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Glycoalkaloids as medicinal agents from callus and regenerated plants of *Solanum nigrum* var. *judiacium*

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Abstract

The main target of this study is production of glycoalkaloids from *in vitro* cultures of *Solanum nigrum* var. *judiacium* Besser. Further, to evaluate their therapeutic effects. *S. nigrum* var. *judiacium* leaves were implanted in solid MS media containing growth regulators for *in vitro* study. HPLC analyses were applied for qualitative and quantitative determination of glycoalkaloids. Cytotoxic effects against human carcinoma cell lines were evaluated. In addition, antiviral, antioxidant, anti-inflammatory and antiparasitic activities of glycoalkaloids were estimated. HPLC data indicated the success of *in vitro* solasodine and solanidine glycosides production. Solasonine represented the highest concentration. Biological assays showed that obtained glycoalkaloids exhibited cytotoxic activity against human carcinoma cell lines that may be attributed to free radical scavenging activity (69.98%). Strong antiherps performance was recorded (94%). In addition, the glycoalkaloids showed *in vitro* schistomicidal (IC_{50} 76.4 ppm) and fasciolicidal (IC_{50} 76.6 ppm) activities. In vivo anti-inflammatory assay revealed potent activity against carrageenan induced edema. Glycoalkaloids were formed 2-5 folds that of intact plant pointed to the efficiency of the cultures. The present findings referred to the pronounced biological performance of the *in vitro* produced glycoalkaloids including antiviral, cytotoxic anti-inflammatory and antiparasitic activities. Botanical derived medication from *S. nigrum* var. *judaicum* could be accomplished guided with the present data.

Key words: Solanum nigrum var. judiacium, tissue culture, biological activity

Introduction

Medicinal plants have been taking place in modern remedy as raw materials for some important medicines (Gheewalaa et al., 2013). Solanaceae, an economically and medicinally important family is characteristic of having alkaloid accumulation (Kumar et al., 2009). *Solanum nigrum* var. *judiacium* Besser belongs to genus Solanum (Night shades), family solanaceae and accumulates steroidal alkaloids called glycoalkaloids.

These glycoalkaloids constitute a paramount class of phytochemicals having distinct medicinal applications including antiviral, anticancer and antiparasitic therapeutic agents. Solanine, an important solanidine glycoalkaloid found in some Solanum species, is reported to have antimicrobial, antidiabetic, antiallergic and anticarcenogenic effects based on apoptotic efficacy (Kumar et al., 2009; Williams, 2013). In previous work, it was reported that *S. tuberosum* glycoalkaloids; a-Chaconine, a-Solanine and Solanidine exhibited noticeable cytotoxic and antiviral activities. The author demonstrated likewise, their antiparasitic performance as antischistomiasis and antifasciolosis (Al-Ashaal, 2010). Solasonine and solamargine glycoalkaloids were reported to have anticarcenogenic effect against different carcinoma cell lines including glioblastoma multiform, liver, basal cell and

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squamous, bladder, and colon rectal cell lines (Tek, 2006). In addition, solamargine and crude *Solanum palinacanthum* ethanolic extract were adduced to have trypanocidal effectiveness against the causative parasite of Chagas' disease (Moreira et al., 2013). Solasonine and solamargine glycoalkaloids as well, are economically important because they are structurally similar to steroidal hormones and have been utilized as a valuable source for medicines, such as contraceptives and steroidal anti-inflammatory medicaments (Tiossi et al., 2012).

Regrettably, there is limited use of herbal drugs because of several reasons such as poor availability and traditional cultivation technology. Moreover, plant secondary metabolites concentration in a given plant often varies during 24-hour period (Tek, 2006). Several factors during growth, harvesting and post-harvest treatment, and maturity can affect glycoalkaloid accumulation (Tek, 2006). Genotype has a major effect on glycoalkaloid levels. It was proposed that any environmental factor which causes a stress in a plant of the *Solanum* species can alter glycoalkaloid content (TeK, 2006). For the medicinal and pharmaceutical importance of glycoalkaloids, and their poor availability in many cases, attempts for *in vitro* production have been documented**.** The production of alkaloids in plant tissue culture by some solanaceous species including solanum have been reported in different studies **(**Lindesy& Yeoman, 1983; Misawa, 1994; El-Ashaalal., 1999; Moreeira et al., 2010; Al-Ashaal et al., 2013).

Due to their significance medical value, the present study focuses on *S. nigrum* var. *judiacium* as a source of glycoalkaloids. Modulating culture conditions to produce considerable concentrations of glycoalkaloids from *in vitro* cultures in good supply for pharmaceutical industry is basic objective of the present study. The other interesting target is to estimate the bioactivity of the *in vitro* produced glycoalkaloids with the intent of producing effective and available therapy from *in vitro* cultures. Consequently, establish the *in vitro* guidelines for handling the medicinal important glycoalkaloids from *S. nigrum* var. *judiacium* for pharmaceutical applications.

Material and Methods

*Experimental tools***.** *Solanum nigrum var. judiacium leaves were obtained from the farm of medicinal plants, Faculty of Pharmacy, Cairo University, Egypt. A voucher specimen of the regenerated plants was identified by Prof. Dr. Mounir Abd El-Ghany and deposited at Cairo University Herbarium (CAI) with registration number CAI 343216.*

Standard solasonine and solamargine, Sigma-Aldrich Co., St Louis, were kindly provided by Dr. Ashgan Zaki Prof. of Pharmacognosy Faculty of Pharmacy Cairo University. Solasodine (≥ 99%), Sigma Co., a-solanine, (≥ N97%) Roth Co.; Cisplatin injectable grade (98–102%) from Merck Co.; Doxorubicin injectable grade (98–102%) from Pharmacia Co.; Taxol injectable grade (98–102%) from Mayne Pharm. Co.; DPPH• (2, 2-Diphenyl-1-picrylhydrazyl — 97%) from Sigma Co.; Vitamin C (N99.5%) from Fluka Co.; Acyclovir (N95%) from Sigma Co.; Braziquantel (97.5–102%) from Alexandria Co. for pharmaceuticals.; Carrageenan (≥ 98%) Sigma Co.; Indomethacin, El- Kahera Pharmaceutical Industry Co., Egypt. Media components and phytohormones for regeneration and in vitro glycoalkaloids production were tissue culture grade. Solvents for analysis were HPLC grade.

