vacuum distilled to remove the solvent completely. The extracts were concentrated under reduced pressure in a rotary evaporator. The leaf extract was 8.92 g (8.92% *w*/*w*), and the extract from stems was 0.49 g (0.49% *w*/*w*), from flowers was 5.30 g (5.30% *w*/*w*), and from seeds was (1.10% *w*/*w*). All the extracts were kept in a nitrogen atmosphere in a refrigerator.

2.4.2 Preparation of methyl ester (FAMEs)

The fatty acid composition was determined by the analysis of their methyl esters. The fatty acids were converted to fatty acid methyl esters (FAMEs) first. Then, they were analyzed according to the method reported by Griffin [32] and by using BF_3-MeOH complex according to the AOAC method [15]. Ten micrograms of the extract of different plant parts was individually used for each case.

2.4.3 Gas chromatograph-mass spectrum analysis

GC-MS analysis of fatty acids' esters of leaves, stems, flowers, and seeds of *Cassia sophera* Linn was carried out on an 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 × 0.25 mm; film thickness 0.25 μm). 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, 12 °C/min to 200 °C for 15 min, and 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 1 μl aliquot was injected automatically. MS was set in the scan mode. 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.

2.5 Determination of phytochemical content

2.5.1 Alkaloid determination [36]

Five grams of leaves, stems, flowers, and seeds of *Cassia sophera* Linn powder sample was taken separately into a 250-ml conical flask, and 200 ml of 10% acetic acid in ethanol was added; the flask was covered by aluminum foil and allowed to stand for 2 days followed by filtration. After filtration, the extract was reduced to one fourth of its original volume on a water bath. Concentrated ammonium hydroxide was added in drops to the reduced volume, until the precipitation was completed. The whole solution was allowed to settle, and the precipitate was collected by filtration, dried, and weighed. Alkaloid content was determined according to the following equation.

$$\%alkaloid = \frac{W2}{W1} * 100\%$$

where $W1$ = initial weight of sample (g) and $W2$ = weight of precipitate (g).

2.5.2 Saponin determination [14]

Five grams of plant samples (leaves, stems, flowers, and seeds) was taken separately in a 250-ml conical flask, 200 ml of 25% ethanol was added, and the suspension was heated with continuous stirring on a water bath at about 60 °C for 4 h. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol and then filtered. The combined extracts were reduced to 40 ml over water bath at 90 °C. The concentrate mixture was transferred into a 250-ml separating funnel, and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered into a 250-ml conical flask, while the ether layer was discarded. The purification process was repeated thrice; 60 ml of n-butanol was added. The combined n-butanol extracts were washed with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight. The saponin content was calculated in percentage.

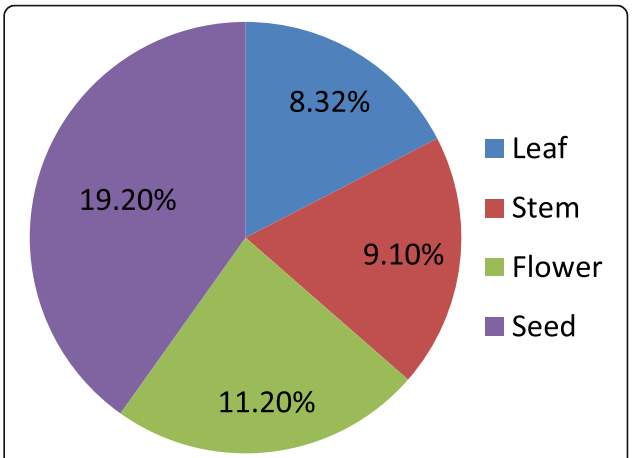

Fig. 1 Protein percentage of different parts of *Cassia sophera* L

2.5.3 Flavonoid determination [6]

Five grams of leaves, stems, flowers, and seeds was extracted separately in 250-ml conical flasks with 150 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper 42 (125 mm). The filtrate was then transferred into a crucible and evaporated to dryness over a water bath and weighed.

