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ANALYSIS OF FATTY ACID COMPOSITION AND PHYSICO-CHEMICAL CHARACTERISTIC OF *NYCTANTHES ARBORTRISTIS* AGED GREEN SEED AND RIPE SEED BY GAS LIQUID CHROMATOGRAPHY

Mohammad Mizanur Rahman[1], Mohammad Shahjahan[2]

[1]Department of Chemistry, Bangladesh Institute of Health Sciences (BIHS), Dhaka, Bangladesh
[2]Department of Chemistry, University of Information Technology and Science, Dhaka, Bangladesh

E-mail of Corresponding Author: pmizan@gmail.com

ABSTRACT

The fatty acid composition of oils extracted from two types (aged green seed and ripe seed) of seed of *Nyctanthes arbortristis* Linn were obtained by gas liquid chromatography. The aged green seed and ripe seed to each individually were successively extracted by light petroleum ether–n-hexane–chloroform (PHCL), which analyze for fatty acid composition. The major saturated and unsaturated fatty acids methyl ester (FAME) of aged green seed PHCL extract were determined palmitic acid ($C_{16:0,}$ 16.3109%), stearic acid ($C_{18:0,}$ 6.6109%) and oleic acid ($C_{18:1,}$ 44.3101%) eicosadienoic acid ($C_{20:2,}$ 13.5202%) respectively. On the other hand the saturated and unsaturated fatty acid of ripe seed PHCL revealed that contain mainly palmitic acid ($C_{16:0,}$ 17.3494%), stearic acid ($C_{18:0,}$ 19.3639%) and oleic acid ($C_{18:1,}$ 36.5009%), eicosadienoic acid ($C_{20:2,}$ 14.8814%) respectively.

Keywords: Seuli, aged green seed, ripe seed, saturated fatty acid, unsaturated fatty acid, *Nyctanthes arbortristis* Linn

INTRODUCTION

Bangladesh has been liberally gifted by nature with diversity in climates, seasons, soils and other ecological factors so very essential for a favorable growth of a large variety of aromatic and medicinal plants. *Nyctanthes arbortristis* Linn (Seuli) is a large shrub which is widely cultivated throughout Bangladesh as a garden plant. It is a C_3 plant[1]. The interests in our country for this plant are mach greater because of the possibility of exportation. Every part of the Seuli tree is used by the local Kobiraj (physicians) as medicinal plants to cure different diseases. The most common use of this plant is as an antihelminthic, cholagogue and laxative[2]. It is used by tribe's people for the treatment of snake bite, bites of wild animals, cachexyia, sores, ulcers dysentery and menorrhagia[3].

The compound of arbortristoside B of seed of *N. arbortristis* was found to have anticancer activity against methylcholanthrene induced fibrosarcoma in rats[4]. The powdered seeds are used to cure scurfy infections of the scalp. The inflorescence and young fruits (seed) powdered in water is used for relieving cough by tribal people of Bangladesh[5]. A 50% alcoholic extract of the Seuli seeds showed a wide spectrum of biological activity, i.e. antiviral, antileishmanial, immunostimulant and hepatoprotective activities[6]. [7]Rahman et al. (2011) reported fourteen fatty acids were identified (include three unknown fatty acid) in pet-ether extract of *Nyctanthes arbortristis* ripe seed. Presently we used twenty seven fatty acids as a standard and studied two type of Seuli seed (aged green seed and ripe seed) for batter investigation and also

find out the dissimilar of fatty acid composition between aged green seed and ripe seed.

MATERIALS AND METHODS

Seed materials

The aged green seeds and ripe seeds were collected from the matured Seuli plants during the months of November, 2002 to March, 2003, at the place of Maymensingh, Bangladesh. The plant was identified by Bangladesh National Herbarium (BNH), Dhaka, Bangladesh. A voucher specimen (DACB Accession Number-35121) of this plant was deposited in the Bangladesh National Herbarium (BNH), Dhaka, Bangladesh. The collected seeds were cleaned, air dried and crushed mechanically.

