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Original Research

The evaluation of biological activity of methanolic extracts of *Solanum nigrum* and molecular docking analysis of selected phytoconstituents against vimentin

Rumana Ahmad[1], Anamika Gupta[1], Afreen Fatima[2], Ishrat Husain[1], Anand Narain Srivastava[2]

[1]Department of Biochemistry, Era's Lucknow Medical College and Hospital, Sarfarazganj, Lucknow, Uttar Pradesh, India, [2]Department of Pathology, Era's Lucknow Medical College and Hospital, Sarfarazganj, Lucknow, Uttar Pradesh, India

Address for correspondence:
Rumana Ahmad, Department of Biochemistry, Era's Lucknow Medical College and Hospital, Sarfarazganj, Hardoi Road, Lucknow - 226 003, Uttar Pradesh, India. E-mail: rumana_ahmad@yahoo.co.in

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ABSTRACT

Background: *Solanum nigrum* L. (SN), commonly known as black nightshade or Makoi in India, is well documented in Ayurveda, the indigenous system of medicine, for its medicinal properties. However, it has still not garnered considerable attention for modern therapeutic use. In this study, *in vitro* activities of methanolic extract(s) of dried roots, stems and leaves of SN were evaluated against human cancer cell lines MDA-MB-231, Hep G2, A549, and normal cell line Vero. **Methods:** The percentage viability of the cell lines was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Antibacterial activity of the extracts was tested against *Staphylococcus aureus* and *Escherichia coli* using disc diffusion method. Molecular docking studies were conducted to assess the inhibitory action of selected phytoconstituents belonging to the class of steroidal glycoalkaloids (SGAs) and steroidal saponins (SSs) against cytoskeletal proteins, namely, actin (G- and F-), tubulin (alpha and beta), and vimentin. **Results:** HepG2 cells were found to be most susceptible to SN extracts. Leaf extract of SN showed significant anticancer activity against HepG2 and MDA cells (IC_{50} values approx. 20 μg/mL). Agarose gel electrophoresis of isolated DNA from treated cancer cells revealed characteristic ladder like fragmentation, a hallmark of apoptosis. High-performance liquid chromatography profiling demonstrated the presence of alpha-solanine in all extracts. Molecular docking analysis revealed that SGAs displayed potent binding to the intermediate filament protein vimentin (K_i 1.0–8.1 μM) whereas the SSs displayed moderate to low binding to vimentin. **Conclusion:** SN methanolic extracts inhibited proliferation and induced apoptosis in human cancer cell lines and thus, warrant further investigation.

KEY WORDS: Antibacterial, anticancer, molecular docking, *Solanum nigrum*, vimentin

INTRODUCTION

India has two mega-biodiversity hotspots in the Western Ghats and the North-East region and also the northern Himalayan region. These biodiversity zones are major sources of herbal products for many Ayurveda based Indian companies. *Solanum nigrum* L. (SN) [Figure 1] is a plant found to grow abundantly in the wild in South Asia, and has been routinely used in Ayurveda since time immemorial. Various parts of this plant are known to possess and exhibit a wide spectrum of biological activities, namely, antitumor, antioxidant,[1] anti-inflammatory,[2] hepatoprotective,[3] diuretic,[2] antipyretic,[2] antimicrobial,[4] antiulcerogenic, and ulcer healing.[5] SN is a major constituent of a number of herbal liver-support formulations. SN possesses an arsenal of active components that are responsible for its diverse activities, namely, glycoalkaloids, glycoproteins, and polysaccharides. Gallic acid, catechin, protocatechuic acid, caffeic acid, epicatechin, rutin, and naringenin[6,7] are the other components reported to occur in SN.

The glycoalkaloids form the major components of SN, the important ones being solanine, solasonine, solamargine, diosgenin, solavilline, and solasdamine[8,9] which are derivatives of parent steroidal aglycones (SAGs) solanidine and solasodine by the addition of carbohydrate residues. Although known to possess antitumor activity, these alkaloids need to be investigated in more detail.[10] Due to a high content of solanine which comprises about 95% of total alkaloid content of the plant,[8]

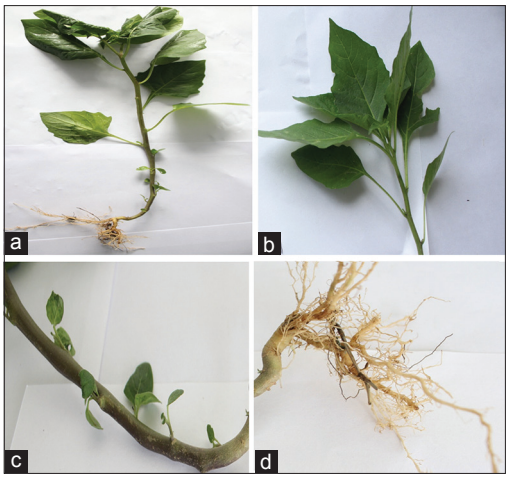

Figure 1: (a) *Solanum nigrum* Linn. Habit, (b) leaves, (c) stem, (d) roots

SN is considered to be toxic.[11-13] Solanine naturally occurs as its alpha form in SN, but can be degraded to its beta- and gamma-forms with selective removal of carbohydrate residues.[14]

The extract(s) of SN fruits are known for their antitumor and neuropharmacological properties, thus allowing their use as a source of antioxidants and cancer chemopreventive agents.[15,16] Solanine is found in high concentrations in nearly every part of SN but the highest content is found in unripe berries. The berries become non-toxic after ripening. As the plant matures, the solanine content of leaves goes on increasing.[11]

