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Investigation of Antimicrobial, DNA Protective and Cytotoxic Activity of Red Cabbage (*Brassica Oleracea* L. *Var. Capitata F. Rubra*) Plant

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Abstract: The need to search for natural products that offer a safe alternative to drugs and industrial products that often cause side effects and harm human health is growing over the years. In this study, the water and methanol extracts of the anthocyanin-rich red cabbage (Brassica oleracea L. var. capitata f. rubra) which is available on markets throughout the year, were examined for their antibacterial activity on Escherichia coli, Staphylococcus aereus and Stenotrophomonas maltophilia, and for their antifungal activity on Yarrowia lipolytica, Candida albicans and Candida maltophilia. The DNA protective activity of extracts on the pBR322 plasmid was evaluated by exposing pBR322 to UV and adding hydrogen peroxide. Furthermore, the MTT assay was performed on the H1299 lung cancer cell line and the HUVEC cell line as a negative control to evaluate the cytotoxicity of the extracts. The red cabbage extracts were found to have no antibacterial or antifungal activity on the tested microorganisms. The results of DNA protective activity have shown that at a concentration of 50 mg/mL there is a DNA protective activity of the extracts. No effect of the red cabbage extracts on the H1299 cell line as shown by MTT assay results. On the other hand, the decrease in the viability of HUVEC cells started at a concentration of 25 µg/mL and above, reaching 70% at 100 µg/ mL for the water extract and 74 % at 100 µg/ mL for the methanol extract. Further studies to investigate the active components of the DNA protective activity in the red cabbage extracts are required. Further studies are also needed to explore the possibility of incorporating these components in cosmetic products such as sunscreens without causing skin damage.

Keywords: Red Cabbage, Antibacterial Activity, DNA Protective Activity, MTT test.

Introduction

Red cabbage (Brassica oleracea L. var capitate f. rubra) is one of the economically important vegetables consumed all over the world as a fresh, cooked and fermented form known for its enriched bioactive constituents (Fernandes et al., 2019). Red cabbage acts as an antioxidant that helps prevent chronic diseases. It is an abundant source of potassium, manganese, iron, and magnesium minerals. It also contains A, B, C, K vitamins and a litany of phyto-chemicals (Draghici et al., 2013). The wonderful purple/red color of red cabbage is due to the presence of anthocyanin. Cabbage leaves vary in color depending on the pH of the soil, red at low pH and violet to green at high pH (Fernandes et al., 2019).

While the search is increasing daily for natural products that provide a healthy alternative to industrial drugs, this study aimed to study the effect of water and methanol extracts of red cabbage as anti-bacterial and anti-fungal, as well as the possibility of protecting DNA and the possibility of working as an anti-cancer drug.

Antimicrobial, DNA protective and anticancer activity of red cabbage

Antibiotic resistance is increasing as a result of its excessive and unconscious use, and this problem is the focus of researchers' attention. Therefore, studies and research are continuing to develop new drugs, especially those of natural origin that are used as antibiotics in order to avoid the side effects that may be caused by industrial drugs.

Reactive oxygen species may result either from internal processes such as metabolism, inflammation and exercise, or from external sources such as environmental pollutants, radiation, smoking and industrial solvents. An imbalance between free radical production and antioxidant defenses leads to what is called oxidative stress. Oxidative stress leads to cell damage, causing a number of cancers, inflammation, aging and neurological disorders. Reactive oxygen species primarily target DNA, proteins, and lipids (Kada et al., 2017; Lobo et al., 2010).

The rapid increase in the number of people with different cancers poses a great challenge for researchers to discover effective natural medicines that can be used safely, whereas many studies have shown that some natural products can effectively affect the prevention of a number of malignant cancers. Some studies have focused on the anti-cancer activity of red cabbage, which is rich in phenolic compounds and anthocyanin (Hafidh et al., 2013). Cytotoxicity tests for extracts are the first commonly used preclinical tests and thus cell culture and potential therapeutic agent screening have become commonplace (Van Tonder et al., 2015).

