



Research Journal of
Phytochemistry

ISSN 1819-3471



Academic
Journals Inc.

www.academicjournals.com

Hepatoprotective Activity and Flavonoids of *Alchornea laxiflora* Leaf Extract

¹Ganiyat K. Oloyede, ¹Patricia A. Onocha, ²Oluwatosin A. Adaramoye and ¹S. Emmanuel Thonda

¹Organic Chemistry Unit, Department of Chemistry, University of Ibadan, Nigeria

²Department of Biochemistry, University of Ibadan, Nigeria

Corresponding Author: Ganiyat K. Oloyede, Organic Chemistry Unit, Department of Chemistry, University of Ibadan, Nigeria Tel: +2348035622238

ABSTRACT

The leaves of *Alchornea laxiflora* have antihistamine, anti-infectious and anti-inflammatory applications in ethno medicine. This study was aimed at investigating the hepatoprotective activity, isolation and structural elucidation of the phytochemical constituents of the leaf ethanolic extract. Hepatoprotective assay was carried out against carbontetrachloride-induced liver damage in rats. The marker enzymes used in assay to assess liver function include: Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkalinephosphatase (ALP) and lactate dehydrogenase (LDH). Isolation and purification of fractions was achieved using standard chromatographic techniques. The structures of the isolated compounds were confirmed by spectroscopic techniques. The marker enzymes registered enhanced activity ($p < 0.05$) in CCl_4 -treated rats as administration of ethyl acetate fraction of the leaves of *A. laxiflora* was found to reduce the elevated levels of AST, ALT, ALP and LDH, thereby protecting the liver from toxic effects of carbontetrachloride. The result was further supported by histopathological studies of liver samples which showed regeneration of hepatocytes by the extract. Two other flavonoids were found for the first time from the Nigerian specie of *A. laxiflora* along with two previously reported flavonoids in this specie. The structures of these flavonoids were confirmed to be quercetin-3-O- β -D-glucopyranoside and quercetin 3, 7, 3', 4'-tetrasulphate while the latter two were identified as quercetin and quercetrin. This study has helped to establish the hepatoprotective influence of the leaves of *A. laxiflora*, isolate and elucidate the structure of the phytochemical constituents of the active fractions and justified the use of the plant in traditional medicine.

Key words: *Alchornea laxiflora*, Euphorbiaceae, hepatoprotective, histopathology, flavonoids

INTRODUCTION

Phytochemicals are of great importance due to their curative properties in healthcare. The rise in resistance of the human body system to a number of curative drugs is redirecting healthcare practices from curative to preventive medicine. Some phytochemicals used in preventive medicine include: flavonoids, polyphenols, saponins, lignoids and alkaloids (Cragg *et al.*, 1997; Groombridge, 1992; Calixto, 2000). Liver diseases can be induced by viruses, various chemicals, pharmaceuticals and environmental pollutant. Search for hepatoprotective agents is on the increase because of the important role that liver plays in detoxification and excretion of both internally and externally

generated toxins. Modern medicine does not so far have a fully effective cure particularly for hepatitis except for that caused by certain viruses which can be treated by corresponding vaccines (Ansari *et al.*, 2011; Prajapati *et al.*, 2003).

Alchornea laxiflora is rich in these phytochemicals, which might justify its use traditionally in ethno medicinal practice of many countries. It is used in folk medicine as a remedy for numerous diseases ranging from inflammation to heart diseases (Burkill, 1998; Dan *et al.*, 2004; Kayode and Omotoyinbo, 2008). The leaves have been reported to be effective in the treatment of kidney, bladder, inflammatory and infectious diseases. They have also been reported to exhibit hypo-sensitive and antihistamine properties (Burkill, 1998; Kayode and Omotoyinbo, 2008; Sofowora, 2008). The antimicrobial and antioxidant activities of *A. laxiflora* has been investigated which make it a suitable plant for this hepatoprotective study (Farombi *et al.*, 2003; Oloyede *et al.*, 2010). *A. laxiflora* belongs to Euphorbiaceae family and is widely spread in African countries especially West Africa. A number of flavonoids have been isolated from *A. laxiflora* and related species among which are quercetin - 7, 4- bisulphate, quercetin - 3', 4'-disulphate, quercetin -3, 4'-diacetate and rutin (Ogundipe *et al.*, 2001; Calvo *et al.*, 2007).

In continuation of the phytochemical and pharmacological investigation of secondary metabolite constituents of plants (Oloyede *et al.*, 2010; Onocha *et al.*, 2011a, b), this study described the hepatoprotective activity against carbontetrachloride-induced liver damage in rats (of the antioxidant active fractions), isolation and structural elucidation of the flavonoids of *Alchornea laxiflora* leaf extract.

