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Research paper

Inhibition of thioacetamide induced liver intoxication and in-vitro antioxidant capacity of the *Hugonia mystax*

MallikarjunaRao.Talluri [1], RajanandaSwamy.Tadi [2]*, Ganga Rao.Battu [2]

[1]Research Associate, MicGene lab, Visakhapatnam, A.P, India-530003
[2]Department of Pharmacognosy and Phytochemistry, A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, A.P, India-530003.
**Corresponding author E-mail:rajaphd2015@gmail.com*

Abstract

Background: The using of synthetic drugs in adequate manner for the treatments of liver diseases are causes the different side effect. Therefore, there is need to search for new bioactive compounds to control liver diseases. In this point of view, we selected the medicinal plant *H. mystax* to evaluate of its antioxidant and hepatoprotective potentiality and isolation of its constituents, because it has been using in traditional medicine in the treatment of different diseases.
Methods: Different extracts were prepared from *H. mystax* using maceration extraction process.Antioxidant activity was carried out using standard test procedures on Superoxide, Hydroxyl and DPPH free radicals. Hepatoprotective was tested using Thioacetamide (TAA) induced liver intoxication in rats.
Results: The *H. mystax* extracts were showed dose dependent antioxidant and hepatoprotective activities. Tested extracts of *H. mystax* have more activity on hydroxyl free radical than superoxide and DPPH radicals and among all extracts hydroalcoholic extract showed more activity. The extracts of *H. mystax* significantly restored the altered biochemical parameters due to TAA induced liver intoxication and hydroalcoholic extract exhibited more significant activity. So, the hydroalcoholic extract was used for the isolation of phytochemical constituents through column chromatography and isolated HM-1 and HM-2 compounds and are identified as stigmasterol and β-sitosterol.
Conclusion: From the results of the study, it could be conclude that *H. mystax* have the capacity in reduction of free radicals and normalization of liver function when it damage due to different liver intoxications and further research is need to evaluate more pharmacological activities and in isolation of most active bioactive compounds from *H. mystax*.

Keywords: *H. mystax; Aerial Parts; Antioxidant Activity; Thioacetamide; Hepatoprotective Activity*.

1. Introduction

Liver is one of the main functional organ in human body, plays an important role in metabolic processes like metabolism of lipids, proteins, and carbohydrates (Senguptaet al., 2011; Alqasoumiet al., 2009; Jamshidzadeh and Nikmahad, 2006). Because of its' multi-dimensional functions, it is major organ easily effect by many diseases. The liver diseases may cause due to the Alcohol consumption, Hepatitis virus, Liver transplantations, Obesity and Non-Alcohol related fatty liver diseases etc. The use of synthetic drugs (Steroids, Inhalants) for the treatments of liver diseases in inadequate manner causes different side effects on their long-term usage. Therefore, there is need to search new fighting strategies to control liver diseases with less or lack of side effects and low cost because the present drugs available in the market in the treatment of diseases are expensive. The medicinal plants are source for new drugs since the ancient times to treat different diseases around world (Bandaranayake, 2002; Ashok et al., 2001, Ganga Rao et al., 2013). But, till now only a small percentage has been investigated phytochemically and submitted to pharmacological screening and large number of plants are unexplored for source of powerful drugs (Thomson, 1978; Stockwell, 1988; Clark and Hufford, 1993). The researchers are turning from manufacturing of synthetic drugs to herbal extracts to isolate new drug molecules have natural origin and synthesis of those drugs derivatives which have less side effects from traditionally used medicinal plants by tribes or societies across the globe (Kroschwitz and Howe-Grant, 1992; Newman et al., 2000; Balandrin et al., 1985). Considering the unbounded potentiality of plants as sources for new drugs, a systematic investigation was undertaken to screen the phytochemical analysis, Antioxidant and hepatoprotective activity of *Hugonia mystax* aerial parts. The plant *H. mystax* has been using in traditional medicine i.e. useful in fever, verminosis and vitiated conditions of vata, externally as a past for inflammations and used swelling due to viper bite (Nambiar, 1995; Santapau and Henry, 1983; Pullaiah and Chennaiah, 1997; Padel et al., 2010; RajanandaSwamy et al., 2016).

2. Material and methods

2.1. Chemicals and drugs

Silymarin, Thioacetamide (TAA) and 1, 1- diphenyl-2-picrylhydrazyl was purchased from Sigma chemicals, USA. Nitrobluetetrozolium was purchased from Sisco Research Laboratories Pvt Ltd., Mumbai. Riboflavin was purchased from LobaChemiePvt Ltd., Bombay. Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Serum Alkaline Phosphatase (ALP), Serum Total bilirubin (T.Bil) and Serum total protein (TP) kits were purchased from

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Span diagnostics Ltd, Gujarat, India. All other chemicals used were of analytical grade.

2.2. Collection of plant material and preparation of extracts

The plant material was collected near Indira Gandhi Zoological park, Visakhapatnam, Andhra Pradesh, India. The authentication of the plant was done by Rtd. Prof. M. Venkaih, Department of Botany, Andhra University, Visakhapatnam. Shade dried powdered plant was extracted using maceration process with hexane, ethyl acetate, and hydroalcoholic (ethanol (70%v/v)) separately and collected solvents were concentrated to dryness under vacuum using rotavapour.

2.3. Animals

Albino rats of either sex weighing between 180-200gm were obtained from M/s. Mahavir Enterprises, Hyderabad. The animals were housed under controlled environmental conditions (temperature of 22 $\pm 1^0$Cwith an alternating 12hr light-dark cycle and relative humidity of 60 ± 5%), one week before the start and also during the experiment. They were fed with standard laboratory diet supplied by M/s. Rayans biotechnologies Pvt. Ltd., Hyderabad, and water ad labium during experiment. Food was withdrawn 12hr before the terminating experiment and water was allowed adlibitum.

2.4. Acute toxicity studies

Acute toxicity study was conducted according OECD Guide lines No.423. The experimental protocol was approved by institutional animal ethical committee of Regd. No. 516/01/A/CPCSEA, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam. After fasting overnight, mices were administered with extracts of *H. mystax* in a single dose up to the highest dose of 2000 mg/kg orally. The animals were observed continuously for 1 h and then hourly for 6 h and finally after every 24 h up to 15 days for any toxicological symptoms or mortality.

