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Hepatoprotective activity against acetaminophen-induced liver dysfunction and GC-MS profiling of a brown algae *Sargassum ilicifolium*

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Abstract

Background: Drug-induced hepatotoxicity is one of the most important causes of liver dysfunction. Acetaminophen (paracetamol) an analgesic-antipyretic drug is generally considered safe but its overdose may cause liver toxicity. Marine macro-algae (seaweeds) especially brown seaweeds possess unique biological activities including hepatoprotective potential. The current study focused on the hepatoprotective effect of different solvent fractions of *Sargassum ilicifolium* and characterization of its *n*-hexane soluble fraction.

Methods: The ethanol extract (20 g) of *S. ilicifolium* was mixed with solvents of increasing polarity, starting with *n*-hexane followed by chloroform and methanol. All three (*n*-hexane, chloroform and methanol) soluble fractions were administered to the rats at dose of 150 mg/kg, b.w. Intraperitoneal administration of acetaminophen (600 mg/kg b.w.) to rats was used to cause liver injury. The hepatic damage was evaluated by liver markers enzymes; aspartate aminotransferases (AST), alanine aminotransferases (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), bilirubin along with other metabolites i.e., triglycerides, cholesterol, urea, glucose and creatinine. Lipid peroxidation and glutathione and were estimated in liver tissue. *n*-Hexane fraction was subjected to GC-MS analysis in order to identify potent compounds.

Results: The oral administration of *n*-hexane and methanol soluble fractions reduced the acetaminophen-augmented liver marker enzymes ALT, AST, ALP, LDH, along with bilirubin, urea, creatinine, glucose and triglycerides. The *n*-hexane and methanol soluble fractions also improved hepatic antioxidant level via enhancing hepatic glutathione and reversing lipid peroxidation. GC-MS spectroscopy of *n*-hexane fraction of *S. ilicifolium* revealed the presence of some new compounds. Among them, fatty acids were found to be in highest concentration followed by halogenated hydrocarbons, benzene derivatives, and sterols. Fatty acid in seaweed may be one of the factors for hepatoprotection from drug-induced hepatotoxicity.

Conclusion: From the results, it is evident that *n*-hexane and methanol soluble fractions of *S. ilicifolium* have the ability to protect the liver against toxicity, which is comparable with silymarin used as a standard drug. *Sargassum ilicifolium* contains bioactive compounds with pharmaceutical importance.

Keywords: *Sargassum ilicifolium*, Hepatoprotective activity, Drug-induced hepatotoxicity, *n*-hexane fraction, Silymarin, GC-MS

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Introduction

Acetaminophen (paracetamol) an analgesic-antipyretic drug is generally considered safe but its overdose may affect liver function [1–3] and causes severe hepatic necrosis to complete hepatic failure [4]. It also affects other organs and may trigger nephrotoxicity [5]. Since medicines used for managing liver diseases may have potential side effects thus progression of chronic liver diseases have not been prevented effectively with any therapy [6].

Marine macro-algae have shown great bioactivity potential including anti-inflammatory, antimicrobial, antiviral, antitumor, hypoglycemic and hypolipidemic activities [7–9], due to the presence of bioactive compounds that may be steroids, terpenoids, isoprenoids and sesquiterpenes [10]. The brown seaweeds, especially *Sargassum* species, are found in the shallow water of tropical and temperate regions [11–13] and contain a considerable amount of these bioactive compounds [14]. *S. ilicifolium* is one of the most commonly and abundantly found *Sargassum* species at Karachi coast. Several researchers evaluated the biological activities of *S. ilicifolium* [15–17]. Ambreen et al. [18] reported that *S. ilicifolium* contains calcium and ascorbic acid and also possesses antifungal activity. Similarly, Rebecca et al. [16] revealed antibacterial activity of ethanol extract of *S. ilicifolium.* The ethyl acetate fraction of *S. ilicifolium* has shown a prominent immune-stimulatory effect [15]. In our previous study, ethanol extract of *S.ilicifolium* (200 mg/kg b.w.) did not show any adverse effect on hepatic and renal function in rats [2, 5]. Intraperitoneal administration of hexane, methanol or butanol extract of *S. wightii* did not cause mortality or showed toxic effect in mice [19]. The current report described the hepatoprotective role of solvent fractions and GC-MS profiling of *n*-hexane fraction of *S.ilicifolium.*

Materials and methods

Chemicals and reagents

Acetaminophen, silymarin, polyethylene glycol, trichloroacetic acid (TCA),thiobarbituric acid, DTNB; 5, 5 dithio-bis-(2-nitrobenzoic acid) were purchased from Sigma Aldrich, U.S.A. Solvents (ethanol, butanol, chloroform, *n*-hexane and methanol) of analytical grades were purchased from Merck (France). All the kits including ALT, AST, ALP, LDH, bilirubin (total & direct), glucose, triglycerides, cholesterol, urea and creatinine were purchased from Merck (France) and Ecoline (Germany).

Collection of seaweed

Sargassum ilicifolium was collected from Buleji beach, Karachi and washed under tap water in the laboratory, dried under shade and grinded to powder in an electric miller. The seaweed powder was stored at room temperature until use. Voucher specimen and herbarium sheet of the seaweed was prepared and kept in the Seaweed Herbarium (KUH-SW-891), MAH Qadri Biological Research Center, University of Karachi for record. Dr. Aisha Begum, Associate Professor, Department of Botany, University of Karachi, Karachi identified the seaweed.

Ethanol extract and solvent fractions of *S. ilicifolium*

Ethanol extract of *S. ilicifolium* was prepared by soaking (500 g), for 1 week in 2 L ethanol at room temperature. Then filtered over cotton wool and concentrated on a rotary vacuum evaporator (Buchi R-200 New Castle, DE) at 35 °C to obtain a gummy mass that gives the yield of 4.8%. A portion (20 g) of ethanol extract of seaweeds was mixed with *n*-hexane (250 mL) in a separating funnel. The *n*-hexane soluble portion was separated and concentrated on a rotary vacuum evaporator at 35 °C, that give an yield of 2.5%. The *n*-hexane insoluble residue was extracted with chloroform, that give an yield of 6.5%. The chloroform insoluble or remaining portion was mixed with methanol (91%). All three (*n*-hexane, chloroform and methanol) soluble fractions were stored in airtight vials at room temperature till used [20].