Table 1 Phytochemical content (%) of different parts of the plant of *Cassia sophera* Linn

Plant part	Phytochemical content (%)		
	Alkaloid (%)	Flavonoid (%)	Saponin (%)
Leaf	6.82	15.27	30.78
Stem	2.33	8.78	31.72
Flower	4.74	8.22	17.78
Seed	2.42	10.05	5.94

The present investigation showed that leaves of this medicinal plant showed higher flavonoid (15.27%) and alkaloid (6.82%) content than the stem, flower, and seed part, whereas for the case of saponin content, the present research showed the stem part showed higher values (31.72%) than the leaf, flower, and seed part

Table 2 GC-MS analysis of fatty acids of the leaves of *Cassia sophera* Linn

Sl no.	Name of the compound	Molecular weight	Molecular formula	Conc. (%)
1.	Caproic acid	116.15	$C_6H_{12}O_2$	0.99
2.	Caprylic acid	144.21	$C_8H_{16}O_2$	1.47
3.	Pelargonic acid	158.23	$C_9H_{18}O_2$	1.42
4.	Capric acid	172.26	$C_{10}H_{20}O_2$	1.23
5.	Undecyclic acid	184.28	$C_{11}H_{22}O_2$	0.56
6.	Lauric acid	200.31	$C_{12}H_{24}O_2$	0.95
7.	Trydecyclic acid	200.31	$C_{13}H_{26}O_2$	0.74
8.	Myristic acid	228.37	$C_{14}H_{28}O_2$	0.96
9.	Pentadecyclic acid (C15:0)	242.39	$C_{15}H_{30}O_2$	0.39
10.	Pentadecenoic acid (C15:1)	242.39	$C_{15}H_{30}O_2$	1.23
11.	Palmitic acid	256.42	$C_{16}H_{32}O_2$	15.92
12.	Stearic acid	284.47	$C_{18}H_{36}O_2$	4.00
13.	Oleic acid	282.46	$C_{18}H_{34}O_2$	4.57
14.	Linoleic acid	280.44	$C_{18}H_{32}O_2$	9.54
15.	α-Linoleic acid	278.43	$C_{18}H_{30}O_2$	8.77
16.	Arachidic acid	312.53	$C_{20}H_{40}O_2$	38.66
17.	Ecosenoic acid (C20:1)	310.51	$C_{20}H_{38}O_2$	1.86
18.	Eicosadienoic acid (C20:2)	308.50	$C_{20}H_{36}O_2$	0.69
19.	Arachidonic acid	304.46	$C_{20}H_{32}O_2$	0.38
20.	Behenic acid	340.58	$C_{22}H_{44}O_2$	3.28
21.	Lignoceric acid	368.63	$C_{24}H_{48}O_2$	0.71
22.	Nervonic acid	366.62	$C_{24}H_{46}O_2$	1.68

3 Results and discussions

3.1 Protein analysis

In *Cassia sophera* Linn, the value of total protein content for the case of leaves (8.32%), stems (9.10%), flowers (11.20%), and seeds (19.20%) is presented in Fig. 1. Protein analysis is of great importance in the nutritive determination, and protein is a complex nitrogen containing organic compounds which are found in all animals and plant cells, characterized by the presence of peptide bonds and formed by the polymerization of amino acids [37]. The storage of protein of different parts of the plant provided amino acids that are readily used for germination and swelling growth [3, 23].

Here in this plant, due to the presence of a higher amount of protein content in the seed part, it indicates that seeds contain nutritive value in appreciable quantity and it can take part in the germination and swelling growth more actively than other parts of the plant. This is the first report of total protein content for different parts of *Cassia sophera* Linn.

