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## ***In vitro* Anticancer Activity and Cytotoxicity of *Solanum nigrum* on Cancers and Normal Cell Lines**

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### **ABSTRACT**

In this study, eighty percent methanol and chloroform extracts of leaves and stems of *Solanum nigrum* were screened for *in vitro* anticancer activity using PC-3 human prostate cancer cell lines and Hela cervical cancer cells and cytotoxicity assays were performed using MTT assay and 3T3 NIH mouse embryo fibroblast cell line and CC-1, a rat Wistar hepatocyte cell line. All extracts in concentration 100 µg mL<sup>-1</sup> showed anticancer activity in PC-3 and Hela and the highest percentage of growth inhibition obtained from stems methanol extracts on Hela 91.11% followed by leaves and stems methanol extracts (74.28 and 80.49, respectively) on PC-3. For cytotoxicity the result obtained indicate that all extracts had non-toxic effect on CC-1 and 3T3 cell lines with IC<sub>50</sub> > 100 µg mL<sup>-1</sup>, except the leaves methanol extract showed the highest percentage of growth inhibition in 3T3 85.63 with IC<sub>50</sub> 17.37 µg mL<sup>-1</sup>. The results obtained indicate that *Solanum nigrum* leaves and stems methanol extracts have anticancer activity on prostate cancer, cervical cancer and have non-toxic effect on 3T3 and CC-1. This result supports the traditional use of *Solanum nigrum* for the treatment of cancer in different regions of the Sudan.

**Key words:** Anticancer activity, medicinal plants, *Solanum nigrum*, cytotoxicity, phytochemical screening, Sudan

### **INTRODUCTION**

Many of the plant materials used in traditional medicine because it's readily available in rural areas at relatively cheaper than modern medicine (Mann *et al.*, 2008). Indeed, Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine (Ibrahim, 1997).

Breast and cervical cancer account for about 50% of all cancers in Sudanese women these two cancers remain the primary cause of death due to cancer (combined crude mortality 18.5/100 000) (Ferlay *et al.*, 2004).

Over 60% of currently used anti-cancer agents are derived in one way or another from natural sources, including plants, marine organisms and microorganisms. Indeed, molecules

derived from natural sources (so-called natural products), including plants, marine organisms and micro-organisms, have played and continue to play, a dominant role in the discovery which leads for the development of conventional drugs for the treatment of most human diseases (Cragg *et al.*, 1997).

The most common causes of cancer are variable due to economical, social and environmental factors which lead to increase in incidence of cancer i.e., from the year 1967-2006 there are about 50.000 cancer patients treated in Radiation and Isotopes Centre in Khartoum. The number of new cases is 10 times more than in year 1976, about 40.7% female and 59% male. The most common cause of cancer in the year 2004, according to the report form Radiation and Isotopes Center of Khartoum is breast cancer with 17.4%. In males, the most common cancers are prostate cancer 3.3% which is found around all Sudan, Non-Hodgkin's Lymphoma (NHL), followed by esophagus cancer which is found mainly in Northern part, bladder cancer 1.9% and stomach cancer 1.4%. In females, breast cancer is still the most common 17.4% while cervical cancer is second 5.5%, followed by ovarian cancer 3.5%, esophagus 3.3%, CML 1.5%, NHL 1.3% and stomach cancers and nasopharynx cancer 0.9% (RICK, 2004).

*Solanum nigrum* L. (Solanaceae) is locally called as Enab el Deib, Elmugad el aswad. Generally, black nightshade is very rich in nutritive values which are capable of supplying minerals, vitamins, proteins and certain hormone precursors (Dhellit *et al.*, 2006). It has been claimed that *Solanum nigrum* fruits in particular are an excellent remedy for liver disorders (Raju *et al.*, 2003). In Sudan there are plenty of medicinal plants used traditionally for cancer treatment which are not *in vitro* investigate to approve it. Therefore, for first time in Sudan this study aims to screen the anticancer activity of leaves and stems extracts of *Solanum nigrum* using two solvents on PC3 prostate cancer and Hela cervical cancer cell line, to study cytotoxicity using 3T3 and CC-1 cell lines by MTT assay and to screen these extracts phytochemically.

