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Isolation, Characterization of Phytochemical Compounds and Hepatoprotective Activity Evaluation in Rats of Various Extracts from *Cyperus esculentus* L. tubers

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Abstract

This study aimed to investigate different phytochemical compounds of *Cyperus esculentus* tubers grown in Egypt, as affected by different extracts (oil, methanol, chloroform), and evaluate the hepatoprotective activity of these various extracts in rats. Methanol fraction was subjected to silica gel column chromatography, resulted in isolation of three compounds reported for the first time from this plant, oleanolic acid, α –Amyrin 3-*0*- glucopyranoside, β -Amyrin3-*0*- glucopyranoside. Their structures were elucidated by spectroscopic methods and comparing data with those reported in literature. GC/MS of oil showed twelve compounds, 9-octadecenoic acid (Z)-,2-hydroxy-1- (hydroxymethyl) ethyl ester represented the major compound, while hexylene glycol was the minor. GC/MS of chloroform gave ten compounds, 9 octadecenoic acid, (E) was the highest peak, and stigmasterol was the lower one. Chloroform, methanol and oil extracts of plant were evaluated for their hepatoprotective activity, in D-galactosamine induced hepatotoxicity in albino rats. The high (500 mg/kg), and low (250 mg/kg) doses of extracts, reduced levels of liver enzymes and total protein towards the normal value, which were comparable to that of reference drug silymarin and D-galactosamine indicating their hepatoprotective activity. Bioactive constituents and hepatoprotective activity of different extracts give this plant its therapeutic effects in addition to nutritional ones.

Keywords: *Cyperus esculentus*, Various extracts, Silica gel column chromatography, Terpenes, GC-MS analysis, |Hepatoprotective activity.

1. Introduction

Cyperaceae is a large family of flowering plants known as sedges, with about 5,500 species described in 90 genera [1]. *Cyperus* genus is about 700 species of sedges, including *Cyperus esculentus*, distributed in tropical and subtropical areas worldwide and extensively in Africa, Asia, and some European countries for their sweetish tubers [2]**.** *Cyperus esculentus* provides edible tubers commonly called tigernut, chufa sedge, nut grass, tigernut sedge, or earth almond [3,4].

It is commonly used as a healthy food for humans and animals in some parts of the world like Africa, Europe and America. It was reported to be rich in carbohydrates, dietary fiber, lipids, oil [5], and some useful mineral elements such as iron, calcium and vitamins C, D, E which are necessary for body growth and development [6,7].

It is very famous in Egypt, and locally named (Hab Alaziz), where it is used as a source of food, medicine, for the manufacture of soap and perfumes, also the oil is used to reduce cholesterol or lose weight [8], and beneficial to diabetics mellitus [9]. Literature revealed that tigernut help in preventing heart attacks, thrombosis and activates blood circulation [10], responsible for preventing and treating urinary tract and bacterial infection, assist in reducing the risk of colon cancer [11]. Antioxidant, antimicrobial [12], and antidiarrhoeal activities of plant was reported [13].

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The phytochemical screening of the *cyperus esculentus* showed the presence of carbohydrates, flavonoids, steroids, reducing sugars, tannins. alkaloids, saponins and resins [14]. Further research needs to be conducted in this area to determine the biologically active compounds present in this plant through their isolation.

2. Experimental

2.1. Plant material

Tigernut tubers were bought from local market at Tanta City, Egypt.

2.2. Instrumentation

NMR spectra were recorded on a Jeol Ex-500 MHz spectrometer: 500 MHz (^{1}H-NMR), 125 MHz (^{13}C-NMR). UV spectrometer (Schimadzu UV-240). EI-MS were recorded on a Finnigan MAT-SSQ 7000 instrument. Column chromatography (CC) was carried out on silica gel F254 (Merck). TLC was performed with silica gel 60 GF254 plates (Merck, Darmstadt, Germany). The spots were detected by spraying vanillin in H_2SO_4 using UV light.

2.3. Preparing extracts

About 500 g of Tigernut tubers were ground into powder with a grinding machine. Extraction was done using chloroform/methanol (3:1) giving oily layer and alcoholic layer. The oil was separated and subjected to GC/MS analysis. Methanol was added to the remaining alcoholic layer producing a brownish ppt., which was separated and dissolved in chloroform giving chloroform extract, and was subjected to GC/MS analysis. The methanol fraction was concentrated and subjected to silica gel column to isolate phytochemical compounds. Parts of oil, chloroform extracts, and methanol fraction were kept for biological study.

