

Flavonoids and some biological activities of *Ailanthus excelsa* leaves

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Abstract

In the present study, the methanolic extract of *Ailanthus excelsa* (Roxb) leaves, a plant used in Egyptian traditional medicine was investigated for phytochemical constituents and some biological activities. In vitro: antioxidant activity and in vivo: Lethal dose (LD₅₀) acute and chronic toxicity and anti-nociceptive and anti-inflammatory effects were determined. It was found that methanolic extract of *Ailanthus excelsa* leaves is nontoxic up to 5 g.kg⁻¹ b. wt in mice. Also rats were given a daily single oral dose of the extract for 7 and 30 successive days for assessment of acute and chronic toxicity. The extract did not induce any significant change in serum levels of ALT, AST, GGT, total protein, albumin, BUN and creatinine as compared with saline control group in rats. The extract exhibited a significant antioxidant, anti-nociceptive activity and no anti-inflammatory effects. Chromatographic separation of *A. excelsa* MeOH extract has yielded eleven known flavonoid compounds. Their structures were established on the basis of chromatographic properties and spectroscopic (UV, ¹H, ¹³C NMR, MS) analyses.

Keywords: *Ailanthus excelsa* leaves, flavonoids, antioxidant, antinociceptive, anti-inflammatory, toxicity.

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Introduction

Ailanthus excelsa is a deciduous tree from the Simaroubaceae family and widely distributed in Asia and northern Australia. Its native origin is China and is known as the "tree of heaven" (Adamik and Brauns 1957). In traditional medicine *A. excelsa* is used to cure wounds and skin eruption and is used in the indigenous system of medicine in febrifuge, bronchitis, asthma and in conditions of diarrhea and dysentery (British Pharmacopoeia 1988). Previous phytochemical studies on *A. excelsa* have demonstrated the presence of quassinoids, flavonoids, alkaloid, terpenoids, and proteins (Ogura et al. 1977; Loizzo et al. 2007; Joshi et al. 2003a; Sherman et al. 1980; Nag and Matai 1994). *A. excelsa* extracts and some isolated

compounds have demonstrated medicinal properties such as significant antileukemic, antibacterial, antifungal and antifertility activities (Ogura et al. 1977; Dhanasekaran et al. 1993; Shrimali et al. 2001; Joshi et al. 2003b). Thus, the present work was designed to calculate the LD₅₀ of methanol extract of *Ailanthus excelsa* (Roxb) leaves and to evaluate the acute and chronic toxicity, antioxidant, antinociceptive and anti-inflammatory activity of the extract as well as its phenolic compounds (flavonoids).

Materials and Methods

Experimental

UV/VIS: Shimadzu UV-visible recording spectrophotometer model-UV 240 (NRC,

Egypt). $^1\text{H-NMR}$ spectra: Varian Unity Inova 400 (400 MHz); $^{13}\text{C-NMR}$ spectra: Varian Unity 400 (100 MHz) (Graz University, Austria). The δ values reported as ppm relative to TMS in $\text{DMSO-}d_6$ (Dimethylsulphoxide) and J values are given in Hz. MS (Finnigan MAT SSQ 7000, 70 eV). Silica gel (E. Merck, type 60-230 mesh, 800 g); Sephadex LH-20 (Pharmacia Fine Chemicals); Solvent mixtures, methanol and ethanol (Merck) and (PC), Whatman No. 3 MM (46 × 57 cm).

Plant material

Ailanthus excelsa leaves were collected from the Zoo Garden, Giza, Egypt in March 2007 during flowering and identified by Dr. Kamal El-Batanony, Professor of Taxonomy and Botany, Faculty of Science, Cairo University. A voucher specimen was deposited in the herbarium of the National Research Centre (CAIRC), Cairo, Egypt.

Animals

Adult pathogen-free Sprague-Dawley rats of both sexes, weighing ranged from 125-150 g, were used for acute and chronic toxicity studies and anti-inflammatory effects. Swiss mice 20-25 g body weight were used for studies of the median lethal dose (LD_{50}) and anti-nociceptive activity. The animals were obtained from the animal house colony of the National Research Centre, Dokki, Giza, Egypt. The animals were housed in standard metal cages in an air conditioned room at $22 \pm 3^\circ\text{C}$, $55 \pm 5\%$ humidity and provided with standard laboratory diet and water *ad libitum*. Experiments were performed between 9:00 and 15:00 p.m. Groups of 6 rats were used for each experiment. All experimental procedures were conducted in accordance with the guide for care and use of laboratory animals and in accordance with the Local Animal Care and Use Committee. Tween 80 (1% in distilled water) was used as a vehicle for the extract used.

Drugs and Chemicals

Indomethacin was obtained from Epico,

Egypt Int. Pharmaceutical Industries Co., ARE under license of MERCK Co. Inc-Rahaway, NJ, USA used at a dose of 25 mg/kg according to Suleyman et al. (2004). Carrageenan (Sigma-Aldrich Chemical Company, USA). Ethyl alcohol (BDH-Chemical, England).

Diagnostic kits:-

-Serum Alanine-aminotransferase (ALT), Aspartateaminotransferase (AST) and Gamma-glutamyl-transpeptidase (GGT) were analyzed by QCA (Quimica Clinica Aplicada S.A) kits (Reitman and Frankel 1957, Szasz 1969).

- Serum total protein, Albumin, Blood urea nitrogen (BUN) and Creatinine were analyzed by Stanbio Laboratory kits (Cannon 1974, Dumas and Biggs 1972, Henry et. al., 1974; DiGiorgio 1974).

Extraction and Isolation

The air-dried powdered leaves of *A. excelsa* (2 kg) were extracted in a Soxhlet apparatus at 50°C with methanol (70%). The extract was concentrated under reduced pressure to dryness to give 260 g. The crude extract was dissolved in 500 ml of distilled water and defatted with *n*-hexane. The residue (215 g) was subjected to silica gel column chromatography (800 g) and eluted with methylene chloride-ethyl acetate-methanol in a gradual manner. One hundred and thirty fractions were collected. Fractions showing similar (PC); Paper Chromatography profiles in (BAW); *n*-butanol:acetic acid: water 4:1:5 upper phase and 15% acetic acid were pooled to provide five combined fractions. The fractions were further purified on preparative paper and Sephadex LH-20 column chromatography to afford 2-3 flavonoids for each fraction.