MATERIALS AND METHODS

General experimental procedure: The Ultraviolet (UV)/Visible Spectra of 0.01% w/v of the fractions were determined with the aid of Genesys 10s.VI.200 UV/Visible spectrophotometer. The samples were scanned between 190 and 400 nm. Data from chart/recorder gave a graph of Absorbance against wavelength (nm). V_{max} (cm^{-1}) from Infra Red (IR) data also confirmed the structures. The Infrared spectrum of the pure compound was determined using a Nicolet Avatar 330 Fourier Transform (FT) Infrared Spectrophotometer. The KBr disc method was used for the preparation of the sample. The spectrophotometer determines the relative strength and position of all absorption in the infrared region and plots the intensity (Transmittance against wave number). The 1H Nuclear Magnetic Resonance (NMR) and ^{13}C NMR spectra of the pure compounds were determined using a 200 MHz machine for 10% (w/v) solutions in deuterio methanol or deuterio chloroform. Pulse irradiation technique employed was FTNMR at ambient temperature. The ethyl acetate soluble fraction was subjected to hepatoprotective activity using carbon tetrachloride induced rats.

Reagents and chemicals: The following chemical and reagents were used: hexane, ethyl acetate, methanol, ethanol, butanol, chloroform (all BDH analar reagents), Fehling's solution A and B, 5% Ferric chloride, concentrated tetraoxosulphate (VI) acid, NH_4OH solution, Dragendroff's reagent, 1% hydrochloric acid, magnesium turnings, glacial acetic acid, iodine vapour, copper acetate, molisch reagent, silical gel F₂₅₄ (Precoated aluminium sheets, Sigma), silica gel (mesh size 70-230, Sigma, Germany), carbon tetrachloride, sodium chloride (BDH) and Randox Laboratory kits for biochemical assays.

Plant materials: Fresh leaves of *A. laxiflora* were collected in June, 2008 at the University of Ibadan Campus, Oyo State, Nigeria. Specimens were identified and authenticated at Botany and

Microbiology Department of University of Ibadan. The leaves were air-dried and ground into fine powder and kept in non-absorptive nylon for subsequent use.

Animals: Adult Wistar rats weighing 130-225 g (14 weeks old) bred in the animal house of the Department of Biochemistry, University of Ibadan were used. The animals were divided into 5 groups. Four animals were used per group. The rats were kept at $27\pm 2^\circ\text{C}$ for 1 week before and during the experiments and were fed with balanced livestock feeds from Pfizer, Plc and water, *ad libitum*. All the animal experimental protocol has been approved by the Institutional Animal Ethics Committee.

Extraction, isolation and characterisation procedures: Air-dried and powdered leaves of *A. laxiflora* (2 kg) was first defatted with hexane. Ethanol (4×5 L) was added to the marc obtained and kept for 72 h before filtration. The filtrates were decanted, combined and concentrated with the aid of a Buchi Rotavapor R110. The crude ethanolic extract (60 g) was first basified with ammonia solution to pH 10. Ethyl acetate was successfully added to the basified aqueous solution of the crude extract in order to remove the moderately polar components while butanol was again added to the mother liquor to remove the polar fractions. The ethyl acetate extract was subjected to column chromatography over silica gel 60 F₂₅₄, 70-230 mesh as adsorbent, eluting with hexane and followed by hexane-EtOAc mixture with increasing polarity. A total of 46 fractions of ca.50 mL each were collected and combined on the basis of TLC analysis giving three mixtures which afforded three compounds (A-C). The butanol extract on the other hand, was subjected to column chromatography over silica gel eluting with Ethyl acetate and followed by EtOAc-methanol mixture with increasing polarity. A total of 56 fractions of ca.50 mL each were collected and combined on the basis of TLC analysis to give three mixtures which also afforded three compounds (D-F). The phytochemical analyses were carried out using standard methods described by Harborne (1993), Egwuiche *et al.* (2011), Satnami and Yadava (2011) and Ganesh and Vennila (2011). Pure compounds were obtained which were characterized by spectroscopic analysis, UV-Visible, IR and NMR spectrometry.

Antihepatotoxicity study: Evaluation experiment of the hepatoprotective activity of *A. laxiflora* was carried out *in vivo*. The extract was suspended in corn oil and subjected to hepatoprotective activity in CCl₄-induced hepatotoxicity. All the animal experimental protocol has been approved by the Institutional Animal Ethics Committee.

Wistar male rats were obtained in Ibadan and bred in the animal house of the Department of Biochemistry, University of Ibadan. The animal were fed with standard feeds and given water daily. The animals were bred to mature body mass before administration was done. A total of five groups of four rats (n = 4) was used for the experiment. Dosage level of 100 mg kg⁻¹ body mass and 200 mg kg⁻¹ body mass of extract and toxicant were given to the rats. The vehicle for the extract was corn oil and CCl₄ was used as the toxicant. Group 1 served as control. Group 2 (CCl₄-treated) rats were injected with a single dose of CCl₄ in corn oil (200 mg kg⁻¹ body weight, standard concentration[s.c.]). Groups 3 and 4 were administered 100 mg kg⁻¹ and 200 mg kg⁻¹ of ethyl acetate extract by intraperitoneal route, respectively once daily for 7 days. The animals were also simultaneously administered a single dose of extract only in Group 5 (200 mg kg⁻¹ body weight, s.c.) (Recknagel, 1983; Okuno *et al.*, 1986; Mankani *et al.*, 2005; Jain *et al.*, 2008).