Table 3 GC-MS analysis of fatty acids of stems of *Cassia sophera* Linn

Sl no.	Name of the compound	Molecular weight	Molecular formula	Conc. (%)
1.	Capric acid	172.26	$C_{10}H_{20}O_2$	0.76
2.	Palmitic acid	256.42	$C_{16}H_{32}O_2$	32.59
3.	Palmitoleic acid	254.41	$C_{16}H_{32}O_2$	0.74
4.	Stearic acid	284.47	$C_{18}H_{36}O_2$	2.54
5.	Oleic acid	282.46	$C_{18}H_{34}O_2$	7.18
6.	Linoleic acid	280.44	$C_{18}H_{32}O_2$	40.12
7.	Ecosenoic acid (C20:1)	310.51	$C_{20}H_{38}O_2$	2.72
8.	Behenic acid	340.58	$C_{22}H_{44}O_2$	2.20

Table 4 GC-MS analysis of fatty acids of flowers of *Cassia sophera* Linn

Sl no.	Name of the compound	Molecular weight	Molecular formula	Conc. (%)
1.	Pelargonic acid	158.23	$C_9H_{18}O_2$	1.18
2.	Capric acid	172.26	$C_{10}H_{20}O_2$	1.54
3.	Palmitic acid	256.42	$C_{16}H_{32}O_2$	12.21
4.	Oleic acid	282.46	$C_{18}H_{34}O_2$	5.37
5.	Linoleic acid	280.44	$C_{18}H_{32}O_2$	17.34
6.	α-Linoleic acid	278.43	$C_{18}H_{30}O_2$	11.13
7	Arachidic acid	312.53	$C_{20}H_{40}O_2$	3.92
8.	Decosadienoic acid	336.56	$C_{22}H_{40}O_2$	27.14
9.	Adrenic acid	332.50	$C_{22}H_{36}O_2$	20.17

3.2 Phytochemical content analysis

The present investigation showed that different parts (leaves, stems, flowers, and seeds) of *Cassia sophera* Linn contain phytochemicals such as flavonoids, alkaloids, and saponins in appreciable quantities, and results are presented in Table 1.

Flavonoids are water-soluble phytochemical and an important plant phenolic. They show antioxidant activities, and they have the property of preventing oxidative cell damage and carcinogenesis. They have anti-cancer and anti-inflammatory activities and a large effect in the lower intestinal tract and heart disease [12]. Alkaloids are complex heterocyclic nitrogen compounds commonly found to possess antimicrobial properties. They are quite useful against viral and protozoan infections. In the case of highly aromatic planar quaternary alkaloids, their mechanism of action is due to their ability to intercalate with DNA [8]. The presence of saponins in higher concentration in the stem part signifies the uses of the plant in wound healing and bleeding treatment [7] in traditional folk medicine. Saponins have the property of coagulating red blood cells, and they also have cholesterol-binding properties, formation of foams in aqueous solutions, and hemolytic activity [29]. It is clear that the plant is very much rich in the above-mentioned phytochemicals. The presence of these phytochemicals signifies that different parts of the plant may be used as an analgesic, antispasmodic, antibacterial, anticancer, anti-inflammatory, and antioxidant.

3.3 Fatty acid analysis

GC-MS analysis of fatty acids of leaves, stems, flowers, and seeds of *Cassia sophera* Linn showed the presence of 22 compounds from leaves, 8 compounds from stems, 9 compounds from flowers, and 12 compounds from seeds. GC-MS analyzed results which include the active principles with their molecular formula, molecular weight, and composition of the fatty acids are presented in Tables 2, 3, 4 and 5.

The analysis of fatty acid from the case of petroleum ether extract of leaves, stems, flowers, and seeds was done. It revealed the presence of saturated fatty acids and unsaturated fatty acids for leaves (73.63% and 26.37% respectively), stem part (38.09% and 50.76%

Table 5 GC-MS analysis of fatty acids of seeds of *Cassia sophera* Linn

Sl no.	Name of the compound	Molecular weight	Molecular formula	Conc. (%)
1.	Caproic acid	116.15	$C_6H_{12}O_2$	0.73
2.	Caprylic acid	144.21	$C_8H_{16}O_2$	0.69
3.	Pelargonic acid	158.23	$C_9H_{18}O_2$	0.97
4.	Capric acid	172.26	$C_{10}H_{20}O_2$	0.51
5.	Trydecyclic acid	200.31	$C_{13}H_{26}O_2$	1.81
6.	Palmitic acid	256.42	$C_{16}H_{32}O_2$	26.97
7.	Stearic acid	284.47	$C_{18}H_{36}O_2$	0.95
8.	Oleic acid	282.46	$C_{18}H_{34}O_2$	15.38
9.	Vacenic acid	282.46	$C_{18}H_{34}O_2$	0.22
10.	Linoleic acid (C18:2)	280.44	$C_{18}H_{32}O_2$	42.40
11.	α-Linoleic acid (C18:2)	278.43	$C_{18}H_{30}O_2$	4.93
12.	γ-Linoleic acid (C18:3)	278.43	$C_{18}H_{30}O_2$	4.93

