JJBS

Volume 13, Number 4, December 2020
ISSN 1995-6673
Pages 567 - 574

Jordan Journal of Biological Sciences

Assessment of Antimicrobial and Anticancer Activity of Radish Sprouts Extracts

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Received: December 31, 2019; Revised: January 29, 2020; Accepted: February 8, 2020

Abstract

People have used plants as medications since the beginning of history. With the advancement in science, people headed more towards using synthetic drugs. However, with the rise of problems like the toxic side effects of these drugs as in the case of chemotherapy, and the origin of drug resistant bacteria, the need has come for developing new drugs. The safest source of developing new drugs is natural products from plants. In this study, antimicrobial and anticancer activity of radish sprouts extracts was tested. Radish seeds were planted and watered until they sprouted. Sprouts were then dried and grinded. Radish sprouts were soaked in three different solvents, (100% water, 100% ethanol, and 80% ethanol) for three hours with sonication, and then solvents were evaporated using rotary evaporator at 40 C°. Radish sprouts extracts were then tested for anticancer activity against MCF7 and HT29 cancer cells. Their antimicrobial activity for different types of positive and negative gram bacteria (*E. coli*, *S. pneumonia*, and *S. aureus*) was also tested using disk-diffusion method. Results showed that radish sprouts have anticancer activity against HT29 and MCF7. The most drastic effect was observed for radish sprouts extracted with 100% ethanol against MCF7 cells where 62% of cells were found dead compared to the control. No antimicrobial activity was observed for the radish sprouts extracts.

Keywords: Anticancer activity, Antimicrobial activity, Radish Sprouts

1. Introduction

Using plants as medicine has been known since ancient ages. Recently, there has been an increased interest in finding therapy for many diseases using natural products; especially the ones derived from plants. Medicinal activities of plants come from their secondary metabolites which include tannins, terpenoids, coumarins, alkaloids and flavonoids (Khamees, 2017). These secondary metabolites could be specifically useful for antimicrobial and antioxidant activity. In recent years, research has been focusing on sprouts and their therapeutic benefits. Sprouts have high nutritional value, and many possible health benefits. For example, mung beans sprouts are used especially in East Asian countries like China, since they have been known for their detoxification activities and are widely used to refresh mentality, ease heat stroke, and reduce swelling in the summer. They also regulate gastrointestinal upset and lipid metabolism (Tang *et al.*, 2014). Also, broccoli sprouts have shown chemoprotective effects, and they can reduce cholesterol or lipid levels. They also have a powerful preventative effect against *Helicobacter pylori* infections. In addition, broccoli sprouts can possibly improve insulin resistance in type II diabetes (Paśko *et al.*, 2018).

Radish is a member of the cruciferous family. Nutritional value of radish sprouts is attributed to the presence of many essential minerals and vitamins, carbohydrates, high content of fiber, and low content of fat. Radish is typically used in traditional medicine as a treatment for many infectious diseases. This made studying its antimicrobial activity of great interest to researchers (Jeries *et al.*, 2016). Researchers who studied radish sprouts have found that they contain alkaloids, flavonoids, saponins, anthraquinons, tannins, steroids, and terpenoids (Angel *et al.*, 2019). This gave them strong antioxidant ability, as these compounds can act as radical scavengers for free radicals, which can sometimes cause cancer. It was also found that radish seeds have important antimicrobial activity against most of the tested microorganisms, most importantly *E. coli*, *S.aureas* and *P.aerugenosa*, with high zone of inhibition (Khamees, 2017).

Most of medicinal benefits of plants are a result of their production of secondary metabolites. Secondary metabolites are chemicals that are not required for the immediate survival of the plant, but they allow the plant to cope with its environment. For example, they are produced

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* **List of abbreviations**: MCF7: breast cancer cell line isolated in 1970 from a 69-year-old Caucasian woman. MCF-7 is the acronym of Michigan Cancer Foundation-7.HT29: human colon cancer cell line used extensively in biological and cancer research. PDA: Photodiode Array detector. MIC: Minimum Inhibitory Concentration. DMSO: Dimethyl sulfoxide

in response to pathogen attacks, herbivore induced damage, or nutrient deficiency. Secondary metabolites can be unique to specific species or genera, depending on the environment in which the plant grows and the environmental challenges it faces (Kennedy and Emma, 2011). Secondary metabolites are usually classified based on their biosynthetic pathways into alkaloids, terpenoids, and phenolics (Tiwari and Rana, 2015). Phenolics are widely spread in plants and include flavonoids, tannins, coumarins, quinones and anthocyanins (Shaik *et al.*, 2011).

An antioxidant is a molecule that is capable of neutralizing a free radical by donating an electron to it, which reduces the free radical capacity to damage. Free radicals can be Oxygen derived (ROS) or Nitrogen derived (RNS). Free radicals are normally produced as a result of normal cellular metabolism such as respiratory chain reactions in the mitochondria (Birben *et al.*, 2012). When ROS concentrations are at high level, oxidative stress is generated, which can result in damage to cell structures. Oxidative stress is a major cause of the development of chronic and degenerative diseases such as cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases. Antioxidants production is one of the mechanisms that the body uses in order to counteract oxidative stress. Antioxidants are either naturally produced inside the body or provided externally through foods or supplements. Antioxidants support immune defenses and decrease the risk of cancer and degenerative diseases by acting as "free radical scavengers" that can prevent and repair damages caused by free radicals (Pham-Huy *et al.*, 2008).