Biochemical assay: The rats were sacrificed by cervical dislocation 24 h after the last treatment and their blood was collected from the carotid artery. The blood was allowed to clot and spin in a centrifuge for 10 min at 4000 r min⁻¹ to obtain the serum and used for the estimation of various biochemical parameters. Also collected was the liver part of which was set aside for histopathology studies. The liver was rinsed and homogenized in buffer solution. The homogenates were spin at 10000 r min⁻¹ using cold centrifuge. The liver and serum for biochemical assay were kept at ice-cold temperature while the liver for histopathology was stored in 10% formalin and then fixed in bovine solution; they were processed for paraffin embedding following the standard micro technique. Sections of liver were stained with haematoxylin-eosin and were observed microscopically for any histopathological changes.

The following biochemical parameters were determined: Alanine aminotransferase (ALT), alkalinephosphatase (ALP), lactate dehydrogenase(LDHP-L) and aspartate aminotransferase (AST) on the serum, using kits manufactured by Randox Laboratories Ltd United Kingdom.

Data analysis: Graphs were plotted from the tables given in the kit (Absorbance Vs U/I) which served as the calibration curve and ALT, ASP, ALP and LDH activities in the serum were obtained by extrapolation from the graph. The Mean±SEM was calculated for each parameter, each parameter was analyzed separately using ANOVA followed by Dunnett's 't' test.

RESULTS AND DISCUSSION

The ethyl acetate and butanol soluble fraction of the ethanolic extract of the finely powdered leaves of *A. laxiflora* was subjected to column chromatography. This gave six mixtures which afforded six pure compounds A-C and D-F, respectively. Compounds (A-F) were obtained and recrystallized in suitable solvents but four were fully characterized while two (C and F) were isolated in minute quantity, with insufficient data to characterize them. These include: Quercetin (A) and Quercetrin (B - (Quercetin-3-O-rhamnoside)) from ethyl acetate fraction; Quercetin-3-O-β-D-glucopyranoside (D) and Quercetin-3,7,3',4'-tetrasulphate (E) from butanol fraction which were identified by their uv, ir, ¹H and ¹³C- nmr in some cases. The spectra data obtained are in complete agreement with literature values of these compounds, Quercetin-3-O-β-D-glucopyranoside (D) and Quercetin-3, 7, 3', 4'-tetrasulphate (E) are encountered for the first time in the leaves of *A. laxiflora*. Structures of the new compounds (D and E) reported here are as shown in Fig. 1.

Spectral data of quercetin (A): Yellow powder; UV [EtOH]nm (log ε): 205, 256 and 372 (4.655, 4.345 and 4.313). IR (KBr) V_{max} cm⁻¹ 3408.62 ((O-H stretch broad, H-bonded), 3220-3095, 1663.53 (C = O stretch conjugated), 1562.57 (C = C stretch), 1562.19, 1529.01, 1463.32, 1460.08, (C-O), 1382.34, 1210.01 and 1132.21 (C-O-C), 942.09, 843.87 and 705.08 (Aromatic system). ¹H NMR (200 MHz; CD₃OD): δppm, J/Hz: 7.63(1H,dd, J = 2.2, 8.5, H-5'), 7.73 (1H, d, J = 2.2, H-2'), 6.89 (1H, d, J = 8.5, H-6'), 6.39 (1H, d, J = 2.1, H-8), 6.19 (1H, d, J = 2.1, H-6); ¹³C NMR (75 MHz, CD₃OD) δ: 94.5(C-8), 99.5(C-6), 104.2(C-10), 116.5(C-5'), 116.1(C-2'), 121.8(C-6'), 123.6(C-), 136.5(C-3), 137.61(C-1'), 145.7(C-3'), 148.1(C-4'), 156.7(C-9), 161.0(C-5), 166.7(C-7), 176.1(C-4).

Spectral data of quercetrin (B-quercetin-3-O-rhamnoside): Yellow shining substance; UV [EtOH]nm (log ε): 220, 259 and 352 (2.031, 2.553 and 2.014) IR (KBr) V_{max} cm⁻¹ 3380.59 (O-H stretch, broad), 1655.59 (C=O conjugated), 1498.27 (C-C stretch), 1202.01 (C-O), 640.90-936.78

