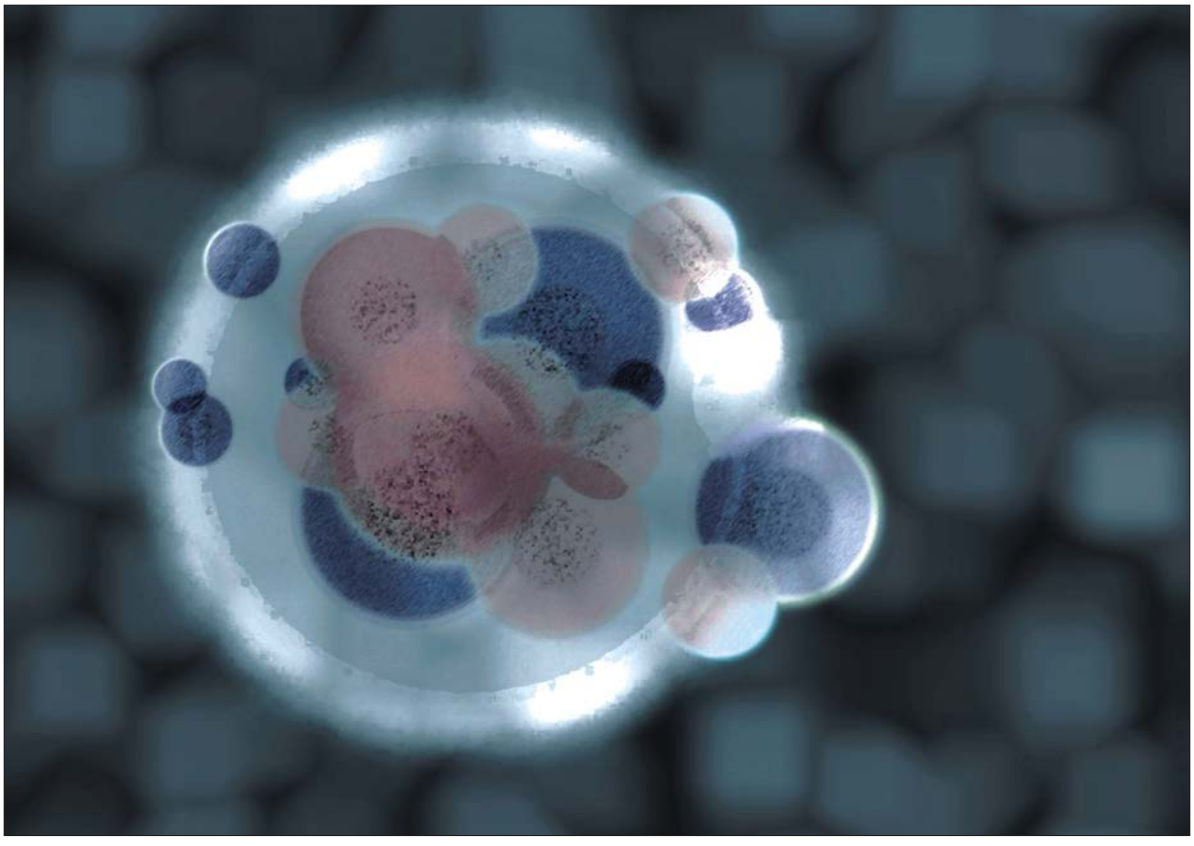

Journal of Biological Sciences

ISSN 1727-3048

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OPEN ACCESS Journal of Biological Sciences

ISSN 1727-3048
DOI: 10.3923/jbs.2018.297.306

Research Article
Prospective Role of *Solanum* Cultures in Producing Bioactive Agents against Melanoma, Breast, Hematologic Carcinomas Cell Lines and Associated Microbiome

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Abstract

Background and Objective: *Solanum* genus (Solanaceae) is well known by a diversity of active phytochemicals with multi disciplinary biological applications. The study aimed to evaluate the activity of methanolic extract and isolated glycoalkaloids of *Solanum torvum* callus as antimicrobial *in vitro* and also to evaluate their cytotoxic role against human carcinomas cell lines. **Materials and Methods:** Callus cultures of *S. torvum* were established on Murashige and Skoog media with plant growth regulators. The main constituents of the methanolic extract were identified using HPLC analysis. Anticandidiasis activity was examined against *candida albicans*. The antibacterial performance was checked versus *Bacillus cereus, Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeroginosa*. Cytotoxic activities were performed contra melanoma, breast and lymphoma cell lines. **Results:** Present outputs illustrated that callus cultures were successfully established and accomplished with biosynthesis of the distinguished *solanum* glycoalkaloids (15.18%). Phenolics were also identified in the *in vitro* cultures as flavonoids (9.80%) and coumarins (3.41%). Regarding anti candidiasis, current results demonstrated high activities of both *S. torvum* callus extract and isolated glycoalkaloids with distinctive effect of the later comparing to nystatin. Present data also, illustrated that glycoalkaloids were more active against Gram +ve bacteria while callus extract was more active against Gram -ve bacteria. Plant materials exhibited promising cytotoxic activity against the examined carcinomas cell. Total callus extract showed higher cytotoxic activity than isolated glycoalkaloids which might return to synergistic effect of other phytochemicals. **Conclusion:** *S. torvum* cultures are promising routes for developing biologically important active compounds that could be applied as favorable antimicrobial and cytotoxic agents.