Cancer is a widespread disease that causes the death of thousands of people every year. Unfortunately, there is no ultimate cure to treat cancer so far. One of the most used treatments is chemotherapy, where chemical anticancer drugs are used to target dividing cells. However, chemotherapy cannot differentiate between cancer cells and normal cells. This creates serious side effects for patients receiving this treatment. These side effects include fatigue, hair loss, easy bruising and bleeding, anemia, appetite changes, nausea and vomiting, and much more. That is why there has been increasing research trying to find a natural alternative to chemotherapy (Vichaya *et al.*, 2015).

Antibiotic resistance is currently another serious problem. Many types of bacteria, whether they were gram negative or gram positive, are now developing resistance to the known and commonly used antibiotics and can grow despite the presence of the drug. This might cause the spreading of dangerous diseases without any possible cure. Thus, new natural antibiotics need to be developed (Zaman *et al.*, 2017).

Plants have been used in therapy since ancient ages, and they can be the solution to all previously mentioned problems by using the different anticancer and antimicrobial products found naturally in plants. Many plants are known to exhibit antioxidant activity, and consuming them reduces the risk of free radicals. Also, plants that possess anticancer activity can be a safer replacement for chemotherapy with fewer side effects (Khamees, 2017; Tang *et al.*, 2014). As for antimicrobial resistance, many plants have antibacterial activity and can be used in the synthesis of new antibiotics (Zaman *et al.*, 2017). In this study, anticancer activity for radish sprouts extract on colon cancer and breast cancer cell line will be tested; in addition, their antimicrobial activity will be tested on some types of both positive and negative grams bacteria.

2. Materials and Methods

2.1. Study design:

Radish seeds were sprouted for 3-4 weeks, then sprouts were collected, dried in shade, and extracted with water/methanol solvent, then the crude extract was obtained after evaporation of the solvent. The crude extract was then analyzed by HPLC and then its activity against bacteria and cancer was tested.

2.2. Sprouting

Radish seeds were purchased from the local market. The seeds were planted on cotton soaked with water and were put in an area with access to sun and air. Seeds were watered every two days until they became sprouts (around 5 cm tall), Figure 1. This process took approximately 3-4 weeks. After that, sprouts were collected and dried in the shade, since UV light from the sun can lead to the loss of active compounds that are present in sprouts.

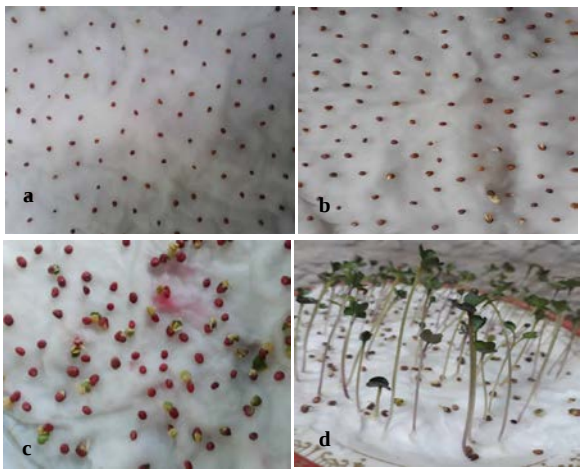


Figure 1. Sprouting process. (a) radish seeds- day 1. (b) radish seeds- day 3. (c) radish seeds- day 7. (d) radish sprouts ready to be collected- day 25.

2.3. Extraction

The dried sprouts were grinded and turned into powder. Five grams of the dried sprouts powder was added separately to 50 ml of three extraction solvents which are 100% water, 80% ethanol, and 100% ethanol. These solvents were then put in a water bath at 37 ◦C and waited for three hours with sonication. The extracts were then filtered. The filtrate was evaporated using rotary evaporator at 40 °C and reduced pressure. The resulting crude extract viscous extracts were stored at 4 ◦C.

2.4. HPLC conditions

The HPLC analysis of the extracts was run on ODS column of Waters (XBridge, 4.6 ID x 150 mm, 5 µm). The mobile phase used is a mixture of acetic acid in water (0.5%) (solvent A) and acetonitrile (solvent B) run in a linear gradient mode. 100% (solvent A) descended to 70% (solvent A) in 40 minutes, then to 40% (solvent A) in 20 minutes and finally to 10% (solvent A) in 2 minutes and stayed there for 6 minutes and then back to the initial

conditions in 2 minutes. The HPLC system was equilibrated for 7 minutes with the initial acidic water mobile phase (solvent A) before injecting the next sample. All the samples were filtered with a 0.45 µm PTFE filter. The PDA wavelengths range was from 210-500 nm. The flow rate was 1 ml/min. Injection volume was 20 µl and the column temperature were set at 25◦C.