Apart from steroidal glycoalkaloids (SGAs), steroidal glycosides also known as steroidal saponins (SSs) are found in SN of which nigrumnins I and II have been recently reported[17] while degalactotigonin was reported earlier. All these SSs have been found to display antineoplastic activity.[18] New saponins continue to be discovered in SN till date, the solanigrosides (II–VII), being among them.[19] Degalactotigonin is one such SS which has been found to be active against a panel of cell lines HepG2, NCI-H460, MCF-7, and SF-268 with IC_{50} values ranging between 0.25 and 4.49 μM[19] with respect to different cell lines. Two furostanol saponins namely, uttroside A and B have also been reported from the stems and roots of SN.[20] Uttroside B has also been isolated and characterized from leaves of SN recently and has been found to be 10 times more active against HepG2 (IC_{50} 0.5 μM) than sorafenib (IC_{50} 5.8 μM), the only FDA-approved drug for liver cancer.[20]

As per the National Cancer Institute, USA, for anticancer screening *in vitro*, an IC_{50} value of <100 μg/mL for medicinal plant extracts is considered to have the potential for further isolation, purification, and characterization of bioactive molecules. In view of the above, our main aim was to assess whether root, stem and leaf extracts of SN possess IC_{50} values <100 μg/mL against the studied cancer cell lines, so as to enable further studies involving isolation and characterization of active components for lead optimization studies. Some selected phytoconstituents known to be present in SN were also screened and assessed *in silico* for their prospective binding to cytoskeletal proteins actin, tubulin and vimentin using molecular docking.

MATERIALS AND METHODS

Reagents

All chemicals used in cell culture were of analytical grade as reported previously.[21,22] Doxorubicin hydrochloride was obtained from Sigma Chemical Co. (St. Louis, MO, USA) and dimethyl sulfoxide (DMSO) from Calbiochem. Alpha-solanine standard was from Sigma-Aldrich. All high-performance liquid chromatography (HPLC) grade reagents were used in HPLC.

Collection of Plant Material

Fresh plants were collected from the region (Sarfarazganj area) around Era's Lucknow Medical College, Lucknow, in the month of February, 2017. A competent botanist from National Botanical Research Institute, Lucknow, identified the plant SN. Fresh plant material was separated into roots, stems, and leaves; washed, shade-dried and then powdered separately using a blender and stored in air tight bottles.

Sample Preparation

Sample preparation was done as per our previously reported studies.[21,22] All extracts were filtered through sterile syringe filter units (0.22 μm, Millipore, Fisher Scientific) before addition to cell culture medium.

Biological Activity Evaluation

Cell lines

Four cell lines, namely, MDA-MB-231 (human breast carcinoma), HepG2 (liver carcinoma), A549 (lung carcinoma), and Vero (normal African green monkey kidney epithelial cells; ATCCCCL-81), obtained from the National Centre for Cell Science, Pune, India, and maintained by serial passaging in Tissue and Cell Culture Lab, Era's Lucknow Medical College, Lucknow, were used in the present study.

Cell culture

For the experiments, cells were trypsinized, seeded and cultured for 24 h in 6-well plates (Linbro, MP Biomedicals) at a density of 0.5×10^5 cells/well for adherence as reported previously.[21,22] The cell lines were incubated with 20–100 μg/mL of root, stem, and leaf extract(s) of SN in 50% DMSO for the next 48 h. The control wells contained cells in presence of the vehicle (50% DMSO). Results obtained were plotted as cell viability versus time period graph based on experiments done in triplicates.

Morphological Analysis

Observations on cellular morphology were done with the help of 10× and 40× objectives of a phase contrast microscope (Nikon Eclipse Ti, Japan).

Cytotoxicity Assays

Trypan blue dye exclusion assay (TBE)

The assay was done as reported previously.[21,22]

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay - Determination of optimal cell number for the assay

MTT was performed as reported previously[21,22] using a Biorad PW41 ELISA plate reader at 550 nm with a reference wavelength of 630 nm for reading absorbance.

Evaluation of Cytotoxicity and Cell Viability

Each cell line was seeded at a frequency of 10,000/200 µl in 96-well microtiter tissue culture plates for 24 h followed by dosing (addition of 20–100 µg/mL of SN extracts), for another 48h. Blanks contained only culture medium. The positive control was a standard anticancer drug doxorubicin hydrochloride. Negative controls contained cells in presence of medium + vehicle (50% DMSO/water). The intensity of color produced was proportional to % cell viability which was obtained by plotting absorbance against the extract dose in µg/mL using the formula $\{(A_T-A_B)/(A_C-A_B)\}\times 100$ where,

A_T = Absorbance of the treatment well

A_B = Absorbance of the blank

A_c = Absorbance of the control well

% cell inhibition = 100-cell survival.

Comparison of the Cytotoxic Activity of Extracts

Vero cells, initially seeded for 24 h were treated with different SN extract(s) at 20–100 µg/mL for the next 48 h followed by MTT assay. Results were interpreted as discussed above.

Antibacterial Activity

The *in vitro* antibacterial activity of SN root, stem, and leaf extracts was evaluated at concentrations of 2–20 mg/disc against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) bacteria by disc diffusion method[23] using Mueller-Hinton agar medium as reported previously.[24] DMSO and tetracycline were used as negative and positive controls, respectively.

HPLC Analysis

Preparation of Standard for HPLC

Alpha-solanine standard was dissolved in ultrapure HPLC grade water at a concentration of 1.0 mg/mL.

Sample preparation

Samples of SN extract(s) were made at 1.0 mg/mL in ultrapure HPLC grade water and filtered through sterile 0.45 µm filters (Millipore) before analysis. Injection volumes were 10 and 25 µl, respectively, for standard and sample extract(s).

Procedure

HPLC was performed as reported before.[21,22] For all separations, an ODS Hypersil Gold C_{18} reverse-phase column (250 mm × 4.6 mm, 5 μM particle size, maintained at 25°C) was used. The mobile phase consisted of water (solvent A) and acetonitrile (solvent B) which was applied in the following gradient for 45 min: 30% A, 70% B for 0-5 min, 70% A, 30% B for 15 min, 80% A, 20% B for 15 min, 30% A, and 70% B for 10 min. The flow rate was set at 0.8 mL/min. All separations were monitored at 280 nm.