Key words: *Solanum torvum*, glycoalkaloids, callus cultures, anticandidiasis, antibacterial cytotoxicity

Received: April 08, 2018 Accepted: June 08, 2018 Published: July 15, 2018

Citation: Hanan Abd Al-Hay Al-Ashaal, Mona Mohamed Hasan, Walid Fayad and Magda Abd El- Ghaffar El-Bendary, 2018. Prospective Role of *Solanum* cultures in producing bioactive agents against melanoma, breast, hematologic carcinomas cell lines and associated microbiome. J. Biol. Sci., 18: 297-306.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Surfaces of the human body in and outside including skin, mouth and intestine are covered with normal microbial flora that causes no harm to the human being. Actually, it could be considered as body own ecosystem against invading harmful pathogens[1]. On contrast, a series of baleful microbes could attack mankind causing dangerous ailments. Candidiasis, a condition mostly found in immuno compromised diseased persons is commonly caused by *Candida albicans* and to less extent by other species[2]. Esophageal candidiasis and recurrent mucocutaneous candidiasis are connected to oral squamous cell carcinomas (OSCC), esophageal carcinomas and lymphoid tumors[2]. Squamous cell carcinomas are widespread all over the body including skin, lung, esophagus, cervix, head and neck carcinomas. In addition to that urinary bladder, vagina prostate and anus carcinomas are usually squamous cell cancers[3]. Another critical type of skin cancer is cutaneous melanoma that has been increased worldwide[4]. *Candida albicans* could be associated to melanoma cell adherence and metastasis in liver through inflammation mediated pathway[5].

On the other hand bacterial infection also, may induce dangerous symptoms and result in skin diseases. Pathogenic bacteria invasions are conventional marker of malfunction local or systemic immune mechanisms. It has been quite reasonable that immune system has a decisive role in cancer and OSCC progress[6]. Among the most invasive bacteria that cause recurrent cellulitis, impetigo, boils, carbuncles and folliculitis are *Staphylococcus aureus*, *Pseudomonas aeruginosa*[7]. In a case study, a strong association was conducted between *S. aureus* and SCC[8]. *Bacillus cereus*, which is uncommon pathogen, may cause bulla skin lesion[9]. In the same line, *bacillus* spp. and *E. coli* could induce severe infection of skin and soft tissue especially in immune suppressed patients[10]. There are also, current studies connected breast cancer evaluation and development with gastrointestinal microbiome[11]. Women having breast cancer had comparatively higher abundances of *Bacillus*, *Staphylococcus* and *E. coli* bacteria comparing to healthy tissues[12]. Of significance importance, the microbiome appears to be directly implicated in the evolution of hematologic malignancies[13]. In addition, some members of human microbiome and their products have been implicated as inductors of pathogenic inflammation connected to colorectal cancer[14].

Fortunately, plant kingdom includes a variety of medicinal plants that could serve as pro drugs having antimicrobial and cytotoxic activities. *Solanum* plants are belonging to these categories. *S. tuberosum* and *S. laciniatum* were reported to have cytotoxic efficacy against different human carcinoma cell lines including brain, liver, breast, larynx and lymphoblastic leukemia carcinomas[15,16]. Extract of *S. incanum* stimulated melanoma apoptosis[17]. As well, Glycosides isolated from *S. torvum* fruits showed cytotoxic effect towards human melanoma[18]. *S. torvum* was also reported for its antifungal and antibacterial activities against *P. oryzae*, *B. oryzae*, *A. alt*ernate, *T. padwickii*, *D. tetramera* and *Xanthomonas campestris*, respectively[19]. However, low yield of active ingredients and lack of constancy, in addition to plant infections, are major problems regarding *solanum* plants[16]. Therefore, an important target of the present study was adopting *S. torvum* plant for producing bioactive compounds employing tissue culture technology. Besides, to appraise the role of *S. torvum* callus extract and isolated glycoalkaloids as Pharmaceutical anti candidiasis, antibacterial and cytotoxic agents against melanoma, lymphoma and breast carcinoma.

MATERIALS AND METHODS

Plant materials: *Solanum torvum* leaves-family Solanaceae- were derived from Medicinal plant farm, Faculty of Pharmacy, Cairo University. The plant was identified by the director of the farm and confirmed by Prof. Dr. Zakaria Foad, Agricultural Research Division; National Research Center, Egypt. This study was carried out during the period from mid. 2016 to the end of 2017.

Standard chemicals: Solasonine, solamargine and solasodine were from Sigma Co. Solanine was from Roth Co. Bergapten., xanthotoxin, quercetin and quercetin rhamnoside were from Sigma Co. and were gifts from Prof. Taheya El Kobasy and Dr. Mohamed El Raiey; National Research Center. Nystatin (100 U) was from Bioanalyse Co. Cephradine antibiotic was from Bristol Myers-Squibb Co.

Equipments

HPLC apparatus for glycoalkaloids analysis: Hewlett Packard 1050 (Wilmington, North Carolina, USA) coupled with UV detector. The column used was C18 (5 μm, 0.4×25 cm).

HPLC for coumarins and flavonoids evaluation: Thermo ultimate 3000 (USA) with (UV) detector. The adopted column was ODS C18 Reprosil column (5 μm, 4.6×25 cm).

Plate reader: Asys, Hitech, GmbH, Austria, Model; Expert plus UV.

Spectrophotometer: Spekol 11; Karl Zeiss, Germany.

Experimental procedures

Callus development, HPLC analyses and glycoalkaloids segregation

Callus cultures: *S. torvum* sterilized leaves were aseptically implanted in static pre-autoclaved Murashige and Skoog media (MS) under 121-130°C and pressure 1.05 kg cm^{-2} for 20 min. The media were provided with sucrose 30%. Different concentrations of growth promoters were also included. The cultures were incubated at 24±2°C and constant white light cycle. The media were renovated monthly[16].