2.4. Isolation of the compounds

The methanol fraction was concentrated and subjected to silica gel CC, eluted with petroleum ether/chloroform (9:1,3:1, 1:1, 1:3), pure chloroform , finally with mixture of chloroform /methanol with increasing polarity (99:1, 97:3,93:7, 91:9). Similar fractions were combined, monitored on the basis of TLC profiles, to afford three major fractions from which 3 compounds were obtained. Three different fractions were eluted by different concentrations of chloroform/ methanol (Table 1), and purified by TLC on silica gel "G" plate using petroleum ether / ethyl acetate (9:1) as developing system, giving compound **1**, and (8:2) developing system giving compound **2**, **3**. The isolated compounds were identified with their physical and spectroscopic data and comparing data with those reported in literature

TABLE 1: Different methanol concentrations

Fractions no.	Different methanol concentrations %
1	3
2	7
3	9

2.5. Acid hydrolysis and identification of sugar

Acid hydrolysis was carried out by dissolving about 3 mg of the compound in 10 ml methanol mixed with 10% HCl refluxed on a boiling water bath for 2 hrs. The solution was diluted with distilled water and extracted with ethyl acetate (3 × 50 ml). The aqueous acidic phase, after separation of the aglycone, was neutralized with barium carbonate, evaporated till dryness and subjected to PC using. *n*-butanol: ethanol: water (4:1:2) as the developing solvent with authentic references from different sugars. The chromatograms were visualized by spraying with aniline phthalate and heated at 105ºC for few minutes. The sugar was identical with that of authentic D-glucose.

2.6.Gas chromatography-mass spectrometry (GC/MS) analysis

The GC-MS was performed using the GC-MS system (Agilent Technologies), equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt. The GC was equipped with HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 μm film thickness). Analysis was carried out using helium as the carrier gas at a flow rate of 1 ml/min at a split-less mode, injection volume of 1 μl and the following temperature program: 60°C for 2 min; rising at 5°C /min to 300°C and held for 10 min. The injector and detector were held at 280°C and 300°C, respectively. Mass spectra were obtained by electron ionization voltage (EI) at 70 eV and using a spectral range of m/z 50-550 and solvent delay 3 min. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

2.7. Animals

In this study, albino rats (100-120 g) were obtained from the animal house, National Research Centre. Rats were grouped and housed in polyacrylic cages (38 x 23 x 10 cm) with not more than six animals per cage and were maintained under standard laboratory conditions (temperature 25± 2ºC) with dark and light cycle (12/12h). The animals were fed with the standard pellet diet and fresh water ad libitum. All animals were acclimatized to laboratory conditions for a week before commencement of experiment. All procedures were described and approved by guidelines of the Institutional Animal Ethical Committee of National Research Centre.

2.8. Biological assay

The acute toxicity study was conducted in accordance with Lorke's method [15]**.** No lethality or any toxic symptoms were observed for the extracts. After estimation of LD_{50} of the extracts, the doses were selected for the hepatoprotective investigations using 5% and 10 % of the highest dose examined for the LD_{50}, which was 5000 mg/kg b.w. So a low dose (250 mg/kg) and a high dose (500 mg/kg) of the extracts were used.

