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Original Research

The Effects of Some Phytotherapeutic Plants on *Escherichia coli* spp. that are Exposed to Different Doses of Gamma Radiation

Mehmet Cihan Yavaş[1], Mustafa Salih Çelik[2]

[1]Department of Biophysics, Kırşehir Ahi Evran University School of Medicine, Kırşehir, Turkey

[2]Department of Nursing, Batman University School of Health, Batman, Turkey

ABSTRACT

Objective: The aim of the present study was to determine the antioxidant effects of phytotherapeutic plants on bacterial groups to which gamma radiation at different doses was applied. By this way, the effects of antioxidant ethanolic extracts of plants and the effect of gamma radiation on *Escherichia coli* spp. *(E. coli)* were investigated.

Methods: In the present study, *E. coli* spp. in the areas of agar and agar+plant extracts (83 µl) was irradiated by increasing gamma radiation (100, 200, 500, 1000, 3000, and 6000 cGy). In our study, six plants (carob (*Ceratonia siliqua* L.), basil (*Ocimum basilicum* L.), ginger (*Zingiber officinale*), rosemary (*Rosmarinus officinalis* L.), yarrow (*Achillea millefolium* L.), and cumin (*Cuminum cyminum* L.)) were found to be consumed by people, and their phytotherapeutic effects were investigated.

Results: In the present study, a decrease in the number of colonies of *E. coli* spp. was demonstrated due to the increasing gamma radiation dose. It has been assumed that the ethyl alcohol components of phytotherapeutic plants cannot be assessed but are distributed and clustered on the Petri dish and plant extracts may show antioxidant effects.

Conclusion: It is understood that some phytotherapeutic plants used in gamma radiation applications may show a protective effect.

Keywords: Antioxidant, *Escherichia coli* spp., gamma radiation, phytotherapeutic plants, plant extract

INTRODUCTION

Radiation is defined as the spreading of energy from a source and is divided into ionizing and non-ionizing radiation. Effective protection measures against the effects of ionizing radiation are very important (1). Gamma radiation is defined as a strong carcinogen due to the potential for oxidative damage. It causes DNA damage that is contained in various bond breaks (2). Microbiological (e.g., colony count and growth rate), biochemical (ATPase activity), and biophysics (H^+ fluxes along the cytoplasmic membrane of bacteria) methods are used to evaluate the effect of radiation on bacteria (3). The intensity of the biological damage that the radiation makes at the cellular level depends on the intensity of ionization of the radiation species. Thus, more intense ionizing radiation can cause more damage (4).

Bacteria are produced in liquid and solid mediums. Bacteria, such as *Escherichia coli*, which show a logarithmic proliferation, cloud the fluid medium in 2-3 h. The growth of bacterial is determined by various methods. These; spectrophotometric turbidity, the volume in centrifuge and total nitrogen determination. (5). Over the past 20 years, it has become increasingly common to work with *E. coli* and organisms that are evolutionarily close to it. Owing to its small size, the absence of pathogens for any living organism and the ease of production in laboratory conditions make *E. coli* the most widely studied organism, except for human beings (6).

Medical plants have been used in developing countries for centuries for the treatment of diseases. Phenolic compounds and flavonoids, which contribute to human health, are used in the treatment and prevention of various diseases (7). It is seen that these plants are used in different regions, such as ancient Egypt, China, India, and Mesopotamia, and in most countries, in kitchens, cosmetics, after medical application, and in traditional medicine applications. Many biological activities of rosemary have been described, including antioxidant, antibacterial, antifungal, and anti-cancer (8). Ginger has been widely used for many years in beverages and foods (9). Cumin is one of the oldest medical

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ORCID IDs of the authors: M.C.Y. 0000–0002–2923–050X; M.S.Ç. 0000–0001–7397–8120

Corresponding Author: Mehmet Cihan Yavaş **E–mail:** mcihanyavas@gmail.com

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food plants grown in Asia, Africa, and Europe and is also cultivated in our country (10). Gamma radiation is used during a safe food processing method, and basil has been reported to increase nutritional value after irradiation (11). Owing to the antioxidant properties of carob, it exhibits many protective properties against oxidative stress (12). Both animal and preclinical studies have highlighted that yarrow has anti-inflammatory, anti-ulcer, hepatoprotective, anxiolytic, and perhaps antipathogenic activities and is widely used worldwide for wounds, digestive problems, respiratory infections, and skin disorders (13).

