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In Vitro and *In Vivo* Anticancer Activity of the Fruit Peels of Solanum *melongena L.* against Hepatocellular Carcinoma

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Abstract

Background: The fruit peels' of *Solanum melongena L.* which is a common vegetable in Egypt, were investigated for biologically active metabolites in an approach to find any medicinal benefits from such waste products.

Methods: The Methanol Extract of the Peels (MEP) was subjected to fractionation and purification for the isolation of its major constituents. Identification of the compounds was carried out on the basis of physico-chemical properties and spectral analysis (^{1}H NMR, ^{13}C NMR, COSY and HMBC). The MEP together with the isolated compounds were tested against five human cancer cell lines representing the most common types of cancer in Egypt: colon cancer cell line (HCT116), larynx cancer cell line (HEP2), breast cancer cell line (MCF7), cervix cancer cell line (HELA) and liver cancer cell line (HEPG2). MEP was tested *in vivo* against the CCl4- induced hepatocelular carcinoma (HCC) in rats at two dose levels (100 and 200 mg/kg.b.wt).

Results: Five steroidal compounds; three steroidal alkaloids: solasodine (S_1), solamargine (S_4) and solasonine (S_5) together with two steroidal glycosides: β-sitosterol-3-O- β-D-glucoside (S_2) and poriferasterol-3-O- β-D-glucoside (S_3) were isolated. The MEP and the five isolated compounds exhibited moderate to potent activities against the tested human cancer cell lines however their pronounced activity was revealed against HEPG2, accordingly, MEP was tested *in vivo* against the CCl_4- induced Hepatocelular Carcinoma (HCC) in rats. The MEP showed a dose dependent anticancer activity through stabilization of the hepato-cells revealed by reduction in α-fitorotein (AFP) (which could considered as tumor marker), it also restored the levels of AST, ALT and albumin in a dose dependent manner. Histopathology of liver tissues treated with MEP strongly supported our results.

Conclusion: Our findings supported the reuse of such waste products as a new remedy for treating cancer

Keywords: S. melongena L.; Peels; Hepatocellular carcinoma; Steroidal alkaloids

Introduction

Cancer is a widespread life-threatening disease that attacks people at all ages, especially those over 65 years. It is an environmental disease related to lifestyle, environmental factors and less commonly genetic factors. According to the cancer profile of the National Cancer Institute (NCI) in Egypt 38.474 patients, visit the NCI every year and about 47.3% of them are confirmed malignant [1].

Various remedies have been reported for the treatment of this disease, but the development of suitable therapeutic is still a major challenge. Many substances are in clinical trial or used in practice to treat cancer. However, they have major drawbacks; their high cost and adverse effects [2-6]. Therefore, our target was to look for alternative ways to develop novel drug candidates with fewer side effects and less cost. Fortunately, fruits and vegetable peels have the advantage over other herbal extracts, as they are easily identifiable, commonly used, rich in various bioactive compounds and some of their compounds have been characterized in terms of their chemical structures and biological properties.

Genus Solanum [family Solanaceae (night shadow plants)] is a very large group of about 1400 species, which are spread throughout the temperate and tropical regions of the world. They are rich in steroidal glycosides, in the form of glycoalkaloids. These compounds are important both ecologically and commercially [7-9]. Economically, they are used instead of the steroidal sapogenin diosgenin as a raw material for the industrial production of corticoids. The fruits of *Solanum melongena* L. (eggplant, Aubergine, Jews apple, mad apple) are well known as a vegetable all over the world. Crude alkaloidal fraction isolated from the leaves of S. melongena exhibited significant analgesic effect and some CNS depressant effect [10].

S. melongena L. fruits are rich in steroidal alkaloids [11-16]. The eggplant's peels were reported to possess antioxidant activity as it contains anthocyanin; delphinidine-3-(p-cumaroylrutinoside)-5-glucoside (nasunin) [14]. The anti carcinogenic effect of steroidal alkaloids isolated from other Solanum species was reported [17-19].