HPLC; Hewlett Packard (Wilmington, North Carolina, *United State)*, series 1050, UV detector, wave length 210 nm, column C18 5 µm, 0.4×25 cm, FR. 1mL/ min. Pressure 6 bar, temp.40°C.

ESR; Bruker, Elexys, (Silberstreifen*, Rheinstetten, Germany),*

X-band modulation frequency 500 MHz. The sample inserted *via* quartz liquid flat cell, average scans 1, average sampling time (s) 0.04096, state of aggregation C, field Mod. Amplitude 0.0002, field Mod. Frequency (Hz) 100,000 microwave frequency (Hz) 9.77568e+o9, microwave power (w) 0.00202637, receiver gain 65, receiver harmonic.

Light microscope; Olympus, Saitama, Japan, Eye piece: 25X, Oil objective: 100X.

Plethysmometer 7150, (UGO, Basil, Italy).

In vitro cultures and qualitative & quantitative analyses of glycoalkaloids. Solanum nigrum var.

judiacium leaves were sterilized with Clorox (10% v/v sodium hypochlorite solution) for 20 min, immersed in 70% ethyl alcohol for 15 seconds, and then rinsed with sterile distilled water twice, in laminar airflow cabinet. The leaves were sliced and aseptically cultured in glass jars containing MS media (Murashige & Skoog, 1962) supplemented with growth regulators including auxins and cytokinins as BA, IAA, NAA and 2, 4-D at different ratios. The cultures maintained at 26 °C and 16/8 light photoperiod. Subcultures of callus cultures on fresh media were performed every 4–6 weeks. Regenerated shoots were subcultured on MS media supplemented with hormones for two subcultures then resubculture in basal media to allow rooting. Acclimatization of regenerated plants was carried according to El-Ashaal et al. (1999).

HPTLC silica plates were used for qualitative identification of *in vitro* glycoalkaloids. Fifty mg of dried calli were extracted with 96% methanol twice (2 x 100 mL). The extract was concentrated under vacuum and the residue was co-chromatographed against standards. Eluting system for the glycoalkaloids was chloroform- methanol-1% ammonia 2:2:1 v/v (the organic layer). While that for aglycone was benzene- methanol 4:1 v/v.

HPLC quantitative analysis was carried using isocratic mode. Plant materials from callus, regenerated shoots, fruits of the acclimatized *in vitro* plants and mother field *Solanum* leaves were dried at a temperature 45°C. Ten mg of the dried materials was crushed, extracted with 25 mL methanol (96%) in water bath at 50°C for 3 h, and subsequent homogenized in methanol using ultra-turrax three times each for 5 min. (3 x 15 mL), the combined extracts were collected and concentrated under reduced pressure and temperature. The residue of the test materials in addition to standard glycoalkaloids were dissolved in methanol (1:1 w/v) and filtered through 0.45 µ Millipore filters then analyzed by HPLC in triplicates (0. 25–1 µL, injection volume depending on the concentration of each sample) using 40% methanol as mobile phase, wave length, 210 nm, flow rate 1 mL/min. Temperature 40 °C. Authentics standard curves were plotted. The concentrations of the glycoalkaloids were determined established on the calculation of percentage peak areas of the samples comparing with that of standards. The regression process was carried out and the following functions for different standards were drawn out:

For solasonine: $Y = 45.55X – 44.25$ $r = 0.972$
For a –solanine: $Y = 5.281X + 2.1485$ $r = 0.994$
For solamargine: $Y = 102.4X – 41.83$ $r = 0.967$
For solasodine: $Y = 129.5X – 230$ $r = 0.999$

Concerning isolation of *in vitro* produced glycoalkaloids; calli were dried at a temperature 45 °C, mashed in coarse powder and then, extracted with 5% acetic acid twice. The combined extracts were filtered and the filtrate was concentrated under vacuum pressure. The concentrate was treated with ammonium hydroxide till pH 10 then pure glycoalkaloids were precipitated upon cooling.

Medicinal investigation

Cytotoxic potential against human carcinoma cell lines. Cytotoxic activity of *in vitro* produced glycoalkaloids was performed against different strains of breast (MCF7-HTB-22), lung (H460), liver (Hepg2-ATCCHB-8065), and brain (U251-NIBIO1F050288) human carcinoma cell lines at the National Cancer Institute, Egypt, according to the reported method of Skehan et al. (1990). While, the tested material was examined for cytotoxicity against lymphoblastic leukemia (1301) at the National Research Center, Egypt, following the described technique (Hansen et al., 1989). IC_{50} values (µg/mL) were determined comparing with standard cytotoxic drugs. Negative control groups were also performed. Cytotoxicity against the selected cell lines was examined in triplicates.

Antioxidant capability. *Free radical scavenging capacity of the isolated glycoalkaloids was measured by spectrophotometric method using electron spin resonance technique (ESR) (Al-Ashaal, 2010) in comparison with a –solanine and vitamin C as positive control against free radical diphenyl picryl hydrazyl (DPPH•). 1 mL of 10−3 MDPPH• was added to 1mg of standard a –solanine, vitamin C or 1mg of the isolated glycoalkaloids. Measurements were taken after*

5 minutes. Antioxidant capacity was function of reduction of integrated areas of DPPH•.

