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RESEARCH ARTICLE

Anti-diabetic activity of *Ficus carica* L. stem barks and isolation of two new flavonol esters from the plant by using spectroscopical techniques

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ABSTRACT

In view of alleged antidiabetic potential, effect of methanolic extract of *Ficus carica* L. (Moraceae) stem bark on fasting blood sugar levels and serum biochemical analysis in streptozotocin-induced diabetic rats were investigated. The resulted extract had shown significant protection and lowered the blood glucose levels to normal in glucose tolerance test. In long term treatment of streptozotocin-induced diabetic rats, the degree of protection was determined by measuring blood glucose, triglycerides, total cholesterol and serum insulin levels. Phytochemical investigation of the stem bark resulted in the isolation of two new flavonol esters characterized as 3,5-dihydroxy-7,4'-dimethoxy-flavonol-3-octadec-9''-en-oxy-5-hexadecanoate **(3),** 3,5,3'-trihydroxy-7,4'-dimethoxyflavonol-3-octadec-9''-en-oxy-5-hexadecanoate **(4),** along with known compounds of β-Amyrin acetate **(1)** and β-sitosterol acetate **(2)**. Their chemical structures were established on the basis of spectral evidence , UV, IR, FAB-MS, 1H-NMR, ^{13}C- NMR as well comparison with literature values

Keywords: *Ficus carica* L., Moraceae, anti-diabetic activity, flavonol esters.

1. INTRODUCTION

Diabetes mellitus is a syndrome associated with hyperglycemia (Georg and Ludvik, 2000; Nyholm et al., 2000) hyperlipidemia, oxidative stress, polyurea, polyphagia, polydypsia, ketosis, nephropathy and cardiovascular disorders (Gandjbakhch et al., 2005). Hyperlipidemia is also associated with diabetes (Pushparaj et al., 2000). *Ficus carica* L. (Moraceae), commonly known as fig, is a small or moderately sized deciduous tree indigenous to Asia Minor, Persia, Seria and the Mediterranean region, and widely found in tropical and sub-tropical regions of India (Anonymous, 2002). The stem bark is thick about 7 inch diameter, knobby, irregular with grayish color (Bentley and Trimen, 1992). In traditional medicine the roots of the plant are used in treatment of leucoderma and ringworm. The bark is antipyretic and vermicidal, and the decoction of bark is used in the treatment of various skin diseases, ulcers and diabetes (Kirtikar and Basu, 1992). The herbal drugs are of less toxicity with fewer side effects in the management of diabetes when compared with synthetic drugs (Geetha et al., 1994; Rao et al., 2003). The petroleum ether and benzene fractions from the alcoholic extract of the leaves of *F. carica* on column chromatography over silica gel afforded β-amyrin, β-sitosterol and β-sitosterol-β -D-glucoside along with the known coumarins like psoralen, bergapten, xanthotoxin and a new coumarin, identified as 6-(2-methoxyvinyl)-7-methylcoumarin (Wasim *et al.*, 1988).

This study was aimed at describing the anti-diabetic activity of *Ficus carica* L. and identifying new chemical constituents isolated from the stem barks by spectral data analyses.

2. MATERIALS AND METHODS

Streptozotocin was purchased from Sigma-Aldrich Co., USA. Glucose, total cholesterol, high density lipoprotein (HDL) cholesterol and triglycerides were assayed by using kits from Ranbaxy Diagnostics, New Delhi, India and the One Touch glucometer (Accu-chek sensor) of Roche

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Diagnostics, Germany. Blood glucose level was estimated by glucose oxidase method, using a commercial diagnostic kit from Span diagnostic Ltd. (Surat, India). Insulin was estimated quantitatively by Enzyme-linked immunosorbent assay (ELISA) kit from Mercodia (Uppsala, Sweden). Other chemicals used were of Analytical Grade. Double distilled water was used in all assay procedures. Melting points were determined on a Perfit melting point apparatus (Ambala, India) and were uncorrected. Infrared (IR) spectra were recorded using KBr pellets, with a Jasco FT/IR-5000 Spectrometer (FTS 135, Hongkong). Ultraviolet (UV) spectra were measured with a Lambda Bio 20 spectrophotometer (Perkin Elmer, Schwerzenbach, Switzerland) in methanol. Proton (1H) and carbon-13 (13C) nuclear magnetic resonance (NMR) spectra were recorded using Bruker ARX- 400 NMR Spectrometer (Rheinstetten, Germany), with tetramethylsilane (TMS) as internal standard. The chemical shifts were measured in δ values (ppm). Fast atom bombardment (FAB) mass, were obtained using a JEOL-JMS-DX 303 Spectrometer (Peabody, MA, USA). Column chromatography was performed on silica gel (Qualigens, Mumbai, India), 60–120 mesh. Thin layer chromatography (TLC) was run on silica gel G (Qualigens) and spots were visualized by exposure to iodine vapors, UV radiation and by spraying with ceric sulphate solution.

