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# PHENOLIC COMPOUNDS FROM *AILANTHUS ALTISSIMA* SWINGLE

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نبات الإيلانسس التيسيماسوينجل المنتمى للعائلة السيماروبيسية هو شجرة كبيرة متوطنة فى عدة أماكن ولها الكثير من الإستعمالات فى الطب الشعبى. وقد تم فصل العديد من المركبات الكوشينودية والقلوانيات من جنس الايلانسس.

تم فصل ستة مركبات من نبات الايلانسس التيسيماسوينجل وهى جلات الإيثيل ، كامبيفيرول ، كوارسيتين ، كامبيفيرول-٣-أ-جليكوزيد ، كوارسيتين-٣-أ-جليكوزيد ، والروتين وقد تم التعرف عليها بإستخدام التحليلات الطيفية المختلفة.

كما تم فصل البيتاسيتوسيترول والكحول السيريلى وبيتا سيتوستيرول جليكوزيد وتم التعرف عليها بدراسة خواصها الطبيعية والكروماتوجرافية ومقارنتها بعينات قياسية.

***The phenolic compounds, ethyl gallate, kampferol, quercetin, kampferol-3-O-glucoside, quercetin-3-O-glucoside and rutin were isolated from the leaves of Ailanthus altissima together with β-sitosterol, ceryl alcohol and β-sitosterol glucoside.***

## INTRODUCTION

*Ailanthus altissima* Swingle Fam. Simaroubaceae is a large tree native to China and naturalized in the Eastern of USA, Europe, Australia and South Africa.[1,2] Many folkloric and medicinal uses have been reported belonging to *Ailanthus altissima*. It is used for treatment of dysentery, gonorrhea and haemorrhoids; it is emmenagogue and a remedy for cough, gastric and intestinal upsets.[3] The extract of the plant has insecticidal and *in-vitro* antibacterial activities.[3] The bark is prescribed to treat anemia, diarrhea, heamorrhage, and spermatorrhea.[3] It is also used as antispasmodic, antiasthmatic, cardiac depressant, astringent and for treatment of epilepsy.[4]

The previous reports showed that many quassinoids, alkaloids and other compounds have been isolated from the genus *Ailanthus*.[5-10]

## EXPERIMENTAL

### Plant material

Leaves of *Ailanthus altissima* were collected in July 1994 from the tree cultivated from the Experimental Station of Faculty of Agriculture, Assiut University, Assiut, Egypt. The plant was kindly identified by Prof. Dr. Abd El-Aziz Fayed, Prof. of Taxonomy, Faculty of Science, Assiut University. The leaves were air-dried and grounded into fine powder. Melting points were determined using Stuart Scientific apparatus. UV specta were measured on Pye Unicam SP 1750 UV spectrophotometer. IR spectra were obtained using Shimadzu infra red 470-spectrophotometer. NMR spectra were carried out by 400 MHz $^1$H-NMR and 100 MHz $^{13}$C-NMR spectrophotometer using TMS as internal standard, DMSO-$d_6$ and $C_5D_5N$ as solvents and chemical shifts were given on (ppm) scale. TLC was carried out on silica gel 60 GF 254 (E-Merck) for TLC and cellulose (Sigma, England) for TLC.

### Solvent systems

The solvent systems used were: System I- 15% HOAc, II- 50% HOAc, III- $CHCl_3$-$CH_3OH$-$H_2O$ (75:25:3), IV- BAW (4:1:5) and V- Hexane-ethyl acetate (95:5).

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**Extracton and fractionation**

About 1 kg of the powdered leaves were percolated with 70% ethanol. The alcoholic extract was concentrated under reduced pressure to a syrupy consistency. The total extract (110 g) was diluted with water and partionated with hexane, chloroform, ethyl acetate and n-butanol respectively. Each extract was dried over anhydrous sodium sulphate, filtered and investigated by silica gel TLC and PC with the above different solvent systems, 5% methanolic $AlCl_3$ and 10% sulphuric acid were used as spraying agents. Both the chloroform and ethyl acetate fractions were similar and mixed together.