2.5. In-vitro antioxidant activity

The extracts of *H. mystax* screened for free radical scavenging activity against Superoxide radical, Hydroxyl and DPPH radicals at different concentrations. The Percentage Inhibition and 50% Inhibition Concentration's (IC_{50}) were calculated.

2.5.1. Superoxide radical scavenging activity

Superoxide scavenging activity of the plant extract was determined by McCord & Fridovich method (1969), this depends on light induced superoxide generation by riboflavin and the corresponding reduction of nitrobluetetrazolium.

2.5.2. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity is commonly used to evaluate the free radical scavenging effectiveness of various antioxidant substances (Elizabeth and Rao, 1990). Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the Fe2+/EDTA/H_2O_2 system (Fenton reaction). The hydroxyl radical attacks deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances (TBARS).

2.5.3. DPPH radical scavenging activity

The scavenging activity for DPPH free radicals was measured according to the standard procedure (Braca et al., 2003). In DPPH assay method is based on the reduction of alcoholic DPPH solution (dark blue in colour) in the presence of a hydrogen donating antioxidant converted to the non-radical form of yellow colored diphenylpicrylhydrazine. Lower the absorbance higher the free radical scavenging activity (Anita Murali et al., 2011).

2.5.4. Calculation of percentage inhibition

The percentage inhibition of superoxide production by the extract was calculated using the formula:

Inhibitory ratio = $(Ao\text{-}A_1) \times 100/A_0$

Where, A_0 is the absorbance of control; A_1 is the absorbance with addition of plant extract/ ascorbic acid.

2.5.5. Calculation of 50% inhibition concentration

The optical density obtained with each concentration of the extract/ascorbic acid was plotted taking concentration on X-axis and percentage inhibition on Y-axis. The graph was extrapolated to find the 50% inhibition concentration of extract/ ascorbic acid.

2.6. Evaluation of hepatoprotective activity on TAA induced hepatotoxicity in rats

Thioacetamide induced hepatotoxicity in rats model was used for evaluation of hepatoprotective activity for the selected plant extracts. Animals were divided into twelve groups consisting of six rats in each group (Mangipudy et al., 1995; Dorğru-Abbasoğlu et al., 2000). The animals of group I served as control (vehicle) were given only Saline (2ml/kg b.w., per orally) for seven days. The animals of group II were administered with Thioacetamide (TAA, 50 mg/kg b. w., s.c.) as a 2% w/v solution in water for injection on day 1st and then normal saline for 21 days. The animals of group III were administered with TAA (50 mg/kg b. w., s.c.) as a 2% w/v solution in water for injection on day 1st and then silymarin (25mg/kg per day, p. o.) for 21 days. Group IV, V and VI animals were administered with TAA (50 mg/kg b .w., s.c.) as a 2% w/v solution in water for injection on day 1st and then Hydro alcoholic extract of *H. mystax* (125, 250 and 500 mg/kg respectively) for 21 days. Group VII, VIII and IX animals were administered with TAA (50 mg/kg b .w., s.c.) as a 2% w/v solution in water for injection on day 1st and then Ethyl acetate extract of *H. mystax* (125, 250 and 500 mg/kg respectively) for 21 days and finally for group X, XI and XII animals were administered with TAA (50 mg/kg b .w., s.c.) as a 2% w/v solution in water for injection on day 1st and Hexane extract of *H. mystax* (125, 250 and 500 mg/kg respectively) for 21 days. Rats of all the groups were anaesthetized by chloroform, 48 hr after the TAA administration. The blood was collected from retro-orbital plexus. The blood was collected from rats were sacrificed and the collected blood samples were immediately centrifuged at 2400rpm for 15 minutes. When serum clearly separated out, the serum was analyzed for AST (SGOT), ALT (SGPT), ALP, total bilirubin and total protein levels using commercial diagnostics reagent kits and Auto analyzer.

2.7. Isolation of phytoconstituents (compounds)

The selected plant extracts (Hexane, Ethyl acetate and hydroalcoholic) on TLC over showed different spots with different retention factor (R_f) values, but on the basis of biological activities (Antioxidant and Hepatoprotective and Antibacterial (RajanadaSwamy et al., 2016), less phytochemical works was reports and availability of the extract quantity hydroalcoholic extract of *H. mystax* was used for separation of compounds using column chromatography.

3. Results

3.1. Antioxidant activity of hugoniamystax

In the present study, hydroalcoholic, ethyl acetate and hexane extracts of *H. mystax* were found to possess concentration depend-

ent scavenging activity on tested free radicals. The results were given in Table 1 and Fig 1.

Table 1: 50% Inhibition Concentrations (IC_{50}) of Different Extracts of *H. mystax* against Superoxide, Hydroxyl and DPPH Radicals.

Name of the extract	50%Inhibition Conc (IC_{50}) Superoxide radical	Hydroxyl radical	DPPH radical
Hydroalcoholic extract	219.58±0.52	283.4±0.40	277.36±0.56
Ethyl acetate extract	281.36±1.22	299.26±0.18	208.66±0.48
Hexaneextract	429.62±0.42	235.84±0.92	335.82±.86
Ascorbic acid	54.4±1.1	68.00±1.3	22.0±0.5

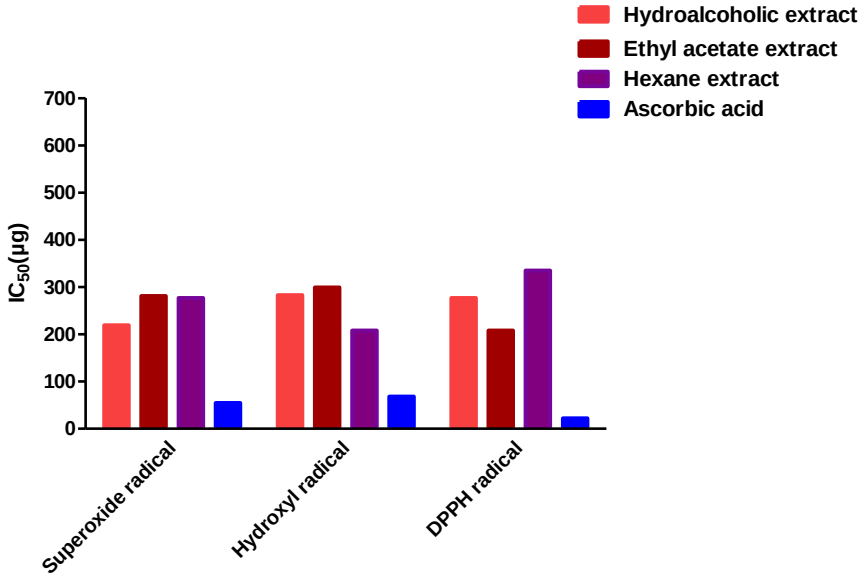


Fig. 1: 50% Inhibition Concentrations (IC_{50}) of Different Extracts of *H. mystax* Against Superoxide, Hydroxyl and DPPH Radicals.

