



Phenolic constituents with promising antioxidant and hepatoprotective activities from the leaves extract of *Carya illinoensis*

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ABSTRACT

The aqueous ethanolic leaf extract of *Carya illinoensis* Wengen. K.Koch (Juglandaceae) showed a significant antioxidant and hepatoprotective activities in a dose of 100 mg/ kg body weight. Fifteen phenolic compounds were isolated from the active extract among which ten were identified for the first time from *Carya illinoensis*. Their structures were elucidated to be gallic acid(1), methyl gallate(2), P-hydroxy benzoic acid(3), 2,3-digalloyl- β -D-⁴C₁-glucopyranoside(4), kaempferol-3-O- β -D-⁴C₁-galactopyranoside, trifolin(8), quercetin-3-O-(6'-O-galloyl)- β -D-⁴C₁-galactopyranoside(9), kaempferol-3-O-(6'-O-galloyl)- β -D-⁴C₁-galactopyranoside(10), ellagic acid(11), 3,3' dimethoxyellagic acid(12), epigallocatechin-3-O-gallate(13). Establishment of all structures were based on the conventional methods of analysis and confirmed by NMR spectral analysis.

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KEYWORDS

Carya illinoensis;
Juglandaceae;
Phenolic compounds;
Hepatoprotective activity.

INTRODUCTION

Family Juglandaceae includes the deciduous genera, *Juglans*(walnuts) and *Carya*(hickories). The species *Carya illinoensis* Wengen. K.Koch, known in English as Pecan, is a large, vase-shaped tree of a 25-45m tall and a trunk of 2.1m in diameter. The leaves are alternate, pinnately compound, 10 to 20 inches long, 11 to 17 leaflets that are 4 to 8 inches long^[1]. Pecan trees produce edible nuts that have a high percentage of fat and are used extensively in candies and cookies^[2]. It was used by the Comanche as a treatment for ringworm^[3]. The Kiowa would consume a concentrate made from the bark of pecan for tuberculosis^[3]. Among the previously identified phenolics of *C. illinoensis*, azaleatin(quercetin-5-methyl ether) and azaleatin glucoside, arabinoside, rutinoside and rhamnoside. In ad-

dition, caryatin(quercetin-3,5-dimethyl ether), caryatin glucoside and rhamnoglucoside were also isolated from the bark^[4], while, quercetin glycoside, galactoside, rhamnoside, arabinoside and kaempferol-3-monomethyl ether were identified in the leaves. The present study describes the isolation and structure elucidation of phenolic metabolites from the aqueous alcohol extract of the leaves *Carya illinoensis*(Juglandaceae) of promising hepatoprotective activity.

EXPERIMENTAL

Plant materials

Fresh leaves of *Carya illinoensis* Wengen. K.Koch(Juglandaceae) were collected on May, 2005, from a mature tree at the yellow mountain area, Cairo, Egypt and identified by Professor Dr.Abdel Salam

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Mohamed AL-Nowiahai, Professor of Taxonomy, Faculty of Science, Ain Shams University. The fresh leaves were collected at (May-August). A voucher specimen has been deposited at the herbarium of the department of pharmacognosy, faculty of pharmacy, Ain-Shams University, Cairo, Egypt.

Plant extract

The fresh leaves were air-dried in shade, reduced to fine powder. The powdered leaves (500g) were exhaustively extracted with water. The extract was filtered and evaporated in vacuo at 45°C till dryness. The dried material was extracted with ethanol (3 times, each with 500ml ethanol for 8hrs, under reflux over hot water bath). The collected ethanol portions were evaporated in vacuum till dryness. Then the dry residual powder (total extract, 55g) were then subjected to phytochemical and biological investigations.

Biological assays

1. Experimental animals

Experimental animals used consisted of albino mice of 25-30g body weight and adult male albino rats of sprague dawley strain of 130-150g body weight. Doses of the drugs used were calculated according to Paget and Barne's^[5] and were administered orally by gastric tubes.

2. Determination of the median lethal dose (LD₅₀)

The LD₅₀ of the aqueous-ethanol extract of *C. Illinoensis* was estimated according to the Karber procedure^[6]. Preliminary experiments were done to determine the minimal dose that kills all animals (LD₁₀₀) and the maximal dose that did not kill any animal. Several doses at equal logarithmic intervals were chosen in between, 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 LD₅₀ was calculated.