% of antioxidant capacity = $A_0 – A_1 / A_0 × 100$

A_0 = Area of DPPH•

A_1 = Area of the tested sample+↰ DPPH•

Antiviral examination. Preliminary antiviral investigation was performed against Herpes simplex virus type 1 (HSV-1) as a model of DNA virus. The virus was isolated and proliferated in the virology Laboratory of the Department of Water Pollution, National Research Center (Egypt). African green monkey cells (Vero) were used as virus host. The non toxic concentration range of the tested sample (in 10% dimethylsulphoxide; DMSO) to Vero cells was determined and the virucidal efficacy was measured (Papageorgiou et al., 2000). The percentage of virus plaques reduction was calculated and taken as measurement of the antiviral activity. Acyclovir was used as positive antiviral control drug and untreated virus and cells in 10% DMSO as negative control.

Antiparasitic prospect. The vermicidal activity of the isolated glycoalkaloids was tested against Schistosoma mansoni and Fasciola gigantica worms. Schistosoma worms were provided from Theodor Belharz Research Institute, Egypt. Fasciola worms were derived from the infected buffalo's livers at Cairo abattoir, Egypt. The experiments were performed in triplicates using RPMI media provided with antibiotics (Hassanain, 1998). Activity of the isolated glycoalkaloids was tested at concentrations (40–200) ppm. Braziquantel was used as positive control drug at concentration of 100 ppm. Negative control without glycoalkaloids was also performed. Observation of mortality was achieved using inverted microscope, after 24, 48, 72 and 96 h for S. mansoni and after 24 h for F. gigantica as the worms are susceptible, and may not remain survival after 24 h. Analysis was performed using probit program and IC_{50} determined at 95% confidence limit (Finney, 1971).

Anti-inflammatory potency. In vivo anti-inflammatory activity was performed using carrageenan induced oedema method (Winter 1962). Male albino rats weighing (70- 80 g) were obtained from the National Research Center animal laboratory (Giza, Egypt). The rats maintained on water and stock commercial pellet diet ad libitum. Handling, procedures were carried out according to the approved institutional and international ethical guidelines for laboratory animals' use and care. Animals were divided into 4 groups each of 5 rats. First control negative group received 0.1 mL saline. Second group each rat received in the left hind paw indomethacin (20 mg/kg i.p) 1h before carrageenan administration. Third and fourth groups each rat received glycoalkaloids in the left hind paw at dose equivalent to 1/4 or 1/2 LD_{50} (Tek, 2006) (8 or 16 mg/kg i.p) 1h before carrageenan administration. All animals except 1st group were given a sub-plantar injection of 0.1 ml of 1 % carrageenan solution in saline in the right hind paw. The paw volume of each rat was measured before carrageenan injection and then hourly at intervals up to four times with Plethysmometer. The oedema rate and inhibition rate of each group were calculated.

Oedema inhibition percentage was calculated according to the following equation:

Inhibition % = Inhibition rate – Rate of edema/ Rate of oedema x 100.

Statistical analysis was carried using one way ANOVA Post Hoc test.

Dependent variable: VAR 00002, LSD at 95% confidence interval.

Results and Discussion

In vitro cultures and glycoalkaloids estimation. Murashige & Skoog (MS) media supplemented with BA and 2, 4-D in a ratio of 1: 4 was the best medium for callus initiation while the best medium for shoot induction was that contained BA and NAA in a ratio of 1:1 with flowers in some cultures (Figures. 1-3).

Rooting was achieved successfully in MS basal media. Fruits were well developed in the acclimatized plants. Media contained 2, 4 –D did not show any differentiation. This is in close agreement with formerly reports elucidated that BA and NAA induced shoots in *S.nigrum* var. *judaicum* cultures but at ratio (0.5: 1) (El-Ashaal, 1999). AS well as, it was reported that roots were developed in MS media contained IAA and BA or NAA and BA in different rates in *S. torvum* cultures (Moreeira et al., 2010).

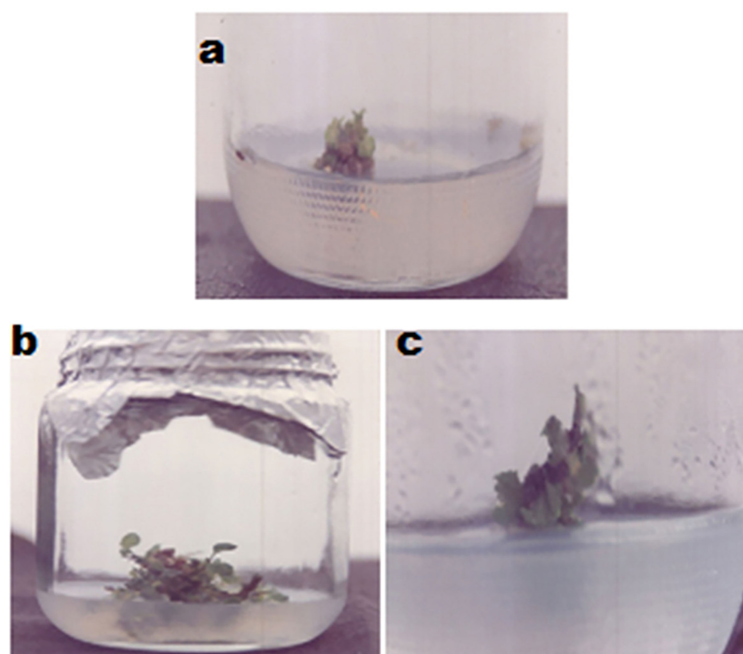

Figure 1. Primordial of regeneration cultures from leaf explants of *S. nigrum* var. *judaicum* after 1 week (a), development of regeneration after 2 weeks (b), regenerated calli after 4 weeks (c).

