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Full Length Research Paper

Two new polyphenolic compounds from *Ficus retusa* L."variegata" and the biological activity of the different plant extracts

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Two new polyphenolic compounds named retusaphenol [2-hydroxy-4-methoxy-1,3-phenylene-bis- (4-hydroxy-benzoate)] and (+)-retusa afzelechin [afzelechin - (4α→8) - afzelechin - (4α→8) - afzelechin] together with ten known compounds: luteolin, (+) - afzelechin, (+) - catechin , vitexin , β-sitosterol acetate, β-amyrin acetate, moretenone, friedelenol, β-amyrin and β-sitosterol were isolated for the first time from the ethanolic extract of the aerial parts of *Ficus retusa* L. "variegata". Their chemical structures were established on basis of spectral evidence; UV, IR, MS, ^{1}H- and ^{13}C-NMR as well as comparison with literature values. Anticancer (IC_{50} 68.64 µg/ml against Hep G2 cells and >100 µg/ml aganist HCT-116 cells), antioxidant (SC_{50} 0.85 µg/ml), antidiabetic, hepatoprotective, anti-inflammatory and antimicrobial activities of the different plant extracts were also studied and significant results were obtained.

Key words: *Ficus retusa* L. "variegata", Moraceae, retusaphenol, retusa afzelechin trimer, anticancer, antioxidant, antidiabetic, hepatoprotective, anti-inflammatory and antimicrobial.

INTRODUCTION

The genus *Ficus* (Moraceae) comprises about 1200 species distributed mainly in tropical and subtropical regions (Sharma et al., 1993). This genus is characterized by its constituents of coumarins, phytosterols, triterpenes, flavonoids as well as alkaloids and tannins (Backheet et al., 2001; Jeong et al., 1999; Zunoliza et al., 2009). Many *Ficus* species have been used as aphrodisiac, antihypertensive, anticancer, antioxidant, hepatoprotective, gastroprotective, antidiabetic, anthelmintic, antimalarial, anti-inflammatory, analgesic and antimicrobial (Chiang et al., 2005; Abraham et al., 2008; Maizatul et al., 2011; Mandal et al., 1999; Rao et al ., 2008; Singh et al., 2009; Hansson et al., 2005; Nguyen et al., 2003; El- Domiaty et al., 2003).

Ficus retusa L. "variegata", is cultivated in Egypt as ornamental plant. No previous reports concerning the chemical constituents or the biological activity of this plant in available literature. In the present work we report the isolation and structure elucidation of two new polyphenolic compounds named retusaphenol and retusa afzelechin, together with ten known compounds. Anticancer, antioxidant, antidiabetic, hepatoprotective, anti-inflammatory and antimicrobial activity of the different plant extracts have been studied and significant results were obtained.

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EXPERIMENTALS

General

Melting points were measured by melting point apparatus SMP3, Digital, Electrothermal LTD, Stuart scientific (England) and were uncorrected; UV spectra were measured in methanol by Schimadzu UV-260 Spectrophotometer (Japan); IR spectra were done on Jasco FT/IR 6100 Spectrophotometer; EIMS were carried out on Jeol JMS-AX 500, 70 *ev* and Shimadzu GC/MS-QP5050A, 70 *ev* ^{1}H- and ^{13}C-NMR spectra were run in $CDCl_3$, DMSO-d_6 and CD_3OD; at 300 or 600 MHz, 150 and 75 MHz, respectively using Varian Mercury-VX-300 NMR Spectrometer; Chemical shifts were reported in δ units relative to TMS; Column chromatography was carried out using silica gel (60 to 120 mesh, Merck); TLC was performed on silica gel coated aluminum plates (Merck kieselgel 60 F_{254}, Germany). Developed chromatograms were visualized under UV

Table 1. UV λ_{max} spectral data for compounds 7 to 12.

Compound No.	MeOH	$NaOCH_3$	$AlCl_3$	$AlCl_3$+HCl	NaOAc	NaOAc+H_3BO_3
7	257		262	260	254	254
8	260,292 (sh), 326(sh.), 348	264, 402	271, 402	263, 357	264, 376	260, 373
9	275	286, 330(sh.)	274	274	275	275
10	280	292, 428 (sh.)	282	279	281	286
11	275	288	275	274	275	275
12	270, 334	279,329(sh.), 395	276,303(sh.), 346, 382(sh.).	277,302(sh), 343,378(sh.).	278, 379	270, 344

light and by spraying with anisaldehyde/sulphuric acid reagent followed by heating at 100°C for 10 min.

For TLC analysis, the following solvent systems were used: Light petroleum- chloroform (7 : 3 , system I), light petroleum - chloroform (3 : 7, system II), light petroleum - chloroform (1: 9 , system III), chloroform - ethyl acetate (9:1, system IV) , benzene - ethyl acetate - formic acid - water (3 : 5 : 1.6 : 0.4, system V).

Plant materials

The aerial parts of *F. retusa* L. "variegata" were collected in July 2007 from trees cultivated in the private garden of "Efficiency Productive Institute, University of Zagazig". The plant was kindly identified by Dr. Abd-Elhalim Abd-Elmagly Mohammed, Agriculture researches centre, Ministry of Agriculture and Land Reclamation, Egypt. Voucher specimen is deposited in the Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University. Fresh samples were used through out the study.

Extraction and isolation

Fresh aerial parts of *F. retusa* L. "variegata" (5 kg) were extracted by percolation with hot 96% ethanol (4 x 20 L) till exhaustion. The combined alcoholic extract was evaporated to a syrupy solution under reduced pressure to give concentrated residue (700 ml). The last residue was successively fractionated with light petroleum (6 x 500 ml), chloroform (4 x 500 ml) then with ethyl acetate (9 x 500 ml). The previous fractions were concentrated under vacuum at 45°C to afford 46 g. (Fraction 1), 2 g (Fraction 2) and 37 g (Fraction 3), respectively.

'Fraction 1' (Pet. ether fraction) was subjected to repeated chromatographic techniques (25 g) on cc (750 g silica gel 60, 110 x 6 cm) eluted with light petroleum, methylene chloride and finally methanol to afford 'compounds 1' (27 mg), 2 (42 mg) , 3 (55 mg) , 4 (403 mg), 5 (87 mg) and 6 (75 mg). The EtOAc 'Fraction 3' (19 g) was applied to a silica gel column (600 g, 110 x 6 cm) , eluted with benzene and increasing polarity with ethyl acetate to afford 'compounds 7' (13 mg) , 8 (95 mg) , 9 (200 mg) and 10 (70 mg), respectively. Finally, fractions eluted with 60 to 75% ethyl acetate in benzene afforded 'compound 11' (220 mg); while 'compound 12' (272 mg) was obtained from fractions eluted with 80 to 85% upon repeated crystallization from MeOH.

Compound (1): white needles (methanol), m.p. 126-128°C, R_f 0.95 (system III); IR (KBr, υ_{max} cm^{-1}): 2922, 2853, 1735, 1631, 1461, 1278. EIMS m/z (% relative abundance): 456 (M^+,1) for $C_{31}H_{52}O_2$, 414 [M^+- (CH_3-CO) +H , 22], 396 (M^+-CH_3COOH, 10). Selected ^{1}H-NMR (300 MHz): δ_H 2.28(3H, s, $COCH_3$), 4.6 (1H, m, H-3) and 5.37 (1H, br,s, H-6).

Compound (2): white needles (methanol), m.p. 241°C, R_f 0.87 (system III); IR (KBr, υ_{max} cm^{-1}): 2923, 2857, 1731, 1458, 1372, 1260, 1023. EIMS m/z (% relative abundance): 468 (M^+, 3) for $C_{32}H_{52}O_2$, 453 (2), 257 (4), 218 (20), 203 (20), 189 (8). Selected ^{1}H-NMR (300 MHz): δ_H 2.3 (3H, s, CH_3CO), 4.05(1H, dd, *J* = 10, 4.5 Hz, H-3) and 5.38(1H, m, H-12).