3. Antioxidant activity

This method depends on measuring the blood glutathione level and was determined according to the method of Beutler et al.^[7]. The method depends on the fact that both protein and non protein SH-groups react with Ellman's reagent [5,5-dithiobis-(2-nitrobenzoic

acid)] to form a stable yellow colour of 5-thio-2-nitrobenzoic acid, which can be measured at 412nm. In order to determine the glutathione level in the blood, precipitation of protein SH-groups was necessary before the addition of Ellman's reagent. Blood sample (0.1 ml) was haemolysed by the addition of 0.9ml bidistilled water. To the haemolysate, 1.5ml of the precipitating solution was added, mixed and allowed to stand for 5 minutes. Centrifugation at 3000 rpm was carried out for 15min. To 1ml of the resulting supernatant, 4ml of phosphate buffer solution was added followed by 0.5ml of Ellman's reagent. The optical density was measured within 5 minutes at 412nm using the Shimadzu double beam spectrophotometer (UV-150-02). The blank solution for the samples was prepared with 4ml phosphate solution, 1ml dilute precipitating solution (3:2) and 0.5ml Ellman's reagent. To 1ml standard glutathione solution, 4ml phosphate buffer solution and 0.5ml Ellman's reagent were added and the optical density was measured at 412nm against blank containing 1ml bidistilled water instead of the standard solution using the following equation:

$$\text{GSH (mg\%)} = \frac{\text{absorbance of sample}}{\text{absorbance of the standard}} \times \frac{37.5}{1000} \times \frac{2.5}{0.1} \times 100$$

All statistical analyses were performed using the student "t" test as described by Spedecor and Cochran^[8].

4. Hepatoprotective activity

Liver damage in rats was induced according to the method of Klassen and Plaa^[9] by intraperitoneal injection of 5ml/Kg of 25% carbon tetrachloride (CCl₄) in liquid paraffin. Animals were randomly divided into three groups each of 10 rats:

1. First group: Represented control group, received a daily oral dose of 1ml saline for one month before and after liver damage.
2. Second group: Liver damaged rats were pretreated with a daily oral dose of 100mg/Kg body weight of the extract for one month. Administration of the extract was continued for one more month.
3. Third group: Liver damaged rats were pretreated with a daily oral dose of 25mg/Kg body weight of silymarin. Administration of the drug (silymarin) was continued after liver damage for one more month.

The whole blood was obtained from the retro-orbital venous plexus through the eye canthus of anaes-

thetized rat after overnight fasting. Blood samples were collected at zero time, one month and 72hrs after CCl_4 injection and after one month intervals. Serum was isolated by centrifugation (Serum ALT, AST^[10] and ALP^[11]).

The data obtained were analyzed using the student's *t* test (Snedecor and Cochran 1991)^[9].

Isolation and structural elucidation

The prepared extract (55g) was fractionated on a sephadex LH-20 column, (100cm×5cm) eluted with distilled water, followed by mixtures of water/methanol of decreasing polarities and finally by acetone. Fractions of 1-2L. were collected and monitored by 2D paper chromatography using solvent systems; BAW/6% acetic acid. Similar fractions were pooled together to give six main fractions (I-VI). 2D-PC, showed that fraction I consisted mainly of carbohydrates, fraction II-V contain mainly phenolic constituents and fraction VI contained condensed tannins. A combination of column fractionation and PPC led to isolation of fifteen pure phenolics from fractions (II-V). Elution with H_2O gave rise to fraction I (25g, 4L). Elution with 20%, 40%, 60%, 80% MeOH afforded fraction II (6L), III (4L), IV (6L), V (5.5L), respectively. Fraction II (9g) was refractionated on polyamide 6s column (\varnothing 2.5×100cm, 120g). Elution was then started with water followed by H_2O -MeOH mixtures of decreasing polarities to afford 3 subfractions were then subjected to PPC to yield pure samples of compounds (1-4). Fraction III (5g) was applied to Sephadex LH-20 column (\varnothing 2×50cm, 30g). Elution with water followed by H_2O -MeOH mixtures of decreasing polarities afforded pure sample of compounds (5-8). Prep.PC over Whatman 3mm of fraction IV (2.2g) using BAW as solvent afforded pure samples of compounds (9 and 10). Fraction V (3.8g) was refractionated Sephadex LH-20 column (\varnothing 2×40cm, 70g). Elution with ethyl acetate water saturated followed by PPC afforded pure sample of (11-15). 3,4,5-trihydroxy benzoic acid, Gallic acid (1) was obtained as an off-white amorphous powder (140 mg); R_f values: 56 (HOAc-6%) and 78 (BAW); UV λ_{MAX} : MeOH (272nm). ¹H-NMR data: δ ppm 6.97(s, H-2 & H-6). ¹³C-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). ¹H- and ¹³C-NMR data were identical to those reported in literature^[12-13].

Methyl 3,4,5-trihydroxybenzoate, Methyl gallate (2) was obtained as an off-white amorphous powder (26mg); R_f values: 48 (HOAc-6%) and 73 (BAW); UV λ_{MAX} : MeOH (279nm), MeOH/KOH (236, 278, 317), AlCl_3 (298sh, nullified by adding HCl). ¹H-NMR data: δ ppm 6.91(2H, s, H-2, H-6), 3.3(3H, s, -CH₃). ¹H-NMR data was identical to that reported in literature^[14-15].

p-Hydroxy benzoic acid (3) was obtained as an off-white amorphous powder (125mg); R_f values: 36 (HOAc-6%) and 84 (BAW); UV λ_{MAX} : MeOH (253). ¹H-NMR data δ ppm 7.8(d, J=9Hz, H-2, H-6), 6.8(d, J=9Hz, H-3, H-5). ¹³C-NMR data: δ ppm 121.6 (C-1), 131.3(C-2 & C-6), 115.1(C-3 & C-5), 160.6 (C-4), 167.7(C=O). ¹H- and ¹³C-NMR data were identical to those reported in literature^[16].