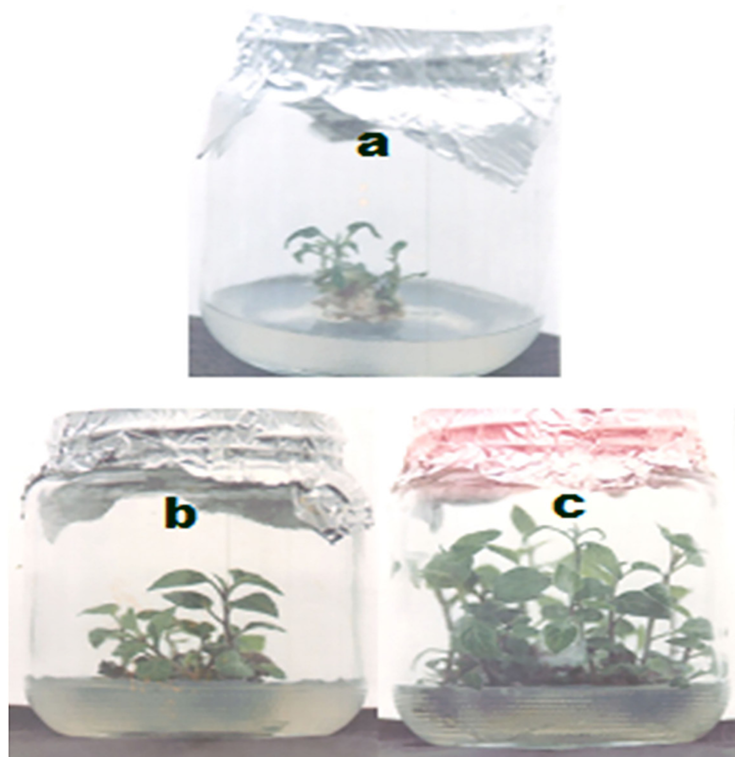

Figure 2. Shoots initiation after 4 weeks (a), 6 weeks (b) and 8 weeks (c) of *S. nigrum* var. *judiacium.*

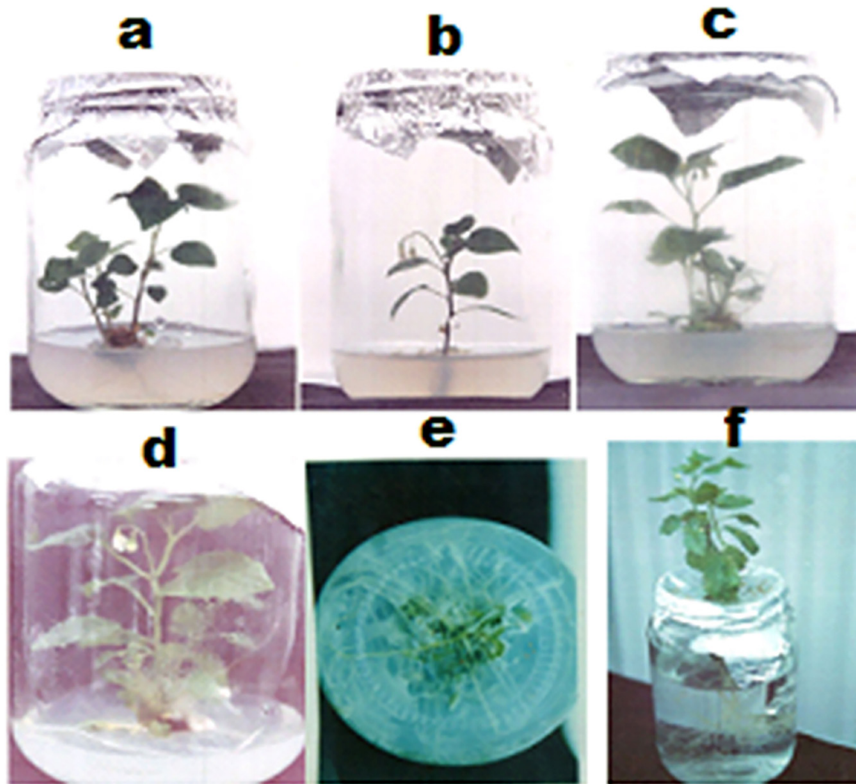

Figure 3. Differentiation from regenerated explants of *S. nigrum* var. *judiacium* after 2 months (a), flowering of subcultured differentiated explants after one subculture (b), two subcultures (c), three subcultures (d), rooting after 3 months (e) and adapted plant (f).

