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# PHCOG MAG.: Research Article
# Phytophenolics from *Peltophorum africanum* Sond. (Fabaceae) with promising hepatoprotective activity

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**ABSTRACT**

The aqueous alcoholic extract of *Peltophorum africanum* Sond. (Fabaceae) exhibited hepatoprotective activity represented by significant reduction of elevated serum liver enzymes and significant reduction in hepatic TBARS levels in hepatotoxicated animals. Fifteen known phenolic metabolites were identified for the first time from the entitled plant. Establishment of all structures were based on conventional methods of analysis, ESI/MS, in addition, complete $^{1}H$ and $^{13}C$ NMR assignments were obtained for the isolated compounds. Their structures were elucidated to be; gallic acid **1**, 3-*O*-methyl gallic acid **2**, quercetin-3-*O*-$^{1}C_{4}$-α-L-rhamnopyranosyl-(1'''→6'')-$^{4}C_{1}$-β-D-glucopyranoside, rutin **3**, kaempferol-3-*O*-$^{1}C_{4}$-α-L-rhamnopyranosyl-(1'''→6'')-$^{4}C_{1}$-β-D-glucopyranoside, nicotiflorin **4**, kaempferol-3-*O*-$^{4}C_{1}$-β-D-glucoside, astralagin **5**, quercetin-3-*O*-$^{4}C_{1}$-β-D-glucoside, isoquercitrin **6**, methyl 3,4,5-trihydroxybenzoate, methyl gallate **7**, kaempferol-3-*O*-(6"-*O*-galloyl)-$^{4}C_{1}$-β-D-galactopyranoside **8**, quercetin-3-*O*-(6"-*O*-galloyl)-$^{4}C_{1}$-β-D-galactopyranoside **9**, myricetin-3-*O*-(6"-*O*-galloyl)-$^{4}C_{1}$-β-D-galactopyranoside **10**, (-)-epigallocatechin-3-*O*-gallate **11,** (+)-gallocatechin-3-*O*-gallate **12**, myricetin **13**, quercetin **14**, kaempferol **15**.

**KEYWORDS:** *Peltophorum africanum* ; Fabaceae; hepatoprotective; Phenolics.

## INTRODUCTION

Five taxa belonging to the genus *Peltophorum* including *P. africanum* Sond. Previously, reported to contain flavonoids (1, 2, 3, 4). However, the phenolic compounds of the leaves of *P. africanum* Sond. were not subjected before, to any comprehensive study. In the present work, the aqueous alcohol extract of the leaves of *P. africanum* has been investigated for hepatoprotective activity represented by significant reduction of elevated serum liver enzymes and significant reduction in hepatic TBARS levels in hepatotoxicated rats with ethanol and carbon tetrachloride. On the other hand, a phytochemical screening, including color reactions and chromatographic analysis (5) of the extract has shown that it contains mainly phenolics. It was therefore, found reasonable to perform a comprehensive structural analysis of these phytophenolics. Fifteen phenolics, **1-15**, were isolated and purified from the extract. All structures were confirmed by conventional methods of analysis, ESI/MS and NMR analysis. *P. africanum* is a small to medium-sized tree of 5 to 15 meters tall, with a spreading green crown, frequently branches from near the ground into 2- or 3- stemmed trunk (6).

## MATERIAL AND METHODS

***Instruments and materials -***

$^{1}H$- NMR spectra were measured on Bruker AMX 400, relative to TMS. $^{13}C$-NMR were measured at 100 MHz, relative to $CDCl_3$ and converted to the TMS by adding 77. Typical conditions = 6000 Hz for $^{1}H$ and 22000 Hz for $^{13}C$, 32 k data points and a flip angle of 45°C.

ESI-MS spectra were measured on SSQ Finnigan MAT 4600 quadropol mass spectrometer (Institut für Chemie, Hamburg Universitiat). Paper chromatography analysis was carried out on Whatmann no. 1 paper, using solvent system: (1) $H_2O$; (2) HOAc; (3) BAW (*n*-BuOH-HOAc-$H_2O$, 4:1:5, upper layer). Solvents (2) and (3) were used for PPC on Whatmann no. 3 MM.

***Plant material***

Fresh leaves of *Peltophorum africanum* Sond. family Fabaceae was collected from Al Ourmann Garden, Giza, during October 2004 and authenticated by Professor Dr. Abdel Salam Mohamed Al-Nowiahi, Professor of Taxonomy, Faculty of Science, Ain -Shams University. A Voucher specimen of the authenticated plant was deposited at Department of Pharmacognosy, Faculty of Pharmacy, Ain-Shams University.