Compound (3): white needles (chloroform-methanol), m.p. 249 to 251°C, R_f 0.72 (system III); IR (KBr, υ_{max} cm^{-1}): 2938, 2861, 1707, 1642, 1456, 1382, 1138. EIMS m/z (% relative abundance): 424(M^+, 95) for $C_{30}H_{48}O$, 409 (M^+ - CH_3, 34), 381 (M^+ - C_3 H_7, 9), 218 (74), 205 (100), 189 (45). Selected ^{1}H-NMR (300 MHz): δ_H 4.5 and 4.7 (2H, H-29, for CH2=).

Compound (4): white plates (chloroform-methanol), m.p. 290 to 292°C, R_f 0.48 (system III); IR (KBr, υ_{max} cm^{-1}): 3474, 2931, 2862, 1454, 1382, 1121, 1082. EIMS m/z (% relative abundance): 428 (M^+, 9) for $C_{30}H_{52}O$, 413 (12), 395 (6), 304 (8), 303 (24), 276 (22), 275 (52), 245 (28), 205 (67), 153 (16), 96 (100). Selected ^{1}H-NMR (300 MHz): δ_H 2.22 (1H, q, H-4) and 3.75 (1H, m, H-3).

Compound (5): white needles (chloroform-methanol), m.p. 195-196°C, R_f 0.34 (system III); IR (KBr, υ_{max} cm^{-1}): 3423, 2921, 2851, 1641, 1465, 1397, 1280 and 1027 cm^{-1}. EIMS m/z (% relative abundance): 426 (M^+, 7) for $C_{30}H_{50}O$, 408 (M^+-H_2O, 7), 218 (cleavage of C-12/C-13, 37), 203 (subsequent loss of CH_3, 18), 189 (8). Selected ^{1}H-NMR (300 MHz): δ_H 3.60 (1H, dd, *J* = 6.6, 6.6 Hz, H-3) and 5.30 (1H, m, H-12).

Compound (6): white needles (methanol), m.p.139 to 140.5°C, R_f 0.17 (system III); IR (KBr, υ_{max} cm^{-1}): 3432, 2934, 2862, 1644, 1460, 1375, 1229, 1060 cm^{-1}. EIMS m/z (% relative abundance): 414 (M^+,14) for $C_{29}H_{50}O$, 399 (M^+ - CH_3, 5) ,396 (M^+ - H_2O,15), 381 (M^+ - H_2O- CH_3, 6), 329 (M^+ - ring A, 6), 303 (M^+ - ring A –CH_2 ,6), 273 (M^+ - side chain, 5), 255 (396 - side chain, 7), 231 (M^+ - C_{13} H_{27}, 4), 213 (7). Selected ^{1}H-NMR (300 MHz): δ_H 3.54 (1H, m, H-3) and 5.35 (1H, t, H-6).

Compound (7): white crystals (methanol), m.p.190-193°C, R_f 0.84 (system V). UV (Table 1). EIMS m/z (% relative abundance): 396 (M^+, 10%) for $C_{21}H_{16}O_8$, 395 (0.5) , 394 (0.5), 393 (0.5) , 381 (M^+ - 15, 0.5), 368 (M^+ -28 , 20) , 340 (M^+ - 2 X28, 20) ,312 (M^+ - 3 X28, 5),, 262 (35) ,236 (22) , 185 (18) , 137 (16) , 129 (35), 124 (21), 94 (58), 83 (84 0, 55 (100). The ^{1}H-NMR (600 MHz): δ_H 7.87 (4H, d, *J* = 8.4 Hz, H-2', 6', 2'', 6''), 7.55 (1H, d, *J* = 8.4 Hz, H-6), 6.81 (5 H, d, *J* = 8.4 Hz, H-3', 5', 3'' 5'' and H-5), 3.9 (3H, s, -OCH_3). The ^{13}C-NMR (150 MHz): δ_C 124.35 (C-1), 152 (C-2), 125 (C-3), 148 (C-4),

114 (C-5), 116 (C-6), 163 (C = O), 133 (C 1',1" , 128 (C-2',2"), 115 (C-3',3"), 152 (C-4', 4"), 114 (C-5',5"), 130 (C-6', 6") and 69.3 (OCH_3).

Compound (8): yellow amorphous powder (methanol), mp >300°C, R_f 0.77 (system V). EIMS m/z (% relative abundance): 286(M^+, 100%) for $C_{15}H_{10}O_6$, 258 (M^+- CO, 39), 153 (23), 152 (5), 137 (3), 134 (11), 124 (8), 123 (4). ^{1}H-NMR (300 MHz): δ_H 7.37 (1H, d, *J* = 8.4 Hz, H-6'), 7.36(1H, s, H-2'), 6.89 (1H, d, *J* = 8.4 Hz, H-5'), 6.44 (1H, s, H-8), 6.43 (1H, s, H-3) and 6.20 (1H, s, H-6).

Compound (9): white needles (methanol), m.p. 225°C, R_f 0.63 (system V). The UV (Table 1). EIMS m/z (% relative abundance): 274 (M^+, 21) for $C_{15}H_{14}O$, 256 (M^+-18, 4), 167 (8), 139 (100), 136 (47), 107 (44), 77 (18), 69 (19). The ^{1}H-NMR (300 MHz): δ_H 2.44 (lH, dd, *J* = 15 and 3.3 Hz, H-4 eq.), 2.67 (lH, dd, *J* = 15 and 4.2 Hz, H-4ax.), 4.02 (lH, m, H-3), 4.80 (lH, s, OH), 4.67 (lH, d, *J* = 4.5 Hz, H-2), 5.72 (l,H, d, *J* = 2.1 Hz, H-6), 5.88 (lH, d, *J* = 2.1 Hz, H-8), 6.72 (2H, d, *J* = 8.1 Hz, H-3', H-5') , 7.23 (2H, d, *J* = 8.4 Hz, H-2', H-6'), 8.92 (lH, s, OH), 9.13 (lH, s, OH), 9.30 (lH, s, OH). The ^{13}C-NMR (75 MHz): δ_C 28.23 (C-4), 64.86 (C-3), 78.06 (C-2), 94.18 (C-8), 95.2 (C-6), 98.51 (C-10), 114.47 (C-3' , 5'), 128.28 (C-2', 6'), 130.02 (C-1'), 155.77 (C-4'), 155.77 (C-9), 156.25 (C-5) and 156.53 (C-7).

Compound (10): buff needles (methanol), mp 175-177°C, R_f 0.50 (system V). The UV (Table 1). EIMS m/z (% relative abundance): 290 (M^+, 2) for $C_{15}H_{14}O_6$, 275 (1.5), 274 (1), 256 (9), 255 (1.5), 228 (6), 185 (8), 167 (4), 152 (4), 151 (7), 149 (20), 140 (6), 139 (5), 138 (8), 123 (19), 111 (33), 110 (13), 107 (67), 57 (100), 55 (87). The ^{1}H-NMR (600 MHz): δ_H 2.53 (lH, dd, *J* = 16, 8.5 Hz, H-4eq.), 2.87 (lH, dd, *J* = 16 and 5.5 Hz, H-4ax.), 3.67 (1H, brs, 3-OH), 4.00 (lH, ddd, *J* = 8.5, 7.8, 5.5 Hz, H-3), 4.58 (lH, d, *J*=7.2 Hz, H-2), 5.86 (lH, d, *J* = 2.4 Hz, H-6), 5.94 (lH, d, *J* = 2.4Hz, H-8), 6.73 (1H, dd, *J* = 8, 1.2 Hz, H-6'), 6.76 (1H, d, *J* = 8 Hz, H-5'), 6.85 (lH, d,*J* = 1.2 Hz, H-2') and 8.05 (2H, s, OH). The ^{13}C-NMR (150 MHz, CD_3OD, δ ppm): δ_C 28.67 (C-4), 68.97 (C-3), 83.00 (C-2), 95.65 (C-6), 96.44 (C-8), 100.96 (C-10), 115.41 (C-2'), 116.23 (C-5'), 120.19 (C-6'), 132.38 (C-1'), 146.37 (C-3'), 146.39 (C-4'), 157.07 (C-7), 157.73 (C-5) and 158.00 (C-9).