In that respect, our aim was to isolate the major bioactive compounds from the MEP and to plot an *in vitro* cytotoxic screening on a wide range of human cancer cell lines. This will be supported by *in vivo* test on the type of carcinoma on which the tested samples will give the best activity. Moreover, we will shed a light on the medicinal benefits of the eggplant's peels as anticancer agents instead of being a useless waste product.

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Materials and Methods

Plant material

The peels of the fruits of garden eggplant (*Solanum melongena* L.) were obtained from the fruits cultivated in the Agricultural Research Center, Giza, Egypt, during summer 2011/2012.

General experimental procedures

Authentic sterols and steroidal alkaloids were obtained from E. Merck, Darmstadt, Germany. Silica gel H (E-Merck, Darmstadt, Germany) for Vacuum Liquid Chromatography (VLC), silica gel 60 (Fluka, 70-230 mesh ASTM, Germany) for Column Chromatography (CC) and Sephadex LH 20 (Pharmacia) were used. Thin-Layer Chromatography (TLC) was performed on silica gel GF254 precoated plates (Fluka, Germany) using solvent systems I: chloroform-methanol (95:5), II: chloroform-methanol (90:10), III: chloroform-methanol-water (80:20:5) and IV: Ethyl Acetate-Methanol-Water (100:16.5:13.5). The chromatograms were visualized after spraying with p-anisaldehyde-sulphuric, ninhydrin and Dragendorff's spray reagents [20]. Melting points (uncorrected) were determined on a D. Electrothermal 9100 (U.K.). 1H NMR (300 MHz) and 13C NMR (75 MHz) were measured on Varian Mercury-VX-300 NMR instrument. The NMR spectra were recorded in DMSO-d6 and chemical shifts were given in δ (ppm) relative to TMS as internal standard.

Extraction and isolation

The air-dried powdered garden eggplant's peels (*Solanum melongena* L.) (500 g) was extracted by cold percolation with methanol (5 × 3 L) till exhaustion. The MEP was evaporated under reduced pressure to give 65 g of brown residue. Forty grams of this residue was chromatographed on a VLC column (220 g silica gel H, 7 × 20 cm) using chloroform, chloroform-ethyl acetate mixtures, ethyl acetate and ethyl acetate-methanol mixtures. Fractions 200 ml each were collected and monitored by TLC to yield five main fractions (A-E).

Fraction A (2 g), eluted with 50% ethyl acetate in chloroform, was purified over silica gel columns using n-hexane-ethyl acetate mixtures to yield compound S_1 (R_f 0.6 in I, 100 mg, white microcrystalline powder, m.p. 245-247°C). Fraction B (3.5 g), eluted with 80-95% ethyl acetate in chloroform, was purified over several silica gel columns using chloroform-methanol mixture as eluent to obtain compound S_2 (R_f 0.3 in I, 73 mg, white microcrystalline powder, m.p. 290-292°C). Fraction C (1 g), eluted with 100% ethyl acetate, was evaporated under reduced pressure and washed with acetone for several times to yield white powder of compound S_3 (R_f 0.28 in I, 51 mg, white microcrystalline powder, m.p. 285-287°C). Fraction D (3 g), eluted with 5-15 % methanol in ethyl acetate, and E (2.5 g), 20-25% methanol in ethyl acetate, upon repeated purification over sephadex LH-20 columns, using methanol and methanol-water mixtures as eluent afforded compounds S_4 (R_f 0.5 in IV, 140 mg, white microcrystalline powder, m.p. 298-300°C) and compound S_5 (R_f 0.3 in IV, 65 mg, white microcrystalline powder, m.p. 269-271°C) from fractions D and E, respectively.

Acid hydrolysis of glycosides

Hydrolysis of compound S_3 was performed according to the method described by Thornton et al. [21].