Antioxidant activity of methanol extract of *Ailanthus excelsa* leaves (in vitro)

The antioxidant activity of *A. excelsa* methanol extract was studied in vitro using the (1,1-Diphenyl-2-picrylhydrazyl (DPPH)

method. The activity of DPPH radical scavenging was investigated according to the method of Peiwu et al. (1999). In this method, a methanol solution of DPPH (2.95 μM) was added to 50- μl sample of different concentrations of the extracts (10-50 mg/ml^{-1}) in disposable cavetti. The absorbance was measured at 517 nm at regular intervals of 15 seconds for 5 min. Ascorbic acid was used as a standard (0.1 M concentration) as described by Govindarajan et al. (2003).

$$\% \text{inhibition (Reactive reaction rate)} = \frac{\text{Abs. (DPPH solution)} - \text{Abs. (sample)}}{\text{Abs. (DPPH solution)}} \times 100$$

Determination of median lethal dose (LD_{50})

The extract was dissolved in 1% tween 80 in distilled water and then given orally in graded doses to mice up to $5\text{g}\cdot\text{kg}^{-1}$ and the control group received the same volumes of the vehicle. The percentage mortality for mice was recorded 24 hours later. No mortality occurred after 24 hours and according to Semler (1992), who reported that in the typical protocol for acute toxicity study if just one dose level at $5\text{g}/\text{kg}$ if this dose is not lethal according agencies no longer require for determination of an LD_{50} value. So the experimental doses used in the present study were 1/20, 1/10 and 1/5 of ($5\text{g}\cdot\text{kg}^{-1}$) of the aqueous methanol extract of *A. excelsa* leaves (250, 500 and $1000\text{mg}\cdot\text{kg}^{-1}$).

Acute and chronic toxicity studies

The rats were divided into eight groups for the two studies: four groups for acute toxicity study and four groups for chronic toxicity study. Each group included six animals and were divided as follows:

A- Acute toxicity study

The first group received one single a daily oral dose of 1 ml vehicle 1% tween 80 in distilled water (normal control group), the remaining three groups were given the *A. excelsa* methanol extract in doses of 250, 500, 1000

$\text{mg}\cdot\text{kg}^{-1}$, one single daily oral dose for 7 successive days. At the end of the 7 days, blood was obtained from all groups of rats from retro-orbital vein plexus under ether anaesthesia (Sorg and Buckner 1964). The blood was allowed to flow into a clean dry centrifuge tube and left to stand 30 minutes before centrifugation to avoid hemolysis. Samples were centrifuged for 15 minutes at 2500 rpm then the clear supernatant serum was separated and collected by Pasteur pipette into a dry clean tube for the following biochemical tests: Alanine-aminotransferase (ALT), aspartateaminotransferase (AST) and gammaglutamyl-transpeptidase (GGT) total protein and albumin for assessment of liver function, blood urea nitrogen (BUN) and creatinine for assessment of kidney function.

B-Chronic toxicity study

The first group received one single daily oral dose of 1 ml vehicle 1 % tween 80 in distilled water (normal control group), the remaining three groups were given the *A. excelsa* methanol extract in doses of 250, 500, $1000\text{mg}\cdot\text{kg}^{-1}$, one single daily oral dose for 30 successive days. At the end of the 30 days, the blood was obtained from all groups as mentioned before for the biochemical tests.

C-Anti-nociceptive activity

This activity was determined by measuring the responses of animals to the thermal and chemical stimulus.

a) Thermal test (Hot-plate test)

The hot-plate test was performed by using an electronically controlled hot plate (Ugo Basile, Italy) heated to 52°C ($\pm 0.1^{\circ}\text{C}$) and the cut-off time was 30s (Woolfe, and MacDonald 1944) for possible centrally mediated analgesics effect. Five groups of mice each of six mice were given the *A. excelsa* methanol extract orally at doses of 250, 500, $1000\text{mg}\cdot\text{kg}^{-1}$ and indomethacin (25 mg/kg) as control vehicle, 60

min prior to the experiment. Latency to lick a hind paw or jump out of the apparatus, Eaton (2003) were recorded for the control and extract treated groups.

b) Chemical test (visceral pain test)

Acetic acid-induced writhing in mice was performed according to the convenient methods published of Collier et al. (1968) and Koster et al. (1959). The mice were divided into six groups of mice each of six mice that were used and received the same doses of extract as mentioned before in case of thermal test, saline as control and indomethacin (25 mg.kg⁻¹) orally (Suleyman et al. 2004). After 60 min interval, the mice received 0.6% acetic acid ip (0.2 ml/mice). The number of writhes in 30 min period was counted and compared.

Carrageenan-Induced Paw Oedema Assay

Paw oedema was induced by injecting 100 µl of a 1% solution of sterile carrageenan lambda in saline in the subplanter region of the right in the hind paw of the rat (Winter et al. 1962). Carrageenan caused visible redness and pronounced swelling that was well developed by 4h and persisted for more than 48h. The rats received vehicle or extract orally 60 min before carrageenan administration. Hind footpad thickness was measured immediately before carrageenan injection and 1-4 h after carrageenan injection with a micrometer caliber (Obukowicz et al. 1998).

The rats received vehicle or *A. excelsa* methanol extract orally at doses of 250, 500, 1000 mg.kg⁻¹ 60 min before carrageenan administration. The oedema component of the inflammation was quantified by measuring the difference in hind footpad thickness before carrageenan injection and 1, 2, 3 and 4 h after carrageenan injection.