DNA Isolation from Treated and Control Cells

DNA was isolated from adherent (live) and floating (dead) cancer (MDA, HepG2, A549) and normal cells (Vero) after 48h of treatment as reported previously.[22]

DNA Fragmentation Assay

Agarose gel electrophoresis (AGE) of isolated DNA from cells treated with SN extract(s) and respective controls was carried out as reported previously.[22]

Molecular Docking Studies

Selected phytoconstituents reported to be present in SN were subjected to molecular docking using AutoDock 4.0.1 docking program (Molecular Graphics Lab, Scripps Research Institute, La Jolla, CA 92037, USA) to understand the drug molecule interaction with prospective protein targets to investigate the potential binding modes and inhibition constants (K_i). The selected phytoconstituents belonged to the class of SGAs (solanine, solasonine, and solamargine) and SSs (degalactotigonin, nigrumnin I, and uttroside B). The criteria for selection were their previously reported structure activity relationships,[9,12,19,25] their demonstrated antitumor effects on various tumor cell lines[7,8,10,12,19,20] and prospective targeted metabolic pathways.[9,20] The structures of protein targets were downloaded from protein data bank (www.rcsb.org/pdb). The PDB IDs of the target proteins were as follows: Vimentin (PDB ID: 1GK4), G-actin (PDB ID: 1J6Z), F-actin (PDB ID: 2ZWH), and tubulin (PDB ID: 1TUB). All protein structures were subjected to refinement and energy optimization before docking analysis. PubChem and chEMBL databases were used for retrieval of 3D structures of the 8 phytoconstituents and 2 reference drugs in SDF format. All ligand structures were optimized using ADT version 1.5.6.

Statistical Analysis

All the experiments were done in triplicates and results were expressed as mean ± standard deviation of experiments done in triplicates.

RESULTS

Methanolic Extract(s) of SN Showed Cytotoxicity against Cancers Cell Lines (MDA, HepG2 and A549) with Little to no Effect on Normal Cells (Vero)

Cytotoxic activity of SN extracts against cancer cell lines was evaluated using two cell viability assays, namely, TBE and MTT assay. Both assays yielded comparable results [Figure 2]. From both TBE and MTT assay, it was found that methanolic extract of SN leaf was the most effective against HepG2 and MDA cells (IC_{50} value approx. 20 μg/mL) with the anticancer activity decreasing in the order leaf>stem>root [Figures 2a and b, 3d-f]. In case of Hep G2 cells, again the leaf extract possessed better activity as compared to that of root and stem [Figured 2b and 4d-f]. However, the stem extract was found to be most active against A549 cells (IC_{50} value of >40 μg/mL) with the activity decreasing in the order of stem>root>leaf [Figure 2c and 5d-f]. The extracts did not exhibit any significant anticancer activity against Vero cell lines at concentrations that were cytotoxic to human cancer cells, namely, 20–100 μg/mL [Figures 2d and 6d-f]. Figure 2 depicts the dose-dependent effect of SN root, stem and leaf extract on cancer and normal cell lines using MTT and TBE assay. Figures 3-6 depict the morphological analysis of untreated versus treated cancer and normal cells with respect to SN methanolic extract(s) at 50 μg/mL. The treated cancer cells displayed an altered morphology under inverted phase contrast microscope at 40× (figure not shown). The treated cells appeared more rounded in contrast to controls; this appearance coincided with the description of classical apoptosis. DMSO at concentration of 0.5% did not have any cytotoxic effect of its own [Figures 3-6a-c].

Doxorubicin HCl, a standard anticancer drug, was tested against all four cell lines in the range of 0.25–1.0 μM and it exhibited potent cytotoxic and dose-dependent inhibition of cell proliferation against cancer cells [Figure 7]. The IC_{50} of doxorubicin was found to be <0.25 μM with respect to MDA, HepG2 and A549 cells [Figure 8]. However, doxorubicin exhibited lesser toxicity against normal (Vero) cells at 0.25 μM