Assessment of *in vitro* cytotoxic activity

The cytotoxicity of the MEP as well as that of the five isolated compounds was measured using the sulphorhodamine B assay (SRB) [22] against five human cancer cell lines: colon cancer cell line (HCT116), larynx cancer cell line (HEP2), breast cancer cell line (MCF7), cervix cancer cell line (HELA) and liver cancer cell line (HEPG2). The assessment was performed in the National Cancer Institute in Egypt (NCI). The IC_{50} (dose which reduces the survival to 50%) and IC_{10} (dose which reduces survival to 10%) values for each tested sample were calculated and recorded in (Table 3) as compared to the reference drug Doxorubicin®, which was used as a positive control in the present study. The results are calculated as a mean of three experiments ± the standard error.

Assessment of *in vivo* hepatocellular carcinoma (HCC)

Animals and grouping: Forty female rats inbred strain of white albino rat of matched weight (120-150 g) were included in the study. Rats were maintained according to the standard guidelines of Institutional Animal Care. Animals were fed a semi-purified diet that contained (g/kg): 200 casein, 555 sucrose, 100 cellulose, 100 fat blends, 35 vitamin mix, and 35 mineral mix. The animals were divided equally into the following groups: group 1: control rats group, group 2: HCC (hepatocellular carcinoma) group induced by diethyl-nitroseamine (DENA) and CCl_4, group 3: (HCC-1) group received MEP (100 mg/kg b.wt) and group 4: (HCC-2) group received MEP (200 mg/ kg b.wt).

Preparation of HCC model: Hepatocarcinogenesis was induced chemically in rats by injection of a single intraperitoneal dose of diethylnitrosamine at a dose of 200 mg/kgb.wt. followed by weekly subcutaneous injections of CCl_4 at a dose of 3 ml/kg.b.wt. for 6 weeks [23]. At the proper time of scarification, blood samples were assessed for α- fetoprotein (AFP) by ELISA (provided by Diagnostic Systems Laboratories, Inc., Webstar, Texas, USA.) according to manufacturer's instruction, Alanine Amino Transferase (ALT), Aspartate Amino Trasferase (AST) and albumin by routine biochemical laboratory test. In addition, liver tissues were collected into Phosphate Buffer Serum (PBS) and fixed overnight in 40 g/l paraformaldehyde in PBS at 4°C. Serial 5- µm sections of the livers were stained with Hematoxylin and Eosin (HE) and were examined histopathologically.

Statistical analysis: All data were expressed as the mean ± S.E.M (n=10). Means were compared by One-Way Analysis of Variance (ANOVA) followed by Tukey-Kramer test. The values were considered to be significantly different when P values were less than 0.01.

Results

Five compounds were isolated from the MEP. The ^{1}H NMR and ^{13}C NMR spectra of the isolated compounds and their assignments are shown in Tables 1 and 2. The compounds were identified as solasodine (S_1), β-sitosterol-3-*O*- β-D-glucoside (S_2), poriferasterol-3-*O*- β-D-glucoside (S_3), solamargine (S_4) and solasonine (S_5). The structures of the isolated compounds are shown in Figure 1. The identification was confirmed by their COSY and HMBC data.

The *in vitro* anticancer potentiality of the MEP was evaluated against five human cancer cell lines (Table 3). The results of the present study indicated that the MEP had shown a remarkable activity against the five tested cell lines; colon cancer cell line (HCT116), larynx cancer cell line (HEP2), breast cancer cell line (MCF7), cervix cancer cell line (HELA) and liver cancer cell line (HEPG2). Consequently, the isolated compounds were tested against the same cell lines. Solasodine (S_1) and solamargine (S_4) revealed the most potent activity against the five tested