2.9. Study design

For each extract, total number of 72 rats were divided into 12 groups of 6 animals each as follows:
Group I: Normal Control: The animals received distilled water (D.W.) 5 ml/kg b.w; orally for 8 days.
Group II: D-galactosamine Group: The amimals were injected intrapreitoneally with 200 mg/kg D-Gal N after 8 days of oral administration of distilled water.
Group III: Positive treatment Group: The animals received 100 mg/kg silymarin orally for 8 days. The animals received a single dose of D-Gal N in D.W. 200 mg/kg i.p. after 1 hour of vehicle on the 8th day.
Group IV: D-Gal N + low dose of chloroform extract of *cyperus esculentus* (250 mg/kg) was administered using stomach tube for 8 days. A single dose of D-Gal N in D.W. 200 mg/kg b.w. was given i.p. after 1hour of vehicle on the 8th day.
Group V: D-Gal N + high dose of chloroform extract of *cyperus esculentus* (500 mg/kg) was administered using stomach tube for 8 days. A single dose of D-Gal N in D.W. 200 mg/kg b.w. was given i.p. after 1hour of vehicle on the 8th day.
GroupVI: High dose of chloroform extract of Cyperus esculentus (500 mg/kg) was administered using stomach tube for 8 days.
Group VII: D-Gal N + low dose of methanol extract of *cyperus esculentus* (250 mg/kg) was administered using stomach tube for 8 days. A single dose of D-Gal N in D.W. 200 mg/kg b.w. was given i.p. after 1hour of vehicle on the 8th day.
Group VIII: D-Gal N + high dose of methanol extract of *cyperus esculentus* (500 mg/kg) was administered using stomach tube for 8 days. A single dose of D-Gal N in D.W. 200 mg/kg b.w. was given i.p. after 1hour of vehicle on the 8th day.
Group IX: High dose of methanol extract of Cyperus esculentus (500 mg/kg) was administered using stomach tube for 8 days.
Group X: D-Gal N + low dose of oil extract of *cyperus esculentus* (250 mg/kg) was administered using stomach tube for 8 days. A single dose of D-Gal N in D.W. 200 mg/kg b.w. was given i.p. after 1hour of vehicle on the 8th day.
Group XI: Group XI: D-Gal N + high dose of oil extract of *cyperus esculentus* (500 mg/kg) was administered using stomach tube for 8 days. A single dose of D-Gal N in D.W. 200 mg/kg b.w. was given i.p. after 1hour of vehicle on the 8th day.
Group XII: High dose of oil extract of Cyperus esculentus (500 mg/kg) was administered using stomach tube for 8 days [16].

2.10. Biochemical studies

At the end of the experiment, the animals were kept fasting for 12 hours before blood sampling. Blood was collected from the retro-orbital venous plexus of the eye using a capillary tube under ether anesthesia [17]. Blood was collected in tubes and livers were removed quickly on ice and homogenized for estimation of liver parameters [18].

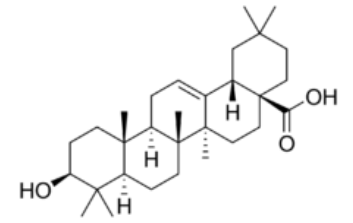
2.11. Biochemical investigations
Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and total protein using Spectrophotometer.

3. Results and Discussion

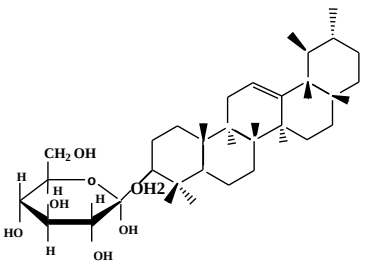
3.1. Elucidation of the compounds

Identification of bioactive compounds from different sources is becoming increasingly important due to their potential application for treating diseases. Isolation and identification of three terpene compounds from methanol fraction of *Cyperus esculentus* tuber using silica gel column chromatography analysis, the compounds were isolated and identified for the first time from this plant (Fig. 1): Oleanolic acid **(1),** *α*-amyrin 3-*0*-*β*-D-glucopyranoside **(2),** and *β*-amyrin3-*0*-*β*-D-glucopyranoside **(3).** The isolated compounds were

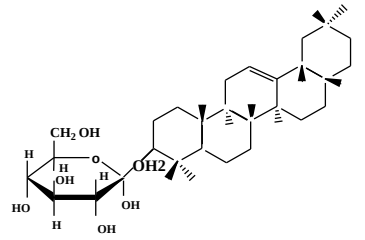
identified with their physical and spectroscopic data and compared with those reported in literature.



Oleanolic acid (1)



***α*-amyrin 3-*0*-*β*-D-glucopyranoside (2)**



***β*-amyrin3-*0*-*β*- D-glucopyranoside (3)**

Fig. 1: Structure of compounds from methanol fraction of *Cyperus esculentus* tuber

Oleanolic acid (**1**): White powder. EI-MS *m/z* 456, molecular formula $C_{30}H_{48}O_3$.^{1}H-NMR[$CDCl_3$.500 MHz.] δ [ppm]:: 3.23(dd, J = 11.2, 4.4 Hz, H-3), 2.82 (dd, J = 3.6, 13.2 Hz, H-18), 5.37 (t, J = 3.5 Hz, H-12), 0.74, 0.77, 0.87, 1.12,0.90, 0.91,0.97 [s, H_3(23,24,25,26, 27,28, 29,30)] respectively. ^{13}C-NMR [$CDCl_3$.125 MHz.]: (C-1 to C-30) 38.2, 27.1,77.2, 38.3, 55.6, 17.7, 32.3, 39.6, 46.1, 37.2,22.7, 122.4, 144.6, 41.2, 27.4, 23.6, 46.5, 42.2,46.3, 31.1, 33.3, 32.2, 28.7, 16.5, 15.5, 17.4,26.1, 179.5, 32.3, 23.8, respectively. The spectral data was compared with those previously reported literature [19].