2,3-digalloyl- β -D-⁴C₁-glucopyranoside, nilocitin (4) was obtained as an off-white amorphous powder (26 mg); R_f values: 73 (HOAc-6%) and 33 (BAW); UV λ_{MAX} : MeOH (253). ¹H-NMR data: δ ppm α -glucose; 5.4(d, J=3.3Hz, H-1), 4.92(dd, J=8, 3.3Hz, H-2), 5.78(t, J=8Hz, H-3), 3.6-4.0(m, H-4, H-5, H-6, H-6'), β -glucose; 4.98(d, J=7.5Hz, H-1), 5.08(dd, J=8, 3.3Hz, H-2), 5.41(t, J=8Hz, H-3), 3.6-4.0(m, H-4, H-5, H-6, H-6'), Galloyl in α - and β -anomer; 7.05(s), 7.07 (s) (H-2, 6). ¹³C-NMR data: δ ppm α -glucose; 89.3(C-1), 72.2(C-2), 72.2(C-3), 68.3(C-4), 72.2(C-5), 60.6(C-6). β -glucose; 94.5(C-1), 73.1(C-2), 75.5(C-3), 68.3 (C-4), 76.7(C-5), 60.6(C-6). Galloyl in α - and β -anomer; 121.38(C-1), 109.97(C-2, 6), 145.64(C-3, 5), 138.9(C-4), 165.4(C=O). ¹H- and ¹³C-NMR data were identical to those reported in literature^[17].

Quercetin-3-O- β -D-⁴C₁-glucopyranoside, isoquercitrin (5) was obtained as dark brown amorphous powder (25mg); R_f values: 60 (HOAc-6%) and 19 (BAW); UV λ_{MAX} : MeOH (258, 267, 356), NaOMe (275, 470), AlCl_3 (263, 430), NaOAc (256, 374, 362), NaOAc/ H_3BO_3 (265, 272, 380, 420sh). ¹H-NMR data: δ ppm 7.57(1H, d, J=2.2Hz, H-2'), 6.88(1H, dd, J=8.3, 2.2Hz, H-6'), 6.8(1H, d, J=8.3Hz, H-5'), 6.22 (1H, s, H-8), 6.0(1H, s, H-6), 5.41(1H, d, J=7.5Hz, H-1'), 3.2-3.8(m, sugar protons). ¹³C-NMR data: δ ppm 177.7(C-4), 167.4(C-7), 159.2(C-5), 158.1(C-2), 155.13(C-9), 149.8(C-4'), 146.3(C-3'), 131.03 (C-3), 121.78(C-6'), 121.2(C-1'), 116.26(C-5'), 115.5 (C-2'), 101.68(C-1''), 106.5(C-10), 99.2(C-6), 94.55(C-8),

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77.78(C-5''), 76.88(C-3''), 74.47(C-2''), 70.21 (C-4''), 61.24(C-6''). ¹H- and ¹³C-NMR data were identical to those reported in literature^[18-19].

Quercetin 3-O-β-D-⁴C₁-galactopyranoside, hyperin(6) was obtained as pale yellow amorphous powder(40mg); R_f values: 58(HOAc-6%) and 36(BAW); UV λ_{MAX}: MeOH(257, 269sh, 299sh, 362), NaOMe(272, 327, 409), AlCl₃(275, 305sh, 331sh, 438), NaOAc(274, 324, 380), NaOAc/H₃BO₃(262, 298sh, 377). ¹H-NMR data: δppm 5.36(1H, d, J=7 Hz, H-1''), 6.19(1H, d, J=2Hz, H-6), 6.40(1H, d, J=2 Hz, H-8), 6.82(1H, d, J=8.5Hz, H-5'), 7.54(1H, d, J=2Hz, H-2'), 7.65(dd, J=2, 8.5Hz, H-6'). ¹³C-NMR data: δppm 60.45(C-6''), 67.99(C-4''), 71.26(C-2''), 73.22(C-3''), 75.91(C-5''), 93.59(C-8), 98.75(C-6), 101.80(C-1''), 104.0(C-10), 115.25 (C-2'), 116.0(C-5'), 121.15(C-1'), 122.20(C-6'), 133.52(C-3), 144.91(C-3'), 148.53(C-4'), 156.30(C-9), 156.37(C-2), 161.30(C-5), 164.19(C-7), 177.57 (C-4). ¹H- and ¹³C-NMR data were identical to those reported in literature^[20].