Proton No.	S_1	S_2	S_3	S_4	S_5
1	1.72 0.98 m	-	-	1.70 1.00 m	1.73 1.01 m
2	2.10 1.85	-	-	2.07 1.85	2.12 1.87
3	3.87 m	3.03 m	3.42 m	3.98 m	3.93 m
4	2.74 m			2.77 m	2.71 m
6	5.37 br.s	5.33 br.s	5.32 br.s	5.32 br.s	5.38 br.s
7	1.35 1.35	-	-	1.50 1.50	1.90 1.51
8	1.50	-	-	1.51	1.54
9	0.89	-	-	0.90	0.90
11	1.45 1.45	-	-	1.45 1.45	1.45 1.45
12	1.73 1.13	-	-	1.73 1.13	1.70 1.10
14	1.08 2.09			1.07 2.07	1.10 2.10
16	4.50	-	-	4.38	4.51 m
17	1.87	-	-	1.85	1.83
18	0.88	0.66	0.69	0.88	0.88
19	1.04	0.96	0.95	1.04	1.06
20	1.91 m 1.09 d (*J*=7)			1.98 m 1.09 d (*J*=7)	2.01 m 1.09 d (*J*=7
21	-	0.92 d (*J*=6.3)	0.98d (*J*=5.7)	-	-
22	-	-	5.16 dd (*J*=8.6,15.1)	-	-
23	1.73 1.73		5.02 dd (*J*=8.6,15.1)	1.73 1.73	1.70 1.70
24	1.65 1.65	-	-	1.63 1.63	1.65 1.65
25	1.48 2.77	-	-	1.50 2.77	1.48 2.83
26	2.82	0.81 d (*J*=6.3)	0.81 d (*J*=5.7)	2.77	2.83
27	0.81 d (*J*=6)	0.90 d (*J*=6.3)	0.78 d (*J*=5.7)	0.82 d (*J*=6)	0.81 d (*J*=6)
29	-	0.78 t (*J*=6.3)	0.89 t (*J*=7.3)	-	-
1`	-	4.21 d (*J*=7.5)	4.21 d (*J*=7.8)	4.95 d (*J*=8)	4.93 d (*J*=8)
1``	-	-	-	6.39 br.s	6.23 br.s
1```	-	-	-	5.85 br.s	5.15 d (*J*=8)

Table 1: ^{1}H NMR chemical shifts (δ ppm) for compound S1 ($CDCl_3$, 300 MHz, *J* in HZ) and S_4-S_5 (DMSO, 300 MHz, *J* in HZ)

cell lines. All the tested samples showed their highest activity against liver cancer cell line (HEPG2). MEP (IC_{50} 2.14 ± 0.35) was tested *in vivo* against CCl_4 induced hepatocellular carcinoma at two dose levels (100 and 200 mg/kg body weight) and it showed a dose-dependent anticancer activity which was indicated by reduction in α-fitorotein (AFP) which is considered a liver tumor indicator (30.98 % at 100 mg/kg.b.wt and 45.77% at 200 mg/kg.b.wt), in addition it also restored the levels of AST (35.97 % at 100 and 48.78 % at 200 mg/kg.b.wt), ALT (31.22 % at 100 and 43.16 % at 200 mg/kg.b.wt) and albumin (26.42 at 100 and 47.64 % at 200 mg/kg.b.wt) in a dose dependent manner (Table 4). Histopathology of liver tissues treated with MEP (Figure 2) confirmed its anticancer activity.

Discussion

Cancer is the uncontrolled growth and spread of cells. It can affect almost any part of the body. The growth often invades surrounding tissue and can metastasize to distant sites. Many cancers can be prevented by avoiding exposure to common risk factors, such as tobacco smoke, diet and obesity. Currently chemotherapy is regarded as one of the most efficient cancer treatment approach. Faced with palliative care, many cancer patients use alternative medicines; including herbal therapies, as such therapy can improve symptoms and quality of life for cancer patients.

In Egypt and according to the cancer profile in the NCI, the most common types of cancer are the breast, cervix and liver cancer [1]. Lung cancer is the leading cause of cancer death in most countries [24]. Colon cancer is also a type of cancer which affects the younger