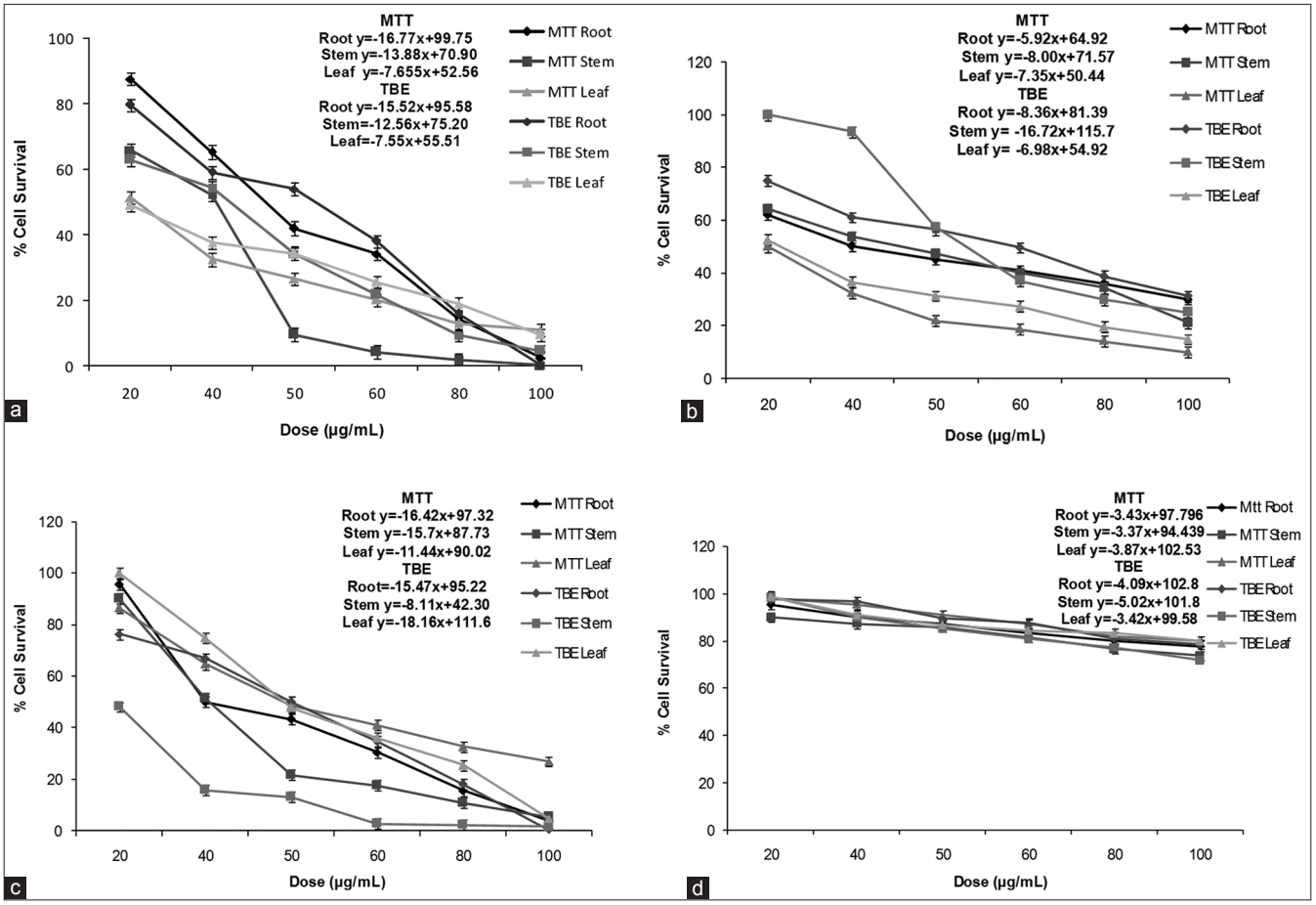

Figure 2: Dose response curves of methanolic extracts of *Solanum nigrum* root, stem, and leaf in 50% dimethyl sulfoxide (DMSO) on viability of (a) MDA (b) HepG2 (c) A549 and (d) Vero cells *in vitro* using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and trypan blue dye exclusion assay. Final concentration of DMSO in each well did not exceed 0.5% (v/v). Results are expressed as mean ± standard deviation of treatments done in triplicates

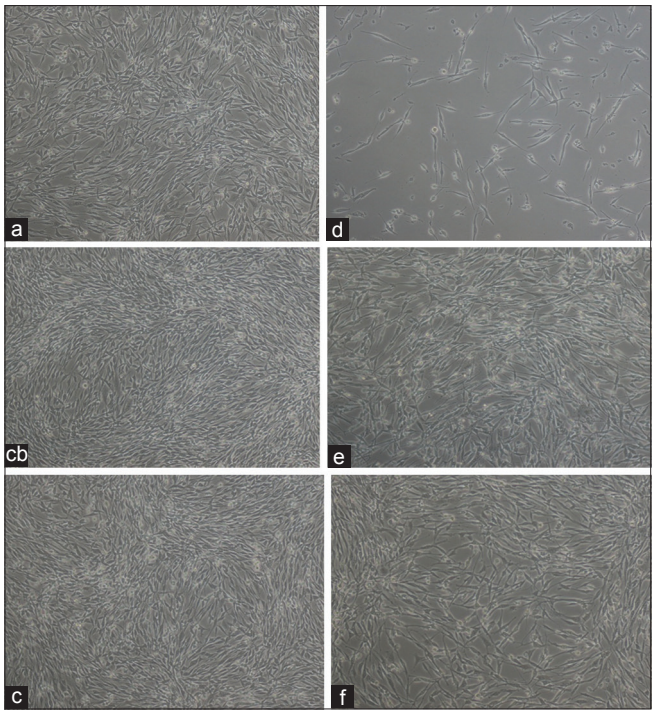

Figure 3: (a-c) Controls showing untreated MDA human breast cancer cells in 50% dimethyl sulfoxide (DMSO) and (d-f) in presence of methanolic extract of *Solanum nigrum* root, stem, and leaf at 50 µg/mL, respectively, in 50% DMSO after 48 h (10x). The final concentration of DMSO in each well did not exceed 0.5% (v/v)

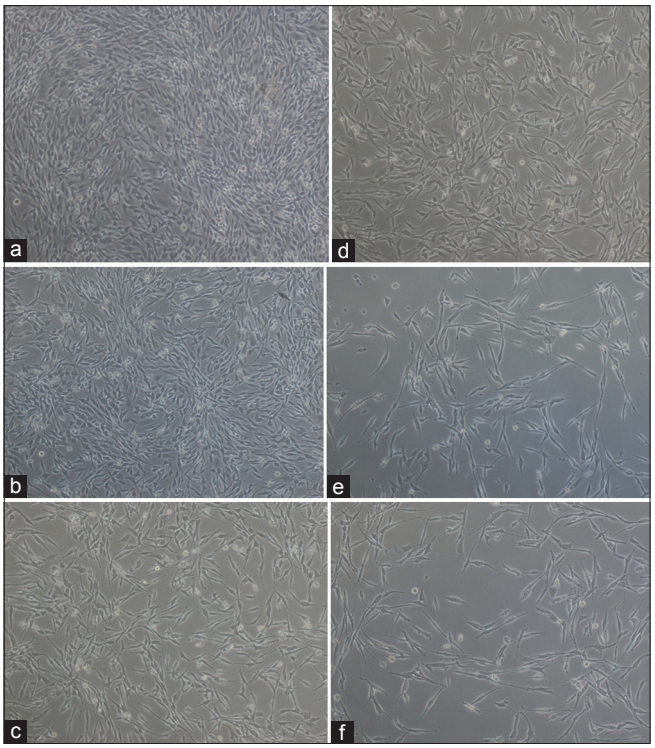

Figure 5: (a-c) Controls showing untreated A549 human lung carcinoma cells in 50% dimethyl sulfoxide (DMSO) and (d-f) in presence of methanolic extract of *Solanum nigrum* root, stem, and leaf at 50 µg/mL, respectively, in 50% DMSO after 48 h (10x). The final concentration of DMSO in each well did not exceed 0.5% (v/v)