Tetrazolium Bromide (MTT) Assay

The MTT test is a common cytotoxicity test that is a colorimetric test based on the reduction of the yellow tetrazolium pigment through the metabolic activity of the enzyme and its conversion to formazan violet crystals in living cells.

Method

Red cabbage collection and extraction preparation

Red cabbage was collected from a local market in Gaziantep, Turkey. The red cabbage leaves were washed well, shade dried for 10 days then were ground into a fine powder. A Gerhardt EV 14 soxhlet apparatus was used to obtain water and methanol extracts of red cabbage leaves. The extracts were placed in Petri dishes and the solvents were left at room temperature 2-3 days until they evaporated (Topçu et al., 2007).

Antimicrobial assay – agar diffusion method

Escherichia coli ATCC 25322, *Staphylococcus aereus* ATCC 29213, *Stenotrophomonas maltophilia, Yarrowia lipolytica* NCAIM Y00591, *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019 are bacterial and fungal strains examined in our research that were obtained from the Molecular Biology laboratory at biology department at Gaziantep University. Standard disk diffusion method recommended by EUCAST (European Committee on Antimicrobial Susceptibility Testing) was used to determine the antimicrobial activity of red cabbage leaf extracts. The red cabbage powder was dissolved in the same solvent, water, methanol to final concentration of 50 mg/mL and loaded in 6 mm sterile blank discs. Screening was carried out by disc diffusion using 100 ml of microbial suspension 0.5 McFarland density and was spread on the surface of Mueller Hinton agar plates. The impregnated discs were placed on the agar surface and incubation for 24 hours was implemented. Inhibition zone diameters were measured in mm. For the control, the results were checked in the EUCAST QC Tables at http://www.eucast.org (EUCAST, 2021).

DNA protective activity

The DNA protective activity of red cabbage extracts was tested on pBR322 plasmid (Vivantis, Czech Republic). pBR322 plasmid (172 ng/µL) was treated with 30% H_2O_2 and UV in the presence and absence of 50 mg/mL of red cabbage extracts. UV irradiation was continued for 5 min by UV transilluminator with intensity 8000 µW/cm^2 at 302 nm under room temperature. The mixture was loaded on 1.5% agarose gel for electrophoresis. Electrophoresis was performed at 100 V for 45 min. samples without extracts were used as controls (Tepe et al., 2011).

Cytotoxic activity

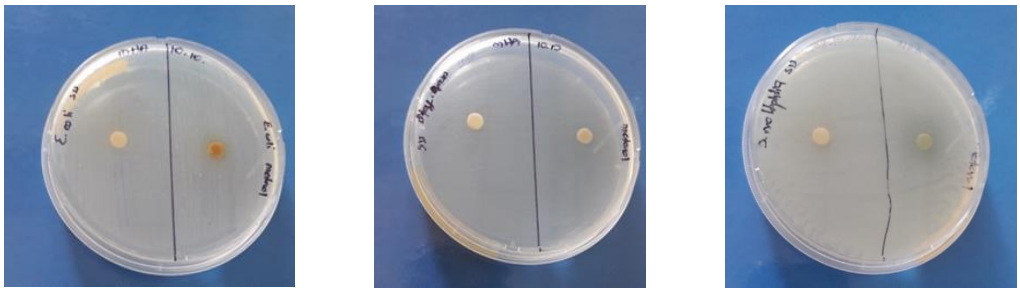
The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to study the cytotoxic activity of red cabbage extracts. H1299 (human non-small cell lung carcinoma cell line) carcinoma cells was tested for cell viability after treating with extracts and HUVEC (Human Umbilical Vein Endothelial Cells) normal cells as a control. The cells were seeded in 96-well plates at a density of 5000 cells/well and incubated for 24 hours at 37 °C in a CO_2 incubator. After incubation, cells were treated with 100 µL of different concentrations of extracts (100, 50, 25, 12.5, 6.25 µg/mL) and incubated in 5% CO_2 for 48 hours at 37 °C. 40 µl of MTT solution was added to wells and incubated again for 4 hours at 37 °C. The medium was removed and 80 µL DMSO was added to dissolved formazan crystals. Finally, the absorbance was measured in a spectrophotometer (Thermo Scientific, USA) at 540 nm (Bahuguna et al., 2017). Cell viability was calculated using the following formula after reading the absorbance values (Kilic, 2020):