Quercetin 3-O-α-L-¹C₄-rhamnopyranoside, quercitrin(7) was obtained as pale yellow amorphous powder(20mg); R_f values: 58(HOAc-6%) and 36(BAW); UV λ_{MAX}: MeOH(259, 297sh, 348), NaOMe(270, 355, 402), AlCl₃(368, 352, 408), NaOAc(276, 372), NaOAc/ H₃BO₃(272, 383). ¹H-NMR data: δppm 5.36(1H, d, J=7Hz, H-1''), 6.19(1H, d, J=2Hz, H-6), 6.40(1H, d, J=2Hz, H-8), 6.82(1H, d, J=8.5Hz, H-5'), 7.54(1H, d, J=2Hz, H-2'), 7.65(dd, J=2, 8.5Hz, H-6'). ¹³C-NMR data: δppm 60.45(C-6''), 67.99(C-4''), 71.26(C-2''), 73.22(C-3''), 75.91(C-5''), 93.59(C-8), 98.75(C-6), 101.80(C-1''), 104.0(C-10), 115.25(C-2'), 116.0(C-5'), 121.15(C-1'), 122.20(C-6'), 133.52(C-3), 144.91(C-3'), 148.53(C-4'), 156.30(C-9), 156.37(C-2), 161.30 (C-5), 164.19(C-7), 177.57(C-4). ¹H- and ¹³C-NMR data were identical to those reported in literature^[21].

Kaempferol-3-O-β-D-⁴C₁- galactopyranoside, trifolin(8) was obtained as bright yellow amorphous powder(30mg); R_f values: 34(HOAc-6%) and 63(BAW); UV λ_{MAX}: MeOH(266,351), NaOMe (273,401), AlCl₃(275, 402), NaOAc(274, 376), NaOAc/H₃BO₃(267, 354). ¹H-NMR data: δppm 6.196(1H, d, J= 2.1Hz, H-6), 6.438(1H, d, J= 2.1Hz, H-8), 8.03(2H, d, J=9.0Hz, H-2', H-6'), 6.937(2H,

d, J= 9.0Hz, H-3', H-5'). 5.425(d, J=7.2Hz, H-1 galactose), 3.08- 3.88(m, six sugar protons). ¹³C-NMR data: δppm 156.423(C-2), 133.06(C-3), 177.78(C-4), 161.176(C-5), 98.927(C-6), 164.51(C-7), 93.857 (C-8), 156.60(C-9), 103.905(C-10), 120.64(C-1'), 130.914(C-2' & C-6'), 115.159(C-3' & C-5'), 160.131(C-4'), 101.968(C-1''), 71.70(C-2''), 73.4 (C-3''), 67.81(C-4''), 75.74(C-5'') 60.44(C-6''). ¹H- and ¹³C-NMR data were identical to those reported in literature^[22].

Kaempferol-3-O-(6''-O-galloyl)-β-D-⁴C₁-galactopyranoside(9) was obtained as dull yellow amorphous powder(100mg); R_f values: 45(HOAc-6%) and 72(BAW); UV λ_{MAX}: MeOH(267, 319, 365). ¹H-NMR data: δppm 6.42(1H, d, J=1.2Hz, H-8), 6.22(1H, d, J=1.2Hz, H-6), 7.93(1H, d, J=7.5Hz, H-2', H-6'), 6.77(1H, d, J=7.5, H-3', H-5'), 5.46(1H, d, J=7.2Hz, H-1''), 3.2-3.8(m, sugar protons), 5.46(1H, d, J=7.2 Hz, H-1''), 6.93(1H, s, H-2''', H-6'''). ¹³C-NMR data: δppm 177.66(C-4,[C=O]), 166.09(C-7'''), 164.48(C-7), 161.68(C-5), 160.3(C-4'), 157.08(C-9), 156.7(C-2), 145.76(C-3''', C-5'''), 139.05(C-4'''), 133.5(C-3), 131.12(C-3', C-5'), 121.0(C-1'), 119.6(C-1'''), 115.44(C-2', C-6'), 108.95(C-2''', C-6'''), 104.15(C-10), 101.77(C-1''), 99.14(C-6), 94.12(C-8), 76.5(C-5''), 74.4(C-2'', C-3''), 69.74(C-4''), 63.12(C-6''). ¹H- and ¹³C-NMR data were identical to those reported in literature^[23-24].

Quercetin-3-O-(6''-O-galloyl)-β-D-⁴C₁-galactopyranoside(10) was obtained as brown amorphous powder(75mg); R_f values: 49(HOAc-6%) and 59(BAW); UV λ_{MAX}: MeOH(252, 319, 372). ¹H-NMR data: δppm 6.37(1H, d, J=2.5Hz, H-8), 6.18(1H, d, J=2.5Hz, H-6), 7.59(1H, dd, J=3.0, 11.0Hz, H-6'), 7.45(1H, d, J=3.0Hz, H-2'), 6.73(1H, d, J=11.0Hz, H-5'), 5.44(1H, d, J=9.0Hz, H-1''), 3.3-3.8(m, sugar protons), 6.90(1H, s, H-2''', H-6'''). ¹³C-NMR data :δppm 177.03(C-4,[C=O]), 165.76(C-7'''), 165.02 (C-7), 161.22(C-5), 160.85(C-9), 156.32(C-2), 148.84(C-4'), 145.52(C-3''', C-5'''), 144.78(C-3'), 138.62(C-4'''), 133.23(C-3), 121.73(C-1'), 120.67 (C-6'), 119.12(C-1''), 115.58(C-2'), 115.19(C-5'), 108.5(C-2''', C-6'''), 103.62(C-10), 101.315(C-1''), 98.88(C-6), 93.6(C-8), 76.17(C-5''), 74.11(C-3''), 73.94(C-2''), 69.34(C-4''), 62.95(C-6''). ¹H- and ¹³C-NMR data were identical to those reported in lit-

erature^[23-24].

