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INHIBITION OF A HeLa CELL LINE WITH THE APPLICATION OF BLACK RADISH (*Raphanus sativus* L. var. niger) EXTRACTS

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Abstract

Raphanus sativus L. var. niger, belongs to the crucifer family, has relevance in traditional medicine for its many uses in ethnobotany. It is used in the treatment of various problems associated with the biliary system (stimulates bile secretion), acts against gallstone, liver and gastrointestinal. The objective of this research article was to determine the percentage of growth inhibition of cervical cancer cells by fine MTT assay from extracts of *Raphanus sativus*.

Keywords:

Black Radish, anticancer, isothiocyanates, HeLa.

Introduction

Raphanus sativus L. var. niger, belongs to the crucifer family, has relevance in traditional medicine for its many uses in ethnobotany. It is used in the treatment of various problems associated with the biliary system (stimulates bile secretion), acts against gallstone [1], liver and gastrointestinal [2]. Phytochemicals containing in *Raphanus* are: sapogenins; polyphenolic compounds such as vanillic acid, protocatechuic acid; enzymes such as catalase, phosphatase, sucrase, amylase, alcohol dehydrogenase and pyruvate carboxylase; amino acids such as lysine and methionine; raphanine antibacterial substances as sulphoraphen (acts against *Streptococcus*, *Pyococcus*, *Pneumococcus* and *Escherichia coli*) [3]. The characteristic elements of the root of black radish are sulfur compounds called glucosinolates about. *Raphanus* major isothiocyanates are: hexyl isothiocyanate (18.4%), 4-methylthiobutyl isothiocyanate (17%), 4-methylpentyl isothiocyanate (8.4%), 4-methylthio- (3E) -butenyl isothiocyanate (5.2%), 4-methylthio-(3Z)-butenyl isothiocyanate (4.7%) and isoamyl isothiocyanate (2.4%). Salts containing horseradish roots trace elements such as aluminum, barium, lithium, manganese, silica, fluorine and iodine (about 18 mg/100 g). The black radish causes moderate gallbladder contraction, which facilitates the removal

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of debris. It is known to be a liver drain. Recent studies have demonstrated the protective effect of extracts of the root of black radish against hepatotoxicity induced lipid peroxidation. The black radish has been used against biliary dyskinesia and hepatobiliary, hepatitis and elevated transaminases disorders. Radish contains glucose as the main sugar and small amounts of fructose and sucrose. The content of pectin in the form of calcium pectate is 0.3%. Recently, there have been profiled radishes as an alternative for the treatment of a variety of mortals diseases such as cancer and AIDS. Also they contain nutraceutical properties. The objective of this work was to determine the percentage of growth inhibition of cervical cancer cells by fine MTT assay from extracts of *R. sativus*.

Materials and methods

Obtaining extracts *R. sativus* three replicates of black radish (*R. sativus* L. var. niger) were used. With a scalpel the fruit rind carried semithin extracted. The extracted cortex was collected in a beaker of 500 mL, previously washed and sterilized. 30 mL Erlenmeyer flasks were weighed on an analytical balance and 2 g of bark *R. sativus* were added to each flask. 10 repetitions were added 50 mL of ethanol (Merck). The flasks were covered with an aluminum cap and labeled. *R. sativus* samples, which were obtained by ethanolic extraction, gravity filtered with a conical funnel and filter paper to remove solid residues. Extractive suspension from each flask was collected in test tubes cap glass and labeled. The contents of the flasks were evaporated with an electric grill. Finally, it was added 1 mL of the dried samples corresponding to fruit and bark of each flask *R. sativus* solvent.