2.1. Plant material

The *F. carica* stem barks were procured from Jammu and Kashmir (India) and authenticated by Dr. Showkat Ara, Head, Department of Environmental Sciences, Sheri-Kashmir Agricultural University of Sciences and Training (SKAUST), Srinagar. A voucher specimen (No. AU/DES/08/252) is deposited in the herbarium section of SKAUST, Srinagar, Jammu and Kashmir, India.

2.2. Animals

Wister albino rats (150 - 250 g) were procured from the Central Animal Facility, Jamia Hamdard and maintained under controlled condition of illumination (12 h light / 12 h darkness) and temperature 20-25°C. They were housed under ideal laboratory conditions, maintained on standard pellet diet (Lipton rat feed, Ltd; Pune) and water *ad libitum* throughout the experimental period. Animals were acclimatized to the conditions before the start of the experiments. The experimental study was approved by the Institutional Animal Ethics Committee (IAEC) of Jamia Hamdard, New Delhi, India. All the extracts and the standard drugs were administered orally.

2.3. Experimental design

Initial screening of the extract for the hypoglycemic activity was performed in normal healthy rats. The antidiabetic affect was studied in diabetic animals by two methods:

(i) By studying the effect of different doses of the aqueous extract on fasting blood glucose (FBG) levels of sub and mild diabetic rats during glucose tolerance test.

(ii) By giving the most effective dose of extract (500 mg/kg) daily once for 21 days to streptozotocin (STZ)-induced severely diabetic rats and observing the changes in fasting blood glucose (FBG) and lipid profile.

2.4. Biochemical estimations

Blood glucose was estimated using one touch glucometer for regular checkup and kit was used for weekly estimations. Blood glucose, total cholesterol (TC), high density lipoprotein cholesterol (HDLc) and triglyceride (TG) levels in serum were measured spectrophotometrically by prescribed methods (Buccolo and David, 1973). Low density lipoprotein cholesterol (LDLc) was calculated from the above measurement using Friedewald formula. Serum insulin level, thiobarbituric acid reactive substances (TBARS) and glutathione (GSH) were evaluated using the commercially available kits.

2.5. Extraction and isolation

F. carica stem barks (2.5 kg) was dried at 45 °C, coarsely powdered and extracted exhaustively with methanol in a Soxhlet apparatus. The methanolic extract was dried under reduced pressure to get dark brown mass (215 g, 8.6% yield). The viscous dark brown mass was dissolved in small quantity of methanol and adsorbed on silica gel (60–120 mesh) for preparation of slurry. It was dried, packed and chromatographed over silica gel column packed in petroleum ether. The column was eluted successively with petroleum ether, chloroform, and methanol, i.e. with solvents of increasing polarity (e.g., petroleum ether, petroleum ether–chloroform in the ratio of 9:1, 3:1, 1:1 and 1:3 v/v, chloroform , chloroform–methanol in the ratio of 99:1, 98:2, 95:5, 9:1, 3:1, 1:1 and 1:3 v/v, and methanol).

3. RESULTS

3.1. Fasting blood glucose level

There was a significant elevation in fasting blood glucose level after a single dose of streptozotocin compared to control. However, this parameter approached the control level after supplementation of methanolic extract of *F. carica* stem barks (Tables 1 and 2).

3.2. Serum lipid profile

Treatment with extracts of *F. carica* stem bark in higher doses of (500 mg/kg) lowered the elevated levels of these cholesterols (TCh, LDL-C and VLDL-C) and triglycerides significantly (P<0.01). However, the HDL-cholesterol levels were significantly (P<0.01) increased when compared with diabetic control.

Groups	Treatment	Blood Glucose (mg/dl)				% Decrease
		0 h	2 h	4 h	6 h	
I	Normal control (2 ml/kg p.o)	89.16 ± 3.31	89.42 ± 2.31	88.17 ± 3.24	87.92 ± 2.31	---
II	Diabetic control (STZ, 60 mg/kg i.p)	320.92 ± 5.98$^{\#\#}$	321.33 ± 7.31$^{\#\#}$	319.96 ± 6.26$^{\#\#}$	322.02 ± 8.01$^{\#\#}$	---
III	STZ + Insulin (5 U/kg)	318.21 ± 5.78	285.66 ± 6.68**	235.45 ± 5.34**	185.56 ± 3.34**	41.68
IV	STZ + *F. carica* (125 mg/kg)	319.23 ± 5.39	306.76 ± 5.35^{ns}	292.78 ± 3.97*	263.89 ± 4.21*	17.33
V	STZ + *F. carica* (250 mg/kg)	321.56 ± 6.01	302.98 ± 4.91^{ns}	285.78 ± 4.91*	251.45 ± 5.01**	21.08
VI	STZ + *F. carica* (500 mg/kg)	322.21 ± 4.66	290.45 ± 4.45**	260.33 ± 4.01**	228.68 ± 3.73**	**29.02**