METHODS

Preparation of Bacteria Used in the Study, Phytotherapeutic Plants, and Irradiation Center

In our study, *E. coli* spp. strains obtained from the Microbiology Laboratory of Dicle University School of Medicine were used. Both liquid and solid mediums were studied. Preparation of the extracts of the phytotherapeutic plants used in our study was performed in the Chemistry Department Laboratory of Dicle University Science Faculty. The gamma irradiation used in the study was held at the Department of Medical Oncology at the Dicle University Oncology Hospital. Ethics committee approval was not taken due to in vitro design of the study. This study does not include human participants. Thus, no consent form was required.

Liquid Mediums (Mueller Hinton Broth: Merck, cat no. 1.05437, KGaA, Darmstadt, Germany)

These materials (beef infusion solids: 4 g/l, starch: 1.5 g/l, casein hydrolysate: 17.5 g/l, distilled water: 1000 mL, pH: 7.1±2) were left in a glass flask at the indicated ratios, then thoroughly mixed, and allowed to boil for 1 h in a water bath. The suitability of the mixture for pH was checked. Cylindrical glass tubes prepared for the study were sterilized at 170°C in a Pasteur oven (Heraeus; Thermo Scientific, Waltham, MA, USA). Then, a 5 cc mixture was transferred to each glass tube through a glass pipette, sterilized in an autoclave (Model HA-300MII, Hirayama, Tokyo Japan), and stored in a refrigerator.

Agar Mediums (Blood Agar Base: Merck, cat no. 1.10886.0500, KGaA, Darmstadt, Germany)

These materials (peptone: 10.0 g, beef extract: 9.9 g, sodium chlorite: 5.0 g, agar: 12.0 g, distilled water: 1000 ml, pH: 7.1±2) were mixed in a glass balloon at the specified ratios and allowed to boil for 1 h in a water bath. The suitability of the mixture for pH was checked. Cylindrical glass tubes were then sterilized in an autoclave at 121°C for 15 min. While hot, the medium is expected to solidify by pouring 15-20 mL into sterile petri dishes before the solidification. Before work, the proliferation was examined for growth in Petri dishes at 37°C, and non-reproductive was used.

Collecting, Drying, and Extracting Plants

Six plants assumed to have antioxidant effects were used in the study. The flowers, leaves, and other parts of the six plants (carob, basil, ginger, rosemary, yarrow, and cumin) were collected, dried, and extracted. Plant specimens were pulverized using a mechanical shredder (hand mill and mixer) after weighing 5 g by precise weighing (Vibra Shinko Denshi, Japan) from each plant sample. The pulverized plants were then dissolved in ethanol and incubated. Filtration was applied to eliminate plant residues. After filtration, the ethanol was removed by an evaporator (Heidolph Laborota 4000; Germany) at 50°C at 200 rpm as seen in Figure 1. The samples were transferred to sterile vial tubes (50 mL) with plastic cap and kept in a refrigerator at +4°C until the examination stage. All these operations were performed indoors and under normal conditions.

Preparation of *E. coli* Cultures and the Addition of Extracts to the Agar Medium

In our study, five cc of *E. coli* spp. bacteria was transferred to glass tubes and left in an incubator for 18 h at 37°C. Then, 0.5 cc was extracted from the bacterial culture via a micropipette, and 5 cc of liquid nutrient was transferred to the tubes under sterile conditions, and a dilution of 10^6 was obtained. It was standardized according to 0.5 McFarland (14). In the present study, a standard curve was drawn, absorbance and transmittance values were determined, and bacterial counts were found in the liquid medium. After incubation, the plant extracts were sterilized and added to 20 mL of uncooled agar medium, separately to give 83 µl.