Inhibition Test

To test the cytotoxicity of the ethanolic extracts of *R. sativus*, the method employed by Villavicencio (2008) in which a culture of the cell line HeLa uterine cervical cancer was used maintained in DMEM (Dulbecco's Modified Eagle Medium was followed) supplemented with 10% fetal bovine serum, in a humidified atmosphere with 5% CO_2 at 37 ° C. In tests 24 well plates, where 5000 HeLa cells per well were seeded in 100 µl of medium, they were treated with ethanolic plant extracts in different concentrations (0.05, 0.1, 0.2, 0.5, 1.0 were used, extract 1.5 and 2.0/100 µl of medium). A control cells not treated with extracts, were added the same amounts of ethanol to the treated cells. Cultures were incubated under the above conditions and at different times, 12, 24 and 48 h, cytotoxicity was determined by estimation of cell viability using the technique of crystal violet, for which the cells with ethanol were set to 70% for five min, then to each well crystal violet was added to 1% dissolved in water, ten minutes after acid acetic 33% was added, the absorbance reading at 570 nm (OD_{570}) are made directly from the wells by placing the plates in an ELISA reader. The tests were conducted with six to seven concentrations. With the data obtained by measuring the OD_{570} percent viability as 100B/A where A and B were the average values of OD_{570} of untreated and treated cells, respectively, were calculated. With the percentage values of cell viability and concentration tested be developed graphic concentration-effect on the curves obtained the 50% (IC_{50}), which is defined as the concentration of the extract and reducing the viability of inhibitory concentration estimate 50% treated cells compared to untreated cells.

Experimental design for obtaining extracts of *R. sativus*

The experimental design consisted of extraction with isothiocyanates by three organic solvents (chloroform, methanol and ethanol), with 20 repetitions (10 for the extraction of fruit and 10 for removing bark) for each type solvent.

Experimental design for the test

The extracts were applied to cells that were seeded in boxes (P100, 100 mm) or 24 well plates, where they were inoculated 50,000 HeLa cells per well in 1 mL of DMEM (+) (Supplemented with fetal bovine serum) and cultured for 24 h at 37 °C in humidified atmosphere with 5% CO_2 in an incubator Thermo Scientific. The extracts were as follows distributed in the plates (Figure 1).

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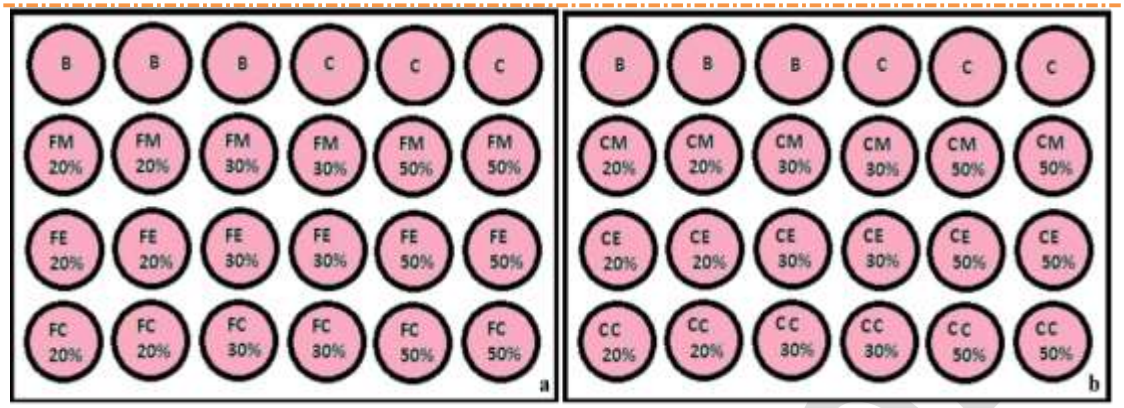

Figure 1. a) Distribution of chloroform extracts, ethanolic and methanolic obtained from the fruit of *R. sativus* on the plate 1 with HeLa cells. b) Distribution of the chloroform extracts, ethanolic and methanolic obtained from the bark of *R. sativus* on the plate 2 with HeLa cells.

Results and discussion

Aliquoting

In total, 6 crude extracts which were three fruit extracts extracted with methanol, ethanol and chloroform, and three peel extracts extracted with methanol, ethanol and chloroform were taken.

HeLa cell culture

HeLa cells, acquired in the Genetics Department CINVESTAV, were cultured in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 mg/mL streptomycin. One day prior to treatment with the extracts, the cells were seeded in boxes (P100, 100 mm) or 24 well plates, where 50,000 HeLa cells per well were inoculated in 1 mL of DMEM (+) (supplemented with serum fetal bovine) and cultured for 24 h at 37 °C in humidified atmosphere with 5% CO_2 in an incubator Thermo Scientific.

