ISSN 0974 – 5211

Journal of Natural Products
Volume 4 (2011)
www.JournalofNaturalProducts.Com

Research Paper

Phytochemical and pharmacological studies of *Ficus auriculata* Lour.

Ahlam El-Fishawy[1], Rawia Zayed[2*], Sherif Afifi[2]
[1] Dept. of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo, Egypt.
[2] Dept. of Pharmacognosy, Faculty of Pharmacy, Sinai University, 55441 North Sinai, Zagazig University, 44519 Zagazig, Egypt.
* Corresponding Author
(Received 12 July 2011; Revised 15 July -11 August 2011; Accepted 14 August 2011)

ABSTRACT

Eight known compounds, including: betulinic acid, lupeol, stigmasterol, bergapten, scopoletin, β-sitosterol-3-O-β-D-glucopyranoside, myricetin, and quercetin-3-O-β-D-glucopyranoside were isolated from the petroleum ether, chloroform and ethyl acetate fractions of alcoholic extracts of the leaves and fruits of *Ficus auriculata* Lour. The structures of these compounds were elucidated on the basis of various spectroscopic methods. This is the first report on compounds separation from *Ficus auriculata* Lour. (*Moraceae*). Both the extracts were effective against gram +ve bacteria (*Staphylococcus aureus*, *Bacillus aureus* and *Bacillus subtilis*) and gram –ve bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) by agar well diffusion method. Meanwhile, the alcoholic extract of leaves at dose of 500mg/kg exhibited anti-inflammatory effect using carrageenin-induced rat hind paw oedema model. Concerning the antioxidant effect the results revealed that increasing the concentrations of the extracts increased the antioxidant effect in a dose dependant manner and the fruits extract has more antioxidant activity than the leaves. Also, the extracts showed slight hepatopratective and antidiabetic activities.

Keywords: *Ficus auriculata*; Phytochemical; Antibacterial; Anti-inflammatory; Antioxidant.

INTRODUCTION

A special feature of higher plants is their capacity to produce a large number of secondary metabolites. Genes *Ficus* (Family *Moraceae*) consists of medicinal and ornamental plants. The chemical review on genus *Ficus*, reveals the presence of sterols and/or terpenes (Kuete, et al., 2008; Djemgou, et al., 2009), flavonoids (Kuete, et al., 2008; Sultana and Anwar, 2008; Oliveira, et al., 2009; Chen, et al., 2010) and coumarins (Oliveira, et al., 2009; Chen, et al., 2010), in addition to minor alkaloids (Subramaniam, et al., 2009).

These compounds are characterized by their physiological and medicinal values such as antioxidant (Channabasavaraj, et al., 2008; Oliveira et al., 2009; Verma, et al., 2010), antihepatotoxic (Channabasavaraj, et al., 2008), antimicrobial (Kuete, et al., 2008; Chen et al., 2010; Subramaniam, et al., 2009), anticancer

(Chiang, et al., 2005; Khan and Sultana, 2005), antidiabetic (Pandit, et al., 2010) antipyretic (Bafor, et al., 2010) and anti-inflammatory (Mandal, et al., 2000). It is worthy to be note that there is almost no report on the chemistry of *Ficus auriculata* Lour. cultivated in Egypt as well as biological screening of this plant. It was deemed of interest and importance to investigate the bioactive compounds of this plant. In the present study, organic extracts of *F. auriculata* were phytochemically analysed and tested for some biological activities.

MATERIALS AND METHODS

Plant materials: *Ficus auriculata* was collected in the fruiting stage from the plant cultivated in Orman Garden, Giza, Egypt. The systematic identification of the plant material was kindly verified by Mrs. Trease Labib, consultant of plant taxonomy at the Ministry of Agriculture and former director of Orman Garden, Giza, Egypt. Identification was further kindly confirmed by Prof. Dr. M. El Gebally, department of plant Taxonomy, Faculty of Science, Cairo University, Egypt. The plant was shade dried at 24-30ºC and ground by electric mill to moderately fine powder.

Extraction and chromatography: The air-dried aerial parts of *F. auriculata* Lour. (1.2kg) were extracted by cold maceration with 70% ethanol (5L. x 3) till complete exhaustion. The combined extract was concentrated under reduced pressure at 50ºC to give 60g of greenish brown residue. The concentrated alcoholic extract was dissolved in about 500ml of MeOH: H_2O mixture (1:9). Then it was extracted with petroleum ether, chloroform, and ethyl acetate. The combined fractions were then washed with distilled water, dried over anhydrous sodium sulphate and then the solvent was distilled off under reduced pressure at 50ºC to afford 12g of petroleum ether, 4g of chloroform and 2g of ethyl acetate extracts.

Vacuum liquid chromatography (VLC) of petroleum ether fraction and isolation of the major compounds: 12g of the petroleum ether soluble fraction was dissolved in the least amount of methanol then adsorbed on 18g of silica gel and subjected to vacuum liquid chromatography (VLC) (11 × 7.5cm). Elution was carried out starting with petroleum ether and the polarity gradually increased with chloroform and methanol. Fractions (200ml each) were collected, monitored by TLC and concentrated. The similar fractions were collected together and subjected to further chromatographic purification. Fractions (2-4) eluted by petroleum ether: chloroform 8:2 gave one major spot R_f 0.63 (TLC, sys.1), subjected to column chromatography eluted by petroleum ether: chloroform 7:3 then purified on silica gel eluted by petroleum ether: chloroform 6:4 to yield compound 1. Fractions (6-7) eluted by petroleum ether: chloroform 1:1 gave two major spots R_f 0.81, 0.64 (TLC, sys.2), subjected to column chromatography to yield two sub fractions eluted by petroleum ether: chloroform 4:6, 2:8, then crystallized from methanol to yield compounds 2 and 3, respectively.