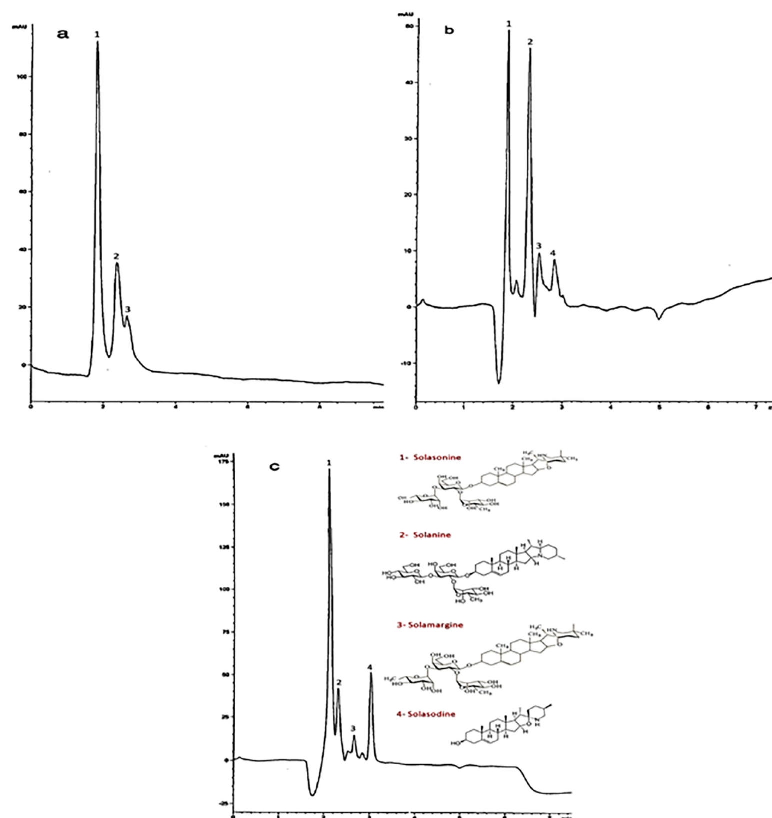

Figure 4. HPLC of *in vitro* glycoalkaloids of *S. nigrum* var. *judiacium*: a; Field plant derived glycoalkaloids, b; regenerated plant fruits derived glycoalkaloids, c; *In vitro* callus derived glycoalkaloids

HPTLC and HPLC analysis showed that calli, differentiated shoots and ripe fruits of acclimatized *in vitro* plants produced solasonine, solanine and solamargine glycoalkaloids in addition to solasodine (Figure 4).

Crystallization of callus methanolic extract showed prisms of solasonine, yellow needles of solamargine and slender transparent needles of solanine (Figure 5).

Complete adapted cycle was represented starting with tissue culture stages and end with extracting glycoalkaloid crystals (Figure 6). Data of HPLC analyses (Table 1) illustrated that solasonine had the highest concentration. In accordance with the present data, solasonine was found to constitute the major glycoalkaloids in *S. nigrum* fruits (Gheewalaa et al., 2013).

The present results likewise, indicated that glycoalkaloids were produced in cultures in much higher concentrations comparing with field plant derived leaves. The increment reached 3.03, 2.48 and 4.10 folds for callus cultures. While, the increments were 2.56, 2.88 and 4.74 folds for regenerated shoots. Finally, for fruits the increments were 2.51, 4.05 and 5.17 folds for solasonine, solanine and solamargine respectively. It is important to know that the concentrations of glycoalkaloids in Solanum plants varied dramatically and sometimes becomes nil (Bhatnagar et al., 2004). In spite that certain Solanum species contain high concentrations of glycoalkaloids (concentrations of some Solanum species were between 110 mg and 890 mg/100 g FW) (Väänäne, 2007) yet, the concentration of glycoalkaloids could be altered due to environmental factors and stress (Tek, 2006).

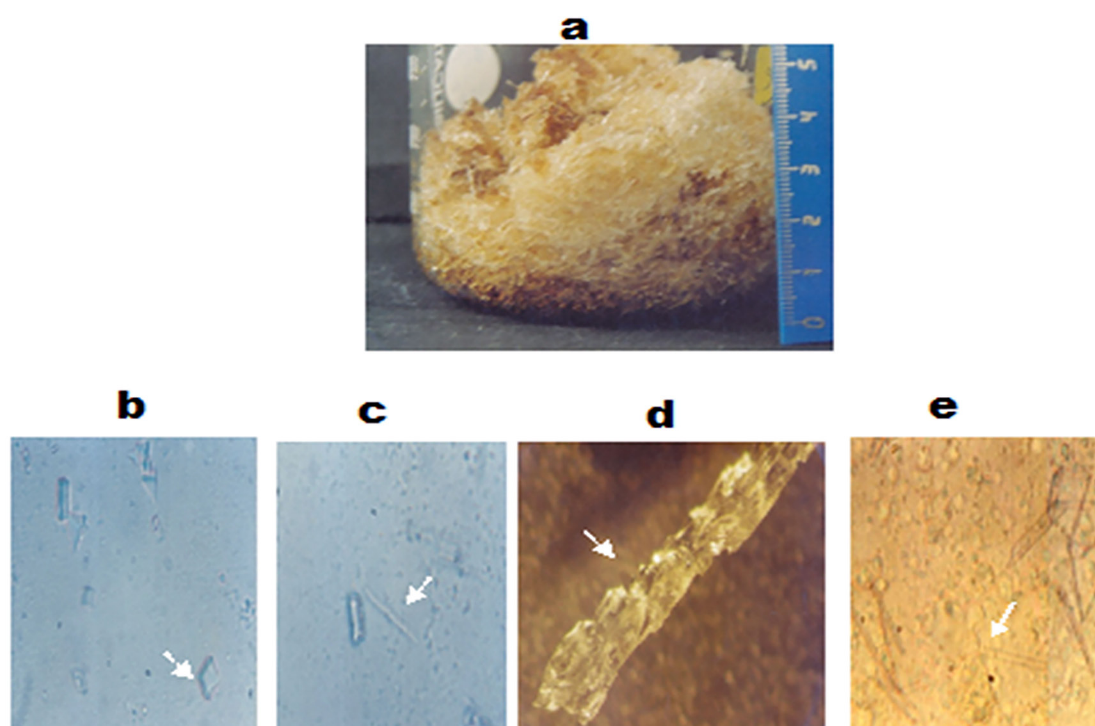

Figure 5. Crystals of *in vitro* glycoalkaloids of *S. nigrum* var. *judiacium:* a ; Collective crystals (Photo), b – Prisms of solasonine (25x100), c ;Transparent needles of solanine (25x100), d ;Long yellow needles of solamargine (25x100) e; Transparent slender needles of solanine

Table 1. *In vitro* glycoalkaloid content of *S.nigrum* var. *judiacium.* (% dry wt.).