Animals

Male rats (140–170 g) of *Wistar* strain, obtained from Dow University of Health Sciences, Karachi were used in this study. They were kept in polyethylene cages, fed with standard pellet diet [21] and water. Animals were kept in the laboratory for 1 week to allow the animals to acclimatize with laboratory conditions. Experiments were conducted with the permission of Institutional Animal Research Ethical Committee.

Induction of hepatotoxicity

Acetaminophen (Sigma Aldrich, U.S.A.) was administered intraperitoneally at 600 mg/kg, body weight (b.w.) and the dose was prepared in 40% polyethylene glycol (Sigma Aldrich, U.S.A.) with constant stirring and mild heating.

Experimental design

Rats were kept in pre bedded polyethylene cages (6 rats/cage) with standard laboratory conditions (temperature 25 ± 2 °C and 12 h light/dark cycle), fed with standard pellet diet prepared in the laboratory following the procedure of NRC (1995) [21] and tap water ad libitum. The animals were kept in the laboratory for 1 week before starting the experiment to acclimatize animals with laboratory conditions. Experiments were conducted with the permission of the Institutional Animal Research Ethical Committee.

Effect of solvent fractions of S. ilicifolium

Rats were divided into six major groups and each group consisted of 6 rats to determine the effect of seaweed fractions in acetaminophen intoxicated rats.

Normal control

Rats were orally administered with distilled water (1 ml/kg b.w.) for 14 days along with the normal diet ($n = 6$).

Sargassum ilicifolium fractions treated group

This group was further divided into 3 subgroups viz.; I, II, III (n = 6 in each sub-group) and rats were administered orally (p.o.) by solvent fractions (hexane, chloroform and methanol separately) of ethanol extract of the *S. ilicifolium* @ 150 mg/kg b.w., dissolved in water, daily for 14 days.

Acetaminophen (AAP) control group

Rats were administered with distilled water (p.o., 1 ml/kg b.w.) for 14 days along with the normal diet. On day 14 rats were intoxicated by a single intraperitoneal injection of acetaminophen (i.p., 600 mg/kg b.w., in saline).

Sargassum ilicifolium fractions + acetaminophen treated group

This group was further divided into 3 subgroups viz.; I, II, III. Each group ($n = 6$) was administered orally with *n*-hexane, chloroform and methanol fractions of *S. ilicifolium* at the dose of 150 mg/kg b.w., daily for 14 days. On day 14th rats were intoxicated by a single intraperitoneal injection of acetaminophen (i.p., 600 mg/kg b.w., in saline) in each subgroup.

Silymarin treated group

Treated with silymarin at the dose of 50 mg/kg b.w., suspended in distilled water and given orally to rats daily for 14 days.

Silymarin treated group + acetaminophen treated group

Treated with silymarin at the dose of 50 mg/kg daily for 14 days and injected with AAP on the last day.

On the 15th day all animals were decapitated after 12 h fasting and blood was collected for assessment of liver marker enzymes and other biochemical parameters.

Assessment of hepatic damage

Liver enzymes ALP, ALT, LDH, AST and metabolites bilirubin, creatinine, urea, triglycerides, cholesterol and glucose in serum were determined by using kits from Merck (France) and Ecoline (Germany). Glutathione and lipid peroxidation (MDA) in liver tissue was determined according to the method of Samarth et al. [22] and Ohkawa et al. [23] respectively.

Characterization of *n*-hexane fraction of *S. ilicifolium* by gas chromatography and mass spectrometry (GC-MS)

n-Hexane fraction was subjected to GC-MS analysis on Agilent 6890 Gas Chromatograph hyphenated with Mass Spectrometer, Jeol, JMS- 600H. The peak of each compound was identified by comparing their retention indices and mass spectra against National Institute of Standards and Technology (mainlib) USA and compared with Science finder [24].

Statistical analysis

Data were analyzed and their means were compared at a significant level ($p < 0.05$) using Duncan's multiple range test [25].

Results

Effect on serum biochemical markers

Acetaminophen intoxication significantly ($p < 0.005$) raised liver function markers like ALP, ALT, LDH, AST and bilirubin level in serum. *n*-Hexane fraction of *S. ilicifolium* showed improvement as evident by considerable decline of serum AST, ALT, ALP, LDH and bilirubin levels. The methanol soluble fraction also significantly ($p < 0.005$) reversed the increased level of ALP, ALT, LDH, AST and bilirubin. Chloroform soluble fraction was found less effective. The acetaminophen also affected kidney function and increased creatinine and urea many fold as compared to control. The creatinine and urea were decreased significantly in *n*-hexane and methanol fraction in comparison with AAP control. The glucose concentration was reached to (169.6) after acetaminophen intoxication and it was reduced up to (– 22%) and (– 30.4%) in *n*-hexane and methanol fractions pretreated rats respectively. The cholesterol level was restored towards normal value in rats pretreated with *n*-hexane fraction (+ 32.5%) of *S. ilicifolium* (Tables 1 & 2).

Administration of chloroform soluble fraction of *S. ilicifolium* to normal rats for 14 days did not influence the liver enzymes except for AST and LDH, while other liver metabolites like bilirubin, creatinine and urea remain unchanged when compared with normal rats. Similarly, *n*-hexane fraction did not alter the ALT, ALP, glucose level and other biochemical markers. The rats pretreated with a methanol fraction of *S. ilicifolium* also not shown any adverse effect on enzymes, but reduced glucose level than control rats. Creatinine, cholesterol and triglyceride level were found increased by the methanol fraction of *S. ilicifolium* (Tables 1 & 2).