Column chromatography of chloroform fraction and isolation of the major compounds: 4g of the chloroform soluble fraction was dissolved in the least amount of methanol and adsorbed on 6g of silica gel for column and applied on packed silica column (2.5 × 70cm). Elution was carried out starting with petroleum ether and the polarity gradually increased with chloroform and ethyl acetate. Fractions (100ml each) were collected, monitored by TLC and concentrated. The similar fractions were collected together and subjected to further chromatographic purification. Fractions (4-5) eluted by petroleum ether: chloroform 1:9 gave one major spot R_f 0.51 (TLC, sys.3), purified on column chromatography eluted by chloroform to yield compound

4. Fractions (6-8) eluted by chloroform: ethyl acetate 9:1 gave one major spot R_f 0.66 (TLC, sys.4), purified on column chromatography eluted by chloroform: ethyl acetate 8:2 to yield compound 5. Fraction 11 eluted by chloroform: ethyl acetate 7:3 gave one major spot R_f 0.37 (TLC, sys.4), crystallized from methanol to yield compound 6.

Column chromatography of ethyl acetate fraction and isolation of the major compounds: 2g of the ethyl acetate soluble fraction was dissolved in the least amount of methanol and adsorbed on 4g of silica gel for column and applied on packed silica column (1.7 × 50cm). Elution was carried out starting with chloroform and the polarity gradually increased with methanol. Fractions (50ml each) were collected, monitored by TLC and concentrated. The similar fractions were collected together and subjected to further chromatographic purification. Fractions (7-8) eluted by chloroform: methanol 4:6 gave one major spot R_f 0.38 (TLC, sys.5), purified on sephadex eluted by methanol to yield compound 7. Fraction (10-11) eluted by chloroform: methanol 2:8 gave one major spot R_f 0.73 (TLC, sys.6), purified on sephadex eluted by methanol to yield compound 8.

Apparatus and Equipments: Melting points were determined by using Melting point apparatus, Digital, electrothermal IA9000. For U.V. spectral analysis, UV-VIS HITACHI U-2900 spectrophotometer was used. Infra-red spectra were carried out on IR spectrophotometer, FT/IR-6300. The ^{1}H and ^{13}C NMR spectra were recorded using JEOL Ex-500 MHz and Ex-125 MHz, respectively. The mass spectra were carried on JEOL model JMS-AX500.

TLC analyses were carried out on silica gel GF_{245} chromatoplates with the developing solvent systems as listed in table1.

Authentic reference materials for TLC (β-Sitosterol, Stigmasterol, Kaempferol, Quercetin and Rutin) were obtained from Merk Co. Darmstadt, Germany (Batch number 715439). For column chromatography, silica gel 60 – 120 mesh and Sephadex LH_{20} were used.

Biological investigation: Ethical clearance for performing the experiments on animals was obtained from Institutional Animal Ethics Committee. The different concentrations of *F. auriculata* extracts were selected according to closely related species (Family *Moraceae*) found in experimental models.

Toxicological Study: The adult mice (weighing 20-25g. b. wt.) were divided into ten groups of six mice each and subjected to series of different concentrations of leaves and fruits alcoholic extracts. The concentrations (1 to 5g/kg, p.o.) were dissolved in 0.1 ml of DMSO then completed to suitable volume with sterile saline. The animals were observed for physical signs of toxicity for 4 days.

Antimicrobial effect: Cup-plate method (Woods and Washington, 1995) was used to detect the preliminary antibacterial activity of different extracts including alcoholic extracts of leaves and fruits. The samples were dissolved in DMSO at different concentrations (50, 100 and 250µg/ml). The nutrient agar was seeded by freshly grown *S. aureus*, *E. coli*, *B. aureus*, *B. subtilis* and *Pseudomonas aeruginosa* bacteria (about 10^6 colony forming units/mL). Each cup was filled by about 100µl from each extract. Ampicillin and gentamicin were used as standards. The plates were incubated overnight at 37ºC. Zones of inhibition were measured (mm) and recorded in table 2.

Anti-inflammatory effect: The anti-inflammatory activity was tested according to the method of Winter et al., 1962. For this purpose, 25 mature swiss albino rats weighing 150-180g b. wt. were used. Eodema was induced in the left hind paw of all rats by subcutaneous (s.c.) injection of 0.1ml of 1% (w/v) carrageenin in distilled water into their footpads. Rats were divided into 5 groups of 5 rats each. The 1st group was kept

as control and received the vehicle only. The 2nd, 3rd, 4th and 5th groups were orally administered separately the leaves and fruits extracts of *F. auriculata* at doses of 500 and 250mg/kg, 1 hour before carrageenin injection. The paw volume of each rat was measured using Plethysmometer; before carrageenin injection and then hourly for 3 hours after induction of inflammation.