Compound (11): whitish-buff amorphous powder (methanol), m.p.235°C, R_f 0.26 (system V). The UV (Table 1). EIMS m/z (% relative abundance): 546 (1%), 274 (28), 256 (10), 185 (5), 149(6), 152 (6), 151 (4), 139 (93), 138 (10), 123 (15), 107 (100), 55 (20). The ^{1}H- and ^{13}C-NMR (Table 2).

Compound (12): yellow granules (methanol), m.p.203-204°C, R_f = 0.12 (system V). UV (Table 1). EIMS m/z (% relative abundance): 432 (M^+,1), 414 (M^+ - H_2O, 84), 396 (M^+ - 2 H_2O, 21), 378 (M^+ - 3H_2O, 37), 366 (4), 336(11), 313 (3), 312(12), 283(100), 270 (M^+ - C-glucosyl + H , 62), 165 (56), 151(1), 118(16), 121 (14). The ^{1}H-NMR (300 MHz): δ_H 4.71-3.50 (5 H, m, glu-H-2''- 6''), 4.95 (1 H, d, *J* = 9.6 Hz, glu-H-1''), 6.26 (1 H, s, H-6), 6.77 (1 H, s, H-3), 6.91 (2 H, d, *J* = 8.4 Hz ,H-3' and 5') 8.03 (2 H, d, *J* = 8.4 Hz, H-2' and 6'), 13.15 (1H, s, 5-OH). The ^{13}C-NMR (75 MHz): δC 163.88 (C-2), 102.38 (C-3), 181.99 (C-4), 156 (C-5), 98.12 (C-6), 162.61 (C-7), 104.54 (C-8), 160.34 (C-9), 104.54 (C-10), 121.54 (C-1'), 128.88 (C-2', 6'), 115.78 (C-3', 5'), 161.06 (C-4'), 73.32 (C-1''), 70.83 (C-2''), 78.60 (C-3''), 70.52 (C-4''), 81.73 (C-5''), 61.25 (C-6'') .

BIOLOGICAL EVALUATION

Anticancer activity

Cytotoxicity of ethyl acetate extract was measured against three human tumor cell lines: Human hepatocarcinoma cell line (Hep-G 2) colon carcinoma cells (HCT-116) and lymphoblastic leukemia cells (1301), while the unsaponifiable matter was tested only against Hep-G2 cell line using MTT cell viability assay. The percentage viability was plotted against the extract concentrations and the 50% cell viability (IC_{50}) was calculated from the curve (Hensen et al., 1989; Gomez et al., 2001). The results are presented in Figure 2.

Antioxidant activity

Using DPPH method, 0.15 ml of the sample and 0.15 ml of DPPH alcoholic solution (0.1 mM) were mixed and incubated in a test plate at 37°C for 10 min. The absorbance of the remaining DPPH was measured at 520 nm using a micro titer plate reader. Its antioxadative activity (%) was calculated in comparison to the blank control (ascorbic acid) (Ratty et al., 1988). For each sample, the radicle scavenging activity was calculated from the equation:

DPPH Inhibition (%) = [$DPPH_{blank}$ – $DPPH_{test.}$] x100 / [$DPPH_{blank}$]

A curve for sample concentration versus DPPH % inhibition was plotted and the half maximal scavenging capacity (SC_{50}) of each tested sample and ascorbic acid were calculated. The results are recorded in Figure 3.

Antidiabetic activity

Twenty-four adult male rats were divided into four groups (n = 6). The first group receiving gum acacia mucilage (10%) and left untreated (control group). The second is diabetic group and received only the vehicle (10% gum acacia). The third and fourth diabetic groups received orally the total extract (400 mg/kg) and glibenclamide (150 mg/kg), respectively by gavage for 5 days once a day (Sokeng et al., 2005). Diabetes was induced in rats by intraperitoneal injection of streptosotozin (STZ) in a single dose of 75 mg/ kg. Rats became diabetic after 5 days of injecting STZ where their blood glucose levels range from 254 to 288 mg/dl. Blood glucose levels were determined using glucomen-glyco® blood glucose meter, 24 sensor strips. The results are recorded in Figure 4.

Hepatoprotective activity

Elevated liver enzyme levels were induced in adult male rats by CCl_4. Liver enzymes (ALT) and proteins (albumin) were measured in the collected plasma (Zhao et al., 2005). The results are recorded in Figures 5a and b.

Anti-inflammatory activity

The anti-inflammatory effect was expressed as the percentage inhibition compared with vehicle –treated animals with respect to a reference group treated with indomethacin (10 mg/ kg) (Winter et al., 1963). The tested extracts (400 mg/kg), were administered orally 1 h before injecting of the phlogistic agent. The results are recorded in Table 5 and Figure 6.

Antimicrobial activity

This included the measurement of anti-bacterial and antifungal activities by the diameter of inhibition zone method utilizing *Salmonella typhi, Escherichia coli* and *Bacillus* spp. as tested bacterial strains. Cefotaxime was used as control and the method

Table 2. NMR spectral data of compound 11 in CD_3OD (δ ppm).

Ring		No.	^{1}H-NMR (δ_H)	^{13}C-NMR (δ_C)
Upper unit	C	2	4.46 (1H, d, *J* = 9.8 Hz)	80.3 (C-2)
		3	4.6 (1H, dd, *J* = 9.8,7.6 Hz)	74.1 (C-3)
		4	4.37 (1H, d, *J* = 7.6 Hz)	38.64 (C-4)
	A	5	`	155.95 (C-5)
		6	5.96 (1H, d, *J* = 2.4 Hz)	96.00 (C-6)
		7		157.67 (C-7)
		8	5.92 (1H, d, *J* = 2.4 Hz)	97.77 (C-8),
		9		157.28 (C-9)
		10		101.78 (C-10)
	B	1'		132.27 (C-1')
		2'. 6'	7.42, 7.36 (1H, each d, *J* = 8.4 Hz)	130.58, 129.7 (C-2',6')
		3', 5'	6.82(2H, dd, *J* = 8.4, 2.5Hz)	116.08,115.97 (C-3',5')
		4'		158.2 (C-4')
Middle unit	F	2	4.46 (1H, d, *J*=9.6 Hz)	80.2 (C-2)
		3	4.6 (1H, dd, *J* = 9.8 , 7.6 Hz)	69.3 (C-3),
		4	4.37 (1H, d, *J* = 7.6 Hz)	38.64(C-4)
	D	5		155.83 (C-5)
		6	6.09 (1H, s)	97.07 (C-6)
		7		157.59 (C-7)
		8		109.05 (C-8)
		9		156.50 (C-9)
		10		102.90 (C-10)
	E	1'		132.34 (C-1')
		2', 6'	7.07 (2H, d, *J* = 8.4 Hz)	130.15, 129.36 (C-2' , 6')
		3', 5'	6.87 (2H, dd, *J* = 9, 2.5 Hz)	116.2, 115.80 (C-3', 5')
		4'		158.00 (C-4')
Lower unit	I	2	4.51 (1H, d, *J* = 7.8 Hz)	83.90 (C-2)
		3	4.29 (1H, ddd, *J* = 9,7.8, 5.6 Hz)	68.70 (C-3)
		4	2.5 (1H, dd, *J* = 16.8, 9 Hz, H-4ax.) 2.95 (1H, dd, *J* = 16.8, 5.6 Hz, H-4eq.)	29.73 (C-4)
	G	5		155.63 (C-5)
		6	6.12 (1H, s)	96.58 (C-6)
		7		157.42 (C-7)
		8		108.60 (C-8)
		9		156.00 (C-9)
		10		99.72 (C-10)
	H	1'		131.93 (C-1')
		2', 6'	7.23, 6.93 (1H, each d, *J* = 8.4 Hz)	130.27, 129.44(C-2' ,6')
		3', 5'	6.82 (2H, dd, *J* = 8.4, 2.5 Hz)	116.00, 115.74 (C-3',5')
		4'		157.82 (C-4')

incubation was continued at 37°C for 24 h (Wood et al., 1995). The results are listed in Table 6. For anti-fungal activity, the same was used but *Candida albicans* and *Mucor* spp. were used as tested fungi and pototo dextrose agar was used as nutritive