2.5. Antimicrobial Activity Testing

In order to test the antimicrobial activity of radish sprouts extracts, disk diffusion method was used. *E. coli*, a gram negative bacteria and *S. pneumonia* and *S. aureus*, which are gram positive bacteria were streaked on Mueller-Hinton agar plates using cotton swaps. After that, sterilized blank disks were dipped in the crude extracts for five minutes, and were then put on the plates using aseptic techniques. Also, antibiotic disks of novobiocin, penicillin, gentamycin, cefepime, and aztreonam were placed on the plates in order to compare the zone of inhibition of these antibiotics and the different sprouts extracts. Plates were then incubated for 24h at 37 ◦C before measuring the zone of inhibition (MIC) on these plates.

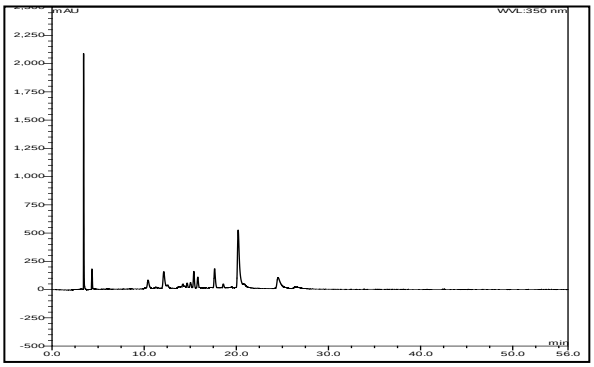
2.6. Anticancer Activity Testing

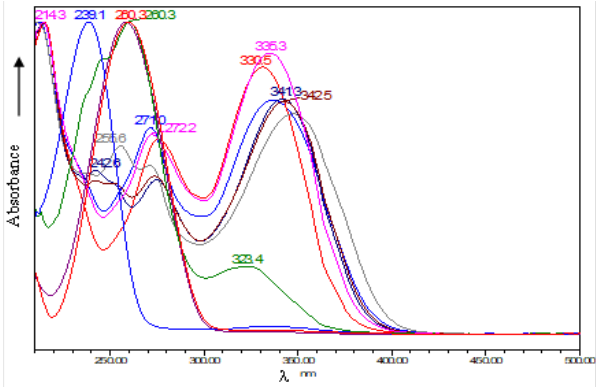
Radish sprouts extracts were tested for anticancer activity on HT29 colon cancer cells, and MCF7 breast cancer cells. HT29 cells and MCF7 were cultured on RPMI media and incubated for 24h before treatment with radish sprouts extracts. After that, 100 µl of the radish sprouts extracts were diluted with DMSO and added separately in four different concentrations 50 µg/ml, 100µg/ml, 300µg/ml, 500µg/ml and 1000µg/ml. Cells were then incubated for 24h, and 48h after treatment.

3. Results and Discussion

3.1. HPLC-PDA profiles of the extracts

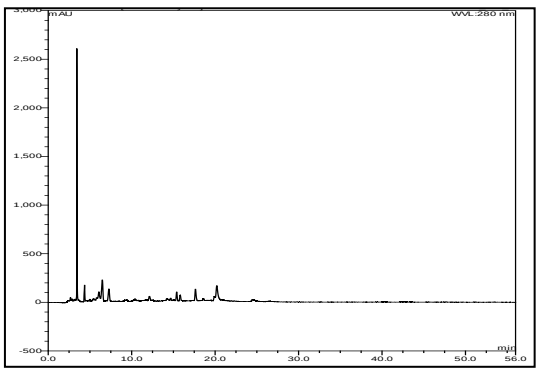
HPLC chromatogram at 350nm and 280nm of crude radish extracts was analyzed; it indicated the presence of polar and non-polar phenolic compounds. Crude radish extracts spectrum showed a maximum absorption at 350nm for the major seven peaks. The eluted compounds were detected in the range of 9-26 minutes; indicating polar and non-polar combination (Fig 2a). At 280nm, new peaks eluted at 5-7 minutes appeared, indicating the presence of polar compounds (Fig 3a). The UV-Vis ranges of these compounds were applied in the range of 220-360nm indicating flavonoids and phenolic compounds abundance (Al-Rimawi *et al.*, 2017; Al-Zereini *et al.*, 2018; Al-Rimawi *et al.*, 2018) (Fig 2b, 3b). Radish sprouts contain polar and non-polar compounds that could possibly possess antimicrobial and anticancer activity.

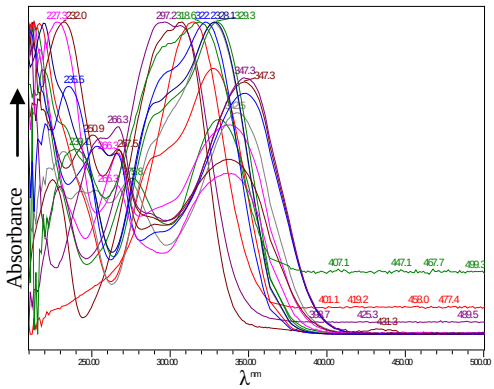




2a 2b

Figure 2. HPLC-PDA chromatogram of radish sprouts extracts at 350 nm (2a), and overlaid UV-Vis spectra at 350nm (2b).