Viability % = 100 × (mean absorbance of cells treated with extract / control cell viability without extract).

Results and Discussion

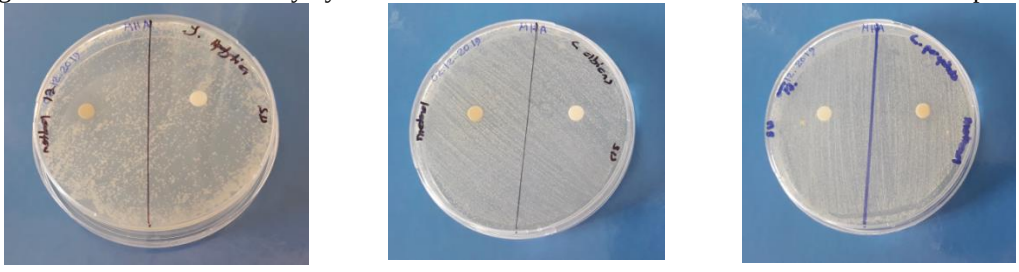
Antimicrobial Effect of Extracts

Red cabbage extracts did not show any antibacterial or antifungal activity whreras pathogens continued growth in petri dishes with zero growth inhibition zone. The results of the disk diffusion test are shown in Figure 1 and Figure 2. Ayshwarya and Rameshwari (2015) have reported that red cabbage extracts (100 µg -200 µg) have antibacterial effects on *E. coli* and *S. aereus*.



A B C

Figure 1. Antibacterial activity by disc diffusion method. A. E. coli. B. S. aereus. C. S. maltophilia.



A B C

Figure 2. Antimicotic activity by disc diffusion method. A. Y. lipolytica. B. C. albicans. C. C. parapsilosis.

The extraction method used in the study depends on homogenizing the ground sample with solvent and keeping the preparation for 72 hours at room temperature then evaporating the solvent may be more effective. In addition, the extract of *B. oleracea* obtained from 2.4 mol/L HCl methanol proved to have antibacterial activity for *E. coli* and *S. aereus* bacteria (Hafidh et al., 2011). It is thought that the solvent used in Hafidh et al. (2011) study made a difference from our study results. According to Santhosh and Vaithyanathan (2018) natural red cabbage dye has a clear antimicrobial activity on *S. aereus* bacteria. It is believed to be related to the red cabbage extract extraction method.

DNA Protective Activity

DNA damage protective activity of red cabbage water and methanol extracts after UV irradiation of DNA plasmid in H_2O_2 presence with 50 mg/mL concentration of the extracts is shown in figure 3. Untreated pBR322 plasmid DNA (K1) showed a bright faster band on agarose gel electrophoresis and conformed to the original shape of supercoiled (scDNA). The slower moving band was the open circular form (ocDNA) and linear form (linDNA) was in the middle. The detrimental effect on treated plasmid DNA (K2) without extracts was clearly visible where only one faint band (scDNA) appeared. (LS) and (LM) were samples in which water and methanol extracts were added to the reaction mixture (DNA plasmid + UV + H_2O_2), respectively. It was noted that adding red cabbage extracts (50 mg/mL) to the DNA with (UV + H_2O_2) induced a good recovery level of DNA especially with methanol extract. These results are consistent with Kada et al. (2017) study which showed the DNA protective effect of *Hertia cheirifolia* extracts. This is the first time that DNA protective activity of red cabbage extracts was demonstrated and there is not any such previous research in the literature till now. Further investigations to search for the active components of the DNA protective activity in the red cabbage extracts are required.