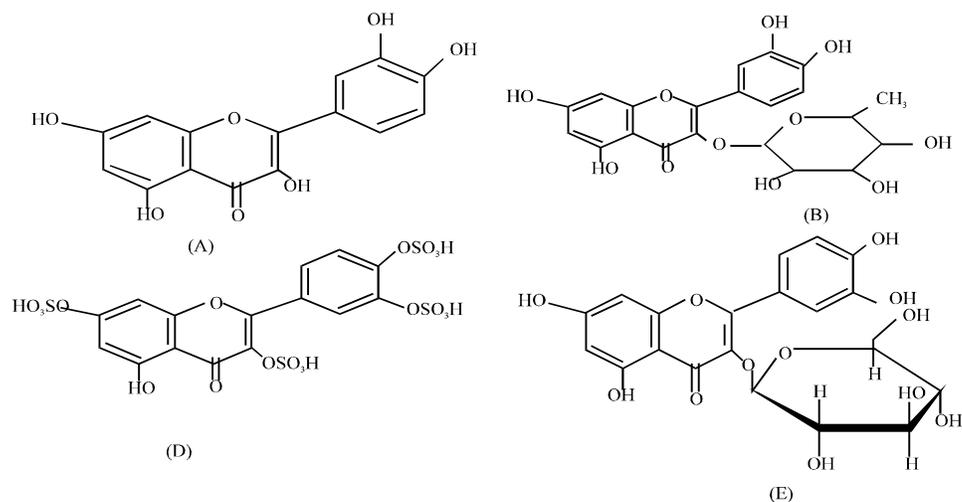


Fig. 1: Structures of Quercetin (A), Quercetrin (B), Quercetin-3,7,3',4'-tetrasulphate (D) and Quercetin-3-O-β-D-glucopyranoside (E). C and F were pure compounds obtained but not fully characterized because of insufficient data therefore no structure has been proposed for them

(Aromatic systems). ¹H NMR (200 MHz; CDCl₃): δ 6.38 (d, 1H, J = 2.05, H-6), 6.21 (d, 1H, J = 2.05, H-8), 7.35 (d, 1H, J = 2.0, H-2'), 6.93 (d, 2H, J = 8.2, H = 5'), 7.32 (dd, 1H, J = 2.0, 8.2, H-6'). ¹³C NMR (50 MHz, CDCl₃): δ 157.33 (C-2), 35.066 (C-3), 178.64 (C-4), 158.13 (C-5), 98.63 (C-6), 164.67 (C-7), 93.54 (C-8), 104.9 (C-4a), 162.02 (C-8a), 121.804 (C-1'), 115.773 (C-2'), 145.217 (C-3'), 148.601 (C-4'), 115.773 (C-5'), 121.713 (C-6'), 102.367 (C-1''), 72.949 (C-2''), 70.949 (C-3''), 70.851 (C-4''), 70.737 (C-5''), 16.475 (C-6'').

Spectral data of quercetin-3, 7, 3', 4'-tetrasulphate (D): Yellowish substance; UV [EtOH]nm (log e): 270, 310 and 340 (4.326, 4.093 and 4.043). IR (KBr) V_{\max} cm⁻¹: 3531.62 ((O-H stretch broad, strong), 1678.59 (C=O stretch conjugated), 1552.17 (C = C stretch) and 1310. ¹H NMR (200 MHz; MeOD): δppm, J/Hz: 6.78 (1H, d, J = 2.5, H-6), 7.13 (1H, d, J = 2.5, H-8), 7.66 (1H, d, J = 8.5, H-5'), 7.96 (1H, d, J = 2.5, H-2'), 8.16 (1H, br, s, H-6').

Spectral data of quercetin-3-O-β-D-glucopyranoside (E): Colourless needles; UV [EtOH]nm (log e): 257, 269, 300 and 361, (4.213, 4.104, 4.023 and 4.349); IR (KBr) V_{\max} cm⁻¹: 3300.11 (O-H), 1650.23 (C = O), 1605-1500 (Aromatic functions); ¹H NMR (200 MHz; CDCl₃): δppm, J/Hz: 7.70(1H,d, J = 2.0 Hz, H-2'), 7.62(1H, dd, J = 8.5,2.0 Hz, H-6'), 6.81(1H, d, J = 8.5 Hz, H-5'), 6.38(1H, d, J = 1.9 Hz, H-8), 6.20(1H, d, J = 1.9 Hz, H-6), 5.20 (1H,d, J = 7.2 Hz, H-1''), 3.72 (1H, m, H-6''a), 3.40 (1H,m, H-6''b), 3.06(1H, m, H-2''), 3.36(1H, m, H-4''), 3.30(1H,m, H-3''), 3.21(1H, m, H-5''); ¹³C NMR (75MHz, CD₃OD) δ: 179.4(C-4),166.2(C-7), 163.0(C-5), 159.0 (C-2), 158.5 (C-9), 149.8 (C-4'), 145.9 (C-3'), 135.0 (C-3), 123.1 (C-6'), 123.0(C-1'), 117.5 (C-5'), 116.0 (C-2'), 105.6 (C-10), 104.0 (C-1''), 99.0 (C-6, 94.7 (C-8)), 76.4 (C-5''), 76.1 (C-3''), 73.7 (C-2''),71.2 (C-4''), 61.5 (C-6'').