3a 3b

Figure 3. HPLC-PDA chromatogram of radish sprouts extracts at 280 nm (3a), and overlaid UV-Vis spectra at 350nm (3b).

3.2. Antimicrobial Activity

In order to test the antimicrobial activity of radish sprouts extracts, disk diffusion method was used. Radish sprouts extracts illustrated no antimicrobial activity for any of the tested bacteria. *S. pneumonia* exhibited no zone of inhibition of any of the tested antibiotics, nor for radish extracts. *S. aureus* also demonstrated no zone of inhibition for any of the tested antibiotics and for radish extracts as well. *E. coli* showed a zone of inhibition for the antibiotics aztreonam and cefepime, but not for novobiocin; however, no zone of inhibition was detected for radish extracts. This indicates that radish sprouts extracts have no antimicrobial activity for any of the tested bacteria. This could possibly be due to the extraction method. Other extraction methods of radish sprouts could give antimicrobial activities as previous studies found that red and white radish seeds have antimicrobial activity against *E. coli*, *S. aureas* with a zone of inhibition ranging between 18mm and 34.2mm (Khamees, 2017).

3.3. Anticancer activity

Anticancer activity of radish sprouts extracts was assessed for HT29 colon cancer cells and MCF7 breast cancer cells. Radish sprouts exhibited anticancer activity against HT29 and MCF7 cells at the higher extract's concentrations. For HT29 cells, radish sprouts extract after 24h showed no effect at 100 µg/ml for any of the solvents (Fig 4d, 4g, 4j). Radish extracted with water demonstrated a change in cell morphology and cell number as there were some few dead cells at 500 µg, and 1000 µg (Fig 4e, 4f). Ethanol 100% extracts at 1000 µg resulted in change in cell morphology, and lowering the density of cells with the presence of few dead cells (Fig 4i). Radish extracts with 80% ethanol resulted in lowering the cells density at 500 µg/ml (Fig 4k), cell density was even lower at 1000 µg with the presence of few dead cells (Fig 4). Radish sprouts exhibit an anticancer activity for HT29 colon cancer cells especially when extracted with water.