The developed calli were dried at 45°C. The dried materials were extracted twice with 96% methanol and then filtered. The combined filtrate was evaporated under reduced pressure and temperature. The methanolic sediment was kept for HPLC analyses. The tested samples were dissolved in methanol (1:1 w/v) then filtered through 0.45 µ Millipore filters. Injection volume was 3-10 µL. Identification of the compounds was achieved by matching peaks area with that of standards.

Analysis and separation of glycoalkaloids: Quantitative determination was achieved adopting isocratic mobile phase (Methanol- H_2O; 60: 100)[20]. Flow rate was 0.9 mL min^{-1} and UV 210 nm. Glycoalkaloids were separated using acid base precipitation[16].

Analysis of coumarins: Isocratic mobile phase was utilized for analysis (MeOH-H2O; 49:51, v/v) acidified with phosphoric acid 0.05% (v/v)[21]. FR 1.0 mL min^{-1} and UV 228 nm.

Analysis of flavonoids: HPLC analysis was carried out using linear gradient mobile phase (acetonitrile and 0.1% acidified water with formic acid)[22]. FR 0.3 mL min^{-1} and UV detector was stabilized at 278 nm.

Antimicrobial study

Anti candidiasis evaluation: *Candida albicans* strains used in the current study were from Microbiology Unit, Veterinary Division at National Research Centre, Egypt. Three strains of *C. albicans* were used in the assay (strain I, II and III). The selected isolates were subcultured on Sabouraud Dextrose agar slopes and spore suspensions were prepared and the turbidity was adjusted to 0.5 McFarland tube. The spore suspensions were then cultured on the surface of Sabouraud dextrose agar plate. Plant extracts including *S. torvum* callus extract and pure *in vitro* biosynthesized glycoalkaloids were diluted at a concentration of 20 µg mL^{-1} in methanol. Sterilized Whatman No. 1 discs were impregnated with 1.25, 2.5 and 5 µL of the extracts to have loading vol. 25, 50, 100 µg $disc^{-1}$ in triplicates. The dried discs were placed on the surface of the plates inoculated with Candida strains and incubated for 24 h and then inhibition zones were measured (mm). The results were compared to nystatin as standard antifungal medicament[23].

Antibacterial examination

Microorganisms and growth conditions: The antimicrobial potential of plant extracts under study was tested using two Gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*), two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeroginosa*). The tested strains were from Microbial Chemistry Dept., Genetic Engineering and Biotechnology Division, National Research Center, Egypt.

Antibacterial assay: The antimicrobial activity was determined by turbidity method[24]. Briefly, the tested bacteria (10^6 colony-forming units (CFU)/mL) were inoculated into nutrient broth medium with each tested plant material separately at concentration 200 µg mL^{-1}, then incubated at 37°C for 24 h under shaking conditions. Antimicrobial activity was evaluated by measuring the optical density (OD) at 600 nm using spectrophotometer and compared to the control then percentage of viability reduction was calculated. The obtained results were compared with the reference antibiotic Cephradine (3.3 µg mL^{-1}). The test was carried out in triplicates.

Cytotoxic examination

Cell culture: MCF7 breast carcinoma, lymphoblastic leukemia (1301), A375 melanoma human tumor cell lines and hTERT-BJ normal human immortalized skin fibroblasts were cultured in 95% humidity, 5% CO_2 and 37°C. MCF-7 and hTERT-BJ were maintained in EMEM, while A375 and lymphoma in DMEM.

Cytotoxicity test: The acid phosphatase assay was used to assess cytotoxicity[25]. Absorbance was measured directly at wavelength 405 nm using plate reader. Cisplatin was used as positive control and DMSO (0.5%) as negative control.

Lymphoblastic leukemia (1301) was tested adopting tetrazolium salt method (MTT)[26]. Taxol was used as positive control.

Statistical analysis: All samples were tested in triplicates. Cytotoxic profiles of the examined plant materials were illustrated by plotting survival fractions for each cell line versus

the tested doses. The data were presented as mean standard error. IC_{50} were determined at $R^2 = 0.9$ using GraphPad Prism 7 software statistics program, Santiago, USA.

RESULTS AND DISCUSSION

Glycoalkaloids, flavonoids and coumarins accumulation in callus cultures: The results showed that calli were grown well on static MS media comprised sucrose as carbohydrate energy source together with auxin and cytokinins including indole butyric acid and benzyl adenine (1, 0.5 mg L^{-1}, respectively) (Fig. 1). These results indicated that growth regulators were essential for callus development and glycoalkaloids production in current study which was compatible with previous findings on other *solanum* species including *S. laciniatum* and *S. nigrum*[16,20].

HPLC analyses illustrated the existence of solasonine, solanine and solamargine glycoalkaloids together with solasodine aglycone (Fig. 2). The analyses also disclosed the presence of quercetin and quercetin rhamnoside flavonoids. Biosynthesized coumarins were identified as bergapten and xanthotoxin (Fig. 3). The concentrations of the *in vitro* biosynthesized alkaloids and phenolics were represented in Table 1. The results illustrated that callus cultures accumulated glycoalkaloids in the highest concentration (15.18%) followed by flavonoids (9.80%) and finally coumarins (3.41%).