***Extraction, isolation, and purification***

Fresh leaves (5 Kg) were exhaustively extracted with aqueous alcohol (75 %), (20 L). The extract was dried in vacuum at low temperature till dryness (155.98 g). 2-DPC of the extract proved the presence of a high

percent of phenolic constituents (intense green $FeCl_3$ color reaction and chromatographic analysis) (5). Repeated fractionation of the extract (90 g) on Sephadex LH-20 columns, using $H_2O$ followed by $H_2O$ / MeOH mixtures of decreasing polarities yielded five fractions **(I – V),** which were individually subjected to 2-DPC. Compound (**1,** 145 mg) and (**2**, 125 mg) were isolated as pure compounds from fraction **I** (eluted with 20 %) by column fractionation over Sephadex LH-20 column using $H_2O$ for elution. Compounds (**3**, 88 mg; **4**, 102 mg and **5**, 54 mg) were individually separated from fraction **II** (eluted with 30 % by prep. PC, using BAW as solvent, while compounds (**6**, 75 mg; **7**, 78 mg; **8**, 63 mg; **9**, 111 mg) were obtained from fraction **III** (eluted with 40 %) through precipitation by ether from the acetone soluble fraction (thrice), and then subsequent prep. PC of the precipitate, using BAW as solvent. Repeated column fractionation of fraction **IV** (eluted with 50 %) on Sephadex LH-20 column, using *n*-BuOH saturated with $H_2O$ yielded pure samples of (**10**, 108 mg). Compounds (**11**, 79 mg; **12**, 134 mg; **13**, 102 mg and **14**, 85 mg and **15**, 90 mg) were isolated from fraction **V** (eluted with MeOH) by repeated prep. PC using BAW as solvent.

***3,4,5-trihydroxybenzoic acid*, *Gallic acid (1)*** was obtained as an off white amorphous powder (140 mg); $R_f$ values: 53 ($H_2O$), 56 (6% AcOH), and 78 (BAW). UV $\lambda_{max}$: MeOH (272 nm). $(M-H)^-$ molecular ion in its negative ESI/MS spectrum, at 169, corresponding to a $M_r$ of 170. $^1$H- NMR data: δ ppm 6.97 (*s*, H-2 & H-6). $^{13}$C-NMR data: δ ppm 121.0 (C-1), 109.0 (C-2 & C-6), 145.9 (C-3 & C-5), 138.3 (C-4), 168.0 (C-7). $^1$H- and $^{13}$C-NMR data were identical to those reported in literature (7, 8).

***4,5-dihydroxy-3-O-methylbenzoic acid*, *3-O-methyl gallic acid (2)*** was obtained as an off white amorphous powder (25 mg); $R_f$ values: 53 ($H_2O$), 56 (6% AcOH), and 78 (BAW). UV $\lambda_{max}$: MeOH (278 nm). ESI/MS indicated a molecular ion at $(M-H)^-$ 183, corresponding to a molecular weight ($M_r$) of 184. $^1$H- NMR data: δ ppm 7.16 (1H, *s*, H-2), 7.05 (1H, *s*, H-6), 3.79 (3H, *s*, -$OCH_3$). $^{13}$C-NMR data: δ ppm 120.9 (C-1), 105.2 (C-2), 148.2 (C-3), 139.5 (C-4), 145.5 (C-5), 111.2 (C-6), 167.1 (C-7, -COOH), 56.1 (-$OCH_3$). $^1$H- and $^{13}$C-NMR data were identical to those reported in literature (7, 8).

***Quercetin-3-O-$^1C_4$-a-L-rhamnopyranosyl-(1'''→6'')-$^4C_1$-β-D-glucopyranoside*, *Rutin (3)*** was obtained as an amorphous light yellow powder (75 mg); $R_f$ values: 57 ($H_2O$), 56 (6% AcOH), and 44 (BAW). UV $\lambda_{max}$: MeOH (259, 266sh, 299sh, 359), NaOMe (272, 327, 410), $AlCl_3$ (275, 303sh, 433), NaOAc (271, 325, 393), NaOAc/$H_3BO_3$ (262, 298, 387). ESI/MS indicated a molecular ion at $(M-H)^-$ 609, corresponding to a molecular weight ($M_r$) of 610. $^1$H- NMR data: δ ppm 6.10 (1H, *brs*, $\Delta v_{1/2}$= 4 Hz, H-6), 6.30 (1H, *brs*, $\Delta v_{1/2}$= 4 Hz, H-8), 6.80 (1H, *d*, *J*=7.5 Hz, H-5'), 7.50 (1H, *brs*, *d*, $\Delta v_{1/2}$= 4 Hz, *J*=7.5 Hz, H-6'), 7.54 (1H, *brs*, $\Delta v_{1/2}$= 4 Hz, H-2'), 5.32 (1H, *d*, *J*= 7.5 Hz, H-1''), 4.30 (1H, *brs*, $\Delta v_{1/2}$= 4 Hz, H-1'''), 3.2-3.8 (*m*, sugar protons), 1.01 (3H, *d*, *J*=6.0 Hz, H-6''', -$CH_3$). $^{13}$C-NMR data: δ ppm 156.2 (C-2), 133.5 (C-3), 177.3 (C-4), 161.2 (C-5), 100.1 (C-6), 164.3 (C-7), 94.5 (C-8), 157.1 (C-9), 102.0 (C-10), 121.1 (C-1'), 115.7 (C-2'), 145.6 (C-3'), 151.5 (C-4'), 116.3 (C-5'), 121.92 (C-6'), 101.1(C-1''), 74.4 (C-2''), 76.8 (C-3''), 70.2 (C-4''), 76.1 (C-5''), 67.3 (C-6''), 100.5 (C-1'''), 70.7 (C-2'''), 70.9 (C-3'''), 72.2 (C-4'''), 68.6 (C-5'''), 18.1 (C-6'''). $^1$H- and $^{13}$C-NMR data were identical to those reported in literature (8, 9, 10).