## **MATERIALS AND METHODS**

**Collection and identification of plant specimens:** Plant specimens were collected from February-May 2012 from Al-Gazira state. Identification was done in Plant Taxonomy, Herbarium Curator, Medicinal and Aromatic Plants Research Institute, National Research Center, Khartoum, Sudan and identified as *Solanum nigrum* L.

The leaves and stems were dried in the shade until a constant weight were obtained and ground to powder using mortar and pestle.

**Extracts preparation:** Fifty gram of the powdered leaves were macerated successively in chloroform, 80% methanol and kept for 5 days at room temperature with occasional shaking. Each mixture was then filtered and the filtrate was evaporated to dryness in an evaporating dish on a steam bath at a temperature of 70°C. The process was repeated four times with intervals of 5 days (Hagerman, 1987). These extracts were stored in screw-capped bottles covered with aluminum foils and kept in the laboratory refrigerator.

**Cytotoxicity assays using 3T3 and CC-1 cell-lines by MTT assay:** The antiproliferative activity of plant extracts was measured using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Lau *et al.*, 2004). The assay detects the reduction of MTT

by mitochondrial dehydrogenase to blue formazan product which reflects the normal function of mitochondria and cell viability.

*In vitro* cytotoxicity assays were performed as described by Lau *et al.* (2004), using the 3T3 NIH mouse embryo fibroblast cell line and CC-1, a rat Wistar hepatocyte cell line, from European Collection of Cell Cultures, (Salisbury, UK).

The CC-1 cells were cultured in Minimum Essential Media (MEM) supplemented with 10% Fetal Bovine Serum (FBS), 2 mM glutamine and 20 mM HEPES. The 3T3 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) formulated with 10% FBS. All of these cells are adherent cells and required to be detached from culture flask surfaces by trypsin/EDTA (Ethylenediaminetetraacetic acid) treatment. The media were removed from the cell culture and sterile Phosphate Buffer Saline (PBS) was added to each flask to wash cells from cell debris. To each flask, 0.25% Trypsin/EDTA solution was added to the attached cells and incubated for 2-3 min at 37°C. The flasks were gently tapped and observed under microscope to check for detachment of cells from flask surfaces followed by addition of media containing 10% FBS. Cells were collected in a 15 mL centrifuge tube and centrifuged at 1200 rpm. The pellet was resuspended in a complete media and cells were enumerated using microscope and Neubauer counting chamber.

The MTT assays on the 3T3 and CC-1 cells were performed using  $6 \times 10^3$  cell/well in a 100  $\mu$ L complete media in a flat-bottomed 96 wells plate. All plates were incubated for 24 h at 37°C in a CO<sub>2</sub> incubator. After attachment of cells, media was replaced by 200  $\mu$ L of media containing the test extracts at variable concentrations (100, 50, 25 and 12.5  $\mu$ g mL<sup>-1</sup>) in triplicates and further incubated for 48 h at 37°C in a CO<sub>2</sub> incubator. Following exposure to each test extracts, cell viability was assessed by using 0.5 mg mL<sup>-1</sup> of MTT in complete media for 4 h followed by the removal of supernatant and addition of 100  $\mu$ L of Dimethylsulfoxide (DMSO) to each well to solubilize the formazan complex formed by the action of mitochondrial dehydrogenases. Untreated cells were used as a negative control while, cells treated with Triton were used as a positive control at the following concentrations 0.01  $\mu$ g mL<sup>-1</sup>. The plates were read at 540 nm after one minute of gentle shaking. The optical density readings were recorded using MS Excel software and the percentage of antiproliferative and/or cytotoxic activity is calculated as:

$$\frac{(A-B)}{A} \times 100$$

where A and B are the OD<sub>540</sub> of untreated and of treated cells, respectively. The results were expressed as Means  $\pm$  SD of triplicate readings.

**Anticancer assays using PC3 and Hela-a cell lines by MTT assay:** *In vitro* anticancer assays were performed as described by Lau *et al.* (2004), using the PC3 prostate cancer cell line and Hela-a cervical cancer cell line, from European Collection of Cell Cultures, (Salisbury, UK).

