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Antimutagenic activity of essential oil from Cinnamomum zeylanicum against Salmonella typhimurium strains TA98 and TA100

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Abstract

Cinnamomum zeylanicum, commonly referred to as Ceylon cinnamon, holds significant value in both medicine and economics. The essential oil (EO) extracted from *Cinnamomum zeylanicum* (*C. zeylanicum*) has been extensively studied for its wide range of pharmacological properties. In this study, we aimed to investigate the antimutagenic activity of EO from C. zeylanicum against *Salmonella typhimurium* (*S. typhimurium*) TA98 and TA100 strains using Ames Salmonella/microsome mutagenicity assays. The mutagenic and antimutagenic effects of *C. zeylanicum* against TA98 and TA100 strains at concentrations of 8, 16, 32 and 64 µg/mL were evaluated using the Ames test. The results demonstrated that the EO from *C. zeylanicum* exhibited antimutagenic activity in a dose-dependent manner against both TA98 and TA100 strains. The EO effectively inhibited mutagenicity induced by the test strains, highlighting its potential as an antimutagenic agent. The present study provides valuable insights into the antimutagenic properties of the EO from *C. zeylanicum* and further supports its potential therapeutic applications in medical fields. The findings highlight the importance of C. zeylanicum as a natural source of antimutagenic compounds and warrant further investigation into its mechanisms of action and potential clinical applications.

Keywords: Cinnamomum zeylanicum, antimutagenicity, salmonella typhimurium strains, TA98 strain, TA100 strain, ames test

Introduction

Essential oils (EOs) are known for their complex mixtures of volatile and lipophilic compounds derived from plants. Extensive research has demonstrated a wide range of bioactive properties, such as antibacterial, analgesic, sedative, anti-inflammatory, antispasmodic, local anesthetic, hepatoprotective, nephroprotective, antidiabetic, antihypertensive, and antiulcer effects. Moreover, they have been traditionally employed in embalming and food preservation [1-3]. They also have antiseptic, bactericidal, virucidal and fungicidal properties. Essential oils are generated by plants in a variety of areas including the stems, leaves, flowers, and roots. They are then stored in specific cells or structures [3]. EOs and their constituents are classified as generally recognised as safe (GRAS) by the Food and Drug Administration (FDA) and are widely used in food preservation, pharmaceuticals and aromatherapy [2,4].

Mutagenicity, a key process associated with carcinogenesis, is commonly assessed using bacterial assays such as the Ames test developed by Dr B. Ames in the 1970s [5,6]. This assay, which uses strains of *Salmonella typhimurium* (*S. typhimurium*) to detect chemically induced mutations, has become a well-established method for assessing both mutagenic and antimutagenic properties of various substances [7,8]. The Ames test is particularly valuable because of the strong correlation between mutagenic activity and carcinogenic potential, making it an essential tool for screening potential carcinogens and exploring the mechanisms underlying mutagenesis [9,10].

Some research focusing on the antimutagenic activity of essential oils has shown antioxidant activity, induction of detoxifying enzymes and inhibition of the activating enzymes of pre-

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carcinogens, particularly CYP450. However, the actual influence of essential oils on such key antimutagenic Deoxyribo Nucleic Acid (DNA) repair processes is poorly understood [11]. The use of bacterial systems for mutagenicity testing is widespread and accepted by many because of the universality of genetic material. This makes these systems effective for testing the mutagenic and antimutagenic potential of many chemicals, either in food or pharmaceuticals [8,12].

