

Phytochemical Studies on *Glinus oppositifolius* (L.) Aug. DC.

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Abstract

The compounds dotriacontyl docosanoate and trilinolein have been isolated from petroleum ether extract and ethyl acetate extract, respectively of the plant *Glinus oppositifolius* (L.) Aug. DC. The compounds were characterised by spectroscopic techniques. Total vitamin C content has been estimated and fatty acid composition has been analysed by GLC.

Key words: *Glinus oppositifolius*, dotriacontyl docosanoate, trilinolein, total vitamin C, fatty acids

I. Introduction

Bangladesh is rich in various plants, herbs and creepers and most of them have medicinal importance and therapeutic value. *Glinus oppositifolius* (L.) Aug. DC. (Locally known as Gima shak) is one of them which are widely used in folk medicine in Bangladesh as well as in Indian subcontinent. The species *Glinus oppositifolius* (L.) Aug. DC. belongs to the Molluginaceae family¹. It is an herb and widely distributed almost all over Bangladesh. This herb is common to use as a non-cultivated food (Herbal food) in Bangladesh on account of its stomachic, appetizer and antiseptic properties. *Glinus oppositifolius* (L.) Aug. DC. is used to treat joint pain, inflammation, diarrhoea, intestinal parasites, fever, and malaria². Anthelmintic and free-radical scavenging potential³, hypoglycemic and hypolipidemic effect⁴, antioxidant activities⁵, analgesic and anti-inflammatory potential⁶ and hepatoprotective activity⁷ and antiprotozoal activity⁸ of *Glinus oppositifolius* (L.) Aug. DC. have been reported. An amino acid derivative, L-(–)-(N-trans-cinnamoyl)-arginine, has been isolated from the whole plant of *Glinus oppositifolius* (L.) Aug. DC. along with kaempferol 3-O-galactopyranoside, isorhamnetin 3-O-β-D-xylopyranosyl-(1→2)-β-D-galactopyranoside, vitexin, vicenin-2, adenosine and L-phenylalanine⁹. Literature survey indicates the presence of oppositifolone, spinasterol, squalene and lutein in the leaves¹⁰. The isolation of triterpenoid saponins, glinosides A and B, from the aerial parts has been also reported⁸. A bioactive pectic polysaccharide has been isolated from this plant which has immunomodulating property¹¹. Six triterpenoid saponins with α-glucosidase inhibitory activity, glinoside C, 3-O-(β-D-xylopyranosyl)-spergulagenin A, spergulacin, spergulin A, spergulacin A and spergulin B have been isolated from *Glinus oppositifolius* (L.) Aug. DC.¹². Although the plant has been investigated by various researchers but further investigation may add its importance and potentialities. A research project has been undertaken to explore its more utility specifically its medicinal importance. Hence, the present study deals with the isolation and characterization of phytochemicals from different extracts of the plant *Glinus*

oppositifolius (L.) Aug. DC. The fatty acid composition and vitamin C content of this plant are also being reported here.

II. Experimental

Sample collection

The whole plants of *Glinus oppositifolius* (L.) Aug. DC. were collected from Gazipur. The collected plants were then washed with water to remove mud and dust particles. The taxonomy of the plant was confirmed in consultation with Prof. Dr. Md. Abul Hassan, Department of Botany, Dhaka University, Bangladesh. A voucher specimen of this plant was deposited in the Bangladesh National Herbarium (BNH) having ACCESSION NO is DACB 35023. The cleaned plant were first dried at room temperature and then in an oven at below 45°C. The dried plants were ground to powder by a Cyclotec grinder (200 meshes) and the powders were stored in air tight bottles and these were used throughout the investigation.

Phytochemical Screening

Phytochemical screening of the plant *Glinus oppositifolius* (L.) Aug. DC. was carried out using standard procedure¹³ to identify the phytochemical constituents. The positive test for tannins, phlobotannins, alkaloids, saponins, flavonoids, steroids, terpenoids and cardiac glycosides was observed.

Extraction

The plant powder (~200 g) was extracted with petroleum ether (b.p 60-80°C) followed by ethyl acetate. These extracts were filtered and evaporated to dryness separately using a rotary evaporator (Stuart, UK) under reduced pressure. The amount of petroleum ether (b.p 60-80°C) extract and ethyl acetate extract were found to be 1.72 g and 1.28 g per 100 g of dry powder, respectively.