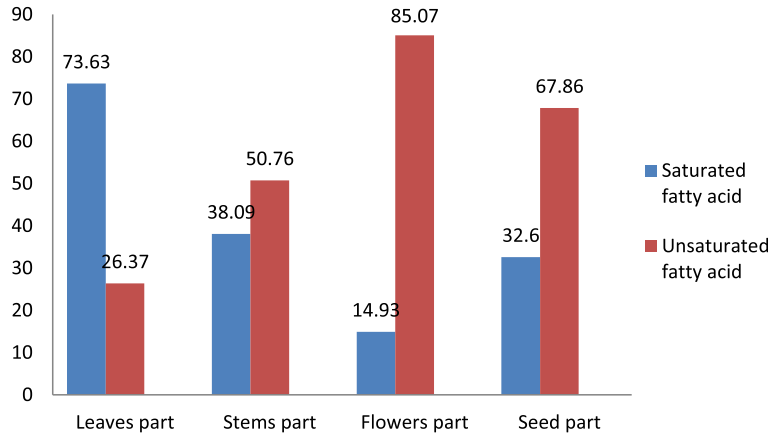

Fig. 2 GC-MS analysis of fatty acid of various parts of *Cassia sophera* Linn

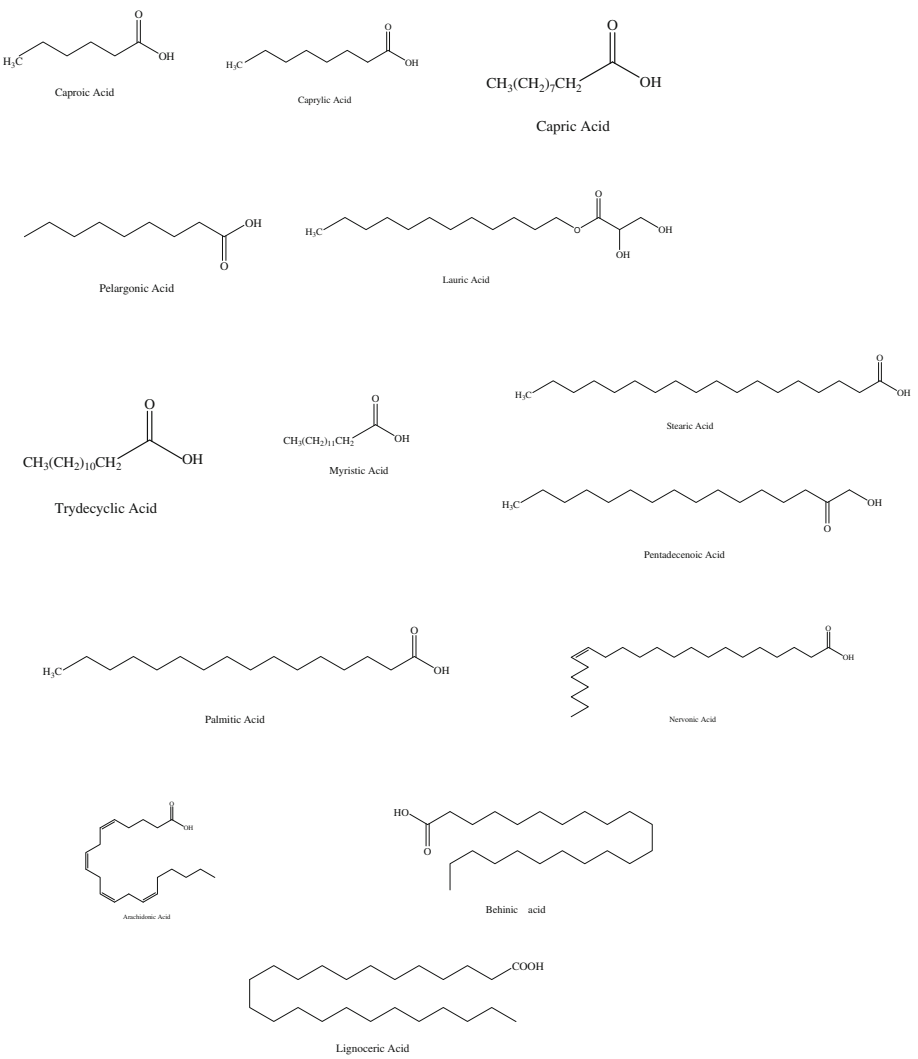

Fig. 3 Structure of the saturated fatty acid identified from GC-MS analysis of different parts of *Cassia sophera* Linn

respectively), flower part (14.93% and 85.07% respectively), and seeds (32.63% and 67.86% respectively) (Fig. 2).

Saturated fats play a key role in cardiovascular health. They are required for calcium to be effectively incorporated into the bone. Saturated fatty acids have been shown to protect the liver from alcohol and medications, including acetaminophen and other drugs commonly used for pain and arthritis (Fig. 3). Certain saturated fatty acids, particularly those found in plants, function directly as signaling messengers that influence metabolism, including such critical jobs as the appropriate release of insulin. Loss of sufficient saturated fatty acids in white blood cells hampers their ability to recognize and destroy foreign invaders, such as viruses, bacteria, and fungi [24]. And unsaturated fatty acids reduce the risk of diabetes (Fig. 4). Some fat-soluble vitamins like vitamins A, D, E, and K are better absorbed in the intestines with unsaturated fat. They are the main sources of energy from food. Eating lots of protein along with unsaturated fat is beneficial for overall brain health [20]. The comparative values of saturated and unsaturated fatty acids of different parts of the *Cassia sophera* Linn are shown in Fig. 2. It clearly indicated that leaf, stem, flower, and seed part contains appreciable amount of saturated and unsaturated fatty acids, which justifies the traditional uses of this important medicinal plant for treatment of various diseases.

The most important findings of the work is that, in the case of petroleum ether extract of the leaf part, the major constituent was arachidic acid (38.66%); for the stem and seed part, the major constituent was linoleic acid (40.12% and 42.40%); and for the flower part, the major constituent was decosadionic acid (27.14%). The so-called essential fatty acids include linoleic acid and α-linolenic acid; they are not synthesized in the human body because of the lack of appropriate enzymes. Linoleic acid is considered the most important of all omega-6 fatty acids, because it can be obtained with other acids of this group such as α-linolenic acid or γ-linoleic acid [33]. Linoleic acid can act as an anti-iflammatory agent [27]. It can also show antiasthmatic