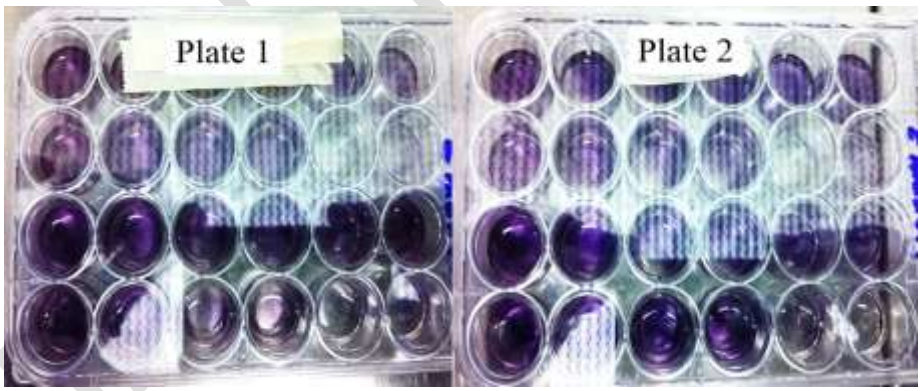

Figure 2. Plates 1 and 2 with cancer HeLa cells.

MTT assay bioassay MTT (3- [4,5-dimethylthiazol-2-yl] 2,5 diphenyltetrazolium bromide) (Sigma, USA) was used to determine growth inhibition of HeLa cells treated with crude extracts already mentioned, each extract was resuspended in 50 mL of dimethylsulfoxide (DMSO) and 110 µL were carried serum DMEM (+). 1 mL culture of HeLa cells contained in each well were added in triplicate volumes afore mentioned extracts per well for growth inhibition assays. In addition, two controls were used, the first one consisted of HeLa cells in DMEM medium (+); the second in HeLa cells or DMEM (+) treated with 15 mL of DMSO. Plates with HeLa cells and extracts were incubated in static conditions at 37 °C in humidified atmosphere with 5% CO_2 for 48 h. Finishing incubation time DMEM (+) was replaced with 500 µL of DMEM mix (-) with MTT reagent (unsupplemented without fetal bovine serum, 0.5 mg MTT/mL DMEM). Plates were again incubated at the same conditions for 30 min. Medium from

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each well was removed (without HeLa cells) using a Pasteur pipet attached to a vacuum pump. To each well they were added 500 mL of isopropanol acid (3.3 mL of concentrated HCl/1 mL isopropanol), then gently agitated for 15 seconds and the supernatant was recovered in an Eppendorf tube of 1.5 mL.

Figure 3. Plates 1 and 2 with cancer HeLa cells and *R. sativus* extracts after incubation.

The latter was centrifuged for three minutes at 10,000 rpm in a centrifuge (Eppendorf AG 22331 Hamburg, Germany). 200 µl of each supernatant were placed in an Elisa plate type and read spectrophotometrically at 595 nm in a spectrophotometer Elisa (BIO-RAD, Japan). The percentage of cell inhibition was obtained with the following equation:

$$\%\ Growth\ inhibition = \frac{Density\ compound}{Density\ compound\ DMSO}\ X\ 100 \qquad (1)$$

In Table 1 and in Figure 4 the results of the percent inhibition of growth of HeLa cells treated with the extracts obtained from the fruit of *R. sativus* present, the % white refers to the percentage of inhibition was extract crude DMSO and DMSO refers to % inhibition percentage which was the crude extract.

Table 1. Percentage growth inhibition in HeLa cells treated with methanol extracts, ethanolic and chloroformic from the fruit of *R. sativus*.

Well	Absorbance 1	Abs 2	Abs 3	Mean	% Control	% DMSO
Control	1.291	0.84	0.844	0.992	-	-
DMSO	0.421	0.48	0.421	0.441	44.437	-
FM 20%	0.739	0.672	-	0.706	71.143	160.098
FM 30%	0.413	0.52	-	0.467	47.042	105.862
FM 50%	0.041	0.122	-	0.082	8.218	18.495
FE 20%	0.694	0.593	-	0.644	64.891	146.029
FE 30%	0.519	0.534	-	0.527	53.092	119.478
FE 50%	0.417	0.621	-	0.519	52.336	117.776
FC 20%	0.626	0.612	-	0.619	62.420	140.469
FC 30%	0.256	0.276	-	0.266	26.824	60.363
FC 50%	0.112	0.139	-	0.126	12.655	28.480