**Phytochemical screening:** The dried extracts were reconstituted in the solvent used for their extraction and subjected to qualitative chemical screening to identify the presence of a variety of phytoconstituents. The methods used have been described by Harborne (1998) and Handa *et al.* (2008), to identify the following chemical classes: alkaloids, saponins, flavonoids, tannins, sterols, triterpenes, coumarins and anthraquinones.

**RESULTS**

The results showed that methanol was a good solvent for extracting anticancer substances from the tested plant (Fig. 1). This finding was based on the percentage of growth inhibition comparing with control positive. The highest percentage of growth inhibition was obtained by stems methanol extract on HeLa-a cell line and PC3 which were 91.11 and 80.49%, respectively in comparison with control positive 89.07%. Followed by leaves methanol extract on HeLa-a cell line and PC3 where the growth inhibition percentages were 84.86 and 74.28%, respectively. Overall, HeLa-a cervical cancer is more sensitive to these extracts than PC-3 prostate cancer. This observation was based on the number of extracts which showed growth inhibition.

*In vitro* cytotoxicity assays using the 3T3 NIH mouse embryo fibroblast cell line and CC-1, a rat Wistar hepatocyte cell line measured by MTT indicate that, all extracts of leaves and stems is non toxic for 3T3 and CC-1 cell line with  $IC_{50} > 100 \mu\text{g mL}^{-1}$ , except the leaves methanol extract showed the highest percentage of growth inhibition in 3T3 85.63 with  $IC_{50} 17.37 \mu\text{g mL}^{-1}$ . The least a viability of CC-1 was 55.20%, whereas the negative control was 100% and the positive control was 34.61% while the least a viability of 3T3 was 14.38% and the positive control was 12.29%, (Fig. 2, 3).

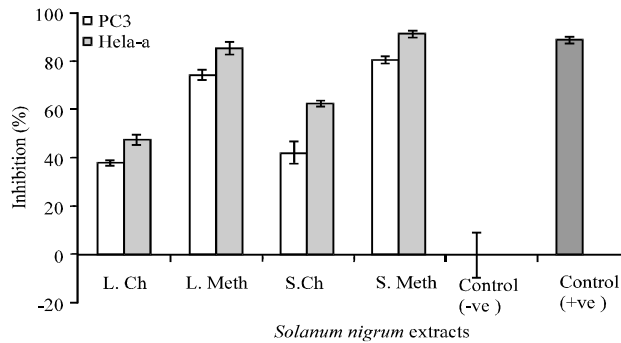


Fig. 1: Anticancer activity of *Solanum nigrum* extracts  $100 \mu\text{g mL}^{-1}$  on PC3 prostate cancer cell line and HeLa-a cervical cancer cell line as measured by the MTT assay, L: Leaves, S: Stems, Ch: Chloroform, Meth: Methanol

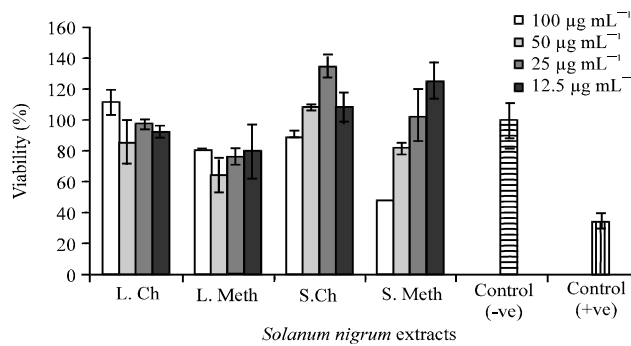


Fig. 2: Cytotoxicity of *Solanum nigrum* extracts on 3T3 NIH mouse embryo fibroblast cell line measured by the MTT assay, L: Leaves, S: Stems, Ch: Chloroform, Meth: Methanol