3,3' Dimethoxyellagic acid(11) was obtained as white crystalline powder(24mg); R_f values: 16(HOAc-6%) and 91(BAW); UV(MeOH): $\lambda_{MAX}(\log \epsilon) = 251, 362, 375$. 1H -NMR data: δ ppm 7.49(s, H-5, 5'), 4.08 (O-CH₃). ^{13}C -NMR data: δ ppm 111.8(C-1, C-1'), 141.1(C-2, C-2'), 140.2(C-3, C-3'), 152.4(C-4, C-4'), 111.4(C-5, C-5'), 112(C-6, C-6'), 158.3(C-7, C-7'), 60.9(O-CH₃). 1H - and ^{13}C -NMR data were identical to those reported in literature^[25-26].

Ellagic acid(12) was obtained as white crystalline powder(24mg); R_f values: 9(HOAc-6%) and 48(BAW); UV λ_{MAX} : MeOH(255, 362). 1H -NMR data: δ ppm 7.48(s, H-5, 5'). ^{13}C -NMR data: δ ppm 112.3(C-1, C-1'), 136.4(C-2, C-2'), 140.2(C-3, C-3'), 153(C-

4, C-4'), 111.4(C-5, C-5'), 107.6(C-6, C-6'), 159.2 (C-7, C-7'). 1H - and ^{13}C -NMR data were identical to those reported in literature^[27].

(-)-Epigallocatechin-3-O-gallate(13) was obtained as buff amorphous powder(45mg); R_f values: 34 (HOAc-6%) and 44(BAW); UV λ_{MAX} : MeOH(269). 1H -NMR data: δ ppm 5.02(1H, d, J=6.5Hz, H-2), 5.23 (1H, m, H-3), 2.70(1H, dd, J=16.4, 4.4Hz, ax.H-4), 3.0(1H, dd, J=16.4, 3.5Hz, eq.H-4), 5.82(1H, d, J=3.0Hz, 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 165.69(C-7'', [C=O]), 157.30(C-7), 156.32(C-9), 155.04(C-5), 146.5(C-3'', C-5''), 145.83(C-3', C-5'), 139.07(C-4''), 133.1(C-4'), 128.9(C-1''), 119.36(C-1'), 109.0(C-2'', C-6''), 105.3

