Chemical Profile and Hepatoprotective Activity of Ethyl Acetate Extracts of *Euphorbia paralias* and *Euphorbia geniculata* (Euphorbiaceae) from Egypt

Afaf E. Abdel Ghani, Sayed A El-Toumy, Wagdi i. A. El-Dougdoug, Ahmed M. Mansour, Wafaa H. B. Hassan, Hanaa M. Hassan

**ABSTRACT**

**Background:** Plants belonging to the genus *Euphorbia* were used traditionally to treat several health disorders and diseases. **Objective:** the aim of this study is evaluation of secondary metabolites and hepatoprotective activity of the ethyl acetate fractions of the aerial parts of *Euphorbia paralias* (Ep) and *Euphorbia geniculata* (Eg). **Materials and Methods:** UPLC-ESI-MS/MS technique was used for identification of the secondary metabolites. The hepatoprotective potential of the two plants was evaluated for the first time in male rats with thioacetamide induced liver injury. **Results:** A total of 32 secondary metabolites were identified in the ethyl acetate fractions of the aerial parts of both species. Ellagitannins such as tetragalloylhexose, ellagic acid, gallic acid, and flavonoids such as kaempferol-3-O-β-D-glucopyranoside, quercetin glycosides (glucoside and arabinoside) were found to be the major components in *Ep* whereas flavonoid glycosides including quercetin rutinoside, quercetin glycosides (glucoside, arabinoside and rhamnoside) and kaempferol glycoside derivatives were highly abundant in *Eg*. Administration of thioacetamide resulted in marked elevation in liver enzymes, elevation of lipid profile and alteration in oxidative stress parameters. While pretreatment of rats with *Ep* and *Eg* ethyl acetate fractions significantly attenuated the hepatic toxicity through reduction of liver biomarkers, improving the redox status of the tissue and so brought down the serum biochemical parameters and lipid profile nearly toward the normal levels. **Conclusion:** The studied fractions show hepatoprotective potential with promising value as hepatoprotective drugs of natural origin in comparison with silymarin as the standard hepatoprotective drug.

**Key words:** Euphorbia, UPLC-ESI-MS/MS, Polyphenolics, Hepatoprotective.

**INTRODUCTION**

Plants have used as an essential source of drugs and remedies on treatment of diseases and health disorders since ancient times1. Flavonoids and phenolic compounds containing plants which are common among medicinal plants were reported for its various health benefits and applications. They also decrease the risk of cardiovascular diseases, enhance regeneration of the liver and increase life expectancy 2,3. As liver damage can be life threatening and its damage is caused by several factors such as alcohol, viruses, organic chemicals, metabolic and genetic abnormalities4. Liver transplantation was improved survival rate of patients in some cases only and is limited to a small number of patients due to non-availability of suitable donors. And so, finding new drugs that are able to enhance liver regeneration and prevent liver failure is a very important need. Natural products as plant extracts exhibiting antioxidant and hepatoprotective activities can be useful in these needs5. The aim of the current study was to identify the polyphenolics and flavonoids in the ethyl acetate (EA) fractions of the aerial parts of *Ep* and *Eg* using UPLC-ESI-MS/MS and to investigate the possible hepatoprotective activities of the studied fractions.

**MATERIALS AND METHODS**

**Plant material and extraction**

Aerial parts of *E. paralias* L. and *E. geniculata* Ortega were collected in the flowering stage on May and August 2015, respectively. *E. paralias* was collected from the North beach of Alexandria, Egypt. While *E. geniculata* was collected from road sides in the vicinity of Banha, Qalubya, Egypt. The identification was kindly verified by Dr. Ahmed Abd El-Razik Lecturer of Plant Taxonomy, Department of Botany, Faculty of Science, Banha University, Egypt. The vouchers specimens (no. 3503 and 3504) were deposited in National Research Centre, Dokki, Cairo, Egypt. The air-dried powdered plant materials *Ep* and *Eg* (500 g of each plant) were extracted by cold maceration with 70 % methanol until complete exhaustion. The methanolic extracts were evaporated under reduced pressure at 45°C. The greenish brown viscous residues (105.0 and 100.5 gm respectively) were separately dissolved in MeOH-H₂O mixture.
Ethyl acetate fractions of Ep and Eg were dissolved in dist. water at the required doses, forty eight rats were divided into eight groups, six animals each, and the following schedule of treatment was adopted: Group 1 (Control group): rats were given normal saline daily (2 ml/kg b.w., orally) for 4 consecutive weeks and served as negative control group. Group 2 (TAA): rats were administered Ep fraction orally (200 mg/kg b.w.) dissolved in normal saline daily for 4 consecutive weeks. Group 3 (Sil): rats were treated with silymarin orally (50 mg/kg b.w.) dissolved in normal saline daily for 4 consecutive weeks. Group 4 (Ep): rats were treated with Eg ethyl acetate fraction orally (200 mg/kg b.w.) dissolved in normal saline daily for 4 consecutive weeks. Group 5 (Eg): rats were treated with Eg ethyl acetate fraction orally (200 mg/kg b.w.) dissolved in normal saline daily for 4 consecutive weeks. Group 6 (Sil+TAA): rats were pretreated orally silymarin then with thioacetamide daily for 4 consecutive weeks. Group 7 (Ep+TAA): rats were pretreated orally with Ep ethyl acetate fraction then with thioacetamide daily for 4 consecutive weeks using the same dose schedules as mentioned above. Group 8 (Eg+TAA): rats were pretreated orally with Eg ethyl acetate fraction then with TAA for 4 consecutive weeks using the same dose schedules as mentioned above, plants fractions and silymarin were administered to the animals orally by gastric intubation for 4 weeks following the procedure of (Dutta et al., 2013)6.