Antioxidant activity: The antioxidant activity was carried out according to the method described by Sharma and Bhat (2009). Aliquots of 3ml methanolic extracts and 1ml of freshly prepared 200M DPPH methanolic solutions were thoroughly mixed and kept for 30 min in the dark. Different concentrations of the extracts (2, 4, 6 and 8mg/ml) were used. The absorbance of the reaction mixture at 517 nm was measured with a spectrophotometer (HITACHI U-2900). Methanol (3ml), replacing the extract, was used as the control. The percentage of free radical scavenging effect was calculated as follows:

% DPPH inhibition = [(absorbance of control – absorbance of test sample)/ Absorbance of control] x 100.

Hepatoprotective activity: Induction of hepatotoxicity in mice was carried out by carbon tetrachloride (in a dose of 0.2ml/kg, p.o.) according to the method of Aghel et al., 2011. Mice (weighing 20-25g b. wt.) were divided into four groups of five mice each. Group one received carbon tetrachloride (positive group). Group two received only olive oil (solvent of CCl_4 as negative group). Groups three and four received crude extracts of leaves and fruits in a dose of 800mg/kg, respectively and one hour later carbon tetrachloride for five consecutive days (test groups). All administrations were made by p.o. in 0.2 ml volume. Then on day six animals were sacrificed and blood samples were taken, serum was obtained for determination of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) enzymes levels. These biochemical parameters were assayed spectrophotometrically using a commercially available assay kits. Livers were removed immediately, weighed and kept in 10% formalin solution, dehydrated in gradual ethanol (50-100%), cleared in xylene and embedded in paraffin. Sections (4-5µm thick) were prepared and then stained with hematoxylin and eosin (H&E) dye for photomicroscopic observations.

Antidiabetic activity: Diabetes was induced in rats according to Prince and Menon, (2000) by i.p. injection of freshly prepared alloxan monohydrate in a single dose of 120 mg/kg b. wt. Eight hours later; blood was withdrawn from the rats and tested for hyperglycemia using diagnostic kits. Rats having blood glucose range 180-350 mg/dl were considered diabetic. 20 diabetic rats (weighing 150-180 g b. wt.) were divided into four groups of five rats each. The first group received normal saline and served as a control group. The second group received diamicron, a standard drug for comparison, in a dose of 4mg/kg. The third and fourth groups received leaves and fruits extracts in a dose of 500mg/kg, respectively.

RESULTS AND DISCUSION

The alcoholic extract of *Ficus auriculata* Lour. cultivated in Egypt on fractionation and repeated chromatographic purification yielded eight compounds (three compounds from the petroleum ether fraction, three from the chloroform fraction and two from ethyl acetate fraction).

Compound 1: was obtained as white needle crystals (11mg) with m.p. 295-296ºC, R_f 0.63 (TLC, sys.1). It gave positive stable violet ring with libermann burchard test indicating a triterpenoid and/or steroid skeleton.

The IR spectrum showed the following absorption frequencies: ν_{max} (KBr) cm^{-1}: 3450, 2939, 2869, 1688, 1644, 1380, 1236, 1145, 1038 and 884. The IR spectrum of compound 1 showed absorption bands at 3450 cm^{-1} attributed to –OH group, 2939, 2869 cm^{-1} indicating C-H stretching, 1688 cm^{-1} for carboxylic group, 1644 and 884 cm^{-1} indicating olefinic bonds.

EI-MS exhibited a molecular ion peak at m/z 456 (M^+) which is compatible with the molecular formula $C_{30}H_{48}O_3$. EI-MS: m/z (%): 456 (M^+, 11), 438 (M^+ - H_2O, 7), 441 (M^+ - Me, 3), 423 (M^+ - H_2O + Me, 7), 411 (M^+ - COOH, 6), 220 (19), 207 (37), 203 (27), 191 (27), 190 (33) and 189 (100) were all consistent with skeletal pattern of lupane series.

The ^{1}H NMR spectrum (500 MHz, DMSO-D6) exhibited the presence of five methyl signals at δ 0.70 (3H, s, Me-24), 0.82 (3H, s, Me-25), 0.92 (3H, s, Me-27), 0.95 (3H, s, Me-23), 0.99 (3H, s, Me-26), allylic methyl 1.70 (3H, s, Me-30), methylene group 4.75 (1H, br. s, Ha-29) and 4.62 (1H, br. s, Hb-29), in addition to 3.04 (1H, m, H-19). The C3 proton at δ 3.34 is comparable to similar protons adjacent to hydroxyl in the lupane series (Muhit et al., 2010).

From the previously mentioned data (Viji et al., 2010) and the available literature it is assumed that this material is betulinic acid (fig. 1).

Compound 2: was crystallized from methanol as white crystals (16mg) with m.p. 214-215ºC, R_f 0.81 (TLC, sys.2). It gave positive stable violet ring with libermann burchard test indicating a triterpenoid and/or steroid skeleton.

The IR spectrum showed absorption peaks at 3367 cm^{-1} indicating the presence of hydroxyl group; peak at 2942 cm^{-1} indicating C-H stretching and peaks at 1644 and 883 cm^{-1} for C=C stretching. The MS fragmentation showed parent ion (M^+) at m/z 426 which is in a good accordance with a molecular formula $C_{30}H_{50}O$ and fragments at m/z 411 (M^+ - Me), 393 (M^+ - Me + H_2O), 385 (M^+ - 41), 220 (M^+ - $C_{15}H_{26}$), 218 (M^+ - $C_{14}H_{24}O$), 207 (M^+ - $C_{16}H_{27}$) and 189 (M^+ - $C_{16}H_{29}O$).