Administration of silymarin in AAP intoxicated rats significantly decreased the liver enzymes and bilirubin level as compared to AAP intoxicated rats. On the other side, kidney profile; creatinine and urea, were also reduced in silymarin + AAP treated group as compared to AAP control rats. Similarly, silymarin in AAP

Table 1 Effect of *n*-hexane, chloroform and methanol soluble fractions of *Sargassum ilicifolium* on liver enzymes and bilirubin in normal and acetaminophen (AAP) dosed rats

Rat groups	Normal rats	AAP dosed rats	Normal rats	AAP dosed rats	Normal rats	AAP dosed rats	Normal rats	AAP dosed rats	Normal rats	AAP dosed rats
	ALT (U/L)		AST (U/L)		ALP (U/L)		LDH (U/L)		Bilirubin (mg/dl)	
Control	$33.6^{ab} \pm 3.5$	$104.6^{a} \pm 9.4$	$134.3^{c} \pm 6$	$239.3^{b} \pm 8.1$	$67.6^{a} \pm 6.5$	$113.6^{a} \pm 10.4$	$328.6^{a} \pm 10$	$396.3^{a} \pm 6$	$0.4^{a} \pm 0$	$0.86^{a} \pm 0.06$
hexane fraction of *S. ilicifolium*	$30.3^{b} \pm 2.3$ (−9.8%)	$63^{b} \pm 2.6$ (− 39.7%)	$166.3^{b} \pm 8.6$ (+ 23.8%)	$178.3^{c} \pm 9.6$ (−25.4%)	$74^{a} \pm 3$ (+ 9.4%)	$87.3^{b} \pm 2.1$ (− 23.1%)	$312.6^{b} \pm 4.5$ (− 4.8%)	$303.3^{b} \pm 22$ (− 23.4%)	$0.46^{a} \pm 0.1$ (+ 15%)	$0.6^{b} \pm 0.1$ (− 30.2%)
Chloroform fraction of *S. ilicifolium*	$38.3^{a} \pm 3.5$ (+ 13.9%)	$99.6^{a} \pm 17$ (− 4.7%)	$237.6^{a} \pm 19.4$ (+ 76.9%)	$295.3^{a} \pm 8$ (+ 23.4%)	$67.6^{a} \pm 3.5$ (0)	$106.6^{a} \pm 6.5$ (− 6.16%)	$298^{b} \pm 7$ (− 9.3%)	$303^{b} \pm 8$ (− 23.5%)	$0.43^{a} \pm 0.05$ (+ 7.5%)	$0.66^{b} \pm 0.06$ (− 23.2%)
Methanol fraction of *S. ilicifolium*	$35.6^{ab} \pm 3.2$ (+ 5.9%)	$66.6^{b} \pm 2.5$ (− 36.3%)	$180.3^{b} \pm 4.5$ (+ 34.2%)	$182.3^{c} \pm 13$ (− 23.8%)	$71^{a} \pm 2.6$ (+ 5.0%)	$79.6^{b} \pm 2$ (− 29.9%)	$312.6^{b} \pm 9$ (− 4.86%)	$331.3^{b} \pm 19.6$ (− 16.4%)	$0.46^{a} \pm 0$ (+ 15%)	$0.53^{b} \pm 0.06$ (− 38.3%)

The values were expressed as means ± Standard error ($n = 6$). The values having the same superscript within the column are not significantly ($p < 0.05$) different according to Duncan's multiple range test. Values in parenthesis are showing percent increased or decreased as compared to their respective control

intoxicated rats also showed protective effect by lowering the glucose level and lipid parameters (cholesterol and triglyceride) as compared to AAP control group (Tables 3 & 4).

Effect on solvent fractions of *S. ilicifolium* on glutathione (GSH) and lipid peroxidation (TBARS)

Glutathione (GSH) level was significantly ($p < 0.05$) decreased in acetaminophen (AAP) treated rats as compared to normal control rats. However, glutathione level was improved and increased in rats pretreated with *n*-hexane, methanol and chloroform fractions of *S. ilicifolium* in acetaminophen intoxicated rats as compared to acetaminophen (AAP) control group. Methanol fraction improved this level and brought almost to the normal control rats, while *n*-hexane fraction increased this level higher than the normal control (Fig. 1).

AAP intoxicated rats significantly elevated TBARS [6.16 μmole/gm wet liver tissue (w.l.t)] level as compared to normal control (2.4 μmole/gm). *n*-hexane (3.19μmole/gm) and methanol (4.1μmole/gm) fraction of *S. ilicifolium* significantly reduced TBARS level as compared to AAP intoxicated rats (Fig. 1). The overall highest hepatoprotective activity was found in *n*-hexane soluble fraction of *S. ilicifolium* followed by methanol fraction. The *n*-hexane fraction was thus characterized on GC-MS considering its hepatoprotective activity.

Glutathione level was significantly improved and TBARS level decreased in rats treated with silymarin (50 mg/kg b.w.) in acetaminophen intoxicated rats as

Table 2 Effect of *n*-hexane, chloroform and methanol soluble fractions of *Sargassum ilicifolium* on glucose, lipid parameters and kidney function markers in normal and acetaminophen dosed rats

Rat groups	Normal rats	AAP dosed rats	Normal rats	AAP dosed rats	Normal rats	AAP dosed rats	Normal Rats	AAP dosed rats	Normal rats	AAP dosed rats
	Glucose (mg/dl)		Triglyceride (mg/dl)		Cholesterol (mg/dl)		Urea (mg/dl)		Creatinine (mg/dl)	
Control	$83.6^{a} \pm 4.7$	$169.6^{a} \pm 8.7$	$74.3^{b} \pm 5.1$	$104.3^{b} \pm 1.5$	$73.6^{b} \pm 6.8$	$55.6^{b} \pm 2.08$	$26.3^{a} \pm 2.5$	$42^{a} \pm 2$	$0.83^{b} \pm 0.06$	$1.3^{a} \pm 0.1$
hexane fraction of *S. ilicifolium*	$81^{a} \pm 5.5$ (− 3.11%)	$131.3^{b} \pm 3.2$ (− 22.5%)	$75.6^{b} \pm 4.9$ (+ 1.7%)	$110.3^{b} \pm 4$ (+ 5.75%)	$75.6^{b} \pm 6.4$ (+ 2.71%)	$73.6^{a} \pm 2.8$ (+ 32.3%)	$25.6^{a} \pm 1.5$ (− 2.66%)	$29^{b} \pm 2.6$ (− 30.9%)	$0.96^{a} \pm 0.06$ (+ 15.6%)	$0.9^{b} \pm 0.1$ (− 30.7%)
Chloroform fraction of *S. ilicifolium*	$71.6^{b} \pm 3.7$ (− 14.3%)	$154.3^{a} \pm 15.6$ (− 9.0%)	$91.6^{a} \pm 3.7$ (+ 23.2%)	$109.6^{b} \pm 4.6$ (+ 5.08%)	$81.3^{b} \pm 3.2$ (+ 10.4%)	$56.3^{b} \pm 5.5$ (+ 1.25%)	$28.6^{a} \pm 1.15$ (+ 8.7%)	$39^{a} \pm 1$ (− 7.14%)	$0.92^{ab} \pm 0.05$ (+ 10.8%)	$1.23^{a} \pm 0.06$ (− 5.38%)
Methanol fraction of *S. ilicifolium*	$63^{c} \pm 3.6$ (− 24.6%)	$118^{b} \pm 5.2$ (− 30.4%)	$87.6^{ab} \pm 11.06$ (+ 17.9%)	$123^{a} \pm 5$ (+ 17.9%)	$92.3^{a} \pm 3.2$ (+ 25.4%)	$68.3^{a} \pm 4.6$ (+ 22.8%)	$26^{a} \pm 2.6$ (− 1.14%)	$25^{c} \pm 2$ (− 40.4%)	$1.03^{a} \pm 0.05$ (+ 24.0%)	$0.96^{b} \pm 0.06$ (− 26.15%)

