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Studies on the Lipid and Glyceride Compositions of *Cassia alata* Seed Oil

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Abstract

The oil composition of *Cassia alata* seed collected from three different districts of Bangladesh was investigated. All the seed samples contain about 3.2 % oil. The triglyceride component of the oil varied from 91.5 to 92.0 % diglyceride from 3.4 to 3.9 % and monoglyceride from 1.5 to 2.1 %. The lipid components were almost the same in all the samples, the neutral lipid accounted for over 93 % of the total lipids present. The analysis of the fatty acid composition showed 25.6 % oleic acid, 45.5 % linoleic acid, 18.7 % palmitic acid, 3.5 % stearic acid, 3.4 % arachidic acid and 3.2 % behenic acid.

Introduction

Cassia alata is an indigenous flowering plant which grows almost in all the districts of Bangladesh but it grows abundantly in the districts of Dhaka, Rajshahi and Dinajpur. *Cassia alat*a is found to grow in the lower Bengal, Western Peninsula, Burma and Malacca, very probably introduced into India, as it does not appear to occur far away from human dwellings. At present it is predominantly found in Indo-Pak-Bangladesh subcontinent.[1] The *Cassia alata* is of considerable importance from a medicinal point of view.[2]

The fresh leaves were often employed to cure ring worm.[2] *Cassia alata* is used in West Africa to treat parasitic skin diseases and pustular skin infections.[3]

In the search for therapeutic agents from natural sources for the treatment of AIDS patients the antibacterial and antifungal activities of water extracts of *Cassia alata* were investigated.[4] *Cassia alata* has wide range of medicinal uses in India and the West Indies for the treatment of various aliments.[5]

Cassia alata plants produce considerable amounts of seeds which are generally left as waste. The physico-chemical properties of oils are believed to be directly related to their glyceride and lipid. So, a first hand knowledge of its chemical composition seems to be very much essential for its potential use as a medicinal plant.

Despite considerable works were done on the isolation of various compounds from leaves by some workers,[2,6] information on the characteristics of the *Cassia alata* seed oil is lacking.

Hence the present investigation was made with a view to finding out the approximate compositions of glyceride and lipid of the oil of the locally available seeds, as the location of Bangladesh is typical in the subcontinent because of seasonal variations of monsoon.

Materials and Methods

Ripe and matured *Cassia alata* seeds were colleted from the districts of Dhaka, Rajshahi and Dinajpur in the month of August. The seeds were deshelled and decorticeted manually and dried in the sunlight for four consecutive days. The sun dried seeds were finally crushed into powder with the help of a grinding machine and dried in the oven at a temperature with the help of a grinding machine and dried in a Soxhlet extractor with n-hexane for an 8 hour. The solvent was removed by using a rotary vacuum evaporator and the percentage of oil content was calculated.

The crude oil thus obtained was purified in a column (Neutral alumina in pet. ether) using pet. ether (70:30) as the eluting solvent. The purity of the oil was checked by normal TLC. The specific gravity of the oil was calculated at 28^{O} C with the help of a Pycnometer. Refractive index of clear oil free from water and air bubbles were determined at 28^{O} C by standard IUPAC method.[7] Moisture and volatile matter in the oil were also determined by IUPAC method.[7] The Free fatty acid (FFA), saponification value, peroxide value and unsaponifiable matters in the oil were determined by the standard AOCS methods.[8] Hanus method[9] was followed to determine the iodine value of the oil.

Separation of glyceride

Each sample of oil of the seeds collected from the above three districts was separated into mono-, di- and triglycerides on silicic acid (E. Merck, Darmstadt, Germany 70-230 mesh) column. The silicic acid was activated at 120^{O} C overnight and again for 1 hour immediately before the column was prepared. Then the silicic acid was hydrated with 5 % (w/w) water. A slurry of silicic acid in chloroform was poured into the column (2.2 cm i.d). One g oil was dissolved in 15 ml of chloroform and quantitatively transferred to the column. The triglyceride components from each of the three samples (0.917, 0.920 and 0.915 g) was eluted with 200 ml of benzene, the diglyceride (0.039, 0.034 and 0.036g) with 200 ml of a 1:9 (v/v) mixture of diethy1 ether and benzene and the monoglyceride (0.021, 0.018 and 0.015g) with 200ml dieth1 ether.[10] The percent compositions of the glyceride are shown in Table II. The elution was controlled at a flow rate of 1.5-2ml/min.