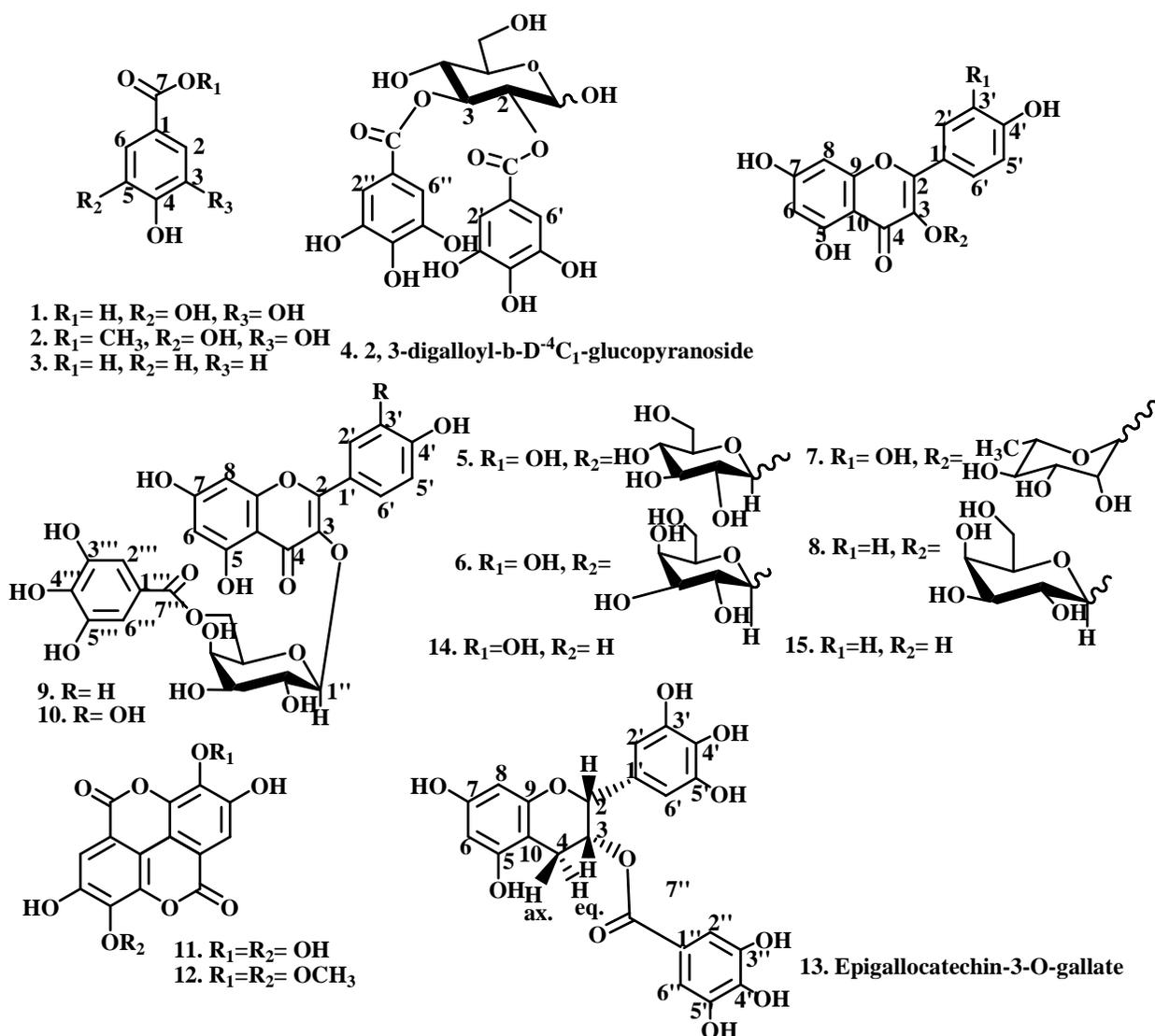


Figure 1: Structures of the isolated compounds(1-15)

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(C-2', C-6'), 97.78(C-10), 95.77(C-8), 94.39 (C-6), 77.325(C-2), 69.31(C-3), 21.42(C-4). ^1H - and ^{13}C -NMR data were identical to those reported in literature^[28-29].

5,7,3',4'-Tetrahydroxyflavon-3-ol, quercetin(14) was obtained as yellow amorphous powder(21mg); R_f values: 7(HOAc-6%) and 57(BAW); UV λ_{MAX} : MeOH(269), NaOMe(274sh, 321), AlCl_3 (272, 304sh, 333), NaOAc(275sh, 274, 329, 390), NaOAc/ H_3BO_3 (261, 303sh, 388). ^1H -NMR data: δ ppm 6.18(1H, d, $J=2.0\text{Hz}$, H-6), 6.4(1H, d, $J=2.0\text{Hz}$, H-8), 7.67(1H, d, $J=2.2\text{Hz}$, H-2'), 6.89(1H, d, $J=8.3\text{Hz}$, H-5'), 7.53 (1H, dd, $J=2.2, 8.3\text{Hz}$, 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'). ^1H - and ^{13}C -NMR data were identical to those reported in literature^[30].

5,7,4'- Tetrahydroxyflavon-3-ol, kaempferol(15) was obtained as dull yellow amorphous powder(24 mg); R_f values: 10(HOAc-6%) and 80(BAW), UV λ_{MAX} : MeOH(253sh, 266, 294sh, 322sh, 367), NaOMe (278, 316), AlCl_3 (260sh, 268, 303sh, 348), NaOAc (274, 303, 3870), NaOAc/ H_3BO_3 (267, 297, 320sh, 372). ^1H -NMR data: δ ppm 6.18(1H, d, $J=2.1\text{Hz}$, H-6), 6.42(1H, d, $J=2.1\text{Hz}$, H-8), 7.88(1H, d, $J=8.5\text{Hz}$, H-2', H-6'), 6.91(1H, d, $J=8.8\text{Hz}$, 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'). ^1H - and ^{13}C -NMR data were identical to those reported in literature^[30].

RESULTS AND DISCUSSION

The aqueous ethanolic extract of the leaves of *C.illinoensis* was shown to possess significant biological activities represented in graphs compared to reference drugs(figures 2-5). The median lethal dose(LD_{50}) of the extract was revealed to be 8.3g/Kg.

Results obtained from the antioxidant activity of the aqueous ethanolic extract of *C.illinoensis*(figure 2) demonstrate its significant antioxidant activity at the dose(100mg/Kg.). The decrease in blood glutathione level was 2.5% in case of the diabetic group treated

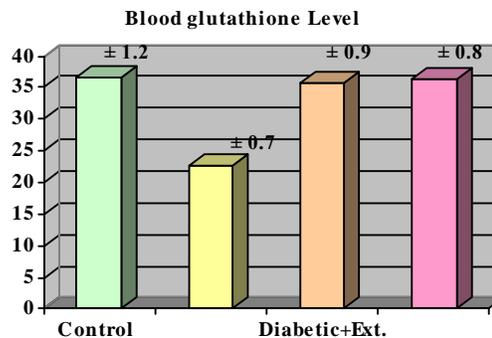


Figure 2: Antioxidant activity of the aqueous ethanolic extract of the leaves of *Carya illinoensis* regarding blood glutathione level in rats, in control(1 ml saline), non-treated diabetic group, diabetic treated with the extract(100mg/kg. b.wt) and diabetic treated with Vit E.