Serum and tissue preparations
Samples of blood were collected retro-orbital venous plexus of rats (under light ether anaesthesia) in non-heparinized tubes and for measuring biochemical parameters; the sera were separated. Later the animals were sacrificed; liver was dissected, washed in saline, blotted between dry filter papers and kept until antioxidants and histopathological examinations.

Biochemical analysis
Serum separated from blood samples was used for the determination of liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol (CH), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and total bilirubin. Part of liver tissue was homogenized and centrifuged at 5000 rpm for 10 min and the resulting supernatant was used for lipid peroxides malondialdehyde (MDA) contents, determination of oxidative enzymes; superoxide dismutase (SOD), catalase (CAT) activities, and reduced glutathione (GSH) 7,8. All tests were carried out using colorimetric spectrum Biodiagnostics6,9 and Diamond® kits (Cairo, Egypt).

Histopathological examination
Autopsy samples were taken from the rats livers in the different groups and fixed in 10% neutral buffered formalin for 24 h. Then serial dilutions of alcohol (ethyl, absolute ethyl and methyl) were used in specimens' dehydration. The specimens were cleaned by xylene and embedded in paraffin in a hot air oven at 56°C for 24 h. Paraffin bees wax tissue blocks were prepared for sectioning at 4 μm thicknesses by sledge microtome. The obtained sections of tissue were embedded between glass slides, deparaffinized, stained by hematoxylin and eosin stain, and another slides from the same samples stained with a specific stain (Masson's trichrom) then all were examined using the light electric microscope.

Statistical analysis of data
All data are presented as mean±SEM. Statistical analysis was performed using GraphPad prism version 7 (GraphPad, San Diego, CA). Group differences were analyzed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer for multiple comparison tests. The difference was considered significant at P ≤ 0.05.

RESULTS
UPLC-ESI-MS/MS identification of secondary metabolites
Structural analysis of different compounds found in the aerial parts of Ep and Eg ethyl acetate fractions resulted in the separation and tentative
Identification of 32 compounds using UPLC-ESI-MS/MS. Identification of compounds was performed using (M-1)^- /MS^2 and comparison with reported data. Ellagitannins and phenolic acids (22.00 and 11.48 %), flavonoids such as quercetin glycosides (35.00 and 39.97 %) and kaempferol glycosides (20.00 and 3.44 %) were found to be the major components in \(Ep\) and \(Eg\), respectively; as ellagittannins (tetragalloyl hexoside, ellagic acid, gallic acid) and flavonoids (kaempferol-3-O-β-(6''-galloyl-O-glucopyranoside), quercetin glycosides (glucose and arabinoside) were found to be the major components in \(Ep\) while quercetin rutinoside and other quercetin glycosides (glucose, arabinoside and rhamnoside) were highly abundant in \(Eg\). As observed; molecular and fragment ions were listed in Table 1. LC-MS/MS profiles for \(Ep\) and \(Eg\) ethyl acetate fractions in the negative ion mode are shown in Figure 1.