Physico-chemical studies

Physico-chemical characteristics of Seuli aged green seed and ripe seed viz. moisture, ash, lignin, crude fibre, refractive index, acid value and iodine value were determined by following the standard procedures[8-10].

Isolation of fatty acids and preparation of methyl ester

The neutral lipids from the air-dry powder about 400g of aged green seed and 450g of ripe seeds to each individually were successively extracted with light petroleum ether (40-60^{0}C), n-hexane and chloroform in a Soxhlet apparatus each for 22h, respectively. Solvents were distilled off and the extracts were concentrated and dried under reduced pressure. The extracts were preserved at 2-4^{0}C in refrigerator. The pet-ether, n-hexane and chloroform extracts were mixed equally weighted (each 5mg) of two types of Seuli seed (aged green seed and ripe seeds) individually to examine for fatty acid analysis. The aged green seed and ripe seed of PHCL extracts was analyzed by Griffin[11] (1960) for esterification and FAME extraction.

Preparation of standard fatty acid methyl ester (FAME)

Twenty seven of standard free fatty acids ($C_{6:0}$, caproic acid; $C_{9:0}$, nonanoic acid; $C_{10:0}$, capric acid; $C_{11:0}$, undecanoic acid; $C_{12:0}$, lauric acid; $C_{13:0}$, tridecanoic acid; $C_{14:0}$, myristic acid; $C_{14:1}$, myristoleic acid; $C_{15:0}$, pentadecanoic acid; $C_{16:0}$, palmitic acid; $C_{16:1}$, palmitoleic acid; $C_{17:0}$, heptadecanoic acid; $C_{18:0}$, stearic acid; $C_{18:1}$, oleic acid; $C_{18:2}$, linoleic acid; $C_{18:3}$, alpha-linolenic acid; $C_{20:0}$, arachidic acid; $C_{20:1}$, eicosenoic acid; $C_{18:4}$, octadecatetraenoic acid; $C_{20:2}$, eicosadienoic acid; $C_{20:4}$, arachidonic acid; $C_{22:0}$, behenic acid; $C_{22:1}$, erucic acid; $C_{20:5}$, timnodonic acid; $C_{24:0}$, lignoceric acid; $C_{24:1}$, nervonic acid and $C_{22:6}$, clupanodonic acid) were individually weighed. About 5mg of each was taken in a reaction tube and BF_3CH_3OH reagent (5ml) was added[11] to it. The mixture was boiled for 5 min. Hexane (5 ml) was added to it and boiled for further 1 min. After cooling the tube a solution of saturated salt was added and vortexed. Then the upper layer containing methyl esters was transferred to a vial with anhydrous sodium sulfate at the bottom. Then the ester was filtered through syringe filter and transferred to a small vial (2ml). The solvent was concentrated by blowing nitrogen gas and stored in a refrigerator before analysis by GLC.

Gas Liquid Chromatography (GLC)

The fatty acid methyl esters were identified and quantified by gas-liquid chromatography method using a capillary glass column (60m × 0.25mm ID) wall coated with 100% cyanopropyl polysiloxane open tubular (WCOT) fused silica (SP2340, Suplco, Bellefonte, Pennsylvania, USA) and flame ionization detector in GC-14A series (shimadzu Co, Tokyo, Japan). Helium gas used as the carrier gas at a flow rate of 30ml/min, air pressure is 2.5kg/cm^2 and hydrogen flow rate 350ml/min. The detector and injector temperatures were chosen as 240^{0}C and 235^{0}C, respectively. The oven temperature was set to 135^{0}C for 14 min and heated to 230^{0}C with a heating rate of 3^{0}C/min and maintained at this temperature for 6 min. Data were analyzed with peak simple chromatography software (Data Apex, Clarity$_{TM}$ software). Each fatty acid methyl ester (FAME) in aged green seed and ripe seed

PHCL was identified by comparing retention times with those of known standard FAME (LIPID STANDARD, Sigma Chemical Co, St Louis, Mo, USA). The results were expressed as percentage composition method. All of the other chemicals used in the experiments were analytical grade (Merck, Darmstadt, Germany).