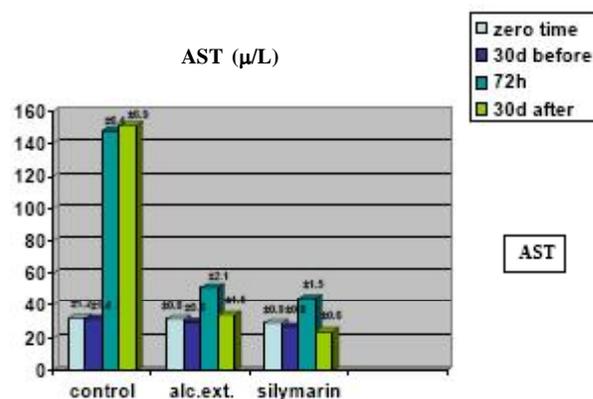


Figure 3: Effect of the aqueous ethanolic extract of the leaves of *Carya illinoensis*, orally administered, on the activities of serum transaminases(AST).

with the extract, while that treated with the reference drug(Vitamin E) had a percentage decrease of 1.4%, both groups showed significant antioxidant activity when compared with the control group. The non-treated diabetic group had decrease of 38.4%, which indicates high oxidative stress. From the previous results, it is evident that the ethanolic extract of *C.illinoensis* at the selected dose has high antioxidant activity approaching that of Vitamin E.

The hepatoprotective activity(figures 3-5) of the aqueous ethanolic extract of *Carya illinoensis* was determined by measuring the level of serum transaminases (AST and ALT) and alkaline phosphatase(ALP) as markers for hepatocellular integrity.

Induction of liver damage by administration of CCl_4 significantly elevated the serum enzymes level(AST, ALT, and ALP) after 72h of administration and the el-

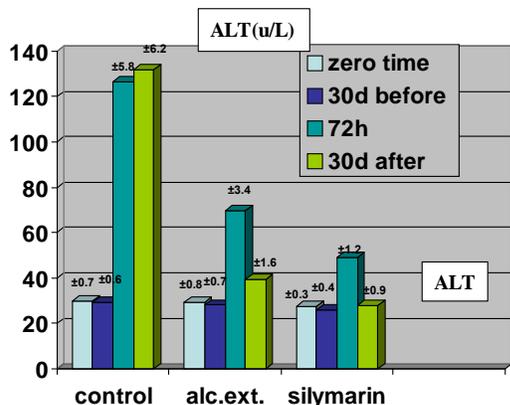


Figure 4: Effect of the aqueous ethanolic extract of the leaves of *Carya illinoensis*, orally administered, on the activities of serum transaminases(ALT).

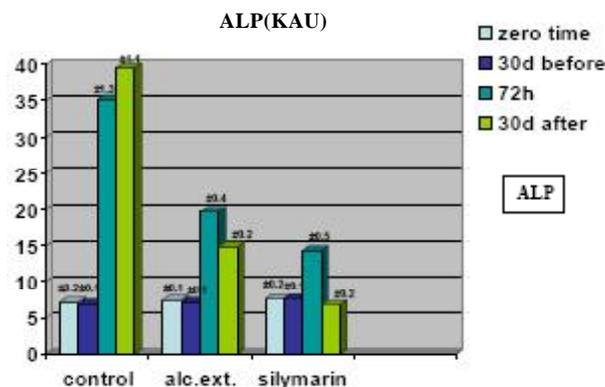


Figure 5: Effect of the aqueous ethanolic extract of leaves of *Carya illinoensis*, orally administered, on the activities of alkaline phosphatase(ALP)

TABLE 1 : Effect of the aqueous ethanolic extract of leaves of *Carya illinoensis*, orally administered, on the activities of serum transaminases(AST and ALT) and alkaline phosphatase(ALP).

Group	AST(u/L)				ALT(u/L)				ALP(KAU)			
	Zero	30d	72h	30d	Zero	30d	72h	30d	Zero	30d	72h	30d
Control (1ml saline)	32.1±1.2	31.6±1.4	148.2±6.4	151.4±6.9	29.8±0.7	29.4±0.6	126.3±5.8	131.6±6.2	7.1±0.2	6.9±0.1	35.2±1.3	39.5±1.1
Extract (100mg/Kg)	31.4±0.8	29.8±0.6	51.3±2.1 ^{o*}	34.1±1.5 ^{o*}	29.3±0.8	28.2±0.7 ^{o*}	69.8±3.4 ^{o*}	39.5±1.6	7.4±0.1	7.1±0.1	19.6±0.4 ^{o*}	14.9±0.2 ^{o*}
Silymarin (25mg/Kg)	29.2±0.9	26.7±0.8	44.3±1.5 ^{o*}	23.9±0.6 ^{o*}	27.4±0.3	26.1±0.4 ^{o*}	48.9±1.2 ^{o*}	27.8±0.9	7.6±0.2	7.1±0.1	14.3±0.5 ^{o*}	6.7±0.2 ^{o*}

*Significantly different from zero time at P < 0.01^oSignificantly different from control group at the same interval at P <0.01

evation present even after one month. While administration of the tested extract, in a dose of 100mg, resulted in significant reduction of the enzymes level even after one month of administration. Also, results showed that silymarin caused a significant reduction in the elevated level of enzymes as compared to the control.