Identification of tannins

Peak 1 with deprotonated molecular ion peak at \(m/z\) 787 [M-H] and MS^2 at \(m/z\) 635, 483, 311, 169, so it was tentatively identified as tetragalloyl hexoside^10. While peak 2 was tentatively identified as gallic acid as its deprotonated molecular ion peak at \(m/z\) 169 [M-H] and MS^2 at \(m/z\) 125 (M−H−COOH) ^10. Peak 5 with molecular ion peak at \(m/z\) 183 [M-H] while MS^2 at \(m/z\) 125, so it was tentatively identified as methyl gallate^10. Peak 24 showed a molecular ion peak [M-H] at \(m/z\) 301, and MS^2 fragmentations at \(m/z\) 151, 211 so it was identified as ellagic acid^11. Peaks 8 and 10 with deprotonated molecular ion peak at \(m/z\) 635 [M-H] and MS^2 at 483 [M−H–galloyl–H_2 O], 331 (M−H–2 (galloyl-H_2 O)), 169 (M−H–hexoside-2 (galloyl-H_2 O)), so they were tentatively identified as trigalloyl hexoside and its isomer respectively^11.

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Identification of phenolic acids and their derivatives

Peak 6 with deprotonated molecular ion peak at m/z 353 [M-H] and MS² at m/z 191 (M–H–caffeoyl, 161) and 179 (M–H–quinic) so it was tentatively identified as chlorogenic acid. Peak 13 with deprotonated molecular ion peak at m/z 325 (M-H), and MS² at m/z 193 (M–H–arabinose) so it was tentatively identified as ferulic acid pentoside.

Identification of flavonoid compounds

Many flavonoid glycosides were identified in the ethyl acetate fractions of E. paralias and E. geniculata. Peak 3 detected at m/z 585 [M-H] with daughter ions at m/z 271, 100% so it was attributed to naringenin–galloylglucoside. Peaks 4, 7, 12, 17-22, 28 and 31 all identified as quercetin derivatives as they have quercetin moieties. They are different in types of sugar moieties according to the m/z lost during fragmentation process as (-glucose or galactose, -162), (-rhamnose, -146), (-xylose or arabinose, -132) and (-acetyl glucose, -206) and some of which identified as galloyl glycoside due to lose of galloyl moiety (-152) as shown in table (1). As the same were peaks 9, 14-16, 23, 25 and 30 were identified as kaempferol derivatives due to presence of kaempferoyl moiety (285 amu); In addition to sugars moieties and galloyl moiety as shown in table (1). Peak 11 identified as diosmetin–hexoside to presence of molecular ion peak at m/z 461, [M-H] and MS² fragments at 299 amu corresponding to diosmetin moiety (M-H- 162 ). Peak 32 identified as dimethoxy apigenin due to molecular ion peak at 329 m/z and daughter ion at 269 amu.

Hepatoprotective

Thioacetamide is hepatotoxic agent known to induce acute or chronic liver disease (fibrosis and cirrhosis) in the experimental animal model. In the present work, TAA is used as potent hepatotoxic agent in rats. A dose of 200 mg/kg ip TAA administration is reported to be the cause of hepatic toxicity. Its effect is due to increased oxidative stress.

Acute toxicity or lethality (LD₅₀) test

The results showed that the animals survived during the 24 h observation and no visible signs of toxicity were observed. According to Hodge and Sterner toxicity scale, the LD₅₀ values of the two fractions were in the practically non-toxic categories.

Antifibrotic effects

Evaluation of liver biochemical parameters

Exposure of animals with the hepatotoxic agent, TAA, resulted in significant (p ≤ 0.05) increase in the liver enzymes (ALT and AST) and total bilirubin in serum, lipid profiles (CH, TG and LDL) and lipid peroxidation (MDA) while significant decrease in HDL, GSH,
Figure 3: The effect of *E. paralias*, and *E. geniculata* on thioacetamid-treated rats on A: Cholesterol, B: triglycerides C: low density lipoprotein D: High density lipoprotein mg/dl in thioacetamid-treated rats on comparison with Silymarin.

Figure 4: The effects *E. paralias*, and *E. geniculata* on malondialdehyde, B: On reduced glutathione C: On catalase activity and D: On superoxide dismutase in thioacetamide-treated rats on comparison with Silymarin.

CAT and SOD), indicative of hepatocytes damage. Pretreatment of experimental animals with (*Ep* and *Eg*) EA fractions reversed the TAA-induced hepatotoxicity and restored the elevated levels of liver biomarkers toward normality in comparison with silymarin treated group as follow; *Ep*, *Eg* and silymarin caused significant decrease in the liver enzymes (ALT, AST and total bilirubin in serum) (Figure 2), lipid profiles (CH, TG and LDL) (Figure 3) and lipid peroxidation (MDA) while significant increase in HDL, GSH, CAT and SOD (Figure 4).

**Histopathological results**

Histopathological examinations of the sections of rat liver exposed to TAA showed (in H and E staining) severe tissue damage and hepatocytes degeneration. *Ep* and *Eg* pre-treatment attenuated the hepatic injury and showed significant protection of the hepatic cells from damage. There were no such alterations in *Ep* and *Eg* groups in compared to normal and silymarin treated groups (Figures 5 and 6).