RESULTS AND DISCUSSION

The amounts of extractive were 6.2298, 0.6878, 4.0714 and 7.9789, 0.6146, 3.8253 percent from pet-ether, n-hexane and chloroform extract of Seuli aged green seed and ripe seed, respectively. From Seuli seed kernels Vasishtha[12] (1938), Tyagi and Vassishtha[13] (1983) and [14]Tumbull et al. (1957) were reported 14%, 14% and 12-16% of oil, respectively. On the other hand [7]Rahman et al. (2011) was reported 7.29% oil found in *Nyctanthes arbortristis* ripe seed.

It has been seen in Table 1 that the higher moisture was found in aged green seed (73.54%) and lower in ripe seed (52.62%) as a fresh matter basis. On the other hand, higher amount of ash (5.16%), lignin (20.83%) and crude fibre (27.46%) was found in ripe seed than aged green seed. The refractive index of the oil depends to some extent on their unsaturation. The higher unsaturation, the greater is the refractive index. In this investigation higher refractive index of pet-ether extract at 29^0C was found in aged green seed (1.4765) and lower in ripe seed (1.4734), which are consistent with the unsaturated oil. The higher acid value of pet-ether extract was found in ripe seed (55.48) than aged green seed (54.97). These values

Table 1. Physico-chemical characteristic of the Seuli aged green seed and ripe seed

Parameters	Aged green seed (DM[a])	Ripe seed (DM)
Moisture	73.54%(FM[b])	52.62% (FM)
Ash	4.23%	5.16%
Lignin	17.36%	20.83%
Crude fibre	24.52%	27.46%
Refractive index (pet-ether extract at 29^0C)	1.4765	1.4734
Acid value (pet-ether extract)	54.97	55.48
Iodine value (Hanus)[c]	48.59	41.10

[a]DM-dry matter basis; [b]FM-fresh matter basis; [c]Iodine value of pet-ether extract

indicated the proportion of free fatty acid is negligible and these oils are inedible. The iodine values (Henus) of aged green seed of pet-ether extract was detected higher value (48.59) than ripe seed (41.10). Both seeds of higher iodine values specify that the both seed oils are highly unsaturated and inedible. [7]Rahman et al. (2011) reported the refractive index, acid value and iodine value of pet-ether extract of Seuli ripe seed oil 1.4737, 55.44 and 41.04 respectively, which was comparable and a negligible variation occur different geographical location.

In Table 2, Oleic acid (C18:1) was the dominant unsaturated fatty acid in both type of seed. Major fatty acids of aged green seed were found palmitic acid (16.3109%), oleic acid (44.3101%) and eicosadienoic acid (13.5202%). On the other hand, ripe seed of fatty acid was identified mainly palmitic acid (17.3494%), stearic acid (19.3639%), oleic acid (36.5009%) and eicosadienoic acid (14.8814%). [7]Rahman et al. (2011) reported[7] the major fatty acid of Seuli ripe seed of pet-ether extract was stearic acid, 39.06%. The lower amount of stearic acid (6.6109%), linoleic acid (8.551%), arachidic acid

(2.3694%), behenic acid (1.9339%) and nervonic acid (1.8267%) was detected in the aged green seed. However the Seuli ripe seed was detected as lower amount of linoleic acid (4.1796%), eicosenoic acid (1.6015%), behenic acid (1.7086%) and lignoceric acid (1.0163%). Trace amount of C9:0, C12:0, C14:0, C14:1, C15:0, C16:1, C17:0, C18:3, C20:1 and C22:6 were identified in the aged green seed PHCL extract. On the other hand, trace amount of C6:0, C9:0, C10:0, C11:0, C12:0, C13:0, C14:0, C14:1, C15:0, C17:0, C18:3, C18:4, C20:4, C22:1, C20:5, C24:1 and C22:6 were detected in the Seuli ripe seed PHCL. However, nine fatty acids (C6:0, C10:0, C11:0, C13:0, C18:4, C20:4, C22:1, C20:5 and C24:0) were not identified in aged green seed. On the other hand, C16:1 and C20:0 fatty acid methyl ester was not identified in Seuli ripe seed. [7]Rahman et al. (2011) reported fourteen fatty acids (caprylic acid, 2.16%; nonanoic acid, 2.83%; undecanoic acid, 1.71%; lauric acid, 4.46%; myristic acid, 0.39%; palmitic acid, 1.79%; linoleic acid, 7.89%; stearic aicd, 39.06%; oleic acid, 7.97%; arachidic acid, 3.72%; behenic acid, 2.5% and three unknown fatty acids) were found in Seuli ripe seed of pet-ether extract. On the other hand [12]Vasishtha (1938) reported the fatty acid viz. linoleic, oleic, lignoceric, stearic, palmitic and myristic acid were found from Seuli seed kernel.