Isolation and characterization of compound from petroleum ether extract

The crude petroleum ether (b.p 60-80°C) extract was subjected to TLC screening to find out the type of compounds present in the extract. TLC analysis of the petroleum ether extract showed several spots in iodine

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chamber and vanillin-sulfuric acid spray on TLC plate. The dry mass of petroleum ether extract (3.45 g) was subjected to column chromatography over column grade silica gel (silica gel 60) using petroleum ether (b.p 40°-60°C) as an eluting solvent with increasing percentage of dichloromethane when twenty seven fractions were obtained. Each of the fractions was monitored by TLC and the fractions of similar behaviors were combined together and marked as F-1 to F-9. From TLC analysis the fraction F-3 was found to be a single compound. The fraction (F-3) was concentrated and allowed to stand for several days when the fraction yielded a white solid compound and it was marked as **A** (16 mg).

Isolation and characterization of compound from ethyl acetate extract

The crude ethyl acetate extract was subjected to TLC screening to investigate the type of compounds present in the extract. TLC analysis of the ethyl acetate extract showed several spots in iodine chamber and vanillin-sulfuric acid spray on TLC plate. The dry mass of ethyl acetate extract (2.55 g) was subjected to column chromatography over column grade silica gel (silica gel 60) using petroleum ether (b.p 40°-60°C) as an eluting solvent with increasing percentage of ethyl acetate when twenty two fractions were obtained. Each of the fractions was monitored by TLC and the fractions of similar behaviors were combined together and marked as T-1 to T-5. From TLC analysis of the fraction T-3 was found to be a single compound. The fraction (T-3) was concentrated and allowed to stand for several days when the fraction yielded semi solid mass and this was marked as **B** (9 mg).

Estimation of total vitamin C

Total vitamin C content (ascorbic acid and dehydroascorbic acid) in the fresh leaves of *Glinus oppositifolius* (L.) Aug. DC. was extracted with meta-phosphoric acid and it was estimated by coupling reaction¹⁴ of 2, 4-dinitrophenylhydrazine with L-dehydroascorbic acid, followed by spectrophotometric determination (at 522 nm). Three determinations were done and the sample was taken for thrice and absorbances were recorded three times for each sample in this method. The average value was taken and the total vitamin C content of this plant was found to be 88.4 g per 100 g of fresh leaves.

Analysis of fatty acids

The free fatty acids (FFA) and bound fatty acids (BFA) of the plant *Glinus oppositifolius* (L.) Aug. DC. were isolated¹⁵ from petroleum ether (b.p 40°-60°C) extracts. The FFA and BFA were found to be 16 mg and 150 mg, respectively per 100 g of petroleum ether (b.p 40°-60°C) extract. A portion (3-5 mg) of both the FFA and BFA were converted¹⁵ into their methyl esters. These methyl esters

were analysed by GLC (Shimadzu 9A, Column BP-50, detector-FID, at 170° to 270°C, rising temperature 4°C/min for 30 minutes). The results are given in Table 1.

III. Results and Discussion

Phytochemical Screening of Glinus oppositifolius

Chemical tests for different class of compounds were carried out on the extract and on the powdered specimen using standard procedures¹ to identify the phytochemical constituents and the results indicated that it contained tannins, phlobatannins, alkaloids, saponins, flavonoids, steroids, terpenoids and cardiac glycosides. The medicinal importance of this plant may be explained on the basis of this finding and hence the plant *Glinus oppositifolius* is used by local people and Kobiraj for the remedy of different diseases.

Characterization of compound A

Compound **A** was a white solid having R_f value 0.48 (over silica gel, Hexane: DCM = 80:20 as the mobile phase) and its m.p. was found to be 65-70°C. It was soluble in dichloromethane. ¹H-NMR (400 MHz, CDCl₃) δ: 0.85-0.89 (s, terminal methyl protons at C-30' and C-22), 1.24 (broad peak, -CH₂- protons at C-29' to C-3', C-4 to C-21), 1.54 (-CO-CH₂-CH₂- protons at C-3), 1.60 (O-CH₂-CH₂- protons at C-2'), 2.272 (-CH₂- protons next to C=O bond of ester group at C-2) and 4.04 (-O-CH₂- protons at C-1'), ppm; ¹³C-NMR (100 MHz, CDCl₃) δ: 174.04 (carboxylate carbon at C-1), 64.43 (-O-CH₂- at C-1'), 34.46 (C-2), 31.96 (C-20 and C-28'), 29.20-29.73 (C-4 to C-19 and C-4' to C-27'), 28.70 (-O-CH₂-CH₂- carbon at C-2'), 25.98 (-CH₂- carbon at C-3'), 25.07 (C-3), 22.72 (C-21 and C-29') and 14.13 (terminal -CH₃ carbons at C-22 and C-30') ppm.

