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Original Research Article

Cytotoxicity Potential of *Nelsonia canescens (Lam.) Spreng* Extracts against Cervical Cancer Cell Lines

Ouattara Nabèrè[1*], Ramdé-Tiendrébeogo Alphonsine[2], Guenné Samson[3], Sumera Zaib[4], Meda Nâg-Tiero Roland[5], Konaté Kiessoun[6], Jamshed Iqbal[4], Hilou Adama[3] and Kiendrébeogo Martin[3]

[1]Laboratory of Applied Biochemistry and Chemistry, UO I, JKZ/ University of Dedougou, BP 176, Burkina Faso
[2]Department of Traditional Medicine and Pharmacopoeia-Pharmacy / Institute for Research in Health Sciences (MEPHATRA-PH / IRSS), 03 BP 7192 Ouagadougou 03, Burkina Faso
[3]Laboratory of Applied Biochemistry and Chemistry, University of Ouaga I, Professor Joseph KI-ZERBO / University of Dedougou, 03 BP 7021 Ouagadougou 03, Burkina Faso
[4]Department of Pharmacy, COMSATS Institute of Information Technology, University Road, Tobe Camp, Abbottabad, Abbottabad, Pakistan
[5]Laboratory for Research and Education in Animal Health and Biotechnology, University of Nazi BONI, 01 BP 1091 Bobo-Dioulasso 01, Burkina Faso
[6]Laboratory of Food Biochemistry, Enzymology, Biotechnology and Bioinformatic, University of Ouagadougou, 03 P.O.Box: 848, Ouagadougou 03, Burkina Faso

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***Corresponding author:** Ouattara Nabèrè

Abstract

Nelsonia canescens is a small perennial herb widely used in Burkina Faso traditional medicine for the treatment of any diseases. In the present study, the purpose was to investigate the potential cytotoxicity and selectivity of different extracts from *N. canescens* on human cervical cancer HeLa and SiHa cell lines. MTT assay revealed higher cytotoxic activity of methanol extract in a dose-dependent manner against both HeLa and SiHa cells with respective IC_{50} values of 21.72 ± 0.08 μg/mL and 25.30 ± 0.44 μg/mL. Methanol extract (ME) showed the best selectivity index value of 7.93 ± 0.03 with HeLa cell line and Ethyl acetate extract (EAE) showed the best selectivity index value of 7.48 ± 0.05 with SiHa. The potential cytotoxicity and selectivity of *N. canescens* extracts against HeLa and SiHa cells line suggest that this species could be exploited further as a potential lead in cervical cancer treatment.

Key words: Cervical cancer, HeLa, SiHa, *Nelsonia canescens* extracts.

INTRODUCTION

Cancer is considered as the second killer of human death in the world [1]. According to the World Health Organization (WHO), the number of people suffering from cancer in the world will continue to grow steadily, and it is estimated that the number of newly-increased cancer patients will reach 19 million or even more by 2025. Especially, cervical cancer is an important public health problem for adult women [2], where 80% of cases occur in the developing world [3]. It is the fourth most common cancer among women globally, with 570,000 new cases and 311,000 deaths (7.5% of all female cancer deaths) in 2018 [4]. The major risk factors associated with cervical cancer development include high-risk human papilloma virus (hrHPV) infection, age, smoking, childbirth, use of oral contraception, and diet [5]. For traitement of various cancers, as the use of concurrent platinum-based chemoradiation often leads to severe toxicity, complementary and alternative medicine is recently becoming popular [6]. In recent years, new drugs have been discovered from natural plant products and it has been proved to have the potential to cure cancer. Invent of natural anticancer drugs paved a way for cancer treatment with no or less side effects. The use of natural extracts that we use in day to day life is an excellent alternative for chemical drugs used in cancer treatment [7].

Nelsonia canescens, a small perennial herb with soft decumbent villous branches [8] is traditionally used for malaria, cancer, gout, cardiovascular and inflammatory diseases treatments[9-12].

Previous studies of *Nelsonia canescens* methanol extract showed a good anti-tumor activity on breast-cancer cell line U373 with a IC_{50} of 27.04 ± 1.55 µg/mL [13] and Phytochemical investigations by HPLC-MS revealed the presence of five phenol acids (p-coumaric acid, caffeic acid, chlorogenic acid, ferulic acid and gentisic acid) and three flavonoids (apigenin,

luteolin, Quercetol) [14]. However, this plant species is widely used traditionally in the treatment of cervical cancer, but little scientific evidence supports its use. In the present study, we investigated the anticancer activities of *Nelsonia canescens* on human cervical cancer HeLa and SiHa cell lines.