Table 2. Fatty acid composition of the Seuli green and ripe seed (percentage composition)

Fatty acid	Structure[a]	Aged green seed (%)	Ripe seed (%)
Caproic acid	C6:0	NF[Ψ]	0.0578
Nonanoic acid	C9:0	0.2135	0.8345
Capric acid	C10:0	NF[Ψ]	0.1429
Undecanoic acid	C11:0	NF[Ψ]	0.061
Lauric acid	C12:0	0.1224	0.0853
Tridecanoic acid	C13:0	NF[Ψ]	0.0573
Myristic acid	C14:0	0.1321	0.1423
Myristoleic acid	C14:1	0.168	0.0903
Pentadecanoic acid	C15:0	0.388	0.1216
Palmitic acid	C16:0	16.3109	17.3494
Palmitoleic acid	C16:1	0.7612	NF[Ψ]
Heptadecanoic acid	C17:0	0.4407	0.2445
Stearic acid	C18:0	6.6109	19.3639
Oleic acid	C18:1	44.3101	36.5009
Linoleic acid	C18:2	8.551	4.1796
Alpha-linolenic acid	C18:3	0.9501	0.4517
Arachidic acid	C20:0	2.3694	NF[Ψ]
Eicosenoic acid	C20:1	0.6212	1.6015
Octadecatetraenoic acid	C18:4	NF[Ψ]	0.5335
Eicosadienoic acid	C20:2	13.5202	14.8814
Arachidonic acid	C20:4	NF[Ψ]	0.1507
Behenic acid	C22:0	1.9339	1.7086
Erucic acid	C22:1	NF[Ψ]	0.1019
Timnodonic acid	C20:5	NF[Ψ]	0.0696
Lignoceric acid	C24:0	NF[Ψ]	1.0163
Nervonic acid	C24:1	1.8267	0.129
Clupanodonic acid	C22:6	0.7697	0.1245

[a]Carbon number with 'zero' double bonds are saturated fatty acids, with 'one' double bonds are monounsaturated and with 'two' and 'three' double bonds are polyunsaturated fatty acid; [Ψ]NF-not found.