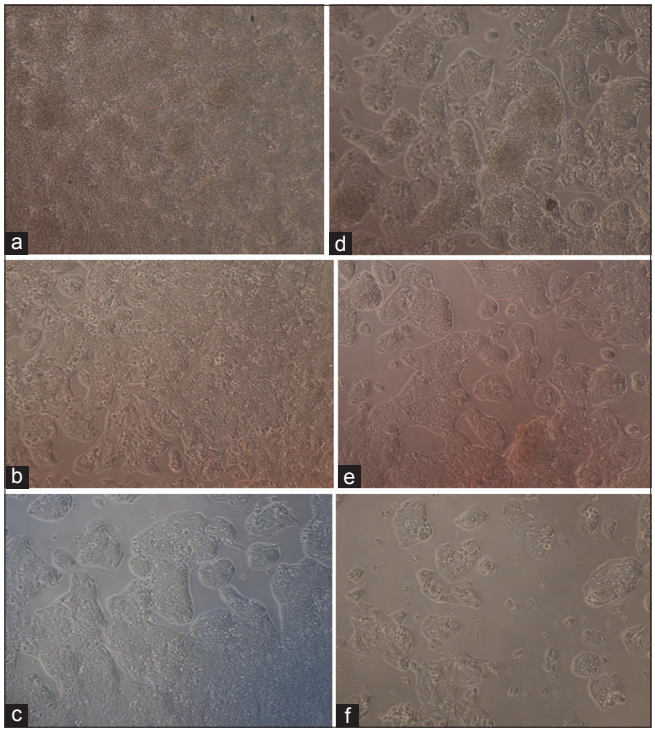

Figure 4: (a-c) Controls showing untreated HepG2 human liver carcinoma cells in 50% dimethyl sulfoxide (DMSO) and (d-f) in presence of methanolic extract of *Solanum nigrum* root, stem, and leaf at 50 µg/mL, respectively, in 50% DMSO after 48 h (10x). The final concentration of DMSO in each well did not exceed 0.5% (v/v)

as compared to cancer cells (IC_{50} < 0.5 µM); although Vero cells were found to be considerably sensitive to doxorubicin at higher concentrations [Figure 8].

The extracts were also evaluated for their probable antibacterial activity against two species of bacteria, namely, *S. aureus* and *E. coli* in the 2–20 mg range. However, none of the extracts showed any appreciable activity against the tested bacterial species (Figure 9a-f; images corresponding to concentration range of 12–20 mg/mL shown). In a study by Zubair *et al.* (2013), methanolic extract of SN leaf has been shown to possess a mild activity against four bacterial strains, namely, *P. multocida*, *E. coli*, *B. subtilis*, and *S. aureus*.[26]

Qualitative phytochemical characterization of extract constituents was carried out using HPLC (Figure 10b-d). The glycoalkaloid alpha-solanine was detected in all three extracts (R_t = 9.884 min).[27] Another SGA solamargine (R_t = 10.799 min) was also tentatively identified in all three extracts.[27] The analytical HPLC method used in the study provided a good baseline resolution of peaks of SGAs present in SN extracts with reference to standard. DNA isolated from HepG2 cells treated with SN root and stem extracts showed characteristic DNA ladder formation on AGE [Figure 11], further corroborating the fact that the methanolic extract(s) of SN induced apoptosis in cancer cells. However, no DNA fragmentation was observed in DNA isolated from Vero cells

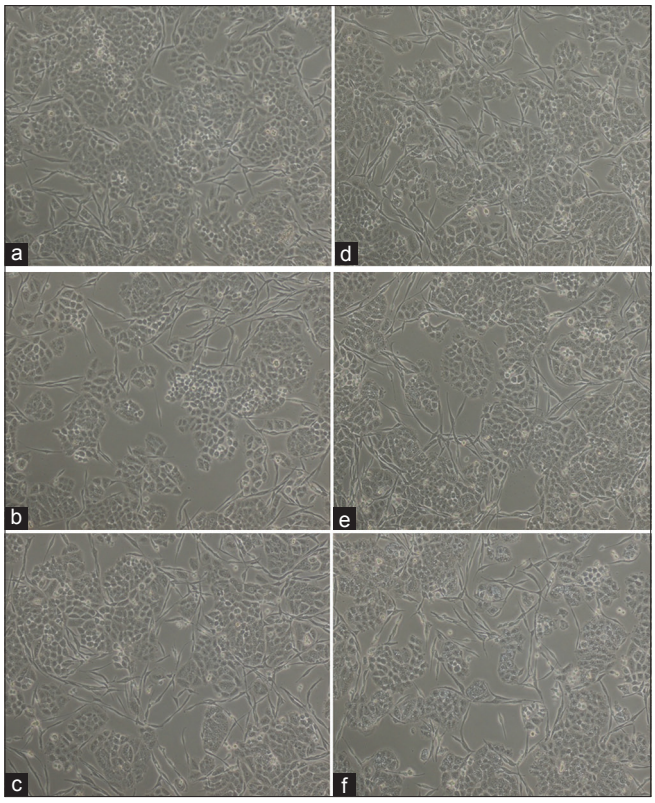

Figure 6: (a-c) Controls showing untreated Vero kidney epithelial cells in 50% dimethyl sulfoxide (DMSO) and (d-f) in presence of methanolic extract of *Solanum nigrum* root, stem, and leaf at 50 µg/mL, respectively, in 50% DMSO after 48 h (10x). The final concentration of DMSO in each well did not exceed 0.5% (v/v)