The UV absorption for the compounds fall into the range of band 1 and 2 absorptions for flavonoids. The proton NMR spectrum of the compounds showed only aromatic signals indicating the absence of the high field protons of C-ring on the flavonoid nucleus. The glycosidic nature of

some of the compounds was indicated by the presence in the ^1H NMR spectrum of sugar multiplets at δ 3-4 ppm. The aromatic proton doublets at δ 6.21 and 6.38 ppm ($J = 2.05$ Hz) were due to the meta coupled protons of a 5, 7-substituted ring A, while the signals at δ 6.93 ppm (d, $J = 8.2$ Hz), δ 7.32 ppm (d, $J = 2.0/8.2$ Hz) and δ 7.35 ppm (d, $J = 2.0$ Hz) were also characteristic of a 3', 4'-substituted ring B. The sugar moiety was inferred to be β -glucopyranoside and rhamnose from the methyl proton signal at δ 0.93 and δ 0.96 ppm respectively, as well as the ^{13}C NMR spectrum that showed the characteristic glucose and rhamnose methyl resonance at δ 16.2 ppm and δ 16.7 ppm. The sugar unit in Quercetin-3-O- β -D-glucopyranoside was assigned as β -D-glucose by comparing the NMR chemical shift values with reported data (Mabry *et al.*, 1970; Agrawal and Bansal, 1989). The β configuration of glucose moiety was assigned on the basis of larger coupling constant of anomeric proton H-1" ($J = 7.2$ Hz). The spectrum of Quercetin-3-O-rhamnoside also showed the other sugar carbon signals at δ 70-72 ppm with the anomeric C1" appearing at δ 102.367 ppm. This was confirmed by comparison of its NMR spectrum with literature (Markham, 1982). Quercetin-3-O- β -D-glucopyranoside, Quercetin-3,7,3',4'-tetrasulphate, quercetin, quercetin-3-O-rhamnoside (quercetrin) are flavonoids that are widely distributed in the plant kingdom (Wollenweber and Dietz, 1981). They gave positive Molisch's test proving they are flavonoids (aglycones) or their glycosides. Hydrolysis of these flavonoids with 0.1N HCl at 100°C produced crystals identified as quercetin by comparison with R_f values thereby further confirming the structures of the flavonoids.

These data are in complete agreement with those reported for these compounds (Miyase *et al.*, 1999; Cruz *et al.*, 2001; Morales *et al.*, 2006).

Antihepatotoxicity studies: Administration of ethyl acetate fraction of *A. laxiflora* leaves showed significant hepatoprotective activity. The results revealed that the ethyl acetate extract of leaves of *A. laxiflora* exhibited ability to counteract the CCl_4 induced hepatotoxicity by decreasing the elevated marker enzymes levels in the blood (18.872, 7.054, 22.864 and 180.321 for ALT, AST, ALP and LDH respectively in group 4) when compared to the CCl_4 group (35.712, 12.513, 27.509 and 480.312 for ALT, AST, ALP and LDH respectively in group 2) ($p < 0.05$). Estimating the activities of serum marker enzymes like AST, ALT, ALP and LDH, can give assessment of liver function. When liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released in to the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage (Venukumar and Latha, 2002; Ali *et al.*, 2011; Solanki and Jain, 2011; Subash *et al.*, 2011; Al-Attar, 2011; Shuid *et al.*, 2011). In this present study, the marker enzymes viz., AST, ALT and LDH registered enhanced activity ($p < 0.05$) in group 2 (CCl_4 -treated) rats when compared to group 1 (control), group 3 ($\text{CCl}_4 + 100 \text{ mg kg}^{-1}$), group 4 ($\text{CCl}_4 + 200 \text{ mg kg}^{-1}$) and group 5 (*A. laxiflora* extract only) (Table 1).

The abnormal high level of serum ALT, AST, ALP and LDH observed in this study (Table 1) are the consequence of CCl_4 induced liver dysfunction and denotes the damage to the hepatic cells. There was no significant difference in group 1, 3, 4 and 5, implying that *A. laxiflora* extract was not toxic and has anti-hepatotoxicity effect on the liver. Treatment with *A. laxiflora* has therefore reduce the enhanced level of serum ALT, AST, ALP and LDH, which is believed to offer the protection and maintain the functional integrity of hepatic cells. CCl_4 has been used as a tool to induce hepatotoxicity in experimental animals (Recknagel, 1983; Okuno *et al.*, 1986; Mankani *et al.*, 2005; Ali *et al.*, 2011; Solanki and Jain, 2011; Shuid *et al.*, 2011). This toxic chemical is known to cause peroxidative degradation in the adipose tissue resulting in fatty

infiltration of the hepatocytes. The increased levels of transaminases and alkaline phosphatase showed that cellular leakage and loss of functional integrity of the cell membrane is as a result of the toxic chemical, carbon tetrachloride (Saraswat *et al.*, 1993).

A comparative histopathological study of liver from the study groups further corroborated the hepatoprotective effect of *A. laxiflora*. Histopathology of liver from normal control group shows prominent central vein, normal arrangement of hepatic cells. Microscopical examination of carbon tetrachloride treated liver section shows various degrees of pathological changes starting from centrilobular necrosis of hepatic cells and central lobular fatty regeneration (Fig. 2, 5).

