Identification of bioactive compounds in ethylacetate fraction of *Tapinanthus bangwensis* leaves that ameliorate CCl₄-induced hepatotoxicity in Wistar rats

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Abstract

The pharmacological effects of medicinal plants are due to the presence of certain chemical compounds present in them. Previous studies have shown that crude extracts of *Tapinanthus bangwensis* (*T. bangwensis*) possess hepatocurative potential. However, the present study aims on evaluating the antioxidant activity as well as identifying the chemical compounds in ethylacetate fraction of *T. bangwensis* that ameliorate carbon tetrachloride-induced hepatotoxicity in Wistar rats. Six subfractions of ethylacetate fraction were obtained by column chromatography. The antioxidant activity was determined by 2,2-diphenyl-1-picrylhydrazine (DPPH), biochemical assay determined by spectrophotometry, and compound elucidation by liquid chromatography–mass spectroscopy (LC-MS) analysis. The DPPH result shows that subfraction F0 exhibited the strongest antioxidant activity followed by F4 while F1 exhibited the lowest. Oral administration of 100 mg/kg body weight of the subfractions of ethylacetate fraction of *T. bangwensis* increases superoxide dismutase and catalase activities and also glutathione level and decreases malondialdehyde level compared to the positive control group. Subsequently, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, and conjugated bilirubin were reduced while albumin and total protein levels increased compared to the positive control group. However, there were no significant differences between the positive control group and the group induced and treated with the subfractions at *p* < 0.05. The histopathology study shows normal hepatocyte distribution with no fat deposit in the induced and treated groups while fatty liver was observed in the positive group. The anti-hepatotoxic effect was higher in F4 than in other subfraction treated groups. Hence, the LC-MS analysis of F4 reveals the presence of 8-hydroxyluteolin-8-glucoside, Avicularin, Fisetin-7-glucoside, Isoscutellarein-7-xyloside, and Isovitexin, respectively, and has been reported to exhibit antioxidant and hepatocurative activities. It can be concluded that the hepatocurative effect could be due to the chemical compounds identified above.

Keywords

*Tapinanthus bangwensis*, liver biomarkers, antioxidant activity, hepatotoxic effect, liquid chromatography–mass spectroscopy

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Introduction

The liver performs over 500 biological and metabolic functions such as detoxification, deamination, synthesis, storage, homeostatic regulation of the body, and so forth.1–3 The susceptibility of the liver to metabolism-dependent drug-induced damage is due to its defective detoxifying function.4,5 This is because active metabolites (free radicals) when produced during xenobiotic biotransformation could periodically cause changes in liver’s microscopic structures, bile acid transporters, and biomolecules consequently causing liver apoptosis/necrosis and metabolic disorders.6,7 Carbon tetrachloride (CCl₄) is one of the widely known hepatotoxic agents that elucidate liver damage by enzymatic activation of CCl₄ by cytochrome P₄₅₀ to produce carbon trichloromethyl free radicals (•CCl₃) within the membrane of the endoplasmic reticulum. These free radicals bind covalently to polyunsaturated membrane lipid resulting in increased formation of lipid peroxide free radicals through lipid peroxidation and influence pathological changes in the liver’s structure and function.8 Due to the harmful effects of these free radicals and the adverse side effects associated with synthetic drugs, researchers considered phytotherapy as an alternative remedy.9 Antioxidants are well-known free radical scavengers and plants are considered to be rich in phytochemical compounds with potent antioxidant properties such as flavonoid, saponin, tannin, and phenol, and the mechanism of action could be by either (1) stimulating the liver to release certain chemicals or enzymes to detoxify agents that potentially cause liver damage (2) improving liver damage repair mechanism or (3) biotransforming active oxygen molecules (species) into nontoxic compounds.10,11 Tapinanthus bangwensis (T. bangwensis) (Engl. & K. Krause) Danser is a citrus-dependent plant of African origin. In Africa, it is popularly called African mistletoe. The English acronyms are “all heal tree,” “bird lime,” or “tree of life.” Traditionally, in Nigeria, T. bangwensis in Hausa is called Kauci (Kanchi), Yoruba—Afomo onisana, and Igbo—Awurusie or Apari. Ethno-medicinally, it has been reported to possess anti-inflammatorv, antimicrobial, anticancer, hypotensive, hypoglycemic effects, and so on.13,14 Previous studies provide limited information on the chemical compounds responsible for the hepatocurative potential of T. bangwensis. Therefore, the present study was undertaken in order to evaluate the antioxidant activity and identify the chemical compounds present in ethylacetate fraction of T. bangwensis that ameliorate CCl₄-induced hepatotoxicity in Wistar rats.