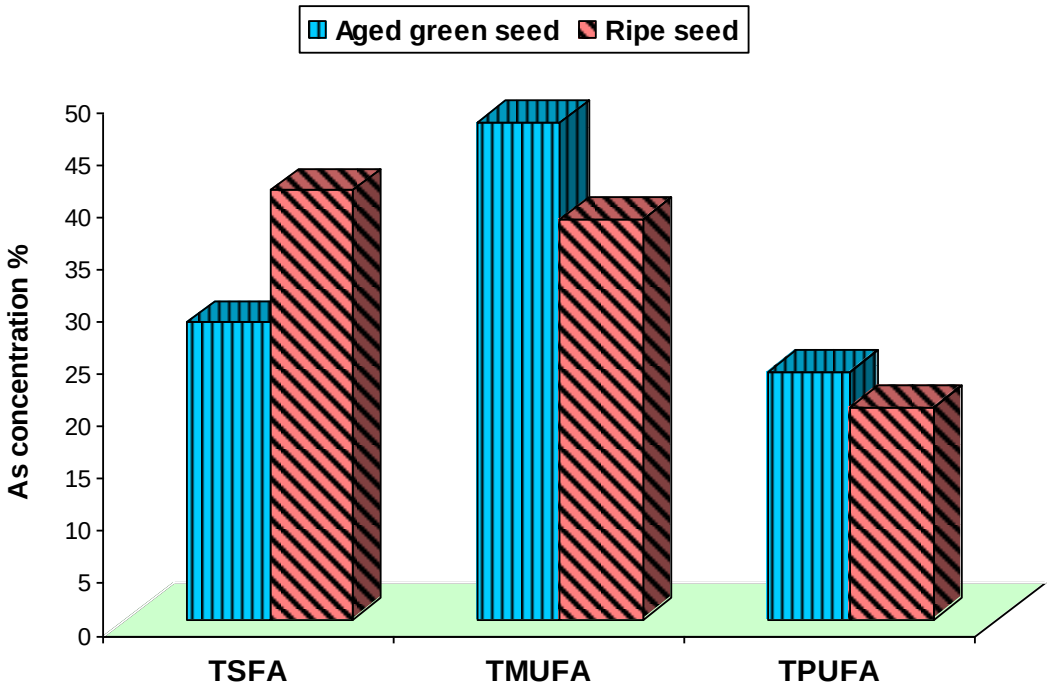

Figure 1. The fatty acid profiles of the Seuli aged green and ripe seed

The fatty acid profiles of the Seuli aged green seed and ripe seed showed in Figure 1. The total saturated fatty acid (TSFA) of ripe seed was detected higher (41.1854%) than aged green seed (28.5218%). On the other hand, higher amount of total monounsaturated fatty acid

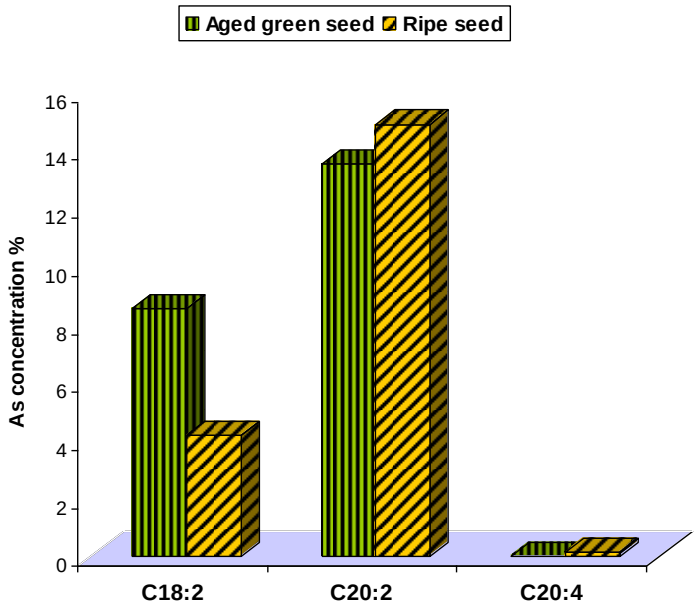

Figure 2. The n-6 polyunsaturated fatty acid composition of *Nyctanthes arbortristis* aged green seed and ripe seed

(TMUFA), 47.6872% and total polyunsaturated fatty acid (TPUFA), 23.791% was found in aged green seed than ripe seed (TMUFA, 38.4236% and TPUFA, 20.391%). However, FAME showed the higher unsaturated/saturated ratio (USR) in aged green seed PHCL (2.5061) than ripe seed (1.4280). It's indicated that the amount of total unsaturated fatty acid of aged green seed was higher than ripe seed. This result showed the order of TSFA increased the aged green seed to ripe seed. In contrast the order of TMUFA and PUFA decreased the aged green seed to ripe seed. This investigation also supported both seed oils of iodine value, occur the more iodine value, the more unsaturated oil.