Statistical analysis

Data were represented as the mean \pm S.D. and the difference between two groups were

analyzed by Student's t-test (Sendocor and Cechran 1971). A probability value less than 0.05 was considered statistically significant.

Results

Chemical characterization of isolated compounds

The structures of the isolated compounds (Fig. 1) were established by means of NMR, MS, and UV spectral analysis as follow:

Apigenin (1): 7.2 mg, yellow powder, PC R_f 0.9 (BAW) and 0.15 (15% HOAc). (-) ESI-MS: m/z 269 [M-H]⁻. UV λ_{max} (MeOH): 268, 337; (NaOMe): 275, 326, 390; (AlCl₃): 274, 303, 355, 390; (AlCl₃/HCl): 274, 300, 342, 386; (NaOAc): 274, 306, 382; (NaOAc/H₃BO₃): 269, 342. ¹H-NMR: δ = 12.8 (s, 1H, 5-OH), 7.6 (d, J = 8 Hz, 2H, H-2',6'), 6.8 (d, J = 8 Hz, 2H, H-3',5'), 6.15 (s, 1H, H-3), 5.83 (d, J = 2 Hz, 1H, H-8), 5.42 (d, J = 2 Hz, 1H, H-6).

Apigenin 7-O- β -glucoside (2): yellow amorphous powder, PC R_f 0.56 (BAW) and 0.23(15% HOAc); (-) ESI-MS: m/z 431 [M-H]⁻. UV λ_{max} (MeOH): 268, 331; (NaOMe): 249, 266, 389; (AlCl₃): 269, 299, 341, 387; (AlCl₃/HCl): 273, 298, 341, 386; (NaOAc): 255, 267, 388; (NaOAc/H₃BO₃): 267, 337. ¹H-NMR: δ = 12.8 (s,1H,5-OH), 7.95 (d, J = 8 Hz, 2H, H-2',6'), 6.90 (d, J = 8 Hz, 2H, H-3',5'), 6.82 (s, 1H, H-3), 6.81 (d, J = 2.2 Hz, 1H, H-8), 6.42 (d, J = 2.2 Hz, 1H, H-6), 5.0 (d, J = 7.5 Hz, 1H, H-1'').

Luteolin (3): yellow powder, PC R_f 0.91(BAW) and 0.07(15% HOAc). EI-MS: m/z 286 (100%). UV λ_{max} (MeOH): 268, 337; (NaOMe): 276, 326, 390; (AlCl₃): 274, 303, 355, 390; (AlCl₃/HCl): 274, 300, 342, 386; (NaOAc): 274, 306, 382; (NaOAc/H₃BO₃): 267, 342. ¹H-NMR: δ = 12.9 (s, 1H, 5-OH), 7.4 (d, J = 8 Hz, 2H, H-6'), 7.38 (d, J = 2 Hz, 2H, H-2'), 6.85 (d, J = 8 Hz, 2H, H-5'), 6.6 (s, 1H, H-3), 6.4 (d, J = 2 Hz, 1H, H-8), 6.15(d, J = 2 Hz, 1H, H-6).

Luteolin 7-O- β -glucoside (4): yellow amorphous powder, PC R_f 0.49 (BAW), 0.2 (15% HOAc). UV λ_{max} (MeOH): 254, 268, 348; (NaOMe): 261, 403; (AlCl₃): 269, 295,

360, 398; (AlCl₃/HCl): 268, 293, 357, 383; (NaOAc): 264, 363, 401; (NaOAc/H₃BO₃): 261, 373. ¹H-NMR: δ = 12.8 (s, 1H, 5-OH), 7.5(d, ²J = 8 Hz, 1H, H-6'), 7.48(d, ³J = 1.2 Hz, 1H, H-2'), 6.85 (d, ²J = 8 Hz, 1H, H-5'), 6.72 (s, 1H, H-3), 6.4 (d, ³J = 2 Hz, 1H, H-8), 6.15 (d, ³J = 2.2 Hz, 1H, H-6), 5.0 (d, ²J = 7.5 Hz, 1H, H-1'). ¹³C-NMR: δ = 182 (C-4), 164.4 (C-2), 162.6 (C-7), 161 (C-5), 158.7 (C-9), 149.8 (C-4'), 145.7 (C-3'), 121.5 (C-1'), 119.3 (C-6'), 116.2 (C-5'), 113.4 (C-2'), 105 (C-10), 103 (C-3), 100 (C-1'), 99.8 (C-6), 94.5 (C-8), 77.2 (C-5'), 76.2 (C-3'), 73.5 (C-2'), 69.6 (C-4'), 61 (C-6').

Kaempferol (5): yellow powder, PC R_f 0.82 (BAW) and 0.05 (15% HOAc). UV λ_{max} (MeOH): 265, 320, 366; (NaOMe): 276, 317, 406; (AlCl₃): 262sh, 269, 310sh, 367; (AlCl₃/HCl): 263sh, 268, 320sh, 344, 425; (NaOAc): 274, 306, 382; (NaOAc/H₃BO₃): 267, 368. ¹H-NMR: δ 8.11 (d, J = 8 Hz, 2H, H-2',6'), 6.96 (2H, d, J = 8 Hz, H-3',5'), 6.47 (d, J = 2 Hz, 1H, H-8), 6.19 (d, J = 2 Hz, 1H, H-6). EI-MS: m/z 286.