***Kaempferol-3-O-$^1C_4$-a-L-rhamnopyranosyl-(1'''→6'')-$^4C_1$-β-D glucopyranoside*, *Nicotiflorin (4)*** was obtained as an amorphous dull yellow powder (70 mg); $R_f$ values: 39 ($H_2O$), 42 (6% AcOH), and 30 (BAW). UV $\lambda_{max}$: MeOH (267, 353), NaOMe (275, 310, 402), $AlCl_3$ (272, 408), NaOAc (273, 355), NaOAc/$H_3BO_3$ (271, 355). ESI/MS indicated a molecular ion at $(M-H)^-$ 593, corresponding to a molecular weight ($M_r$) of 594. $^1$H-NMR data: δ ppm 6.17 (1H, *brs*, $\Delta v_{1/2}$= 4 Hz, H-6), 6.37 (1H, *brs*, $\Delta v_{1/2}$= 4 Hz, H-8), 6.83 (1H, *d*, *J*=7.5 Hz, H-3', H-5'), 7.53 (1H, *d*, *J*=7.5 Hz, H-2', H-6'), 5.31 (1H, *d*, *J*=7.5 Hz, H-1''), 4.38 (1H, *brs*, $\Delta v_{1/2}$= 4 Hz, H-1'''), 3.2-3.8 (*m*, sugar protons), and 1.16 (3H, *d*, *J*=6.2 Hz, -$CH_3$). $^{13}$C-NMR data: δ ppm 156.7 (C-2), 134.3 (C-3), 177.2 (C-4), 161.1 (C-5), 98.6 (C-6), 164.2 (C-7), 93.7 (C-8), 159.8 (C-9), 103.8 (C-10), 120.8 (C-1'), 115.0 (C-2', C-6'), 130.8 (C-3', C-5'), 160.0 (C-4'), 101.2 (C-1''), 74.1 (C-2''), 76.2 (C-3''), 69.8 (C-4''), 75.6 (C-5''), 66.8 (C-6''), 100.7 (C-1'''), 70.2 (C-2'''), 70.5 (C-3'''), 71.7 (C-4'''), 68.1 (C-5'''), 17.6 (C-6'''). $^1$H- and $^{13}$C-NMR data were identical to those reported in literature (8, 10).

***Kaempferol-3-O-$^4C_1$-β-D-glucoside*, *Astralagin (5)*** was obtained as an amorphous light brown powder (56 mg); $R_f$ values: 40 ($H_2O$), 52 (6% AcOH), and 20 (BAW). UV $\lambda_{max}$: MeOH (267, 353), NaOMe (275, 310, 402), $AlCl_3$ (272, 408), NaOAc (268, 355), NaOAc/$H_3BO_3$ (271, 355). ESI/MS indicated a molecular ion at $(M-H)^-$ 477, corresponding to a molecular weight ($M_r$) of 448. $^1$H-NMR data: δ ppm 6.23 (1H, *brs*, $\Delta v_{1/2}$= 4 Hz, H-6), 6.42 (1H, *brs*, $\Delta v_{1/2}$= 4 Hz, H-8), 6.92 (2H, *d*, *J*= 8 Hz,, H-3', H-5'), 8.08 (2H, *d*, *J*= 8 Hz, H-2', H-6'), 5.42 (1H, *d*, *J*= 7.5 Hz, H-1''), 3.2-3.8 (*m*, sugar protons). $^{13}$C-NMR data: 156.3 (C-2), 133.0 (C-3), 177.4 (C-4), 161.1 (C-

5), 98.7 (C-6), 164.1 (C-7), 93.6 (C-8), 156.3 (C-9), 104.1 (C-10), 121.0 (C-1'), 130.7 (C-2'), 115.0 (C-3'), 159.8 (C-4'), 115.0 (C-5'), 130.7 (C-6'), $^1$H-NMR data were identical to those reported in literature (8, 9, 11)

***Quercetin-3-O-$^4C_1$-β-D-glucoside*, *Isoquercitrin (6)*** was obtained as a dark brown amorphous powder (55 mg); $R_f$ values: 38 ($H_2O$), 60 (6% AcOH), and 19 (BAW). UV $\lambda_{max}$: MeOH (258, 267, 356), NaOMe (275, 470), $AlCl_3$ (263, 430), NaOAc (256, 374, 362), NaOAc/$H_3BO_3$ (265, 272, 380, 420sh). ESI/MS indicated a molecular ion at $(M-H)^-$ 463, corresponding to a molecular weight ($M_r$) of 464. $^1$H- NMR data: δ ppm 6.0 (1H, *brs*, $\Delta v_{1/2}$= 4 Hz, H-6), 6.22 (1H, *brs*, $\Delta v_{1/2}$= 4 Hz, H-8), 6.8 (1H, *d*, *J*=8.3 Hz, H-5'), 6.88 (1H, *dd*, *J*=8.3, 2.2 Hz, H-6'), 7.57 (1H, *d*, *J*=2.2 Hz, H-2'), 5.41 (1H, *d*, *J*=7.5 Hz, H-1"), 3.2-3.8 (*m*, sugar protons). $^{13}$C-NMR data: δ ppm 158.1 (C-2), 131.1 (C-3), 177.7 (C-4), 159.2 (C-5), 99.2 (C-6), 167.4 (C-7), 94.6 (C-8), 155.1 (C-9), 106.5 (C-10), 121.2 (C-1'), 115.5 (C-2'), 146.3 (C-3'), 149.8 (C-4'), 116.3 (C-5'), 121.8 (C-6'), 101.7 (C-1"), 74.5 (C-2"), 76.9 (C-3"), 70.2 (C-4"), 77.8 (C-5"), 61.2 (C-6"). $^1$H- and $^{13}$C-NMR NMR data were identical to those reported in literature (8, 9).