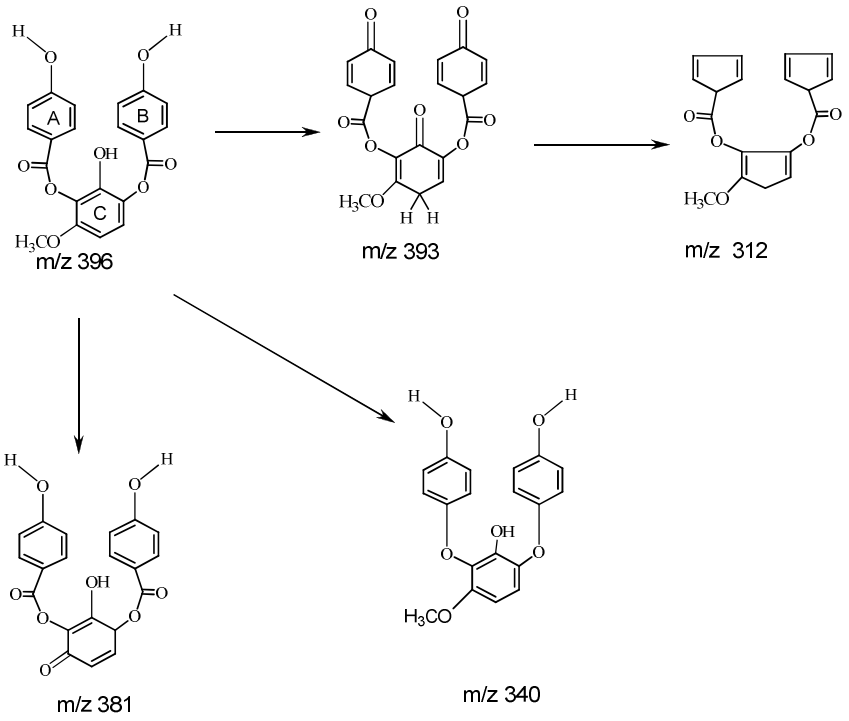

Figure 1. Mass fragmentation pattern of Retusaphenol.

medium at a dose of 100 mg of test extract. The incubation was continued at 28°C for 72 h. The results are listed in Table 6. Amphotericin B was used as control.

RESULTS AND DISCUSSION

Column chromatography of the petroleum ether extract of *F. retusa* L. "variegate" afforded compounds 1 to 6. These compounds gave positive Liebermann's and Salkowski's tests indicating their steroidal or triterpenoidal nature. The structures were determined by direct comparison of their mps, ms, ir, ^{1}H-NMR, and tlc with authentic samples as well as with available published data (Goad and Akihisa, 1997; Yamaguchi, 1970; Gedara et al., 2005) confirming their identity as β-sitosterol acetate (1), β-amyrin acetate (2), moretenone (3), friedelinol (4), β-amyrin (5) and β-sitosterol (6) (Figure 7).

Furthermore, column chromatography of the ethyl acetate extract and repeated chromatographic methods afforded two new compounds (7 and 11) and four known compounds (8 to 10 and 12). The konwn compounds were identified by analyzing their spectroscopic data (UV, MS and NMR) as well as comparison with available published data to be luteolin (8) (Darwish et al., 2002; Harborne et al., 1975; Kang et al., 2010) , (+) - afzelechin (9) (Hwang et al., 1989; Sayed et al., 1991), (+) - catechin (10) (Hye et al., 2009; Donovan et al ., 1999) and vitexin (12) (Colombo et al., 2008; Kim et al., 2005; Zhang et al., 2002). This finding represents the first isolation of these compounds from *F. retusa* L."variegata".

Retusaphenol (7): white crystals (methanol), m.p.190-193 °C, R_f 0.84 (system V). Its formula was established as $C_{21}H_{16}O_8$ by EIMS with M^+ at m/z 396 (20), followed by 395 (M^+ -H ,5), 394(M^+ - 2H ,5), 393 (M^+ - 3H, 5) The most important fragments being associated with loss of methyl group at m/z 381 (5) and successive losses of three 28 mass units at m/z 368 (20), 340 (20) and 312 (5), respectively indicating the presence of three phenolic moieties and confirmed by the fragment at m/z 94(30%) for the phenolic ring indicating the presence of 4 hydroxybenzoate (Yamaguchi, 1970) or to the losses of two carbonyl groups (intense δ_C 163) from ring C and confirmed by fragment at m/z 340 (20) (Figure 1). The attachment of the two hydroxy benzoates to the ring C was determined through the ^{1}H-NMR data, δ 7.55 , 6.81 (each 1 H, d, *J* = 8.4 Hz) for two ortho coupled aromatic H-6 and H-5 ,respectively and δ 3.9 (1 H,s) for one methoxyl group. ^{1}H-NMR also showed two pairs of doublets at δ 6.81, 7.87 (each 4 H, *J* = 8.4 Hz) for H-3', 5', 3", 5" and H-2', 6',2", 6" ,respectively for bis hydroxybenzoate moieties . ^{13}C-NMR spectrum established that 7 contained one methoxyl , ten olefinic methines and ten quaternary carbons, including two carbonyl and six oxygenated olefinic carbons. Finally the structure of compound 7 was proven to be 1,3- bis(4-hydroxybenzoate) - 2-hydroxy-4-methoxy-benzene from its ^{1}H-and^{13}C-NMR data. To our knowledge, this represents the first report for isolation of this compound from the plant and genus *Ficus.*