Materials and methods

Chemical reagents

All the chemical reagents used in this work such as methanol, ethylacetate, hexane, n-butanol, glacial acetic acid, CCl₄, potassium acetate, Folin–Ciocalteu reagent, hydrogen peroxide (H₂O₂), 2,2-diphenyl-1-picrylhydrazine (DPPH), 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB) (Ellman’s reagent), sodium pyrophosphate, methosulfate p-nitroblue tetrazolium chloride, nicotinamide dinucleotide (NADH), 1,1,3,3-tetraethoxypropane, trichloroacetic acid (TCA), thiobarbituric acid (TBA), ascorbic acid, and potassium chloride (KCl) were of analytical grade from Zigma in the United Kingdom.

Preparation of plant material

Fresh leaves of T. bangwensis were obtained from Lagos, botanically identified by Mr Adeleke, Department of Pharmagony, University of Lagos, and deposited at the University’s herbarium with the voucher number LUH 3856. The leaves were washed, dried for 5 days under dry climatic condition at room temperature, and pulverized into a fluffy mass using electric blender.

Extraction of plant material

About 2.15 kg of T. bangwensis ground leaves was extracted with 10 L of 100% methanol solvent using cold maceration method. The mixture was left for 48 h with intermittent shaking at intervals to increase the surface area for readily solvent penetration. The mixture was filtered using Whatman paper and the filtrate concentrated by evaporation at room temperature until a constant weight of 187.5 g (8.71%) was obtained. About 60 g of the concentrated methanol extract was further extracted by solvent partition method using separating funnel. The solution mixture was ethylacetate–water in 3:2. Two layers were observed. The upper portion was ethylacetate fraction which was collected and air-dried at room temperature until a constant weight of 20.45 g was obtained.

Column chromatographic extraction

The method of Vivex et al.15 was adopted. A cylindrical glass column (55 × 22 cm²) was packed with 100 g of silica gel (mesh size of 60–120) (stationary phase) dissolved in hexane solution. A 15 g of the dried ethylacetate fraction was mixed with 30 g of the silica gel to form a slurry particle. The slurry particle was then placed on top of the prepacked silica gel column and the sample covered with a layer of cotton wool. Four solvents of different polarities were used, namely hexane, ethylacetate, methanol, and water. The solvent mixtures were hexane (100%), hexane–ethylacetate (80:20, 60:40, 40:60, 20:80, and 100%), ethylacetate–methanol (80:20, 60:40, 40:60, 20:80, and 100%), methanol–water (80:20, 60:40, 40:60, 20:80, and 100%), and finally water only (100%). A total of 105 fractions were collected in 100 mL bottles and numbered consecutively. The fractions were covered with perforated aluminum foil and concentrated by air-drying. The various concentrated fractions were then pooled together based on their retention factors via an activated thin layer...
chromatography (TLC) and a total of six subfractions were obtained labeled as F0, F1, F2, F3, F4, and F5, respectively.

**Animal model and management**

Forty apparently healthy male Wistar rats weighing 100 g were used. They were maintained under standard laboratory conditions according to the animal welfare regulation. The rats were acclimatized for 2 weeks under standard environmental conditions (temperature, 25°C; humidity, 55%; and photoperiod, 12:12 h light:dark cycle) in airy plastic cages and were fed with rat feed and distilled water.

**Experimental design**

The rats were divided into eight groups with five rats in each group:

- **Group A**: Rats received feed and water only (normal control group).
- **Group B**: Rats induced with 120 mg/kg body weight (bwt) of CCl4 intraperitoneally (positive control group).
- **Group C**: Rats induced with 120 mg/kg bwt of CCl4 and then administered orally 100 mg/kg bwt of subfraction (F0) of ethylacetate fraction of T. bangwensis daily for 14 days.
- **Group D**: Rats induced with 120 mg/kg bwt of CCl4 and then administered orally 100 mg/kg bwt of subfraction (F1) of ethylacetate fraction of T. bangwensis daily for 14 days.
- **Group E**: Rats induced with 120 mg/kg bwt of CCl4 and then administered orally 100 mg/kg bwt of subfraction (F2) of ethylacetate fraction of T. bangwensis daily for 14 days.
- **Group F**: Rats induced with 120 mg/kg bwt of CCl4 and then administered orally 100 mg/kg bwt of subfraction (F3) of ethylacetate fraction of T. bangwensis daily for 14 days.
- **Group G**: Rats induced with 120 mg/kg bwt of CCl4 and then administered orally 100 mg/kg bwt of subfraction (F4) of ethylacetate fraction of T. bangwensis daily for 14 days.
- **Group H**: Rats induced with 120 mg/kg bwt of CCl4 and then administered orally 100 mg/kg bwt of subfraction (F5) of ethylacetate fraction of T. bangwensis daily for 14 days.