The ¹H-NMR and ¹³C-NMR spectral data of the compound **A** were analysed and the different protons and carbons were assigned (as indicated in the given data). Finally the ¹H-NMR and ¹³C-NMR spectral data of the compound **A** were compared with the published data¹⁶ of dotriacontyl ester of docosanoic acid and the compound **A** was identified as dotriacontyl ester of docosanoic acid or dotriacontyl docosanoate (Fig. 1).

Characterization of compound B

Compound **B** was a light yellowish semi-solid mass having R_f value 0.62 (over silica gel, Hexane: DCM = 60:40 as the mobile phase) and its m.p. was found to be 50-55°C. It was soluble in dichloromethane. ¹H-NMR (400MHz, CDCl₃) δ: 0.870 (s, terminal methyl protons at H-18, 18', 18''), 1.246 (broad peak, -CH₂- protons at C-4 to 6, 16, 17, C-4' to 6', 16', 17', C-4'' to 6'', 16'', 17''), 1.293 (C-7, 7', 7'', 15, 15', 15''), 1.589 (-CO-CH₂-CH₂- protons of C-3, 3', 3''), 2.032 (-CH₂- protons next to the olefinic bond at C-8, 8', 8'', 14, 14', 14''), 2.299 (-CH₂- protons next to C=O bond of ester

group at H-2, 2', 2''), 2.760 (=C-CH₂-C= at C-11, 11', 11''), 4.141 (-CH₂- H_b protons at C-1*, 3*), 4.272 (-CH₂- H_a protons at C-1*, 3*), 5.103 (>CH-O- proton at C-2*) and 5.336 (olefinic protons at C-9, 10, 12, 13, 9', 10', 12', 13', 9'', 10'', 12'', 13'') ppm; ¹³C-NMR (100MHz, CDCl₃) δ: 173.26 (carboxylate carbon at C-1 and C-1''), 173.26 (carboxylate carbon at C-1'), 130.03 & 128.11 (olefinic carbons at C-9, 9', 9'' and C-10, 10', 10''), 127.92 & 130.25 (olefinic carbons at C-12, 12', 12'' and C-13, 13', 13''), 62.13 (-O-CH₂- at C-1*, 3*), 68.93 >CH-O- at C-2*), 34.06 (C-2, 2''), 34.23 (C-2'), 31.56 (C-16, 16', 16''), 29.15-29.72 (C-4 to 7, 15, C-4' to 7', 15', C-4'' to 7'', 15''), 27.23 (C-8, 8', 8'',

14, 14', 14''), 25.66 (C-11, 11', 11''), 24.90 (C-3, 3', 3''), 22.60 (C-17, 17', 17'') and 14.13 (terminal methyl carbons at C-18, 18', 18'') ppm.

The ¹H-NMR and ¹³C-NMR spectral data of the compound **B** were analysed and the different protons and carbons were assigned (as indicated in the given data). Finally the ¹H-NMR and ¹³C-NMR spectral data of **B** were compared with the published data¹⁷ of trilinolein and the compound **B** was identified as trilinolein or 1,2,3-propanetriyl tris(cis,cis-9,12- octadecadienoate) (Fig. 2).

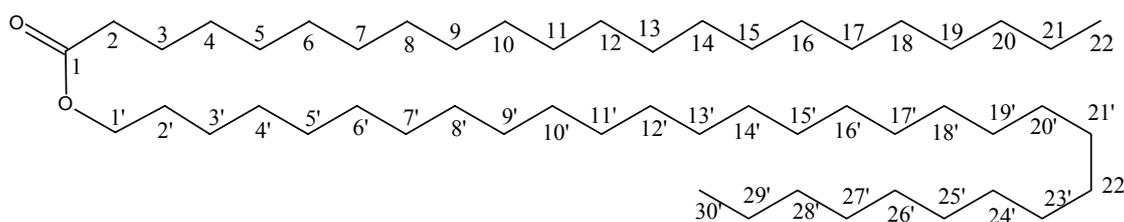


Fig. 1. Structure of **A** as dotriacontyl docosanoate.