Compound (9): white needles (methanol), mp 225°C, R_f

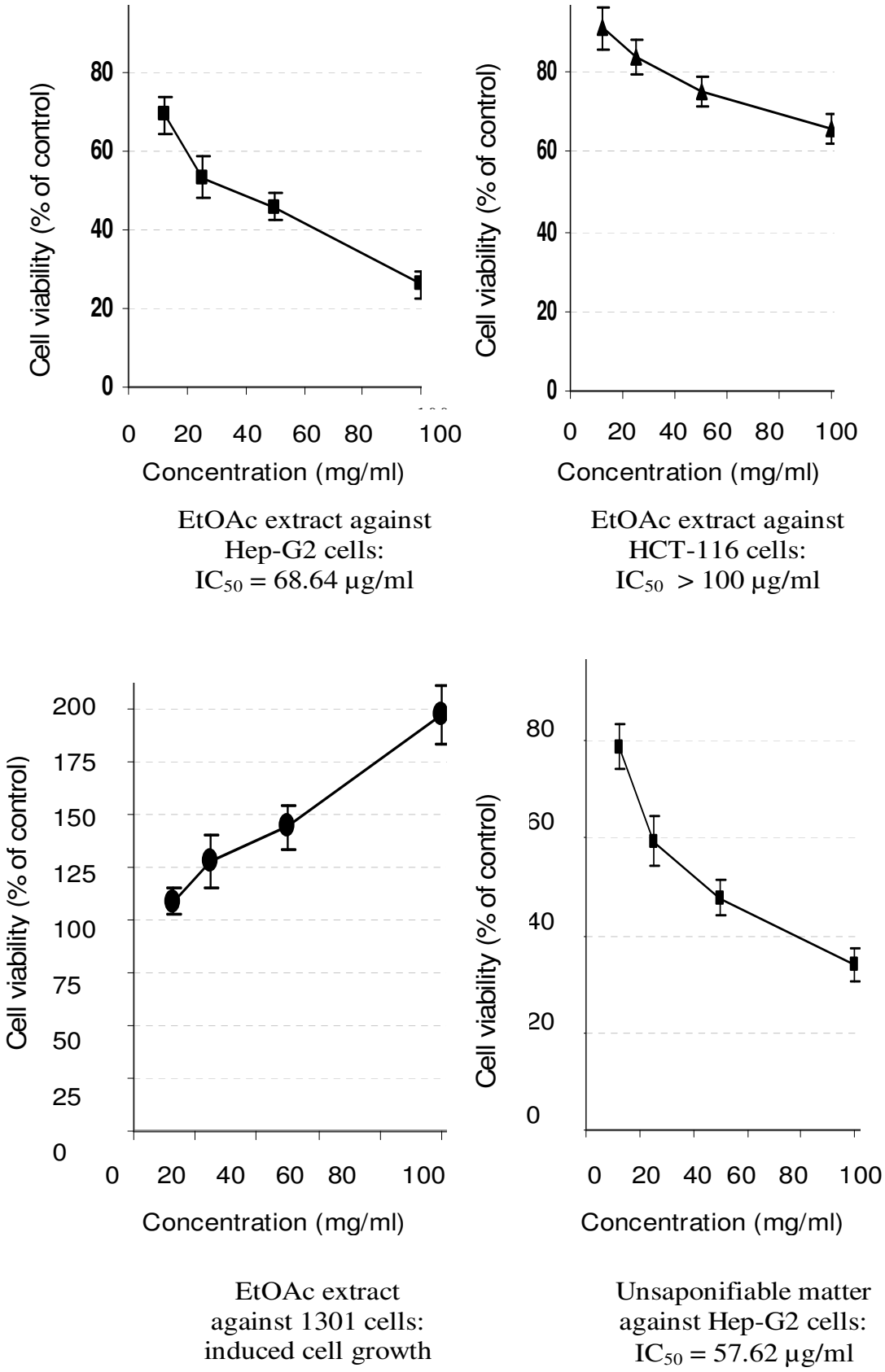

Figure 2. Anti-tumor activity of ethyl acetate extract and unsaponifiable matter of *Ficus retusa* L."variegata" against different tumor cell lines.

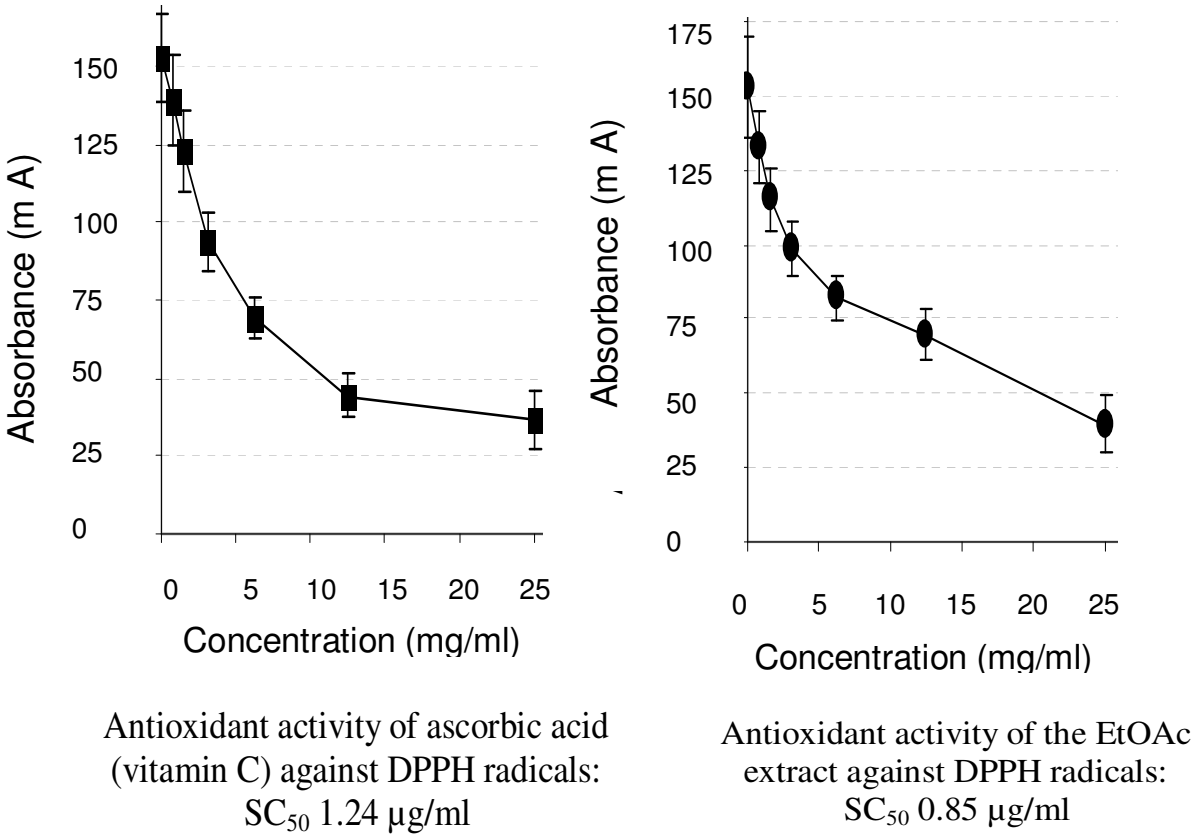

Figure 3. Anti-oxidant activity of ethyl acetate extract of *Ficus retusa* L."variegata".

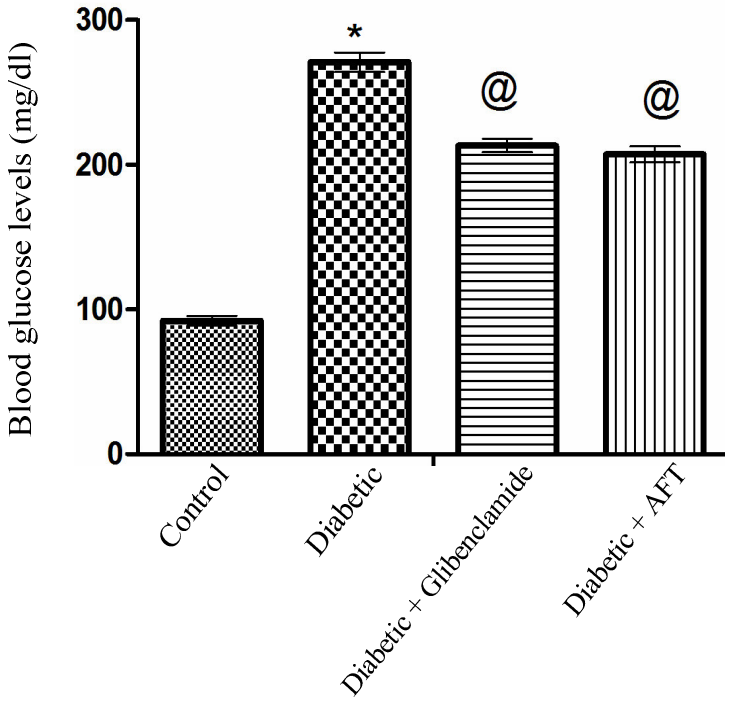

Figure 4. The effect of total extract (AFT extract) of *Ficus retusa* L."variegata" on blood glucose level.

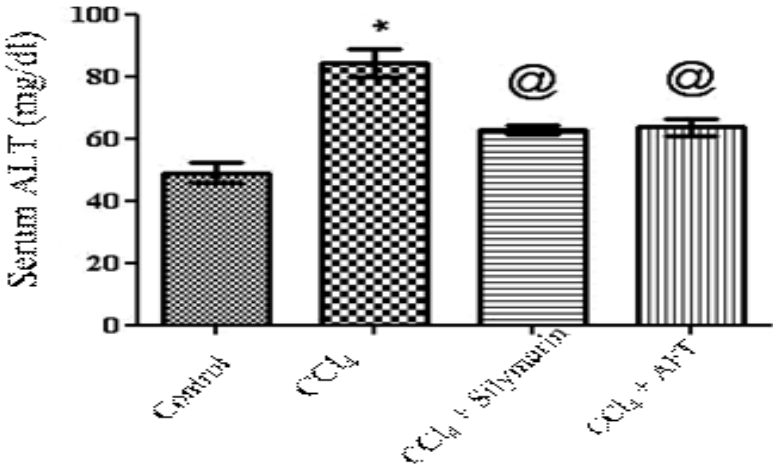

Figure 5a. The effect of total extract (AFT extract) of *Ficus retusa* L."variegata" on serum ALT level.