The ^{1}H NMR spectrum revealed the presence of seven tertiary methyl groups (singlets at δ 0.74, 0.77, 0.81, 0.92, 0.95, 1.01 and 1.66), along with two peaks at δ 4.67 and 4.55 which were assigned to the olefinic protons. The signal at δ 3.17 (1H, dd, *J*=11.4, 4.9Hz, H-3) was attributed to a proton geminal to alcoholic group (Thanakijcharoenpath and Theanphong, 2007).The previous spectral analysis; IR, MS and ^{1}H NMR confirmed that this compound is lupeol (fig. 1).

Compound 3: was crystallized from methanol as white crystals (6mg) with m.p. 165-166ºC, R_f 0.64 (TLC, sys.2). It gave positive stable violet ring with libermann burchard test indicating a triterpenoid and/or steroid skeleton.

The IR spectrum showed a hydroxyl stretching band at 3363 cm^{-1}. Also it showed an absorption band at 2942 cm^{-1} for C-H stretching and 1637 cm^{-1} for C=C stretching. The identity of this compound as stigmasterol (fig. 1) has been confirmed by Co-TLC with authentic specimen.

Compound 4: was isolated obtained as white powder (9mg) with m.p. 187-188ºC, R_f 0.51 (TLC, sys.3). It gave yellow colour with alkali. The IR spectrum showed a hydroxyl stretching band at 3413 cm^{-1}. Also it showed an absorption band at 1728 cm^{-1} for carbonyl group besides another band at 1625 cm^{-1} for aromaticity. The MS pattern is a suggestive one and showed parent ion peak at m/z 216 (M^+) corresponding to the molecular formula $C_{12}H_8O_4$, the fragments at m/z 201 (M^+ - Me), 188, 145 and 89.

The ^{1}H NMR exhibited the presence of two doublets with coupling constant of 9.8Hz at δ 6.27 and 8.16, which were assigned as H-3 and H-4, respectively,

characteristic for coumarins and another two doublets with coupling constant 2.4Hz at δ 7.57 and 7.01, which were assigned as H-2' and H-3', respectively. The spectrum showed a methoxyl group singlet at δ 4.27 and one proton singlet (H-8) at δ 7.13 (Bergendorff, et al., 1997).The data confirmed that compound 4 is bergapten (fig. 1).

Compound 5: was obtained as pale yellow crystals (12mg) with m.p. 204ºC, R_f 0.66 (TLC, sys.4). It gave yellow colour with alkali. The IR spectrum showed a hydroxyl stretching band at 3336 cm^{-1}. Also it showed an absorption band at 1702 cm^{-1} for carbonyl group besides another bands at 1611, 1565 and 1509 cm^{-1} for aromaticity.

The MS fragmentation showed parent ion (M^+) at m/z 192 which is in a good accordance with a molecular formula $C_{10}H_8O_4$ and fragments at m/z 177 (M^+ - Me), 164, 149, 121 , 79 and 69.

The ^{1}H NMR spectrum displayed two doublets (*J*=9.5Hz) centered at δ 6.28 (1H) and 7.61 (1H), which were typical for H-3 and H-4 of a coumarin nucleus. The spectrum also showed a singlet at δ 6.93, 6.86 and a broad singlet at δ 6.22, each of single proton intensity; these could be assigned to H-8, H-5 and a hydroxyl group proton at C-7, respectively. A three proton singlet in the spectrum at δ 3.97 revealed the presence of a methoxyl group (Lee et al., 2004).On this basis, compound 5 (fig. 1) was characterized as 7-hydroxy-6-methoxy coumarin (scopoletin).

Compound 6: was crystallized from methanol, obtained as white crystals (23mg) with m.p. 290-292ºC, R_f 0.37 (TLC, sys.4). It developed a stable violet ring with libermann burchard test indicating a triterpenoid and/or steroid skeleton.

The IR spectrum showed a hydroxyl stretching band at 3420 cm^{-1}. Also it showed an absorption band at 2939 cm^{-1} for C-H stretching and 1664 cm^{-1} for C=C stretching beside another three bands at 1450, 1376 and 838 cm^{-1}.

The mass spectrum (EI-MS) exhibited a fragment at m/z 414 (M^+ - $C_6H_{10}O_5$) which is in a good accordance with the aglycone of this compound. Fragments at m/z 396, 382, 381, 255, 147 and 44 confirmed the structure of the aglycone.

The ^{1}H NMR spectrum of this material confirmed the presence of glycoside through a doublet signal localized at δ 4.94 (*J*= 5.0Hz) assigned for anomeric proton. The spectrum also showed peaks at δ 3.00-3.80 characteristic for other protons of the sugar part, 5.40 for the H-6, 1.00 (3H, s, H-19), 0.81-9.40 (12H, H-21, 26, 27, 29) and 0.70 (3H, s, H-18) (Lee et al., 2004).