Table 1: Phytochemical screening of *Solanum nigrum* extracts

Parts used	Solvents used	Fla	Alk	Sap	Tan	An	Cou	Tri	Ste
Leaves	Chloroform	-	-	-	-	-	+	+++	+++
	Methanol 80%	-	+	+++	+	-	-	+	-
Stems	Chloroform	-	-	+	-	-	+	++	-
	Methanol 80%	-	+	+++	++	-	+	+	-

Fla: Flavonoids, Alk: Alkaloids, Sap: Saponins, Tan: Tannins, Ste: Sterols, Cou: Coumarins, Tri: Triterpens, An: Anthraquinones, +: Positive reaction trace concentration, ++: Moderate concentration, +++: High concentration -: Negative reaction

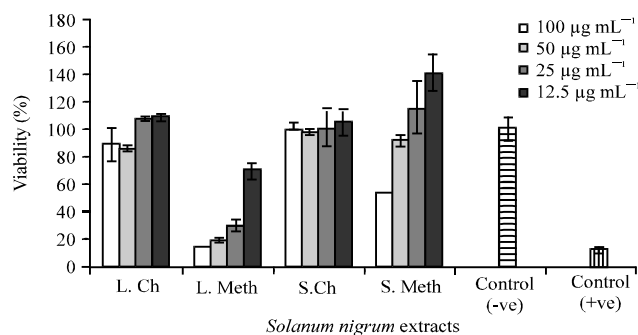


Fig. 3: Cytotoxicity of *Solanum nigrum* extracts on CC-1 a rat Wistar hepatocyte cell line as measured by the MTT assay, L: Leaves, S: Stems, Ch: Chloroform, Meth: Methanol

From Table 1, the phytochemical screening result showed that alkaloids, saponins, tannins, sterols, triterpenes and coumarins were present in which extracts with difference in concentrations, whereas flavonoids and anthraquinones were absent.

## DISCUSSION

Anticancer activity showed that the methanol extracts were more active than chloroform extracts. This may indicate that the polar active principles are responsible for the anticancer activity in *Solanum nigrum*. This finding agreed with Patel *et al.* (2009) who evaluated the anticancer activity of the fruits of *Solanum nigrum* on the Hela cell line. The fruits of *Solanum nigrum* methanolic extract were tested for its inhibitory effect on Hela cell line and has significant cytotoxicity effect on Hela Cell Line in concentrations ranged between 10-0.0196 mg mL<sup>-1</sup> by using SRB assay and the study also showed that the inhibitory action on Hela cell line in concentrations ranged between 10-0.0196 mg mL<sup>-1</sup> by using MTT assay.

*S. nigrum* elaborated a wide spectrum of medicinal properties such as anticancer, antioxidant (Al-Qirim *et al.*, 2008), neuroprotective (Jainu and Devi, 2006; Caragay, 1992) reported that plants contain several phytochemicals which possess strong antioxidant activities. The antioxidants may prevent and cure cancer and other diseases by protecting the cells from damage caused by free radicals, the highly reactive oxygen compounds. Many plant-derived products have been reported to exhibit potent antitumor activity against several rodent and human cancer cell lines. Moreover, phytochemicals such as vitamins (A, C, E and K), carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, pigments, enzymes and minerals have been found to elicit anticancer activities. These chemicals block various hormone actions and metabolic pathways that are associated with the development of cancer (Prajapati *et al.*, 2003).

Several studies evaluated the relationships between the antiproliferative activity of plant products and their phenolic content. A correlation exists between the structural oxidation state and the position, number and nature of the substituents of the polyphenolic compounds and their antiproliferative effects (Yanez *et al.*, 2004). Since phenolics act as antiproliferative agents through the cell cycle, they may liquidate the tumor cells by this mechanism (Conforti *et al.*, 2008).

Furthermore, triterpenes and saponin ingredients of *Solanum nigrum* may contribute to the strong anticancer activities (Dzhambazov *et al.*, 2002).

The result supports the traditional use of *Solanum nigrum* for the treatment of cancer and various infectious diseases in different regions of the Sudan. More investigation is needed to know the mechanism of growth suppression of tumor cell lines and whether it induces apoptosis or not and an intensive work is needed to isolate the bioactive components of these plants.

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