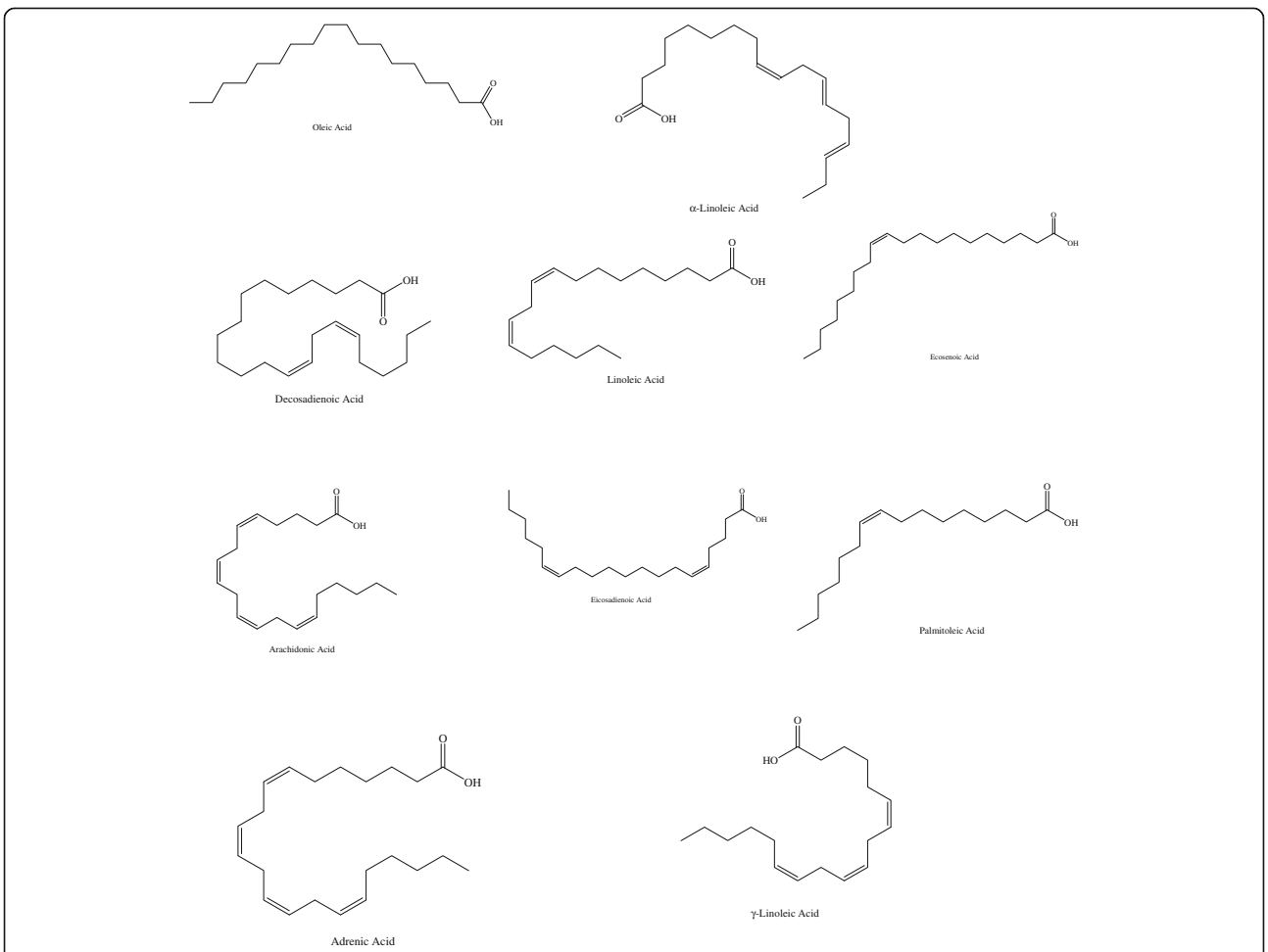

Fig. 4 Structure of the unsaturated fatty acid identified from GC-MS analysis of different parts of *Cassia sophera* Linn

activity [31]. Arachidic acids (saturated fatty acids) have C_{20}. This acid and its metabolites play an important role in a variety of biological process, including signal transduction, contraction, chemotaxis, cell proliferation and differentiation, and apoptosis [33]. Decosadionic acid is a polyunsaturated fatty acid (PUAF). PUAFs are necessary for overall health. The proportion of PUAFs in serum and erythrocyte phospholipids, which depends on endogenous metabolism controlled by genetic polymorphisms and dietary intake, is an important determinant of both health and disease. PUAFs are cardio-protective, perhaps though their anti-inflammatory, anti-arrhythmic, lipid-lowering, and anti-hyper sensitive effects [28].

4 Conclusion

Due to the presence of a good number of fatty acids and protein content and appreciable quantities of secondary metabolites in different parts of the plant studied here, the plant can be seen as a potential source of useful drugs. The presence of these fatty acids in a considerable amount might serve to recognize the potential pharmacological importance of this plant in disease control. The medicinal value of plants lies in some chemical substances that have a definite physiological action on human body. It also justifies the folklore medicinal uses and claims about the therapeutic values of this plant as curative agent. We therefore suggest further the isolation, purification, and characterization of the bioactive compounds from leaf, stem, flower, and seed of *Cassia sophera* Linn with a view to obtain useful chemotherapeutic agents.