Kaempferol 3-O-α-arabinoside (6): yellow powder, PC R_f 0.79 (BAW) and 0.3 (15% HOAc). UV λ_{max} (MeOH): 265, 320, 366; (NaOMe): 276, 317, 406; (AlCl₃): 262sh, 269, 310sh, 367; (AlCl₃/HCl): 263sh, 268, 320sh, 344, 425; (NaOAc): 274, 306, 382; (NaOAc/H₃BO₃): 267, 368. ¹H-NMR: δ = 12.65 (s, 1H, 5-OH), 8.08 (d, J = 8.8 Hz, 2H, H-2',6'), 6.88 (d, J = 8.4 Hz, 2H, H-3',5'), 6.42 (d, J = 2 Hz, 1H, H-8), 6.19 (d, J = 2 Hz, 1H, H-6), 5.33 (d, J = 5.2 Hz, 1H, H-1'), 3.65 (1H, m, H-4'), 3.74 (1H, dd, J = 5.3, 6 Hz, H-2'), 3.56 (1H, dd, J = 5.8, 11.6 Hz, H-5'), 3.52 (1H, dd, J = 2.7, 6.9 Hz, H-3'), 3.19 (1H, dd, J = 2, 11.6 Hz, H-5'). ¹³C-NMR: δ = 177 (C-4), 164.9 (C-7), 161.3 (C-5), 160.1 (C-4'), 156.2 (C-2, C-9), 133.6 (C-3), 131 (C-2', 6'), 120.8 (C-1'), 115.3 (C-3',5'), 103.9 (C-10), 101.4 (C-1'), 98.9 (C-6), 93.8 (C-8), 64.3 (C-5'), 71.7 (C-3'), 70.9 (C-2'), 66.1 (C-4').

Kaempferol 3-O-β-galactoside (7): yellow powder, PC R_f 0.76 (BAW) and 0.4 (15% HOAc). UV λ_{max} (MeOH): 266, 326sh, 350; (NaOMe): 274, 324, 395; (AlCl₃): 269, 305,

349, 407; (AlCl₃/HCl): 270, 302, 347, 397; (NaOAc): 274, 308, 387; (NaOAc/H₃BO₃): 267, 301, 352. ¹H-NMR: δ = 12.57 (s, 1H, 5-OH), 8.04 (d, J = 8.9 Hz, 2H, H-2',6'), 6.88 (d, J = 8.9 Hz, 2H, H-3',5'), 6.44 (d, J = 2.1 Hz, 1H, H-8), 6.21 (d, J = 2.1 Hz, 1H, H-6), 5.34 (d, J = 7.8 Hz, 1H, H-1'), 3.65 (s, 1H, H-4'), 3.54 (t, J = 8.9 Hz, 1H, H-2'), 3.45(dd, J = 5.5, 9.6 Hz, 1H, H-6'), 3.37 (dd, J = 2.8, 9.8 Hz, 1H, H-3'), 3.33 (m, 1H, H-5'), 3.29 (m, 1H, H-6'). ¹³C-NMR: δ = 177.7 (C-4), 164.5 (C-7), 161.2 (C-5), 160 (C-4'), 156.7(C-2, C-9), 133 (C-3), 130.9 (C-2', 6'), 121.1(C-1'), 115.2(C-3',5'), 104.1(C-10), 101.9 (C-1'), 99.3 (C-6), 93.8 (C-8), 75.6 (C-5'), 73.7 (C-3'), 71.2 (C-2'), 67.8 (C-4'), 60.5 (C-6').

Quercetin (8): yellow powder, PC R_f 0.91 (BAW) and 0.12 (15% HOAc). UV λ_{max} (MeOH): 255, 267, 371; (NaOMe): 270, 320, 420; (AlCl₃): 270, 455; (AlCl₃/HCl): 264, 303sh, 315sh, 428; (NaOAc): 257, 274, 318, 383; (NaOAc/H₃BO₃): 259, 387. ¹H-NMR: δ = 7.74 (d, J = 8, 2 Hz, 1H, H-2'), 7.55 (d, J = 2 Hz, 1H, H-6'), 6.92 (d, J = 8 Hz, 1H, H-5'), 6.42 (d, J = 1.2 Hz, 1H, H-8), 6.15 (d, J = 1.2 Hz, 1H, H-6). EI-MS: m/z 302.

Quercetin 3-O-α-arabinoside (9): yellow powder, PC R_f 0.67 (BAW) and 0.14 (15% HOAc). UV λ_{max} (MeOH): 257, 266, 358; (NaOMe): 272, 328, 408; (AlCl₃): 271, 299sh, 402; (AlCl₃/HCl): 268, 298, 364, 400; (NaOAc): 273, 324, 389; (NaOAc/H₃BO₃): 261, 379. ¹H-NMR: δ = 12.65 (s, 1H, 5-OH), 7.66 (dd, J = 2.2, 8 Hz, 1H, H-6'), 7.5 (d, J = 2.3 Hz, 1H, H-2'), 6.83 (d, J = 8.6 Hz, 1H, H-5'), 6.38 (d, J = 2.3 Hz, 1H, H-8), 6.18 (d, J = 2.3 Hz, 1H, H-6), 5.26 (d, J = 5.3 Hz, 1H, H-1'), 3.63 (m, 1H, H-4'), 3.75 (dd, J = 5, 6.8 Hz, 1H, H-2'), 3.6 (d, J = 5.9, 11.3 Hz, 1H, H-5'), 3.51 (dd, J = 2.8, 6.8 Hz, 1H, H-3'), 3.21 (d, J = 11.3 Hz, 2H, H-5'). ¹³C-NMR: δ = 177.65 (C-4), 164.54 (C-7), 161.37 (C-5), 156.3 (C-2), 159.39 (C-9), 148.79 (C-4'), 145.16 (C-3'), 133.9 (C-3), 122.17 (C-6'), 121.04 (C-1'), 115.93(C-2'), 115.54 (C-5'), 104(C-10), 98.86 (C-6), 93.67 (C-8), 71.81 (C-1'), 70.88 (C-2'), 66.2 (C-4'), 64.39 (C-5').