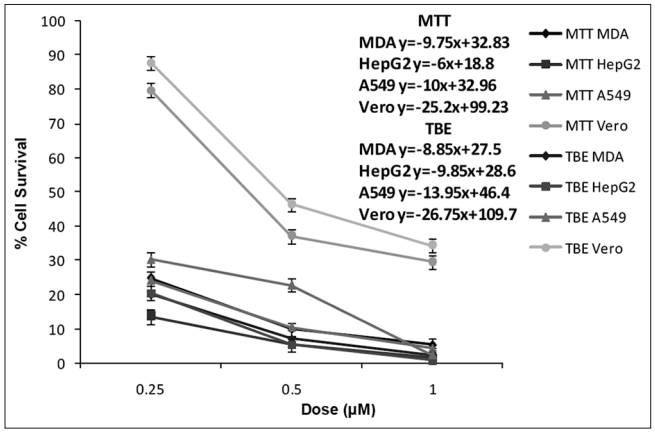

Figure 7: Dose response curves of doxorubicin HCl on cancer and normal cell lines using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and trypan blue dye exclusion assay. Results are expressed as mean ± standard deviation of treatments done in triplicates

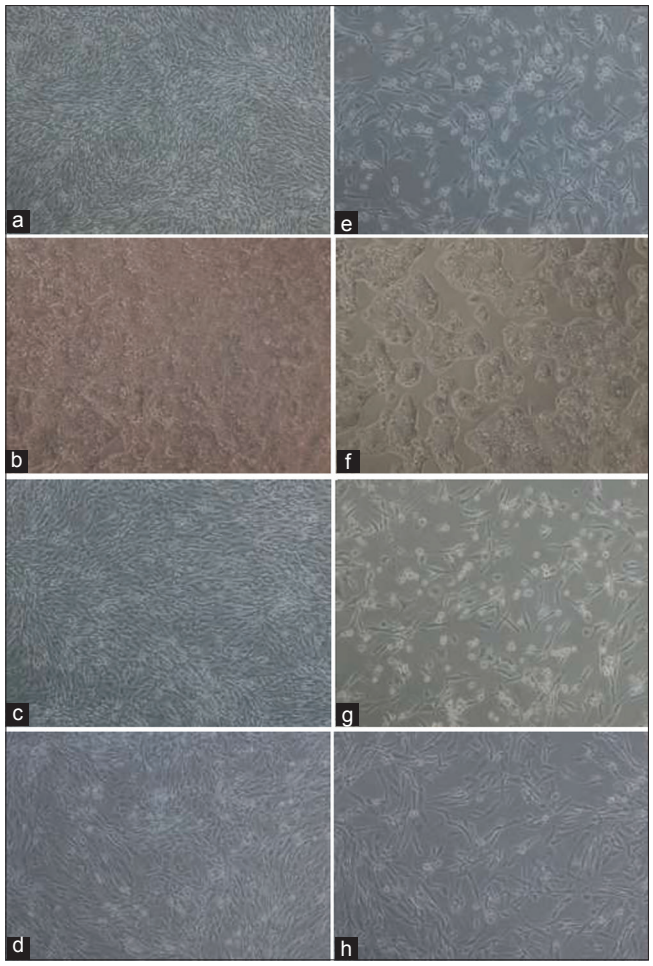

Figure 8: (a) Controls showing untreated MDA (b) Hep G2 (c) A549, and (d) Vero cells in presence of water (vehicle) and (e-h), respectively, show the cytotoxic activity of doxorubicin chloride at 0.5 µM after 48 h (10x)

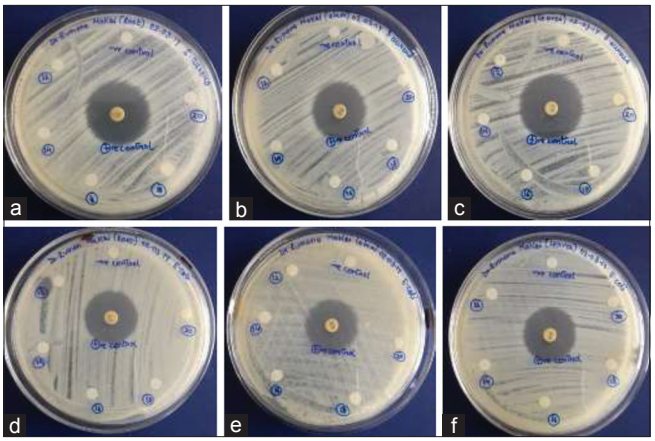

Figure 9: Antibacterial activity of methanolic extracts of *Solanum nigrum* root (a and d) stem (b and e) and leaf (c and f) against *Staphylococcus aureus* and *Escherichia coli* using disc diffusion method

treated with SN extracts and neither were they found to affect the survival and viability of normal cells [Figures 6d-f and 11].

Molecular docking analysis revealed that of the SAGs, solasodine had greater binding affinity to vimentin than solanidine and among the SGAs, solasonine had the greatest affinity for vimentin [Figure 12 and Table 1]. Of the SSs, nigrumnin-I displayed better binding to vimentin

Table 1: Docking results of major components in SN extract(s) with vimentin (PDB ID: 1GK4)

Phytoconstituent name	Chemical class	Binding energy (kcal/mol)	Inhibition constant (K_i)	Interacting amino acid (s)
Solanidine	SAG	−7.59	2.71 μM	Arg381, Ser339, Asn350, Arg342, Glu346, Asn388, Gln384, Glu349
Solasodine	SAG	−8.18	1.0 μM	Arg381, Asn350, Glu346, Glu349, Asn357, Asp385, Val353, Asn388
alpha-Solanine	SGA	−7.12	5.99 μM	Asn357, Ala356, Val353, Asp360, Glu349, Glu346, Arg381, Asn350
Solasonine	SGA	−7.82	1.87 μM	Ser339, Gln343, Leu380, Gln384, Ala377, Asn350, Arg381, Asp385
Solamargine	SGA	−6.95	8.1 μM	Asn 357, Ala377, Arg381, Leu380, Ser339, Val353, Thr361, Asp360
Degalactotigonin	SGA	−1.83	45.68 mM	Gln384, Met347, Asn360, Leu387, Asn388, Glu348
Nigrumnin I	SG	−3.81	1.61 mM	Leu380, Arg381, Ser339, Arg342, Gln343, Glu346, Val353, Glu354
Uttroside B	SG	−1.89	40.84 mM	Val353, Asn357, Glu374, Arg378, Ala377, Arg381, Leu380, Gln343
Doxorubicin HCl	Anthracycline	−9.42	124.64 nM	Asp385, Gln384, Arg381, Glu349, Glu346, Asn350, Glu346, Gln343
Tetracycline	Tetracycline	−6.88	9.11 μM	Arg381, Glu384, Asp385, Glu349, Asn350, Glu346, Asn350, Asn388