Carbon No.	S_1	S_2	S_3	S_4	S_5
1	37.4	37.3	37.2	37.5	37.0
2	32.0	31.6	31.4	30.2	29.6
3	71.0	71.8	70.1	78.1	77.6
4	42.5	42.3	41.8	39.0	38.3
5	141.5	140.8	140.4	140.8	140.4
6	120.7	121.7	121.1	121.8	121.3
7	32.0	31.9	31.9	32.4	31.8
8	31.5	31.6	31.9	31.7	31.2
9	50.4	50.2	49.6	50.4	49.8
10	36.7	36.5	38.7	37.2	36.6
11	20.9	21.1	21.1	21.2	20.6
12	39.9	39.8	39.8	40.1	39.6
13	40.4	42.3	41.8	40.6	40.1
14	56.5	56.8	56.1	56.7	56.1
15	31.6	24.3	23.8	32.6	32.0
16	78.7	28.3	28.7	78.8	78.5
17	63.2	56.1	55.4	63.6	62.9
18	15.9	11.9	11.8	16.5	16.0
19	18.9	19.4	19.1	19.4	18.9
20	41.4	36.2	40.3	41.6	41.2
21	14.7	18.8	20.9	15.7	15.2
22	97.9	34.0	137.9	98.3	98.0
23	34.2	26.1	128.7	34.7	34.0
24	30.4	45.9	50.5	31.0	30.4
25	31.0	29.2	31.3	31.6	30.8
26	47.5	19.1	20.6	48.1	47.3
27	18.9	19.8	18.9	19.8	19.2
28	-	23.1	25.5	-	-
29	-	12.0	12.1	-	-
1`	-	100.3	100.8	100.3	99.9
2`	-	77.8	73.4	77.8	75.6
3`	-	78.0	76.7	78.0	84.6
4`	-	76.9	73.3	78.7	69.3
5`	-	78.7	76.9	76.9	74.3
6`	-	61.3	61.1	61.3	61.9
1``	-	-	-	102.0	101.5
2``	-	-	-	72.5	71.7
3``	-	-	-	72.7	72.0
4``	-	-	-	73.9	73.4
5``	-	-	-	69.5	68.9
6``	-	-	-	18.6	18.0
1```	-	-	-	102.9	105.1
2```	-	-	-	72.8	74.2
3```	-	-	-	72.5	77.6
4```	-	-	-	74.1	70.9
5```	-	-	-	70.4	77.3
6```	-	-	-	18.5	61.5

Table 2: 13C NMR chemical shifts (δ in ppm) for compounds S1 (CDCl3, 75 MHz) and S4-S5 (DMSO, 75 MHz)

Tested compounds/ extract	IC50 (Mean ± S.E.)				
	HCT116	HEP2	MCF7	HELA	HEPG2
MEP	4.36 ± 0.32	4.99 ± 0.36	6.56 ± 0.32	4.90 ± 0.21	2.14 ± 0.35
S1	2.97 ± 0.38	3.43 ± 0.49	2.97 ± 0.29	3.43 ± 0.50	2.51 ± 0.21
S2	2.23 ± 0.35	12 ± 0.79	2.36 ± 0.41	13.4 ± 1.55	2.12 ± 0.90
S3	5.20 ± 0.70	11.2 ± 0.69	4.51 ± 0.21	10.01 ± 0.21	3.52 ± 0.21
S4	2.97 ± 0.57	3.13 ± 0.26	3.13 ± 0.11	2.97 ± 0.22	2.67 ± 0.44
S5	6.79 ± 1.41	6.94 ± 0.27	8.46 ± 0.45	9.07 ± 0.41	4.34 ± 0.31
Doxorubicin®	0.69 ± 0.21	1.10 ± 0.30	0.7 ± 0.12	0.91 ± 0.10	0.67 ± 0.21

MEP: Methanol Extract of S. melongena peels; HCT116: Colon cancer cell line; HEP2: Lung cancer cell line; MCF7: Breast cancer cell line; HELA: Cervix cancer cell line; HEPG2: Liver cancer cell line; SD: Standard error for n = 3