The ^{13}C NMR spectrum indicated 35 carbon signals, of which 29 were attributed to the aglycone moiety and six to the sugar moiety. The aglycon signals were at δ 140.41 (C-5), 121.20 (C-6), 76.85 (C-3), 56.13 (C-14), 55.38 (C-17), 49.55 (C-9), 45.09 (C-24), 41.82 (C-13), 39.28 (C-4), 38.26 (C-12), 36.79 (C-1), 36.18 (C-10), 35.45 (C-20), 33.29 (C-22), 31.38 (C-7), 31.26 (C-8), 29.23 (C-2), 28.64 (C-25), 27.76 (C-16), 25.36 (C-23), 23.83 (C-15), 22.56 (C-28), 20.56 (C-11), 19.69 (C-26), 19.07 (C-19), 18.89 (C-27), 18.58 (C-21), 11.75 (C-29) and 11.64 (C-18). The carbon signals of the sugar moiety at δ 100.73 (C-1'), 76.86 (C-3'), 76.72 (C-5'), 73.42 (C-2'), 70.04 (C-4') and 61.04 (C-6') were well consistent with those of glucose (Lee et al., 2004). These data confirmed that compound 6 is β-sitosterol-3-O-β-D-glucoside (fig. 1).

Compound 7: was obtained as yellow crystals (15mg) with m.p. 354-355ºC, R_f 0.38 (TLC, sys.5). It dissolved in dilute solution of alkali producing intense yellow colour, indicating the flavonoidal nature of the material. Alcoholic solution developed a yellow colour with 0.1 M $AlCl_3$ solution and a bluish green colour with neutral ferric chloride solution indicating the presence of phenolic compound.

The IR showed absorption bands at 3800-3200 cm^{-1} indicating the presence of several hydroxyl groups. It showed, also the presence of carbonyl group at 1661 cm^{-1}. Compound 7 was recognized as a flavonol from its UV absorption maxima at 253 nm (band II) and 376 nm (band I). The mass spectrum (EI-MS) exhibited a molecular ion at m/z 318 (M^+) which is in a good accordance with a molecular formula $C_{15}H_{10}O_8$ and fragments at m/z 301, 166, 152, 135 and 107. Two doublets in the ^1H NMR spectrum with coupling constants 2Hz at δ 6.17 and 6.37 were consistent with meta-substitution of ring A. A 2H-singlet at δ 7.34 was assigned to H-2' and H-5' of ring B (Korulkina et al., 2004). It can be concluded that this material is myricetin (fig.1).

Compound 8: was obtained as yellow crystals (21mg) with m.p. 255-257ºC, R_f 0.73 (TLC, sys.6). It dissolved in dilute solution of alkali producing intense yellow colour, indicating the flavonoidal nature of the material. Alcoholic solution developed a yellow colour when a few drops of 0.1 M $AlCl_3$ solution were added and a bluish green colour with neutral ferric chloride solution indicating the presence of phenolic compound.

The compound had characteristic IR absorption frequencies at 3800-3400 cm^{-1} (Phenolic -OH) and 1653 cm^{-1} (C=O). The UV spectrum showed λ_{max} (methanol) at 256 and 358 nm which implied the presence of a flavonol structure. The mass spectrum (EI-MS) showed a parent ion at m/z 302 which is in a good accordance with the aglycon (M^+ - $C_6H_{10}O_5$) of this compound and fragments at m/z 285, 273, 153, 150 and 137.

The ^1H NMR spectrum confirmed flavonol structure and displayed the presence of protons H-6 at δ 6.21 (1H, d, *J*= 2.0Hz), H-8 at δ 6.38 (1H, d, *J*= 2.0Hz), H-2' at δ 7.59 (1H, d, *J*= 2.2Hz), H-5' at δ 6.85 (1H, d, *J*= 9.0Hz) and H-6' at δ 7.57 (1H, dd, *J*= 2.2, 9.0Hz). An anomeric proton signal of the compound appeared at δ 5.42 (1H, d, *J*= 7.4Hz, Glu-H-1) and the resonances in the region of δ 3.10-3.65 (6H, m, Glu-H) suggested the presence of glucopyranose unit (Liu et al., 2010).

The ^{13}C NMR spectrum of this compound showed signals for five (C-7, C-5, C-4′, C-3′ and C-3) carbons with OH at δ 164.25, 161.22, 148.54, 144.93 & 133.68 and C=O carbon at δ 177.67 in addition to the other characteristic chemical shift for carbon at δ 156.67 (C-9), 121.87 (C-6′), 121.22 (C-1′), 116.40 (C- 5′), 115.22 (C-2′), 104.28 (C-10), 101.16 (Glu-C-1), 98.86 (C-6), 93.86 (C-8), 77.61 (Glu-C-5), 76.58 (Glu-C-3), 74.15 (Glu-C-2), 70.05 (Glu-C-4) and 61.46 (Glu-C-6) (Jin et al.,2009).

The IR, NMR and EI-MS data led to the identification of the compound 8 (fig. 1) as quercetin 3-O-β-D-glucopyranoside (quercetrin).

Biological and pharmacological studies

Toxicological Study: The dose was up to 5g/kg (maximum soluble dose for mice) with no signs of toxicity.

Antibacterial activity: As shown in table 2, 50µg/ml extracts had least activities against all organisms. Though 250µg/ml extracts showed most activities on the organisms, these were less than those of the reference drugs. The extract of leaves showed more antibacterial activities than the extract of fruits except in case of *Bacillus subtilis*.

Anti-inflammatory activity: The anti-inflammatory activity (table 3) of the extracts of *F. auriculata* was evaluated on carrageenin-induced rat hind paw oedema model. The results revealed that increasing the concentrations of the extracts reduced the inflammation in a dose dependant manner. The leaves extract (500mg/kg) has been found to possess significant anti-inflammatory activity on the tested experimental model.