The potent biological activities of the extract including the antioxidant and hepatoprotective activities are probably attributed to the richness in phenolics compounds (fraction II-V), a total of fifteen compounds were identified from four fractions of the extract, as well as condensed tannins that contributed most of the last fraction collected(fraction VI).

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REFERENCES

- [1] L.F.Merritt; ;'Gray's Manual of Botany', D.Van Nastrand Company, 528 (1970).
- [2] W.C.Evans; 'Trease and Evans Pharmacognosy', Ediburgh, London, New York, Philadelphia, St. Louis, SydmeY and Toronto, 21, (2002).
- [3] D.E.Moerman; 'Native American Ethnobotany', Timber Press, Oregon, (1998).
- [4] T.Sasaki; J.Pharm.Soc.Jpn, 84, 47-51 (1964).
- [5] G.Paget, E.Barnes; 'Toxicity tests in evaluation of drug activities sited in the laboratory rat', Academic Press, London, (1964).
- [6] G.Karber; Arch.Exp.Pathol.Pharmacol., 162, 480 (1931).
- [7] E.Beutler, O.Duron, B.Kelly; J.Lab.Clin.Med., 61, 882-888 (1963).
- [8] W.G.Spedecor, G.W.Cochran; 'Statistical methods', 10th Edition. Iowa State Univ. Press, USA, (1982).
- [9] C.D.Klassan, G.L.Plaa; Toxic.Appl.Pharmacol., 18, 2019 (1969).
- [10] W.Thewfweld; Deutsch Med., 99, 343 (1974).
- [11] P.R.Kind, E.G.King; J.Clin.Path., 7, 322 (1954).
- [12] M.K.Sakar, F.Petereit, A.Nahrstedt; Phytochemistry,

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- 33, 171- 174 (1993).
- [13] M.A.M.Nawwar, S.A.M.Hussein, J.Bouddrus, M. Lincheid; *Phytochemistry*, **35**, 1349 (1994).
- [14] H.H.Barakat, S.A.M.Hussein, M.S.Marzouk, I. Merfort, M.Linscheid, M.A.M.Nawwar; *Phytochemistry*, **46**, 935-941 (1997).
- [15] J.Mendez, M.C.Matov; *Phytochemistry*, **44**, 41-43 (1997).
- [16] N.Durust, S.Ozden, E.Umer, Y.Durust, M.Kucukis-lamoglu; *Turk.J.Chem*, **25**, 93 (2001).
- [17] M.A.M.Nawwar, A.M.A.Souleman, J.Buddrus, H. Bauer; M.Linscheid; *Tetrahedron Letters*, **25**, 49-52 (1984).
- [18] G.Hunber, V.Wray, A.Nahrstedt; *Planta Medica*, **65**, 636-642 (1999).
- [19] K.Kazuma, N.Noda, M.Suzuki; *Phytochemistry*, **62**, 229-237 (2003).
- [20] I.I.Mahmoud, F.A.Moharam, M.S.Marzouk, M.W.Linscheid, M.I.Saleh; *Pharmazie*, **57(7)**, 494-496 (2002).
- [21] M.A.M.Nawwar, A.D.El-Mousallamy, H.H. Barakat, J.Bouddrus, M.Linscheid; *Phytochemistry* **28**, 3201-3206 (1989).
- [22] L.Foo, D.Molan, D.Woodfield, W.Mcnabb; *Phytochemistry*, **54**, 1755-1758 (2000).
- [23] H.H.Barakat, A.M.Souleman, S.A.M.Hussein, O.A.Ibrahiem, M.A.M.Nawwar; *Phytochemistry*, **51**, 139-142 (1999).
- [24] L.Y.Foo, Y.Lu, A.L.Molan, D.R.Woodfield, W.C. McNabb; *Phytochemistry*, **54**, 539-548 (2000).
- [25] K.R.Markham, B.Ternai, R.Stanely, H.Geiger, T.J.Mabry; *Tetrahedron*, **34**, 1389-1397 (1978).
- [26] M.A.M.Nawwar, J.Buddrus, H.Bauer; *Phytochemistry*, **21(7)**, 1775-1758 (1982).
- [27] T.Sato; *Phytochemistry*, **26(7)**, 2124-2125 (1987).
- [28] M.A.M.Nawwar, S.A.M.Hussein, I.Merfort; *Phytochemistry*, **36(3)**, 793-798 (1994).
- [29] F.Petereit, H.Koldodziej, A.Nahrstedt; *Phytochemistry*, **30**, 981-985 (1991).
- [30] J.B.Harborne, T.J.Mabry, H.Mabry; 'The Flavonoids', Academic Press, New York, San Francisco, (1975).