The values were expressed as means ± Standard error ($n = 6$)
The values having the same superscript within the column are not significantly ($p < 0.05$) different according to Duncan's multiple range test
Values in parenthesis are showing percent increased or decreased as compared to their respective control

Table 3 Effect of silymarin at dose of 50 mg/kg b.w., on liver enzymes and bilirubin in normal and acetaminophen (AAP) dosed rats

Rat groups	ALT (U/L)	AST (U/L)	ALP (U/L)	LDH (U/L)	Bilirubin (mg/dl)
Normal control	$35.6^{b} \pm 2.08$	$94.6^{c} \pm 6.8$	$53^{c} \pm 7$	$216.3^{c} \pm 13.05$	$0.56^{c} \pm 0.057$
Silymarin (50 mg/kg b.w.)	$34.3^{b} \pm 3.5^{a}$ (– 3.6%)	$91.6^{c} \pm 14.5^{a}$ (– 3.1%)	$43.6^{c} \pm 4.7^{a}$ (– 17.7%)	$176.3^{d} \pm 20.1^{a}$ (– 18.4%)	$0.46^{c} \pm 0.115^{a}$ (– 17.8%)
Acetaminophen	$91.3^{a} \pm 10.9^{a}$ (+ 156.4%)	$214.6^{a} \pm 20.5^{a}$ (+ 126.8%)	$112^{a} \pm 13.5^{a}$ (+ 111.3%)	$374^{a} \pm 11.1^{a}$ (+ 72.9%)	$0.9^{a} \pm 0.1^{a}$ (+ 60.7%)
Silymarin (50 mg/ kg b.w.) Acetaminophen	$44.3^{b} \pm 6.8^{b}$ (– 51.4%)	$120.6^{b} \pm 8.3^{b}$ (– 43.8%)	$79^{b} \pm 5.2^{b}$ (– 29.4%)	$250.6^{b} \pm 10.2^{b}$ (– 32.9%)	$0.73^{b} \pm 0.057^{b}$ (– 18.8%)

The values were expressed as means ± standard error ($n = 6$)
[a] Compared with normal control,
[b] Compared with acetaminophen control
Values in parenthesis are showing percent increased or decreased as compared to their respective control
The values having the same superscript within the column are not significantly ($p < 0.05$) different according to Duncan's multiple range test

compared to acetaminophen (AAP) control group (Fig. 2).

GC/MS profiling of *n*-hexane fraction of *S. ilicifolium*

The GC/MS characterization of *n*-hexane soluble portion of *S. ilicifolium* revealed the presence of different volatile compounds like normal hydrocarbon, alcohols, fatty acid, aliphatic compounds, benzene derivatives, aldehyde and terpenoid. Total fifty one compounds were isolated and identified in which forty six compounds were found new from this source (Table 5). According to the results the highest concentration of hexadecanoic acid was found in our extract followed by octadecenoic acid. However the hexadecanoic is already a known compound from this source while octadecenoic acid is a new compound from *S. ilicifolium*. Our results showed the presence of steroids; spiro (1, 3-dioxolane)-2, 3′-(5′-androsten-16′-ol) and estra-1, 3, 5(10)-trien-17β-ol in *S. ilicifolium* (Table 5 & Fig. 3; Fig. S- 1, S-2, S-3, S-4).

Discussion

In this study, pretreatment with *n*-hexane fractions of *S. ilicifolium* significantly decreased the toxin (acetaminophen) induced raised in serum transaminases, alkaline phosphatase, lactate dehydrogenase and bilirubin level. The methanol soluble fraction also reversed the increased level of ALT, AST, ALP and LDH but to a lower extent than *n*-hexane fraction. Raghavendran et al. [26] and Raghavendran and Srinivasan [27] have reported the protective role of ethanol and water extracts of *S. polycystum* against AAP induced hepatic damage. The drug induced hepatotoxicity may trigger nephrotoxicity as well [5]. An increase in urea and creatinine concentration in serum is indicative of nephrotoxicity [28]. In this study, adverse effect of acetaminophen was also found in AAP dosed rats, which was attenuated in rats pretreated with *n*-hexane fraction of *S.ilicifolium* evident from decreased concentration of urea and creatinine. Hepatotoxicity may affect lipid and glucose metabolism. In our study, *n*-hexane and methanol fractions of *S. ilicifolium* lowered the raised triglycerides level and improved decreased level of cholesterol in AAP dosed rats. Taj et al. [29] reported attenuation of adverse effect of AAP on liver and kidney function and glucose metabolism by the ethanol extract of a brown alga *Stokeyia indica.* Similarly Sohail et al. [5] reported the hepatoprotective and nephroprotective role of ethanol extract of *S.ilicifolium* in drug induced hepatotoxicity and nephrotoxicity in

Table 4 Effect of silymarin at dose of 50 mg/kg b.w., on glucose, lipid parameters and kidney function markers in normal and acetaminophen (AAP) dosed rats

Groups	Glucose (mg/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Normal control	$95^{c} \pm 7.5$	$95^{b} \pm 6.2$	$97^{a} \pm 8.8$	$31.6^{c} \pm 2.08$	$0.90^{b} \pm 0.1$
Silymarin (50 mg/ kg b.w.)	$89^{c} \pm 5.2^{a}$ (–1.3%)	$82^{b} \pm 6^{a}$ (– 8.6%)	$88^{a} \pm 4.3^{a}$ (– 6.2%)	$24.3^{d} \pm 2.08^{a}$ (– 45.2%)	$0.66^{c} \pm 0.11^{a}$ (– 26.6[a])
Acetaminophen	$151^{a} \pm 3.6^{a}$ (+ 63.9%)	$133.6^{a} \pm 9.5^{a}$ (+ 45.6%)	$70^{b} \pm 8.7^{a}$ (– 24.8%)	$44^{a} \pm 1.7^{a}$ (+ 39.2%)	$1.3^{a} \pm 0.1^{a}$ (+ 44.4%)
Silymarin (50 mg/ kg b.w.) + Acetaminophen	$119.3^{b} \pm 5.6^{b}$ (– 71.9%)	$89.6^{b} \pm 8.7^{b}$ (– 66.5%)	$60.3^{b} \pm 2.08^{b}$ (– 16.1%)	$37.3^{b} \pm 2.08^{b}$ (– 15.2%)	$0.93^{b} \pm 0.15^{b}$ (– 28.4%)