The existing outcomes demonstrated that total glycoalkaloids were biosynthesized in callus cultures at high concentration of 15.18% on dry wt bases However it was reported that best results concerning solasodine accumulation from *S. torvum* were obtained by using MS basal medium devoted of any growth regulators and reached only 1.34% at the highest concentration expressed as solasodine[27]. Whereas, the reported total contents of glycoalkaloids from *S. torvum* natural plants were 0.038% and concentrations of solasonine and solamargine were 0.0043 and 0.0028%, respectively[28]. Thus current results indicated that *in vitro* accumulated glycoalkaloids were 11.33 folds the reported ones from *in vitro* cultures and 399.47 folds the reported concentrations from intact natural *S. torvum* plants. This makes the present outputs of notable importance to overcome the very low yield of such substantial bioactive metabolites. The current

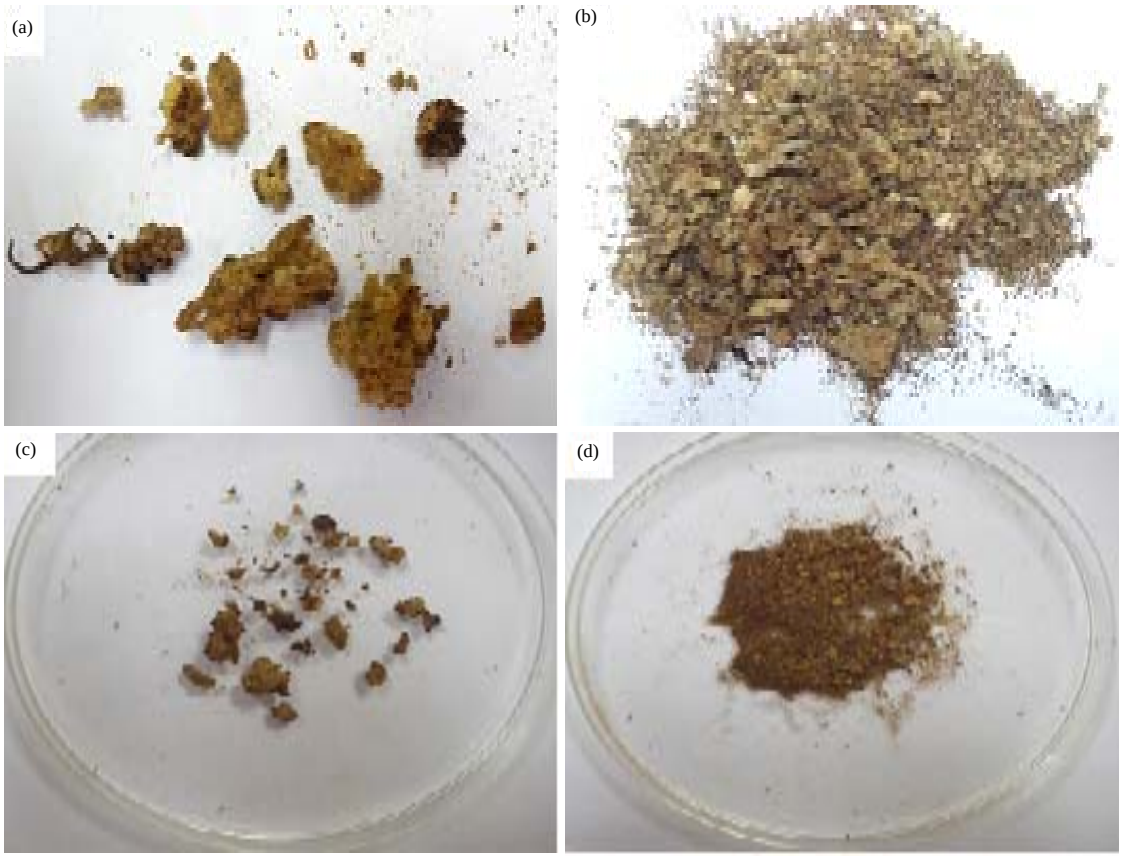

Fig. 1(a-d): (a, c) *Solanum torvum* intact and crushed (b, d) calli

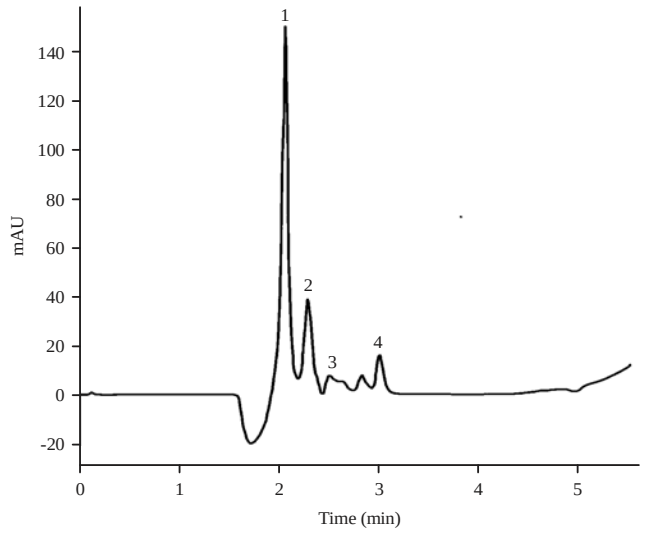

Fig. 2: *In vitro* glycoalkaloids of *S. torvum* callus culture where 1: Solasonine, 2: Solanine, 3: Solamargine and 4: Solasodine