**Blood collection and storage**

After the 14th day of treatment with the subfractions of ethylacetate fraction of T. bangwensis, rats were fasted overnight for 12 h. They were anesthetized and blood collected through the jugular vein in the neck region. The blood samples were centrifuged at 3000 r/min for 5 min, supernatants (sera) collected using a Pasteur pipette and then stored at 4°C before use for the determination of liver function indices such as alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) activities. Total protein (TP), albumin (ALB), total bilirubin (T.Bil), and conjugated bilirubin (C.Bil) levels were also analyzed.

**DPPH assay**

The method of Burtis and Buca17 was adopted. A 0.5 mL of the extract (or subfraction) in methanol at different concentrations (100, 300, 400, and 600 µg/mL) was added to 2.0 mL of DPPH solution (4 mg DPPH/100 mL methanol). The mixture was incubated at 25°C for 30 min and centrifuged at 3000 r/min for 15 min. The supernatant was collected and absorbance read at 517 nm. The blank contained 2.0 mL of DPPH only.

\[
\text{Scavenging activity} = \frac{\text{Absorbance of blank} - \text{Absorbance of extract}}{\text{Absorbance of blank}} \times 100
\]

**Liver tissue homogenate**

The excised rat livers were washed in 1.15% KCl solution blotted and weighed. Then, 0.5 g of the liver was homogenized gently with 5.0 mL of 0.1 M phosphate buffer at pH 7.2 using ceramic mortar and pestle. The resulting homogenate was centrifuged at 2500 r/min for 15 min and the supernatant collected by Pasteur pipette and stored at −20°C for analyses of liver biomarkers of oxidative stress such as glutathione (GSH) and malondialdehyde (MDA) levels as well as superoxide dismutase (SOD) and catalase (CAT) activities, respectively.

**Determination of GSH level**

The GSH level was estimated using the method of Zerargul et al.18 This method is based on the reduction of DTNB (Ellman’s reagent) by sulphydryl group to form 2-nitro-5-mercaptobenzoic acid. A 50 µL of liver homogenate was diluted in 1.0 mL of 0.1 M phosphate buffer at pH 8.0; 20 µL of 0.01 M DTNB was mixed with 3.0 mL of the mixture of dilution. A yellow color developed and absorbance was read at 412 nm after 5 min. The GSH level was then determined by the formula as shown below

\[
2\text{GSH} + \text{H}_2\text{O}_2 \xrightarrow{\text{glutathione peroxidase}} \text{GSSG} + 2\text{H}_2\text{O}
\]

\[
\text{GSH level (µmol/ml)} = \frac{\Delta \text{Abs} \times 100}{1.34} \times \frac{\text{Total volume of extract (ml)}}{\text{Weight of tissue (g)} \times 1000}
\]

**Determination of SOD activity**

SOD activity was measured according to the method of Kakkar et al.19 A 0.05 mL of the liver homogenate was
mixed with 1.2 mL of 0.05 M sodium pyrophosphate buffer at pH 8.3, 0.1 mL of 0.186 mM methosulfate phenazine, 0.3 mL of 0.3 mM nitroblue tetrazolium chloride, and 0.2 mL of 0.78 mM reduced NADH. The reaction was stopped after 90 s by the addition of glacial acetic acid. The color intensity of the chromogen was extracted in 20 mL butanol with vigorous shaking. The mixture was then centrifuged at 3000 r/min for 10 min. The supernatant was collected and the absorbance read at 500 nm.

$$2\text{HO}_2 \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$$

**superoxide dismutase**

$$\text{SOD Activity} = \frac{\Delta \text{Absorbance} \times 100}{\text{Amount of protein (mg)} \times \text{Time (min)}}$$

**Determination of CAT activity**

CAT activity was measured by the method of Claiborne. This method is based on the breakdown of H$_2$O$_2$ to water and oxygen by CAT. Briefly, the assay mixture contained 1.95 mL of 0.05 M phosphate buffer at pH 7.4, 1.0 mL of 19 mM H$_2$O$_2$, and 0.05 mL liver homogenate in a total volume of 3.0 mL. The absorbance was read at 240 nm and CAT activity expressed as

$$2\text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O} + \text{O}_2$$

$$\text{CAT Activity} = \frac{\Delta \text{Abs} \times 100}{\text{Amount of protein (mg)} \times \text{Time (min)}}$$

**Determination of MDA level**

The level of MDA was determined by the method of Okhawa et al. This method is based on the interaction of MDA with 1,1,3,3-tetraethoxypropane in different concentrations was plotted from where the MDA concentration was determined.

**Liver histology**

The method of Million et al. was followed. After the rats were sacrificed, livers were excised and fixed in 10% formalin solution for 72 h, dehydrated through ascending alcohol gradient (50, 70, 90, and 100%), and cleared in two changes of xylene I and II and embedded in paraffin wax. Serial transverse section of 4–5 μm thickness was prepared using the Leica microtome (Leica Biosystems, USA) and then stained with hematoxylin and eosin. The samples were then viewed under light microscope with high resolution and observations recorded.