***Methyl 3,4,5-trihydroxybenzoate*, *Methyl gallate (7)*** was obtained as an amorphous off white powder (26 mg); $R_f$ values: 35 ($H_2O$), 48 (6% AcOH), and 73 (BAW). UV $\lambda_{max}$: MeOH (279), MeOH/KOH (236, 278, 317), $AlCl_3$ (298sh, nullified by adding HCl). ESI/MS indicated a molecular ion at $(M-H)^-$ 183, corresponding to a molecular weight ($M_r$) of 184. $^1$H- NMR data: δ ppm 6.91 (2H, *s*, H-2, H-6), 3.60 (3H, *s*, $-CH_3$). $^1$H-NMR data were identical to those reported in literature (12).

***Kaempferol-3-O-(6''-O-galloyl)-$^4C_1$-β-D-galactopyranoside (8)*** was obtained as an amorphous dull yellow powder (100 mg); $R_f$ values: 55 ($H_2O$), 45 (6% AcOH), and 72 (BAW). UV $\lambda_{max}$: MeOH (267, 319, 365). ESI/MS indicated a molecular ion at $(M-H)^-$ 599, corresponding to a molecular weight ($M_r$) of 600. $^1$H-NMR data: δ ppm 6.22 (1H, *d*, *J*=2.0 Hz, H-6), 6.42 (1H, *d*, *J*=2.0 Hz, H-8), 6.77 (2H, *d*, *J*=8.5, H-3', H-5'), 7.93 (2H, *d*, *J*=8.5 Hz, H-2', H-6'), 5.46 (1H, *d*, *J*=7.5 Hz, H-1"), 4.25 (1H, *d*, *J*=12.5 Hz, $H_a$-6"), 4.32 (1H, *dd*, *J*=3.5, 12.5 Hz, $H_b$-6"), 3.2-3.8 (*m*, sugar protons), 6.93 (1H, *s*, H-2'", H-6'"). $^{13}$C-NMR data: δ ppm 156.7 (C-2), 133.5 (C-3), 177.7 (C-4, (C=O)), 161.7 (C-5), 99.1 (C-6), 164.5 (C-7), 94.1 (C-8), 157.1 (C-9), 104.2 (C-10), 121.0 (C-1'), 115.4 (C-2', C-6'), 131.1 (C-3', C-5'), 160.3 (C-4'), 101.8 (C-1"), 74.4 (C-2", C-3"), 69.7 (C-4"), 76.5 (C-5"), 63.1 (C-6"), 119.6 (C-1'"), 108.9 (C-2'", C-6'"), 145.8 (C-3'", C-5'"), 139.1 (C-4'"), 166.1 (C-7'"), $^1$H- and $^{13}$C-NMR NMR data were identical to those reported in literature (9).

***Quercetin-3-O-(6''-O-galloyl)-$^4C_1$-β-D-galactopyranoside (9)*** was obtained as an amorphous brown powder (75 mg); $R_f$ values: 52 ($H_2O$), 49 (6% AcOH), and 59 (BAW). UV $\lambda_{max}$: MeOH (252, 319, 372). ESI/MS indicated a molecular ion at $(M-H)^-$ 615, corresponding to a molecular weight ($M_r$) of 616. $^1$H-NMR data: δ ppm 6.18 (1H, *d*, *J*=2.5 Hz, H-6), 6.37 (1H, *d*, *J*=2.5 Hz, H-8), 6.73 (1H, *d*, *J*= 8.0 Hz, H-5'), 7.45 (1H, *d*, *J*= 3.0 Hz, H-2'), 7.59 (1H, *dd*, *J*= 3.0, 8.0 Hz, H-6'), 5.44 (1H, *d*, *J*=7.5 Hz, H-1"), 4.25 (*d*, *J*= 12 Hz, $H_a$-6"), 4.32 (*dd*, *J*= 5Hz and 12 Hz, $H_b$-6"), 3.3-3.8 (*m*, sugar protons), 6.90 (1H, *s*, H-2'", H-6'"). $^{13}$C-NMR data: δ ppm 156.3 (C-2), 133.2 (C-3), 177.0 (C-4, (C=O)), 161.2 (C-5), 98.9 (C-6), 165.0 (C-7), 93.6 (C-8), 160.9 (C-9), 103.6 (C-10), 121.7 (C-1'), 115.6 (C-2'), 144.8 (C-3'), 148.8 (C-4'), 115.2 (C-5'), 120.7 (C-6'), 101.3 (C-1"), 73.9 (C-2"), 74.1 (C-3"), 69.3 (C-4"), 76.2 (C-5"), 62.9 (C-6"), 119.1 (C-1'"), 108.5 (C-2'", C-6'"), 145.5 (C-3'", C-5'"), 138.62 (C-4'"), 165.8 (C-7'"), $^1$H- and $^{13}$C-NMR NMR data were identical to those reported in literature (12).