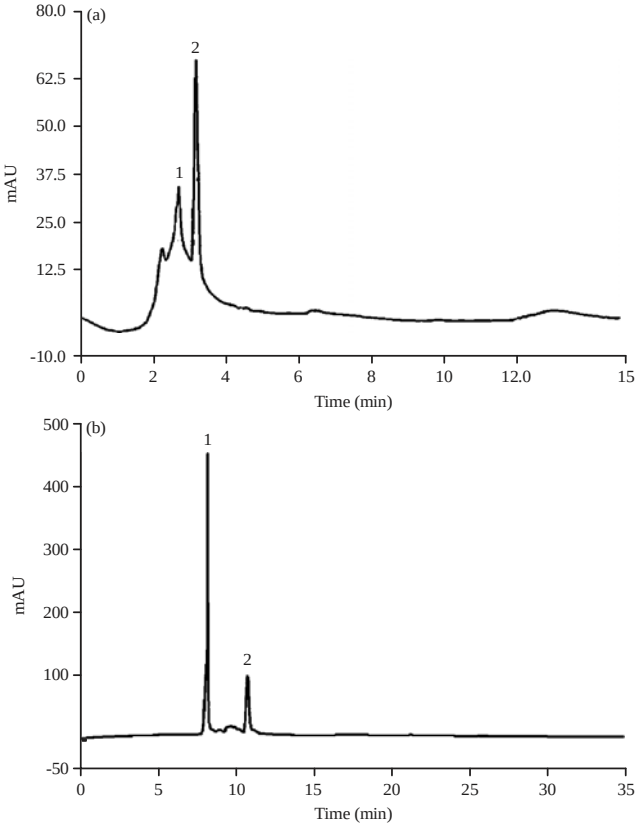

Fig. 3(a-b): *In vitro* phenolics of *S. torvum* callus culture where a (coumarins; 1: Bergapten, 2: Xanthotoxin) and b (flavonoids; 1: Quercetin rham.., 2: Quercetin.)

Table 1: *In vitro* glycoalkaloids of *S. torvum* callus extract

Secondary metabolites	Molecular formula	Dry weight (%)
Glycoalkaloids		
Solasonine	$C_{45}H_{73}NO_{16}$	9.52
Solanine	$C_{45}H_{73}NO_{15}$	3.07
Solamargine	$C_{45}H_{73}NO_{15}$	1.12
Solasodine	$C_{27}H_{43}NO_2$	1.47
Total		15.18
Flavonoids		
Quercetin	$C_{15}H_{10}O_7$	2.02
Quercetin rham.	$C_{21}H_{20}O_{11}$	7.78
Total		9.80
Coumarins		
Bergapten	$C_{l2}H_8\ o_4$	1.98
Xanthotoxin	$C_{l2}H_8\ o_4$	1.43
Total		3.41

increments are of multifactorial purposes including auxin and cytokinins concentrations, sucrose concentration and parent plant strain. The results are in good compatibility with the increments of glycoalkaloids from *S. nigrum* cultures[20]. High yield of alkaloids from *Thalictrum minor* cultures that reached1000 folds the parent plant was also documented[29]. Regarding flavonoids and coumarins phenolics little was mentioned about *in vitro* production of these secondary metabolites from *solanum* species; almost the majority of the reported *in vitro* studies concerned with glycoalkaloids bio-production. However, qualitative production of flavonoids and coumarins from other *solanum* species as *S. xanthocarpum* were reported from *in vitro* cultures[30]. Cultures from other plants as *Digitalis lanata* and *Ruellia tuberose* were also found to produce flavonoids and coumarins[31,32].

Anti candidiasis and antibacterial potential: The current data illustrated that both callus methanol extract and pure isolated glycoalkaloids showed potent anti candidiasis property that was dose dependent. The results also illustrated that pure glycoalkaloids were more effective than total callus extract (Table 2). The results showed that the tested plant materials were approximately active as equal as standard nystatin at their highest doses.

Table 3 illustrated that *S. torvum* callus methanol extract and pure biosynthesized glycoalkaloids showed antibacterial performance against Gram +ve and Gram -ve selected bacteria species. The data also clarify that, pure glycoalkaloids were more effective against *B. cereus* and *S. aureus* (Gram positive bacteria). While, calli extract which, included flavonoids and coumarins phenolics displayed higher effectiveness against *E. coli* and *P. aeroginosa* (Gram negative bacteria). Table 3 also demonstrated that the activity of callus extract was as much as the activity of

Table 2: Effect of pure glycoalkaloids and callus methanol extract of *S. torvum* on *Candida albicans*

		Inhibition zones (mm) Mean±SE					
		Pure glycoalkaloids (µg/disc)			Total callus extract (µg/disc)		
Candida albicans	Nystatin (100 U)	100	50	25	100	50	25
Strain I	1.68±0.12	1.75±0.04	1.45±0.06	1.10±0.04	1.73±0.08	1.25±0.02	1.00±0.03
Strain II	2.85±0.09	2.58±0.08	2.12±0.06	1.73±0.07	2.45±0.11	1.58±0.09	1.37±0.05
Strain III	2.87±0.10	2.75±0.08	2.40±0.12	1.73±0.08	2.17±0.07	1.87±0.06	1.48±0.07

Table 3: Antibacterial activity of *in vitro* pure glycoalkaloids and callus methanol extract of *S. torvum*