Antioxidant activity: As shown in table 4, the antioxidant activity of the ethanolic extracts of *F. auriculata* leaves and fruits was assayed by using DPPH radical. The results revealed that increasing the concentrations of the extracts increased the antioxidant effect in a dose dependant manner. Also the fruits extract has more antioxidant activity than the leaves.

Hepatoprotective activity: Animals treated with toxic dose of carbon tetrachloride had markedly elevated levels of the serum AST and ALT, compared to normal mice, indicating acute hepatocellular damage. Serum enzyme values of the crude extracts of *Ficus auriculata* Lour. were nearly at the same levels as those of toxic control (table 5). The histopathological examination of the liver sections of positive control group showed necrosis associated with vacuolization of cytoplasm and fatty degeneration of hepatocytes. The liver sections of the mice treated with the crude extracts exhibited slight improvement with slight vacuolization of hepatocytes.

Antidiabetic activity: As shown in table 6, the total extracts of *Ficus auriculata* induced 8.2% reduction of blood glucose level of diabetic rats as compared to the values before treatment. Diamicron as standard induced 39.8% reduction of blood glucose level of diabetic rats.

Generally the results from the biological studies revealed that the extract of leaves showed more antibacterial activities than the extract of fruits except in case of *Bacillus subtilis*. The same extract reported a significant anti-inflammatory activity this effect may be mainly due to the presence of sterols and triterpenoids as betulinic acid, lupeol, stigmasterol and β-sitosterol-3-O-β-D-glucoside (Perez, 2001).

Also the fruits extract has more antioxidant activity than the leaves. This effect may be mainly due to the presence of flavonoids and phenolic compounds (Hopia and Heinonen, 1999). Concerning the hepatoprotective and antidiabetic activity the results showed slightly effect of the ethaanolic extract.

REFERENCES

Aghel, N., Kalantari, H., Rezazadeh, S., (2011): Hepatoprotective Effect of *Ficus carica* Leaf Extract on mice Intoxicated with carbon tetrachloride. *Iranian J. Pharmace. Research*, 10: 63–68.

Bafor, E.E., Uwumarongie, H.O., Idiake, J.O., (2010): Antipyretic effects of the aqueous, ethyl acetate and hexane leaf extracts of *Ficus exasperata* (Moraceae) in mice. *J. Thermal Biology*, 35: 275–279.

Bergendorff, O., Dekermendjian, K., Nielsen, M., Rudong, S., Witt, R., Ai, J., Sterner, O., (1997): Furanocoumarins with affinity to brain benzodiazepine receptors in vitro. *Phytochemistry*, 44: 1121–1124.

Channabasavaraj, K.P., Badami, S., Bhojraj, S., (2008): Hepatoprotective and antioxidant activity of methanol extract of *Ficus glomerata*. *J. Nat. Med.*, 62: 379–383.

Chen, L.W., Cheng, M.J., Peng, C.F., Chen, I.S., (2010): Secondary metabolites and antimycobacterial activities from the roots of *Ficus nervosa*. *Chemistry and Biodiversity*, 7: 1814–1821.

Chiang, Y.M., Chang, J.Y., Kuo, C.C., Chang, C.Y., Kuo, Y.H., (2005): Cytotoxic triterpenes from the aerial roots of *Ficus microcarpa*. *Phytochemistry*, 66: 495–501.

Djemgou, P.C., Ngandeu, F., Hegazy, M.F., Nkanwen, E.R., Neguim, G., Chosson, E., Verite, P., Tane, P., (2009): GC-MS Analysis of terpenes from *Ficus mucuso*. *Phcog. Res.*, 1: 197–201.

Hopia, A., Heinonen, M., (1999): Antioxidant activity of flavonol aglycones and their glycosides in methyl linoleate. *JAOCS*, 76: 139–144.

Jin, H.Z., Chen, G., Li, X.F., Shen, Y.H., Yan, S.K., Zhang, L., Yang, M., Zhang, W.D., (2009): Flavonoids from *Rhododendron decorum*. *Chemistry of Natural Compounds*, 45: 85–86.

Khan, N., Sultana, S., (2005): Chemomodulatory effect of *Ficus racemosa* extract against chemically induced renal carcinogenesis and oxidative damage response in wistar rats. *Life Sciences*, 77: 1194–1210.

Korulkina, L.M., Shults, E.E., Zhusupova, G.E., Abilov, Z.A., Erzhanov, K.B., Chaudri, M.I., (2004): Biologically active compounds from *Limonium gmelinii* and *L. popovii*. *Chemistry of Natural Compounds*, 40: 465–471.

Kuete, V., Ngameni, B., Simo, C.C.F., Tankeu, R.K., Ngadjui, B.T., Meyer J.J.M., Lall N., Kuiate, J.R., (2008): Antimicrobial activity of the crude extracts and compounds from *Ficus chlamydocarpa* and *Ficus cordata* (Moraceae). *Ethnopharmacology*, 120: 17–24.

Lee, K.H., Min, Y.D., Choi, S.Z., Kwon, H.C., Cho, O.R., Lee, K.C., Lee, K.R., (2004): A new sesquiterpene lactone from *Artemisia rubripes* Nakai. *Arch Pharm Res*, 27: 1016–1019.

Liu, H., Mou, Y., Zhao, J., Wang, J., Zhou, L., Wang, M., Wang, D., Han, J., Yu, Z., Yang, F., (2010): Flavonoids from *Halostachys caspica* and their antimicrobial and antioxidant activities. *Molecules*, 15: 7933–7945.