***Myricetin-3-O-(6''-O-galloyl)-$^4C_1$-β-D-galactopyranoside (10)*** was obtained as an amorphous pure brown powder (108 mg); $R_f$ values: 30 ($H_2O$), 28 (6% AcOH), and 35 (BAW). UV $\lambda_{max}$: MeOH (252, 319, 372). $(M-H)^-$ molecular ion in its negative ESI/MS spectrum, at 631, corresponding to a $M_r$ of 632. $^1$H- NMR data: δ ppm 6.18 (1H, *d*, *J*=1.5 Hz, H-6), 6.37 (1H, *d*, *J*=1.5 Hz, H-8), 7.19 (2H, *s*, H-2', H-6'), 5.46 (1H, *d*, *J*=7.5 Hz, H-1"), 4.2 (*d*, *J*= 12 Hz, $H_a$-6"), 4.3 (*dd*, *J*= 5Hz and 12 Hz, $H_b$-6"), 3.3-3.8 (*m*, sugar protons), 6.88 (1H, *s*, H-2'", H-6'"). $^{13}$C-NMR data: δ ppm 155.9 (C-2, C-9), 133.5 (C-3), 177.7 (C-4, (C=O)), 161.1 (C-5), 98.6 (C-6), 164.1 (C-7), 93.3 (C-8), 103.8 (C-10), 121.8 (C-1'), 108.5 (C-2', C-6', C-2'", C-6'"), 145.2 (C-3', C-5', C-3'", C-5'"), 136.6 (C-4'), 101.1 (C-1"), 73.8 (C-2"), 69.7 (C-4"), 76.3 (C-5"), 63.7 (C-6") 120.0 (C-1'"), 74.4 (C-3"), 138.4 (C-4'"), 165.7 (C-7'"). $^1$H- and $^{13}$C-NMR NMR data were identical to those reported in literature (13).

***(-)-Epigallocatechin-3-O-gallate (11)*** was obtained as a buff amorphous powder (45 mg); $R_f$ values: 38 ($H_2O$), 34 (6% AcOH), and 44 (BAW). UV $\lambda_{max}$: MeOH (269). ESI/MS indicated a molecular ion at $(M-H)^-$ 457, corresponding to a molecular weight ($M_r$) of 458. $^1$H-NMR data: δ ppm 5.02 (1H, *d*, *J*=6.5 Hz, H-2), 5.23 (1H, *m*, H-3), 2.70 (1H, *dd*, *J*=16.4, 4.4 Hz, ax.H-4), 3.0 (1H, *dd*, *J*=16.4, 3.5 Hz, eq.H-4), 5.82 (1H, *d*, *J*=3.0 Hz, H-6), 5.93 (1H, *d*, *J*=3.0 Hz, H-8), 6.27 (2H, *s*, H-2',

H-6'), 6.86 (2H, *s*, H-2", H-6"). $^{13}$C-NMR data: δ ppm 77.3 (C-2), 69.3 (C-3), 21.4 (C-4), 155.0 (C-5), 94.4 (C-6), 157.3 (C-7), 95.8 (C-8), 156.3 (C-9), 97.8 (C-10), 119.4 (C-1'), 105.3 (C-2', C-6'), 145.9 (C-3', C-5'), 133.1 (C-4'), 128.9 (C-1"), 109.0 (C-2", C-6"), 146.5 (C-3", C-5"), 139.1 (C-4"), 165.7 (C-7", (C=O)), , $^{1}$H- and $^{13}$C-NMR NMR data were identical to those reported in literature (14).

***(+)-Gallocatechin-3-O-gallate (12)*** was obtained as a buff amorphous powder (30 mg); $R_f$ values: 42 ($H_2O$), 39 (6% AcOH), and 40 (BAW). UV $\lambda_{max}$: MeOH (269). ESI/MS indicated a molecular ion at $(M-H)^-$ 457, corresponding to a molecular weight ($M_r$) of 458. $^{1}$H-NMR data: δ ppm 5.03 (1H, *d*, *J*=4.8 Hz, H-2), 5.25 (1H, *m*, H-3), 2.60 (1H, *dd*, *J*=16.4, 4.4 Hz, ax.H-4), 2.85 (1H, *dd*, *J*=16.4, 3.5 Hz, eq.H-4), 5.83 (1H, *d*, *J*=3.0 Hz, H-6), 5.94 (1H, *d*, *J*=3.0 Hz, H-8), 6.28 (2H, *s*, H-2', H-6'), 6.87 (2H, *s*, H-2", H-6"). $^{13}$C-NMR data: δ ppm 77.1 (C-2), 69.1 (C-3), 22.9 (C-4), 154.6 (C-5), 94.2 (C-6), 156.6 (C-7), 95.6 (C-8), 156.1 (C-9), 97.6 (C-10), 119.2 (C-1'), 105.2 (C-2', C-6'), 145.3 (C-3', C-5'), 132.6 (C-4'), 128.7 (C-1"), 108.7 (C-2", C-6"), 145.7 (C-3", C-5"), 138.7 (C-4"), 165.4 (C-7", [C=O]). $^{1}$H- and $^{13}$C-NMR NMR data were identical to those reported in literature (14).