**Elucidation of chemical compounds**

The chemical compounds present in subfraction (F4-A(2)) of ethylacetate fraction of T. bangwensis were elucidated by liquid chromatography–mass spectroscopy (LC-MS) technique. The instrument used was Agilent 1290 infinity liquid chromatography system coupled to Agilent 6520 (Agilent Technologies Inc., USA) Accurate mass Q-TOF mass spectrometer with dual ESI source. A 3.0 mL liquid sample of subfraction (F4-A(2)) was diluted with methanol in 1:1. Subsequently, 1.0 μL of the resulting solution was injected into the column and read against a methanol as blank.

**Statistical analysis**

The data obtained were statistically analyzed as mean ± standard deviation. One-way analysis of variance and Tukey multiple comparison test were used, respectively. The confidence limit was at $p < 0.05$.

**Results**

Table 1 shows the result of the recovery profile of the subfractions of ethylacetate fraction of T. bangwensis. A total of 105 column chromatographic fractions were obtained in 100 mL bottles. They were pooled together into six subfractions and labeled as F0, F1, F2, F3, F4, and F5, respectively. The subtraction F1 indicated the highest yield of 3.63 g (24.76%) with a retention factor of 0.3, while subfraction F5 showed the lowest yield of 1.46 g (9.96%) with a retention factor of 0.7. Figure 1 shows the result of the inhibition of oxidized DPPH free radical (DPP*) with a retention factor of 0.7. Table 1 shows the result of the recovery profile of the subfractions of ethylacetate fraction of T. bangwensis. A total of 105 column chromatographic fractions were obtained in 100 mL bottles. They were pooled together into six subfractions and labeled as F0, F1, F2, F3, F4, and F5, respectively. The subtraction F1 indicated the highest yield of 3.63 g (24.76%) with a retention factor of 0.3, while subfraction F5 showed the lowest yield of 1.46 g (9.96%) with a retention factor of 0.7. Figure 1 shows the result of the inhibition of oxidized DPPH free radical (DPP*). The inhibition concentration (IC$_{50}$) result revealed that subfraction F0 exhibited the strongest antioxidant activity followed by subfraction F4, while subfraction F1 exhibited the lowest antioxidant activity. However, the antioxidant activity of ascorbic acid was higher compared to other subfractions. The effects of the subfractions of ethylacetate fraction of T. bangwensis on liver function indices of oxidative stress such as GSH and MDA levels as well as SOD and CAT activities were evaluated as shown in Figure 2. The GSH level increased in the induced and treated groups except in the group treated with subfractions F0 and F1 compared to the positive control group. However,
subfraction F4 effect was higher compared to other induced and treated groups. SOD activity reduced in the positive control group but increased in the induced and treated groups except in the groups treated with subfractions F2 and F3. Conversely, subfraction F4 treated group improved SOD activity than in other induced and treated groups. There was no significant difference between the positive control group and the group induced and treated with subfractions at \( p < 0.05 \). CAT activity was found to be reduced in the positive control group. On treatment with 100 mg/kg bwt of the subfractions, CAT activity increased. However, the group treated with subfractions F1 and F3 improved CAT activity compared to other treated groups (Figure 2). There was no significant difference between the positive control group and the induced and treated groups at \( p < 0.05 \). The MDA level increased in the subfraction F2, F3, and F5 treated groups compared to the positive control group, however, the group induced and treated with subfractions F0, F1, and F4 showed reduction in MDA level compared to the positive control group. However, there was no significant difference between the positive control group and the induced and treated groups at \( p < 0.05 \) (Figure 2). The liver function indices assayed were AST, ALT, and ALP activities as well as ALB, TP, T.Bil, and C.Bil levels (Table 2). The AST activity after treatment was found to be lower in the groups induced and treated with the subfractions compared to the positive control group. The ALT activity reduced in the groups induced and treated with the subfractions compared to the positive control group, nevertheless the effect was higher in the group induced and treated with subfraction F3. Furthermore, ALP activity was reduced in the groups induced and treated with subfractions F0 and F4, however, the ALP activity was higher in the other induced and treated groups compared to the positive control group. There were increase in the levels of serum ALB and TP in the induced and treated groups and normal control group except in the subfraction F1 and F2 treated groups compared to the positive control group, while the levels of T.Bil and C.Bil decreased in the induced and treated groups compared to the positive control group; however, there were no significant differences between the positive control group and the induced and treated groups in the liver function indices at \( p < 0.05 \). The histopathology study shows liver steatosis in the positive control group (plate B), but after 14 days of oral administration of subfractions F0, F1, F2, F3, F4, and F5, normal hepatocyte distributions and no fatty changes were observed as shown in plates C, D, E, F, G, and H, respectively, nonetheless, sinusoidal congestion was observed in plate G. The normal control group shows normal hepatocyte distribution, no fatty