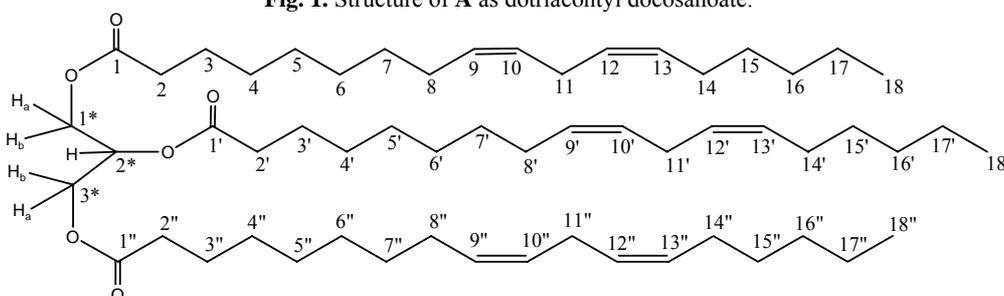


Fig. 2. Structure of **B** as trilinolein.

This investigation indicated the presence of dotriacontyl docosanoate in petroleum ether extract and trilinolein in ethyl acetate extract in the species *Glinus oppositifolius* (L.) Aug DC. and these are the common fatty ester of plant and it has been also reported from the other species of the genus. It appears from literature survey that the isolation of dotriacontyl docosanoate and trilinolein from *Glinus oppositifolius* (L.) Aug. DC. as natural compounds has not been reported prior to this work. Trilinolein is a natural triacylglycerol which has linoleic acid as the fatty acid residue. Trilinolein has been demonstrated to have anti-ischemic property e.g. improvement of erythrocyte deformability and inhibition of platelet aggregation, antiarrhythmic and antioxidant properties¹⁸.

Estimation of total vitamin C

The amount of total vitamin C obtained from 100 g of *Glinus oppositifolius* (L.) Aug. DC. plant was 88.4 mg. Recommended¹⁹ dietary allowance of adult male is 90 mg/day and adult female is 75 mg/day. The amount of total vitamin C content of the plant indicates that the plant is the good sources of vitamin C (ascorbic acid). About 100-150 g

of *Glinus oppositifolius* (L.) Aug. DC. plant may fulfill our daily requirement of vitamin C. Vitamin C is essential for the function of our immune system as well as to repair our tissues and cells. In term of nutritional status, this plant is beneficial for our health.

Fatty acid analysis

Both the free and bound fatty acids of the plant were converted to their methyl esters and analyzed by GLC. The fatty acids present in the plant was identified and their relative percentages were determined (Table 1) by comparing the retention time of the standard samples.

The proportion of BFA (150 mg/100 g of petroleum ether extract) was found to be higher than the FFA (16 mg/ 100 g of petroleum ether extract) which indicated that only a small percentage of fatty acids existed as free state and the remaining fatty acids were associated with lipids or esterified with other organic compounds. The analysis of bound fatty acids (BFA) showed (Table 1) that *Glinus oppositifolius* (L.) Aug. DC. contained highest proportion of palmitic acid (46.75 %) and lowest proportion of myristic

acid (0.95 %). The proportion of stearic acid (18.45 %) and oleic acid (19.93 %) were also significant. Palmitic acid is major saturated fatty acid in leaf lipids and also occurs in some seeds oils like palm oil²⁰. Oleic acid is common in soybean, palm, corn, linseed, coconut and cottonseed oil²⁰. Stearic acid is the most common fatty acid of plant and animal lipid²⁰. The proportion of unsaturated fatty acids (oleic and linoleic acid) was also significant. The analysis of free fatty acids fraction (FFA) indicated (Table 1) that palmitic acid (49.75 %) and lignoceric acid (29.70 %) were the major fatty acids. The presence of unsaturated acid was not detected as free fatty acid. The fatty acid composition

analysis of the plant *Glinus oppositifolius* (L.) Aug. DC. indicated that palmitic acid was the major constituent in both FFA and BFA in this plant. The fatty acids composition is very much similar to edible oils²⁰. So this plant may be very useful as an herbal food for general people.

From all of these studies it can be concluded that this plant is beneficial for the health as a diet and as its medicinal property. So further investigation on this plant may contribute more to the field of medicine.

Table 1. Relative percentage of free fatty acids (FFA) and bound fatty acids (BFA) in *Glinus oppositifolius* (L.) Aug. DC.

Fatty acid→	Lauric	Myristic	Palmitic	Stearic	Oleic	Linoleic	Arachidic	Behenic	Lignoceric
FFA	2.03	1.60	49.24	-	-	-	-	18.21	29.70
BFA	-	0.95	46.75	18.45	19.53	5.48	3.70	2.47	2.14

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