***α*-amyrin 3-*0*-*β*- D-glucopyranoside(2):** Colourless crystal . EI-MS *m/z* 588 [M]$^+$, molecular formula $C_{36}H_{60}O_6$. UV λ_{max} : 208 nm. ^{1}H-NMR($CDCl_3$.500 MHz.) : 5.31 (t, J =2.8 Hz , H-12), 3.39 (dd, J =5.1,9.5 Hz, H-3), 2.01 (d, J =10.8 Hz,H-18), 5.02 (d,J =,6.0 Hz, H-6´), 4.73 (d, J =8.1 Hz, H-1´), 3.47 (dd, J = 4.0, 9.6 Hz, H-2´), 4.49 (m,H-3´, H-4´),3.09 (m, H-5´), 3.06 (t , J =6.4; 12.8 Hz, H-6´), 0.86, 0.65,0.69, 0.59, 1.11, 1.23, 0.89, 0.86 [s,H (23,24,25,26,27,28, 29,30)] respectively. ^{13}C-NMR [$CDCl_3$.125 MHz.]: 124.1 (C-12),139.5 (C-13), 103.5 (C-1´),79.0 (C-3), 38.0, 23.9, 39.8, 57.3, 19.3 [C (1,2,4,5,6)] respectively, 32.6 , 40.5,55.7, 36.7, 18.9 , [C (7,8,9,10,11)] respectively, 42.5, 28.9 , 23.9, 29.8,50.4[C (14,15,16,17,18)] respectively, 46.3, 29.7, 34.6, 37.2 [C(19,20,21,22)] respectively, 29.1, 12.4, 12.1, 19.1, 21.5, [C(23,24,25,26,27)] respectively, 26.5 , 32.5 , 20.1, [C (28,29,30)], 75.5,78.2, 72.1, 79.1,63.2 [C (2´, 3´ ,4´,5´, 6´)] respectively. Further confirmation was achieved by acid hydrolysis of compound **2** giving glucose in the sugar portion. The spectral data was compared with those previously reported literature [20].

***β* –amyrin 3-*0*- *β* -D-glucopyranoside (3)**: Colourless crystal. EI-MS *m/z* 588 [M]$^+$,molecular formula $C_{36}H_{60}O_6$. UV λ_{max} : 208 nm. ^{1}H-NMR ($CDCl_3$.125 MHz.) : 5.35 (t, J = 2.8 Hz, H-12), 3.14(dd,J = 5.6,8.1 Hz, H-3), 2.73 (d, J =11.3,6.4 Hz,H-18), 5.06 (d , J =6.0 Hz, H-6´), 4.57 (d, J = 8.0 Hz, H-1´), 4.47 (dd, J =4.0, 9.6 Hz , H-2´), 4.29 (m,H-3´, H-4´), 3.99 (m, H-5´),4.06 (t ,J =6.4; 12.8 Hz, H-6´), 0,98 , 0.66, 0.89, 0.87, 0.91, 0.94, 0.99, 0.85 [s,H (23,24,25,26,27,28,29,30)]. ^{13}C-NMR[125 MHz: $CDCl_3$] :122.6 (C-12),141.4 (C-13), 103.0 (C-1´), 79.2 (C-3), 38.5 , 24.9,39.5 ,57.2 , 19.7 [C (1,2,4,5,6)] respectively, 32.9 , 39.8 , 56.7 , 36.9 , 19.9 [C (7,8,9,10,11)] respectively, 43.0 , 29.0 , 24.0 , 30.8 , 50.9 , 46.5 [C14,15,16,17,18,19)] respectively, 30.0 , 34.8 , 37.4 , 29.0 , 12.6 , 12.4 [C (20,21,22,23,24,25)] respectively,19.5, 21.8, 26.9, 32.7, 20.4 [C (26,27,28,29,30)] respectively, 75.8 ,78.6 ,72.2 ,79.0 , 63.4 [C(2´, 3´ ,4´,5´, 6´)] respectively. . Further confirmation was achieved by acid hydrolysis of compound **3** giving glucose in the sugar portion. The spectral data was compared with those previously reported [21].

