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Computational and experimental pharmacology reveals hepatoprotective effect of *Cucurbita pepo* in isoniazid-induced liver cirrhosis

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Supplementary Material

Table S1: Physico-chemical investigations of *C. pepo*

Parameters	*C.pepo* mean % W/W
LOD (Loss on Drying)	14%
Total Ash	3%
Acid Insoluble Ash Value	0.5%
Water Soluble Ash Value	2%
Alcohol Soluble Extract	10%
Water Soluble Extract	8%

Table S2. Preliminary Phytochemical screening of *C. pepo*

Sl. No.	Phytoconstituents	Test	Result
1	Alkaloid	Mayer's test	+ve
		Wagner's test	+ve
		Dragendroff's test	+ve
		Hager's test	+ve
2	Glycosides	Molisch test	+ve
		Fehling's test	+ve
		Barfoed's test	+ve
3	Saponins	Foam test	+ve

4	Steroids	Salkowski test	+ve
5	Tannins	FeCl3	+ve
6	Flavonoids	FeCl3	-ve
		NaOH solution test	+ve
		Lead acetate test	+ve
		Mineral acid test	-ve
7	Proteins	Biurette test	-ve

Table S3: LC-MS identified molecules from *C. pepo*

S. No	**COMPOUND**	**MOL.FORMULA**	**MOL. WEIGHT CALCULATED** (g/mol)	**MOL. WEIGHT FOUND**(g/mol)
1.	Alpha-Linolenic-Acid	$C_{18}H_{30}O_2$	278.4	278.90
2.	Cucurbic acid	$C_{12}H_{20}O_3$	212.28	-
3.	Octadecanoic acid	$C_{18}H_{36}O_2$	284.5	-
4.	**Schottenol**	**$C_{29}H_{50}O$**	**414.7**	**-**
5.	**Alpha-Spinasterol**	**$C_{29}H_{48}O$**	**412.7**	**412.40**
6.	Chondrillasterol	$C_{29}H_{48}O$	412.7	412.40
7.	(+)-Dehydrovomifoliol	$C_{13}H_{18}O_3$	222.28	-
8.	Clerosterol	$C_{29}H_{48}O$	412.7	412.40
9.	Codisterol	$C_{28}H_{46}O$	398.7	397.35
10	Isofucosterol	$C_{29}H_{48}O$	412.7	412.40
11	Cucurbitacin-D	$C_{30}H_{44}O_7$	516.7	-
12	Cucurbitacin-I	$C_{30}H_{42}O_7$	514.6	-

13	Dihydrobrassicas terol	$C_{28}H_{48}O$	400.7	399.4
14	Cryptoxanthin	$C_{40}H_{56}O$	552.9	553.40
15	Eicosadienoic-Acid	$C_{20}H_{36}O_2$	308.5	-
16	Delta-7-Ergostenol	$C_{28}H_{48}O$	400.7	-
17	Abscisic acid	$C_{15}H_{20}O_4$	264.32	-
18	7-beta,13-Dihydroxykaurenolide	$C_{20}H_{28}O_4$	332.4	-

SHIMADZU, LCMS-8040 SAMPLE RUN CONDITIONS used are:

HPLC CONDITIONS:

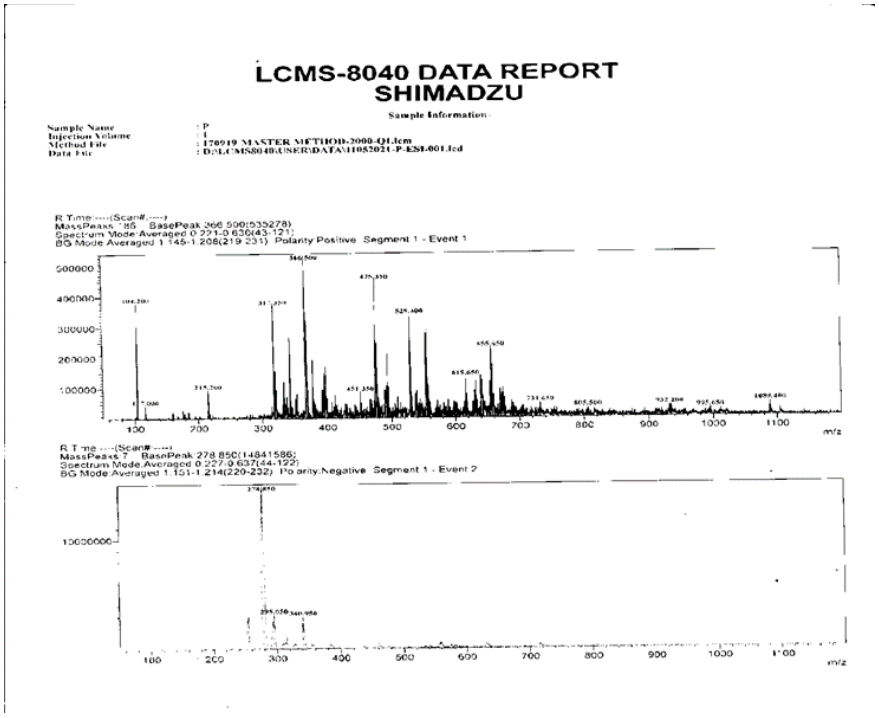
Wave Number: 254 nm (D2)

Coloum: C18 Mobile phase: Methanol Water 80:20 Total flow: 0.2 ml/min

MS CONDITIONS:

Probe: ESI (Electron Spray lonization) which gives M+1 value and M+2 value

After carrying out LCMS of Plant extracts we found that Alpha-Spinasterol was found be present in the Plant which has the calculated molecular mass of ($C_{29}H_{48}O$) 412.40g/mol and Found molecular mass is LC-MS; m/*z* 412.4(M), 413 (M+1), 414 (M+2).



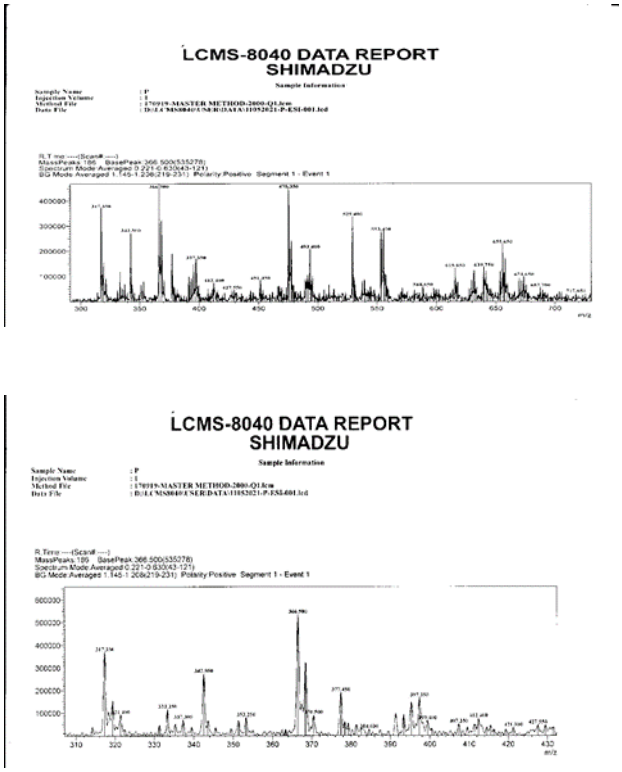


Figure S1: LC-MS profile of identified compounds of *C.pepo*

Table S4: Effect of *C. pepo* on body weight and liver weight.

GROUPS	Body weight		Liver weight
	Initial	Before sacrifice	
I (Normal)	190.14	192.54	2.99±0.04
II (control)	192.19	195.12^{ns}	4.66±0.14****
III(Standard)	189.16	190.16 ^{ns}	3.43±0.13*
IV (*C. pepo* fraction)	190.44	192.16 ^{ns}	3.42±0.12*
V (*C. pepo* extract)	195.79	196.19 ^{ns}	3.24±0.06^{ns}

I: Normal, II: control (INH, 50 mg/kg, p.o.), III: Silymarin (50 mg/kg, p.o.) + INH, IV: *C. pepo* fraction (50 mg/kg, p.o.) +INH, VI: *C. pepo* Extract (500mg/kg p.o.). ns: not significant.