Flavonoids are the major phytochemical constituents found in this plant and may have been responsible for the observed activity. Also, it has been reported by many workers that the flavonoid constituents of plants are known to possess antioxidant properties and possibly found to be useful in the treatment of liver damage (Urquiaga and Leighton, 2000; Li *et al.*, 2005; Yang and Kong, 2008). The presence of alkaloids, tannins, flavonoids, saponins, carbohydrate, glycosides and phenols *and* the antioxidant property of the leaf extract and fractions of *A. laxiflora* have been established in a previous study (Oloyede *et al.*, 2010).

Histological profile of the control animals showed normal hepatocytes as observed in Fig. 2. Group 2 animals which are the CCl₄ treated rats exhibited intense centrilobular necrosis, vacuolization and macrovesicular fatty change (Fig. 3). The animals treated with ethyl acetate fraction exhibited significant liver protection against the toxicant as evident by the presence of normal hepatic cords, absence of necrosis and lesser fatty infiltration (Fig. 4, 5).

Since the results of hepatoprotective activity showed a significant decrease in the elevated levels of serum enzymes and histopathological results showed a significant regeneration of hepatocytes, it can be concluded that the ethyl acetate extract of the leaves of *A. laxiflora* can be used as a hepatoprotective agent.

Table 1: Effect of ethylacetate extract of *A. laxiflora* in different biochemical parameters in CCl₄ induced-hepatotoxic rats

Animal control parameters	Group 1 normal	Group 2	Group 3	Group 4	Group 5
Serum (U/L)	control group	CCl ₄ group	CCl ₄ + AL ₁	CCl ₄ + AL ₂	AL
ALT	14.101±1.05**	35.712±1.69**	24.120±3.57**	18.872±1.43**	12.608±3.21**
AST	5.930±0.89**	12.513±2.34**	8.623±0.08**	7.054±2.67**	5.253±1.07**
ALP	29.912±0.56**	27.509±0.33**	27.509±1.28**	22.864±0.51**	27.345±0.02**
LDH	150.013±1.35**	480.312±0.45**	185.156±2.48**	180.321±1.54**	145.457±1.41**

Values are expressed as Mean±SEM (n = 4), CCl₄: Carbon tetrachloride, AL: *A. laxiflora* AL₁: CCl₄ +100 mg kg⁻¹, AL₂: CCl₄ +200 mg kg⁻¹, **: The mean difference is significant at p<0.05 when compared with control and toxicant (CCl₄), respectively

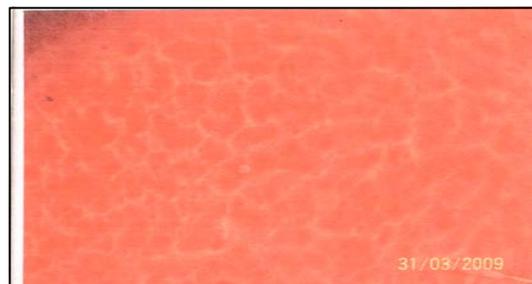


Fig. 2: Section of the liver tissue of control rats showing normal histology, portal triad consisting of portal vein, portal artery, hepatic duct (Mag, 400x)

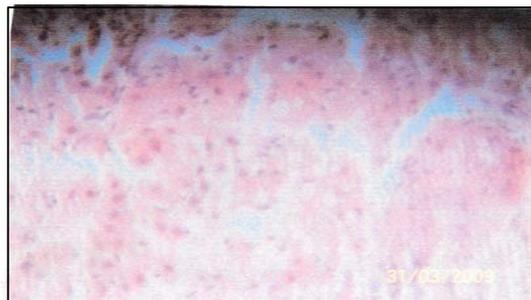


Fig. 3: Section of the liver tissue of rats treated with CCl_4 showing necrosis and fatty vacuole (Mag, 400x)

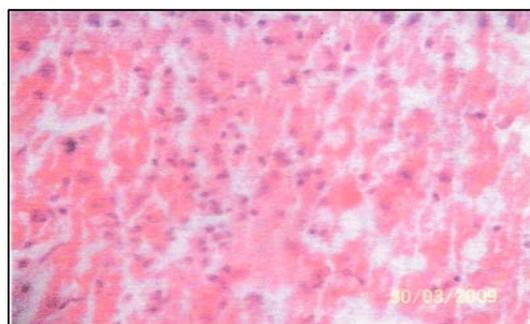


Fig. 4: Section of the liver tissue of ethylacetate-treated rat showing normal hepatocytes, necrosis, portal triad showing portal vein, portal artery, hepatic duct (Mag, 400x)



Fig. 5: Section of the liver tissue of ethylacetate extract-treated rat at 200 mg kg^{-1} showing moderate normal arrangements of hepatocytes around the central vein, absence of necrosis and fatty vacuoles (Mag, 400x)