***5,7,3',4',5'- pentahydroxyflavon-3-ol, Myricetin (13)*** was obtained as a yellow amorphous powder (18 mg); $R_f$ values: 12 ($H_2O$), 4 (6% AcOH), and 29 (BAW). UV $\lambda_{max}$: MeOH (254, 272sh, 301sh, 374), NaOMe (262sh, 285sh, 322), $AlCl_3$ (271, 316sh), NaOAc (269, 335), NaOAc/$H_3BO_3$ (258, 304sh, 392). ESI/MS indicated a molecular ion at $(M-H)^-$ 317, corresponding to a molecular weight ($M_r$) of 318. $^{1}$H- NMR data: δ ppm 6.18 (1H, *d*, *J*=2.1 Hz, H-6), 6.37 (1H, *d*, *J*=2.1 Hz, H-8), 7.24 (2H, *s*, H-2', H-6'). $^{13}$C-NMR data: δ ppm 146.8 (C-2), 135.8 (C-3), 175.7 (C-4), 160.7 (C-5), 98.1 (C-6), 163.8 (C-7), 93.2 (C-8), 156.1 (C-9), 103.0 (C-10), 120.8 (C-1'), 107.2 (C-2', C-6'), 145.7 (C-3', C-5'), 135.8 (C-4') (15).

***5,7,3',4'- tetrahydroxyflavon-3-ol, Quercetin (14)*** was obtained as a yellow amorphous powder (21 mg); $R_f$ values: 20 ($H_2O$), 7 (6% AcOH), and 57 (BAW). UV $\lambda_{max}$: MeOH (255, 269sh, 301sh, 370), NaOMe (247sh, 321), $AlCl_3$ (272, 304sh, 333), NaOAc (257sh, 274, 329, 390), NaOAc/$H_3BO_3$ (261, 303sh, 388). ESI/MS indicated a molecular ion at $(M-H)^-$ 301, corresponding to a molecular weight ($M_r$) of 302. $^{1}$H- NMR data: δ ppm 6.18 (1H, *d*, *J*=2.0 Hz, H-6), 6.4 (1H, *d*, *J*=2.0 Hz, H-8), 7.67 (1H, *d*, *J*=2.2 Hz, H-2'), 6.89 (1H, *d*, *J*=8.3 Hz, H-5'), 7.53 (1H, *dd*, *J*=2.2, 8.3 Hz, H-6'). $^{13}$C-NMR data: δ ppm 146.8 (C-2), 135.6 (C-3), 175.7 (C-4), 160.6 (C-5), 98.1 (C-6), 163.8 (C-7), 93.3 (C-8), 156.1 (C-9), 103.0 (C-10), 121.9 (C-1'), 115.1 (C-2'), 145.0 (C-3'), 147.6 (C-4'), 115.5 (C-5'), 119.9 (C-6') (15).

***5,7,4'- trihydroxyflavon-3-ol, Kaempferol (15)*** was obtained as a yellow amorphous powder (18 mg); $R_f$ values: 31 ($H_2O$), 10 (6% AcOH), and 80 (BAW). UV $\lambda_{max}$: MeOH (253sh, 266, 294sh, 322sh, 367), NaOMe (278, 316), $AlCl_3$ (260sh, 268, 303sh, 350), NaOAc (274, 303, 387), NaOAc/$H_3BO_3$ (267, 297sh, 320sh, 372). ESI/MS indicated a molecular ion at $(M-H)^-$ 285, corresponding to a molecular weight ($M_r$) of 286. $^{1}$H- NMR data: δ ppm 6.18 (1H, *d*, *J*=2.1 Hz, H-6), 6.42 (1H, *d*, *J*=2.1 Hz, H-8), 7.88 (1H, *d*, *J*=8.5 Hz, H-2', H-6'), 6.91 (1H, *d*, *J*=8.8 Hz, H-3', H-5'), 12.43 (1H, *s*, OH-5). $^{13}$C-NMR data: δ ppm 146.8 (C-2), 135.2 (C-3), 175.7 (C-4), 160.4 (C-5), 98.1 (C-6), 163.8 (C-7), 93.4 (C-8), 156.1 (C-9), 103.0 (C-10), 122.7 (C-1'), 115.1 (C-2', C-6'), 135.0 (C-3', C-5'), 161.1 (C-4') (15).

***Evaluation of hepatotoxic activity***

***Animals:***

Male albino rats, their individual body weight ranging between 175-200 g. They were used for determination of median lethal dose ($LD_{50}$) of the aqueous ethanolic extract of leaves of *Peltophorum africanum* Sond. family Fabaceae.

***Determination of the median lethal dose ($LD_{50}$).***

It was performed according to the procedure described by (Hardison *et al*. 16*)*. Preliminary experiments were done to determine the minimal dose that kills all animals ($LD_{100}$) and the maximal dose that fails to kill any animal. For that purpose, several doses at equal logarithmic intervals were chosen in between these two doses, each dose was injected in a group of 6 animals by subcutaneous injection. The mice were then observed for 24 hours and symptoms of toxicity and mortality rates in each group were recorded and the $LD_{50}$ was calculated.

It revealed that the median lethal dose ($LD_{50}$) of the aqueous ethanolic extract of the leaves of *P. africanum* was **7.6** g / Kg b.wt.