SN: *Solanum nigrum*, SAG: Steroidal aglycones, SGA: Steroidal glycoalkaloids

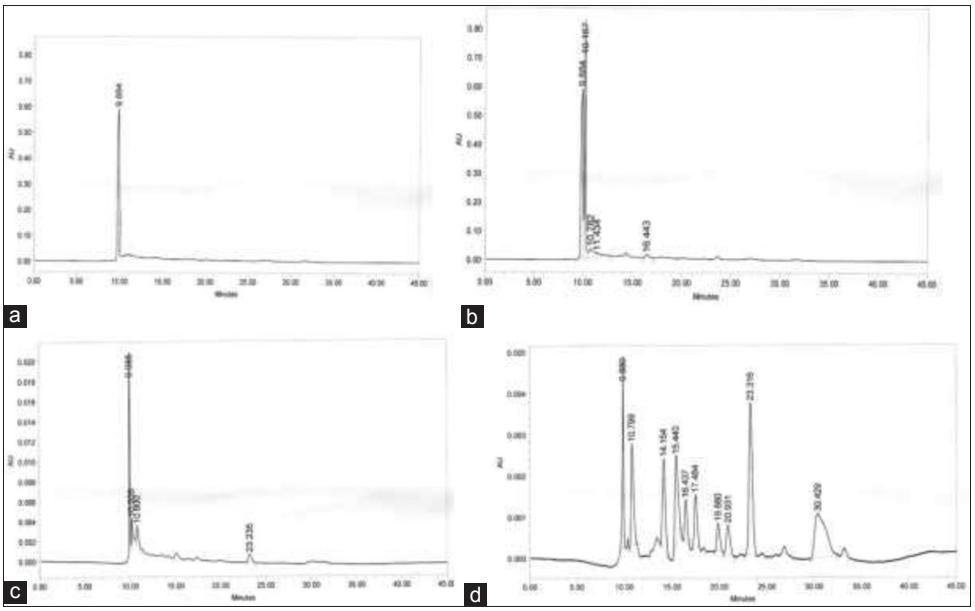

Figure 10: (a) high-performance liquid chromatography (HPLC) profile of alpha-solanine reference standard (R_t = 9.884 min) (b) HPLC profile of methanolic extract(s) of *Solanum nigrum* root (c) stem and (d) leaf under identical conditions

(K_i 1.61 mM) than the others. However, no significant binding of any of the phytoconstituents to actin and tubulin was detected *in silico*.

DISCUSSION

Medicinal plants are indispensable to human health. Traditional medicine comprises about 80% of the world population's need. Data review of medicinal plants reveals that many valuable phytochemicals are obtained from them many of which serve as panaceas and remedies for several ailments. Phytochemicals have structurally and sterically more complexity and diversity as compared to synthetic libraries of compounds.

Both crude extracts and isolated components of SN have been reported to possess significant antiproliferative activity against various cancer cell lines. Aqueous extracts have generally been reported to be prepared with dried berries, but other plant parts can also be used. The antiproliferative activities of the crude organic solvent-based extracts of SN and isolated compounds thereof have been studied on tumor cell lines of liver (HepG2),[28,29] colon (HT29 and HCT-116),[28,29] breast (MCF-7),[30] and cervical (U14 and HeLa).[15,25,31] DNA fragmentation assays have been used to analyze the extent of apoptosis in treated cells. Cytotoxic effects of a number of glycoalkaloids have also been studied on various cancer cell lines such as HepG2. Solanine has been reported to facilitate the opening of the permeability transition channels of mitochondria by lowering the membrane potential. This causes an increase of the intrinsic calcium ion level that culminates in apoptosis. Solanine also causes Bcl-2 inhibition leading to an increase in cytochrome c, which activates caspases and triggers apoptosis.[14] Very little work has been done regarding the structure and antiproliferative activity of other glycoalkaloids from SN. This paper reports a possible mechanism for the antiproliferative activity of these SGAs through inhibition of the intermediate filament (IF) protein vimentin.

Antimicrobial activity of methanolic extract and different fractions of SN leaves has been determined by disc diffusion

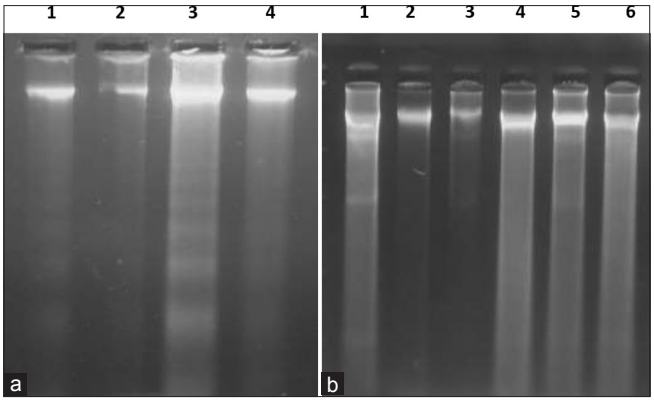

Figure 11: DNA fragmentation assay for detection of apoptosis, (a) Gel electrophoresis of DNA isolated from HepG2 cells (live+dead) in control and treated wells Lane 1, 3: DNA from HepG2 cells treated with 100 µg/mL of root and stem extracts of *Solanum nigrum* (SN) in 50% dimethyl sulfoxide (DMSO), respectively, Lane 2, 4: DNA from matched control cells treated with 50% DMSO, respectively. SN extracts induced DNA ladder formation, a hallmark property of apoptosis (Lane 1, 3), while no apoptosis was detected in DNA from control cells (Lane 2, 4), (b) gel electrophoresis of DNA isolated from Vero cells (live + dead) in control and treated wells Lane 2,4,6: DNA from Vero cells treated with 100 µg/mL of root, stem and leaf extracts of SN in 50% DMSO, respectively, Lane 1, 3, 5: DNA from matched control cells treated with 50% DMSO, respectively. No apoptosis was detected in DNA from control and SN extract(s) treated Vero cells

method as reported previously.[26] Leaf extract and fractions of SN were found to be mildly potent as antibacterial agents[26] while in our study, none of the methanolic extracts displayed any significant activity against the tested microbes.