CONCLUSION

Two flavonoids quercetin-3-O- β -D-glucopyranoside and quercetin 3, 7, 3', 4'-tetrasulphate are newly reported in this specie in addition to other flavonoids that have been previously reported by other workers. The result of the hepatoprotective activity revealed that the ethyl acetate soluble fraction of *A. laxiflora* showed significant hepatoprotective activity by reducing elevated levels of

biochemical parameters, ALT, AST ALP and LDH P-L levels at a dose of 100 mg and 200 mg kg⁻¹ body weight. The extract at 100 mg kg⁻¹ body weight showed better hepatoprotective influence than at 200 mg kg⁻¹ body weight. A comparative histopathological study of the liver from the different study groups (1-5) further corroborated the hepatoprotective efficacy of *A. laxiflora*. This present study has been able to establish the hepatoprotective influence of the leaves of *A. laxiflora* and has helped to isolate and elucidate the structure of some of the phytochemical constituents of the active fractions. Further work will be needed to fully characterize the other pure isolates. Additional work is also necessary to subject the pure isolated compounds from ethyl acetate soluble fraction to hepatoprotective activity to determine the particular isolates that are responsible for the activity in this promising plant. This study has therefore justified the use of this plant in traditional medicine.

REFERENCES

- Agrawal, P.K. and M.C. Bansal, 1989. Flavonoid Glycosides. In: Carbon-13 NMR of Flavonoids, Agrawal, P.K. (Ed.). Elsevier, Amsterdam, Netherlands, pp: 283-364.
- Al-Attar, A.M., 2011. Hepatoprotective influence of vitamin C on thioacetamide-induced liver cirrhosis in wistar male rats. *J. Pharmacol. Toxicol.*, 6: 218-233.
- Ali, S.A., A.A. Gameel, A.H. Mohamed and T. Hassan, 2011. Hepatoprotective activity of *Capparis decidua* aqueous and methanolic stems extracts against carbon tetrachloride induced liver histological damage in rats. *J. Pharmacol. Toxicol.*, 6: 62-68.
- Ansari, J.A., M. Sayyed and F. Sayeed, 2011. Management of non alcoholic fatty liver diseases and their complications. *Int. J. Pharmacol.*, 7: 579-588.
- Burkill, H.M., 1998. The Useful Plants of West Tropical Africa. 2nd Edn., Royal Botanical Gardens, Kew, UK.
- Cahxto, J.B., 2000. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicine (Phytotherapeutic agents). *Braz. J. Med. Biol. Res.*, 33: 179-189.
- Calvo, T.R., Z.P. Lima, S.J. Silva, K.V. Ballesteros and C.H. Pellizzon *et al.*, 2007. Constituents and antiulcer effect of *Alchornea glandulosa*: Activation of cell proliferation in gastric mucosa during the healing process. *Biol. Pharm. Bull.*, 30: 451-459.
- Cragg, G.M., D.J. Newman and K.M. Snader, 1997. Natural products in drug discovery and development. *J. Nat. Prod.*, 60: 52-60.
- Cruz, F.G., J.T. da Silva-Neto and M.L.S. Guedes, 2001. Xanthenes and coumarins from *Kielmeyera lathrophyton*. *J. Braz. Chem. Soc.*, 12: 117-122.
- Dan, B., C. Steven, S. Erich and G. Andrew, 2004. Chinese Herbal Medicine: Materia Medica. 3rd Edn., Plenum Press, New York, pp: 22-25.
- Egwuche, R.U., A.A. Odetola and O.L. Erukainure, 2011. Preliminary investigation into the chemical properties of *Peperomia pellucida* L. *Res. J. Phytochem.*, 5: 48-53.
- Farombi, O.E., O.O. Ogundipe, E.S. Uhunwangbo, M.A. Adeyanju and J.O. Moody, 2003. Antioxidant properties of extracts from *Alchornea laxiflora* (Benth) Pax and Hoffman. *Phytother. Res.*, 17: 713-716.
- Ganesh, S. and J.J. Vennila, 2011. Phytochemical analysis of *Acanthus ilicifolius* and *Avicennia officinalis* by GC-MS. *Res. J. Phytochem.*, 5: 60-65.
- Groombridge, B., 1992. Global Biodiversity Status of the Earth's Living Resources. Chapman and Hall, London, pp: 350-355.