Gamma Irradiation

In our study, gamma radiation was applied at doses of 100 cGy, 200 cGy, 500 cGy, 1000 cGy, 3000 cGy, and 6000 cGy. For the groups outside the control group, gamma radiation was applied using the Co 60 teletherapy device (General Electric Alcyon II, Buc,. France) in the Oncology Hospital of the School of Medicine of Dicle University. The activity of the device was 195 cGy/min, and the surface area of the samples was 80 cm on a 32×32 cm^2 area. *E. coli* spp. was irradiated in logarithmic phase (1-8 min). After incubation, incubation was allowed for 18 h at 37°C. Colony counts were made at the end of the incubation period. Figure 2 shows the irradiation process.

Streaming

In the study, seven groups including one control and six experimental groups were formed. Then, the groups were created to in-

Figure 1. Removal of ethanol by an evaporator after filtration (Evaporator, Heidolph Laborota 4000, Germany)

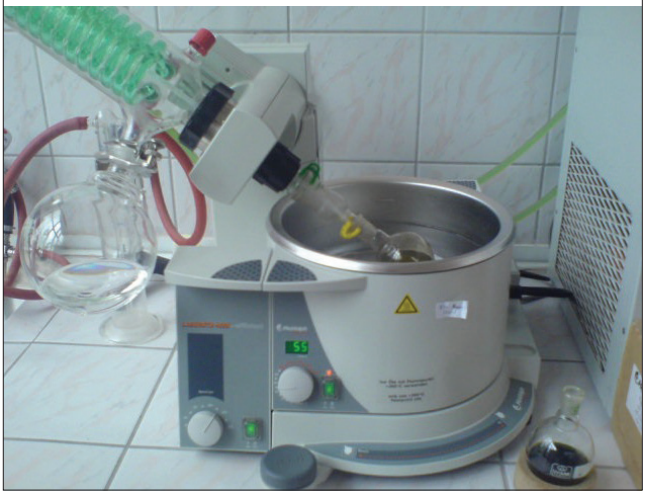


Figure 2. The device that the irradiation is applied to (General Electric Alcyon II, Buc,. France)

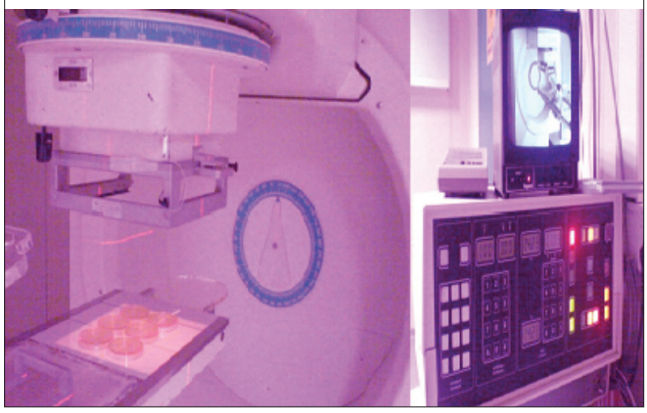
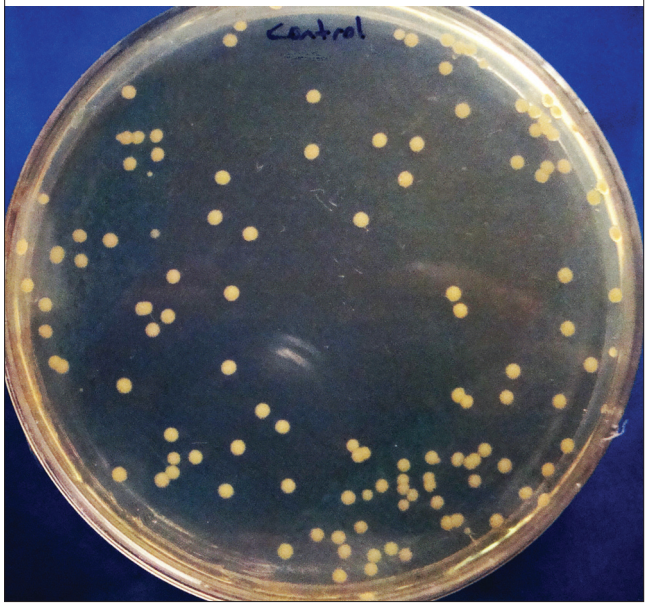