The data are expressed in mean ± SEM; n=6 in each group. $^{\#\#}$ (P <0.01) compared with the corresponding value for normal control animals (group I). * (P <0.05), ** (P <0.01) compared with the corresponding value for diabetic control animals (group II). ns – not significant

Table 1: Hypoglycemic effect of single dose treatment of *F. carica* stem bark extracts on blood glucose levels in STZ-induced diabetic Wister rats.

Groups	Treatment	Blood Glucose (mg/dl)				% Decrease
		Day 1	Day 7	Day 14	Day 21	
I	Normal control (2 ml/kg)	88.79 ± 2.34	87.22 ± 2.63	86.34 ± 2.59	88.78 ± 3.01	---
II	Diabetic control (STZ, 60 mg/kg i.p)	322.56 ± 5.89$^{\#\#}$	316.45 ± 6.12$^{\#\#}$	319.66 ± 7.01$^{\#\#}$	321.89 ± 5.87$^{\#\#}$	---
III	STZ + Glibenclamide (3 mg/kg)	320.68 ± 4.98	160.46 ± 2.78**	125.87 ± 2.63**	108.64 ± 2.12**	66.12
IV	STZ + *F. carica* (500 mg/kg)	323.82 ± 7.03	286.66 ± 5.78**	232.82 ± 3.44**	174.76 ± 3.98**	**46.03**

The data are expressed in mean ± SEM; n=6 in each group. $^{\#\#}$ (P <0.01) compared with the corresponding value for normal control animals (group I). *(P <0.05), ** (P <0.01) compared with the corresponding value for diabetic control animals (group II).

Table 2: Hypoglycemic Effect of 21 days treatment of *F. carica* stem bark extracts on blood glucose levels in STZ-Induced diabetic Wister rats.

On the other hand, glimepiride treatment also significantly reduced elevated levels of all these cholesterols (TCh, LDL-C and VLDL-C) and triglycerides. Atherogenic index was calculated for diabetic control group, which was found to be significantly higher than normal control rats. The extract treatment resulted in a significant decrease in the atherogenic index found to be significantly increased by the STZ treatment (Table 3).

Groups	Treatment	HDL-C mg/dl	VLDL-C mg/dl	LDL-C mg/d	TG Mg/dl	TCh mg/dl	Atherogenic Index
I	Normal control (2 ml/kg)	46.34 ± 1.43	13.95 ± 0.98	8.15 ± 0.42	69.79 ± 3.24	68.45 ± 3.97	0.47
II	Diabetic control (STZ, 60 mg/kg i.p)	32.47 ± 1.27$^{\#\#}$	29.55 ± 1.35$^{\#\#}$	96.79 ± 4.56$^{\#\#}$	147.78 ± 6.05$^{\#\#}$	158.82 ± 2.05$^{\#\#}$	3.89
III	STZ + Glibenclamide (3 mg/kg)	43.46 ± 0.78**	13.77 ± 0.67**	14.88 ± 0.76**	68.89 ± 3.98**	72.12 ± 3.02**	0.65
IV	STZ + *F. carica* (500 mg/kg)	42.75 ± 1.37**	16.29 ± 0.43**	19.31 ± 0.59**	81.45 ±5.03**	78.35 ± 2.02**	**0.83**

The data are expressed in mean ± SEM; n=6 in each group. $^{\#}$(P<0.05) $^{\#\#}$ (P <0.01) compared with the corresponding value for normal control animals (group I). ** (P <0.01) compared with the corresponding value for diabetic control animals (group II).

Table 3: Effect of *F. carica* stem bark extracts on lipid profile in STZ-induced diabetic Wister rats.

3.3. Serum insulin, TBARS and GSH levels

The experimental animals showed a marked reduction in serum insulin levels and tissue glutathione, with increase in the level of TBARS. When compared with diabetic control rats, administration of the methanolic extracts of *F. carica* stem bark (500 mg/kg) elevated serum insulin levels, but the level of significance was found to be P < 0.05 (Table 4).