Table 3: In vitro anticancer effect of MEP and the isolated compounds

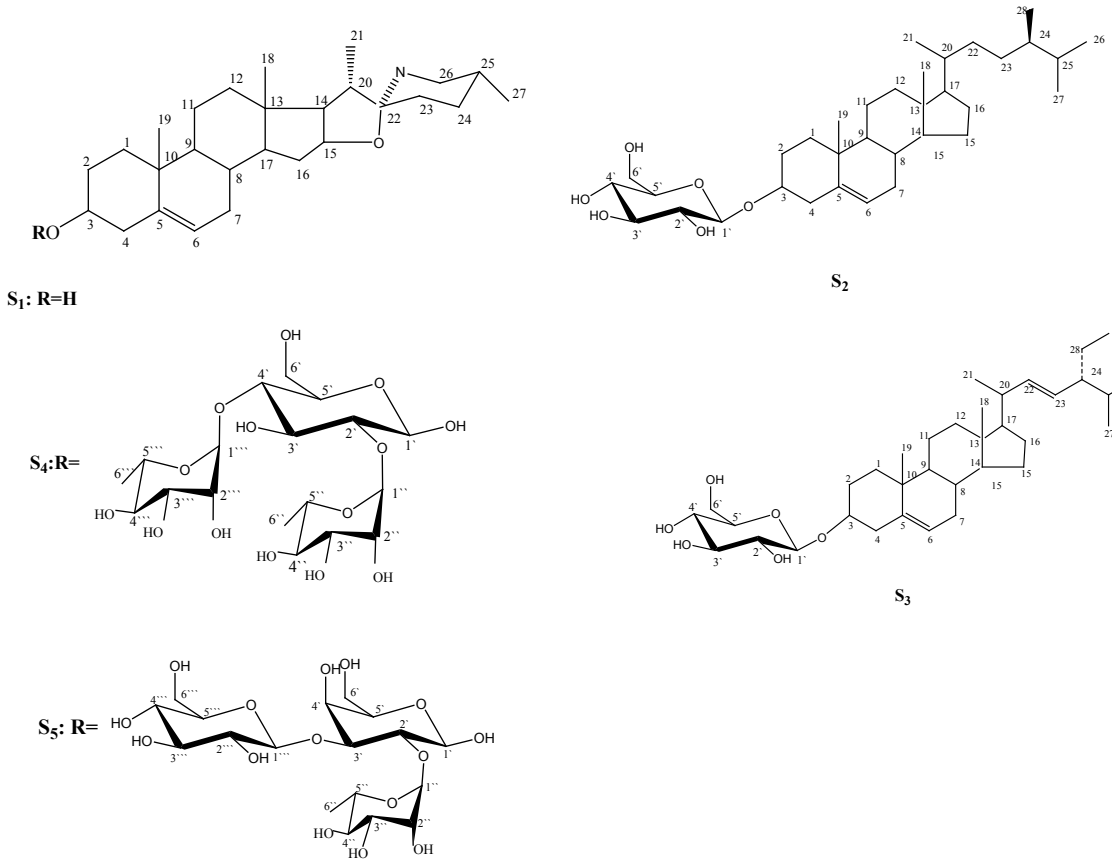

Figure 1: Structures of the isolated compounds

population with an incidence of 2 to 6%. An increasing number of young colorectal carcinoma patients attending at Mansoura University Hospital, Mansoura, Egypt, were noted in the last few years [25]. In the view of cancer prevalence in Egypt, and from the above reported data these five main types of cancer were the interest of our research.

S. melongena L. fruit is rich in steroidal alkaloids, which were reported to possess anti-cancer activity against skin cancer, liver cancer and leukemia [11-16]. Accordingly, in the present study our aim was to investigate the presence of such bioactive compounds in the fruit peels and to evaluate their anticancer activity against the most common types of cancer in Egypt.

Investigation of the methanol extract of the dried powdered fruit peels of *S. melongena* L. afforded five compounds. Compounds S_1, S_4 and S5 responded positively to Dragendorff's spray reagent indicating their alkaloidal nature. They also gave positive Lieberman's and Salkowiski's tests confirming the presence of a steroidal nucleus. Additionally, compounds S_4 and S_5 gave positive response to Molisch's test which supports their presence in a glycosidic form. Based on these positive chemical reactions, we could conclude that these compounds are glycoalkaloids (steroidal alkaloids). The chemical shift values of compound S_1 as well as the aglycones of compounds S_4 and S_5 (Tables 1and 2) are in good agreement with those published for solasodine [26]. On the other hand, it is obvious from the number of anomeric signals in the ^{1}H NMR and ^{13}C NMR spectra that compounds S_4 and S_5 have three attached sugars. The sugars of compound S_4 were identified from the chemical shifts and coupling constants of their anomeric protons in ^{1}H