Figure 3. Colony appearance of the control group



vestigate the effect of radiation without adding any plant extracts. The control group was not irradiated, whereas the other groups were irradiated with gamma radiation of 100 cGy, 200 cGy, 500 cGy, 1000 cGy, 3000 cGy, and 6000 cGy in the form of agar+plant extracts (carob: *Ceratonia siliqua* L., basil: *Ocimum basilicum* L., ginger: *Zingiber officinale*, rosemary: *Rosmarinus officinalis* L., yarrow: *Achillea millefolium* L., and cumin: *Cuminum cyminum* L.).

Statistical Analysis

Data were assessed using the Statistical Package for Social Sciences software, version 11.5 (SPSS Inc.; Chicago, IL, USA) for statistical analysis. The average and standard deviations of the colony counts produced by the bacteria in the Petri dish were calculated. Kruskal-Wallis ANOVA test, a non-parametric test, was used for statistical evaluation between the control group and the experimental groups. Mann-Whitney U test, a non-parametric test, was used to compare each of the experimental groups with the control group.

Figure 4. Colony appearance of *E. coli* spp. after gamma irradiation (group 1, 100 cGy)

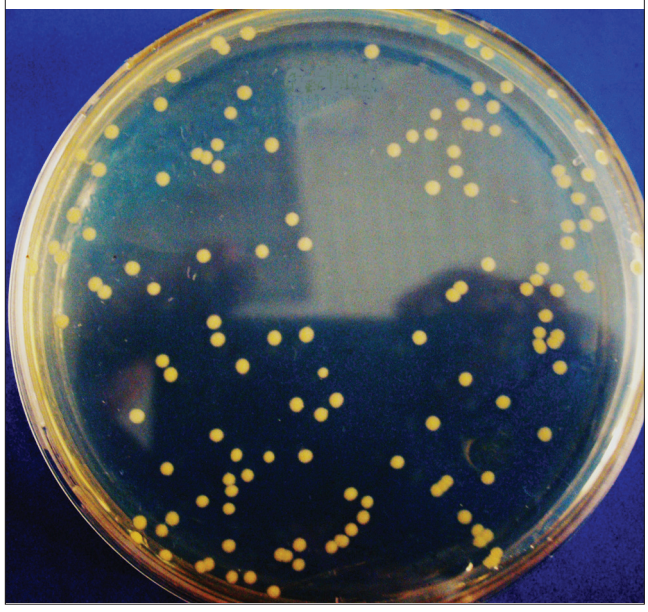
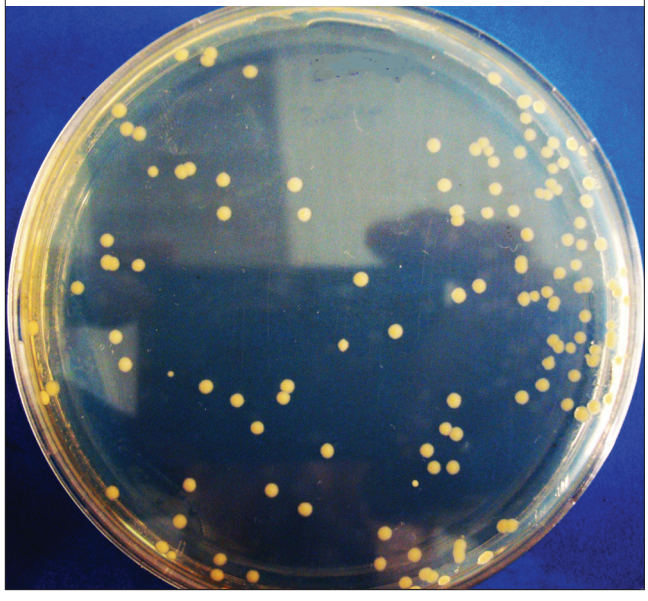