The elution of each fraction was monitored by micro slide thin layer chromatography (TLC) to ensure complete separation of each class of the glycerides during slilicic acid chromatography and the eluted solvents were collected in weighed flasks. The fractions thus obtained were evaporated in a rotary vacuum evaporated in a rotary vacuum evaporator and were dried under reduced pressure before being weighed. The purity of the different glyceride components was further checked by TLC using silica gel developed with n-hexene : diethy1 ether checked by TLC using silica gel developed with n-hexene: diethy1 ether (80/20 v/v) and identified by chromic-sulphuric acid spot tests at 180^{O} C. The glyceride were identified by comparison of Rf values with standard references. The weight percentage of each glyceride class was based on total glyceride recovered, which averaged to 99.3 of the total glyceride present.

Fractionation of *Cassia alata* seed lipid

Total lipid extracted by Bligh and Dyer method[11] was fractionated into three major lipid groups, neutral lipid, glycolipid and phospholipid by silicic acid chromatography on about 150mg of *Cassia alata* seed lipids Neutral lipids were eluted with chloroform, glycolipid with acetone and phospholipid. with methanol.[12] The elution was controlled at a flow rate of 0.5ml-1.0ml/min. The complete elution of each fraction was monitored by micro slide TLC during silicic acid column chromatography and eluted solvents were collected in weighed vials. The percentages of these fractions were determined by gravimetric method.

Separation of saturated and unsaturated fatty acid present in the seed oil

Separation of saturated and unsaturated fatty acids was carried out by lead-salt-ether method.[14] 50gm of the oil was saponified with alcoholic caustic soda to obtain soap solution. A slight excess of lead acetate solution was added to the soap solution to form lead salt of fatty acid which were then separated. Ether was added to the mixture of lead salt and the whole mixture was added to the soap solution to form lead salt of fatty acid which were then separated. Ether was added to the mixture of lead salts and the whole mixture was boiled and then cooled at O^{O} C for 24 hours. The upper ether layer was decanted off. The lead of the unsturated fatty acid were obtained by removing the ether from the upper ethereal solution. The precipitated part contained lead salts of the saturated fatty acids. Each group of lead salts was suspended in water and treated with sufficient hydrochloric acid and extracted with ether. On evaporating the ether, the fatty acid were obtained mainly in two groups. Finally masses of saturated and unsaturated fatty acids were obtained by weighing them separately.

Fatty acid composition of *Cassia alata* seed oil

Fatty acid composition of *Cassia alata* seed oil was analysed as their methy1 ester, which was prepared by the Boron trifluoride

methanol method.[14] A GCD Pye Unicam gas chromatograph equipped with a flame ionization detector was used to determine the fatty acid methy1 esters. Nitrogen carrier gas was used at a flow rate of 30ml/min. Fatty acid were separated on a 18X $^{1}/_{8}$ I. d glass column packed with 6 % BDS (Butanediol Succinate Polyesters) on solid support Anakorm ABS (100/120) mesh. Analysis was carried out at isothermal column temperature 190^{O} C, injector and detertor temperatures for all GLC analyses were 230^{O} C. Gas chromatographic peaks were identified by comparison with standard methy1 ester with respect to retertion times against equivalent carbon length (ECL). Peak areas were measured by a Pye Unicam electronic integrator. The percentage of each peak was calculated as the percentage of total area of all the peaks.

Results and Discussion

The solvent extraction of *Cassia alata* seed yielded 3.2 % light yellow coloured oil. Purification of the oil was carried out by column chromatography and the purity was verified by normal TLC. The physico-chemical characteristics of purified oil were determined by the conventional methods and results were given in Table I. No. appreciable change in physical and change in physical and chemical characteristics among the three samples collected from three different districts was observed. Specific gravity and refractive index were normal in comparison with other vegetable oils.[13]

The whole oil was fractionated into mono-, di- and triglycerides by means of column chromatography and the results were presented in Table II. From the results in Table II, it is evident that triglycerides in all the three samples irrespective from where they were collected accounted for over 91.5 % of the total weight of the oil.