Cinnamomum zeylanicum (*C. zeylanicum*), widely known as Ceylon cinnamon, is a species from the Lauraceae family, indigenous to Sri Lanka, India, and certain regions of Southeast Asia. The essential oil from the inner bark of the species is highly valued for its distinctive taste and smell, as well as its anti-inflammatory, anti-diabetic, antimicrobial and antioxidant properties [13]. It shows strong antioxidant activity and significant antibacterial potential, with an interesting profile dominated by the compounds cinnamaldehyde, eugenol and linalool, particularly against Gram-positive bacteria such as *Staphylococcus aureus* and *Listeria innocua*, by damaging bacterial cell membranes, leading to cell lysis and death. In addition, the EO has shown antiproliferative effects on certain cancer cell lines, attributed to its ability to scavenge free radicals and inhibit oxidative processes, suggesting its potential application in cancer therapy [11]. In addition, EO from *C. zeylanicum* may serve as a natural preservative in food products. Given its diverse therapeutic potential and culinary applications, further research is warranted to explore its role in food safety and medicine. Thus, we aimed to evaluate the mutagenic and antimutagenic properties of the EO extracted from *C. zeylanicum* by employing Ames Salmonella/microsome mutagenicity assay, utilizing the standard plate incorporation technique.

Material and Methods

Materials

Bacto-agar (Difco; BD BioSciences, Franklin Lakes, NJ, USA), bacteriological agar (Thermo Scientific™ Oxoid™ Agar No.1, Hampshire, England), Nutrient Broth No: 2 (Oxoid), D-Biotin (Merck, Darmstadt, Germany), L-histidine hydrochloride (Merck, Darmstadt, Germany) and ampicillin trihydrate (Merck, Darmstadt, Germany) were used to prepare the medium plates.

In this study, the essential oil from *C. zeylanicum* was obtained from the Biology Department of the Faculty of Science at Anadolu University and has been cited in the work by Unlu et al. Additionally and the essential oil from *C. zeylanicum* were purchased as a commercial product (Talya Herbal LLC) from Eskişehir in Türkiye.

Genotype Control of *S. Typhimirium* Bacterial Strains

The potential effects of the EO from *C. zeylanicum* were evaluated using two *S. typhimurium* strains (TA98 and TA100), which were generously provided by Dr. Bruce N. Ames (University of California, Berkley, USA).

The genetic markers and spontaneous reversion patterns of test strains were regularly monitored, and the strains were preserved at -80°C for long-term storage. Sodium azide for strain TA100 and 4-nitro-o-phenylenediamine (NPA) for strain TA98 were used as positive controls. Sodium azide (5 µg/petri) and NPA (2.5 mg/petri) were prepared by dissolution in Dimethylsulfoxide (DMSO). Stock solutions of positive control mutagens were stored at +4°C. Genotypic controls of bacterial strains were performed as described below. The strains that did not revert to the ancestral form were stored and then the frequency of spontaneous revertant colonies was checked in each set of experiments as negative and positive controls only.

Histidine Requirement Control

Strains were incubated in nutrient broth at 37°C for 12-16 hours. After incubation, the strains were inoculated onto minimal glucose agar (MGA) and histidine/biotin (HB) plates and incubated for 48 hours. While growth was observed on HB plates, no growth was observed on MGA plates.

Crystal Violet Sensitivity Test to Confirm Rfa Mutation

The strains were cultured in nutrient broth at 37°C for 12-16 hours. Subsequently, 100 µL of the nutrient broth (NB) was mixed with 2 mL of top agar, which had been heated to 45°C in a water bath, and then poured onto nutrient agar (NA) plates. Once the top agar solidified on the plates, a sterile filter paper disk with a 0.5 cm diameter was positioned at the center of each plate.10 µl of crystal violet was dropped into the centre of the plate. After the paper had absorbed the dye, the plates were incubated at 37°C for 24 hours.

Uvr B Mutation Control

The DNA repair mechanism necessary for the correction of replication errors caused by ultraviolet (UV) light is inhibited in these strains. The presence of this mutation is detected by UV light sensitivity test. For this purpose, the strains grown in NB were inoculated parallel to NA plates and half of the plate was covered with a plastic sheet (so as to cut the lines). The plates were exposed to a 15-watt UV lamp from a height of 33 cm for 8 seconds. Following irradiation, the plates were incubated at 37°C for 24 hours.