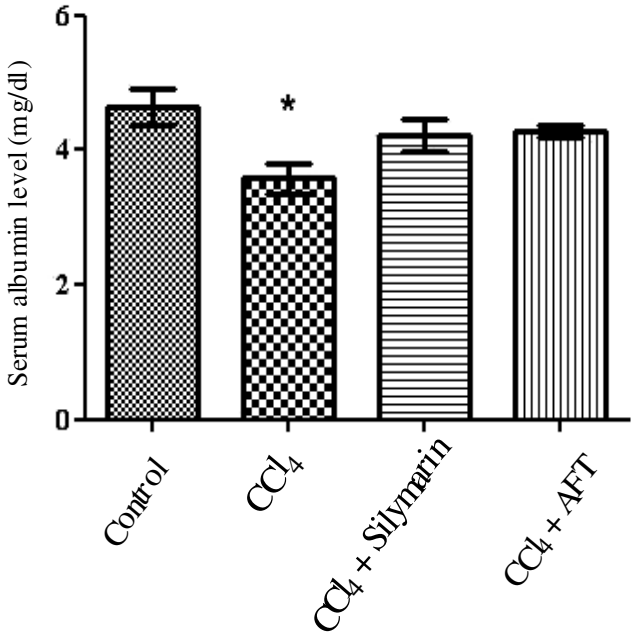

Figure 5b. The effect of total extract (AFT extract) of *Ficus retusa* L."variegata" on serum albumin level.

0.63 (system V). Its UV spectrum in MeOH showed only one absorption band at 275 nm which was not affected by the shifting agents indicating a flavan derivative (Hwang et al., 1989; Sayed et al., 1991). The ^{1}H-NMR spectrum exhibited two pairs of doublets at δ 6.72 and 7.23, two protons each (J = 8.1 Hz) characteristic for para substituted benzene ring system .The chemical shifts and the coupling constants of H-6 and H-8 indicated that position 5 and 7 were dihydroxylated, in addition the chemical shifts and the coupling constants of H-2, H-3 and H-4 evidenced the orientation of the 3-hydroxyl group [4.80 (lH, s, OH)] (Silverstein et al ., 1974). The ^{13}C-NMR spectrum verified the previous conclusions which showed three sp^3 carbons (C-2 - C-4) and twelve sp^2 carbons (C-5 -C-10 and C-1'– C-6') (Silverstein et al., 1974). The mass spectrum of the investigated compound showed a molecular ion peak at m/z 274 and base peak at m/z 139 suggesting a molecular formula $C_{15}H_{14}O_5$ and confirmed the hydroxyl substitution at C_5 and C_7 in addition to characteristic and significant peaks at m/z 136 and 107 (Darwish et al., 2002). From the previously mentioned data, the structure of compound 9 was identified as (+)

afzelechin. This was confirmed by direct comparison of (MS, ^{1}H-NMR, ^{13}C-NMR) with the available published data (Silverstein et al., 1974; Hwang et al., 1989; Sayed et al., 1991).

Retusa afzelechin trimer (11): Whitish-buff amorphous powder (methanol), m.p.235°C, R_f 0.26 (system V). It showed UV spectrum similar to that of compound **9** indicating presence of a flavan-3-ol skeleton. Having unambiguous structure for compound 9 greatly expected structural solution of compound 11 relative to compound 9. ^{13}C-NMR spectrum of compound 11 (Table 2) showed trible the number of flavonoid resonances of compound 9. These included the readily identifiable carbons of afzelechin/ afzelechin/ afzelechin trimer. The ^{1}H-NMR spectrum (Table 2) showed signals at δ 6.82 (4 H, d, *J*=8.4 Hz, H-3',5' u and L), 6.87 (2H,dd, *J*= 9 ,2.5 Hz, H-3',5' m), , 7.36, 7.42 (1H each, d, *J* = 8.4 Hz, H-2',6' u), 7.07 (2H, d, *J* = 8.4 Hz, H-2', 6' m), 7.23, 6.93 (1H each, d, *J* = 8.4 Hz, H-2', 6' L) suggested a flavanol ring B in accordance with a 4' -hydroxylation in a trimer form, while the chemical shifts and the coupling constants of H-6 [δ 5.96 (1H, d, *J* = 2.4 Hz, H-6 u), 6.09 (1H, s, H-6 m), 6.12 (1H, s, H-6 L)], H-8 [δ 5.92 (1H, d, *J* = 2.4 Hz, H-8 u)] and H-4 [2.5 (1H, dd, *J* = 16.8 , 9 Hz, H-4ax. L), 2.95 (1H, dd, *J* = 16.8, 5.6 Hz, H-4 eq. L), 4.37 (2H, d, *J* = 7.6 Hz, H-4 u, m)] indicated that position 5 and 7 were dihydroxylated. The ^{1}H-NMR spectrum also permitted determination of the interflavonoid bond position, the lower unit C-8 attached with middle unit C-4. In addition C-8 of the middle unit was attached to C-4 of the upper unit. Thus establishing the linkage as C-4→C-8/ C-4→C-8. However, we found an important ^{13}C signal at δ 38.64 which indicated a linked C-4 (Lauro et al., 2008). ^{13}C-NMR spectrum verified the above conclusions which showed nine sp^3 and thirty-six sp^2 carbons. The ^{13}C-NMR shifts of 80.30, 80.20 and 83.90 ppm, for the position 2 carbons of the upper, middle and lower flavonoid subunits, respectively as well as 7.8-9 Hz doublets for the attached protons, indicated 2,3 trans stereochemistry for the three subunits. The EIMS displayed ion peaks at m/z 546 (1) for afzelechin dimer, 274 (28) for afzelechin and 256 (10) for afzelechin $–H_2O$, confirming that the compound has an afzelechin nucleus Compound 11 was identified as (+) Afzelechin-(4α→8) -afzelechin-(4α→8)-afzelechin. This was confirmed by direct comparison of (UV, ^{1}H-and ^{13}C-NMR) with the available published data (Darwish et al., 2002; Zhang et al., 2003). However, this represents the first isolation of this compound from the plant and from genus *Ficus*.

Biological evaluation

Anticancer activity

Regarding the anti-tumor activity using cell lines Figure 2, the ethyl acetate fraction showed a moderate anti-proliferative activity against hepatoma cells (Hep-G2 cells) with IC_{50} value 68.64 µg/ml, low lymphoproliferative activity against colon carcinoma (HCT-116) cells with IC_{50} > 100 µg/ml and a significant lymphostimulatory activity towards lymphocytes which might be promising as immunostimulant agent in the immunosuppressing diseases. On the other hand, the unsaponifiable matter showed a moderate cytotoxic activity against (Hep-G2 cells) with IC_{50} value 57.62 µg/ml.

Antioxidant activity

Considering the antioxidant activity Figure 3, the ethyl acetate fraction possessed a strong antioxidant activity with SC_{50} 0.85 µg/ml compared to ascorbic acid solution (SC_{50} 1.24 µg/ml).

Antidiabetic activity

As shown in Table 3 and Figure 4, the results indicated that the total alcoholic extract of plant (400 mg/kg) and glibenclamide (150 mg/kg), reduced blood glucose levels of the diabetic rats significantly as compared to the diabetic group.

Hepatoprotective activity

As shown in Table 4 and Figures 5a and b, results indicated that the total extract of the plant (400 mg/kg) and standard silymarin (150 mg/kg) significantly reduced the ALT enzyme compared to the CCl_4 - treated group. The total extract and standard silymarin also decrease serum albumin levels but the reduction is not significant when compared with the CCl_4- treated group.

Anti-inflammatory activity

The results recorded in Table 5 and Figure 6, indicated that the petroleum ether fraction and unsaponifiable matter in a dose (400 mg/kg) were more potent than indomethacin (10 mg/kg) as anti-inflammatory drugs.

Antimicrobial activity

As shown in Table 6, the four extracts showed mild antimicrobial activity against *Candida albicans, Mucor* spp. *Salmonella typhi*, *Escherichia coli* and *Bacillus* spp.