The mean IC_{50} values for superoxide radical of hydroalcoholic, ethyl acetate and hexane extracts of *H. mystax* were found to be 219.58±0.52 µg, 281.36±1.22µg and 429.62±0.42µg respectively. The mean IC_{50} value of ascorbic acid was found to be 54.4±1.1µg. Among all extracts hydroalcoholic extract at a concentration of 640µg showed the better scavenging activity on superoxide free radical i.e. 70.3±1.2%.

The mean IC_{50} values for hydroxyl radical of hydroalcoholic, ethyl acetate and hexane extracts of *H. mystax* were found to be 283.4±0.40µg, 299.26±0.18µg and 235.84±0.92µg respectively. The mean IC_{50} value of ascorbic acid was found to be 68.00±1.3µg. Among all extracts hexane extract at a concentration of 640µg showed the better scavenging activity on hydroxyl free radical i.e. 66.8±0.16%.

The mean IC_{50} values for DPPH radical of hydroalcoholic, ethyl acetate and hexane extracts of *H. mystax* were found to be 277.36±0.56µg, 208.66±0.48µg and 335.82±.86µg respectively. The mean IC_{50} value of ascorbic acid was found to be 22.0±0.5µg. Among all extracts ethyl acetate extract at a concentration of 640µg showed the better scavenging activity on DPPH free radical i.e. 68.4±0.48%.

The selected plant extracts showed more activity on hydroxyl free radical than other two radicals. The order of activity in the following manner: ascorbic acid >hydroalcoholic extract > ethyl acetate extract> hexane extract.

3.2. Acute toxicity studies

There were no visible sign of toxicity and mortality after oral administration of single dose (2000 mg/kg) of selected medicinal plant extracts. The results (Table 2) clearly indicated non toxicity/safety of the extracts at a dose of 2000 mg/kg. Hence there is no lethal dosage (LD_{50}) and all the tested extracts were considered safe and non toxic. This prompted the author to select different doses of selected three plants extracts for evaluation of biological activities.

Table 2: Acute Toxicity of *H. mystax* Extracts Administered by Orally to Mices.

Doses (mg/kg)	Sex	D/T	Mortality latency	Toxic symptoms
2000 (H. m EtOHE)	M	0/6	-	None
2000 (H. m EAE)	M	0/6	-	None
2000 (H. m HE)	M	0/6	-	None

D/T = dead/treated rats; none = no toxic symptoms during the observation period; mortality latency = time to death (in hours) after administered by orally.
H. m EtOHE: *H. mystax* Hydroalcoholic extract; H. m EAE: *H. mystax* Ethyl acetate extract; H. m HE: *H. mystax* Hexane extract.

3.3. Hepatoprotective activity

The increase in various biochemical parameters like SGOT, SGPT, ALP, TP and TBIL levels were recorded due to TAA. The effects of test extracts of all the plants were compared against the standard drug silymarin which was orally administered to the rats at a dose of 25mg/kg body weight. The doses of the extracts were determined from the drug tolerance studies and the respective plant extracts were accordingly administered. Administration of the extracts of selected medicinal plants orally in doses of 125, 250 and 500mg/kg followed by TAA after administration of the test sample prevented the rise of SGOT, SGPT, ALP, TP and TBIL levels and percentage protection of the extracts were recorded. The tested extracts against Thioacetamide intoxication, hydroalcoholic extracts of selected plants showed maximum hepatoprotective activity in terms of reduction in the elevated levels of serum biochemical parameters. The ethyl acetate extract, hexane extract follow next in order.

Group I was treated with vehicle showed no significant changes in the biomarkers of liver enzymes (AST (SGOT), ALT (SGPT), ALP, total bilirubin and total protein) levels. Group II was treated TAA, there is significant changes in levels of biomarker enzymes (Table 3). The animals of group III were administered with TAA (50 mg/kg b. w., s.c.) as a 2% w/v solution in water for injection

on day 1st and then silymarin (25mg/kg per day, p. o.) for 21 days. There is significant changes in biomarker enzymes levels compared to group II rats enzymes levels (Table 3) and the percentage protection offered by the silymarin against the changes in AST (SGOT), ALT (SGPT), ALP, total bilirubin and total protein levels were 96.24%, 95.25%, 93.90%, 97.83% and 96.14% respectively (Table 4).

Table 3: Enzymes Levels of the Animals Due to the Effect of *H. mystax* Extracts

.	Name of the drug											
	Normal (Drug Vehicle)	Thio-acetamide	Sylimarin(mg/kg b.w)	*H. mystax* Hexane extract (mg/kg b.w)			*H. mystax* Ethyl acetate extract (mg/kg b.w)			*H. mystax* 70% ethanol extract (mg/kg b.w)		
			25	125	250	500	125	250	500	125	250	500
SGOT (U/L)	96.17± 6.97	330.5± 6.53	87± 2.83	249.5± 5.24	221.5± 4.46	195.3± 4.46	233.3± 4.27	210.2± 3.13	187.67± 5.32	229.5± 6.25	199± 5.87	152.17± 2.48
SGPT (U/L)	56± 3.58	173± 5.22	50.17± 4.96	137.3± 3.93	122.5± 4.64	106.5± 4.09	129.8± 2.93	116.5± 5.82	102± 3.95	120± 4.15	105.17± 2.79	82.5± 1.87
ALP (U/L)	217.5± 2.59	548.67± 21.60	196.00± 5.10	457.3± 3.88	422± 5.97	378.5± 4.59	442± 7.01	405.5± 3.89	367.67± 5.09	434.3± 4.93	380.5± 6.53	321.3± 6.62
Total bilirubin (mg/dl)	0.17± 0.01	2.123± 0.27	0.13± 0.05	1.75± 0.22	1.4± 0.28	1.12± 0.20	1.65± 0.22	1.38± 0.30	1.07± 0.20	1.57± 0.23	1.27± 0.16	0.93± 0.22
Total protein (g/dl)	7.40± 0.46	4.87± 0.78	7.50± 0.37	5.43± 0.29	5.78± 0.29	6.22± 0.30	5.4± 0.23	5.78± 0.29	6.25± 0.28	5.5± 0.33	5.95± 0.44	6.43± 0.27