**Table 1. Recovery profile of the subfractions of ethylacetate fraction of *Tapinanthus bangwensis.***

| Subfractions | Solvent mixtures | Fraction size | Mass recovery (g) | Percentage recovery (%) | Retention factor (\( R_f \)) |
|--------------|-----------------|---------------|-------------------|-------------------------|-----------------------------|
| F0           | 100% Hx         | F1–F5         | 2.80              | 19.10                   | 0.5                         |
| F1           | Hx:ETOAc 80:20  | F6–F12        | 3.63              | 24.76                   | 0.3                         |
| F2           | Hx:ETOAc 80:20  | F13–F36       | 1.89              | 12.89                   | 0.6                         |
| F3           | Hx:ETOAc 80:20  | F37–F60       | 1.94              | 12.23                   | 0.3                         |
| F4           | Hx:ETOAc 40:60  | F61–F86       | 2.94              | 20.05                   | 0.4                         |
| F5           | ETOAc:MeOH 80:20| F87–F105      | 1.46              | 9.96                    | 0.7                         |
|              |                 | Total         | 14.66             | 99.99                   |                             |

Hx: hexane; ETOAc: ethylacetate; MeOH: methanol; H2O: water.

**Figure 1.** DPPH antioxidant activity of subfractions of ethylacetate fraction of *Tapinanthus bangwensis.* DPPH: 2,2-diphenyl-1-picrylhydrazine.
change, and no sinusoidal congestion, respectively (plate A) (Figure 3). The result of the DPPH bioguided assay used for screening the preparative-TLC (Prep-TLC) bands of subfraction (F4) of ethylacetate fraction of *Tapinanthus bangwensis* for structural elucidation, as shown in Figure 4, shows that band A(2) exhibited stronger antioxidant activity compared to band B(1) though ascorbic acid exhibited the strongest antioxidant activity compared to the bands (Figure 4). Therefore, LC-MS analysis of subfraction (F4-A(2)) as shown in Table 3 reveals the presence of five chemical compounds such as 8-hydroxyflavone-8-glucoside, Avicularin, Fisetin-7-glucoside, Isoscantellarein-7-xyloside, and Isovitexin, respectively.

**Discussion**

CCl₄ hepatotoxic effect is caused due to the release of carbon trichloromethyl free radicals (*CCl₃*) resulting from the metabolic activation of CCl₄ by cytochrome P₄₅₀ dependent mixed oxidase present in the liver endoplasmic reticulum. These free radicals attack lipoprotein-deoxyribonucleic acid membrane in the presence of oxygen forming trichloromethylperoxyl free radicals (*CCl₃OO*) which induce lipid peroxidation. This metabolic action leads to liver damage, loss of metabolic enzyme activation, protein inhibition, and lipoprotein secretion. The decrease observed in SOD and CAT activities in the positive control group may be due to accumulation of superoxide anion radicals and H₂O₂, respectively, in the system. Furthermore, the decrease in GSH level could be due to the extrusion of GSH from the liver or increased utilization of GSH for free radical detoxification, while the increase in MDA level could be due to the attack by lipid peroxyl free radicals on biological membrane produced via lipid peroxidation. These abnormalities in liver function indices of oxidative stress were ameliorated after oral administration of 100 mg/kg bwt of CCl₄ in rats.
the different subfractions of ethylacetate fraction of *T. bangwensis*. The improvement in these liver parameters suggests that the plant possess chemical compounds that exhibit strong antioxidant activity against the effect of lipid peroxyl free radical activity. Another attributable evidence of hepatocytic damage is the increase in the activities/levels of hepatic biomarkers (or liver function indices) found in the circulatory system. The elevated serum enzyme activity indicates tissue perforation, hepatocellular leakage, and loss of functional integrity of liver’s plasma-lemma and could be due to the stimulation of lipid peroxidation and depletion of antioxidant reservoirs. Furthermore, elevated levels of serum T.Bil and C.Bil indicate either decrease uptake and conjugation of bilirubin, decrease secretion by the liver or obstruction of the bile acids. The reduction in these serum liver biomarkers observed in this study indicates the antioxidative effect of *T. bangwensis* against lipid peroxyl free radical attack.