R Factor Control

Whether or not the R-factor carrying the drug resistance plasmid (pKM101) present in the strains have been lost is determined by measuring ampicillin resistance. For this purpose, strains grown in NB are inoculated on histidine/biotin/ampicillin (HBA) plates. They are incubated at 37°C for 24 hours. Mutant bacteria containing the plasmid are observed to grow in ampicillin medium.

Control of the Frequency of Spontaneously Revertant

To determine the number of revertant colonies, the strains grown in NB were suspended on 100µl of top agar. Subsequently, 200 µL of a 0.05 mM histidine-biotin solution was added to the plates.

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After gentle agitation, MGA plates were placed and incubated at 37°C for 48 hours. Colonies were then counted and the number of revertant colonies determined according to the type of strain. TA98 30-50 revertant/plate and TA100 120-200 revertant/plate are expected.

Cytotoxicity Test

The amount of test component used for mutagenicity analyses was determined by a cytotoxicity assay. For this purpose, a pre-incubation experiment between 0-30 min was performed and the OD600 value was measured in a spectrophotometer. According to the data obtained, it was decided to apply the test substance to the strains cultured without incubation for 15 hours and then the cytotoxicity test was performed. For this purpose, 0.1mL of bacterial strains were prepared in Oxoid Nutrient Broth No: 2 medium overnight for 15 hours, and 0.1mL of bacterial strains, different concentrations of test components were prepared in serial dilutions and added to 2 mL of overlay agar. The mixture in the test component tube was then transferred to nutrient agar plates and incubated at 37°C for 24 hours. The colonies on the plates were counted and the toxic concentration range was determined by comparison with the control plates.

Mutagenicity Test

After determination of the non-cytotoxic concentration range, mutagenicity testing was performed using the Salmonella microsome test system. The mutagenic properties of EO from *C. zeylanicum* were assessed utilizing the plate incorporation method as outlined by Maron and Ames (1983) [5,14]. In the Salmonella microsome test, data are analyzed by observing a consistent doubling of the spontaneous/revertant reversal frequency, which is further validated by a dose-response relationship. A positive result can still be concluded, even if the number of induced revertant is less than double, as long as a reproducible dose-dependent increase is observed [15,16]. For this purpose, 200µL of histidine/biotin solution, 200µL of bacterial strain culture and 100µL of test component were added to 2mL of top agar. The mixture in the tube was inoculated onto MGA plates and incubated at 37°C. The mutagenicity test was performed for 72 hours. In each experimental group, spontaneous control, solvent control and diagnostic controls were performed and 3 independent experiments were repeated. Three parallel plates were used in each experimental group.

Antimutagenicity Test

The antimutagenic properties of EO from *C. zeylanicum* were evaluated using the plate incorporation method outlined by Maron and Ames (1983) [5,14]. Following mutagenicity testing of the test component, antimutagenicity testing was performed using the Salmonella microsome test system and the number of revertant colonies was determined. For this purpose, a pre-incubation experiment was performed for 5, 10, 15, 20, 25 and 30 minutes to determine the application time. Then 100 µL of the test, bacterial strain culture, 100 uL of the test component, 100 uL of the test component were incubated with 100 uL of positive mutagen until the determined application time and added to the chemical soft agar, then plated on MGA plates and incubated at 37°C. The antimutagenic activity of the test component was compared with that of the positive control and the inhibitory effects were determined by counting the revertant colonies at the end of the 72-hour incubation.