Table 4: Percentage (%) Protection on Enzymes Levels Due To the Effect of *H. mystax* Extracts at Different Doses on TAA-Induced Liver Toxicity

Name of the extract	Amount of the extract 125mg/kg b.w					250mg/kg b.w					500mg/kg b.w				
	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Total bilirubin (mg/dl)	Total protein (g/dl)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Total bilirubin (mg/dl)	Total protein (g/dl)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Total bilirubin (mg/dl)	Total protein (g/dl)
Sylimarin	96.24	95.25	93.90	97.83	96.14	96.24	95.25	93.90	97.83	96.14	96.24	95.25	93.90	97.83	96.14
Hydro-alcoholic	43.10	45.30	34.52	28.55	26.28	56.12	57.98	50.78	43.93	42.75	76.10	77.35	68.65	61.03	61.86
Ethyl acetate	41.47	36.89	32.21	24.27	21.67	51.35	48.29	43.23	37.95	36.17	60.95	60.68	54.66	54.19	54.61
Hexane	34.57	30.48	27.58	19.15	22.33	46.51	43.16	38.25	37.09	36.17	57.68	56.84	51.38	51.62	53.29

Groups IV, V and VI were treated with hydroalcoholic extract of *H. mystax* orally at doses of 125mg/kg, 250mg/kg and 500 mg/kg b.w. The percentage protection produced by the extract on the enhancement of AST (SGOT), ALT (SGPT), ALP, total bilirubin and total protein levels were 43.10%, 45.30%, 34.52% 28.55% and 26.28%, 56.12%, 57.98%, 50.78%, 43.93% and 42.75%, 76.10%, 77.35%, 68.65%61.03% and 61.86% respectively.

Groups VII, VIII and IX were treated with ethyl acetate extract of *H. mystax* orally at doses of 125mg/kg, 250mg/kg and 500 mg/kg b.w. The percentage protection produced by the extract on the enhancement of AST (SGOT), ALT (SGPT), ALP, total bilirubin and total protein levels were 41.47%, 36.89%, 32.21% 24.27%and 21.67%, 51.35%, 48.29%, 43.23% 37.95%and 36.17%, 60.95%, 60.68%, 54.66% 54.19%and 54.61% respectively.

Groups X, XI and XII were treated with hexane extract of *H. mystax* orally at doses of 125mg/kg, 250mg/kg and 500 mg/kg b.w. The percentage protection produced by the extract on the enhancement of AST (SGOT), ALT (SGPT), ALP, total bilirubin and total protein levels were 34.57%, 30.48%, 27.58% 19.15% and 22.33%, 46.51%, 43.16%, 38.25% 37.09% and 36.17%, 57.68%, 56.84%, 51.38% 51.62% and 53.29% respectively.

3.4. Isolation of compounds form hydroalcoholic extract

Column chromatography was carried out for the hydroalcoholic extract using different combination of solvents polarity increasing from hexane (100%) to Ethyl acetate (100%) and then to Ethanol. We isolated the compounds HM-1 and HM-2 at different concentrations.

3.4.1. Structural elucidation and characterization of compound HM-1

This compound was obtained from hexane: ethyl acetate (95:05) fractions as color less crystalline solid was identified as Stigmasterol by spectrosopic analysis. The molecular formula is $C_{29}H_{48}O$. The melting point was recorded at 140^{o}C. The IR spectrum showed absorption bands at 3424, 2960,2937,2867,1464 and 1381cm-^{1} (Fig 2). This compound showed very strong color reaction with sulphuric acid indicating its steroidal nature.

The ^{1}H NMR (Fig 3) spectrum of the compound MH-1 revealed a one-proton multiplet at δ 3.23, the position and multiplicity of which was indicative of H-3 of the steroidal nucleus. The typical signal for the olefinic H-6 of the steroidal skeleton was evident from the multiplet at δ 5.04 integrating for one proton. The olefinic protons (H-22 andH-23) appeared as characteristic downfield signals at δ 5.03 and δ 5.18 respectively in the ^{1}H NMR spectrum. Each of the signal was observed as double doublets (J=14.4, 8.4 Hz) which indicated coupling with the neighboring olefinic and methane protons. The spectrum further revealed the signals at δ 0.67 and δ 1.00 (three proton each) assignable to two tertiary methyl groups at C-13 and C-10, respectively. The ^{1}H NMR spectrum also showed two doublets centered at δ 0.80 (J = 7.4 Hz) which could be attributed to the two methyl groups at C-25. The doublet at 0.91 (J=6.4 Hz) was demonstrative of a methyl group at C-20 (Table 5).

Table 5: ^{1}H (400 MHz), ^{13}C (100 MHz) NMR Spectral Data of Compound MH-1 (Stigmasterol) In $Cdcl_3$.