R f	Rsh	Ca.	Ml	Glyc.
35.14	35.84	42.35	14.00	Solasonine
29.42	20.91	18.00	7.26	Solanine
8.90	8.15	7.06	1.72	Solamargine
11.47	8.75	9.45	-	solasodine

Glyc.: glycoalkaloid; Ml : mother derived leaves; Ca: callus; Rsh: regenerated shoots; Rf: regenerated plant fruits

Convenient with the present results, *In vitro* alkaloids production has been documented (Misawa, 1994; El-Ashaal et al., 1999; Moreeira et al., 2010). The current data showed that 2, 4-D enhanced glycoalkaloids production which is consistence with previous finding (Lindesy& Yeoman, 1983) illustrated that 2, 4-D enhanced alkaloid production in *Datura innoxia* cultures. Although, no shoots were developed in media contained 2, 4-D in the present study, other researchers reported regeneration of *S. nigrum* cultures on MS media containing 1.0 mg/L 2, 4-dichlorophenoxyacetic acid, under dark condition (Xu et al., 2014). The current work showed that auxins and cytokinins were essential for glycoalkaloids production. On contrast, it was found that *S. torvum* plants grown in MS media produced more solasodine than those grown in MS with auxins and cytokinin added (Moreeira et al., 2010). The high glycoalkaloids accumulation in the present work is compatible with the reported high yield of *in vitro* anthraquinones at concentration of 18% on dry weight bases in cultures of *Morinda citrifolia* comparing with 0.3% of mother plant that represents 60 folds (Misawa, 1994). Higher yield of glycoalkaloids from *S. tuberosum* cultures comparing with field tubers was also reported (Al-Ashaal, 2010). The author accused the high yield to good selection of the original strain, combination of growth regulators and adjustment of cultured conditions which may also be the case in the current study. The present work enables high glycoalkaloids yield from *S. nigrum* var. *judiacium* cultures. This achievement could be helpful for providing these important phytochemicals for pharmaceutical drugs in stabilized manner.

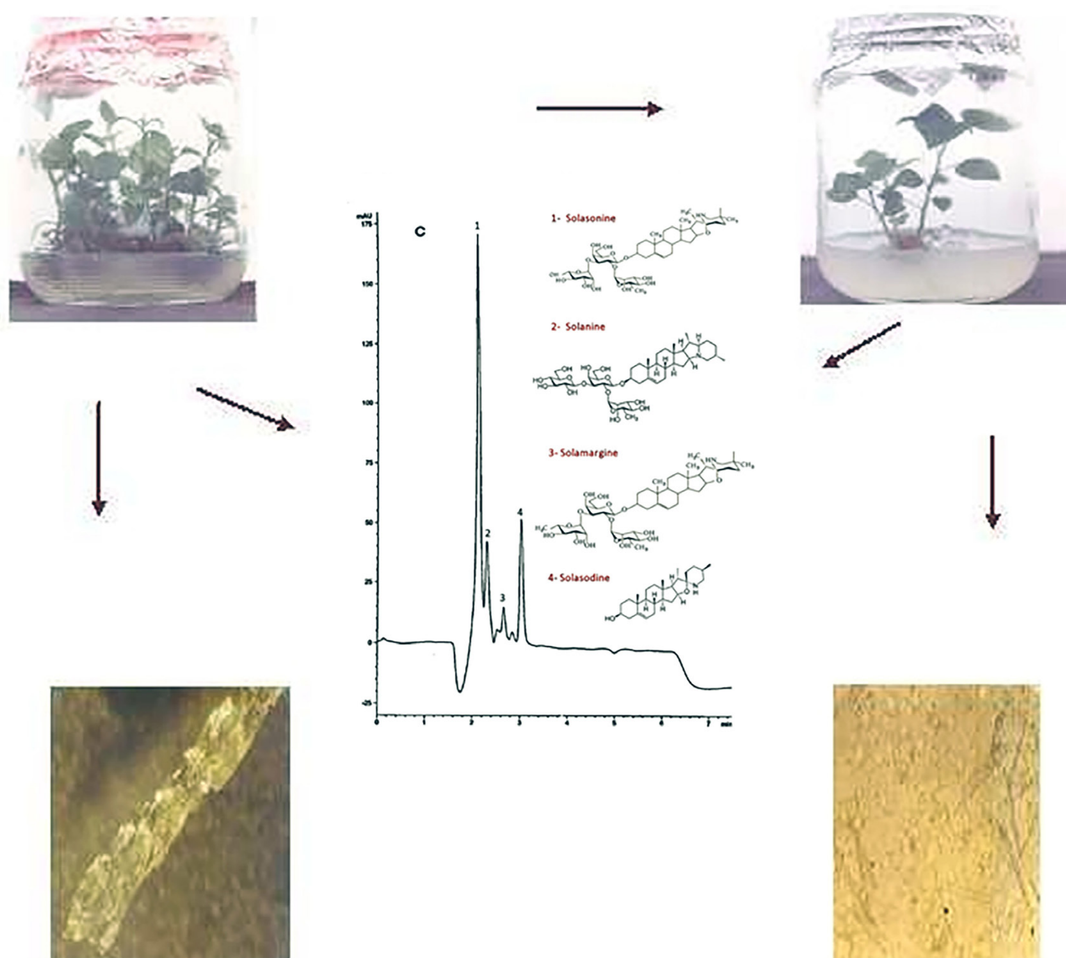

Figure 6. Illustration of whole *in vitro* process beginning with tissue culture cycle and end with glycoalkaloid crystals.