Group	Treatment	Serum Insulin (mU/L)	Tissue GSH (µmole/ mg protein)	TBARS (ηmoles of MDA/mg protein)
I	Normal control (2 ml/kg)	1.86 ± 0.038	1.51 ± 0.028	0.68 ± 0.032
II	Diabetic control (STZ, 60 mg/kg i.p)	0.43 ± 0.023$^{\#\#}$	0.55 ± 0.040$^{\#}$	1.66 ± 0.038$^{\#}$
III	STZ + Glibenclamide (3 mg/kg)	0.87 ± 0.029**	0.62 ± 0.035	0.75 ± 0.041
IV	STZ + *F. carica* (500 mg/kg)	0.64 ± 0.019**	1.13 ± 0.042$^{\#***}$	**1.19 ± 0.030**$^{\#***}$

The data are expressed in mean ± SEM; n=6 in each group. $^{\#}$(P<0.05) $^{\#\#}$ (P <0.01) compared with the corresponding value for normal control animals (group I) * (P <0.05), ** (P <0.01), *** (P <0.001) compared with the corresponding value for diabetic control animals (group II).

Table 4: Effect of *F. carica* extracts stem bark on Insulin, TBARS and GSH levels in STZ-induced diabetic Wister rats.

3.4. Isolated Compounds

3.4.1. β- Amyrin acetate (1)

Elution of the column with petroleum ether-chloroform (1:1) yielded colorless amorphous powder of **1**, recrystallized from acetone; yield: 2.35 g (0.094 %); R_f : 0.89 (petroleum ether-chloroform, 1:1); m.p: 240-241 °C; UV λ_{max} (MeOH): 221 nm (log ε 5.2); IR ν_{max} (KBr): 2924, 2852, 1732, 1642, 1462, 1374, 1246, 1090, 1027, 982 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.13 (1H, d, *J* = 5.7 Hz, H-12), 4.54 (1H, dd, *J* = 9.2, 4.8 Hz, H-3 α), 2.04 (3H, brs, $COCH_3$), 1.27 (3H, brs, Me-27), 1.03 (3H, brs, Me-23), 0.99 (3H, brs, Me-24), 0.89 (3H, brs, Me-25), 0.86 (3H, brs, Me-28). 0.82 (3H, brs, Me-29), 0.80 (3H, brs, Me-30), 0.78 (3H, brs, Me-26); +ve ion FAB MS *m/z* (*rel. int.*): 469 $[M+H]^+$ ($C_{32}H_{53}O_2$) (21.9), 453 (26.0), 410 (38.2), 408 (20.5), 393 (15.6), 250 (5.1), 218 (19.1), 203 (32.4), 191 (24.7), 190 (27.0), 188 (23.3), 176 (18.3), 175 (20.5), 173 (18.1), 161 (19.3), 160 (26.3), 158 (28.4), 146 (48.5), 145 (36.1), 130 (27.6), 119 (49.81), 109 (53.6), 95 (100.0).

3.4.2. β-Sitosterol acetate (2)

Elution of the column with petroleum ether-chloroform (1:1) yielded white needles of **2**, recrystallized from methanol); yield: 2.35 g (0.094 %); R_f : 0.95 (petroleum ether-chloroform, 1:1); m.p. 126-128°C; IR ν_{max} (KBr): 2922, 2853, 1735, 1631, 1461, 1278; ^1H NMR ($CDCl_3$): δ 2.28(3H, brs, $COCH_3$); 4.60 (1H, m, H-3) and 5.37 (1H, brs, H-6); +ve ion FAB MS (*rel. int.*): 456 (M+H) for $C_{31}H_{52}O_2$.

3.4.3. Caricaflavonol diester A (3)

Elution of the column with chloroform-methanol (99:1) gave pale yellow amorphous powder of FC-3, recrystallized from acetone; yield: 1.35 g (0.054 %); R_f : 0.88 (chloroform-methanol, 99:1); m.p: 94-95 °C; UV λ_{max} (MeOH): 205, 268, 297, 335 nm (log ε 4.8, 1.9, 2.3, 1.8); IR ν_{max} (KBr): 2917, 2849, 1725, 1715, 1680, 1632, 1462, 1285, 1160 cm^{-1}; ^1H NMR (DMSO-*d6*): Table: 5; ^{13}C NMR (DMSO-*d6*): Table: 5; +ve ion FAB MS *m/z* (*rel. int.*): 817 $[M+H]^+$ ($C_{51}H_{77}O_8$) (1.3), 577 (22.1), 551 (2.5), 523 (1.5), 412 (8.7), 398 (19.0), 312 (14.6), 265 (5.2), 239 (6.1), 147 (21.6), 132 (20.2), 107 (55.2).