Abbreviations

BCSIR: Bangladesh Council of Scientific and Industrial Research; FAME: Fatty acid methyl ester; GC-MS: Gas chromatography and mass spectrum; IFST: Institute of Food Science and Technology; NIST: National Institute of Standards and Technology; PUAF: Polyunsaturated fatty acid

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Authors' contributions

SA designed and outlined the study. TKM supervised the collection of plant materials and carried out the determinations. SA managed the GC-MS meas urement through the support of IFST personnel. SA and TKM conducted the computational study. SA drafted and revised the manuscript. Both authors have approved the manuscript for submission.

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Availability of data and materials

The data that support the findings of this study are available from IFST, BCSIR, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with the permission of IFST, BCSIR.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Aminabee S, Rao AL (2012) A plant review of Cassia sophera Linn. Int J Pharm Chem Biol Sci 2(3):408–414
2. Ayeni M, Oyeyemi S, Kayode J, Peter G (2015) Phytochemical, proximate and mineral analyses of the leaves of Gossypium hirsutum L. and Momordica charantia L. J Nat Sci Res 5:99–107
3. Aziz S, Saha K, Sultana N, Ahmed NU, Amin R, Al-Mansur A (2015) Comparative studies on proximate analysis and amino acid composition of leaf and flower proteins conducted on medicinal plant, Catharanthus Roseus: available in Bangladesh. World J Pharm Res 4(11):296–309
4. Banso A, Adeyemo S (2007) Evaluation of antibacterial properties of tannins isolated from Dichrostachys cinerea. Afr J Biotechnol 6(15):1785–1787
5. Bilal A, Khan NA, Ghufran A, Inamuddin H (2005) Pharmacological investigation of Cassia sophera linn. var. purpurea, roxb. Med J Islamic World Acad Sci 15(3):105–109
6. Boham B, Kocipai-Abyazan R (1974) Flavonoids and condensed tannins from leaves of Hawaiian vaccinium vaticulatum and V. calycinium. Pacific Sci 48:458–463
7. Choudhury A, Rahmatullah M (2012) Ethnobotanical study of wound healing plants among the folk medicinal practioners several district in Bangladesh. Am Eurasian J Sustain Agric 6(4):371–377
8. Cowan MM (1999) Plant products as antimicrobial agents. Clin Microbiol Rev 12(4):564–582
9. Cragg GM, Newman DJ (2001) Natural product drug discovery in the next millennium. Pharm Biol 39(sup1):8–17
10. Drever BD, Anderson WG, Riedel G, Kim DH, Ryu JH, Choi D-Y, Platt B (2008) The seed extract of Cassia obtusifolia offers neuroprotection to mouse hippocampal cultures. J Pharm Sci 107(4):380–392
11. Fatima S, Zaman R, Haider N, Shamsi S, Alam A (2017) Design and development of Unani anti-inflammatory cream. J Ayurveda Integrative Med 8(3):140–144
12. Graefe E, Derendorf H, Veit M (1999) Pharmacokinetics and bioavailability of the flavonol quercetin in humans. Int J Clin Pharmacol Ther 37:219–233
13. Guo H, Chang Z, Yang R, Guo D, Zheng J (1998) Anthraquinones from hairy root cultures of Cassia obtusifolia. Phytochemistry 49(6):1623–1625
14. Harbone J (1973) Phytochemical methods. A guide to modern technique of plant analyses by Chapman and Hall, New York
15. Herborne AJ 1984 Phytochemical methods: a guide to modern techniques of plant sciences. Ass Of OfficAnalut Chem,SPRINGER.
16. Idu M, Obaruyi G, Erhabor J (2008) Ethnobotanical uses of plants among the binis in the treatment of ophthalmic and ENT (ear, nose and throat) ailments. Ethnobotanical Leaflets 2009(4):9
17. Ivanova D, Gerova D, Chervenkov T, Yankova T (2005) Polyphenols and antioxidant capacity of Bulgarian medicinal plants. J Ethnopharmacology 96(1-2):145–150
18. Kirtikar KR, Basu BD (1918) Indian medicinal plants Vol-3: Bishen Singh Mahendra Pal Singh And Periodical Experts
19. Kuo LC (2001) Medicinal plants. J Chinese Chem Sosciety 48(6):1053–1058
20. Lunn JE, Feil R, Hendriks JH, Gibon Y, Morcuende R, Osuna D, Scheible W-R, Carillo P, Hajirezaei M-R, Stitt M (2006) Sugar-induced increases in trehalose 6-phosphate are correlated with redox activation of ADPglucose pyrophosphorylase and higher rates of starch synthesis in Arabidopsis thaliana. Biochem J 397(1):139–148
21. Mandal V, Mohan Y, Hemalatha S (2007) Microwave assisted extraction—an innovative and promising extraction tool for medicinal plant research. Pharmacogn Rev 1(1):7–18
22. Misra A (2009) Studies on biochemical and physiological aspects in relation to phyto-medicinal qualities and efficacy of the active ingredients during