Total extracted *Cassin alata* seed lipid were separated into neutral lipid, glycolipid and

Table I. Physical and chemical characteristics of *Cassia alata* seed oil

Physical and chemical characteristics	Name of the districts from where seeds were collected		
	Rajshahi	Dinajpur	Dhaka
1. Specific gravity at 28 ^{O} C	0.9215	0.9226	0.9248
2. Refractive index at 28 ^{O} C	1.4590	1.4628	1.4625
3. Moisture and volatile matter (%)	0.1171	0.1173	0.1170
4. Melting point	28-29^{O} C	28-29^{O} C	29-30^{O} C
5. Free fatty acid as oleic (%)	2.1	2.5	2.7
6. Iodine value (Hanus method)	94.3	94.5	95.0
7. Saponification value	189.0	188.4	188.7
8. Unsaponifiable matter (%)	1.5	1.8	2.0
9 Peroxide value (m.e.v/mg)	1.80	1.82	1.85

Mean results of three experiments for each sample

Table II. Glyceride composition of *Cassia alata* seed oil (weight %)

Name of the districts from where seeds were collected	Monoglyceride	Diglyceride	Triglyceride	FFA
Rajshahi	2.1	3.9	91.7	2.1
Dinajpue	1.8	3.4	92.0	2.5
Dhaka	1.5	3.6	91.5	2.7

Mean results of three experiments results for each sample

phosphopid by slilicic acid column and presented in Table III. The results indicate that no significant change in the lipid composition among the three samples was noticed. From the results shown in Table III, it is observed that neutral lipids in all the three samples were in Table III, it is observed that neutral lipid in all the three samples were found to be over 93 % of the total weights of the lipid.

The saturated and unsaturated fatty acid present in the oil were separated by lead salt- ether method and the results were depicted in Table IV. From the results in Table IV, it is seen that the percentage of saturated and unsaturated fatty acid present in the oil collected from Rajshahi and Dinajpur districts were almost similar. But the percentage of fatty acid composition of the oil in the sample collected from Dhaka district differs from fatty acid composition of the samples from other two districts.

The fatty acid composition of the oil was determined by GLC and the results were presented in Table V. Gas chromatographic analysis showed that unsaturated fatty acid present in *Cassia alata* seed oil were mainly oleic (25.6 %) and linoleic (45.5 %) which altogether accounted for over 70 % of the total fatty acid.

Table III. Lipid composition of *Cassia alata* seed oil (weight %)

Name of the districts from where seeds were collected	Neutral lipid (%)	Glycolipid (%)	Phospholipid (%)
Rajshahi	93.2	4.6	2.6
Dinajpur	94.3	3.3	2.1
Dhaka	92.8	4.6	2.3

Mean results of three experiments results for each sample

Table IV. Percentage of saturated and unsaturated fatty acid

Name of the districts from where seeds were collected	Saturated fatty acid	Unsaturated fatty acid
Rajshahi	25.5	70.7
Dinajpur	23.7	72.5
Dhaka	29.0	67.2

Table V. The fatty acid composition of *Cassia alata* seed oil of Rajshahi variety (weight %)

Fatty acid	Weight percent
$C_{16:0}$ (Palmilic acid)	18.7
$C_{18:0}$ (Stearic acid)	3.5
$C_{18:1}$ (Oleic acid)	25.6
$C_{18:2}$ (Linoleic acid)	45.5
$C_{20:0}$ (Arachidic acid)	3.4
$C_{22:0}$ (Behenic acid)	3.2

Conclusion

The *Cassia alata* seed has been found to contain about 3.2 % oil. The oil is mainly composed of unsaturated fatty acid (over 70 % of which 25 % accounts for oleic acid and 45.5 % linoleic acid.

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