Molecular docking has now become an indispensable tool in structural biology and computer-aided drug design. Its primary goal is to predict the binding of a putative ligand (mostly virtual) to a protein of known 3D structure and investigate the potential binding modes and inhibition constants (K_i) of the ligand thereof. The ligand-protein conformation having the lowest binding energy and K_i value is chosen as the best docking pose of the ligand in the binding site of the docked protein target as it is considered energetically favorable. Molecular docking is routinely employed in virtual screening of large libraries of compounds *in silico* as well as in lead optimization studies.

SGAs have been reported to bind to intracellular receptors by direct diffusion across the plasma membrane of target cells.[32] This binding might further activate the receptors, which then regulate the transcription of apoptosis-relative genes and as such, can be candidates for development of low toxicity antitumor agents. Probable inhibition of mammalian target of rapamycin (mTOR) enzymatic activity by SN leaf extracts needs to be investigated in detail.[9] Uttroside B isolated from SN leaf has also been reported to inhibit MAPK and mTOR signaling in Hep G2 cells.[20] Treatment of HepG2 cells with crude ethanolic extracts of SN leaf has shown enhancement in levels of proapoptotic proteins like caspase-3.[33]

IFs are one of three filament systems comprising the cytoskeleton of metazoa. IFs being highly dynamic are critical for proper

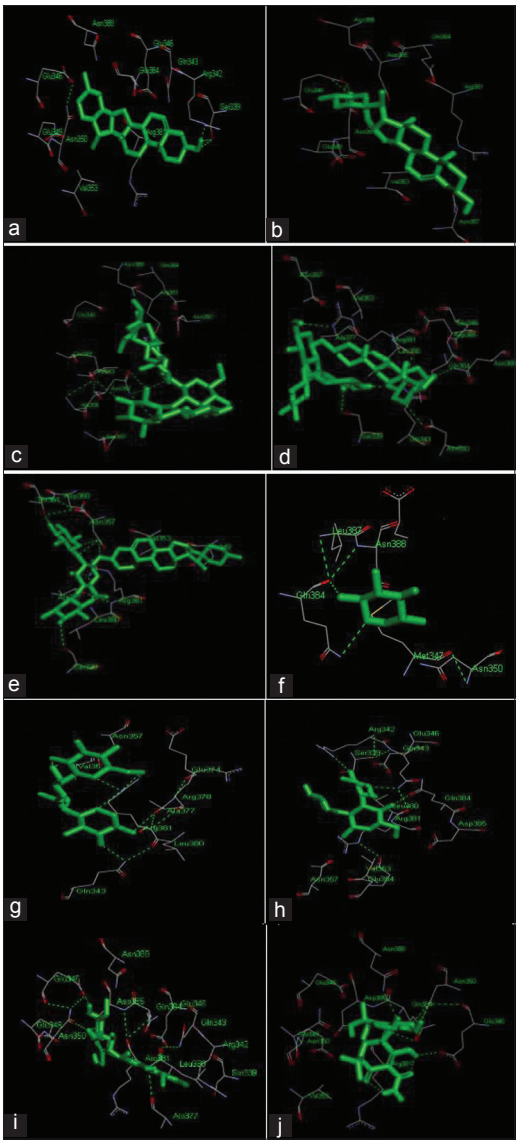

Figure 12: Best docking poses for binding of (a) solanidine (b) solasodine (c) alpha-solanine (d) solasonine (e) solamargine (f) degalactotigonin (g) uttroside B and (h) nigrumnin I versus (i) doxorubicin HCl and (j) tetracycline to vimentin

organization of the actin and tubulin filament systems and regulating cell motility, shape, structure, signaling, and adhesion during interphase and mitosis.[34] The most common IF is vimentin. Vimentin is a type III IF protein found in cultured and tumor cells as well as the majority of cells of mesenchymal origin. Vimentin filaments are considered a crucial marker of epithelial to mesenchymal transition (EMT),[35,36] a critical step in cancer metastasis.

Steroidal lactones and alkaloids have been shown to bind and inhibit vimentin, leading to aggregation of vimentin filaments *in vitro*.[37] Furthermore, it is known that cytoplasmic IFs, such as vimentin, are phosphorylated at mitosis, leading to their disassembly and reorganization in dividing cells.[38-45] As vimentin is abundantly expressed by mesenchymal cells and plays a critical role in angiogenesis, and in spread and growth

of cancer,[46,47] it is hypothesized that possible binding of glycoalkaloids (viz., alpha-solanine) to vimentin might inhibit IF disassembly leading to apoptosis, thus elucidating another possible mechanism of action of the well-known cytotoxic activity of the SGAs and saponins. This premise warrants further investigation *in vitro* and *in vivo*.

CONCLUSION

The present study evaluated the apoptotic activity of SN root, stem, and leaf extracts on cancer and normal cell lines. Antibacterial activity was tested against two bacterial species. Molecular docking was used to predict binding of selected phytoconstituents previously reported to be present in SN to cytoskeletal proteins actin, tubulin and vimentin *in silico*. IF protein vimentin was elucidated as a novel prospective target for SN aglycones and glycoalkaloids. Antiapoptotic/anticancer activity of SN extract(s) might be due to structural alteration/modification of cytoskeletal network, inhibition of IF disassembly and EMT inhibition along with induction of apoptosis. Methanolic extracts of SN could be potentially beneficial in treatment of cancers of liver, breast, and lung and may be of interest in future studies for developing integrative cancer therapy against proliferation, metastasis, and migration of cancer cells.

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