Mandal, S.C., Maity, T.K., Das, J., Saba, B.P., Pal, M., (2000): Anti-inflammatory evaluation of *Ficus racemosa* Linn. leaf extract. *Ethnopharmacology*, 72: 87–92.

Muhit, M.A., Tareq, S.M., Apu, A.S., Basak, D., Islam, M.S., (2010): Isolation and identification of compounds from the leaf extract of *Dillenia indica* Linn. *Bangladesh pharmaceutical*, 13: 49–53.

Oliveira, A.P., Valentao, P., Pereira, J.A., Silva, B.M., Tavares, F., Andrade, P.B., (2009): *Ficus carica* L.: Metabolic and biological screening. *Food and Chemical Toxicology*, 47: 2841–2846.

Pandit, R., Phadke, A., Jagtap, A., (2010): Antidiabetic effect of *Ficus religiosa* extract in streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology*, 128: 462–466.

Perez, R.M. (2001): Anti-inflammatory activity of compounds isolated from plants. *The Scientific World J.*, 1: 713–784.

Prince, S.M., Menon, V.P., (2000): Hypoglycemic and other related actions of *Tinospora cardifolia* roots in alloxan induced diabetic rats. *J. Ethnopharmacol.*, 70: 9–15.

Sharma, O.P., Bhat, T.K., (2009): DPPH antioxidant assay revisited. *Food Chemistry*, 113: 1202–1205.

Subramaniam, G., Ang, H., Ng, S., Bus, D., Butler, S., (2009): A benzopyrroloisoquinoline alkaloid from *Ficus fistulosa*. *Phytochemistry Letters*, 2: 88–90.

Sultana, B., Anwar, F., (2008): Flavonols (kaempeferol, quercetin, myricetin) contents of selected fruits, vegetables and medicinal plants. *Food Chemistry*, 108: 879–884.

Thanakijcharoenpath, W., Theanphong, O., (2007): Triterpenoids from the stem of *Diospyros glandulosa*. *Thai J. Pharm. Sci.*, 31: 1–8.

Verma, A.R., Vijayakumar M., Rao, C.V., Mathela, C.S., (2010): In vitro and in vivo antioxidant properties and DNA damage protective activity of green fruit of *Ficus glomerata*. *Food and Chemical Toxicology*, 48: 704–709.

Viji, V., Shobha, B., Kavitha, S.K., Ratheesh, M., Kripa, K., Helen, A., (2010): Betulinic acid isolated from *Bacopa monniera* (L.) Wettst suppresses lipopoly-saccharide stimulated interleukin-6 production through modulation of nuclear factor-κB in peripheral blood mononuclear cells. *International Immunopharmacology*, 10: 843–849.

Winter, C.A., Risley, E.A., Nuss, G.W., (1962): Carrageenin-induced oedema in hind paw of the rat as an assay for anti-inflammatory drugs. *Experimental Biology and Medicine*, 111: 544–547.

Woods, G.L., Washington, J.A., (1995): Antibacterial Susceptibility Test: Dilution and Disk Diffusion Methods in: *Manual of Clinical Microbilogy*, 6th ed.: 1327– 1332.

Table-1: Solvent systems used for TLC.

System No.	Composition
1	petroleum ether: ethyl acetate (9:1)
2	petroleum ether: ethyl acetate (8:2)
3	petroleum ether: ethyl acetate (6:4)
4	chloroform: methanol (47:3)
5	benzene: ethyl acetate: formic acid: water (55:45:5:10)
6	ethyl acetate: acetic acid: methanol: water (60:15:15:10)

Table -2: Antimicrobial activity of different concentrations of *F. auriculata*.

Microorganism	Inhibition Zones (mm)							
	Antibiotics (10 µg/well)		Leaves (µg/ml)			Fruits (µg/ml)		
	Ampicillin	Gentamicin	50	100	250	50	100	250
Staphylococcus aureus	24	19	10	14	21	9	12	17
Esherichia coli	17	25	8	11	16	8	10	15
Bacillus aureus	-	13	-	9	11	-	8	10
Bacillus subtilis	12	20	8	12	17	9	15	19
Pseudomonas aeruginosa	-	21	9	14	18	8	12	15

Table-3: Effect of *Ficus auriculata* on carrageenin induced inflammation in mice.

Treatment	Dose mg/kg	Percentage change in paw volume		
		1 hour after induction	2 hours after induction	3 hours after induction
Control	-	27.23	42.10	65.24
Alcoholic extract of leaves	250	22.88	46.49	52.95
Alcoholic extract of leaves	500	18.87	34.99	50.37
Alcoholic extract of fruits	250	34.40	44.05	55.55
Alcoholic extract of fruits	500	27.65	43.71	53.57

- Values are given as means of 5 measurements.

Table-4: Antioxidant effect of *Ficus auriculata* extracts on DPPH radical.

Extracts	Alcoholic extract of leaves				Alcoholic extract of fruits			
Conc. (mg/ml)	2	4	6	8	2	4	6	8
% DPPH inhibition	40.78	54.31	75.88	85.49	44.90	74.90	82.35	88.24

Table-5: ALT and AST enzyme activities in different test groups.