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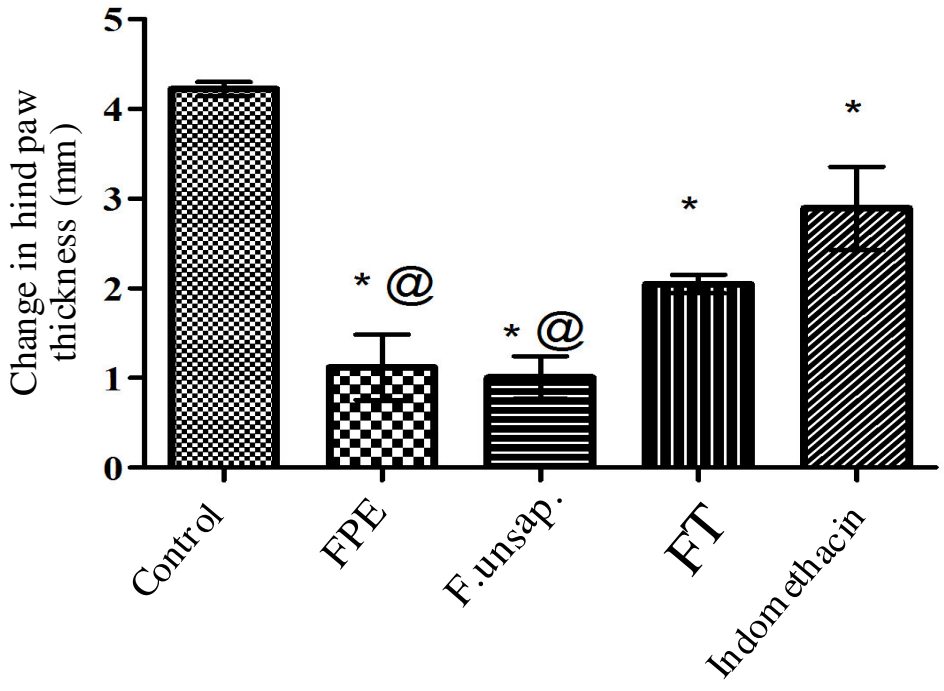

Figure 6. The anti-inflammatory effect of total extract (FT), petroleum ether fraction (FPE) and unsaponifiable matter (F.unsap) of *Ficus retusa* L."variegata" on baker's yeast-induced paw.

Table 3. Effect of total extract (400 mg/kg) of *Ficus retusa* L. "variegata" and glibenclamide (150 mg/kg) on blood glucose levels of STZ-induced diabetic rats.

Treatment	Blood glucose level (mg/dl) (Mean±SEM)	Relative potency
Control	92.17 ± 3.5	-
Diabetic	270.9 ± 6.7 *	-
Diabetic + Glibenclamide	213.2 ± 4.6 @	1
Diabetic + total extract	207.1 ± 5.6 @	1.10

* Significantly different from control group at P < 0.05, @ Significantly different from diabetic group at P < 0.05.

Table 4. Effect of total extract of *Ficus retusa* L."variegata"(400 mg/kg) on liver enzymes (ALT enzyme) and serum proteins (albumin).

Treatment	Serum ALT levels (Mean±SEM)	Serum albumin levels (Mean±SEM)
Normal rats	49 ± 3.9	4.6 ± 0.3
Control cirrhotic liver rats (CCl_4)	84.3 ± 4.3*	3.6 ± 0.2*
CCl_4 + Silymarin standard	63 ± 1.5@	4.2 ± 0.24
CCl_4 +Total extract	63.7 ± 2.6@	4.3 ± 0.1

* Significantly different from control group at P < 0.05, @ Significantly different from CCl_4 group at P < 0.05.

Table 5. The anti-inflammatory effect of total extract, petroleum ether fraction and unsaponifiable matter of *Ficus retusa* L."variegata" on baker's yeast-induced paw edema in rats.

Group paw	Dose (mg/kg)	Increase in paw volume in ml (Mean±SEM)	% Inhibition of edema
Control	-	4.224±0.078	-
Unsaponifiable matter	400	1.006±0.23*@	76.2
Pet.ether fraction	400	1.12±0.36*@	73.5
Total extract	400	2.05±0.102*	51.5
Indomethacin	10	2.89±0.46*	31.5

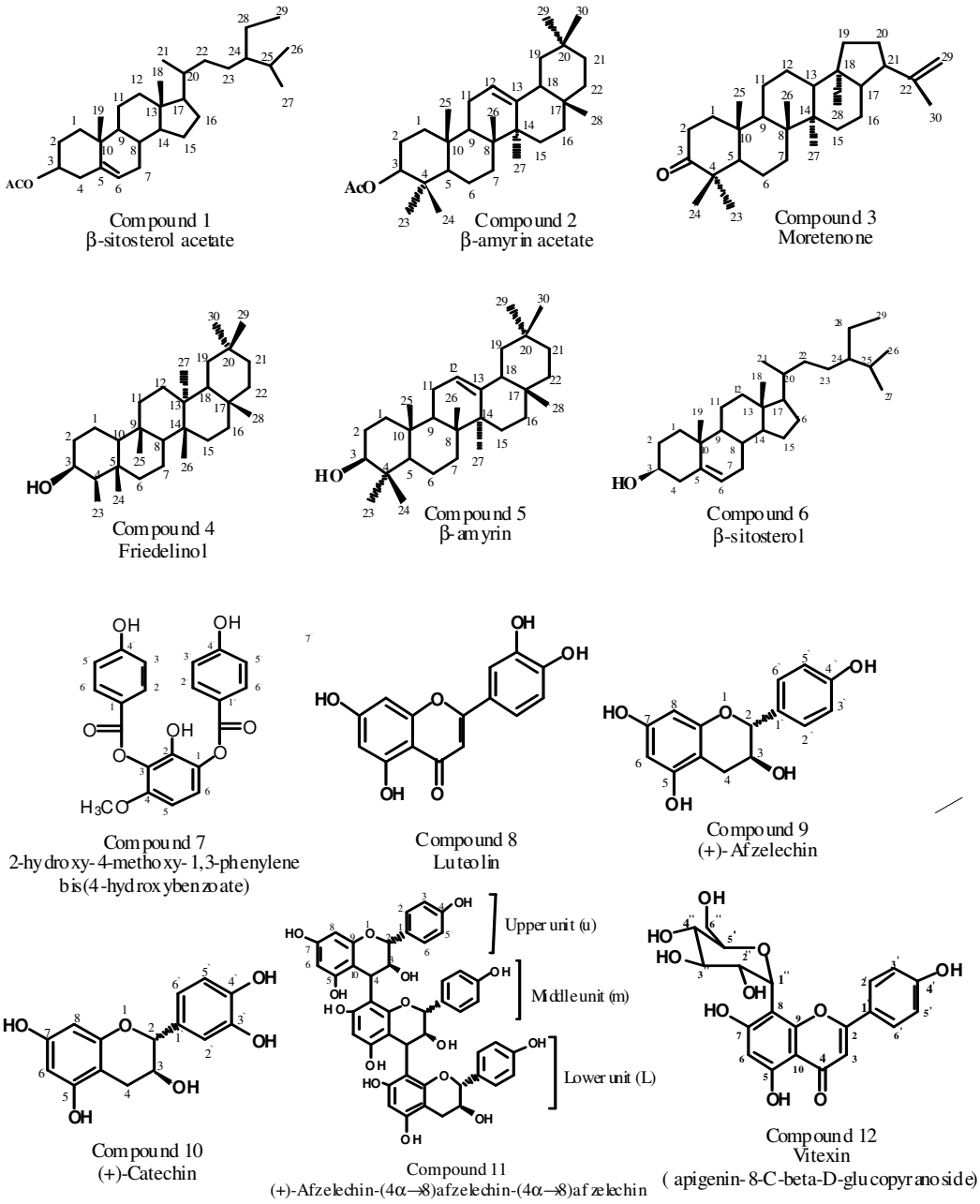

Figure 7. Compounds isolated from *Ficus retusa L."variegata".*

Table 6. Results of antimicrobial activity of different extracts of *Ficus retusa* L."variegata".