Quercetin 3-O- β -galactoside (10): yellow powder, PC R_f 0.46 (BAW) and 0.41 (15% HOAc). UV λ_{max} (MeOH): 257, 269sh, 299sh, 362; (NaOMe): 272, 327, 409; (AlCl₃): 275, 305sh, 331sh, 438; (AlCl₃/HCl): 268, 299sh, 366sh, 405; (NaOAc): 274, 324, 380; (NaOAc/H₃BO₃): 262, 298sh, 377. (+)ESI-MS/MS: m/z 487 [M+Na]⁺; (-)ESI-MS/MS: m/z 463 [M-H]⁻; ¹H-NMR: δ = 7.82 (d, J = 2 Hz, 1H, H-2'), 7.57 (dd, J = 2.0, 7.5 Hz, 1H, H-6'), 6.85 (d, J = 8.0 Hz, 1H, H-5'), 6.3 (d, J = 1.9 Hz, 1H, H-8), 6.12 (d, J = 1.9 Hz, 1H, H-6), 5.04 (d, J = 7.6 Hz, 1H, H-1''), 3.85 (d, J = 2.0 Hz, 1H, H-4''), 3.82 (m, 1H, H-2''), 3.65 (dd, J = 11, 4.0 Hz, 1H, H-6''), 3.58 (dd, J = 11, 7.0 Hz, 1H, H-6''), 3.54 (m, 1H, H-3''), 3.45 (m, 1H, H-5'').

Rutin; Quercetin 3-O-rutinoside; Quercetin 3-O- α -rhamnosyl (1'' \rightarrow 6'')- β -glucoside (11): yellow amorphous powder, PC R_f 0.5(BAW) and 0.64(15% HOAc). (-) ESI-MS: m/z 609 [M-H]⁻; (+) ESI-MS: m/z 633 [M+Na]⁺; (+)ESI-MS/MS: m/z 633, 487 [M-rhamnosyl+Na]⁺, 330 [M-quercetin+Na]⁺. UV λ_{max} (MeOH): 257, 266, 358; (NaOMe): 272, 328, 408; (AlCl₃): 271, 299sh, 402; (AlCl₃/HCl): 268, 298, 364, 400; (NaOAc): 273, 324, 389; (NaOAc/H₃BO₃): 261, 379. ¹H-NMR: δ = 12.5 (s, 1H, 5-OH), 7.5 (d, J = 8 Hz, 2H, H-2', 6'), 6.8 (d, J = 8 Hz, 2H, H-5'), 6.35 (d, J = 2.2 Hz, 1H, H-8), 6.15 (d, J = 2.2 Hz, 1H, H-6), 5.34 (d,

J = 7.5 Hz, 1H, H-1''), 4.38 (d, J = 1.2 Hz, 1H, H-1''), 1.1 (d, J = 6 Hz, 1H, CH₃-rhamnosyl). ¹³C-NMR: δ = 177.4 (C-4), 164 (C-7), 161 (C-2), 156.6 (C-5), 156 (C-9), 148.5 (C-4'), 145 (C-3'), 133.5 (C-3), 121.7 (C-6'), 121 (C-1'), 116.5 (C-5'), 115 (C-2'), 101 (C-1''), 100.05 (C-1''), 104 (C-10), 98.5 (C-6), 93 (C-8), 77.2 (C-5''), 76.5 (C-3''), 74 (C-2''), 72 (C-4''), 70.5 (C-4''), 70.4 (C-2''), 71.9 (C-3''), 68 (C-6''), 67.3 (C-5''), 17.8 (CH₃-rhamnosyl).

Pharmacological results

Study of antioxidant activity

The antioxidant activity of methanol extract of *A. excelsa* was studied *in vitro* using the DPPH method; the results of the kinetics of DPPH scavenging reaction of tested extract and L-ascorbic acid were demonstrated in Figure (2) and revealed that the extract in different concentrations (10, 20, 30, 40 and 50 mg/ml) showed a marked significant scavenging activity, the maximum reactive reaction rate after 5 minutes was 18.8, 27.9, 35, 38.1, 43.5 % respectively when compared to L-ascorbic acid which was 86.8%.

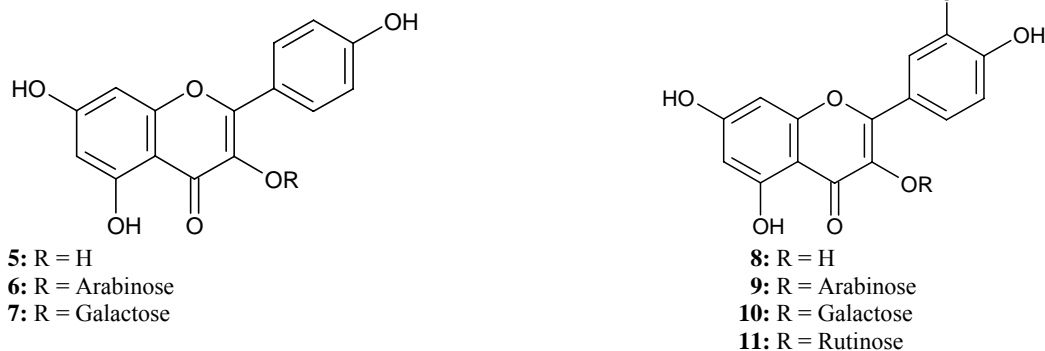


Figure 1. Antioxidant activity of extracts of *A. excelsa* (10, 20, 30, 40 and 50 mg/ml) and ascorbic acid (0.1 M concentration) *in vitro*, using DPPH radical scavenging activity method.

LD₅₀

Results of the LD₅₀ determination revealed that the investigated extract was non toxic up to 5 g.kg⁻¹

Acute and chronic toxicity studies

The methanol extract of *A. excelsa* leaves when given one single daily oral dose (250, 500, 1000 mg.kg⁻¹) either for 7 or 30 successive days showed non-significant change in serum levels of ALT, AST, GGT, total protein, albumin BUN and creatinine as compared with saline control group (Tables 1 and 2).