Treatment	ALT (U/ml)	AST (U/ml)
CCl_4 (positive group)	116.75	94.5
Olive oil (negative group)	39.75	28.5
Leaves	107.5	81.5
Fruits	109.25	85.25

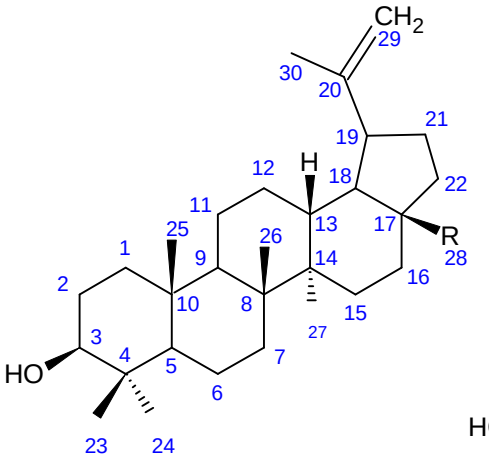
- Values are given as means of 5 measurements.

Table-6: Effect of extracts and diamicron on blood glucose levels of diabetic rats.

Treatment	Blood glucose levels mg/dl		Reduction %
	Before treatment	After treatment	
Control	267.6	254.8	4.8
Diamicron	268.6	161.6	39.8
Leaves extract	269.6	247.4	8.2
Fruits extract	266.2	244.4	8.2

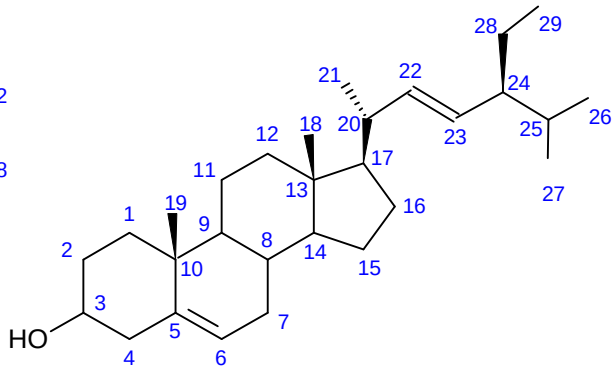
- Values are given as means of 5 measurements.

Figure-1: The suggested structures of the isolated compounds from *F. auriculata* Lour.

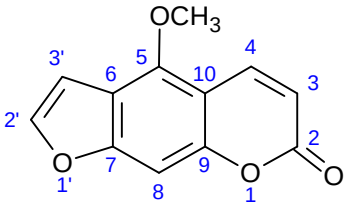


Compound 1 R= COOH
Betulinic acid

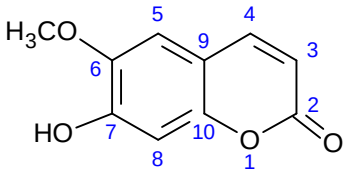
Compound 2 R= Me
Lupeol



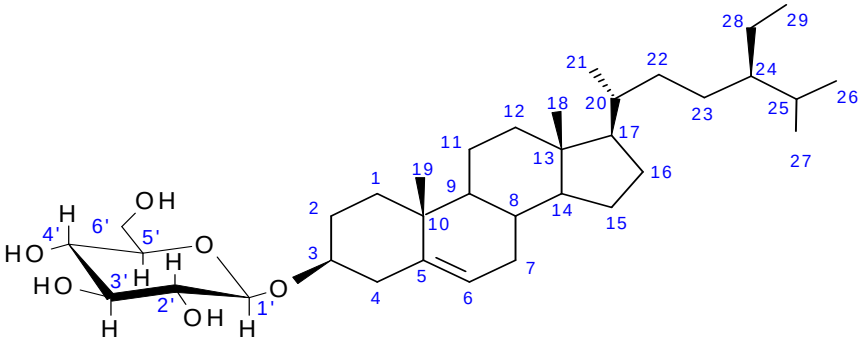
Compound 3
Stigmasterol



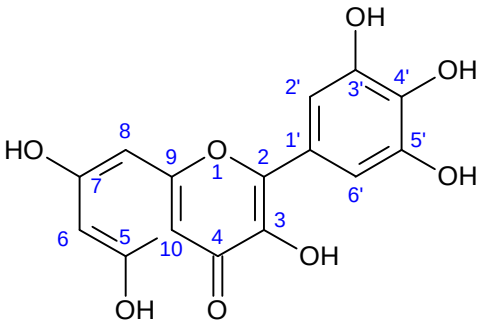
Compound 4
Bergapten



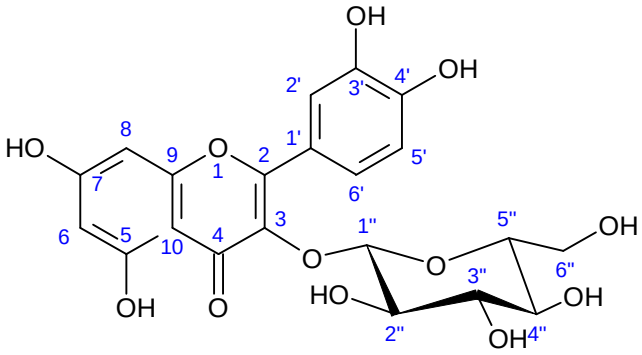
Compound 5
Scopoletin



Compound 6
β-Sitosterol-3-O-β-D-glucopyranoside



Compound 7
Myricetin



Compound 8
Quercetin-3-O-β-D-glucopyranoside