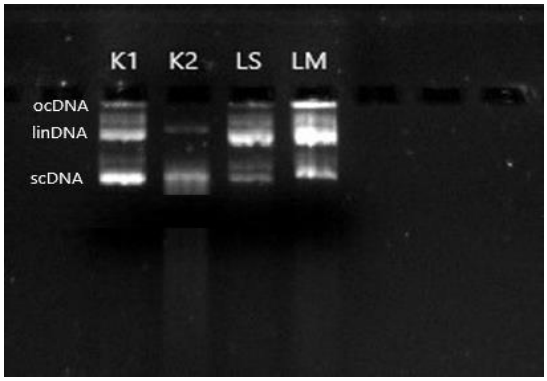


Figure 3. DNA protective activity

K1: Control: DNA plasmid, **K2:** Control: DNA plasmid + UV + H_2O_2,

LS: DNA plasmid + red cabbage water extract + UV + H_2O_2 , **LM:** DNA plasmid + red cabbage methanol extract + UV + H_2O_2

Cytotoxic Activity

The cytotoxic effect of red cabbage water and methanol extracts was investigated on H1299 cancerous cell line and HUVEC normal cell line as a control. The viability (%) graphs are given in Figure 4 and Figure 5. Cancer cells (H1299) maintained a vitality rate of over 90 % even at the highest applied concentration (100 µg/mL) of the extracts. While the vitality rate of HUVEC normal cells, which used as a control, decreased gradually with increasing concentration of extracts until it reached about 74.16% and 69.54% at the concentration of 100 µg/mL of water and methanol extracts, respectively. These results are in agreement with Farag and Abdel Motaal study (2010) which showed weak anticancer activity of red cabbage aqueous extracts against A-549 cell line (human lung carcinoma) as cell vitality was 73% at 500 µg/mL concentration. In Devi and Thangam study (2012), anticancer activity of isolated sulforaphane (SFN) from red cabbage was approved against HEp-2 (human epithelial carcinoma) and Vero cells. Ye et al. (2020) purified antifungal protein from red cabbage seeds and demonstrated its antiproliferative activity on nasopharyngeal cancer NE-1 cells and hepatoma HepG2 cells. Three cancerous cell lines Caco-2 (human epithelial colorectal adenocarcinoma cell), KYSE-30 (human esophageal squamous carcinoma cell) and MCF-7 (human adherent and epithelial breast cancer cell line) were

searched for their anticancer effect and compared with HFF-3 (human adherent and fibroblast normal cell from foreskin tissue). The prepared concentrations in the study ranged from 625-20000 µg/mL, and the study showed the presence of anti-cancer activity for the studied cancerous cell lines, especially at higher doses. Red cabbage extract also decreased the cell viability of HFF-3 normal cells (IC_{50} = 6.4 mg/mL) thus red cabbage extract should not be used at higher concentration than 6.4 mg/mL to avoid normal cell damage (Tajalli et al., 2020).