- Harborne, J.B., 1993. Phytochemical Methods: A Guide to Modern Technique in Plant Analysis. Chapman and Hall, New York.
- Jain, A., M. Soni, L. Deb, A. Jain, S.P. Rout, V.B. Gupta and K.L. Krishna, 2008. Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of *Momordica dioica* Roxb. leaves. J. Ethnopharmacol., 115: 61-66.
- Kayode, J. and M.A. Omotoyinbo, 2008. Conservation and botanicals use for dental and oral Health care in Ekiti State, Nigeria. Ethnobotanical Leaflets, 12: 7-18.
- Li, J.C., L. Feng, B.H. Sun, T. Ikeda and T. Nohara, 2005. Hepatoprotective activity of the constituents in *Swertia pseudochinensis*. Biol. Pharm. Bull., 28: 534-537.
- Mabry, T.J., K.R. Markham and M.B. Thomas, 1970. The Systematic Identification of Flavonoids. Springer-Verlag, New York, USA., pp: 128-302.
- Mankani, K.L., V. Krishna, B.K. Manjunatha, S.M. Vidya and S.D. Jagadeesh Singh *et al.*, 2005. Evaluation of hepatoprotective activity of stem bark of *Pterocarpus marsupium* Roxb. Indian J. Pharmacol., 37: 165-168.
- Markham, K.R., 1982. Technique of Flavonoids Identification. Academic Press, London, UK., ISBN-13: 9780124726802, pp: 37-85.
- Miyase, T., H. Noguchi and X.M. Chen, 1999. Sucrose esters and xanthone C-glycosides from the roots of *Polygala sibirica*. J. Nat. Prod., 62: 993-996.
- Morales, G., P. Sierra, A. Paredes, L.A. Loyola and J. Borquez, 2006. Constituents of *Helenium atacamense* Cabr. J. Chil. Chem. Soc., 51: 134-140.
- Ogundipe, O.O., J.O. Moody, P.J. Houghton and H.A. Odelola, 2001. Bioactive chemical constituents from *Alchornea laxiflora* (Benth) Pax and Hoffman. J. Ethnopharmacol., 74: 275-280.
- Okuno, H., H. Hazama, T. Murase, Y. Shiozaki and Y. Sameshima, 1986. Drug metabolizing activity in rats with chronic liver injury induced by carbon tetrachloride: Relationship with the content of hydroxyproline in the liver. Jpn. J. Pharmacol., 41: 363-371.
- Oloyede, G.K., P.A. Onocha, J. Soyinka, O.W. Oguntokun and E. Thonda, 2010. Phytochemical screening, antimicrobial and antioxidant activities of four Nigerian medicinal plants. Ann. Biol. Res., 2: 114-120.
- Onocha, P.A., G.K. Oloyede and Q.O. Afolabi, 2011a. Chemical composition, cytotoxicity and antioxidant activity of essential oils of *Acalypha hispida* flowers. Int. J. Pharmacol., 7: 144-148.
- Onocha, P.A., G.K. Oloyede and Q.O. Afolabi, 2011b. Cytotoxicity and free radical scavenging activities of hexane fractions of Nigeria specie of African pear (*Dacryodes edulis*). Int. J. Biol. Chem., 5: 143-149.
- Prajapati, N.D., S.S. Purohit, A.K. Sharma and T.A. Kumar, 2003. Handbook of Medicinal Plants: A Complete Source Book. Agrobios Publication, India.
- Recknagel, R.O., 1983. Carbon tetrachloride hepatotoxicity: Status quo and future prospects. Trends Pharmacol. Sci., 4: 129-131.
- Saraswat, B., P.K. Visen G.K. Patnaik and B.N. Dhawan, 1993. Anticholestatic effect of picroliv, active hepatoprotective principle of *Picrorhiza kurrooa*, against carbon tetrachloride induced cholestasis. Ind. J. Exp. Biol., 31: 316-318.
- Satnami, D.K. and R.N. Yadava, 2011. Potential phytochemical from *Caesalpinia crista* Linn. Res. J. Phytochem., 5: 22-31.
- Shuid, A.N., L.K. Siang, T.G. Chin, N. Muhammad, N. Mohamed and I.N. Soelaiman, 2011. Acute and subacute toxicity studies of *Eurycoma longifolia* in male rats. Int. J. Pharmacol., 7: 641-646.

- Sofowora, A., 2008. Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd., Ibadan, Oyo State, Nigeria.
- Solanki, Y.B. and S.M. Jain, 2011. Hepatoprotective effects of *Clitoria ternatea* and *Vigna mungo* against acetaminophen and carbon tetrachloride-induced hepatotoxicity in rats. *J. Pharmacol. Toxicol.*, 60: 30-48.
- Subash, K.R., K.S. Ramesh, B.V. Charian, F. Britto, N.J. Rao and S. Vijaykumar, 2011. Study of hepatoprotective activity of *Solanum nigrum* and *Cichorium intybus*. *Int. J. Pharmacol.*, 7: 504-509.
- Urquiaga, I. and F. Leighton, 2000. Plant polyphenol: Antioxidants and oxidative stress. *Biol. Res.*, 33: 55-64.
- Venukumar, M.R. and M.S. Latha, 2002. Hepatoprotective effect of the methanolic extract of *Curculigo orchioides* in CCl₄-treated male rats. *Indian J. Pharmacol.*, 34: 269-275.
- Wollenweber, E. and V.H. Dietz, 1981. Occurrence and distribution of free flavonoid aglycones in plants. *Phytochemistry*, 20: 869-932.
- Yang, M.H. and K.L. Kong, 2008. Flavonols and flavonolglycosides from *Rhododendron irroratum*. *Chem. Nat. Compd.*, 44: 98-99.