**Isolation of the components of the hexane fraction**

The fraction was investigated by TLC using silica gel plates, solvent system No. V and 10% sulphuric acid as spraying agent. The hexane extract (5 g) was subjected to column chromatography packed with silica gel (200 g) and eluted with hexane and ethyl acetate using stepwise gradient elution with increasing order of polarity.

ß-sitosterol and ceryl alcohol were isolated from this fraction and purified by repeated cyrstallization from methanol.

**Isolation of the components of the chloroform / ethyl acetate mixed fraction**

The fraction was subjected to chromatographic screening using silica gel TLC, developed in system no. III and sprayed with 10% sulphuric acid. Cellulose plates were also used, developed in systems I and II. The chromatoplates were examined in visible light and under UV light, then after spraying with 5% methanolic $AlCl_3$. The fraction (20 g) was column chromatographed on silica gel (E. Merck, 800 g) and eluted with chloroform and methanol in the manner of increasing polarities. Five pure compounds were isolated from this fraction (Compounds **1-5**), in addition to ß-sitosterol glucoside.

**Isolation of the components of n-butanol fraction**

The fraction was investigated by silica gel and cellulose TLC with I and III solvent systems using 10% sulphuric acid (for silica gel TLC) and 5% methanolic $AlCl_3$ (for silica gel and cellulose TLC) as spraying agents. Eight g of this fraction were subjected to silica gel column chromatography (E. Merck, 300 g) using ethyl acetate and ethanol as eluent in the manner of increasing polarities. Compounds **4**, **5** and **6** were separated in pure forms.

**Compound 1:** White crystals (methanol) m.p 151-153°. It gave blue colour with $FeCl_3$ solution. UV: $\lambda_{max}^{MeOH}$ 280 nm. IR (KBr), $\nu_{max}$ 3445-3290, 3055, 1694, 1600-1523, 1251, 1197 and 1040 $cm^{-1}$. $^1$H-NMR ($C_5D_5N$) δ: 7.8 (2H, s, H-2 and H-6), 4.27 (2H, q, J= 7.1 Hz, O.<u>CH<sub>2</sub></u>.CH$_3$), 1.88 (3H, t, J= 7.1 Hz, O.CH$_2$.<u>CH<sub>3</sub></u>). $^{13}$C-NMR ($C_5D_5N$) δ: 167.4 (C-7, C=O), 147.64 (2C, C-3 and C-5), 140.49 (C-2), 121.49 (C-1), 110.22 (d, 2C, C-2 and C-6), 60.64 (t, CH$_2$), 14.45 (q, CH$_3$).

**Compound 2:** Yellow needle crystals (methanol), m.p 284-286°. UV: The UV data with different ionizing and complexing reagents are shown in Table (1). $^1$H-NMR (DMSO-$d_6$) δ: 8.03 (2H, d, J= 8.7 Hz, H-2´,6´), 6.93 (2H, d, J= 8.7 Hz, H-3´,5´), 6.43 (1H, d, J= 1.7 Hz, H-8), 6.22 (1H, d, J= 1.7 Hz, H-6). $^{13}$C-NMR (DMSO-$d_6$) δ: 176.1 (C-4), 164.2 (C-7), 160.9 (C-5), 159.5 (C-4´), 156.5 (C-9), 147.2 (C-2), 135.8 (C-3), 129.7 (C-2´,6´), 121.9 (C-1´), 115.7 (C-3´,5´), 103.3 (C-10), 98.5 (C-6), 93.8 (C-8).

**Compound 3:** Yellow needle crystals (methanol), m.p 315-317°. UV: Table (1). $^1$H-NMR (DMSO-$d_6$) δ: 7.84 (1H, d, J= 1.8 Hz, H-2´), 7.74 (1H, dd, J= 8.5, 1.8 Hz, H-6´), 7.20 (1H, d, J= 8.5 Hz, H-5´), 6.70 (1H, d, J= 1.2 Hz, H-8), 6.49 (1H, d, J= 1.2 Hz, H-6). $^{13}$C-NMR (DMSO-$d_6$) δ: 175.7 (C-4), 163.8 (C-7), 160.6 (C-5), 156.1 (C-9), 147.6 (C-4´), 146.7 (C-2), 145.0 (C-3´), 135.6 (C-3), 121.9 (C-1´), 119.9 (C-6´), 115.5 (C-5´),

115.0 (C-2´), 102.9 (C-10), 98.1 (C-6), 93.3 (C-8).