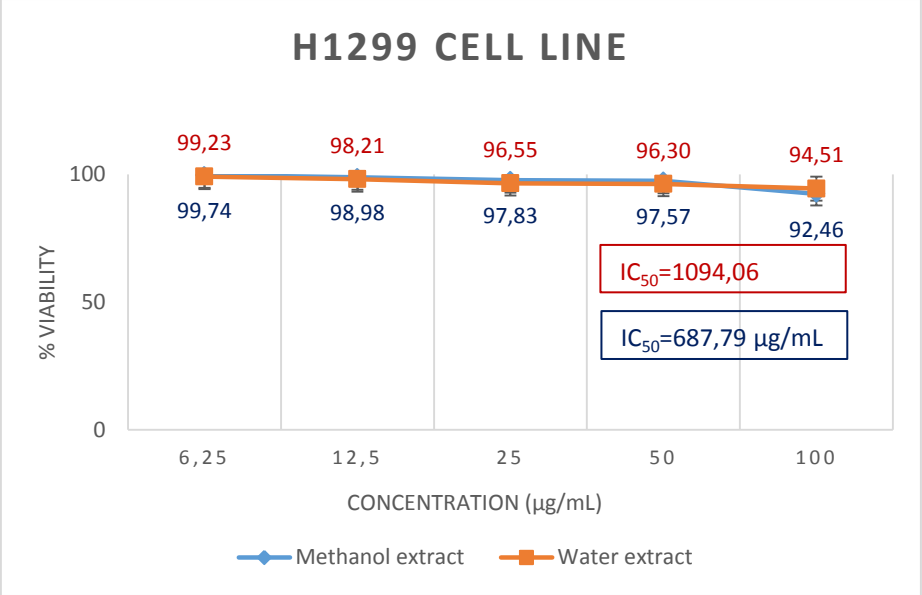


Figure 4. Viability (%) graph of red cabbage extracts (H1299).

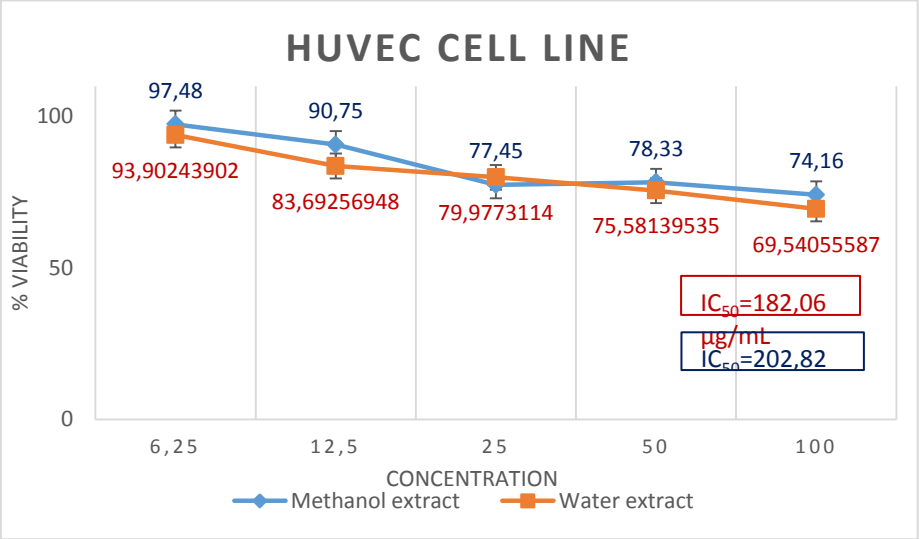


Figure 5. Viability (%) graph of red cabbage extracts (HUVEC)

Conclusion

According to our study and reviewing of previous literature, red cabbage extracts do not have any anti-fungal activity, but they may have anti-bacterial activity against some types of bacteria and that by using 2.4 mol/L HCl methanol as a solvent and extracting by soaking the plant powder in solvent, then filtering the extract and evaporating the solvent at room temperature. Red cabbage water and methanol extracts have a DNA protective activity at a concentration of 50 mg/mL of the extracts. Previous literature did not report any study on the effect of red cabbage extracts on DNA protection activity and this study opens new horizons for research in this field. There is no anticancer activity of red cabbage extracts. However, some isolated components from red cabbage such as sulforaphane and antifungal protein possess anti-cancer activity against some types of cancer.

Recommendations

Further studies are needed to investigate the optimal concentration of red cabbage extracts that gives the best DNA protection effect as well as further studies to explore the effective ingredients in this process and the possibility of incorporating these ingredients into cosmetics such as sunscreens without causing skin damage.

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Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

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