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Regeneration, *in vitro* glycoalkaloids production and evaluation of bioactivity of callus methanolic extract of *Solanum tuberosum* L.

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ABSTRACT

Callus and differentiated shoots initiated from *Solanum tuberosum* L. on MS media containing BA, IAA, and Kin. Glycoalkaloids are produced in callus and shoots in concentrations higher than original tubers using HPLC. Callus methanolic extract had promising anticancer activity with low IC_{50} values against human carcinoma cell lines of breast, lymphoplastic leukemia, larynx, liver, cervix, colon, and brain, IC_{50} (μg/mL) were 2.7, 3.7, 6, 6.7, 10, 13.6, and 22.3 respectively. Antioxidant capacity of the extract (76.4%) performed using ESR. Preliminary screening showed that the extract exhibited *in vitro* virucidal activity against Herpes simplex. The extract possessed *in vitro* schistomicidal and fasciolicidal activity.

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1. Introduction

Solanum tuberosum L. (potato) Family Solanaceae contain steroidal glycoalkaloids including solanidin an important precursor for hormone synthesis and they can be used to obtain a potential preparation that may protect against skin cancer [1].

Glycoalkaloids are of great importance from both ecological and human aspects having multidiscipline pharmacological applications as antiviral and anticancer [1–3]. *S. tuberosum* is also a source of anti diabetic and antioxidant agents [4–6]. In addition, it is a source of lectins that could be used as a means of "anchoring" a drug delivery system to the mucosal surface of the eye [7]. Unfortunately, potato sprouts are susceptible to fungal and viral diseases [8,9]. On the other hand, plant tissue culture technique performs a potential source of valuable phyto pharmaceuticals in well controlled closed systems. Previous *in vitro* studies of potato were concerned with agricultural point of view. Potato micro tubers are available source for *in vitro* germplasm conservation and disease free germplasm exchange [10]. Cell culture is also used for selection of frost tolerant potato cell lines [11]. In addition, *in vitro* cultures are used to produce potato plantlets resistant to pathogenic fungi, virus infections and for micro propagation [12–14]. Therefore, the new scope of this work is the enhancement of *in vitro* glycoalkaloids production through manipulation of culture media from both callus and shoots of *S. tuberosum* L. local cultivar and evaluation of biological activity of callus methanolic extract as anticancer against human carcinoma cell lines, antioxidant, antiviral and antiparasitic.

2. Materials and methods

2.1. Plant material

S. tuberosum L. local cultivar (Spunta) was identified by Dr. Zakaria Fouad at the Agricultural and Biological Division, National Research Center. A voucher specimen of the regenerated plants was deposited at Cairo University Herbarium (CAI) with registration number CAI-343456 and identified by Prof./Mounir Abd El-Ghany.

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2.2. Chemicals and standard samples

Standard α-solanine and solanidine (approx. >97%) were purchased from Roth Company, α-chaconine (approx. 95%) from Sigma 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$^{\bullet}$ (2,2-Diphenyl-1-picrylhydrazyl — 97%) from Sigma Co. Vitamin C (>99.5%) from Fluka Co. Acyclovir (>95%) from Sigma Co. Braziquantel (97.5–102%) from Alexandria Co. for pharmaceuticals. Media components and phytohormones for regeneration and *in vitro* alkaloids production were tissue culture grade. Solvents for analysis were HPLC grade.

2.3. Instruments

- HPLC; HEWLETT PACKARD series 1050, UV detector, wave length 210 nm, column C18 5 μm, 0.4×25 cm, FR. 0.9 mL/min. Pressure 6 bar, temp. 32 °C.
- ESR; Bruker, Elexys, 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 1.

2.4. Callus and shoot initiation

Potato eye buds were sterilized with 10% v/v sodium hypochlorite solution (clorax) for 20 min, immersed in 70% ethyl alcohol for seconds, then rinsed with sterile distilled water twice, in laminar airflow cabinet. The buds were sliced and aseptically cultured in glass Jars containing MS media [15] (3% sucrose) and supplemented with benzyl adenine (BA) as auxin and indole acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), naphthalene acetic acid (NAA) and Kinetin (Kin.) as cytokinins either individually or in combination at different concentrations. The cultures maintained at 26 °C and 16/8 light dark cycle (fluorescent light). Resubculture on fresh media was performed every 4–6 weeks to avoid depletion of the nutrients.