**Compound 4:** Yellow amorphous powder, m.p 242-244°. UV: Table (1). $^1$H-NMR (DMSO-$d_6$) δ: 8.08 (2H, d, J= 8.2 Hz, H-2´,6´), 6.90 (2H, d, J= 8.2 Hz, H-3´,5´), 6.45 (1H, d, J= 2.0 Hz, H-8), 6.20 (1H, d, J= 2.0 Hz, H-6), 5.45 (1H, d, J= 7.4 Hz, H-1 glucose), 3.10-3.45 (6H, m, other glucose protons). $^{13}$C-NMR (DMSO-$d_6$) δ: 177.5 (C-4), 164.3 (C-7), 161.3 (C-5), 160.4 (C-4´), 156.5 (C-2), 133.0 (C-3), 131.0 (C-2´), 121.0 (C-1), 115.1 (C-3´,5´), 104.0 (C-10), 98.8 (C-6), 93.7 (C-8). Sugar carbons 101.0 (C-1´´), 77.5 (C-5´´), 76.5 (C-3´´), 74.3 (C-2´´), 70.0 (C-4´´), 61.0 (C-6´´).

**Acid hydrolysis:** 10 mg of compound **4** in 10 ml methanol were added to an equal volume of N/2 $H_2SO_4$ and refluxed in a water bath for 2 hrs. The hydrolysate was extracted with ether (3x50 ml). The combined ethereal extract was dried over anhydrous $Na_2SO_4$ and the residue was crystallized from methanol to give the aglycone. The mother liquor was neutralized with barium carbonate, filtered and the filterate was concentrated under reduced pressure. The residue was subjected to PC using Whatman No. 1 and solvent system No. I. It was identified as glucose. The obtained aglycone was investigated and co-chromatographed with authentic samples on silica gel TLC using solvent system No. III and cellulose TLC using solvent system No. II.

The UV data (Table 1) of the aglycone in methanol with different ionizing and complexing reagents showed that the aglycone was kampferol.

**Compound 5:** Yellow amorphous powder, m.p 205-207°: Table (1). $^1$H-NMR (DMSO-$d_6$) δ: 7.67 (1H, dd, J= 8.5, 2.2 Hz, H-6´), 7.51 (1H, d, J= 2.2 Hz, H-2´), 6.86 (1H, d, J= 5.8 Hz, H-5´), 6.41 (1H, d, J= 2.0, H-8´), 6.21 (1H, d, J= 2.0 Hz, H-6), 5.28 (1H, d, J= 7.5 Hz, H-1-glucose), 3.1-3.8 (6H, m, other glucose protons).

**Acid hydrolysis:** As compound **4**, the sugar moiety was identified as glucose while the obtained aglycone was investigated and co-chromatographed with authentic samples on silica gel TLC using solvent system No. III and cellulose TLC using solvent system No. II.

The UV data (Table 1) of the aglycone in methanol with different ionizing and complexing reagents showed that the aglycone was quercetin.