Statistical Analysis

The statistical analysis of the experimental data was conducted using GraphPad Prism version 9.5. Data were analyzed using one-way ANOVA, as the data distribution was assumed to be parametric based on the experimental design. Post-hoc comparisons were performed using Dunnett's test to compare treatment groups with the control group. Statistical significance was determined as follows: (*) $p \leq 0.05$, (**) $p \leq 0.01$, (***) $p \leq 0.001$, (****) $p \leq 0.0001$

Results

The present study was conducted using both a commercially available essential oil from *C. zeylanicum* and the essential oil extracted from cinnamon bark and analyzed by Ünlü et al. [17]. Due to the high similarity between the results of the commercial oil and the essential oil used by Ünlü et al. (2010), the study was conducted with the commercially purchased oil, and the results obtained from this oil are presented in the study. The study of the EO was conducted using a non-toxic dose range.

Genotype controls for the bacterial strains *S. typhimurium* TA98 and TA100 were assessed through control tests that evaluated histidine requirement, UvrB mutation, Rfa mutation, presence of R factors, and spontaneous revertant frequency. According to our results, approximately 20-30 revertant/plate for strain TA98 and approximately 120-150 revertant/colony for TA100 were counted as negative control plates. The anticipated number of revertant colonies in the negative and positive control groups was determined by assessing whether the bacterial strains reverted to their ancestral form at each stage of the experiment. After bacterial genotype control, bacterial strains were treated with different concentrations of the EO from *C. zeylanicum* to determine the cytotoxic effect. The cytotoxicity test was performed for the non-lethal concentration range. According to these results, it was considered appropriate to perform mutagenicity test in the concentration range of 8, 16, 32, 64 ug/mL.

The colony counts obtained in the mutagenicity test with *S. typhimurium* TA98 and TA100 at 8, 16, 32 and 64 ug/mL concentrations of the EO from *C. zeylanicum* were averaged and the results compared with the number of spontaneous revertant in the DMSO control. It was found that the concentrations determined were not lethal for both TA98 and TA100 strains and that there were approximately as many hist+ revertant colonies as there were revertant colonies in the DMSO control group (Figure 1). As a result of three independent experiments

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with three replicates, the number of revertant colonies in the experimental groups was found to be comparable to that in the DMSO control group. Consequently, the antimutagenic activity was subsequently evaluated.

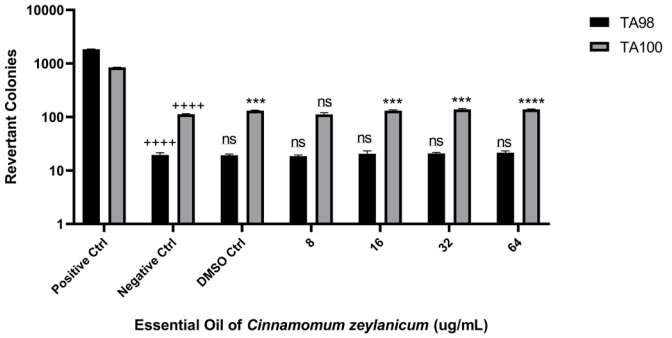

Figure 1. Investigation of mutagenic activities of essential oil from *C. zeylanicum*; spontaneous reversion TA98 30±12 TA100 170±30 ***p<0.001; each data point represents the average of nine replicates across three independent experiments; the (*) symbol represents significant differences between the control and experimental groups, while the (+) symbol indicates significant differences between the negative and positive control groups; the significance value was evaluated as (*) p≤0.05, (**) p≤0.01, (***) p≤0.001, (****) p≤0.0001, and non-significant (ns) (p>0.05), differences that do not show significance

The potential antimutagenic effects of the EO of *C. zeylanicum* were examined against NPA and sodium azide in the same test strains utilizing the plate association assay. For this purpose, the pre-incubation experiment of the EO (500 µl/plate) was performed in both strains and the application time was determined. Accordingly, the pre-incubation time was selected to be 30 minutes and it was found to be effective in inhibiting the mutagenic activity induced by sodium azide and NPA.

The antimutagenic activity of the essential oil is shown in Figure 2. The essential oil dose-dependently reduced the mutagenic activity of both sodium azide (for TA100 strain) and NPA (for TA98 strain). The significant difference compared to the control is clearly seen in Figure 2 with the decrease in colony number at the dose of 64 µg/mL in the TA98 strain.