2.5. Extraction and HPLC analysis

Plant materials from callus, shoots and field potato tubers were dried at a temperature 45 °C. 10 mg of the dried materials was crushed, extracted with 100 mL methanol (96%) in water bath at 50 °C for 3 h, and subsequent homogenized three times each for 5 min. (ultra-turrax) in methanol (3 x 15 mL), the combined extracts were concentrated in vacuum. 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 filter then analyzed by HPLC in triplicates (0. 25–1 μL, injection volume depending on the concentration of each sample) using 60% methanol as mobile phase, wave length, 210 nm, flow rate 0.9 mL/min. The concentrations of the glycoalkaloids based on the calculation of percentage peak areas of the samples comparing with that of standard using linear regression equation for each standard compound

The regression equation for solanidine,
$Y = 24.54X + 0.26$, $r = 0.999$.

The regression equation for α – chaconine,
$Y = 33.685X - 18.06$, $r = 0.994$.

The regression equation for α – solanine,
$Y = 4.667X + 6.14$, $r = 0.991$.

2.6. Biological investigation

2.6.1. Cytotoxic activity

Potential cytotoxic activity of the dried callus methanolic extract of different concentrations (0–25 μg/mL) was performed against breast (MCF7-HTB-22), cervix (Hela-CCL2), liver (Hepg2-ATCCHB-8065), larynx (Hep2-CCL-23), colon (HT116-CCL-247) and brain (U251-NIBIO1F050288) human carcinoma cell lines at the National Cancer Institute, Egypt, according to the method of Skehan et al. [16], while the tested material was examined for cytotoxicity against lymphoplastic leukemia (1301) at concentrations (0–100 μg/mL) at the National Research Center, Egypt, according to Hansen et al. [17]. IC50 values (μg/mL) of the extract were determined comparing with cisplatin as standard cytotoxic agent against brain carcinoma cell line, Doxorubicin against breast, larynx, liver cervix, and colon cell lines while Taxol was used as standard cytotoxic agent against lymphoplastic leukemia. Negative control groups without callus methanolic extract were also performed. Cytotoxicity against the selected cell lines was examined in triplicates.

2.6.2. Free radical scavenging capacity

Spectrophotometric method using electron spin resonance technique (ESR) [18] was performed to determine the antioxidant capacity of the callus methanolic extract in comparison with standard α-solanine and vitamin C as positive control against free radical diphenyl picryl hydrazyl (DPPH$^{\bullet}$). 1 mL of 10^{-3} M DPPH$^{\bullet}$ was added to 1 mg of standard α-solanine, standard vitamin C or 1 mg of the callus extract. The reduction of integrated areas of DPPH$^{\bullet}$ represented the antioxidant capacity; measurements were taken after 5 minutes.

$$\% \text{ of antioxidant capacity} = A_0 - A_1 / A_0 x\ 100$$

$$A_0 = \text{Area of DPPH}^{\bullet}$$

$$A_1 = \text{Area of the tested sample} + \text{DPPH}^{\bullet}$$

2.6.3. Antiviral screening

Herpes simplex virus type 1 (HSV-1) was used as a model of DNA virus for preliminary antiviral screening. The virus was isolated and propagated in the virology Laboratory of the Department of Water Pollution, National Research Center.

African green monkey cells (Vero) were used as virus host. Cells grew in minimum essential medium with Hank's buffer (HMEM) supplemented by 1% antibiotic–antimycotic mixture (GIBCO-BRL), 8% fetal bovine serum and the pH adjusted to (7.2–7.4) by 7.5% sodium bicarbonate solution. Cells grew as monolayer sheets dissociated by trypsin–versine solution (0.15% trypsin and 0.04% ethylene diamine tetraacetic acid,

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