Data are presented as Mean±SEM (n=6), one-way ANOVA followed by Tuckey multiple comparison test.

***p < 0.01 compared with normal group

Table S5: Effect of *C. pepo* on plasma hepatic markers against Isoniazid induced hepatotoxicity in rats.

GROUPS	SERUM BIOCHEMICAL PARAMETERS				
	ALT(U/ml)	AST(U/ml)	ALP(U/ml)	SERUM	TOTAL

				BILIRUBIN (mg/dl)	BILIRUBIN (mg/dl)
I (Normal)	49.90±0.82	77.85±0.88	93.23±1.24	0.47±0.0045	1.68±0.0024
II (control)	174.00±1.34***	234.60±0.52***	309.4±2.74***	1.06±0.0055***	4.64±0.019***
III(Standard)	66.04±0.50$^{###}$	96.46±0.42$^{###}$	107.00±0.60$^{###}$	0.52±0.0062$^{###}$	1.89±0.0036$^{##}$
IV (*C. pepo* fraction)	102.90±0.84$^{###}$	152.8±0.34$^{###}$	109.69±0.64$^{##}$	0.62±0.0034$^{##}$	2.62±0.0089$^{###}$
V (*C. pepo* extract)	120.40±0.24$^{##}$	164.6±0.89$^{##}$	124.34±0.22$^{#}$	0.92±0.0069$^{#}$	2.94±0.0019$^{##}$

Data are presented as Mean±SEM (n=6), one-way ANOVA followed by Tuckey's multiple comparison test.

**p < 0.05, ***p < 0.01 compared with normal group, $^{##}$p < 0.05, $^{###}$p < 0.01 compared with Isoniazid.

Hepatoprotective effect of hydroalcoholic extract and steroid fraction of *C. pepo* studied on Isoniazid (50 mg/kg, p.o) induced hepatotoxicity in rats. Hepatoprotective activity was monitored by estimating the serum transaminases such as alkaline phosphatase and Bilirubin in serum in the livers of experimental rats. Pre-treatment of rats with different doses of test drugs and standard leads to normalization of Isoniazid-induced changes in the levels of all the biochemical parameters, the present study suggests that extract and fraction was found to be effective in combating liver injury induced due to oxidative stress. Findings of this study provide evidence in support of medicinal value of drugs may be used to alleviate Isoniazid induced liver injury. Bioactive guided fraction show better effect compared to the extract.

Table S6: Effect of *C. pepo* on Total cholesterol, Total protein, Triglyceride, High density lipoprotein, Low density lipoprotein, and Very low density lipoprotein. against Isoniazid induced hepatotoxicity in rats.

GROUPS	SERUM BIOCHEMICAL PARAMETERS (mg/dl)					
	TC	TP	TG	HDL	VLDL	LDL

I (Normal)	95.15±0.36	4.3±0.13	85.23±0.4	40.53±0.16	18.43±0.18	38.35±0.09
II (control)	123.84±0.32	2.57±0.14	119.80±0.20	22.65±0.15	25.52±0.12	74.76±0.23
III(Standard)	102.92±0.16	3.34±0.10	96.25±0.27	31.59±0.10	20.67±0.20	52.85±0.35
IV (*C. pepo* fraction)	108.24±0.312	3.80±0.03	102.62±0.15	33.06±0.58	21.6±0.12	59.36±0.27
V (*C. pepo* extract)	106.24±1.95	4.12±0.02	109.03±0.77	35.73±0.64	23.43±0.21	61.60±0.21

Data are presented as Mean±SEM (n=6), one-way ANOVA followed by Tuckey's multiple comparison test.

**$p < 0.05$, ***$p < 0.01$ compared with normal group, $^{\#\#}p < 0.05$, $^{\#\#\#}p < 0.01$ compared with Isoniazid.