3.4.4. Caricaflavonol diester B (4)

Elution of the column with chloroform-methanol (19:1) furnished greenish crystals of **4**, recrystallized from chloroform-methanol (1:1), yield: 2.20 g (0.088 %), R_f : 0.95 (chloroform-methanol, 19:1); m.p: 178-180 °C; UV λ_{max} (MeOH): 213, 268, 309, 335 nm (log ε 4.8, 2.1, 1.7, 1.3); IR ν_{max} (KBr): 3510, 2923, 2852, 1736, 1710, 1632, 1463, 1246, 961 cm^{-1}; ^1H NMR (DMSO-*d6*): Table. 5; ^{13}C NMR (DMSO-*d6*): Table.5; +ve ion FAB MS *m/z* (*rel. int.*): 833 $[M+H]^+$ ($C_{51}H_{77}O_9$) (1.5), 598 (3.8), 576 (5.6), 550 (2.3), 428 (3.3), 413 (3.6), 404 (3.1), 328 (5.3), 313 (20.1), 300 (2.6), 298 (4.0), 285 (4.1), 270 (3.80), 265 (3.7), 239 (6.2) 163 (6.2), 149 (19.8).

Position	^1H NMR		^{13}C NMR	
	3	4	3	4
2	----	----	147.13	145.24
3	----	----	138.63	138.70
4	----	----	173.63	173.49
5	----	----	159.47	159.50
6	7.04 d (1.1)	6.98 d (1.8)	104.86	104.92
7	----	----	155.44	157.43
8	7.62 d (1.1)	7.68 d (1.8)	98.65	92.73
9	----	----	151.29	151.85
10	----	----	106.15	105.57
1′	----	----	120.12	122.48
2′	8.16 d (9.9)	7.10 d (2.3)	129.29	129.33
3′	6.30 d (9.9)	----	114.99	154.83
4′	----	----	144.19	149.19
5′	6.38 d (9.6)	6.29 d (9.6)	113.62	112.36
6′	8.09 d (9.6)	8.13 dd (2.3, 9.6)	111.87	111.85
1''	----	----	172.95	171.05
2''	2.73 brs	2.78t (7.2)	48.35	33.37
3''	1.50 m	1.64 m	28.53	30.06
4''-6''	1.24 brs	1.32 brs	28.53	28.54
7''	1.24 brs	1.64 m	26.21	28.14
8''	1.71 m, 2.10 m	2.03 m	44.70	30.37
9''	5.32 m	5.35 m	127.30	127.35
10''	5.30 m	5.35 m	124.16	124.86
11''	2.01m, 1.65 m	2.02 m	44.67	33.34
12''-13''	1.24 brs	1.64 m	28.16	28.54
14''	1.24 brs	1.26 brs	26.21	28.14
15''	1.24 brs	1.26 brs	24.10	26.24
16''	1.24 brs	1.26 brs	21.53	24.09
17''	1.24 brs	1.26 brs	18.25	21.80
18''	0.85 t (6.3)	0.88 t (6.1)	13.20	13.31
1'''	----	----	172.90	169.89
2'''	2.19 t (6.9)	2.36 t (7.2)	48.32	33.35
3'''	1.48 m	2.05 m	33.34	30.06
4'''	1.24 brs	1.64 m	30.80	30.06
5'''	1.24 brs	1.32 brs	28.53	30.47
6'''	1.24 brs	1.32 brs	28.53	33.34
7'''	1.24 brs	1.32 brs	28.53	28.54
8'''-9'''	1.24 brs	1.26 brs	28.16	28.14
10'''-12'''	1.24 brs	1.26 brs	28.16	26.24
13'''- 15'''	1.24 brs	1.26 brs	18.59	24.09
16'''	0.83 t (6.1)	0.83 t (6.3)	15.38	13.26
OMe	3.05 brs, 4.24 brs	4.26 brs, 4.24 brs	59.93, 55.94	61.83, 59.93

Coupling constants in Hertz are given in parentheses.

Table 5: ^1H and ^{13}C NMR spectral values of Caricaflavonol diester A (3) and Caricaflavonol diester B (4).