3.2. Identification of compounds in oil etract from Cyperus esculentus tuber using GC/MS analysis

Twelve fatty acid and sterol compounds were identified from the oil extract *of Cyperus esculentus* tubers which was analyzed by GC/MS (Figure 2 , Table 2), reported for the first time from this plant. The total peak areas of the detected compounds represent (100%). The major peak areas (represent 65.05% of the total peak areas) are 9-octadecenoic acid (Z)-, 2-hydroxy-1(hydroxymethyl) ethyl ester (41.7%) and 9-octadecenoic acid (Z)-, methyl ester (23.72%), the lowest peak area was hexylene glycol (0.7%). Identification of different compounds was

based on the peak area, retention time and molecular formula and by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

TABLE 2: GC/MS analysis of oil extract from *Cyperus esculentus* tubers

Peak	Compound Name	Molecular Formula	RT	Area %
1	Hexylene glycol	$C_6H_{14}O_2$	4.913	0.7
2	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	29.197	9.14
3	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	32.35	4.52
4	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	32.573	23.72
5	Methyl stearate	$C_{19}H_{38}O_2$	32.939	4.38
6	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	36.372	0.83
7	----------	----------	37.156	0.77
8	Octadecanoic acid, 9,10-dihydroxy-, methyl ester	$C_{19}H_{38}O_4$	37.803	0.86
9	2,3-Dihydroxypropyl elaidate	$C_{21}H_{40}O_4$	38.787	0.96
10	Hexadecanoic acid, 2,3-dihydroxypropyl ester	$C_{19}H_{38}O_4$	39.359	8.38
11	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{40}O_4$	42.26	41.7
12	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{42}O_4$	42.443	3.1
13	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{40}O_4$	42.546	0.94

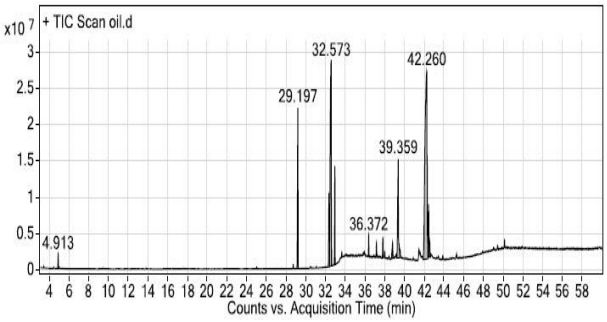


Fig. 2: GC- MS chromatogram of oil extract from *Cyperus esculentus* tubers

3.3. Identification of compounds in chloroform extract of Cyperus esculentus tubers using GC/MS analysis

Ten compounds were identified from the chloroform extract of *Cyperus esculentus* tubers which was analyzed by GC/MS, seven of them reported for the first time from this plant, with three reported before in literature stigmasterol, beta-sitosterol [22], and n-Hexadecanoic acid [2], (Figure 3, Table 3). The total peak areas of the detected compounds represent (100%). The major peak areas represent 63.81% of the total peakes, 9-Octadecenoic acid, (E)- had the highest peak area (32.34%), the lowest was Stigmasterol (0.33%). Identification of different compounds was based on the peak area, retention time and molecular formula and by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

TABLE 3: GC/MS analysis of chloroform extract of *Cyperus esculentus* tubers

Peak	Compound Name	Molecular Formula	RT	Area %
1	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	13.714	14.85
2	n-He	$C_{16}H_{32}O_2$	14.424	6.67
3	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	15.969	3.25
4	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	16.1	31.47
5	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	16.409	5.48
6	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	16.919	32.34
7	unidentified	----------	17.056	2.62
8	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	18.836	1.21
9	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	23.242	0.57
10	unidentified	----------	24.243	0.37
11	Stigmasterol	$C_{29}H_{48}O$	28.5	0.33
12	beta-Sitosterol	$C_{29}H_{50}O$	42.443	0.84

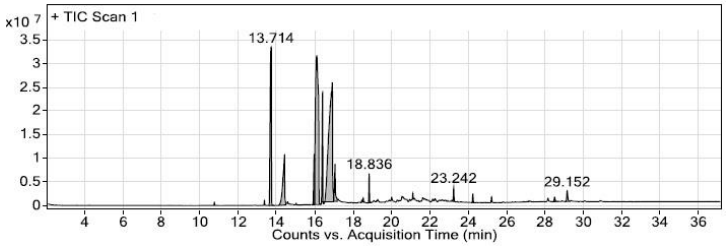


Fig. 3: GC- MS chromatogram of chloroform extract of *Cyperus esculentus* tubers

3.4. Hepatoprotective activities of different extracts of Cyperus esculentus tubers

To detect liver damage we determine the level of some enzymes in blood. Normally, the enzymes reside within the cells of the liver. However, when there is injury to the liver, these enzymes are spilled into the blood stream, raising the enzyme levels in the blood, thus signaling liver damage. The liver functional enzymes GOT, GPT and TP activities in experimental animal bloods are considered the excellent marker of liver dysfunctions and damages which caused by exposure to the toxic substances [18].