Figure 5. Colony appearance of E. coli spp. after gamma irradiation (group 2, 200 cGy)



RESULTS

In the study, gamma radiation was applied at doses of 100 cGy, 200 cGy, 500 cGy, 1000 cGy, 3000 cGy, and 6000 cGy, and the number of colonies and images formed after incubation were obtained.

Gamma radiation significantly reduces the number of bacterial colonies at increasing doses, and radiation at high doses appears to negatively affect bacterial proliferation. It is assumed that the phytotherapeutic plant-containing extracts we use are not fully

Figure 6. Colony appearance of *E. coli* spp. after gamma irradiation (group 3, 500 cGy)

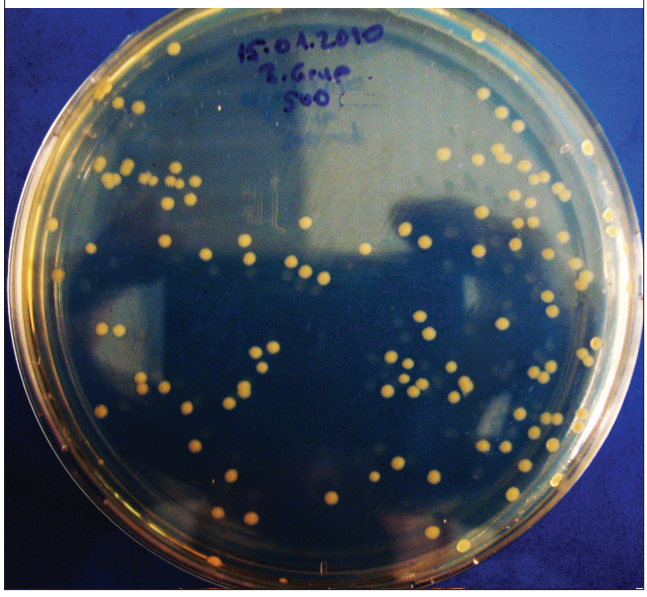


Figure 7. Colony appearance of *E. coli* spp. after gamma irradiation (group 4, 1000 cGy)

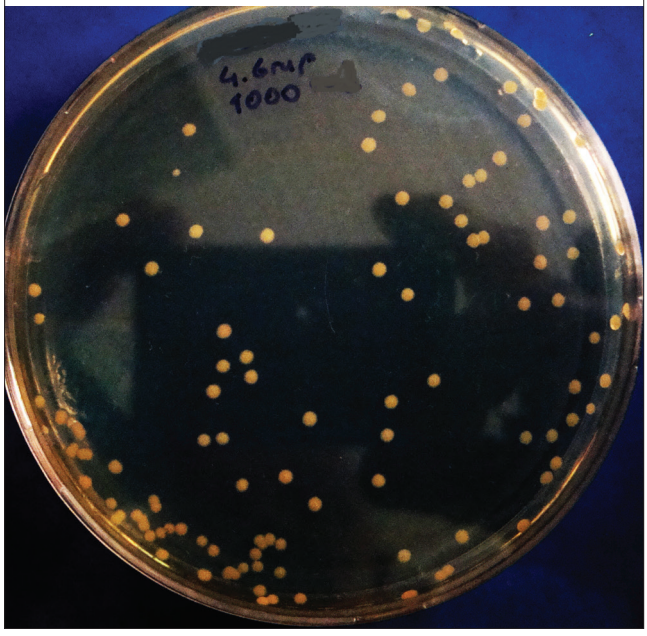


Figure 8. Colony appearance of *E. coli* spp. after gamma irradiation (group 5, 3000 cGy)