4. DISCUSSION

The present study dealt with two dimensions for antidiabetogenic effects of the methanolic extract of *F. carica* stem bark in separate manner. In one dimension, the fasting blood glucose levels at different doses were

measured. In the other dimension, antihyperlipidemic potency was studied as there was a close correlation between hyperglycemia and hyperlipidemia (Cho et al., 2002). Here, we selected the STZ-induced hyperglycemia as an experimental model because it was one of the best models to study the effect of the antidiabetogenic agent (Carter et al., 1971). The specific doses of the extract used and duration of the treatment adopted here were selected by trial and error where good promising results were noted without any metabolic toxicity induction. Supplementation of the separate extract of the plants parts resulted a significant correction of fasting blood glucose (FBG) level with respect to STZ-induced diabetic group and this recovery was more effective when treatment of extracts of *F. carica* stem bark in higher doses of 500 mg/kg was used, which primarily focused on the antidiabetic activity of the plant products. Meanwhile, the actual mechanism of such antidiabetogenic activity is not clear from this study but following possible dimensions may be enlighten. The aforementioned parameters recovered showed a more potent correction after extract of *F. carica* stem bark treatment and this was equal to control, which may be due to the insulinotropic effect of this extract. *F. carica* treated animals showed a significant increase in the levels of GSH and significant decrease in the levels of TBARS elevation. The reduction in lipid peroxide levels might be due to the electron and H+ donating capacity of *F. carica*, causing termination of lipid peroxidation chain reaction or interacting with cell membranes, improving their fluidity, thereby protecting them against lipid peroxidation. The *F. carica*, has beneficial effects on blood glucose levels in STZ-induced diabetes, as well as in improving hyperlipidemia due to diabetes. The active ingredient(s) present here may recover the disorders in carbohydrate metabolism noted in diabetic state by stimulating existing β-cell or by increasing the rate of β-cell regeneration or by modulating intracellular glucose utilization.

In phytochemical evaluation compound **-3**, designated as Caricaflavonol diester A, was obtained as a pale yellow amorphous powder from chloroform-methanol (99:1) eluent. The UV absorption maxima at 268 nm and 335 cm^{-1} indicated that the compound was a 3-hydroxy substituted flavonol. There was no shifting of the UV absorption maxima with shift reagents suggesting the presence of bound hydroxyl groups in the molecule (Markham, 1982). Its IR spectrum displayed characteristic absorption bands for ester groups (1725, 1715 cm^{-1}), keto group (1680 cm^{-1}) and unsaturation (1632 cm^{-1}). On the basis of FAB mass and ^{13}C NMR spectra, the molecular ion peak of **3** has been established at *m/z* 817 $[M+H]^+$ corresponding to the molecular formula of the dimethoxyflavonol diester, $C_{51}H_{77}O_8$ **(Figure 1)**.

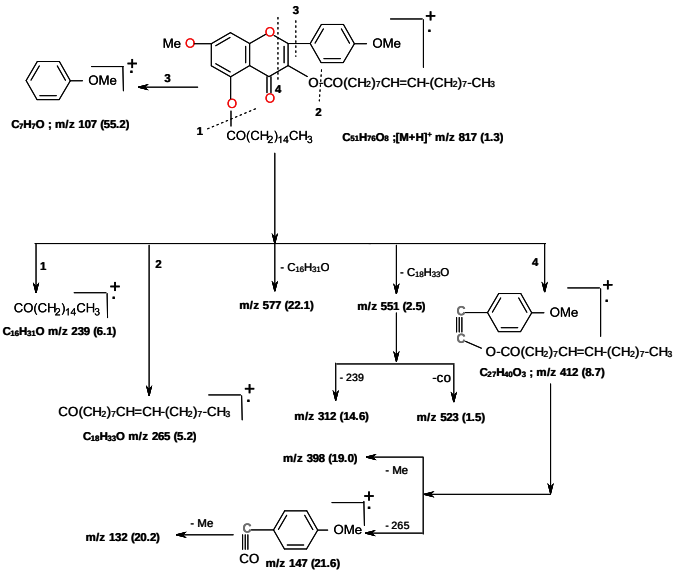

Figure 1: Mass fragmentation of Caricaflavonol diester A (3)

The ion fragments arising at *m/z* 239 $[CH_3 (CH_2)_{14}CO]^+$, 577 $[M-239]^+$, 265 $[CH_3 (CH_2)_7CH=CH(CH_2)_7CO]^+$, 551 $[M-265]^+$ and 523 $[551-CO]^+$ supported the presence of palmityl and oleiyl moieties esterified with the flavonol unit. The ion peaks generating at *m/z* 412 due to retro-Diels-Alder fragmentation and the expulsion of the mass unit 265 yielding an ion fragment at *m/z* 147 $[C_9H_7O_2]^+$ and formation of ion fragment at *m/z* 132 $[147-Me]^+$ indicated that oleiyl moiety was located at C-3 of the flavonol. An ion fragment produced at *m/z* 107 $[C_6H_4OMe]^+$ suggested location one of the methoxy group in ring B. The ^1H NMR spectrum of **3** showed two one-proton doublets at δ 7.04 (*J* = 1.1 Hz) and δ 7.62 (*J* = 1.1 Hz) assigned to *meta*-coupled H-6 and H-8, respectively. Four one-proton doublets, at δ 8.16 and 6.30 with coupling interactions of 9.9 Hz each and at δ 6.38 and 8.09 with coupling interactions of 9.6 Hz each were accounted to aromatic H-2', H-3' and H-5', H-6' respectively, supporting AA´, BB´ system of ring B. Two one-proton multiplets at δ 5.32 and 5.30 were ascribed to vinylic H-9'' and H-10'' protons, respectively.