	OD±SE/inhibition (%)			
Tested materials	*E. coli*	*S. aureus*	*P. aeroginosa*	*B. cereus*
Callus extract	0.86±0.06 (29.51%)	0.82±0.04 (21.15%)	0.89±0.03 (25.83%)	0.95±0.02 (19.49%)
Glycoalkaloids	0.98±0.07 (19.67%)	0.77±0.08 (25.96%)	1.00±0.09 (16.67%)	0.91±0.07 (22.88%)
Cephradine	0.77±0.01 (36.89%)	0.69±0.02 (33.65%)	0.74±0.02 (38.33%)	0.95±0.02 (19.49%)
Control	1.22±0.10	1.04±0.05	1.20±0.09	1.18±0.04

E. coli: *Escherichia coli, S. aureus*: *Staphylococus aureus, P. aeroginosa*: *Pseudomonas aeroginosa* and *B. cereus*: *Bacillus cereus*, OD: Optical density. Inhibition (%): Percentage of viability reduction

cephradine antibiotic while glycoalkaloids were more active than cephradine towards *B. cereus* at the tested doses.

With respect to antimicrobial performance recorded in the present research, both pure glycoalkaloids and total *S. torvum* callus methanol extract exhibited promising anticandidiasis and antibacterial activities. The results are in agreements with previous studies reported that different *solanum* spp. showed antimicrobial leverage. Glycoalkaloids derived from *S. tuberosum* and *S. lycopersicum* showed antimicrobial efficacy against *S. typhimurium*, *S. aureus* and *Candida*[33]. Glycoalkaloids from S. *habrochaites* were documented to have antimicrobial activity versus *E. coli*, *S. aureus* and *C. albicans*, which was the most susceptible microbe to the tested glycoalkaloids[34]. In the same approach with present results, compounds of *S. tuberosum* extract showed potent anticandidiasis and antimicrobial performance[33]. Glycoalkaloids might exert their antibiotic action *via* membrane sterol binding property that lead to microbial membrane leakage and damage of cell membrane entirety[35]. Likewise, saponin identified from *S. chrysotrichum* showed anticandidiasis against Candida species including *C. albicans* via morphological fungal variation and could be used in the treatment of Vulvovaginal candidiasis[36]. Similarly, hexane extract of *S. xanthocarpum* exhibited antifungal activity against *C. albicans*[37]. Presence of coumarins and flavonoids phenolics herein in the callus extract, could also account for the recorded antimicrobial activity. Generally, phenolics are natural defense against invasive bacteria in the plants[38]. Xanthotoxin was reported to have antibacterial performance versus *S. aureus* (Gram +ve) and *E. coli* and *S. typhimurium* (Gram -ve) bacteria[39]. Further, it was acquainted that quercetin exerted synergistic antibacterial effect collectively with antibiotics[40].

The results showed that, glycoalkaloids were more effective against Gram +ve bacteria whereas, callus extract contained phenolics was more efficient against Gram -ve bacteria. These current results are in good consistence with previous studies reported that phenolics demonstrated antimicrobial properties and had diverse ability to alter membranes particularly in Gram-negative bacteria[41].

The present outcomes are of special advantages as *Candida* spp. including *C. albicans* were reported to have natural resistance against synthetic antifungals as flucytosine, flukonazole and ketoconazole[42]. In addition, according to WHO, Antimicrobial resistance, Fact sheet; resistance to microbes is a serious problem that impendence the effectiveness of major surgery and cancer treatment with chemotherapy.

Cytotoxicity evaluation: The data herein illustrated that *S. torvum* callus extract and isolated glycoalkaloids exhibited remarkable cytotoxic performance against melanoma, breast and lymphoblastic leukemia cell lines at different concentrations with different susceptibility of the examined cell lines to the plant materials. (Fig. 4-6). The data also illustrated that the inhibition response was nonlinear versus the tested doses mostly. Callus methanol extract showed more potent cytotoxicity comparing to pure glycoalkaloids fraction regarding all the selected carcinoma cell lines which was represented by lower IC_{50} values (Table 4). Figure 4 also, illustrated that the callus extract and the glycoalkaloids were safe and exert no cytotoxic effect to normal human skin fibroblasts.

Concerning the recorded cytotoxic effects of *S. torvum* callus and the glycoalkaloids herein, existence of glycoalkaloids might account for these outcomes. The current

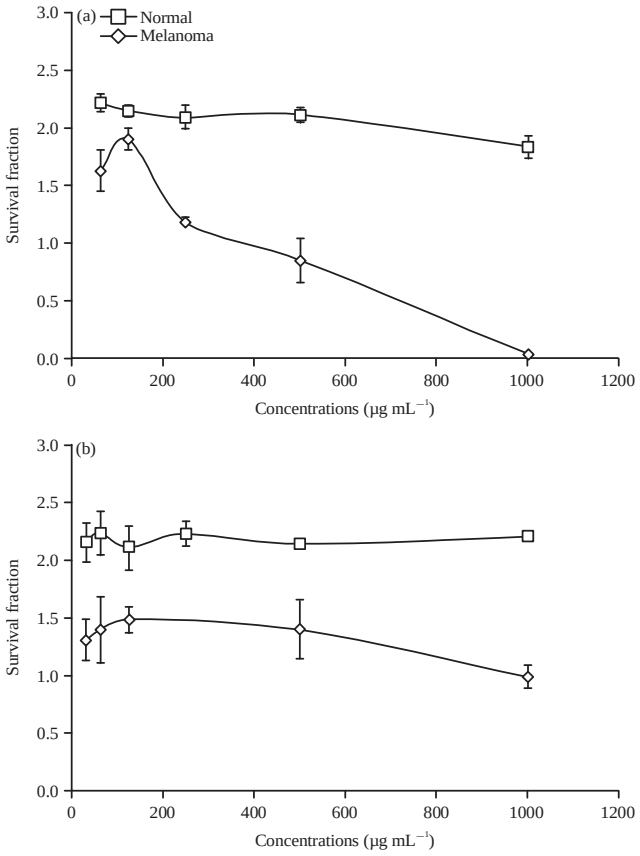

Fig. 4(a-b): Cytotoxicity profile of *S. torvum*, (a) Total callus extract (T.ext.) and (b) Glycoalkaloids (Glyc.) on normal human skin fibroblast and A375 melanoma human carcinoma