the handling, cultivation and harvesting of the medicinal plants. J Med Plants Res 3(13):1140–1146
23. P.R. Shewry JAN (1985) Energy and protein requirements. WHO, Geneva, pp 13–205
24. Peichev M, Naiyer AJ, Pereira D, Zhu Z, Lane WJ, Williams M, Oz MC, Hicklin DJ, Witte L, Moore MA (2000) Expression of VEGFR-2 and AC133 by circulating human CD34+ cells identifies a population of functional endothelial precursors. Blood 95(3):952–958
25. Ranganna S (1986) Handbook of analysis and quality control for fruit and vegetable products. Tata McGraw-Hill Education
26. Rasool Hassan B (2012) Medicinal plants (importance and uses). Pharmaceut Anal Acta 3:e139
27. Reifen R, Karlinsky A, Stark AH, Berkovich Z, Nyska A (2015) α-Linolenic acid (ALA) is an anti-inflammatory agent in inflammatory bowel disease. J Nutr Biochem 26(12):1632–1640
28. Ristić-Medić D, Vučić V, Takić M, Karadžić I, Glibetić M (2013) Polyunsaturated fatty acids in health and disease. J Serb Chem Soc 78(9):1269
29. Sodipo O, Akinniyi J, Ogunbameru J (2000) Studies on certain characteristics of extracts of bark of Pausinystalia johimbe and Pausinystalia macroceras (K. Schum.) Pierre ex Beille. Global J Pure Appl Sci 6(1):83–87
30. Sofowora E (1993) Medicinal plants and medicine in Africa 2nd eds. In.: John Wiley and Sons;. hibueze U, Akubugwo E: Nutritive values and phytochemical contents of some leafy vegetables grown with different fertilizers. Agricultural Biology Journal of North America 2011; 2:1437-1444.
31. Wendell SG, Baffi C, Holguin F (2014) Fatty acids, inflammation, and asthma. J Allergy Clin Immunol 133(5):1255–1264
32. Winterkorn HF, Fang H-Y (1991) Soil technology and engineering properties of soils. In: Foundation engineering handbook. edn. Springer, pp 88–143
33. Zielińska A, Nowak I (2014) Kwasy tłuszczowe w olejach roślinnych i ich znaczenie w kosmetyce. Chemik 68(2):103–110
34. Ukpabi Chibueze, E. Akubugwo, (2011) Nutritive values and phytochemical contents of some leafy vegetables grown with different fertilizers. Agriculture and Biology Journal of North America 2(12):1437–1444
35. Hussein Al Marzoqi Ali, Yahya Hadi Mohammed, Hadi Hameed Imad, (2016) Determination of metabolites products by Cassia angustifolia and evaluate antimicobial activity. Journal of Pharmacognosy and Phytotherapy 8(2):25-48
36. Baxter H, Harborne JB and Moss GP: Phytochemical Dictionary: a handbook of bioactive compounds from plants: CRC press: 1998
37. Natalie Diether, Benjamin Willing, (2019) Microbial Fermentation of Dietary Protein: An Important Factor in Diet–Microbe–Host Interaction. Microorganisms 7(1):19

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