A two-proton broad signal at δ 2.37 and a two-proton triplet at δ 2.19 (*J* = 6.9 Hz) were attributed to methylene H_2-2'' and H_2-2''' protons, respectively, adjacent to the ester groups. Two three-proton triplets at δ 0.85 (*J* = 6.3 Hz) and 0.83 (*J* = 6.1 Hz) were ascribed to primary methyl H_3-18'' and H_3-16''' protons, respectively. Two three-proton broad signals at δ 3.05 and 4.24 were associated with methoxy protons. The remaining methylene protons resonated between δ 2.10-1.24. The ^{13}C NMR spectrum of **3** displayed signals for ester carbons at δ 172.95 (C-1'') and 172.90 (C-1''') ; carbonyl carbon at 173.63 (C-4); flavonol

carbons between δ 159.47-98.65; vinylic carbons at δ 127.30 (C-9'') and 124.16 (C-10''); methoxy carbons at δ 59.93 and 55.94; methyl carbons at δ 13.20 (C-18'') and 15.38 (C-16''') and methylene carbons from δ 48.35 to 18.25. The ^{13}C NMR values of the flavonol nucleus were compared with the reported data (Schlienmann, *et al*; 2006: Brun *et al*; 1999). On the basis of all these data, the structure of **3** has been established as 3,5-dihydroxy-7,4'-dimethoxy-flavonol-3-octadec-9''-en-oxy-5-hexadecanoate. This is a new flavonol ester derivative isolated from the plant.

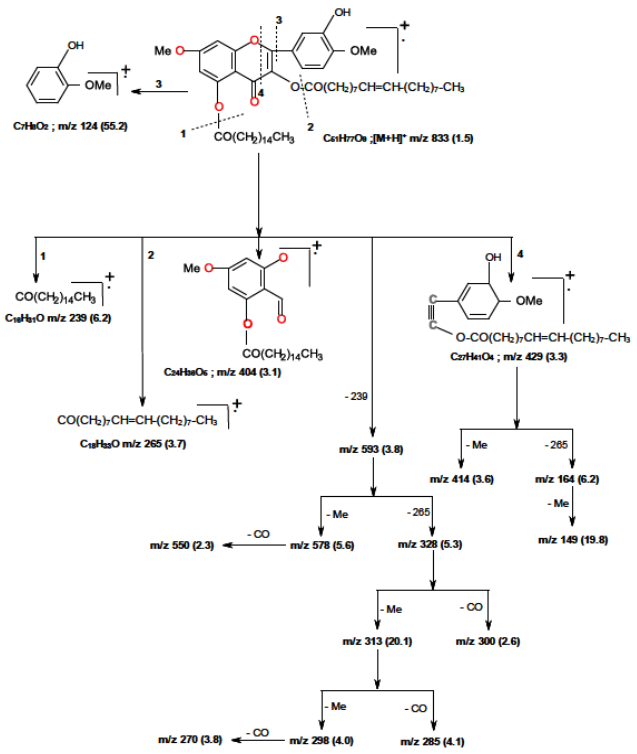

Figure 2: Mass fragmentation of Caricaflavonol diester B (4)

Compound **4**, designated as Caricaflavonol diester B, was obtained as a greenish crystalline mass from chloroform-methanol (19:1) eluent. The U.V absorption maximum at 268 nm and 335 cm^{-1} indicated that the compound was a 3-hydroxy substituted flavonol (Markham, 1982). Its IR spectrum displayed characteristic absorption bands for hydroxyl group (3510 cm^{-1}), ester group (1736, 1719 cm^{-1}) and keto group (1675 cm^{-1}). On the basis of FAB mass and ^{13}C NMR spectra, the molecular weight of **4** has been established at *m/z* 833 $[M+H]^+$ corresponding to the molecular formula of the dimethoxyflavonol diester, $C_{51}H_{77}O_9$ **(Figure 2)**. The ion fragments arising at *m/z* 239 $[CH_3(CH_2)_{14}CO]^+$, 265 $[CH_3(CH_2)_7CH{=}CH(CH_2)_7CO]^+$, 593 $[M-239]^+$, 578 $[593-Me]^+$, 550 $[578-CO]^+$, 578 $[593-265]^+$, 300 $[328-CO]^+$, 313 $[328-Me]^+$, 298 $[313-Me]^+$, 285 $[313-CO]^+$, and 270 $[298-CO]^+$ supported the presence of palmityl and oleiyl moieties as esterified with the flavonol unit. The ion peaks generating at m/*z* 404 $[C_{24}H_{36}O_5]^+$ and 428 $[C_{27}H_{40}O_4]^+$ due to retro-Diels-Alder fragmentation and at *m/z* 413 $[428-Me]^+$, 163 $[428-C_{18}H_{33}O]^+$ and 148 $[163-Me]^+$ suggested existence of the palmityl unit in ring A and oleiyl unit in ring C.