The values were expressed as means ± standard error (ign = 6)
[a] Compared with normal control
[b]Compared with acetaminophen control
Values in parenthesis are showing percent increased or decreased as compared to their respective control
The values having the same superscript within the column are not significantly ($p < 0.05$) different according to Duncan's multiple range test

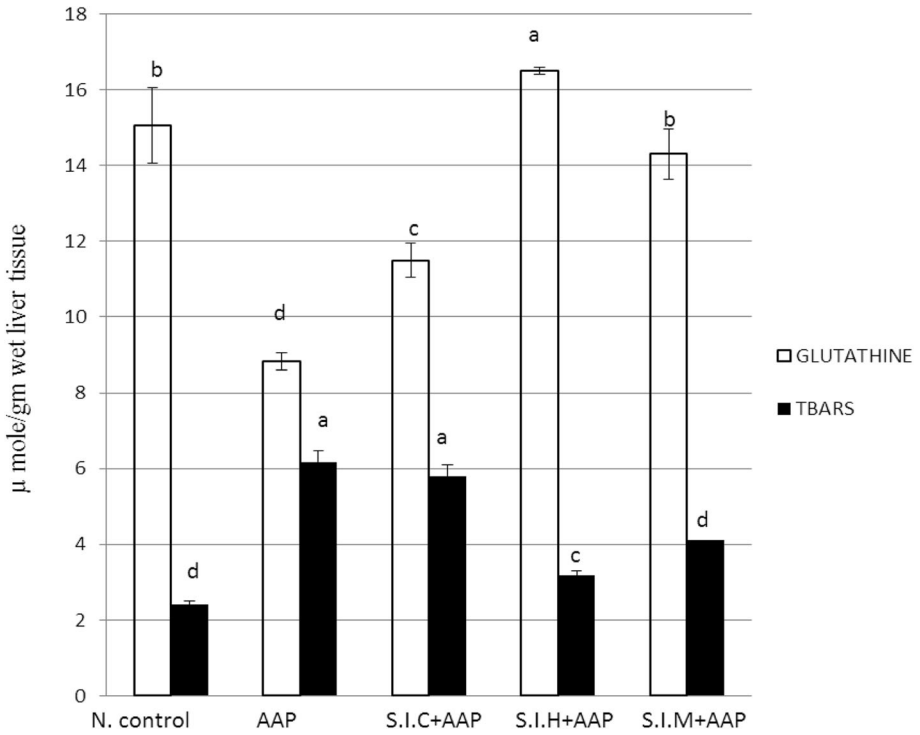

Fig. 1 Effect of chloroform, *n*-hexane and methanol soluble fractions of *Sargassum ilicifolium* on glutathione (GSH) and lipid peroxidation (TBARS) in acetaminophen (AAP) dosed rats. The bars in the graph showing mean ± Standard error. The values having the same superscript on the bars are not significantly ($p < 0.05$) different according to Duncan's multiple range test

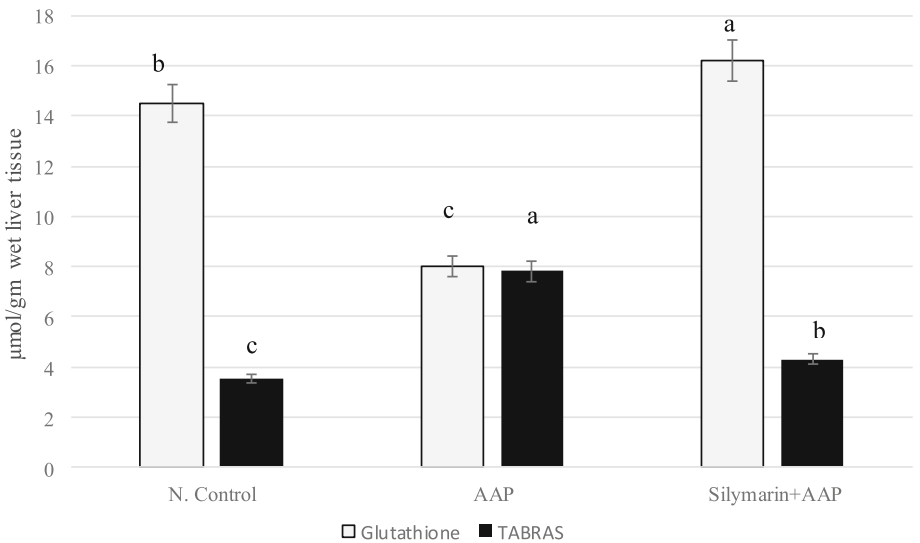

Fig. 2 Effect of silymarin at the dose of 50 mg/kg b.w., on glutathione and lipid peroxidation (TBARS) in acetaminophen dosed rats. The bars in the graph showing mean ± Standard error ($n = 6$). The values bearing the same superscript on bar are not significantly ($p < 0.05$) different according to Duncan's multiple range test. N. Control = Normal Control; AAP = Acetaminophen; SM = Silymarin