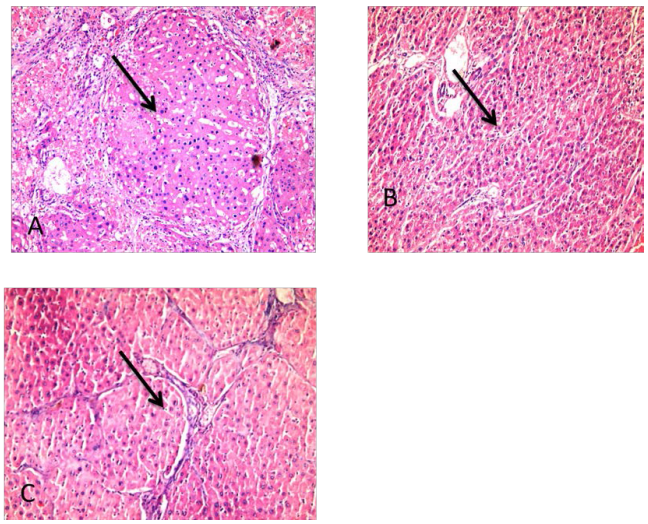

Figure 2: Histopathological picture of liver tissues
A. Characterized by large anaplastic carcinoma cells with eosinophilic cytoplasm, large hyperchromatic nuclei and prominent nucleoli. The normal trabecular structure of the liver is distorted. B and C. No nodularity & liver cells and lobules appear normal with ballooning degeneration. Normal portal tracts. No fibrosis. No inflammation.

	AST (U/l)	ALT (U/l)	Albumin (gm/ml)	AFP (pg/ml)
Control	17.33 ± 2.94	17.33 ± 3.56	3.61 ± 0.48	0.47 ± 0.03
Untreated group (HCC)	61.17 ± 5.27	47.5 ± 6.71	2.12 ± 0.53	1.42 ± 0.29
HCC + 100 mg/kg MEP	39.17 ± 4.35[a]	32.67 ± 4.88[a]	2.68 ± 0.31[a]	0.98 ± 0.08[a]
HCC + 200 mg/kg MEP	31.33 ± 5.39[a]	27 ± 3.58[a]	3.13 ± 0.23[a]	0.77 ± 0.12[a]

MEP: Methanol Extract of S. melongena peels; HCC: Hepatocellular Carcinoma; AST: Alanine Amino Transferase; ALT: Aspartate Amino Transferase; AFP: α-fetoprotein, results are expressed as mean ± standard error
a Statistically significant from HCC group at p< 0.01

Table 4: The in vivo anticancer activity of MEP against hepatocellular carcinoma

NMR and from the chemical shifts of their carbons in ^{13}C NMR as β-D-glucose and two terminal molecules of α-L-rhamnose. The sugars of compound S_5 were identified as β-D-galactose, α-L-rhamnose and β-D-glucose. Therefore, compounds S_4 and S_5 were identified as solamargine"3 β-{O-α-L-rhamnopyranosyl-(1→2)-[O-α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosyloxy}-22α-N-spirosol-5-ene" (S_4) and solasonine "3 β-{O-α-L-rhamnopyranosyl-(1→2)-[O-β-D-glucopyranosyl-(1→3)]-β-D-galacto-pyranosyloxy}-22α-N-spirosol-5-ene" (S_5) by comparing their spectra with the published data [27-29]. Compounds (S_2) and (S_3) gave positive Lieberman's, Salkowiski's and Molisch's test. The data of compound S_2 was in good agreement of the published data of stigmast-5-en-3 β-O- β-D-glucoside (β-sitosterol-3-O- β-D-glucoside) [30]. Also, data of the aglycone part of compound S3 were in accordance with those published for (24E)-poriferasta-5, 22-dien-3 β-ol (poriferasterol) aglycone [31-33], in addition to an attached β-glucose moiety. Thus, compound S_3 was identified as (24E)-poriferasta-5, 22-dien-3 β-O-β-D-glucoside (poriferasterol-3-O-β-D-glucoside). The nature of the aglycone was further confirmed by measuring its melting point after acid hydrolysis which was found to be 156°C (c.f. its 24 α isomer; stigmaserol which has m.p. of 170°C) and this is considered as the main difference between the two isomers) [31].