Table 4: Cytotoxic activity of *S. torvum* callus extract and pure glycoalkaloids

	IC_{50} (µg mL^{-1})			
Cell line	T.ext.	Glyc.	Cis.	Tax.
Melanoma	2.92	3.12	0.39	
Breast	2.22	28.36	2.89	
Lymphoblastic leukemia	1.40	1.44		0.60

T.ext: Total callus extract, Glyc.: Glycoalkaloids, Cis.: Cisplatin, Tax.: Taxol

results were in the same approach with previous studies explore the cytotoxic effects of *solanum* plants. Glycoalkaloids; *solanum* main components, were documented to have anticancer efficacy through different mechanisms including cytotoxicity, apoptosis. Hence the presence of solamargine, solasonine and solanine in addition to solasodine in the present study may account for the observed cytotoxic performance. Solasonine and solamargine isolated from *S. laciniatum* were acquainted for their cytotoxic effect on different human cell lines including lung, liver, brain, lymphoma and breast carcinomas[16]. As well, solanine and

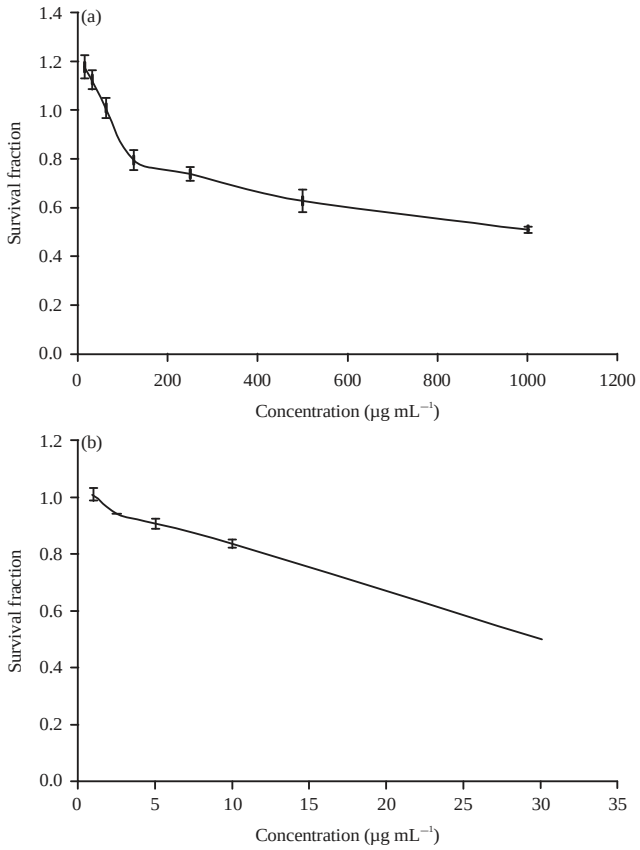

Fig. 5(a-b): Cytotoxicity profile of *S. torvum*, (a) Total callus extract (T.ext.) and (b) Glycoalkaloids (Glyc.) on MCF7 breast carcinoma

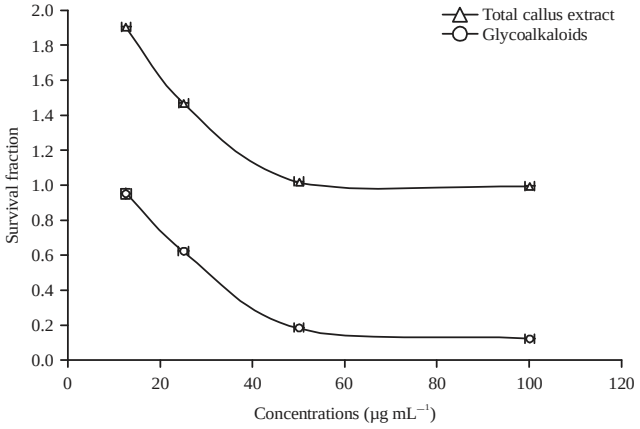

Fig. 6: Cytotoxicity profile of *S. torvum*, Total callus extract (T.ext.) and Glycoalkaloids (Glyc.) on 1301 lymphoblastic leukemia carcinoma

chaconine from *S. tuberosum* were also documented to have cytotoxic activities against carcinoma cell lines of breast, cervix, liver, larynx, colon, brain and lymphoblastic

leukemia[15,33]. Concerning the IC_{50} of the isolated glycoalkaloids herein towards MCF7 breast carcinoma cell line (28.36 μg mL^{-1}), it seems to be in close agreements with that previously recorded of solasodine glycoalkaloids from *S. laciniatum* (23.40 μg mL^{-1})[16]. Whereas, total callus extract in the current research, induced potent cytotoxic activity with lower IC_{50} (2.22 μg mL^{-1}) approximately as potent as callus extract of *S. tuberosum* (2.70 μg mL^{-1})[15]. Whilst, solamargine glycoalkaloid was reported to possess the lowest IC_{50} (1.80, 1.87 and 2.60 μg mL^{-1}) on HBL-100, ZR-75-1 and SK-BR-3 breast carcinomas respectively[43]. With respect to lymphoblastic leukemia, the data showed pronounced activity with low IC_{50} (1.40, 1.46 μg mL^{-1}) for *S. torvum* callus extract and glycoalkaloids, respectively. The results are in consistence with the documented IC_{50} of *S. tuberosum* callus extract (3.70 μg mL^{-1})[15]. Solamargine exhibited K562 leukemia carcinoma cells cytotoxicity at elevated concentration (IC_{50}; 7.38 μg mL^{-1})[43]. There is no doubt that endocrine remedies of some cancers as breast and lymphoma carcinomas are associated with significant hazards. Therefore the present data are of marker importance in cancer therapies.