**DISCUSSION**

The liver is the largest gland in the human body and susceptible to almost many different diseases including hepatitis, cirrhosis, alcohol related disorders and liver cancer. A major cause of these disorders is due to exposure to different environmental pollutants and xenobiotics. Also, the exposure to a lot of chemicals, such as carbon tetrachloride, bromobenzene, ethanol, thioacetamide and polycyclic aromatic hydrocarbons have been implicated in the etiology of liver diseases. It is fundamentally known that the regulation of apoptosis is a potential mechanism through which many agents such as polyphenolic compounds; can prevent toxicity and carcinogenesis. Silymarin is a mixture of natural flavanolignans contains at least seven compounds. The hepatoprotective and antioxidant activities of silymarin were attributed to control free radicals (FR), produced by the hepatic metabolism of toxic substances. The present study revealed that both *Ep* and *Eg* contain several types of gallotannins, phenolic
Acids and flavonoids which tentatively identified through UPLC-ESI-MS/MS analysis of ethyl acetate fractions of \textit{Ep} and \textit{Eg}. Polyphenolic compounds including flavonoids constitute 77.10 and 51.51\% of the ethyl acetate fractions of \textit{Ep} and \textit{Eg}, respectively. Quercetin and its derivatives in \textit{Eg} (39.97\%) represent about twice the kaempferol compounds in \textit{Ep} (20.00\%). These compounds were known to have high antioxidant activity that attributed to their ability to control FR demonstrated by high hepatoprotective activity in comparison with standard drug silymarin. That was evidenced by the significant improvement of liver enzymes (ALT and AST), total bilirubin, lipid peroxidation (MDA), oxidative stress related parameters (CAT, GSH and SOD) and lipid profile (CH, LDL, TG and HDL). According to the study, the potency strength of the fractions and silymarin on the liver enzymes differs from one to other; \textit{Ep+TAA} gp show more improvement in ALT and CH, than \textit{Eg+TAA} gp but nearly equal to \textit{Sil+TAA} gp, while \textit{Eg+TAA} gp show more improvement in GSH and CAT in comparison with \textit{Sil-gp} but the both plant fractions were equally in improvement degree in the rest of liver enzymes. Histopathologically, parallel structural improvement was elicited by either plants extracts as compared to silymarin. This is evidenced by the views of rat’s liver tissues treated with both plants as showing no collagen aggregation around central veins, no inflammatory infiltrate and no fibrosis. The results of the study on liver were indicating the promising values of these plants as hepatoprotective herbs in the future with more flow up.

**CONCLUSIONS**

This study revealed the identification of 32 polyphenolic compounds in the ethyl acetate fractions of the \textit{Ep} and \textit{Eg} using UPLC-ESI-MS/MS analysis; mainly tannins and flavonoid glycosides. Hepatoprotective activity exhibited by the studied extracts might be attributed to the high

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**Figure 5:** Light photomicrograph of H & E stain of liver sections tissues as A: (Cont gp): showing average central vein (CV), and hepatocytes arranged in single cell cords (black arrow) with intervening blood sinusoids. B: (TAA-gp): mildly expanded portal tract with mild portal and peri-portal inflammatory infiltrate (black arrows) and scattered apoptotic hepatocytes in peri-portal area (yellow arrow). C: (Sil+TAA gp): average central vein with apoptotic hepatocytes in peri-venular area (black arrows). D: (Ep+TAA gp): average portal tract with average portal vein (PV) and average hepatocytes in peri-portal area (black arrow). E: (Eg+TAA gp): dilated central veins, and scattered apoptotic hepatocytes in peri-venular area (black arrow) (×400 except D×200).

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**Figure 6:** Light photomicrograph Masson’s trichrome as: A: (Cont gp): liver sections showing average collagen distribution in portal tract (black arrows). B: (TAA gp): excess collagen bundles with complete nodule formation (black arrow). C: (Sil+TAA gp): high power view showing excess collagen in portal tracts with fibrous septa extending into hepatic lobule (yellow arrow). D: (Ep+TAA gp): average collagen around central vein (black arrow). E: (Eg+TAA gp): average collagen in portal tracts (black arrow) (×400).
content of these compounds. These findings need more explored and investigated through further set of experiments to recommend the ethyl acetate extracts of the two plants as hepatoprotective drugs of natural origin.

ACKNOWLEDGEMENTS

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REFERENCES

GRAPHICAL ABSTRACT

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