Table 5 Spectral data of *n*-hexane soluble fraction of *Sargassum ilicifolium*

Peak #	Systemic name	Molecular formula	Mol. wt.	t_R (min)	Conc.	Characteristic mass fragments, m/z (rel.% BP)	Area Sum (%)
1.	Methyl 3,3-dichloropropenoate	$C_4H_4Cl_2O_2$	154	6.104	0.092	123(99), 125(66), 60(20), 63(15), 127(14), 95(13), 154(99), 97(92), 156(77), 62(76)	0.092
2.	Benzaldehyde	C_7H_6O	106	8.132	0.092	106(99), 105(94), 77(92), 51(36), 50(18), 78(16), 52(98), 107(77), 74(63), 39(60)	0.092
3.	3, 3, 5, 5-Tetramethylcyclohexanol	$C_{10}H_{20}O$	156	10.201	0.16	123(99), 41(44), 57(42), 81(29), 55(28), 56(26), 85(25), 29(20), 43(19), 39(18)	0.16
4.	Benzyl Alcohol	C_7H_8O	108	11.457	0.242	79(99), 108(89), 107(69), 77(54), 51(22), 91(18), 78(11), 50(10), 39(10), 80(95)	0.242
5.	1-Propene, 2, 3-dibromo-	$C_3H_4Br_2$	198	12.987	0.107	119(99), 121(98), 39(90), 200(71), 198(37), 202(35), 40(30), 38(21), 37(13), 93(98)	0.107
6.	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	14.737	0.071	57(99), 73(91), 43(84), 60(74), 71(63), 55(57), 69(44), 41(43), 129(43), 85(41)	0.071
7.	2-Oxepanone	$C_6H_{10}O_2$	114	15.122	0.109	42(99), 55(96), 41(49), 84(44), 56(30), 28(26), 27(22), 70(21), 39(20), 114 (20)	0.109
8.	Palmitic anhydride	$C_{32}H_{62}O_3$	494	15.612	0.1	98(99), 256(37), 55(36), 43(27), 57(27), 83(26), 73(25), 112(24), 84(23), 239(23)	0.1
9.	Cyclopropane, 1, 1-dichloro-2-methyl-3-pentyl-	$C_9H_{16}Cl_2$	194	16.419	0.086	110(99), 41(97), 43(96), 39(77), 56(74), 112(65), 55(60), 42(48), 69(36), 65(30)	0.086
10.	Cyclopropane, 1,1-dichloro-2,2-dimethyl-	$C_5H_8Cl_2$	138	18.954	0.089	103(99), 123(96), 41(63), 125(61), 87(59), 67(43), 39(34), 105(33), 42(25), 89(24)	0.089
11.	2-Hexanone, 6-(acetyloxy)	$C_8H_{14}O_3$	158	19.297	0.14	43(99), 98(17), 56(10), 55(70), 41(60), 101(60), 58(50), 61(50), 83(50), 42(30)	0.14
12.	Bis-(2,2,6,6-tetramethyl,-4-piperidyl) amide oxalic acid	$C_{20}H_{38}N_4O_2$	366	20.732	0.149	124(99), 98(66), 77(56), 105(56), 58(44), 42(39), 122(31), 41(21), 51(21), 141(17)	0.149
13.	2-Decenal, (E)-	$C_{10}H_{18}O$	154	22.112	0.104	41(99), 43(99), 55(71), 70(55), 29(53), 39(48), 27(45), 57(43), 83(34), 69(30)	0.104
14.	Benzeneacetic acid, 4-tetradecyl ester	$C_{22}H_{36}O_2$	332	24.939	0.116	91(99), 57(59), 43(42), 41(37), 71(33), 55(30), 39(21), 85(20), 44(19), 196(17)	0.116
15.	5,5,10,10-Tetrachlorotricyclo[7.1.0.0(4,6)]decane	$C_{10}H_{12}Cl_4$	272	25.683	0.066	109(99), 91(90), 111(73), 65(67), 39(65), 77(61), 127(53), 51(46), 79(38), 101(36)	0.066
16.	4-(3-Hydroxy-2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept-1-yl)-but-3-en-2-one	$C_{13}H_{20}O_3$	224	26.612	0.187	43(99), 123(97), 109(50), 41(19), 55(18), 124(17), 165(16), 101(14), 125(12), 83(11)	0.187
17.	1-Nonene, 2-ethyl-3-(methoxymethoxy)	$C_{13}H_{26}O_2$	214	29.205	0.075	45(99), 129(36), 83(15),55(10), 41(73), 97(64), 69(63), 43(55), 70(49), 99(49)	0.075
18.	2-(2-Chloromethylene-1-hydroxycyclohexyl) propionic acid, methyl ester	$C_{11}H_{17}ClO_3$	232	29.628	0.085	145(99), 109(75), 81(45), 88(44), 57(35), 147(35), 197(32), 79(19), 41(19), 91(19)	0.085
19.	1-Ethyl-1-(undec-10-enyloxy)-1-silacyclohexane	$C_{18}H_{36}OSi$	296	30.614	0.072	115(99), 41(85), 55(61), 45(54), 29(43), 87(41), 43(39), 27(33), 39(27), 267(26)	0.072
20.	1-Methyl-1-(2-tridecyl)oxy-1-silacyclopentane	$C_{18}H_{38}OSi$	298	30.911	0.131	143(99), 41(73), 43(69), 39(42), 55(39), 99(36), 45(27), 57(25), 42(21), 69(17)	0.131
21.	1-Pentene, 1,1,5-trichloro-	$C_5H_7Cl_3$	172	31.971	0.068	65(99), 101(90), 136(47), 109(46), 138(31), 111(30), 39(29), 103(28), 73(18), 96(15)	0.068
22.	2-Oxa-7-thiatricyclo[4.4.0.0(3,8)]decan-4-ol, 4-methylbenzenesulfonate, stereoisomer (1-α, 3-α, 4-β, 6-α, 8-α)-	$C_{15}H_{18}O_4S_2$	326	33.222	0.074	91(99), 97(83), 154(68), 113(55), 41(55), 81(52), 65(46), 326(46), 119(38), 45(36)	0.074
23.	Octahydrobenzo[b]pyran, 4a-acetoxy-5,5,8a-trimethyl-	$C_{14}H_{24}O_3$	240	33.496	0.077	111(99), 124(85), 43(69), 69(49), 41(45), 180(44), 55(28), 97(23), 71(22), 96(22)	0.077
24.	1-Octyne, 3-(methoxymethoxy)-	$C_{10}H_{18}O_2$	170	33.78	0.086	45(99), 26(71), 99(56), 84(15), 41(14), 67(10), 56(99), 55(97), 39(884), 43(82)	0.086
25.	Spiro(1,3-dioxolane)-2,3′-(5′-androsten-16′-ol)	$C_{21}H_{32}O_3$	332	33.974	0.091	99(99), 55(13), 100(10), 91(88), 79(59), 105(56), 41(51), 77(46), 86(43), 332(41)	0.091
26.	Oleic Acid	$C_{18}H_{34}O_2$	282	35.904	0.146	55(99), 41(60), 69(56), 57(47), 43(35),	0.146