Therapeutic evaluation

Regarding cancer cell cytotoxicity, the results illustrated that *in vitro* isolated glycoalkaloids, exhibited significant cytotoxic activity against the examined strains from human carcinoma cell lines of lymphoplastic leukemia, lung, liver, and brain (Figure7).

Table 2. Cytotoxicity of *in vitro* glycoalkaloids from *S. nigrum* var. *judiacium.*

Cell line	IC_{50} (µg/mL)			
	Glyc.	Cis	Dox	Tax
Lung (H460)	3.96	4.77		
Brain (U251)	10.59	2.30		
Liver (Hepg2)	14.1	5.99		
Lymphoblastic leukemia	18.7			0.60
Breast (MCF7)	25.90		6.71	

Glyc: glycoalkaloids; Cis: cisplatin; Dox: Doxorubicin; Tax: Taxol.

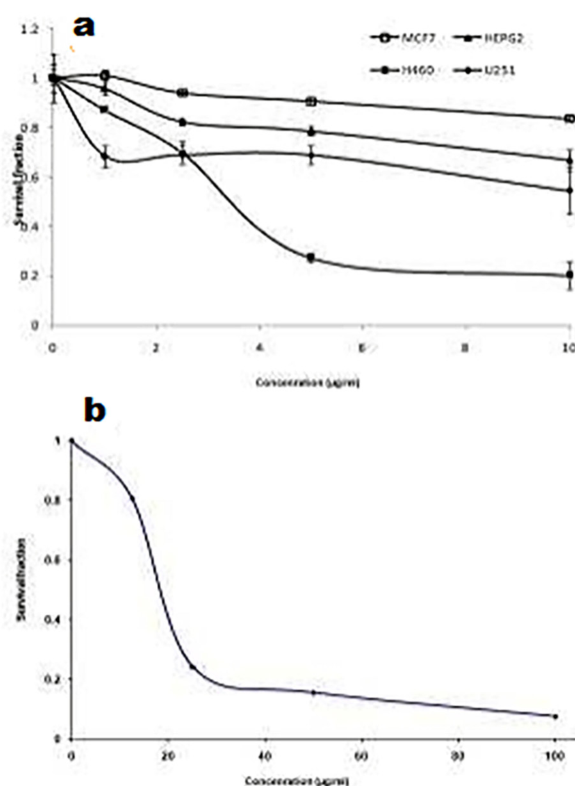

Figure 7. Cytotoxic activity of tissue culture derived glycoalkaloids of *S. nigrum* var. *judiacium* against: a– breast, liver, lung and brain carcinoma cell lines; b – lymphoblastic leukemia cell line

IC_{50} values comparing with standard cytotoxic drugs are presented (Table 2). The examined cell lines were inhibited in different degrees while control normal cells were unaffected. Lung carcinoma cells were the most susceptible cells to inhibition. Cytotoxic activity might be a virtue to the presence of solasonine, solamargine and solanine. This present outcome is compatible with studies reported that solasonine, β1-solasonine, solamargine and solanigroside P purified from *S. nigrum* have cytotoxicity to MGC-803 cells and may be potential candidates for the treatment of gastric ulcer (Ding et al., 2013). In the same line, callus methanolic extract of *S. tuberosum* including solanine exhibited pronounced cytotoxicity against different human carcinoma cell lines (Al Ashaal, 2010). Supporting the current data, a-solanine, had shown inhibitory effect on multiple cancer cells, such as clone, liver, melanoma cancer cells in addition to its beneficial effects on pancreatic cancer *in vitro* and *in* vivo (Lv et al., 2014). In addition, in vitro

cytotoxicity of chloroform fruit extract of *Solanum nigrum* L. against Non Hodgkin Lymphoma (SR) cells was performed and IC50 was 669.27µg/mL (Huda et al., 2015). Meanwhile, in the present study, purified *in vitro* glycoalkaloids have potent cytotoxic effect against lymphoma (1301) cell lines with IC_{50} 18.7µg/mL.

Concerning with inflammation, the current study showed that, isolated glycoalkaloids displayed outstanding anti-inflammatory activity (Table 3). The activity was 1.2 folds increase than standard indomethacin at lower glycoalkaloids dose (8 mg/kg) comparing with indomethacin (20 mg/kg). At the higher dose (16 mg/kg) the activity was 1.45 folds that of reference drug indomethacin. Relative to the present data, decoction of the *S.nigrum* plant depressed the CNS and reflexes of the spinal cord while, the whole plant was used as anti-inflammatory and aqueous plant extracts possessed antiproliferative activity as demonstrated by growth inhibition of cervical carcinoma (Ramalingum& Mahomoodally, 2014). As well, the anticancer activity of *S. muricatum* fruit extract was associated with immunomodulatory and anti-inflammatory activities (Shathish, & Guruvayoorappan, 2014).

Table 3. Anti-inflammatory activity of *in vitro* glycoalkaloids of *S. nigrum* var. *judiacium*.

	%of inhibition			
	1st h	2nd h	3rd h	4th h
Glycoalkaloids (8mg /kg)	36.231	93.43	90.04	94.74
Glycoalkaloids (16mg /kg)	58.23	86.85	93.54	113.85
Indomethacin (20mg /kg)	38.10	67.89	86.20	78.48

Thus the anti-inflammatory activity of the glycoalkaloids in the present study, support the detected cytotoxic activity. Furthermore, the present results could be useful for developing natural treatment for psoriasis whereas, psoriasis is a chronic, proliferative, and inflammatory skin disease with reactive abnormal epidermal differentiation and hyper proliferation that affects 2–3% of the global population (Garg et al., 2014).