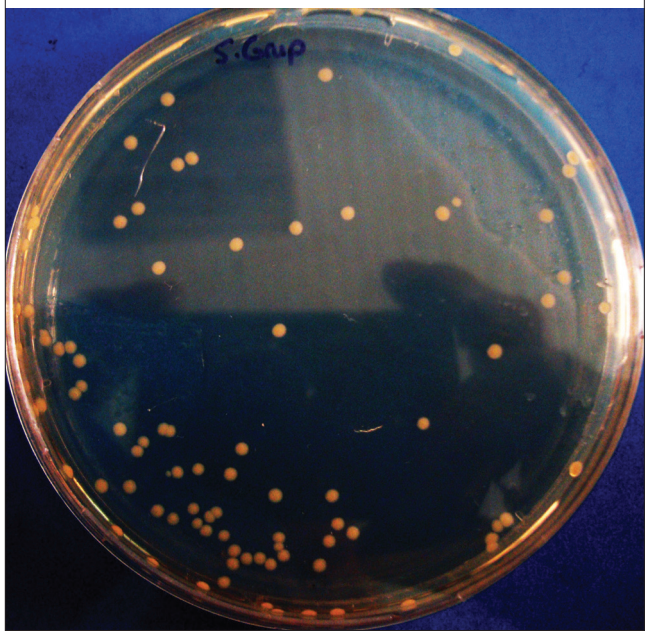
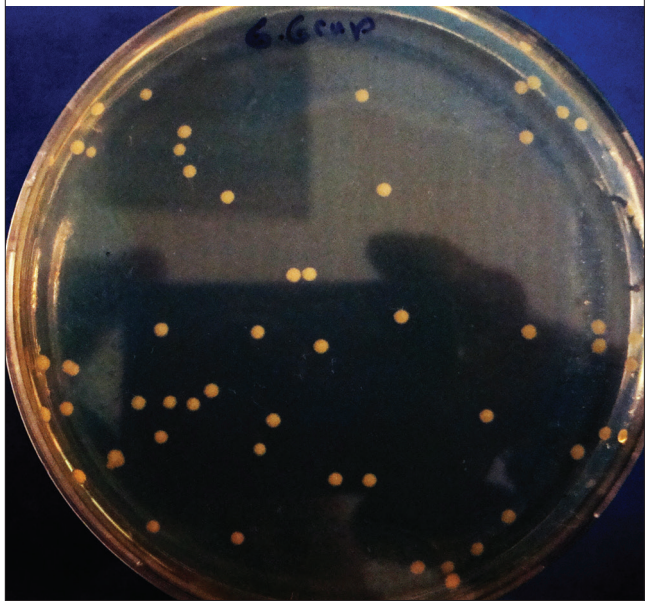


Figure 9. Colony appearance of *E. coli* spp. after gamma irradiation (group 6, 6000 cGy)



counted in the colony counts but may be antioxidant by looking at colony appearance and colony spreads. The number of colonies after irradiation is seen in Table 1. The image of the control group is shown in Figure 3. Figures 4-9 show the number of colonies after 100-6000 cGy radiation.

DISCUSSION

In recent years, serious work has been done on cancer that threatens human life. It has been known for a long time that antioxidant substances are used in cancer treatment. In our study, the effects of changes of gamma radiation we applied on *E. coli* spp. and the possible protective effects of phytotherapeutic plants (basil, carob, rosemary, ginger, cumin, and yarrow) were investigated.

For many years, studies have concluded that ionizing radiation forms reactive oxygen species in cells, which in turn damages the biological system (15). Free radicals are composed of different physiological and pathological conditions and are formed by normal metabolism. When free radicals are formed, the condition between the antioxidant and the oxidant balance deteriorates in