MATERIALS AND METHODS

Cell Cultures.

In this research, two human cervical cancer cell lines HeLa (ATCC® CCL-2™) and SiHa (ATCC® HTB-35™) and one normal fibroblast (L929) cell line are used. These cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), L-glutamine, streptomycin (100 μg/mL), and penicillin (100 U/mL). All cells were incubated in humidified air with 5% CO_2 incubator at 37°C.

Preparation of Nelsonia canescens Extracts.

The plant material is constituted of the whole plant of *Nelsonia canescens*. This plant was selected basing on the ethnobotanic investigation of the professor Nacoulma [15]. The plant was identified and authenticated by professor Millogo, botanist at the Ouagadougou University. Whole plants of *N. canescens* collected in August 2017 in Loumbila, 15 km north of Ouagadougou, capital of Burkina Faso, were dried at room temperature and ground to fine powder. Twenty five grams of powdered plant material was macereted successively with 250 mL thrice of hexane, of dichloromethane, of ethyl acetate, of Methanol and acidified water. Thereafter, extract solutions were concentrated under reduced pressure in a rotary evaporator (BÜCHI, Rotavopor R-200, Switzeland) and dried at ambient temperature. The extracts obtained were weighed and packed in waterproof plastic flasks and stored at 4°C until use.

Then, extracts were dissolved with dimethyl sulfoxide (DMSO) with a final concentration of DMSO in extracts less than 0.5% for preparation of various concentrations for cytotoxicity and other assays.

Cytotoxicity and Selectivity Assay

The cell viability was evaluate by the MTT (3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide) dye reduction assay (Sigma, USA). This colorimetric assay utilizes the enzymatic activity of mitochondrial succinate dehydrogenase enzyme found in living cells. The enzyme reduces the yellow water-soluble MTT to formazan insoluble purple colored crystals that are measured using a spectrophotometer [16] and adapted by Kamini *et al.* 2015 [17]. Then, the activity of the enzymes to produce formazan is inversely proportional to the level of cell inhibition and directly proportional to the level of cell viability [18].

Briefly, Cells were maintained as a monolayer culture in their culture media supplemented with 10% fetal bovine serum and incubated at 37°C in a humidified incubator at 5% CO_2. The cells were seeded in 96-well plates to a final volume 100 µl media containing 2.4×104 cells per well, enumerated using the trypan blue dye exclusion method. The plates were incubated for 24 hours, then the extracts were added in row H and serially diluted up to ow B in their respective wells at concentrations of 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml, 15.625 µg/ml and 7.813 µg/ml. Rows A, of the 96 well plates were left untreated to serve as a negative cell control. After 48 hours of incubation, the culture medium in the plates was discarded, followed by washing step using phosphate buffered saline (PBS). A volume of 10 µl of PBS solution containing 5 mg/ml MTT dye (5 mg of MTT, dissolved in 1 ml serum-free PBS) was added to all the wells and incubated for another 4 hours after which 100 µl of 100% DMSO was added. The plates were read using a scanning multiwell spectrophotometer (Multiskan Ex lab systems) at 562 nm and 690 nm as reference. The proliferation rate of the cells was calculated using the following formula [19, 20].

$$Proliferation\ rate = \frac{At\text{-}Ab}{Ac\text{-}Ab}$$

$$Pourcentage\ of\ viability = \frac{At\text{-}Ab}{Ac\text{-}Ab}\ x100$$

$$Pourcentage\ of\ inhibition = 100\text{-}\frac{At\text{-}Ab}{Ac\text{-}Ab}\ x100$$

At = Absorbance value of test extract
Ab = Absorbance value of blank

Ac =Absorbance value of negative control (cells plus media)

The selectivity index (SI) was determined by the ratio of the EC_{50} value of the extracts on normal cells (L929) to the IC_{50} value of the extracts on cancer cells (HeLa or SiHa). Samples with an SI greater than 3 were considered to have a high selectivity towards cancer cells [21].