Position	Chemical Shifts (δ) ^{1}HNMR	^{13}C NMR
1	-	37.2
2	-	31.6
3	-	71.8
4	-	39.7
5	-	140.7
6	5.35(1H,s)	121.6
7	-	31.9
8	-	31.6
9	-	50.2
10	-	36.5
11	-	21.1
12	-	39.8
13	-	42.3
14	-	56.8
15	-	24.3
16	-	28.2
17	-	56.1
18	0.79(3H,s)	11.8
19	0.95(3H,s)	19.3
20	-	34.0
21	1.06(3H, d, J=8.0Hz	19.7
22	5.03(1H,m)	138.3
23	5.18(1H,m)	129.2
24	-	45.9
25	-	31.9
26	0.86(3H,d,J=8.6Hz)	19.0
27	0.84(3H,d,J=7.5Hz)	29.2
28	-	25.3
29	0.89(3H,d,J=8.0 MHz)	12.1

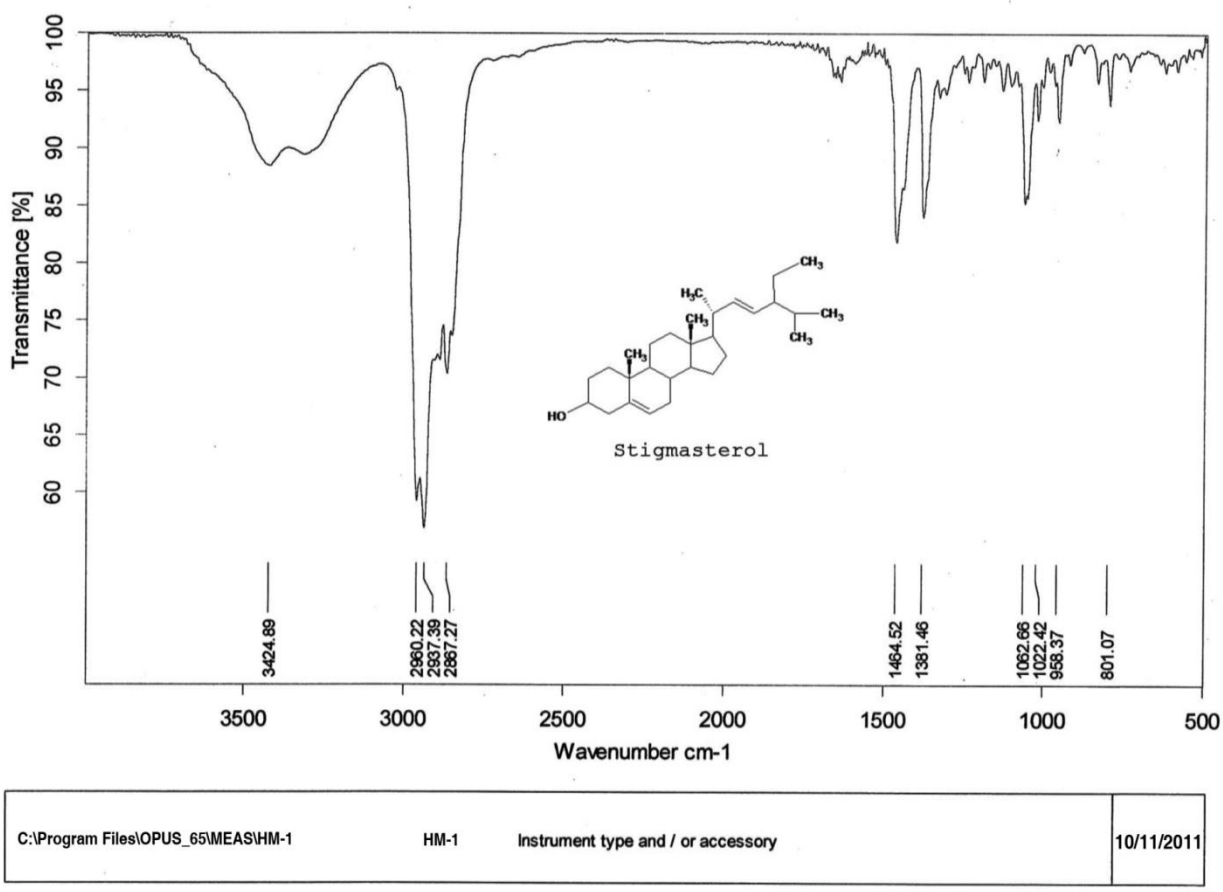


Fig.2:IR Spectrum of the Compound HM-1.

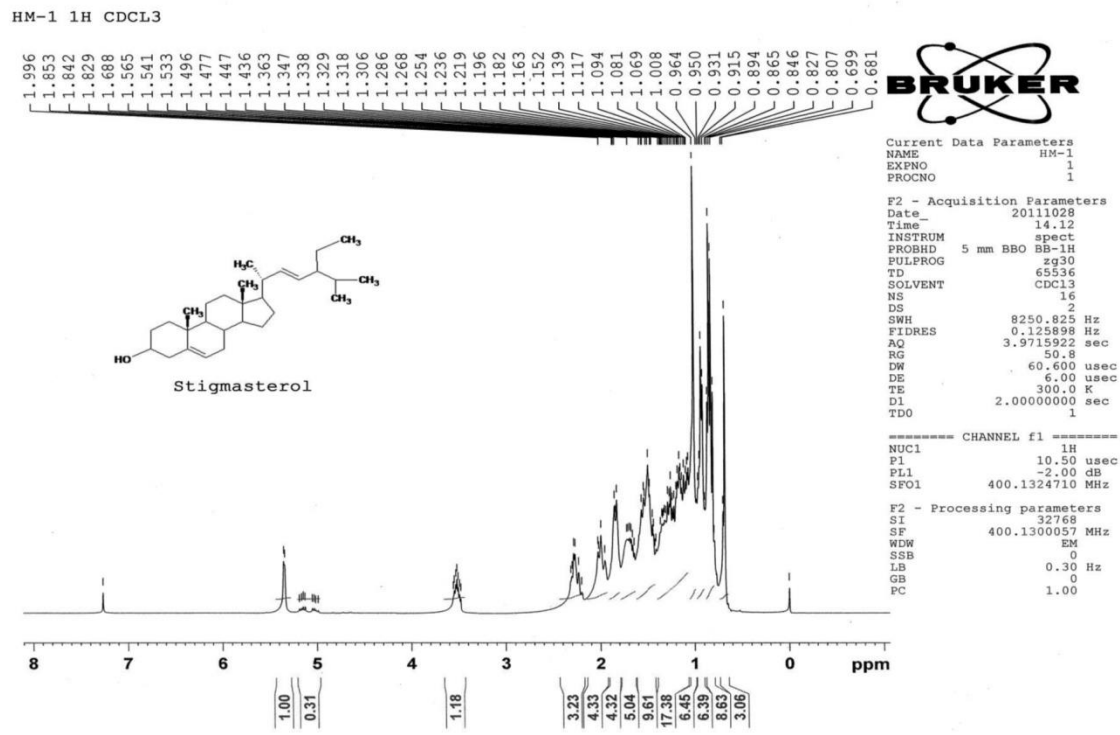


Fig.3: ^{1}H NMR Spectrum of the Compound HM-1.