This result correlates with reported findings. Some studies suggest that decrease in ALB and TP levels could be attributed to reduction in protein biosynthesis caused by disruption and dissociation of polyribosemes on rough endoplasmic reticulum. This phenomenon was observed in the positive control group as shown in Table 2. However, oral treatment with the subfractions of ethylacetate fraction of *T. bangwensis* elevated ALB and TP levels and subsequently decreased AST, ALT, and ALP activities and T.Bil and C.Bil levels, respectively, compared to the positive control group. Oxidant substances (free radicals) generated from chemicals, drugs to mention a few cause oxidative stress. Free radicals are unstable substances as a result of the presence of unpaired electron(s), thus are called electron-deficient molecules. This instability makes them to attack membrane macromolecules resulting in membrane disruption/damage. The DPPH assay which is a stable and widely used method for evaluating antioxidant power of medicinal plants shows that *T. bangwensis* exhibits strong antioxidant activity and may be attributed to the antioxidant activities of Isoscutellarein-7-xyloside and Fisetin-7-glucoside as reported by research findings. The liver fatty deposit observed in the positive control group indicates defective lipid metabolism and excretion thereby leading to impaired blood flow through the portal vein resulting in the onset of hepatic hypertension. After oral treatment with the subfractions of ethylacetate fraction of *T. bangwensis*, ameliorative effects were observed. The LC-MS analysis of the 8-hydroxyluteolin-8-glucoside, Avicularin, Fisetin-7-glucoside, Isoscutellarein-7-xyloside, and Isovitexin, respectively. These chemical compounds...
identified are phytochemical compounds of flavonoid derivatives in which a sugar moiety is attached at different positions of the flavonoid ring at either position 3, 7, or 8, respectively. The 8-hydroxy-luteolin-8-glucoside (also called Hypolaetin-8-O-D-glucoside) is a flavonoid glycoside and has been reported to exhibit both antioxidant and hepatocurative activities.\(^{48}\) Avicularin (also called Avicularine, Avicularoside, fenicularin, or Quercetin-3-O-L-arabinofuranoside) is also a flavonoid glycoside in which an O-L-arabinofuranosyl residue is attached at position 3 of quercetin via a glycosidic linkage and has been reported to possess both antioxidant and hepatocurative properties.\(^{49}\) Furthermore, Fisetin-7-glucoside (also called 2-[3',4'-dihydroxyphenyl]-3,7-dihydroxy-4H-chromen-4-one-7-O-L-glucoside) is a flavonol glycoside with a reported antioxidant activity\(^{46}\) while Isoscutellarein-7-xyloside, a novel flavone glycoside compound in which xylose sugar residue is attached at position 7 of the flavonoid ring. It exhibits antioxidant property.\(^{45}\) Finally, Isovitexin (also called homovitexin or saponaretin) which is a flavone glycoside phytochemical compound, rich in polyphenols has been reported to exhibit antioxidant and hepatocurative properties.\(^{50}\)

### Conclusion

In lieu of the life-threatening effects of hepatotoxicity, huge financial cost associated with liver transplant, and adverse side effects of synthetic drugs, the present study validates that the antioxidant activity and hepatocurative potential of ethylacetate fraction of *Tapinanthus bangwensis* is due to the presence of the chemical compounds mentioned above. Hence, it can be concluded that *T. bangwensis* has a promising medicinal potential against CCl\(_4\)-induced hepatotoxicity in Wistar rats.

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**Table 3.** Bioactive compounds in subfraction (F4-A(2)) of ethylacetate fraction of *Tapinanthus bangwensis* using LC-MS.

| Peaks | RT (min) | Formula | Mass | m/z  | Compounds | Structure |
|-------|----------|---------|------|------|-----------|-----------|
| 3     | 9.164    | C\(_{21}\)H\(_{30}\)O\(_{12}\) | 464.09657 | 463.08891 | 8-Hydroxy-luteolin-8-glucoside |
| 4     | 9.424    | C\(_{20}\)H\(_{18}\)O\(_{11}\) | 434.08578 | 433.07848 | Avicularin |
| 5     | 9.517    | C\(_{21}\)H\(_{30}\)O\(_{11}\) | 448.10141 | 447.09421 | Fisetin-7-glucoside |
| 6     | 9.704    | C\(_{20}\)H\(_{18}\)O\(_{10}\) | 418.09076 | 417.0834 | Isoscutellarein-7-xyloside |
| 8     | 9.973    | C\(_{21}\)H\(_{30}\)O\(_{10}\) | 432.10602 | 431.0988 | Isovitexin |

LC-MS: liquid chromatography–mass spectrometry.
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Author contributions
GOI, AJA, and MSS designed and supervised the work while GOI and CJO prepared the manuscript and analyzed the data obtained. All authors proofread the manuscript and made corrections where appropriate.