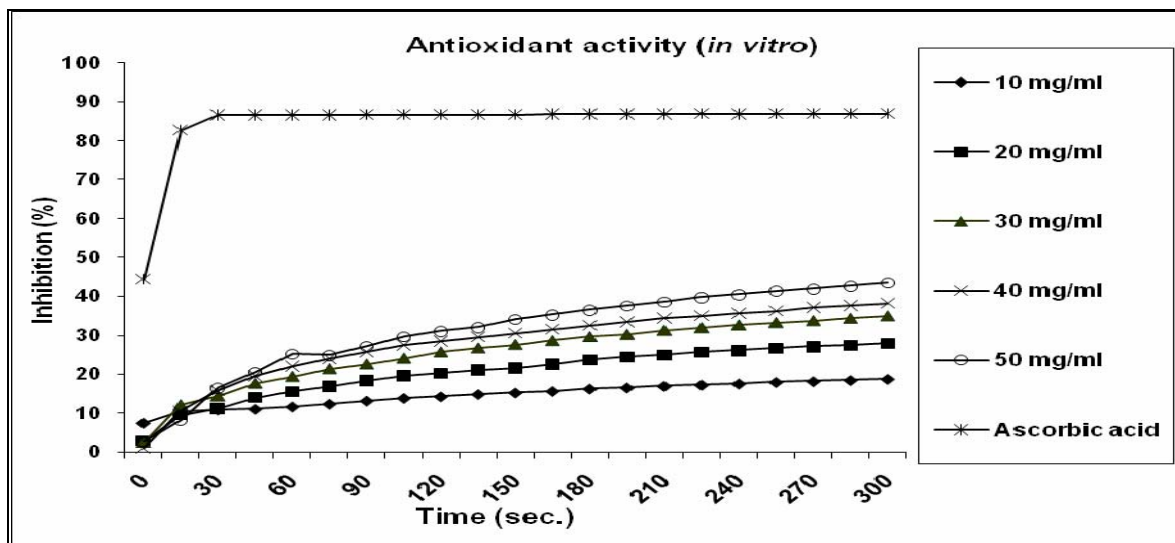


Figure 2. Antioxidant activity of extracts of *A. excelsa* (10, 20, 30, 40 and 50 mg/ml) and ascorbic acid (0.1 M concentration) in vitro, using DPPH radical scavenging activity method.

Anti-nociceptive activity**a) Thermal test (Hot-plate test)**

The mean reaction time on the hot plate was significantly prolonged one and two hours in a dose-dependent manner after the oral administration of methanol extract of *Ailanthus excelsa* at doses of 500, 1000 mg.kg⁻¹ by 18.5 & 29.71% and 63.37 & 82.67 %, respectively as compared with basal values, denoting decreased nociception. While in oral administration of methanol extract of *A. excelsa* at a dose of 250 mg.kg⁻¹ there was significant prolongation of

the mean reaction time on the hot plate after two hour only by 29.71% as compared with basal values (Table 3). So our result in this study revealed that the tested extract in the high dose (1000 mg.kg⁻¹) was more potent than indomethacin as analgesic for thermal pain. The latency time for indomethacin after one and two hours was 35.69% and 60.29%, respectively, while the latency for *A. excelsa* extract at dose of 1000 mg.kg⁻¹ was 63.37% and 82.67%, respectively as compared with basal values.

b) Chemical test (visceral pain test)

In the chemical test (visceral pain test) the given extract demonstrated a significant decrease in the number of writhes in mice after acetic acid injection in dose-dependant manner. Methanol extract of *A. excelsa* leaves showed significant decrease in the number of writhes by -29.9 and -40.6 % at dose of 500 and 1000 mg.kg⁻¹, respectively as compared with saline control group. While indomethacin treated group showed a significant decrease of number of writhes by -89.3% (Table 4).

Anti-inflammatory activity

The subplanter injection of 100 µl of 1% sterile carrageenan into the rat hind paw elicited an inflammation (swelling and erythema) and a time-dependent increase in paw oedema that was maximal at 4 h post-carrageenan. In the control group, the paw thickness increased by 107.12 ± 1.76 % 4 h after carrageenan injection as compared with pre-carrageenan control values.

Table 1: The effect of oral administration of methanol extracts of *A. excelsa* leaves (250, 500 and 1000 mg/kg) for 7 days on the serum activity of, ALT, AST, GGT, total protein, albumin, BUN & creatinine in rats, (n=6).

Groups	Dose (mg/kg)	ALT (IU/L)		AST (IU/L)		TGG (IU/L)		Total protein (g/dl)		Albumin (g/dl)		BUN (mg/dl)		Creatinin (mg/dl)	
		X- ± S.E	% of change	X-± S.E	% of change	X-± S.E	% of change	X-± S.E	%of change	X-± S.E	%of change	X-± S.E	%of change	X-± S.E	%of change
Control	1ml saline	36.7 ± 1.2	---	68.3 ±1.6	---	2.66 ±0.3	----	8.32 ±0.5	---	4.5 ±0.3	---	23.1±1.4	---	0.58 ±0.05	----
Extract	250	35.8 ± 0.7	-2.5	68.8 ±1.6	0.7	2.70 ±0.9	1.5	7.5 ±0.6	-9.9	4.6 ±0.5	2.2	20.7±0.9	-10.4	0.57 ±0.05	-1.7
	500	36.9 ± 1.4	0.5	68.6 ±2.8	0.4	2.8 ±0.4	5.3	7.71 ±0.5	-7.3	4.9 ±0.4	8.9	22.9±2.1	-0.9	0.58 ±0.03	----
	1000	37.5 ± 1.0	-0.5	69 ±1.0	1	2.2 ±0.5	-17.3	8.82 ±0.4	6	5.2 ±0.3	15.6	23.6±1.6	2.2	0.56 ±0.02	-3.4

Data represent the mean value ± SE of six rat per group. Statistical comparison of difference between saline control group and treated groups were done by (Student's t test). No significant difference at P ≤ 0.05

Table 2: The effect of oral administration of methanol extracts of *A. excelsa* leaves (250, 500 and 1000 mg/kg) for 30 days on the serum activity of, ALT, AST, GGT, total protein, albumin, BUN & creatinine in rats, (n=6).