Bacteria sample	Diameter of inhibition zone (mm)				
	Fungi		Gram –ve bacteria		Gram +ve
	Candida albicans	*Mucor* spp	*Salmonella typhi*	*Escherichia coli*	*Bacillus* spp
Cefotaxime	--	--	40	40	38
Amphotericin B	22	22	--	--	--
Light pet. fraction	13	12	13	14	14
Chloroform fraction	18	12	14	16	14
Ethyl acetate fraction	13	12	15	14	21
Total ethanolic extract	17	15	17	16	16

REFERENCES

Abraham LCN, Masakuni T, Isao H, Hajime T (2008). Antioxidant flavonoid glycosides from the leaves of *Ficus pumila* L. Food Chem., 109: 415-420.

Backheet EY, Ahmed AS, Sayed HM (2001). Phytochemical study of the constituents of the leaves of *Ficus infectoria* Roxb. Bull.Pharm.Sci., Assiut Univ., 24(1) : 21-27.

Chiang YM, Chang JY, Kuo CC, Chang CY, Kuo YH (2005). Cytotoxic triterpenes from the aerial roots of *Ficus microcarpa* L.f. Phytochemistry, 66(4): 495-501.

Colombo R, Yariwake JH, Mc Cullagh M (2008). Study of C-and O-glycosylflavones in Sugarcane extracts using liquid chromatography - Exact Mass Measurement Mass Spectrometry. J. Braz. Chem. Soc., 19(3): 483-490.

Darwish Faten MM (2002). Phytochemical study of *Ficus bengalensis* L. Bull. Fac. Pharm. (Cairo Univ.), 40(2): 249-258.

Donovan JL , Luthria DL , Stremple P, Waterhouse AL (1999). Analysis of (+)-catechin, (-)-epicatechin and their 3' - and 4'-O- methylated analogs, a comparison of sensitive methods. J. Chromatogr. B., 726: 277-283 .

El-Domiaty MM, Abdel Aal MM, Abou-Hashem MM, Abd Alla RH (2003). A Pharmacognostical study of *Ficus elastica* Roxb. var.decora (Family Moraceae) cultivated in Egypt . MSc. Thesis, Zagazig, Egypt.

Gedara SR, Zaghloul MG (2005). The constituents of the leaves of *Ficus benjamina* L. Zagazig J. Pharm. Sci., 14(1): 43-47.

Goad LJ, Akihisa T (1997). Analysis of Sterols. Blackie Academic & Professional, an imprint of Chapman & Hall, London, New York, Tokyo, Melbourne, Madras, 1st Ed.

Gomez MA, Garcia MD, Saenz MT , Ahumada MC , Aznar J (2001). Cytotoxic activity of *Achillea ageratum* against cultures Hep-2 and Mc Cog cells. Pharm. Biol., 39: 79-81.

Hensen MB, Nielsen SE, Berg K (1989). Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. J. Immunol. Methods**,** 119: 203-210.

Hansson A, Zelada JC, Noriega HP (2005). Reevaluation of risks with the use of *Ficus insipida* Willd. latex as a traditional anthelmintic remedy in the Amazon. J. Ethnopharmacol**.,** 98: 251-257.

Harborne JB, Mabry TJ, Mabry H (1975). The Flavonoids. Chapman and Hall Ltd., London, pp. 49, 118-119, 516-517.

Hwang T, Kashiwada Y, Nonaka G , Nishioka I (1989). Flavan-3- ol and proanthocyanidin allosides from *Davallia divaricata*. Phytochemistry, 28(3): 891-896.

Hye MA, Taher MA , Ali MY , Ali MU , Zaman S (2009). Isolation of (+)-catechin from *Acacia catechu* (Cutch tree) by a convenient method. J. Sci. Res., 1(2): 300-305.

Jeong S, Jun K, Kang T, Ko E, Kim Y (1999). Flavonoids from the fruits of *Opuntia Ficus-indica* var. saboten. Korean J. Pharm., 30: 84-86.

Kim J Hwa , Lee BC, Kim JHu , Sim GS , Lee DH , Lee KE, Yun YP, Pyo HB (2005). The isolation and antioxidative effects of vitexin from *Acer palmatum* . Arch. Pharm. Res., 28(2): 195-202.

Kang J, Li Z, Wu T, Jensen GS, Schauss AG, Wu X (2010). Antioxidant capacities of flavonoid compounds isolated from *Acai* pulp (*Euterpe oleracea* Mart.). Food Chem., 3: 20.

Lauro M deS, Thales RC, Marcello I, Philip A JG, Guilherme LS (2008). HPLC/ESI-MS and NMR analysis of flavonoids and tannins in bioactive extract from leaves of *Maytenus ilicifolia* . J. Pharmaceut. Biomed. Anal. , 47: 59–67.

Maizatul HO, William M, Alan C (2011). Identification of proanthocyanidin dimer and trimers, flavone C-glycosides, and antioxidants in *Ficus deltoidea*, a Malaysian herbal tea. J. Agric. Food Chem., 59: 1363-1369.

Mandal SC, Maity TK, Das J, Saha BP, Pal M (1998). *Ficus racemosa* L. affords antihepatotoxic activity against paracetamol-induced acute liver damage in rats. Nat. Prod. Sci., 4(3): 174-179.

Nguyen VT, Nguyen VH, Le MH, Le TX (2002) . Anti-malarial principles of *Ficus fistulosa* Reinw ex Blume. Tap Chi Hoa Hoc., 40(4): 75-78.

Rao CV, Verma AR, Vijaykumar M, Rastogi S (2008). Gastroprotective effect of standardized extract of *Ficus glomerata* Roxb. fruit on experimental gastric ulcers in rats. J. Ethnopharmacol., 115: 323-326.

Ratty AK, Sunamoto J, Das NP (1988). Interaction of flavonoids with 1,1-diphenyl-2-picrylhydrazyl free radical, liposomal membranes and soybean lipoxygenase-1. Biochem. Pharmacol., 37(6): 989-995.

Sayed HM, Backheet EY (1991). Study on the constituents of *Ficus nitida* (Thumb). Bull. Fac. Pharm. (Cairo Univ.), 29(2): 99-102.

Sharma OP (1993). Plant Taxonomy. Tata MC Graw-Hill Publishing Company Ltd., New Delhi, p. 386.

Silverstein RN , Bassler GC, Mcrill (1974). Spectrophotometric Identification of Organic Compounds. John Wiley and Sons, Inc., N.Y.,L Sydney, IIIrd Ed.

Singh RK , Mehta S , Jaiswal D, Rai PK , Watal G (2009). Anti-diabetic effect of *Ficus bengalensis* L. aerial roots in experimental animals. J. Ethnopharmacol., 123: 110-114.

Sokeng SD, Rokeya B , Mostafa M , Nahar N , Mosihuzzaman M , Ali L, Kamtchouing P (2005). Antihyperglycemic effect of *Bridelia ndellensis* ethanol extract and fractions in streptozotocin-induced diabetic rats. Afr. J. Trad. CAM., 2(2): 94–102.

Winter CA, Risle EA, Nuss GW (1963). Anti-inflammatory and antipyretic activities of indomethacin. J. Pharmacol. Exp. Ther., 141: 369-376.

Woods GL, Washington JA (1995). Antibacterial susceptibility tests: Dilution and disk diffusion methods in manual of clinical microbiology, Ed. by Marray PR, Bason GJ, Pfaller MA , Tenover FC, Yolken RH, ASM. Press Washington D.C. 6th Ed., p. 1327.

Yamaguchi K (2005). Spectral Data of Natural Products 1. Elsevier Publishing Company, Amsterdam, London & New York.

Zhang CF , Sun QS , Wang ZT , Hattori M (2003). One new A-type proanthocyanidin trimer from *Lidera aggregate* (Sims) Kosterm. Chin. Chem. Lett., 14**(**10): 1033-1036.

Zhao DC, Lei Jxy, Chen R, Yu WH, Zhang XM, Li SN. (2005). Bone marrow derived mesenchymal stem cells protect against experimental liver fibrosis in rats. World J. Gastroentrol., 14: 3431-3440.

Zunoliza A, Khalid H, Zhari I, Rasadah M A, Mazura P, Fadzureena J, Rohana SE (2009). Evaluation of extracts of leaf of three *Ficus deltoides* varieties for antioxidant activities and secondary metabolites. Pharmacogn. Res., 1: 216–223.