With respect to antioxidant activity, the isolated glycoalkaloids extract manifested emphatic antioxidant capacity (69.98 %) comparing with standard a-solanine (44%) and standard vitamin C (100%). Thus combined glycoalkaloids exerted synergetic antioxidant activity comparing with individual a-solanine. The antioxidant capacity of the isolated glycoalkaloids might be an additional factor explaining their cytotoxic activity against the examined carcinoma cell lines. The key element in linking environmental toxicity to the multistage carcinogenic process is oxidative stress (Tarlovsky, 2013). Besides, *S.nigrum* herb and its phenolics content were reported to elaborate antioxidant properties (Kumar et al., 2012; Noumedem et al., 2013; Sharma et al., 2014). Based on the present results the glycoalkaloids could act as defensive and protective agent against oxidative hazards. Due to the scavenging power, glycoalkaloids may neutralize free radicals that cause cell damage and are responsible for oxidative stress and harmful diseases including cancer.

Related to virucidal activity, the safe non toxic concentrations of the glycoalkaloids towards vero cells ranged between 10–50 µg/mL. Table 4 illustrates that purified glycoalkaloids had noticeable inhibitory effect against HSV-1 virus (88.9. %) at concentration 20 µg/mL. Acyclovir at the same concentration exhibited no virucidal activity. At higher concentration (50 µg/mL) glycoalkaloids had 94.0% virucidal inhibitory effect comparing with 89.9% activity of acyclovir. This result is remarkable due to risky effects of HSV1which is the cause of cornea ulcer and brain damage (Lennerra, 1992). The current antiviral activity of established *in vitro* glycoalkaloids may be a result of their antioxidant property. In the same line, the antiviral activity of Morus alba extract against Herpes simplex virus type 1 and 2 was returned to its antioxidant activity (Dkhil, 2015). The present results are of special interest for developing new natural antiviral treatment from *S.nigrum* var. *judiacium*. *Herpes simplex* viruses 1 and 2 are the cause of dangerous diseases as oral herpes and genital lesions. Infection of HSV is the most common causes of meningitis and

encephalitis when it infects the central nervous system (Dkhil, 2015). HSV infection may be lethal in immunocompromised patients. Globally, 45% to 98% of the world population are infected or previously infected with *Herpes simplex* virus type 1(Dkhil, 2015). Furthermore relative high side effects and emergence of drug- resistant virus strains are major complications associated with treatment of Herpes infections.

Table 4. Virucidal effect of *in vitro* glycoalkaloids of *S. nigrum* var. *judiacium.* against HSV-1.

	HSV-1		
	Initial count (PFU/mL)	Treated count (PFU/mL)	% of virus reduction
Glyc. 20 µg	9.76×10^4	1.08×10^4	88.9 %
Acy. 20 µg	9.76×10^4	9.76×10^4	0%
Glyc. 50 µg	9.76×10^4	0.58×10^4	94.0%
Acy. 50 µg	9.76×10^4	1.08×10^4	88.9%
N.C	9.76×10^4	9.76×10^4	-

PFU/mL; plaque forming unit/mL. HSV-1 ; *Herpes simplex* virus type 1. Glyc..; Glycoalkaloids from of *S. nigrum* var. *judiacium* callus cultures. Acy. ; acyclovir. N.C.; negative control.

As Regard to hepatic vermfuge assay, the glycoalkaloids showed schistomicidal activity against *S. mansoni* with LC_{50} values 132.8, 83.9, 76.4 and 69.3ppm. While LC_{90} values were179.7, 120.1, 104.9 and 89.5 ppm, respectively after 48, 72, and 96 and 120 h. Concerning fasciolicidal effect of the glycoalkaloids, LC_{50} and LC_{90} were 76.60 and 114.41 ppm, respectively after 24 h. Braziquantel as positive control induced 100%mortality after 24 h at 100 ppm for both worms, while negative control groups remained survival all over the experiment period. Analysis was performed using probit program and LC_{50} determined at 95% confidence limit. The results of this part of study could be considered as a promising strategy to control the disease and are in close agreements studies which reported that dry powdered *S. nigrum* leaf exhibited potent effect in disturbing the intermediate host for *S. mansoni* (Biomphalaria snail) biochemistry which may render them physiologically unsuitable for the developing of schistosome parasite (Al-Daihan, 2008). Antifasciolosis effect of pure glycoalkaloids in the current research is in consistence with the reported potent molluscicidal activity of hydro methanol immature fruit extract of *Solanum nigrum* var. *villosum* against *Fasciola hepatica* intermediate host *Galba truncatula* (Hammami et al., 2011). The current results may be a guide for elaborating anti parasitic remedies where fasciolosis and schistomiasis are common diseases.

Conclusion

The present outcomes ultimately encourage glycoalkaloids production from *Solanum nigrum* var. *judiacium via* tissue culture way. The increments ranged from 2.51 to 5.17 folds that of the parent plant. This overflow production allows amalgamation of these potent metabolites from botanical origin in pharmaceutical industry. The derived glycoalkaloids possessed anti-inflammatory activity represents 1.2- 1.5 folds that of indomethacin. The most affected cell line is that of lung (IC_{50} is 0.83 that of cisplatin). The produced glycoalkaloids also performed antiparasitic effect against liver parasites (*S. mansoni* and *F. gigantica*). The confirmed positive biological results illustrate substantially possible future formulation of these high potency glycoalkaloids as prodrugs from botanical origin that serve in treatments of critical diseases and could help to overcome the problems of resistance to drugs currently in use.

Conflict of interest

The author has no conflict of interest.

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