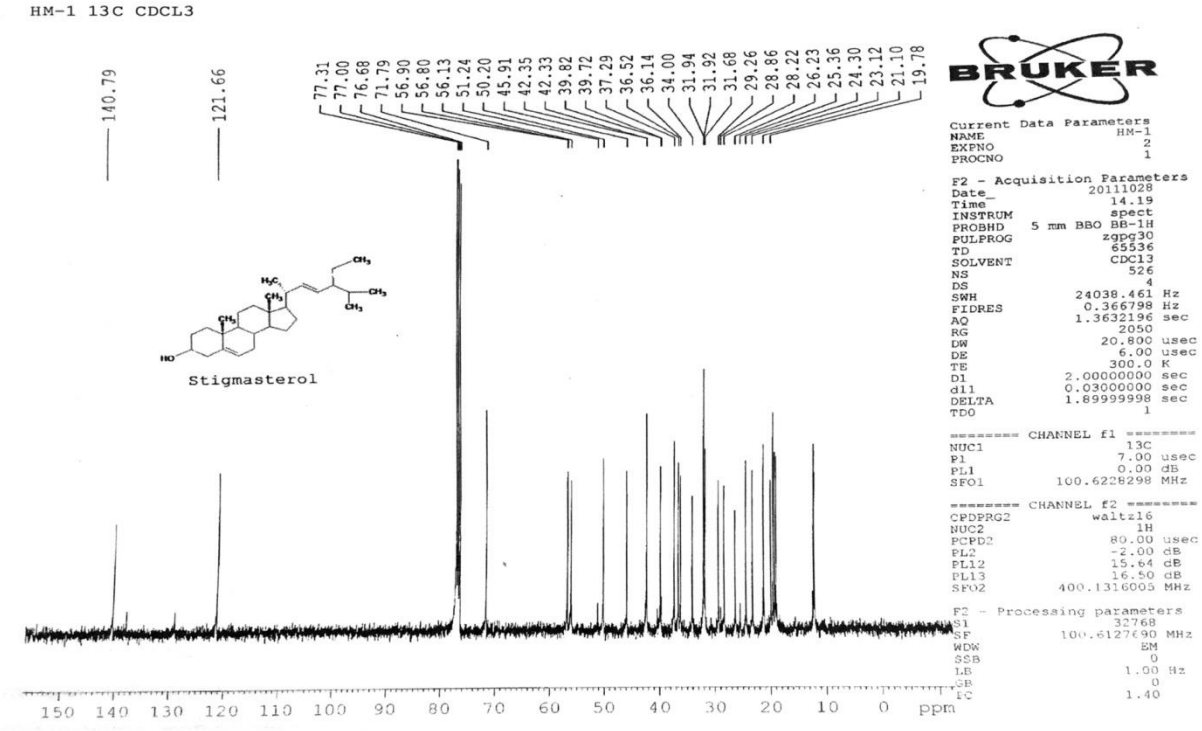


Fig.4: ^{13}C NMR Spectrum of the Compound HM-1.

The ^{13}C NMR spectrum (Fig 4) showed 29 carbons. Among 29 carbon resonances six were CH_3, nine CH_2, eleven CH and three quaternary carbons. These special features are in close agreement to those observed for stigmasterol (Khan, 1991). On the basis of NMR assignment and literature data, the compound was identified as stigmasterol (Pouchert and Behnke, 1968; Grecaet al., 1990; Habib et al., 2007).

3.4.2. Structural elucidation and characterization of compound HM-2

Compound HM-2 is color less or white needles with little impurities. The compound showed m.p. at 136-137^{o}C, with R_f value: 0.50 (hexane; ethyl acetate, 93:07). The compound was freely soluble in hexane, chloroform, ether and chloroform and sparingly soluble in alcohol with molecular formula is $C_{29}H_{50}O$. The compound gave positive color reaction with Liebermann Burchard reaction and Solkowski reaction test for steroids.

IR spectrum showed a strong and broad absorption band at 3438cm^{-1}, which corresponded to –OH stretching. Two bands were seen at 2931 and 2854 cm^{-1}, which corresponded to C=C stretching vibrations. Absorption bands were seen at 1462 and 1378 cm^{-1}, which corresponded to C-O stretching vibrations (Fig 5).

The ^{1}HNMR spectrum (Fig 6) showed the presence of 6 methyl groups at δ 0.8 -1.25 and 6 methylenic protons at δ 1.2 to 2.04. The proton attached to C-3 carbon bearing the hydroxyl group appeared δ3.52 as a multiplet. The protons attached to the olefenic carbon appeared to δ value of 5.1 as multiplet. The ^{1}H NMR spectrum ofthe compound KCHE-8(400 MHz, $CDCl_3$) exhibited a broad doublet at δ5.36(1H,d,J=5.1 Hz) attributed to be a double bonded proton typical for a multiplet at δ3.55(1H,m) integrated for one proton which could be H-3 of a steroidal skeleton. Other signals appeared between δ0.9 – δ2.4 was due to the methylene and methane protons.

The ^{13}C NMR spectra (Fig 7) showed the presence of 6 quartets, 11 triplets, 9 doublets and three singlets corresponding to 6 methyl, 11 methylene, 9 methine and 3 tertiary carbons. The presence of two C=C was inferred by the presence of a singlets and 3 doublets at δ 140.85 and 121.72. The mass spectrum showed a peak at 414(molecular ion peak) which corresponds to the molecular weight, a peak at 396(M-H_20) $^{-}$ which confirmed the presence of hydroxyl group (Table 6). Mass spectrum indicates (M^{+} 414) the molecular formula $C_{29}H_{50}O$. Comparison of ^{1}H and ^{13}C NMR data with literature reports and Co-TLC identified it as β-sitosterol (Ahmad et al., 1992). These features were in close agreement with those of β-sitosterol reported in the literature (Hill et al., 1999).