According to the criteria set by the US NCI, 20 µg/ ml is regarded as the upper IC_{50} limit considered promising for purification and bio-guided study of a crude extract and 4 µg /ml for the pure compounds [34]. In that respect, the MEP could be considered potent cytotoxic agent against the five tested cell lines. Solasodine and solamargine were the most potent among the tested compounds as represented by their low IC_{50} values (Table 3). Mechanisms previously proposed for the cytotoxicity of these compounds is through interfering with the cell membrane by the special branched connection of the connected sugars and disrupting the integrity of the cells by changing of cell morphology and DNA content leading to cell apoptosis [11,12]. Furthermore, the promising activity of solamargine may be attributed to the fact that solamargine is the only compound having the glucose moiety directly attached to the 3-β-OH and also it has two attached rhamnose moiety which is thought to bind itself and taken up into the cancer cell, where it ruptures the lysosomes that when ruptured, can eat up any cell from within [16]. Other reports mentioned that solamargine induces apoptosis, measured as generation of cell fragments with low DNA content and the 2' rhamnose moiety of solamargine was suggested to be essential for apoptosis induction [16]. Solasonine which has an attached galactose moiety, one rhamnose and a terminal glucose revealed a lower activity represented by higher IC50 values. Moreover, β-sitosterol-3-O-β-D-glucoside and poriferasterol-3-O- β-D-glucoside showed also promising cytotoxic activities especially against the tested breast (MCF7) and colon (HCT116) cancer cell lines. MEP and the isolated compounds showed significant activity against the hepatocellular carcinoma. Accordingly, MEP was tested *in vivo* against hepatocellular carcinoma with the aim of confirming its anticancer activity. MEP showed a dose-dependent anticancer activity through stabilization of the hepato-cells verified by reducting α-fitorotein (AFP) (a liver tumor indicator), in addition to restoring the levels of AST, ALT and albumin in a dose dependent manner. Histopathology of liver tissues treated with MEP (Figure 2) showed also normal liver cells, normal portal tracts and absence of fibrosis and inflammation.

Hepatocellular carcinoma (HCC) is one of the most common malignancies responsible for an estimated one million death annually especially in developing countries. Among the major contributing factors to the development of HCC are chronic infections with hepatitis B (HBV) or hepatitis C (HCV) virus, and food contaminated with aflatoxins. The high burden of HCV-associated liver cancer in Egypt was largely because of the extensive spread of the virus through contaminated injection equipment during mass treatment campaigns against Schistosoma hematobium [35,36]. In view of the fact that; HCC and its treatment imposes a large burden on the developing countries' economy, it was our deem interest to test the *in vivo* anticancer activity of the peel extract on HCC in an attempt to find an alternative cheaper and safe remedy.

Our findings showed that MEP, which is a source of steroidal alkaloids and sterol glycosides exhibited potential anticancer activity against hepatocellular carcinoma both *in vitro* and *in vivo* and this supports the reuse of waste products as it could be a source of a novel antitumour agent new remedy for treating major diseases.

Conclusion

Although peels are considered to be waste and are believed to adversely affect the cleanliness of the environment, so their utilization in pharma or nutraceuticals will certainly offer the potential for cost effective new generation therapeutics and also enhance the value

of fruits and vegetables. Here we report that the peels of *Solanum melongena* L. fruits have a promicing anticancer activity against hepatocellular carcinoma and also supplied steroidal alkaloids and sterol glycosides with anticancer activity. This findings support the use of such waste products as a new pathway for treating major diseases.

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