Groups	Dose (mg/kg)	ALT (IU/L)		AST (IU/L)		GGT (IU/L)		Total protein (g/dl)		Albumin (g/dl)		BUN (mg/dl)		Creatinin (mg/dl)	
		X-± S.E	%of change	X-± S.E	%of change	X-± S.E	%of change	X-± S.E	%of change	X-± S.E	%of change	X-± S.E	%of change	X-± S.E	%of change
Control	1ml saline	35.4±1.9	---	64.6 ± 1.1	---	1.5±0.4	---	8.5 ± 0.9	---	3.9±0.2	---	16.2 ±0.9	---	0.73±0.04	---
Extract	250	33.3±2.4	-5.9	65.5 ± 0.9	1.4	1.7±0.5	13.3	8.12±0.6	-4.5	3.12 ±0.1	-20	15.9±1.0	-1.9	0.70±0.05	-1.4
	500	35.6±1.7	0.6	66.9±1.1	3.6	2.0±0.4	33.3	8.89±0.9	4.6	3.2±0.09	-17.9	16.3±0.6	0.6	0.75±0.02	2.7
	1000	35.5±0.8	0.3	65.5±1.2	1.4	2.7±0.14	80	7.1±0.8	-16.5	3.5±0.22	-10.3	15.9±0.7	-1.9	0.75±0.02	2.3

Table 3: Analgesic effect of oral administration of alcoholic extract of *A. excelsa* and indomethacin (25 mg/kg) on thermal pain by using hot plate test.

Treatment groups	Latency(s)		
	basal	1 st h (% change)	2 nd h (% change)
Saline control	21.62 ± 1.9	22.21 ± 2.1	22.56 ± 0.9
Indomethacin (25mg/kg)	20.12 ± 1.4	27.3 ± 1.2 (35.69 %)*	32.25 ± 1.6(60.29%)***
<i>A. excelsa</i> extract 250 mg/kg	21.78±1.5	25.81±1.9 (18.50 %)	28.25 ± 1.5(29.71 %)*
<i>A. excelsa</i> extract 500 mg/kg	18.38±0.9	23.24 ± 1.2(27.42%)*	26.65± 2 (44.99%)**
<i>A. excelsa</i> extract 1000 mg/kg	17.72 ± 1.2	28.95± 1.5 (63.37 %)***	32.55 ± 2.1 (82.67 %)***

Data represent the mean value ± SE of six mice per group .Statistical comparisons between basal, 1st h and 2nd h of each group are made by (Student's t test) significant at * = P<0.050, ** = P<0.010, *** = P<0.001

Table 4: Analgesic effect of oral administration of alcoholic extract of *A. excelsa* and indomethacin (25 mg/kg) on visceral pain in mice by using writhing test (N=6).

Groups	Dose(mg/kg)	Number of Writhing / 30 min (X ± S. E)	Inhibition of writhing%	Potency
Saline control	1 ml	93.3 ± 3.5	---	---
Indomethacin	25 mg/kg	10.00 ± 0.3*	- 89.3 %	1
Alcoholic extract of <i>Ailanthus excelsa</i>	250 mg/kg	76.24 ± 2.8	- 18.3 %	0.2
	500 mg/kg	65.43 ± 2.4*	- 29.9 %	0.3
	1000 mg/kg	55.43 ± 1.3*	- 40.6 %	0.5

Data represent the mean value ± S.E. of six mice per group and percentage inhibition of number of writhing/30 min. Statistical comparison of the difference between saline control group and treated groups was done by using (Student's t test) * = P < 0.05. - Potency was calculated as regard the percentage change of the indomethacin.

The oral administration of methanol extract of *A. excelsa* in all doses used in this study exhibited a non significant change of the oedema formation at 1st, 2nd, 3rd and 4th hours ($P > 0.05$) post-carrageenan injection as compared with saline treated control group at the same time post carrageenan injection.

While indomethacin treatment showed a significant decrease of the paw legs oedema formation induced by carrageenan by -27.2, -44 and -36.8% at 2, 3 and 4h, respectively, post carrageenan injection as compared with saline treated control group at the same time post carrageenan injection (Table 5).

Table 5: Time course of the effect of oral administration of alcoholic extract of *A. excelsa* and indomethacine on rat paw oedema formation induced by sub-plantar injection of 100 μ of 1% carrageenan.

Drugs (mg/kg Oral)	Zero min (basal)	1h oedema (cm) (% increase)	2h oedema (cm) (% increase)	3h oedema (cm) (% increase)	4h oedema (cm) (% increase)
Saline control	0.39 \pm 0.004	0.54 \pm 0.02 (37.24 \pm 2.3%)	0.67 \pm 0.04 (71.97 \pm 5.34%)	0.79 \pm 0.02 (101.66 \pm 4.82%)	0.81 \pm 0.02 (107.12 \pm 1.76%)
<i>A. excelsa</i> extract (250 mg/kg)	0.39 \pm 0.003	0.68 \pm 0.01** (71.67 \pm 2.95%)	0.78 \pm 0.01 ^{NS} (77.12 \pm 4.9%)	0.80 \pm 0.001 ^{NS} (103.88 \pm 1.63%)	0.83 \pm 0.02 ^{NS} (110.97 \pm 3.45%)
<i>A. excelsa</i> extract 500 500 mg/kg	0.39 \pm 0.006	0.65 \pm 0.08** (67.66 \pm 2.47%)	0.74 \pm 0.01 ^{NS} (76.55 \pm 3.73%)	0.76 \pm 0.02 ^{NS} (95.78 \pm 3.4)	0.79 \pm 0.03 ^{NS} (102.66 \pm 1.8%)
<i>A. excelsa</i> extract 1000 mg/kg	0.39 \pm 0.005	0.61 \pm 0.03** (57.43 \pm 3%)	0.7 \pm 0.04 ^{NS} (73.64 \pm 5.9%)	0.73 \pm 0.03 ^{NS} (90.34 \pm 5.9%)	0.77 \pm 0.03 ^{NS} (100.44 \pm 5.8%)
Indomethacin (25mg/kg)	0.39 \pm 0.002	0.56 \pm 0.01** (42.36 \pm 3.49%)	0.59 \pm 0.01** (52.39 \pm 4.92%)	0.61 \pm 0.02*** (56.78 \pm 2.8%)	0.65 \pm 0.02*** (67.22 \pm 6.1%)

Data represent the mean value \pm S.E. of six rats per group and the percent changes versus basal value (zero min), Data were analyzed using (Student's t test), significant difference from control group at same time point post carrageenan injection is denoted by ** = $P < 0.010$, *** = $P < 0.001$ and **NS** = non significance.