Chloroform, methanol and oil extracts of *Cyperus esculentus* tubers were evaluated for their hepatoprotective activities**.** Table 4 demonstrates that D-galactosamine significantly increased the levels of serum (GOT, GPT and TP) in untreated group compared to control group as it caused severe liver injury. D-Gal N could be taken as an index value of liver damage, that might be due to the fact that its administration disturbed plasma membrane permeability causing leakage of liver enzymes from the cell, which led to elevation in serum enzymes levels [23]. The values of liver enzymes were significantly restored by high dose (500 mg/kg) followed by low dose (250 mg/kg) of different plant extracts (Table 4)**.** The levels of serum (GOT, GPT and TP) were reduced in chloroform extract (90.1±7.8, 40.5± 5.1& 6.1±0.84), while (110.9±7.1, 55.4± 1.9 & 6.9±0.20) in methanol extract and (120.9±7.9, 47.4± 1.9 & 5.9±0.20) in oil extract, relative to the galactosamine group (215.61±2.21, 81.64±3.66 & 8.6±0.21), and to silymarin group (84.39±18.81, 31.08± 2.54& 6.05±0.18). The reduction in the levels of enzymes and total protein towards the normal value was an indication of regeneration process and hepatoprotective activity of different plant extracts of *Cyperus esculentus* tubers.The enhancement in liver function enzymes activities with addition of tiger nut extracts could be due to their higher content of triterpenes and fatty acids. These results indicated the presence of bioactive phytochemical compounds and the ability of the used plant in maintaining liver health and functions confirming its hepatoprotective activity.

Table 4: Effect of different plant extracts of *Cyperus esculentus* on liver function

Groups	Parameters		
	GOT (U/L)	GPT (U/L)	T. Protein (g/dL)
Control	87.9 ±6.17	30.4±1.91	5.5±0.27
Galactosamine	215.61±2.21^{a}	81.64 ± 3.66^{a}	8.6±0.21^{a}
Silymarin	84.39±18.81^{b}	31.08±2.54^{b}	6.05±0.18^{b}
Chloroform extract of Cyperus (low dose)	4.5b±120.3	1.99ab±60.3	0.99a±7.8
Chloroform extract of Cyperus (high dose)	90.1±7.8^{b}	40.5±5.1^{ab}	6.1±0.84^{ab}
Chloroform extract of Cyperus	80.9 ± 6.17^{b}	35.4± 1.91^{b}	5.9± 0.28^{b}
Methanol extract of Cyperus (low dose)	180.9 ±11.17^{b}	90.4±5.4^{ab}	7.9± 0.28^{ab}
Methanol extract of Cyperus (high dose)	110.9 ±7.17^{b}	55.4±1.9^{ab}	6.9±0.20^{ab}
Methanol extract of Cyperus	90.9 ± 6.67b	30.4± 2.11b	5.9± 0.28^{b}
Oil extract of Cyperus (low dose)	150.9 ±9.17^{b}	80.4±6.4^{ab}	7.9±0.28^{ab}
Oil extract of Cyperus (high dose)	120.9 ±7.97^{b}	47.4±1.9^{ab}	5.9±0.20^{ab}
Oil extract of Cyperus	85.9 ± 6.07^{b}	31.4± 1.11^{b}	5.2± 0.28^{b}

Data presented as mean ± SE, a Significant at P< 0.05 compared to control group, b Significant at P< 0.05 compared to Galactosamine group. NS Non significant compared to Galactosamine group

Conclusion

This research had focused on phytochemical compounds of different extracts and their hepatoprotective activities of *Cyperus esculentus* tubers. The results showed the presence of rich variety of phytochemical compounds, for the first time reported for this plant, and also, hepatoprotective activities of these extracts. So, tiger nut has been unexploited, despite its biological active phytochemicals, which could serve as potential sources of drugs. Further investigation of bioactive compounds are important to give this plant therapeutic effects in addition to nutritional ones**.**

Conflicts of interest

The authors declare no conflicts of interests.

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