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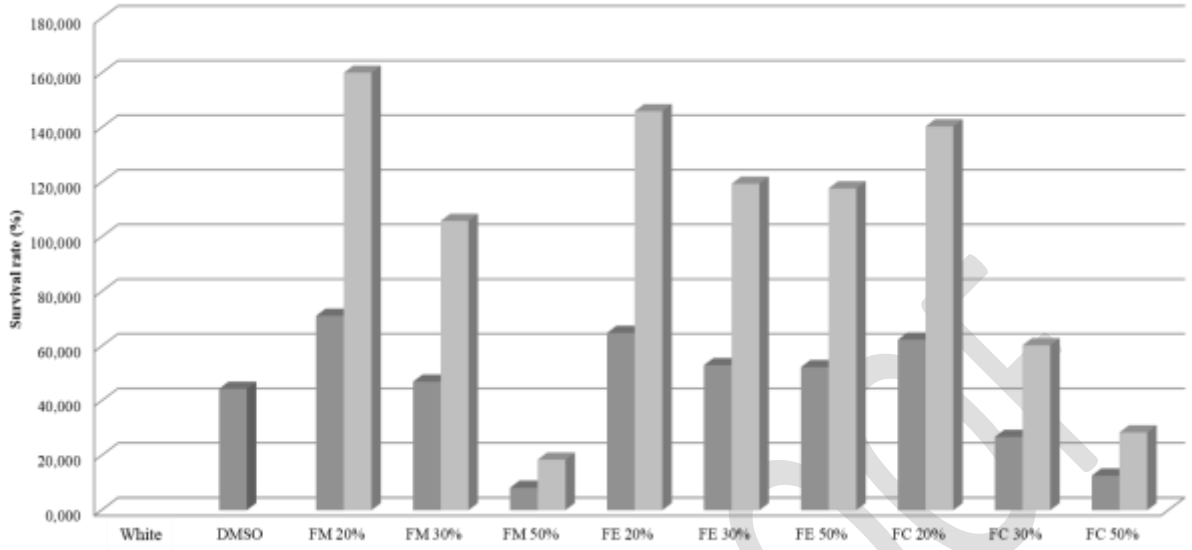

Figure 4. Percentage growth inhibition of cancer HeLa cells treated with the methanolic extracts, ethanolic and chloroform from the fruit of *R. sativus* (Plate 1).

In Table 2 and Figure 5 the results of the percentage of growth inhibition of HeLa cells treated with the extracts obtained from *R. sativus* of crust present, the % white refers to the percentage of inhibition was the crude extract DMSO and DMSO refers to % inhibition percentage which was the crude extract.

Table 2. Percentage growth inhibition in HeLa cells treated with methanolic extracts, ethanolic and chloroformic from *R. sativus* bark.

Well	Abs 1	Abs 2	Abs 3	Mean	% Control	% DMSO
Control	0.995	1.126	1.058	1.060	-	-
DMSO	0.701	0.849	0.841	0.797	75.212	-
CM 20%	0.638	0.602	-	0.620	58.509	77.792
CM 30%	0.4	0.442	-	0.421	39.729	52.823
CM 50%	0.038	0.229	-	0.134	12.598	16.750
CE 20%	0.716	0.661	-	0.689	64.973	86.386
CE 30%	0.524	0.577	-	0.551	51.950	69.072
CE 50%	0.396	0.374	-	0.385	36.332	48.306
CC 20%	0.566	0.708	-	0.637	60.113	79.925
CC 30%	0.649	0.706	-	0.678	63.935	85.006
CC 50%	0.176	0.098	-	0.137	12.929	17.189