***Experimental procedures***

2 g of the aqueous ethanolic extract were completely dissolved into about *5* ml of distilled water yielding an aqueous solution (400 mg/ml), which was sterilized by filtration through sterile 0.22i micropore membrane filter, *25* mm in diameter (Microfiltration systems (MFS), Japan) and dispensed into (1.0 ml) aliquots into sterile screw-capped plastic vials, which were stored at 4°C till being tested.

Thirty two male albino rats were used to study hepatoprotective activity as described by (17) with

little modification. Animals were divided into four groups; eight rats in each group. Group (I) represented control group; vehicle-treated group. In group (II), rats were given a single oral dose of 40 % ethanol (2 ml/100 g body weight); daily for 21 consecutive days. On the 20th day, animals were injected subcutaneously with 0.1 ml of 50 % (v/v) solution of carbon tetrachloride ($CCl_4$) in olive oil per Kg body weight, and then they were sacrificed 48 hours later.

Animals of groups (III) and (IV) were put to similar protocol as those of group (II). Moreover, rats of groups (III) and (IV) were received an oral dose of the aqueous ethanolic extract of leaves of *P. africanum* equivalent to (250 mg / Kg body weight / day, orally) and silymarin (25 mg / Kg body weight / day, orally) daily from the l5th day onward till the 21st day, respectively.

For scarification, on the 22nd day from the beginning of the experiment, rats of all groups of the experiment were anaesthetized with ether; orbital blood was collected using heparinized microcapillaries. Serum was separated for determination of activities of serum transaminases (AST and ALT). Then, all animals were killed by cervical dislocation and the livers were dissected out, washed with saline, plotted dry on filter paper, and weighed. Afterwards, liver homogenate was prepared in ice cold 0.1 M potassium chloride, KCl, and used for the determination of GSH and TBARS.

***Analytical method:***

Serum transaminase; Alanine aminotransferase (ALT) and aspartate aminotransferase (AST); activities were determined using Quimica Clinica Aplicada (QCA) diagnostic kits (Spain) and measured spectrophotometrically using the methods described by (Wroblewski and Due, 18). Reduced glutathione (GSH) was estimated following the method described by (Beutler *et.al*. 19). The lipid peroxidation products were estimated by the determination of the level of thiobarbituric acid reactive substances (TBARS) that were measured as malondialdehyde (MDA) following the method described by (Yoshioka *et al.*, 20).

**RESULTS AND DISCUSSION**

the aqueous ethanolic extract of the leaves showed promising hepatoprotective, was inspected for its constitutive phytochemicals (5) to prove the presence of a high percent of phenolic constituents (intense blue $FeCl_3$ color reaction and paper chromatographic analysis). The extract was then subjected to repeated Sephadex LH-20 column fractionation, using $H_2O$ followed by $H_2O$ / MeOH mixtures of decreasing polarities followed, in some cases, by prep.PC of the received desorbed fractions, to afford compounds **1-15.** The compounds gave chromatographic, UV, ESI-MS, $^1H$, and $^{13}C$ NMR data identical with those reported for gallic acid **1** (7, 8, 21) 3-*O*-methoxy gallic acid **2** (7, 8, 22) ; quercetin 3-*O*-$^1C_4$-α-rhamnopyranosyl-(1"→6")-$^4C_1$-ß-glucopyranoside, (rutin) **3** (8, 9, 10); kaempferol 3-*O*-$^1C_4$-α-rhamnopyranosyl-(1"→ 6")-$^4C_1$-ß - glucopyranoside, (nicotiflorin) **4** (8, 10); kaempferol 3-*O*-$^4C_1$-ß-glucoside **5** (8, 9, 10, 11); quercetin-3-*O*-$^4C_1$-ß - D-glucopyranoside, (isoquercitrin) **6** (8, 9, 10, 11 ); methyl gallate **7** (12) , kaempferol-3-O-(6"-O-galloyl)-$^4C_1$-ß-D-galactopyranoside **8** (9); quercetin3-*O*-(6"-galloyl)-$^4C_1$-ß-D galacto-pyranoside **9** (10, 23); Myricetin-3-O-(6"-O-galloyl)-$^4C_1$-ß-D-galactopyranoside **10** (13); (-)- epigallocatechin-3 - *O*-gallate **11** (14); (+)-gallocatechin-3 -*O*-gallate **12** (14, 24) ; myricetin **13**; (15) quercetin **14** (15); kaempferol **15** (15), respectively.

The tested extract rich in phytophenols showed a significant hepatoprotective activity when orally administered at a dose of 250 mg / Kg b.wt. in comparison to an oral dose of 25 mg / Kg b.wt. of silymarin as a reference drug (Table 1). Serum transaminases (AST and ALT) are reliable markers of liver function. Indeed they were significantly increased in group (II), treated with ethanol and $CCl_4$. However, the elevated activities of AST and ALT have decreased significantly in animals of group (III) that were treated with the tested extract. This finiding suggests that the hepatoprotective action might be due to the effects of the used extract of *P. africanum*.

In the present study, the observed decrease in content of reduced glutathione (GSH) in group (II), ethanol+$CCl_4$ treated rats may be due to non-enzymatic interaction of GSH with excessive free radicals generated by the toxic insult in rat liver (25).