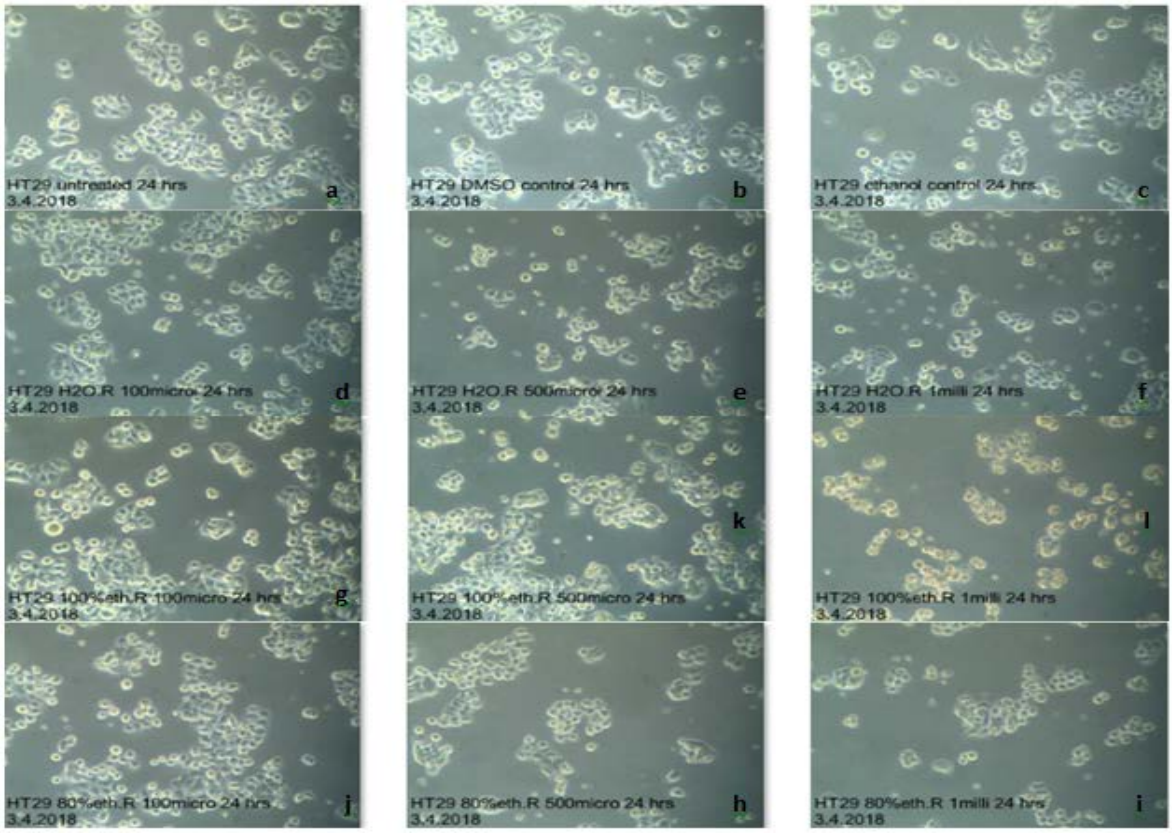


Figure 4. Anticancer activity of radish sprouts extracts against HT29 colon cancer cells. HT29 colon cancer cells cultured were on RPMI media and incubated for 24h at 37 C°. (a) Cells untreated. (b) DMSO control. (c) Ethanol control. (d) water radish extracts 100 µg/ml (e) water radish extract 500 µg/ml. (f) water radish extract 1000 µg/ml. (g) 100% ethanol radish extract 100 µg/ml. (e) 100% ethanol radish extract 500 µg/ml. (i) 100% ethanol radish extract 1000 µg/ml. (j) 80% ethanol radish extract 100 µg/ml. (k) 80% ethanol radish extract 500 µg/ml. (l) 80% ethanol radish extract 1000 µg/ml.

When anticancer activity of radish sprouts extracts was tested against MCF7 cells, it showed the presence of dead and floating cells at high extracts concentrations. After 24h of MCF7 cancer cells incubation and treatment with radish sprouts extracts, extracts in water resulted in a change in cell morphology, and lowering cell density with presence of floating cells at a concentration of 300 µg/ml. At 500 µg/ml, the effect was more drastic as most cells were round and floating. Yet, there was no effect for the extract at low concentrations of 50 µg/ml and 100 µg/ml (Fig 5). Radish extracts in 100% ethanol also showed no effect at 50 µg/ml, but there were very few floating cells at 100 µg/ml. At 300 µg/ml, there were more floating cells. At 500 µg/ml, most cells were floating and cells density became less, and less cells numbers were present as a result of cells death (Fig 6). The effect of 80% ethanol radish extracts was less than that of 100% ethanol at 300 µg/ml and 500 µg/ml, and no effect was observed at 100 µg/ml and 50 µg/ml (Fig 7). Radish sprouts possess a strong anticancer activity against MCF7 breast cancer cells.

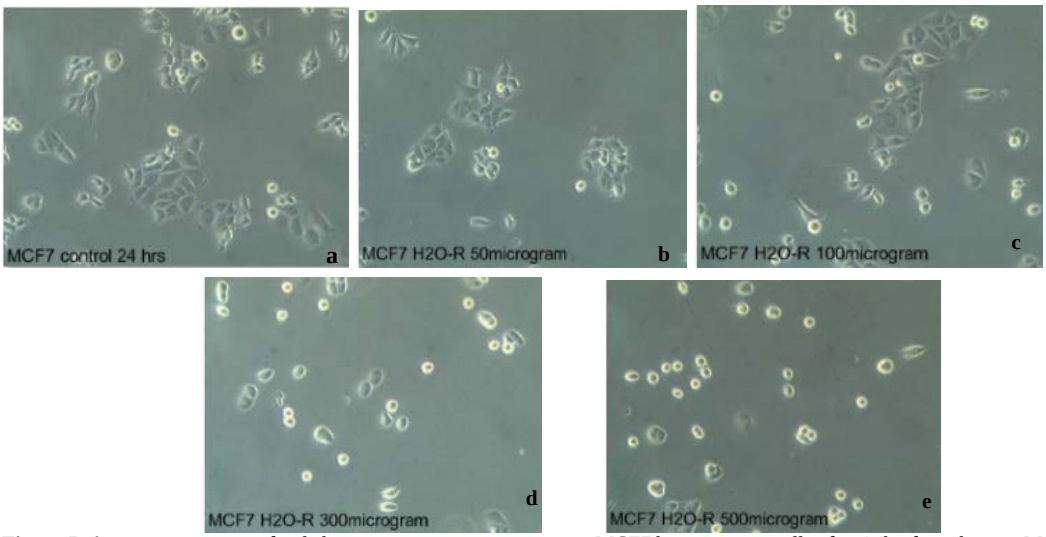


Figure 5. Anticancer activity of radish sprouts water extracts against MCF7 breast cancer cells after 24h of incubation. MCF7 breast cancer cells were cultured on RPMI media and incubated for 24h at 37 C°. (a) control. (b) water radish extracts 50 µg/ml. (c) water radish extract 100 µg/ml. (d) water radish extract 300 µg/ml. (e) water radish extract 500 µg/ml.