Discussion

Flavonoids are diphenyl propanoids that occur everywhere in plant foods and form important constituents of human diet.

The methanol extract of the *A. excelsa* leaves was chromatographed on silica gel column followed by successive separation on preparative paper chromatography and Sephadex LH-20 affording eleven pure known flavonoids identified as four flavones, apigenin (1), apigenin 7-*O*- β -glucoside (2), luteolin (3) and luteolin 7-*O*- β -glucoside (4) and seven

flavonols, kaempferol (5), kaempferol 3-*O*- α -arabinoside (6), kaempferol 3-*O*- β -galactoside (7), quercetin (8), quercetin 3-*O*- α -arabinoside (9), quercetin 3-*O*- β -galactoside (10), quercetin 3-*O*-rutinoside (11). Compounds 2, 5, 8, 10, 11 were isolated here for the first time from *A. excelsa* plant.

All compounds appeared as dark purple spots on PC under UV light, changing to yellow when exposed to ammonia vapour except compounds (5 and 8) where the two compounds showed a yellow spot on PC under UV light.

Chemical investigation as complete acid hydrolysis for O-glycosides were carried out, and followed by paper co-chromatography with authentic samples to identify the hydrolytic flavonoid glycoside products whether aglycons and sugar moieties. All the structures were determined from UV, MS and NMR spectral data, identical with those previously reported (Nakasugi and Komai 1998; Sanbongi et al. 1998; Yun-Lian et al. 2000; Foo et al. 2000; Flamini et al. 2001).

In the present study the *in vitro* antioxidant activity and the *in vivo* study for determination of LD₅₀, acute & chronic toxicity, and the assessment of some pharmacological activities of methanol extract of *A. excelsa* (Roxb) leaves as anti-nociceptive and anti-inflammatory in different experimental animal models were done.

The result of LD₅₀ determination revealed that the investigated extract was non toxic up to 5 g/kg and also when the extract was given as a single daily oral dose of (250, 500, 1000 mg.kg⁻¹) to rats for either 7 or 30 days to assessment the acute and chronic toxicity showed non significant change in the serum levels of ALT, AST, GGT, total protein, albumin, BUN and creatinine as compared with saline control group.

The anti-nociceptive activity results in the present study showed that the tested extract has the ability to prolong the latency time on hot plate test and to inhibit the abdominal contraction induced by acetic acid and these results suggested that the extract inhibits the thermally and chemically induced noxious stimuli and the ability to inhibit both types of stimuli indicates that the extract has a characteristic strong centrally and peripherally mediated analgesic activity (Hunnskaar and Hole 1987).

In general, some mechanism of action may explain the observed antinociceptive activity of the tested extract in the current study; namely the ability to inhibit/reverse the centrally synthesized prostaglandins or COX (Uzcategui et al. 2004) or to block the neurogenic pain. This activity was dose-dependent that reached a

maximum at dose of 1000 mg.kg⁻¹ which is equal to that antinociceptive activity of indomethacin. Moreover, the extract showed a decrease in the number of writhes in mice after acetic acid ip (0.2 ml/mice) injection, so it reduced the intensity of the peritoneal inflammation induced by acetic acid injection, thus indicating its ability to inhibit the permeability of the small blood vessels (El Batran 2005).

The results of the kinetics of DPPH scavenging reaction of tested extracts and L-ascorbic acid revealed that the extract in different concentrations (10, 20, 30, 40 and 50 mg.ml⁻¹) showed a marked significant scavenging activity, the maximum reactive reaction rate after 5 minutes was (18.8, 27.9, 35, 38.1, 43.5%) respectively when compared to L-ascorbic acid which was 86.8 %.

The major bioactive compounds of defatted alcoholic extract of *A. excelsa* leaves was found to be flavonoids compounds such as quercetin, kaempferol and rutin. The main function of these compounds is antioxidant activity (Siddhuraju and Becker 2003). The anti-oxidant property of the *A. excelsa* extract may be due to quercetin glycosides compounds, and this was in agreement with Katsube et al. (2008), Mancini et al. (2008). Thus, the estimated antioxidant effect of the investigated extract is in accordance with these data Ramizi et al. (2008).

The methanol extract of *A. excelsa* leaves showed a non significant anti-inflammatory activity on carrageenan animal model. This fact may be related to the presence of volatile or unstable substances which undergo alteration during the time needed for drying or extraction even at room temperature. The development of oedema in the paw of the rat after injection of carrageenan is a biphasic event. The initial phase of the oedema is due to the release of histamine and serotonin and the oedema is maintained during the plateau phase by kinin like substance (Chauhan et al. 1998) and the second accelerating phase of swelling due to the release of prostaglandin like substances. The inflammatory processes are accompanied with

an increase of free radicals activity (Sulaiman et al. 2008) and, as the in vitro antioxidant activity in the present study showed, that with an increase of the concentration of the extract the antioxidant activity was decreased so we can explain the lack of the anti-inflammatory effects of the *A. excelsa* methanol extract may also be due to the dose of extract used. While Selvam (2008) reported that the bark part of the *Ailanthus excelsa* (Roxb) plant was used for treating asthma and bronchitis.

In conclusion, it was shown that the methanol extract of *A. excelsa* appears to be safe when given to rats orally for either short or long term use without the appearance of any toxic manifestation. It also exhibited a significant antioxidant and anti-nociceptive activity, but with slight anti-inflammatory effects and these may be due to the presence of flavonoid compounds in the extract.

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