Declaration of conflicting interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval
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References
1. Shani S. Evaluation of hepatoprotective efficacy of APCL-A polyherbal formulation in vivo in rats. Indian Drugs 1999; 36: 628–631.
2. Subramoniam A and Pushpangadan P. Development of phytomedicine for liver diseases. Indian J Pharmcol 1999; 31: 166–175.
3. Ward FM and Daly MJ. Hepatic disease: In: Walker R and Edward C (eds) Clinical pharmacy and therapeutics. New York: Churchill Livingstone, 1999, pp. 195–212.
4. Sahreen S, Khan MR and Khan RA. Hepatoprotective effect of methanol extract of Carissa opaca leaves on CCl₄-induced damage in rats. BMC Complement Altern Med 2011; 11(1): 48.
5. Salama E, Tousson T, Elfetoh EM, et al. Effects of Egyptian plant (Silybum marianum) on the kidney during the treatment of liver fibrosis in female albino induced by alcohol in comparison to the medical silymarin from China. Int J Curr Microbiol Appl Sci 2015; 4(3): 557–570.
6. Cullen J. Mechanistic classification of liver injury. Toxicol Pathol 2005; 33(1): 6–8.
7. Nikoletopoulov V, Markaki M, Palikaras K, et al. Crossstalk between apoptosis, necrosis and autophagy. BBA Med Cell Res 2015; 1833(12): 3448–3459.
8. Reilly PM and Bulkley GB. Tissue injury by free radicals and other toxic metabolites. Brit J Surg 1990; 77(12): 1323–1324.
9. Kyung JL, Jea HC and Hye GJ. Hepatoprotective and antioxidant effects of the coffee diterpenes kahweol and cafestol on carbon tetrachloride-induced liver damage in mice. Food Chem Toxicol 2007; 45: 2118–2125.
10. Suresh-Kumar SV and Mishra SH. Hepatoprotective effect of Pergularia daemia (Forsk) ethanol extract and its fraction. Indian J Exp Biol 2008; 46: 447–452.
11. Moselby SS and Ali HK. Hepatoprotective effects of cinnamon extract against carbon tetrachloride induced oxidative stress and liver injury in rats. Biol Res 2009; 42: 93–98.
12. Oluwole O, Osungunna MO and Abimbola Y. Phytochemical and antimicrobial screening of Globimetula oreophila (Oliv) Van Tiegh and Phragmanthera capitata (Spreng) Balle. Int J Green Pharm 2013; 7(2): 127–130.
13. Ekhaise FO, Ofuezie VG and Enobakhare DA. Antibacterial property and preliminary phytochemical analysis of methanolic extract of mistletoe (T. bangwensis). Bayero J Pure Appl Sci 2010; 3(2): 65–68.
14. Patrick-Iwuanyanwu KC, Onyeike EN and Wegwu MO. Hepatoprotective effects of methanolic extract and fractions of African mistletoe Tapinanthus bangwensis (Engl. & K. Krause) from Nigeria. Excil J 2010; 9: 187–194.
15. Vivek KB, Rajib M and Jae GP. Isolation and purification of plant secondary metabolites using column chromatographic techniques. Bangladesh J Pharmacoal 2016; 11: 844–848.
16. OECD. Acute oral toxic class method. Guideline 423, adopted 23 March 1996. In: Eleventh addendum to the OECD guidelines for the testing of chemicals. Paris: OECD, June 2000.
17. Burtis M and Bucar F. Antioxidant activity of Nigella sativa essential oil. Phytother Res 2000; 14: 323–328.
18. Zerargul F, Baghiani A, Khennouf S, et al. Antioxidant activity assessment of Tamus communis L. roots. Int Pharm Pharm Sci 2016; 8(12): 64–71.
19. Kakkar P, Das B and Viswanthan PM. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biol 1984; 21: 130–132.
20. Clai borne AL. Handbook of methods or oxygen radical research. London: CRC Press, 1985.
21. Okhawa H, Ohishi N and Yagi K. Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 105: 351–358.
22. Schmidt E and Schmidt FW. Das Verteilungsmuster einiger Enzyme in der menschlichen Leber und seine Veränderungen unter der Zeil-Schädigung. Enzymol Biol Clin 1963; 3: 73–79.
23. Babson LA, Greeley SJ, Coleman CM, et al. Phenolphthalein monophosphate as a substrate for serum alkaline phosphatase. Clin Chim Acta 1966; 12: 482–490.
24. Doumas BT, Watson WA and Biggs HG. Albumin standards and the measurement of serum albumin with bromresol green. Clin Chim Acta 1971; 31: 87–96.
25. Tietz NW. Fundamentals of clinical chemistry. 3rd ed. Philadelphia, PA: W.B. Saunders Company, 1976, pp. 874–897.
26. Tietz NW. Clinical guide to laboratory tests. 3rd ed. Philadelphia, PA: W.B. Saunders Company, 1996, pp. 518–519, 1028.
27. Million L, Abay M, Solomon MA, et al. Acute and subacute toxicity of methanol extract of Syzygium guineense leaves on the histology of the liver and kidney and biochemical
composition of blood in rats. *Evid Based Complement Altern Med* 2019; 2019: 1–15.

28. Recknager RO, Glende RA and Hruszkewycz AM. In: Pryor EA (ed) *Free radicals in biology*. Vol. II. New York: Academic Press, 1976, pp. 97–132.