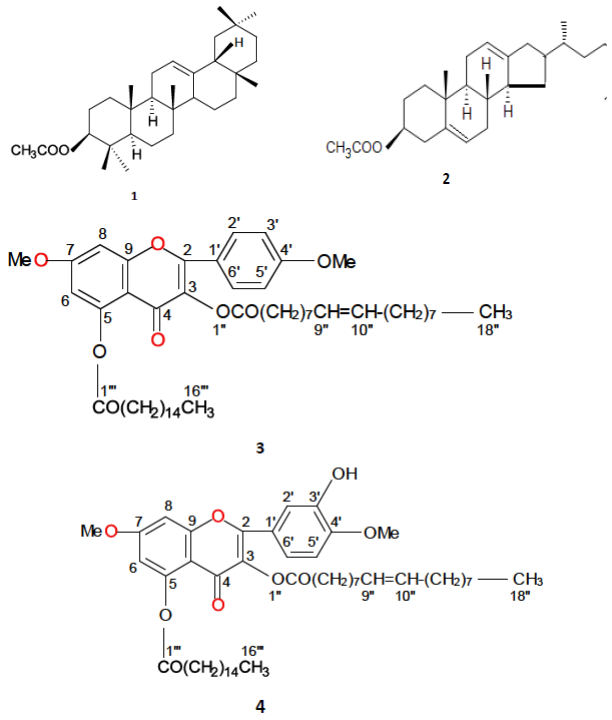

Figure 3. Structure of Isolated Compounds 1, 2, 3 and 4

The ^{1}H NMR spectrum of **4** exhibited three one-proton doublets at δ 6.98 (*J* = 1.8 Hz), 7.68 (*J* = 1.8 Hz) and 7.10 (*J* = 2.3 Hz); a one-proton doublet at δ 6.29 (*J* = 9.6 Hz), and a one-proton double-doublet at δ 8.13 (*J* = 2.3, 9.6 Hz) assigned to H-6, H-8, H-2′, H-5′ and H-6′ aromatic protons, respectively, indicating ABX system for ring B. A two-proton multiplet at δ 5.35 was ascribed to vinylic H-9'' and H-10'' protons. Two two-proton triplets at δ 2.78 (*J* = 7.2 Hz) and 2.36 (*J* = 7.2 Hz) were accounted to methylene H_2-2'' and H_2-2''' protons, respectively, adjacent to the ester group. Two three-proton broad signals at δ 4.26 and 4.24 were associated with methoxy protons. Two three-proton triplets at δ 0.88 (*J* = 6.1 Hz) and 0.83 (*J* = 6.3 Hz) were ascribed to primary methyl Me-18'' and Me-16''' protons, respectively. The remaining methylene protons resonated between δ 2.20-1.26. The ^{13}C NMR spectrum of **4** displayed signals for ester carbons at δ 171.05 (C-1'') and 169.89 (C-1'''), flavonol carbons between δ 173.49-92.73; vinylic carbons at δ 127.35 (C-9'') and δ 124.86 (C-10''); methoxy carbons at δ 61.83 and 59.93; methyl carbons at δ 13.31 (C-18'') and δ 13.26 (C-16'') and methylene carbons between at δ 48.35 - 18.25. The ^{13}C NMR values of the flavonol nucleus were compared with the related flavonol carbons (Schlienmann, *et al*; 2006: Brun *et al*;

1999). On the basis of foregoing account, the structure of **4** has been established as 3,5,3'-trihydroxy-7,4'-dimethoxyflavonol-3-octadec-9''-en-oxy-5-hexadecanoate. This is a new flavonol diester isolated from the plant.

5. CONCLUSION

The present work described the anti-diabetic activity of the stem bark of *Ficus carica w*ith the isolation of new phytoconstituents, which may be useful in the medicinal properties of the drug.

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Conflict of Interest: None Declared

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