Table 5 Spectral data of *n*-hexane soluble fraction of *Sargassum ilicifolium (Continued)*

Peak #	Systemic name	Molecular formula	Mol. wt.	t_R (min)	Conc.	Characteristic mass fragments, m/z (rel.% BP)	Area Sum (%)
						73(32), 83(32), 29(25), 56(22), 60(19)	
27.	Hexanoic acid, 2-ethyl-, oxybis (2,1-ethanediyloxy-2,1-ethanediyl) ester	$C_{24}H_{46}O_7$	446	36.478	0.267	171(99), 7(63), 127(45), 99(32), 87(30), 55(21), 73(21), 43(18), 114(17), 172(14)	0.267
28.	E-9-Methyl-8-tridecen-2-ol, acetate	$C_{16}H_{30}O_2$	254	36.586	0.037	43(99), 55(57), 97(31), 41(29), 109(17), 69(15), 71(14), 123(12), 112(11),57(11)	0.037
29.	Cyclohexanol, 2-methyl-3-(1-methylethenyl)-, acetate, (1α,2α,3α)-	$C_{12}H_{20}O_2$	196	38.058	0.065	43(99), 136(24), 93(18), 121(18), 41(17), 55(13), 107(12), 95(12), 81(12), 94(10)	0.065
30.	Tert-Hexadecanethiol	$C_{16}H_{34}S$	258	38.515	0.067	57(99), 69(76), 43(71), 71(68), 55(61), 83(57), 41(54), 85(47), 97(35), 70(28)	0.067
31.	Methyl-2-methoxyoct-2-enoate	$C_{10}H_{18}O_3$	186	39.112	0.082	43(99),41(47), 115(44), 55(38), 85(36), 45(34), 69(27), 109(25), 29(24), 42(21)	0.082
32.	7-(1-Hydroxy-cyclohexyl)-2,2-dimethyl-hept-5-en-3-one	$C_{15}H_{26}O_2$	238	41.82	0.202	57(99), 85(44), 99(40), 81(35), 41(26), 55(21), 29(17), 83(14), 86(11), 43(10)	0.202
33.	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	43.242	0.046	73(99), 60(84), 43(68), 57(60), 41(46), 55(45), 129(37), 71(33), 69(28), 85(24)	0.046
34.	Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl-	$C_9H_9F_3O_2$	206	43.987	0.105	149(99),83(29),43(27),151(26),57(24), 55(23), 69(16), 71(15), 77(14), 41(13)	0.105
35.	Estra-1,3,5(10)-trien-17β-ol	$C_{18}H_{24}O$	256	44.366	0.071	43(99), 57(99), 55(71), 41(55), 73(47), 71(44), 45(39), 85(35), 83(35), 256(34)	0.071
36.	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	44.936	0.077	43(99), 58(89), 71(45), 57(42), 59(40), 41(37), 55(34), 69(24), 85(22), 95(20)	0.077
37.	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	45.054	0.061	73(99), 60(87), 43(86), 57(81), 41(73), 55(64), 71(49), 129(45), 69(40), 29(36)	0.061
38.	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	45.987	0.091	73(99), 60(87), 43(86), 57(81), 41(73), 55(64), 71(49), 129(45), 69(40), 29(36)	0.091
39.	1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$	256	46.126	0.073	57(99), 43(88), 55(67), 41(63), 69(58), 56(57), 71(51), 83(46), 97(38), 70(34)	0.073
40.	2-Butenal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	$C_{14}H_{22}O$	206	46.683	0.077	107(99), 123(96), 41(72), 206(72), 95(70), 81(64), 55(64), 191(63), 121(59), 135(59)	0.077
41.	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	$C_{16}H_{28}O_3$	268	47.671	0.077	43(99), 97(48), 69(48), 55(44), 41(36), 82(30), 67(29), 81(29), 84(27), 83(27)	0.077
42.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	53.368	0.496	60(99),73(98), 57(84), 43(81), 55(76), 41(57), 129(43), 71(37), 69(35), 83(26)	0.496
43.	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	62.93	0.436	55(99), 69(54), 41(46), 83(43), 97(39), 84(24), 43(24), 56(24), 67(22), 96(21)	0.436
44.	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282	63.897	0.244	55(99), 97(85), 83(82), 69(75), 84(64), 96(62), 81(61), 57(54), 264(49), 67(48)	0.244
45.	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268	69.535	0.076	43(99), 55(66), 41(50), 81(26), 57(24), 85(23), 67(22), 69(22), 99(21), 83(20)	0.076
46.	Z-5-Methyl-6-heneicosen-11-one	$C_{22}H_{42}O$	322	70.771	0.085	43(99), 55(87), 57(81), 41(75), 71(57), 83(47), 169(44), 85(43), 81(40), 69(40)	0.085
47.	Hexadecanoic acid, 3-[(trimethylsilyl) oxy]propyl ester	$C_{22}H_{46}O_3Si$	386	72.529	0.058	57(99), 43(86), 239(76), 73(68), 71(60), 130(52), 55(50), 41(48), 75(46), 85(38)	0.058
48.	Heptacosane	$C_{27}H_{56}$	380	72.942	0.051	57(99), 43(79), 71(62), 85(41), 55(28), 41(27), 69(17), 99(14), 56(13), 29(13)	0.051
49.	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	73.918	0.06	149(99), 167(35), 57(34), 70(26), 41(22), 71(22), 55(21), 43(20), 150(10), 83(10)	0.06
50.	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	390	74.505	0.05	149(99), 167(54), 57(44), 71(35), 70(28), 43(27), 279(21), 41(21), 113(20), 55(16)	0.05
51.	1,3,2-Dioxaborolane, 4,4′-(1,4-butanediyl)bis[2-ethyl-	$C_{12}H_{24}B_2O_4$	254	79.581	0.049	99(99), 98(32), 43(27), 41(17), 57(17), 83(13), 138(12), 29(11), 42(10), 55(10)	0.049