STATISTICAL ANALYSIS

The experiments were repeated in three times and data were expressed as means ± standard deviation (SD). One-way ANOVA was used to compare means. A p value < 0.05 was considered to indicate a statistically significant difference.

RESULTS AND DISCUSSION

Using the MTT assay, we evaluated the antiproliferative activity of *Nelsonia canescens* extracts against humain cervical cancer cells (HeLa and SiHa) and normal fibroblast cells (L929). These cancer and normal cells were tested using five different extracts sus as Hexane, Dichloromethane, Ethyl Acetate, Methanol and Acidified water extracts at various concentrations (7,816-500 μg/mL). In this study, the finding is that all the extracts presented an inhibition, but the best inhibitions were obtained with polar extracts (table 1). Then, it's obtained that both HeLa and SiHa cancer cells line showed remarquable dose-dependent susceptibility with the different concentrations of extracts (Figure1). When the concentration of the extract increase, the number of viable cancer cells decrease. Interresting, the IC_{50} value obtained are respectively 21.72 ± 0.08 µg/ml and 25.30 ± 0.44 µg/ml with methanol extracts (ME), 54.95 ± 1.13 µg/ml and 43.87 ± 0.81 µg/ml with acidified water extracts (AWE) and 421.83 ± 3.40 µg/ml and 64.93 ± 0.85 µg/ml with ethyl acetate extracts (EAE).

The selectivity index (SI) is a value who indicates the safety of an extract used for anticancer therapy. It was calculated based on the ratio of the EC_{50} value of different extracts on normal L929 cells to the IC_{50} value of the same extracts on human cervical cancer cells (HeLa and SiHa). So, a SI value greater than 3 was regarded as highly selective [21]. Considering hour extracts, Methanol extract (ME) displayed high selectivity with a SI value of 7.93 ± 0.03 following by Acidified Water Extract (AWE) with 3.04 ± 0.05 for HeLa cells. It's showed in this study that SiHa cells were more selected with Ethyl acetate extract (EAE), Methanol extract (ME) and Acidified Water Extract (AWE) with the respective values of 7.48 ± 0.05, 6.81 ± 0.05 and 3.81 ± 0.05 (Table 2).

Table-1: Inhibition concentration 50% (IC_{50}) of extracts of *Nelsonia canescens* inoculated in HeLa and SiHa cell lines. All the results presented as Inhibition concentration at 50% mean ± SD (n=3)

Extract	HeLa	SiHa
HE	>500	>500
DCME	>500	>500
EAE	421.83 ± 3.40	64.93 ± 0.85
ME	21.72 ± 0.08	25.30 ± 0.44
AWE	54.95 ± 1.13	43.87 ± 0.81
Key: HE= *Hexane Extract* ; DCME = *Dichloromethane Extract* ; EAE = *Ethyl Acetate Extract* ; ME = *Methanol Extract* and AWE = *Acidified Water Extract*		

Table-2: Anti-proliferative activity and selectivity index (SI) of Nelsonia canescens extracts against HeLa, SiHa and L929 cell line. The results are presented as anti-proliferative activity at 50% mean ± SD (n=3)

Extracts	L929	HeLa		SiHa	
	EC_{50}	IC_{50}	SI	IC_{50}	SI
HE	>500	>500	nd	>500	nd
DCME	>500	>500	nd	>500	nd
EAE	485.80 ± 3.29	421.83 ± 3.40	1.15 ± 00	64.93 ± 0.85	7.48 ± 0.05
ME	172.23 ± 1.43	21.72 ± 0.08	7.93 ± 0.03	25.30 ± 0.44	6.81 ± 0.05
AWE	167.12 ± 0.35	54.95 ± 1.13	3.04 ± 0.05	43.87 ± 0.81	3.81 ± 0.05
Key: HE= *Hexane Extract* ; DCME = *Dichloromethane Extract* ; EAE = *Ethyl Acetate Extract* ; ME = *Methanol Extract* and AWE = *Acidified Water Extract* nd = not determinated					