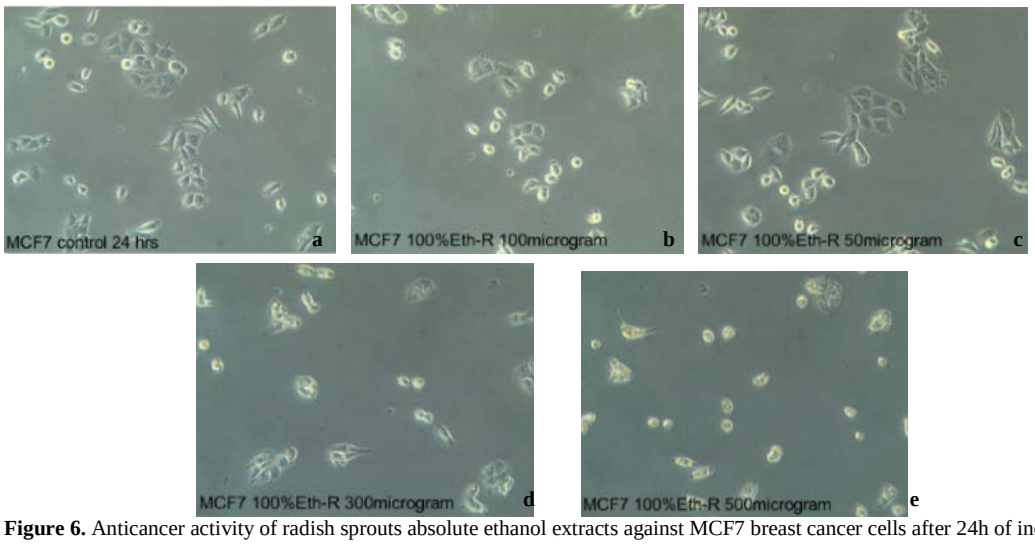


Figure 6. Anticancer activity of radish sprouts absolute ethanol extracts against MCF7 breast cancer cells after 24h of incubation. MCF7 breast cancer cells were cultured on RPMI media and incubated for 24h at 37 C°. (a) control. (b) 100% ethanol radish extract 50 µg/ml. (c) 100% ethanol radish extract 100 µg/ml. (d) 100% ethanol radish extract 300 µg/ml. (e) 100% ethanol radish extract 500 µg/ml.

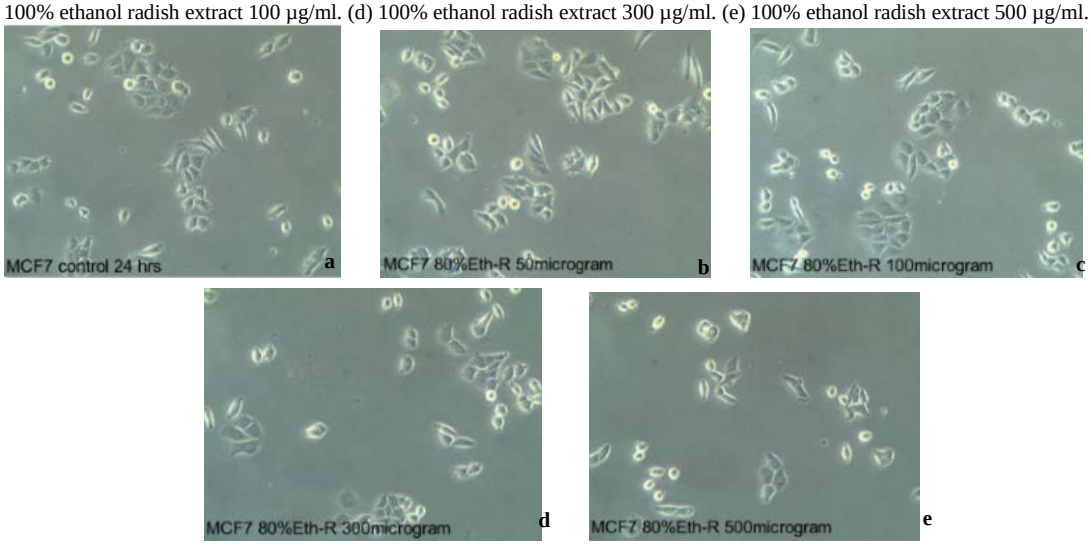


Figure 7. Anticancer activity of radish sprouts 80% ethanol extracts against MCF7 breast cancer cells after 24h of incubation. MCF7 breast cancer cells were cultured on RPMI media and incubated for 24h at 37 C°. (a) control. (b) 80% ethanol radish extract 50 µg/ml. (c) 80% ethanol radish extract 100 µg/ml. (d) 80% ethanol radish extract 300 µg/ml. (e) 80% ethanol radish extract 500 µg/ml.

In order to further examine anticancer activity of radish sprouts extracts, MCF 7 cells were incubated for 48h after treatment with the extracts. Upon doing that, stronger effect of the extracts was spotted. Water extracts still showed no effect at 50 µg/ml, but few floated cells were observed at 100 µg/ml. A much stronger effect was

observed at 300 µg/ml, and 500 µg/ml where cell count was noticeably lower than control, and existing cells were round and floating, and cell density was less, which indicates cell death (Fig 8). Cells treated with 100% ethanol showed no effect at 50 µg/ml and 100 µg/ml; however, at concentrations of 300 µg/ml and 500 µg/ml, radish sprouts extracts displayed the severest effect of all extracts, where most cells were dead (Fig 9), and cell death percentage was approximately 62% (Fig 11). 80% ethanol extracts also showed no effect at 50 µg/ml and 100 µg/ml. There was a slight effect at 300 µg/ml and 500 µg/ml and a presence of few floating and few dead cells (Fig 10). Radish sprouts extracted with ethanol have a strong anticancer potential for breast cancer as they resulted in the death of more than half of MCF7 cells cultured on the plate.