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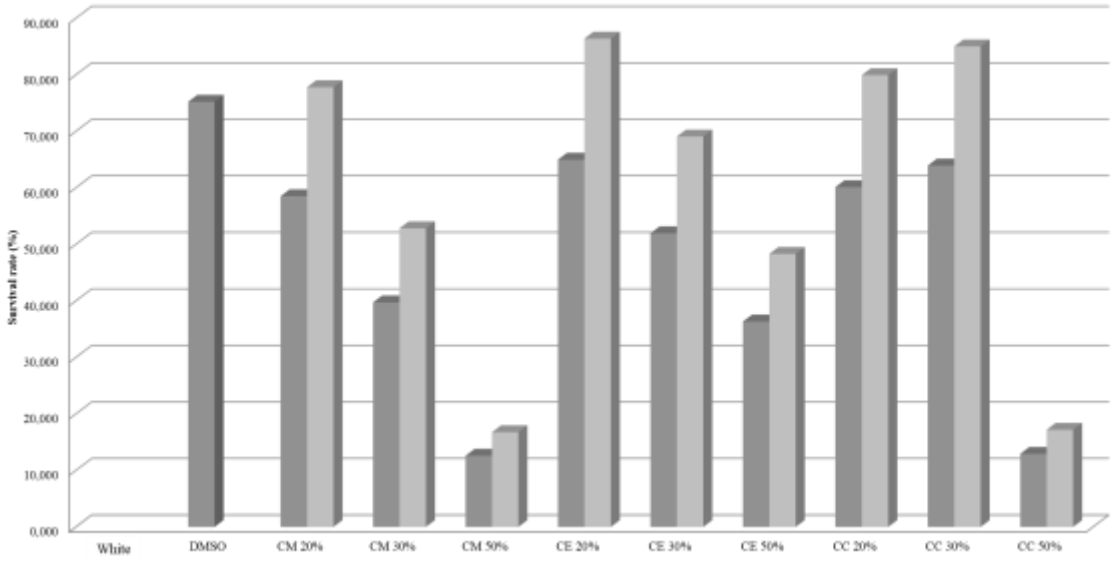

Figure 5. Percentage growth inhibition of cancer HeLa cells treated with the methanolic extracts, ethanolic and chloroformic from *R. sativus* bark (Plate 2).

R. sativus has been used in traditional medicine to treat various ailments. Isothiocyanates are formed from precursors of glucosinolates of cruciferous vegetables. Many isothiocyanates have anticancer activity because they reduce the activation of carcinogens and increase their detoxification [4]. Abd-Elmoneim *et al*. (2013) analyzed the anticarcinogenic effect of *R. sativus* seed extracts from colon chemically induced with 1,2-dimethylhydrazine (DMH) [5]. Beevi *et al*. (2010) evaluated the chemopreventive efficacy of different parts of *R. sativus*, such as root, stem and leaves, extracted with solvents of variable polarity and investigated the molecular mechanism that leads to growth arrest and apoptotic cell death in human cancer cell lines. From the different parts, a significant growth inhibitory effect was observed with the hexane extract from *R. sativus* root. The root extract of *R. sativus* induced cell death both in cell lines competent in p53 and deficient in p53 by inducing the apoptotic signaling pathway independently of the p53 status of the cells [6].

Conclusion

They could obtained pure isothiocyanates from the three solvents used. The extracts obtained according colorations presented different solvent used for the extraction. Crude extracts methanolic and chloroformic 50% *R. sativus* fruit showed antiproliferative activity on HeLa cells of a 81.50 % 71.52 % and respectively. Crude extracts methanolic chloroform and 50% bark *R. sativus* with antiproliferative activity in HeLa cells of a 83.25 % and 82.81 % respectively were obtained.

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References

1. López-Herranz G.P., Arroyo-Valerio A.G. Consumo de hierbas medicinales en pacientes prequirúrgicos en el Hospital General de México. Revista Médica del Hospital General. 2008;71(1):6-10.
2. Castro-Torres I.G., Minarda De la O-Arciniega, Gallegos-Estudillo J, Naranjo-Rodríguez E., Domínguez-Ortiz M.A. *Raphanus sativus* L. var niger as a source of phytochemicals for the prevention of cholesterol gallstones. Phytother. Res. 2013;28(2):167-171.
3. Singh P, Singh J. Medicinal and therapeutic utilities of *Raphanus sativus.* International Journal of Plant, Animal and Environmental Sciences. 2013;3(2):103-105.
4. Anubhuti S., Ashok S., Prashant Y., Dhiraj S. Isothiocyanates in *Brassica*: Potential Anti Cancer Agents. Asian Pac J Cancer Prev 2016;17(9):4507-4510.
5. Abd-Elmoneim M.A., Bakar A.A., Awad I.M., Mohamed E.M., Moharib S.A. Anticarcinogenic Effect of *Raphanus sativus* on 1, 2 Dimethylhydrazine (DMH) Induced Colon Cancer in Rats. Department of Supplementary Medical Science-Faculty of The Egyptian Journal of Hospital Medicine. 2013;51:473- 486.
6. Beevi SS, Mangamoori LN, Subathra M, Edula JR. Hexane extract of *Raphanus sativus* L. roots inhibits cell proliferation and induces apoptosis in human cancer cells by modulating genes related to apoptotic pathway. Plant Foods Hum Nutr. 2010;65(3):200-229.