**Compound 6:** Yellow needle crystals (methanol), m.p 190-192° UV: Table (1). $^1$H-NMR (DMSO-$d_6$) δ: 7.55 (1H, d, J= 2.1 Hz, H-2´), 7.53 (1H, dd, J= 8.4, 2.1 Hz, H-6´), 6.84 (1H, d, J= 8.4 Hz, H-5´), 6.39 (1H, d, J= 2.0 Hz, H-8), 6.19 (1H, d, J= 2.0 Hz, H-6), 5.35 (1H, d, J= 7.5 Hz, H-1-glucose), 4.38 (1H, s, H-1 rhamnose), 3.10-3.75 (10H, m, other protons of sugars), 1.0 (3H, d, J= 6.2 Hz, $CH_3$ of rhamnose). $^{13}$C-NMR (DMSO-$d_6$) δ: 177.3 (C-4), 164.1 (C-7), 161.2 (C-5), 156.5 (C-9), 156.4 (C-2), 148.4 (C-4´), 144.7 (C-3´), 133.3 (C-3), 121.5 (C-5´), 121.1 (C-1´), 116.2 (C-5´), 115.1 (C-2´), 103.9 (C-10), 98.6 (C-6), 93.5 (C-8). Glucose: 101.1 (C-1´´), 77.9 (C-3´´), 76.4 (C-5´´), 74.0 (C-2´´), 70.3 (C-4´´), 66.9 (C-6´´). Rhamnose: 100.7 (C-1´´´), 71.8 (C-4´´´), 70.5 (C-2´´´), 70.3 (C-3´´´), 68.2 (C-5´´´), 17.7 (C-6´´´).

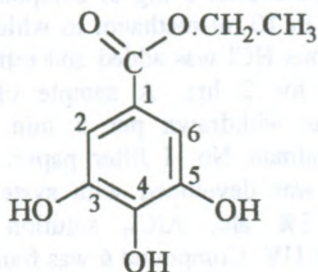
**Partial acid hydrolysis:** 5 mg of compound **6** were dissolved in 10 ml methanol to which 10 ml of 2% aqueous HCl was added and refluxed in water bath for 2 hrs. A sample of the hydrolysate was withdrawn per 5 min. and spotted on Whatman No. I filter paper. The chromatogram was developed with system I, sprayed with 5% alc. $AlCl_3$ solution and visualized under UV. Compound **6** was found to be bioside. Complete acid hydrolysis revealed the presence of glucose, rhamnose and the aglycone which was identified, by its UV data in methanol with different ionizing and complexing reagents and co-chromatography using silica gel TLC in solvent system III and cellulose TLC in solvent system II, as quercetin.

## RESULTS AND DISCUSSION

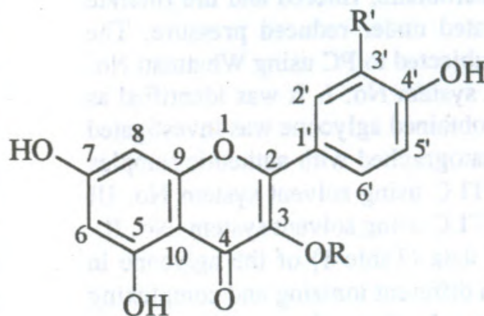
Five flavonoids were isolated and identified as kampferol, quercetin, kampferol-3-O-glucoside, quercetin-3-O-glucoside and rutin

**Table 1: The UV spectral data of the isolated compounds with different ionizing and complexing reagents.**

| Compd. | $\lambda_{max}$ nm | | | | | |
|---|---|---|---|---|---|---|
| | +MeOH | +NaOMe | +NaOAc | +NaOAc/ $H_3BO_3$ | +$AlCl_3$ | +$AlCl_3$/ HCl |
| **2** | 374, 270 | 442, 288 | 378, 276 | 374, 270 | 334, 358, 276 | 434, 358, 274 |
| **3** | 374, 254 | 414, 273 | 380, 260 | 382, 256 | 456, 272 | 430, 266 |
| **4** | 356, 272 | 410, 356, 280 | 376, 310, 280 | 356, 272 | 402, 356, 278 | 402, 356, 280 |
| **5** | 358, 258 | 414, 272 | 364, 268 | 378, 262 | 436, 274 | 402, 272 |
| **6** | 359, 260 | 416, 274 | 370, 270 | 378, 266 | 436, 274 | 402, 362, 272 |




**Compound 1**




| | **R** | **R'** |
|---|---|---|
| **Compound 2** | **H** | **H** |
| **Compound 3** | **H** | **OH** |
| **Compound 4** | **gl.** | **H** |
| **Compound 5** | **gl.** | **OH** |
| **Compound 6** | **gl. rh.** | **OH** |

together with ethyl gallate, ß-sitosterol, ceryl alcohol and ß-sitosterol glucoside.