Table 6: ^{1}H, (400 MHz) ^{13}C NMR (100 MHz) Spectral Data of Compound B-Sitosterolin $Cdcl_3$.

Position	Chemical Shifts (δ) ^{1}HNMR	^{13}C NMR
1	-	36.1
2	2.03(2H,m)	31.7
3	3.24(1H,m)	71.8
4	-	42.4
5	-	140.8
6	5.28(1H,s)	121.7
7	-	29.3
8	-	31.9
9	-	50.2
10	-	36.5
11	-	21.1
12	-	40.4
13	-	42.4
14	-	56.8
15	-	24.3
16	-	29.3
17	-	56.8
18	0.68(3H,s)	12.0
19	1.01(3H,s)	12.2
20	-	34.0
21	-	21.1
22	-	34.0
23	5.02(1H,dd,J=15.2, 8.6Hz)	28.2
24	-	51.2
25	-	26.2
26	0.83(3H,d,J=6.6Hz)	19.1
27	0.83(3H,d,J=6.6Hz)	19.8
28	-	24.3
29	0.84(3H,m)	11.9

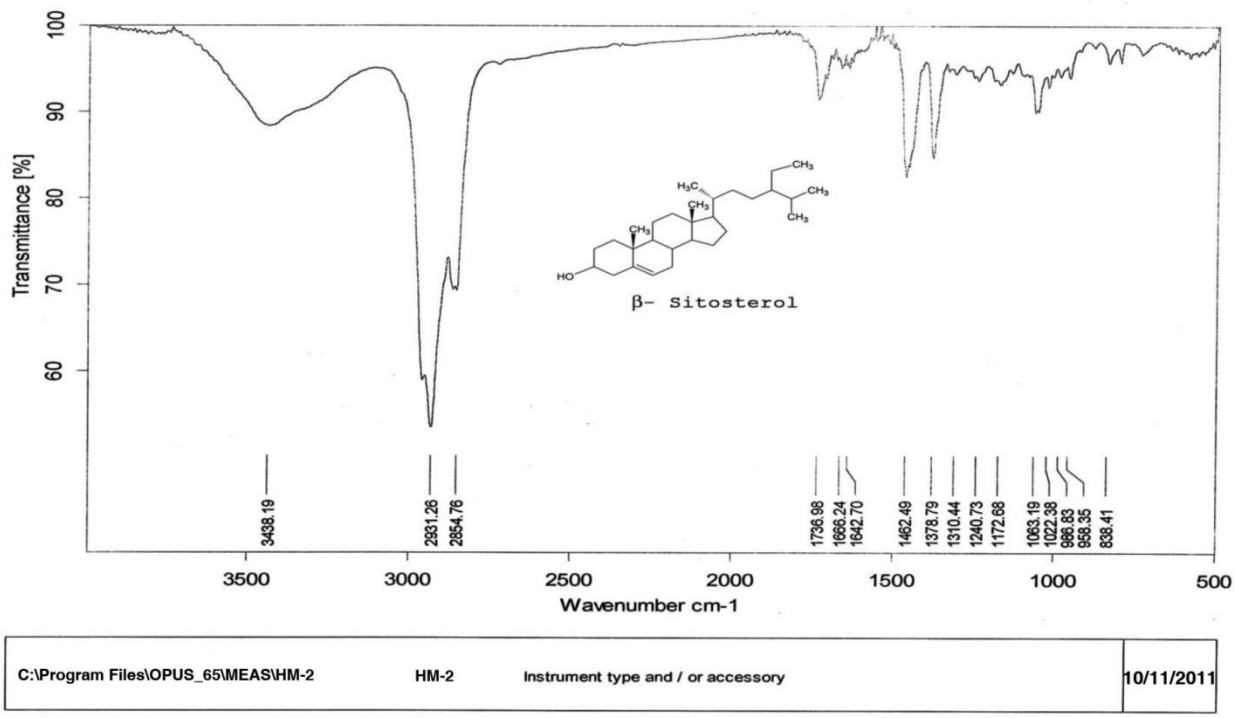


Fig.5:IR Spectrum of Compound HM-2.