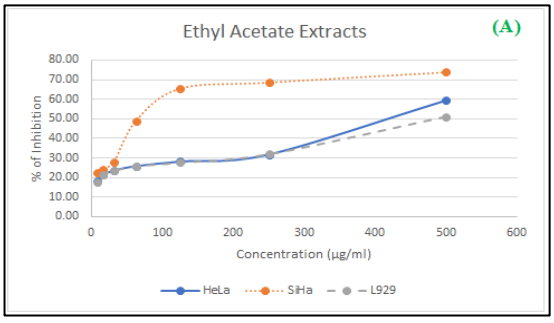
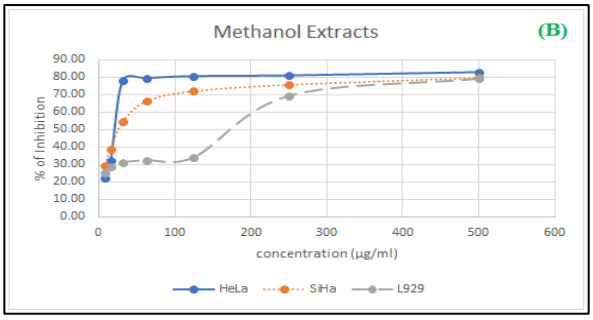
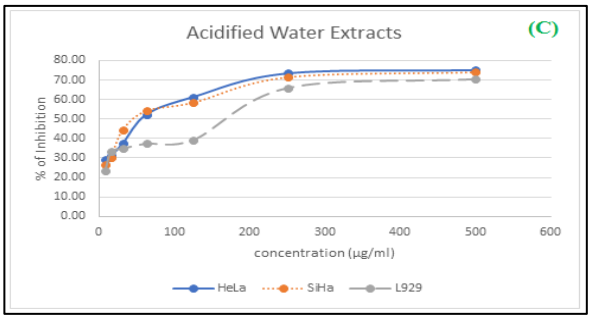


Figure-1: Dose-dependent Cell survival curves on HeLa, SiHa and L929 cell lines determined by MTT assay for 72h of treatment with Ethyl acetate extract (A), Methanol extract (B) and Acidified water extract (C) at concentrations of 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml, 15.625 µg/ml and 7.813 µg/ml. Data are shown as mean ± SD of three independent experiments (p<0.05; one-way ANOVA).





In addition to the present study, previous studies of *Nelsonia canescens* methanol extract showed a good anti-tumor activity on breast-cancer cell line U373 with a IC_{50} of 27.04 ± 1.55 µg/mL [13]. However, according to the American National Cancer Institute (NCI), a plant extract with an IC_{50} less than 30 µg/mL was interesting for research of antitumor compounds[22]. These two different studies using methanol extracts show that the most anticancer compounds in this medicinal plant were extractible with polar solvent including methanol. Interesting, in previous phytochemical investigations by HPLC-MS using methanol extract of this plant, we revealed the presence of five phenol acids (p-coumaric acid, caffeic acid, chlorogenic acid, ferulic acid and gentisic acid) and three flavonoids (apigenin, luteolin, Quercetol) [14]. Numerous researches noted the anticancer potential of these various molecules from herbal. So, it is demonstrated by any studies the potential anticancer activities Apigenin against cervical cancer [23, 24]. in addition, this compound associated with interferon gamma (IFNγ) is a good combination therapy against cervical cancer cell line HeLa and SiHa [25]. According to numerous studies, Luteolin is known for it potent prevention and inhibition against cervical cancer [26]. Quercetin decreased cervical cancer cell proliferation and induced cell death [27]. It is showed that caffeic acid isolated from several kinds of vegetables and herbals had anti-proliferative effects on cervical cancer cell lines, this compound can significantly reduce the proliferation of HeLa cells in a time-dependent manner[28]. In another study, it was demonstrated that Ferulic acid significantly inhibit cell proliferation and invasion in Hela and Caski cells[29]. All these researches about compounds presented in *N. canescens* supported the potential cervical anticancer of this plant extracts. Regarding the selectivity index (SI) in this study, the high selectivity in cytotoxic response between cancer and normal cell lines could be due to unique or combinate effect of compounds in *N. canescens* which could serve as a novel anticancer drug.

CONCLUSION

Serial dilution of different extracts from *Nelsonia canescens* showed in a dose-dependent manner high cytotoxicity on HeLa and SiHa cervical cancer cells and less cytotoxicity with the normal fibroblast cells L929. But, particularly, methanol extract exhibited a remarkable higher cytotoxic activity by MTT assay. The selectivity index obtained showed that the species contain active compounds with anticancer potential. Further molecular investigations are necessary in order to isolate the main active compound for any biological tests.

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