Regarding the cytotoxic activity versus melanoma, the data showed that both *S. torvum* callus extract and pure isolated glycoalkaloids possessed hopeful cytotoxic effects against melanoma with no harmful hazards on normal cells. Solamargine detected in the callus extract and the biosynthesized glycoalkaloids was reported to prohibit fastly, particularly and efficiently the development of malignant melanoma *via* cell necrosis *in vitro*[44]. In the same line with present results, solasonine, solamargine and solanine glycoalkaloids from *S. sodomaeum* and *S. incanum* were documented to restrain melanoma, basal and squamous carcinomas in addition to keratosis *via* cell apoptosis[44]. Furthermore, pure glycoalkaloids chaconine, tomatine, solanine and solasonine in addition to solamargine which was isolated from *S. americanum*, each induced A375 melanoma cytotoxicity of IC_{50} 1.99, 2, 00, 5.49, 7.00 and 7.51 μg mL^{-1}, respectively[45]. These results are compatible with the present data (IC_{50}: 2.92, 3.12 μg mL^{-1}) against A375 melanoma for *S.torvum* callus extract and glycoalkaloids. The data in the current study, illustrated that generally total callus extract was more active than the glycoalkaloids against melanoma, breast and hematological lymphoblastic leukemia carcinomas that was illustrated by comparing IC_{50} values. The synergistic effect of phenolics and glycoalkaloids disclosed in callus extract might account for the prevalence of the cytotoxic activity of the extract. Flavonoids were reported to modulate vascularization and cell proliferation, differentiation and apoptosis and to repress various processes associated with cancer as oxidative stress and angiogenesis thus, restrain cancer upgrowth and development[46-48]. Quercetin, detected in the present study, was documented to induce cell apoptosis in cancer cell lines and tumor tissues through interacting with DNA[49]. Moreover, xanthotoxin and bergapten furanocoumarins that found in callus extract were reported to exert malignant cell cytotoxicity and could be a prospect combination remedy for malignant cancers[50].

In addition to the pronounced cytotoxicity of the *S. torvum* callus extract and glycoalkaloids, the antimicrobial activities registered in the current study could be beneficial in breast cancer and lymphoblastic leukemia treatment as it seems to be direct relations between microbiome and breast and hematologic carcinomas development[12,13]. Besides, the anticandidiasis and antibacterial activities illustrated herein, could be very important as drug therapies for melanoma and squamous carcinomas. There are evidences demonstrated that bacterial infection as well as, candidiasis are implicated in OSCC, through suppressing the immune system[8,2,6]. Besides, candidiasis is implicated in melanoma metastasis in liver[5].

CONCLUSION

The results deduced that tissue culture technology was efficient in glycoalkaloids and phenolics biosynthesis from *S. torvum* cultures in concentrations surpassed that reported from plant growing naturally. *S. torvum* callus extract and pure isolated glycoalkaloids, under investigation showed pronounced anticandidiasis versus *candida albicans* showing more effect of pure glycoalkaloids. Besides, the glycoalkaloids showed more efficacy regarding gram positive bacteria. Meanwhile, callus extract was more effective concerning gram negative bacteria. Both callus extract and glycoalkaloids stimulated cell cytotoxicity against human melanoma, breast and lymphoblastic leukemia carcinomas cell lines demonstrating more effectiveness of the callus extract. As microbiomes are directly linked to several malignancies, accordingly current outcomes could be helpful in seeking for fast and effective treatments of the examined carcinomas and to modulate the related microbiome before and during the malady.

SIGNIFICANT STATEMENTS

The data indicated the significance of *S. torvum in vitro* cultures as effective tool for producing bioactive alkaloids, flavonoids and coumarins and thus help to dominate the scarce and low yield of these metabolites. Also, the results substantially demonstrate the activity of these materials as remarkable cytotoxic, anticandidiasis and antibacterial agents.

The results are benefits as guide for producing these secondary metabolites continuously in sufficient amounts applicable in pharmaceutical industry for producing cytotoxic and antimicrobial drugs from natural origin that has become a focus for active research. The study is important to help researchers for continual investigations on *S. torvum* to attain natural remedies to overcome antimicrobial resistance and carcinoma recurrence of the tested cell lines.

ACKNOWLEDGMENTS

This work was supported by National Research Centre (Egypt) to whom the authors are thankful. The authors are also grateful for Professor Stig Linder, Karolinska Institute, Sweden, for generously providing us with A375, MCF7 and hTERT-BJ cell lines. Many thanks are due to Dr Ahmed El-Beih; Pharmaceutical and Drugs Industries Division, NRC and to Mr. Ahmed Ganem; assistant researcher, Chemical Industries Research Division, NRC; for their valuable assistance.

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