Radish sprouts extracts showed stronger anticancer activity for MCF7 breast cancer cells compared to HT29 colon cancer cells. This might be due to the presence of more targets of the active ingredients extracted from radish sprouts in MCF7 cells than the targets present in HT29 cells.

Radish sprouts extracted with 100% ethanol showed stronger anticancer activity compared to sprouts extracted with other solvents (water and 80% ethanol). The reason for this might be that ethanol is able to extract a variety of polar and non-polar phenolic compounds present in radish sprouts compared to other solvents. HPLC results confirmed the presence of polar and non-polar compounds in radish sprouts extracts. These polar and non-polar compounds were probably the most efficient in targeting MCF7 and HT29 cancer cell lines.

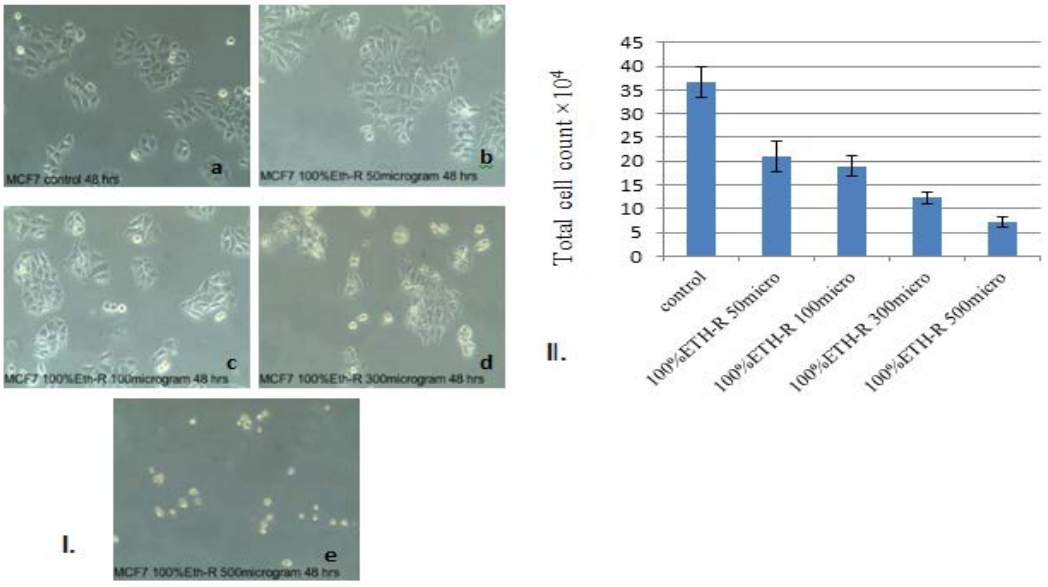


Figure 8. Anticancer activity of radish sprouts water extracts against MCF7 breast cancer cells after 48h of incubation. I. MCF7 breast cancer cells were cultured on RPMI media and incubated for 48h at 37 C° after extracts treatment. (a) control. (b) water radish extract 50 µg/ml. (c) water radish extract 100 µg/ml. (d) water radish extract 300 µg/ml. (e) water radish extract 500 µg/ml. II. Total cell count after treatment with water radish sprouts extracts for 48h.

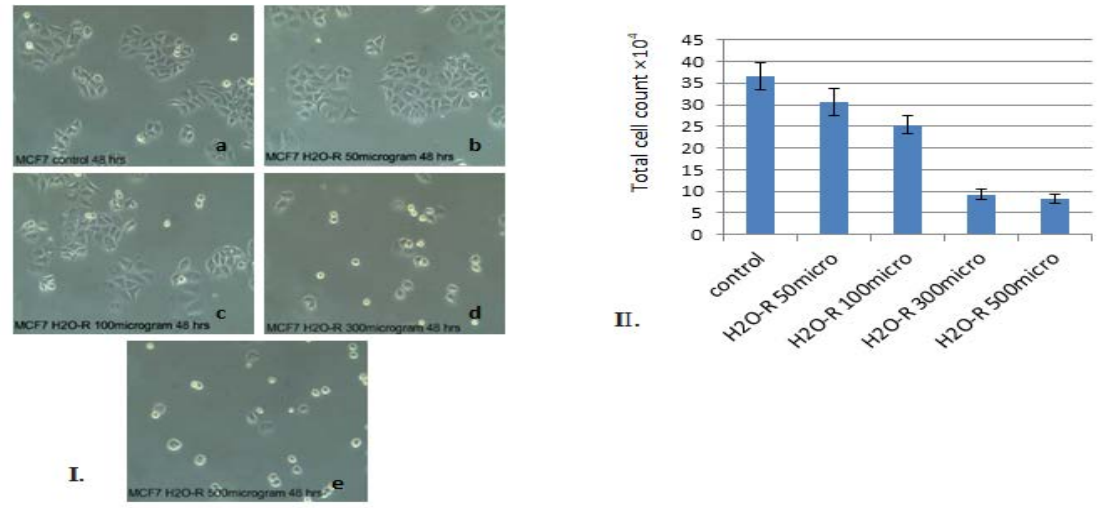


Figure 9. Anticancer activity of absolute ethanol extracts against MCF7 breast cancer cells after 48h of incubation. I. MCF7 breast cancer cells were cultured on RPMI media and incubated for 48h at 37 C° after extracts treatment. (a) control. (b) 100% ethanol radish extract 50 µg/ml. (c) 100% ethanol radish extract 100 µg/ml. (d) 100% ethanol radish extract 300 µg/ml. (e) 100% ethanol radish extract 500 µg/ml. II. Total cell count after treatment with 100% ethanol radish sprouts extracts for 48h.