The demonstrated protective antioxidant properties of the tested extract of *P. africanum* can be attributed to its content of flavonoids and polyphenolics (26).

In fact, there is a direct correlation between GSH depletion and enhanced lipid peroxidation (27). This was also observed in this study, as depletion of liver GSH was accompanied by significant elevation in hepatic tissue content of TBARS. Also, a significant reduction in hepatic TBARS levels in rats of group (III), treated with the tested extract.

**CONCLUSION**

The results of the present study suggest that the aqueous ethanolic extract of leaves of *Peltophorum africanum* Sond. family Fabaceae possesses a potential activity against hepatotoxicity induced by oral

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*Table 1: Results of hepatoprotective activity study*

| Parameters | Group (I) | Group (II) | Group (III) | Group (IV) |
|---|---|---|---|---|
| **AST** (IU / L) | 13.39 ± 1.28 | 34.41 ± 2.96[a] | 25.48 ± 2.26[a,b] | 20.93 ± 1.97[b] |
| **ALT** (IU / L) | 6.61 ± 0.56 | 13.43 ± 1.2[a] | 7.93 ± 0.66[b] | 7.5 ± 0.41[b] |
| **GSH** (µmol /g ) | 1.68 ± 0.25 | 0.74 ± 0.14[a] | 1.42 ± 0.08[a] | 1.53 ± 0.07[b] |
| **TBARS** (µmol/g) | 90.56 ± 2.51 | 282.67 ± 16.6[a] | 158.63 ± 5.76[a,b] | 120.47 ± 2.06[a,b] |

*Data are presented as (means ± SE, n=8).*

*Multiple comparisons were done using one way ANOVA followed by Tukey-Kramer as post-ANOVA test.*

[a] *Significantly different from corresponding control at ($p < 0.05$).*

[b] *Significantly different from corresponding group (II), ($EtOH+CCl_4$)-treated, at ($p<0.05$).*

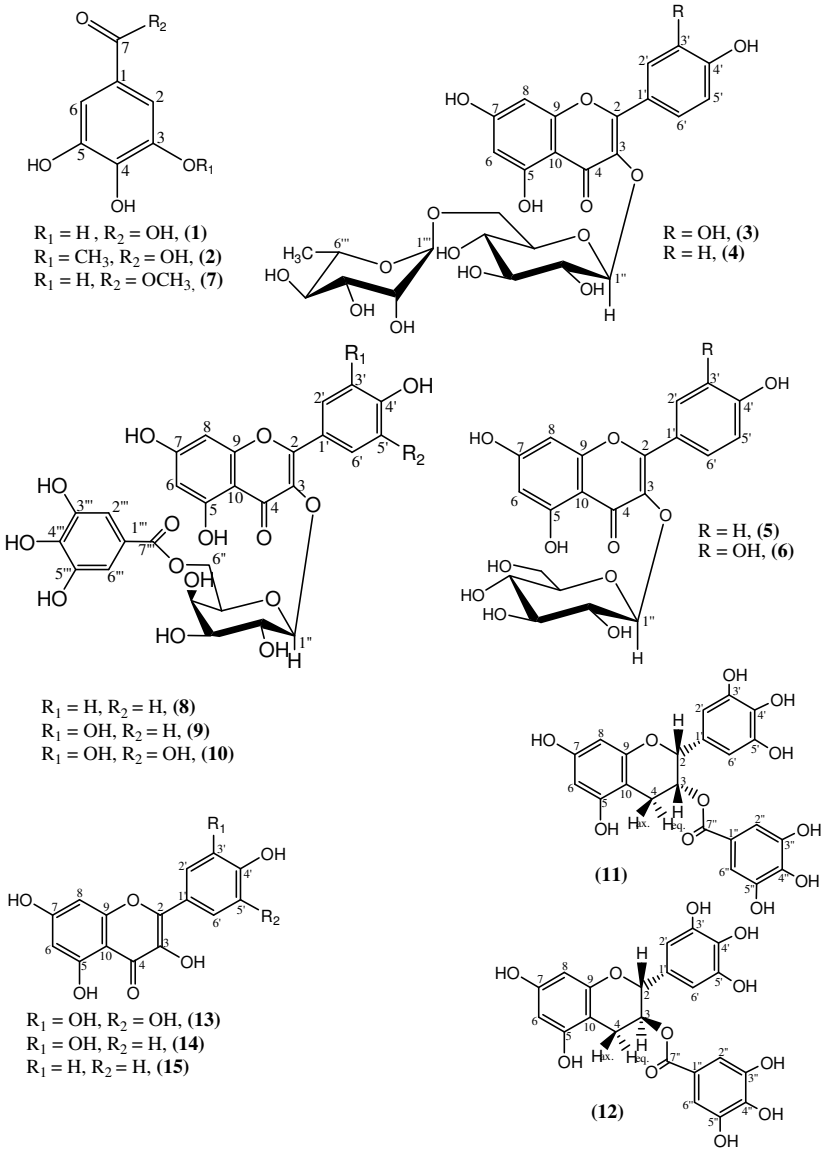


*Figure (1) Structures of isolated compounds (1-15)*

administration of Ethanol+$CCl_4$ in rats. This encourages further and more clinical studies on *Peltophorum* extract. This is the first report concerning the hepatoprotective activity and phenolics metabolites from active extract of the *P. africanum*.

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