29. DeGrot H and Noll T. The crucial role of low steady state of oxygen partial pressure of halogen free-radical mediated lipid peroxidation. *Biochem Pharmacol* 1986; 35: 15–19.

30. Gravel E, Albano E, Dianzani MU, et al. Effects of carbon tetrachloride in isolated rat hepatocytes: inhibition of protein and lipoprotein secretion. *Biochem J* 1979; 178: 509–512.

31. Rajagopal SK, Manickam P, Periyasamy V, et al. Activity of *Allium sativum* leaf extract in rats with alcoholic liver injury. *J Natr Biochem* 2003; 14: 450–458.

32. Srinivasan R, Chandrasekar MJN, Nanjan MJ, et al. Antioxidant activity of *Caesalphia digyna*. *J Ethnopharmacol* 2007; 113: 284–291.

33. Kepekci RA, Palat S, Celik A, et al. Protective effect of *Spirulina platensis* enriched in phenolic compounds against hepatotoxicity induced by CCl4. *Food Chem* 2013; 141: 1972–1979.

34. Li G, Yang S and Lu F. Effect of *P. quinquefolius* saponin on the platelet aggregation rate and superoxide dismutase activity in the hyperlipidemic rats. *Banquen Yike Daxue Xuebao* 1996; 22: 3.

35. Hvattum E. Determination of phenolic compounds in rose hip (*Rosa canina*) using liquid chromatography coupled to electrospray ionization tandem mass spectroscopy and diode array detection. *Rapid Commun Mass Spectrom* 1996; 116: 655–662.

36. Fecka I. Qualitative and quantitative determination of hydrolysable tannins and other polyphenols in herbal products from meadow sweet and dog rose. *Phytochem Anal* 2009; 20: 177–190.

37. Osman NN. The role of antioxidant properties of celery against lead induced hepatotoxicity and oxidative stress in irradiated rats. *Arab J Nucl Sci Appl* 2013; 46(1): 339–346.

38. Oyetayo VO and Osibo B. Assessment of properties of strains of *Lactobacillus plantarum* isolated from fermenting corn slurry (Ogi). *J Food Agric Environ* 2004; 2(1): 132–134.

39. Sharma A, Sharma V and Kansal L. Therapeutic effects of *Allium sativum* on lead-induced biochemical changes in soft tissues of Swiss albino mice. *Pharmacogn Mag* 2009; 5(20): 364–371.

40. Sharma V and Pandey D. Protective role of *Tinospora cordifolia* against lead-induced hepatotoxicity. *Toxicol Int* 2010; 17(1): 12–17.

41. Farombi EO. Locally derived natural antioxidant substances in Nigeria. Potential role as a new chemotherapeutic agents. In: Bahoun T and Gurib-Fakim A (eds) *Molecular and therapeutic aspects of redox biochemistry*. UK: OICA Int. Ltd, 2003, pp. 208–225.

42. Thabrew MI, Joice PDTM and Rajatiss WA. Comparative study of efficacy of *Paetta indica* and *Osbeckia octandra* in the treatment of liver dysfunction. *Planta Med* 1987; 53: 239–241.

43. Asadollahi A, Sarir H, Omidi A, et al. Hepatoprotective potential of *Prosopis farcta* beans extracts against acetaminophen-induced hepatotoxicity in Wistar rats. *Int J Prev Med* 2014; 5(10): 1281–1285.

44. Sanchez-Moreno C. Methods used to evaluate the free scavenging activity in foods and biological systems. *Food Sci Technol Int* 2002; 8: 121–137.

45. D’Abrosca B, Scognamiglio M, Graziani V, et al. Polar bioactive constituents from aerial parts of *Thymus longicaulis* C. Presl. *Lett Health Biol Sci* 2016; 1(1): 1–4.

46. Khan N, Syed DN, Ahmad N, et al. Fisetin: a dietary antioxidant for health promotion. *Antioxidant Redox Signal* 2013; 19(2): 151–162.

47. Shannon M. Pathology of the liver and biliary act-2: developmental, circulatory and metabolic disorders. *Develop Anomalies* 2017; 1: 23.

48. El-Touny SA, Omara EA, Nada SA, et al. Flavone C-glycosides from *Montanoa bipinnatifida* stems and evaluation of hepatoprotective activity of extract. *J Med Plant Res* 2011; 5(8): 1291–1296.

49. Kim SM, Kangi K, Jho EH, et al. Hepatoprotective effect of flavonoid glycosides from *Lespedeza cuneate* against oxidative stress induced by tert-butyl hydroperoxide. *Phytother Res* 2011; 25(7): 1011–1017.

50. Mamiya-Kumar K, Ganga-Rao B and Padmaja V. Role of vitexin and isovitexin in hepatoprotective effect of *Alysicarpus monilifer* Linn. against CCl4 induced hepatotoxicity. *Phytotherapic* 2012; 3(2): 273–285.