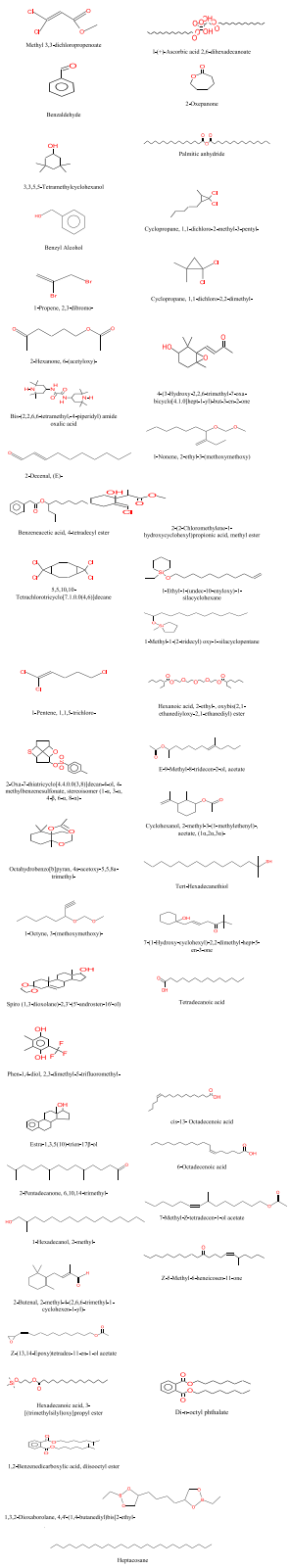

Fig. 3 Compounds identified from *n*-hexane soluble fraction of *Sargassum ilicifolium*

rats. The present findings further investigated and confirmed the hepatoprotective and nephroprotective role of *n*-hexane fraction of *S.ilicifolium.*

In the biological system oxidative stress may result in increased lipid peroxidation thus indicating cellular damage [30]. Excess production of free radicals may damage cell membranes and lipoproteins by a process called lipid peroxidation, resulting in the production of malondialdehyde (MDA). MDA is an end product of membrane damage, can bind with thiobarbituric acid, hence also called thiobarbituric acid reactive substances (TBARS). Glutathione can help in reducing the free radicals [31]. A decrease in GSH and increase in MDA is considered as a sign of liver dysfunction [3]. Reduction in GSH level of kidneys and lungs was reported by Taye and Abdel-Raheem [32], along with CCl_4 intoxication. Ohta et al. [33] also reported GSH reduction in various organs of rats injected with CCl_4. In this study glutathione (GSH) was improved and TBARS (MDA) was decreased in rats pretreated with *n*-hexane and methanol fractions of *S. ilicifolium* in AAP intoxicated rats as compared to AAP control group.

The hepatoprotective activity of different phytoconstituents like flavonoides, triterpenes, saponins and alkaloids and fatty acids has been reported earlier [31, 34]. In present study GC-MS analysis of *n*-hexane extract showed that hexadecanoic acid was found in highest concentration followed by cis-13- octadecenoic acid, hexanoic acid 2-ethyl-oxybis (2,1-ethanediyloxy-2,1-ethanediyl) ester, 6-octadecenoic acid respectively. The fatty acids related compounds present in *Sargassum fulvellum* and *S. thunbergii* possess anti-inflammatory activities, as these are competitive inhibitors of cyclooxygenase and/or lipoxygenase, hence decrease the production of prostaglandins and leukotrienes [35, 36]. Biological activities of fatty acids from a brown alga *Spatoglossum asperum* has been reported earlier [37]. Hepatoprotective potential of *S. ilicifolium* may be due to presence of fatty acids that have been reported to ameliorate liver function enzymes in rats, which ultimately lead to reduced liver necrosis and inflammations [29, 38]. The *n*-hexadecanoic acid is known as an anti-inflammatory compound [34]. Seaweeds also contain diterpenes, triterpenes and halogenated compounds with diverse biological activities as antibacterial, antioxidant, insecticidal and cytotoxic activities [39–41]. In this study, besides fatty acids, hydrocarbons, alcohols, aliphatic compounds, benzene derivatives, aldehyde and terpenoid were also found in *n*-hexane fraction of *S.ilicifolium.* Hepatoprotective activity of *S.ilicifolium* might be due to the presence of individual compounds or combinations of more than one compound. Like other natural products, presumably, seaweed acts as an antioxidant agent, increasing intracellular concentration

of glutathione [42, 43]. It may enhance protein synthesis and regeneration of liver cells [44]. Polysaccharide from *Sargassum* sp., has been reported to decrease the MDA and increased glutathione in acetaminophen intoxicated rats [3].

Conclusion

Present study described that *n*-hexane and methanol soluble fraction of a brown seaweed *S. ilicifolium,* exhibited hepatoprotective activity via reducing liver marker enzymes and enhancing hepatic antioxidant level. Characterization of *n*-hexane soluble fraction of *S. ilicifolium* confirmed the presence of different volatile compounds, in which fatty acids were found to be in highest concentration followed by halogenated hydrocarbons, fatty acid derivatives and sterols. Further investigation is needed to examine the hepatoprotective effect of individual compounds identified from *n*-hexane soluble fraction of *S. ilicifolium*.

Abbreviations

LDH: Lactate dehydrogenase; AST: Aspartate aminotransferases; ALP: Alkaline phosphatase; ALT: Alanine aminotransferases; GC-MS: Gas chromatography-mass spectrometry; *S. ilicifolium*: *Sargassum ilicifolium*; AAP: Acetaminophen; TBARS: Thiobarbituric acid reactive substances; GSH: Glutathione; MDA: Malondialdehyde

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40816-021-00274-4.

Additional file 1 Figure S1. Concentration of different compounds in *n*-hexane soluble fraction of *Sargassum ilicifolium*. **Figure S2**. Concentration of different compounds in *n*-hexane soluble fraction of *Sargassum ilicifolium* (expansion of S-1). **Figure S3**. Concentration of different compounds in *n*-hexane soluble fraction of *Sargassum ilicifolium* (expansion of S-1). **Figure S4**. Concentration of different compounds in *n*-hexane soluble fraction of *Sargassum ilicifolium* (expansion of S-1). *(DOCX 129 kb)*

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Authors' contributions

KH & JA conceived and designed the study. KH & NS performed experimental work, analyzed and interpreted the results. GC-MS profiling was done by HF & MA. KH wrote the manuscript. SE & JA improved the manuscript. All authors read the final manuscript.

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Availability of data and materials

Research data (Lab notebook) and materials can be provided on request.

Declarations

Ethics approval and consent to participate

The experiment was conducted according to the rules of Institutional Animal Ethics Committee (IAEC)/ Board of Advanced Studies and Research (BASR/No./0584/Sc. dated 06-08-2010), University of Karachi.

Consent for publication

Not Applicable.

Competing interests

The authors declare that they have no competing interests.

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