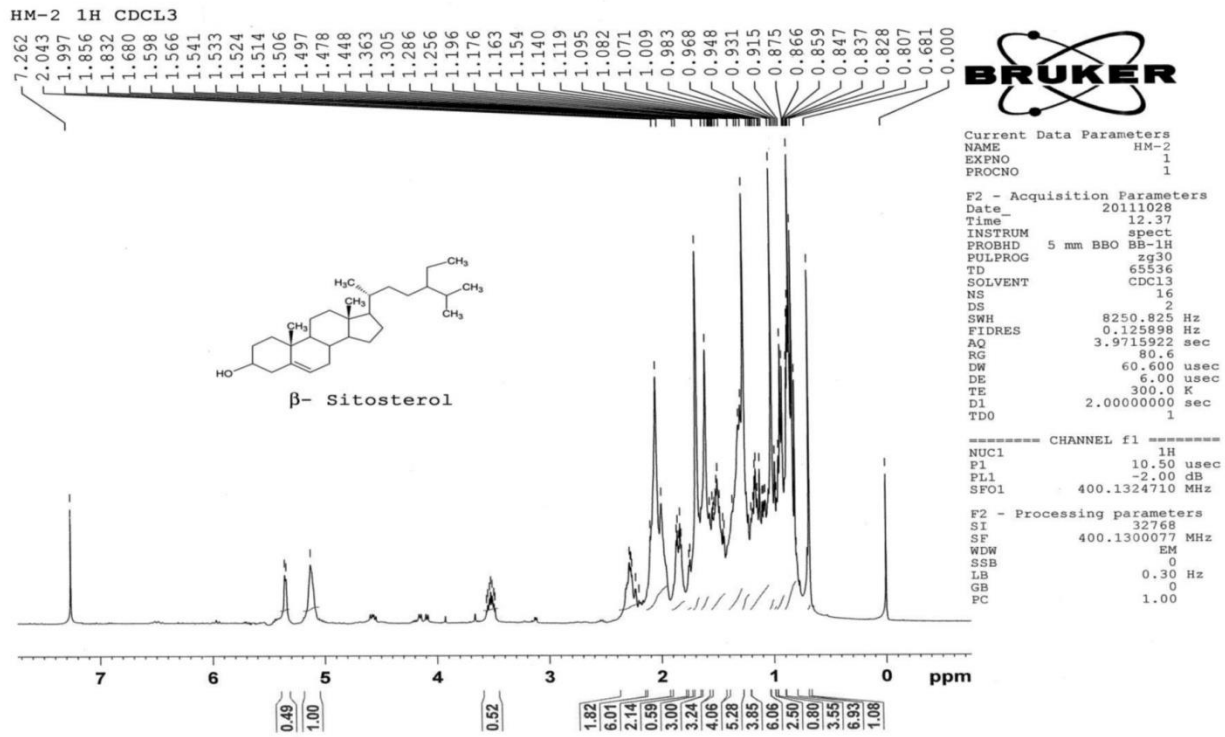


Fig. 6: [1]HNMR Spectrum of Compound HM-2.

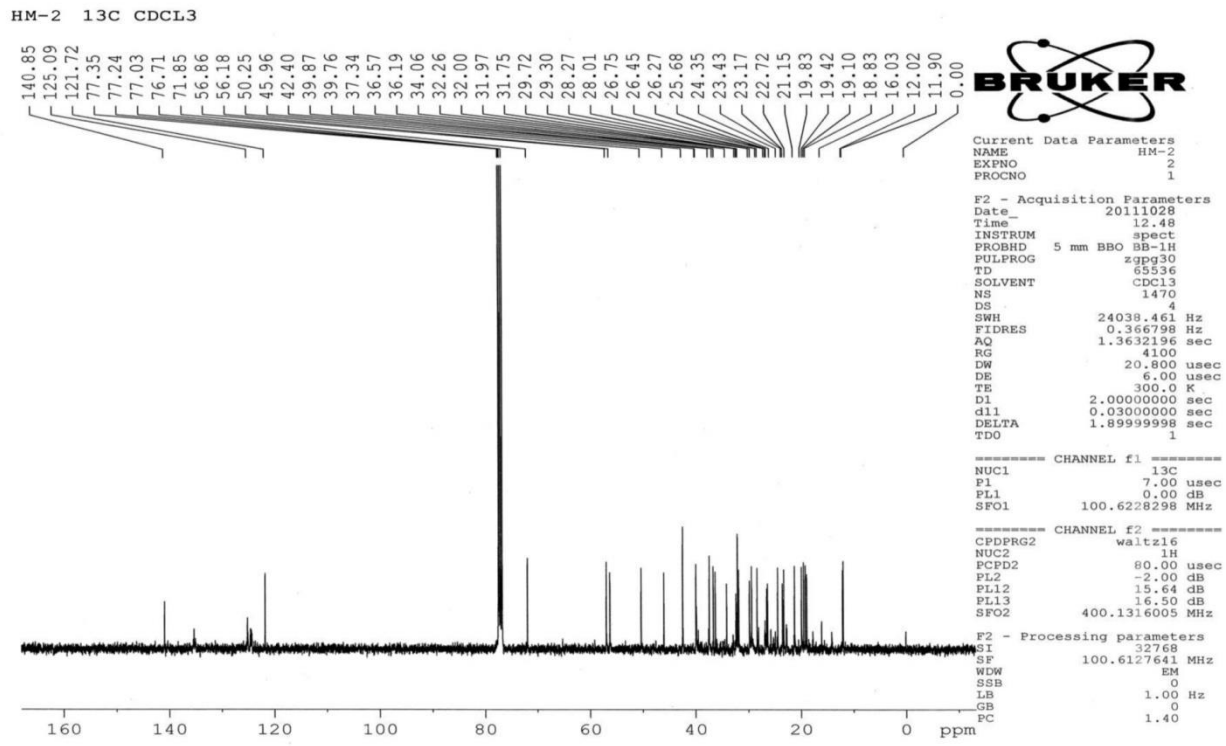


Fig.7: [13]C NMR Spectra of Compound HM-2.

4. Discussion

The remedies available in modern medicine provide only symptomatic relief, without any significant changes to the etiological causes of the disease process (Okada and Okada, 1998; Pen-Der-Duh, 1998), there are increasing harmful effects of modern medicines and diseases causing microorganisms getting resistance towards the drugs using in the treatment of diseases (Aktar et al., 2009; Ganga Rao et al., 2013). Moreover, as many therapeutic agents are known to cause conditions such as liver cirrhosis and fulminate hepatic failure, the development or identification of new molecules effective in treating or preventing hepatic damage remains a challenge in the field of drugs development (Ajith et al., 2007; Kappus, 1991; Khan and Shahidi, 2001). All these problems, insisting the scientists to search for the discovery of new drugs with more efficacy, less side effects and low economic costs. The present study aimed to evaluate the antioxidant and hepatoprotective capacity of the *H. mystax* aerial parts extracts and results reveal that the plant posses significant capacity in reduction of free radicals and significant restoration of the altered biochemical parameters towards normal in TAA intoxicated rat i.e. TAA causes the hepatic injury and subsequently to its failure, thus severely affecting the body's entire metabolism by producing the different free radicals formation in the cells metabolism process (Charlotte et al., 2013; PavlaStanˇková et al., 2010; James et al., 2014; Stephen et al., 2013). The effects of the tested extracts were increased as concentration was increases in ameliorate the levels of free radicals, enzymes levels due to intoxication and also the presence of compounds stigamsterol and β-sitosterol in the selected plant from the hydroalcoholic extract which are previously reported different biological activities (Hogg John, 1992; Salvador et al., 2004; Panda et al., 2009; Gabay et al., 2010), so further investigations are needed to isolate novel bioactive compounds.

5. Conclusion

It could be conclude that *H. mystax* have the capacity in reduction of free radicals produced in the body due to different physiological mechanisms either normal or abnormal conditions (Diseases).The isolated compounds stigamsterol and β-sitosterol are evidence about the presence of different bioactive molecules in *H. mystax*.Further research is need to evaluate more pharmacological activities and in isolation of the more bioactive compounds.

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Conflicts of interest

There are no conflicts of interest.

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