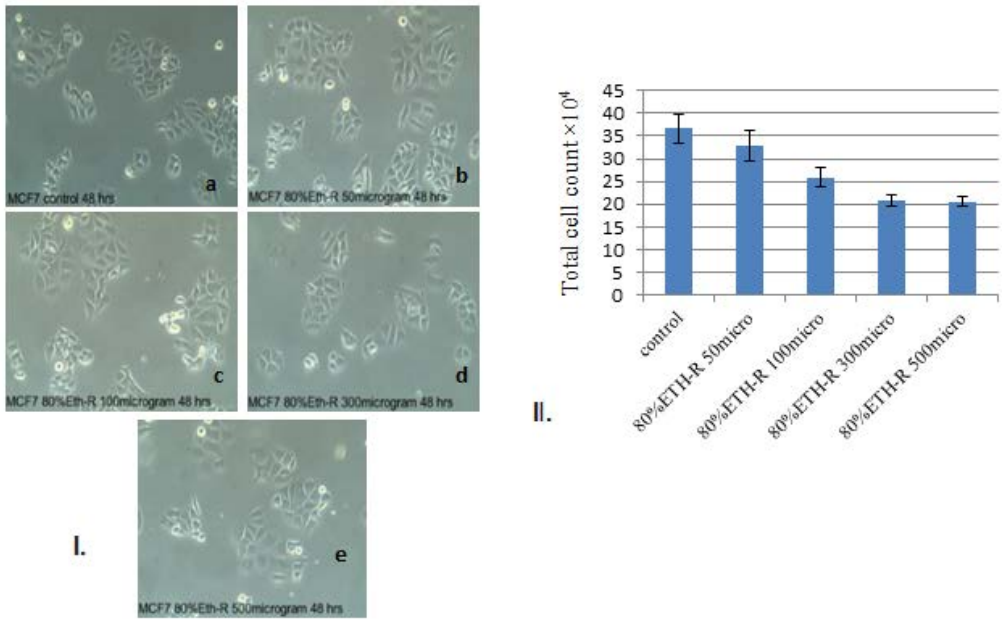


Figure 10. Anticancer activity of radish sprouts 80% ethanol extracts against MCF7 breast cancer cells after 48h of incubation. I. MCF7 breast cancer cells were cultured on RPMI media and incubated for 48h at 37 C° after extracts treatment. (a) control. (b) 80% ethanol radish extract 50 µg/ml. (c) 80% ethanol radish extract 100 µg/ml. (d) 80% ethanol radish extract 300 µg/ml. (e) 80% ethanol radish extract 500 µg/ml. II. Total cell count after treatment with 80% ethanol radish sprouts extracts for 48h.

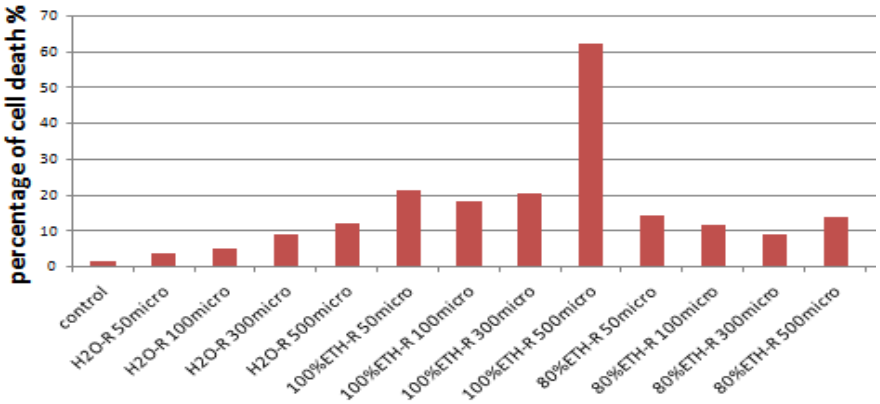


Figure 11. Percentage of MCF7 dead cells after 48h of being treated with different concentrations of radish sprouts extracts of water, 100% ethanol, and 80% ethanol.

4. Conclusion

Radish sprouts have anticancer activity against MCF7 breast- and of HT29 colon-cancer cell lines, especially when extracted with ethanol. Radish sprouts extracts exhibited stronger anticancer activity against MCF7 breast cancer cells than that of HT29 colon cancer cells. Ethanol extracts showed the fiercest effect against MCF7 cells, as 62% of cells were found dead after 48h of incubation. Radish sprouts have negative antibacterial activity against *E. coli*, *S. aureas* and *S. pneumonia*. In conclusion, radish sprouts extracts can be a possible target for developing anticancer drugs, especially for breast cancer.

Conflict of Interest

The authors have no conflicts of interest to declare.

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