Table 1. Colony counts after incubation of *E. coli* spp. after irradiation

Groups	No. of colonies Mean±SD	Radiation dose (cGy)	p	p
Control	130.3±5.13	0	–	–
Group 1	129±2.00	100	1.00^{ns}	0.008**
Group 2	118±20.22	200	0.376^{ns}	
Group 3	117±2.00	500	0.05*	
Group 4	93.3±9.45	1000	0.05*	
Group 5	75.6±3.78	3000	0.05*	
Group 6	64±6.08	6000	0.05*	

ns: p>0.05, insignificant; *=p<0.05, significant; **=p<0.01, very important

the organism. Thus, lipids, macromolecules, proteins, and nucleic acids will lead to strong damage. It is stated that it may cause different diseases, such as cancer and tissue damage (16). In recent years, extracts and essential oils of many plant species have been used in the treatment of different diseases. Owing to the bioactive nature of these plants, many nutrient and drug applications are increasingly being used. They contain a wide variety of free radical scavenging molecules, such as plants (fruits, vegetables, and medicinal herbs), terpenes, vitamins, nitrogen compounds, phenolic compounds, and other endogenous metabolites. This is due to their rich antioxidant properties (17).

The study of agar+phytotherapeutic plant groups was prepared on the same conditions (e.g., quantity and pH). We observed that irradiation of 6000 cGy on agar+basil group bacteria changed the radiation effect of basil in the medium after comparing with the control group. Carob, rosemary, ginger, cumin, and yarrow also appear to reduce the effect of radiation. Radiation exposure was applied to the bacteria that we received at the same dilution rate. In the case of agar+phytotherapeutic plant, it was observed that the colons dispersed and aggregated on the Petri surface, whereas the columns that formed in the agar environment were countable and fell into a single Petri dish.

Fathiazad et al. (18) stated that ethanolic extracts made from basil leaf extracts may have a cardioprotective effect due to their rich antioxidant. Mahtout et al. (12) reported that the carob is a natural antioxidant because of its potential health benefits. Scientific studies on rosemary and its compounds have been increasing in recent years. It was emphasized that rosemary extracts were effective in modulating irregular signaling pathways in blood cancers (19). Ginger extract (GE) has been reported to have antioxidant properties. GE was evaluated for its activity at different temperatures. It has been found that it has a strong antioxidant effect at high temperatures (20). Owing to the antioxidant effects of essential oils obtained from cumin, it is accepted that it is a useful antioxidant compound in the food industry (21). Navaie et al. (22) used the extracts of the *A. millefolium* L. (yarrow) on breast cancer cell lines. Yarrow extracts can have a potential chemotherapeutic activity for breast cancer treatment. Scientists have shown that they prevented the proliferation of ovarian cancer cell lines by affecting the cell cycle at multiple phases (23). Traditionally, the use of ginger has attracted the attention of scientists, and it has been emphasized that ginger has chemopreventive and anticancer effects due to studies performed in the laboratory environment (9).

In our study, we found that gamma radiation significantly reduces the number of colonies at increased doses, and that radiation is harmful to high doses in bacterial growth. At the same time, plant extracts were added to the agar medium and exposed to gamma radiation at increasing doses. In the agar+plant extract applications, rosemary and yarrow can show antioxidant effects, and carob relatively, basil, ginger, and cumin can have significant phytotherapeutic effects at a certain level. As a result, the size, spread, and distribution of the colonies suggest that these plants have protective effects and are consistent with the literature.

CONCLUSION

The effects of radiation and plants are shown on the bacterium (*E. coli*). It did not fully reflect the effects on human beings. More studies are necessary before clinical use of these plants. The uncontrolled use of herbal products may have unintended consequences. After being proven to be harmless and usable in the clinic, the benefits to human health come before economic benefits.

Ethics Committee Approval: Ethics committee approval was not taken due to in vitro design of the study.

Informed Consent: This study does not include human participants. Thus, no consent form was required.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - M.C.Y., M.S.Ç.; Design - M.C.Y., M.S.Ç.; Supervision - M.C.Y., M.S.Ç.; Materials - M.C.Y.; Data Collection and/or Processing - M.C.Y.; Analysis and/or Interpretation - M.C.Y., M.S.Ç.; Literature Search - M.C.Y.; Writing Manuscript - M.C.Y.; Critical Review - M.C.Y.

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Conflict of Interest: The authors have no conflicts of interest to declare.

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