Contents of n-6 and n-3 polyunsaturated fatty acids of aged green seed and ripe seed are shown in Figure 2 and 3. The Polyunsaturated fatty acids showed the most observable differences in the content of essential fatty acid, linoleic acid (C18:2 n-6) and alpha-linolenic acid (C18:3 n-3). Another polyunsaturated fatty acids showed the secondarily observable differences in the content of fatty acid, eicosadienoic acid (C20:2 n-6) and clupanodonic acid (C22:6 n-3). The n-6 and n-3 fatty acid of arachidonic acid (C20:4 n-6), octadecatetraenoic acid (C18:4 n-3) and timnodonic acid (C20:5 n-3) were found only ripe seed. The ration of n-6/n-3 was higher (16.2908) in ripe seed than aged green seed (12.8336). It indicated n-6 fatty acid was higher in aged green seed than ripe seed. This result indicated, in order of polyunsaturated fatty acids of linoleic acid (C18:2 n-6), alpha-linolenic acid (C18:3 n-3) and clupanodonic acid (C22:6 n-3) were decreased in aged green seed to ripe seed and increased only eicosadienoic acid (C20:2 n-6) in aged green seed to ripe seed of *Nyctanthes arbortristis* Linn.

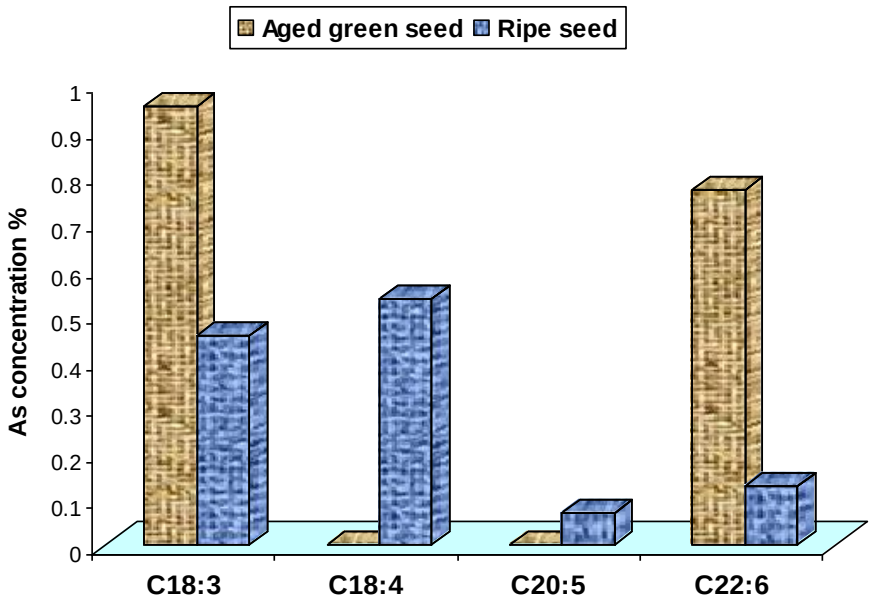

Figure 3. The n-3 polyunsaturated fatty acid composition of *Nyctanthes arbortristis* aged green seed and ripe seed

CONCLUSION

This study revealed the fatty acid composition of Seuli aged green seed and ripe seed PHCL extracts, which have not previously been studied. Seuli ripe seed was detected 13 saturated fatty acids (SFA) and 12 unsaturated fatty acids (UFA). On the other hand 9 SFA and 9 UFA were found in aged green seed. In this study, palmitic acid, stearic acid, oleic acid, eicosadienoic acid was indicated as a main source

of fatty acid both of the Seuli aged green and ripe seed. Interesting finding in our study, the highest total saturated fatty acid (41.1854%) was identified in the Seuli ripe seed. In contrast, the highest total unsaturated fatty acid (71.4782%) was found in the Seuli aged green seed. So, it identically observable that the total unsaturated fatty acid was decreased from aged green seed to ripe seed. In contrast, the saturated fatty acid was increased from aged green seed to ripe seed. α-linoenic acid (n-3) and linolenic acid (n-6) are an essential fatty acid for human. Fatty acids contents and their composition depended on the kinds of the plant seeds. *Nyctanthes arbortristis* seeds are found the potential source of n-3 and n-6. We suggest the *Nyctanthes arbortristis* seeds powder to be used as a supplementary food or drug for deficiency of essential fatty acids.

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