ß-Sitosterol was identified by its physical properties, chemical tests and IR spectrum while ceryl alcohol was identified by comparing its IR. $^{1}$H-NMR and $^{13}$C-NMR data with those reported in the literatures. ß-Sitosterol glucoside was identified by its physical properties, IR and acid hydrolysis.

**Compound 1:** was proved to be ethyl gallate by UV, IR, $^{1}$H-NMR and $^{13}$C-NMR and this represents the first report in the genus *Ailanthus*.

**Compound 2:** was proved from the UV data (MeOH/NaOMe) to have the flavonol structure[11] with free OH at C-4´ and at C-7 (MeOH/NaOAc) and from $^{1}$H-NMR to contain two protons in ring A ($\delta$ 6.43, d, H-8 and $\delta$ 6.22, d, H-6) and monosubstituted B-ring[12] ($\delta$ 8.03, d, H-2´,6´ and $\delta$ 8.93, d, H-3´,5´). These data together with the $^{13}$C-NMR data, co-chromatography, m.p and m.m.p. confirmed that compound **2** is kampferol[13] which is firstly reported in the genus *Ailanthus*.

**Compound 3:** The UV data showed a flavonol structure with free OH at C-4´ (NaOMe), orthodihydroxy in ring B and free OH at C-7 (NaOAc).[11] The $^{1}$H-NMR and $^{13}$C-NMR showed the characteristic pattern of quercetin[12,13] which was confirmed by m.m.p. and co-chromatography with authentic samples.

**Compound 4:** has the UV data of flavonol with blocked hydroxy group at C-3 ($\lambda_{max}$ 356 nm and brown colour under UV), free OH at C-4´ and free OH at C-7.[11] The $^{1}$H-NMR and $^{13}$C-NMR showed signals characteristic for kampferol and ß-D-glucose. The structure was completely confirmed as kampferol-3-O-ß-D-glycoside by acid hydrolysis and identification of the aglycone part by UV data and co-chromatography. The sugar part was identified as glucose by PC using systems I and IV. This is the first report for kampferol-3-O-ß-D-glucoside in the genus *Ailanthus*.

Concerning **compound 5**, from the UV data, it is proved to be a flavonol containing glycosylated OH at C-3 ($\lambda_{max}^{MeOH}$ 358 nm), a free OH at C-4´ (NaOMe), orthodihydroxy groups in ring B ($AlCl_3$/HCl and NaOAc/$H_3BO_3$) and free OH at C-7 (NaOAc).[11] $^{1}$H-NMR showed 5 signals characteristics to quercetin pattern in addition to sugar protons.

Acid hydrolysis of compound **5** gave aglycone which was identified as quercetin by UV and co-chromatography with authentic samples, the sugar was identified as glucose. From these data, compound **5** was proved to be quercetin-3-O-ß-D-glucoside.

On the basis of the UV data **compound 6** was identified as a flavonol containing glycosylated OH at C-3 ($\lambda_{max}^{MeOH}$ 359 nm and brown colour under UV), free OH at C-4´ (NaOMe), orthodihydroxy groups in ring B ($AlCl_3$/HCl and NaOAc/$H_3BO_3$) and free OH at C-7 (NaOAc).[11] Both $^{1}$H-NMR and $^{13}$C-NMR gave signals reported for glycosylated quercetin and rutinoside sugar.

Partial acid hydrolysis of compound **6** gave the aglycone in two steps indicating that it is bioside while complete acid hydrolysis proved the sugar moiety to be glucose and rhamnose by using PC and solvent systems I and IV. The obtained aglycone was identified by UV data, co-chromatography using silica gel TLC (system III) and cellulose TLC (system II) with authentic sample quercetin. From these data, compound **6** was proved to be rutin which is reported for the first time from the genus *Ailanthus*.

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