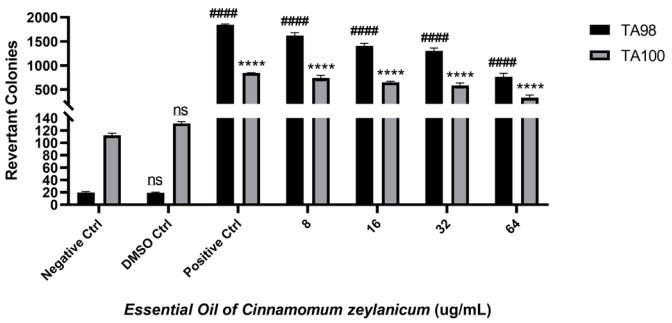

Figure 2. Investigation of antimutagenic activities of essential oil from *Cinnamomum zeylanicum*; spontaneous reversion TA98 30±12 TA100 170±30 ***p<0.001; each data point represents the mean of nine replicates obtained from three independent experiments; the symbol (*) indicates significant differences compared to the control group for the TA100 strain, while (#) denotes significant differences for the TA98 strain; the significance value was evaluated as (*) p≤0.05, (**) p≤0.01, (***) p≤0.001, (****) p≤0.0001, and non-significant (ns) (p>0.05), differences that do not show significance

Discussion

The present study highlights the antimutagenic potential of the EO from *C. zeylanicum* against *Salmonella typhimurium* TA98 and TA100 strains using the Ames Salmonella/microsome mutagenicity assay, a standard plate incorporation method. It was shown that the EO of *C. zeylanicum*, applied at different concentrations, had no mutagenic effects on the TA98 and TA100 strains. The antimutagenic effect of the essential oil on the strains was then examined, given that no mutagenic activity was found at the indicated dosages. Our results show that the EO of *C. zeylanicum* has antimutagenic activity against NPA and sodium azide in *S. typhimurium* TA98 and TA100 strains, respectively. Furthermore, the strongest antimutagenic activity was observed at a concentration of 64 µg/mL of the EO against *S. typhimurium* TA98 strain. This strain, which has a frame-shift mutation, showed slightly greater the antimutagenic activity than the TA100 strain, which has a base-substituted mutation [8]. This result suggests that the EO has stronger antimutagenic properties against the NPA mutagen. According to studies, the essential oil showed a dose-dependent antimutagenic effect and successfully inhibited the mutagenicity induced in the test strains.

In another study investigating the mutagenic activity of cinnamon oil, only the mutagenic activity was evaluated using TA98 and TA100 mutant strains and it was suggested that doses of 2 and 4 µg/mL may have a low mutagenic potential [18]. According to our results, no mutagenic activity was detected in the dose range of 8-64 µg/mL. In our investigation of its antimutagenic activity, the EO showed a dose-dependent protective effect against substances with established mutagenic properties. These results suggest that the EO may have antimutagenic effects at higher concentrations, but its protective effects may be reduced at lower concentrations. This is consistent with previous findings showing that certain phenolic compounds can exhibit mutagenic activity at low doses, possibly due to their pro-oxidant effects [19]. Furthermore, despite differences in the commercial cinnamon oils used in the different studies, these variations suggest that the biological effects of herbal products may vary depending on the strain and the test protocol. In our current study, mutagenic activity was induced by pre-incubation with the mutagenic substances NPA and sodium azide for 30 minutes, followed by testing for antimutagenic activity.

Moreover, a comparative experimental analysis was performed using the cinnamon oil from this study and the essential oil extracted from cinnamon bark, both of which yielded comparable results. In the study yielding similar results, Ünlü et al. analyzed the composition of essential oil extracted from *C. zeylanicum* bark and identified nine distinct compounds, with (E)-cinnamaldehyde being the predominant component at 68.75%. The other identified constituents included benzaldehyde (9.94%), (E)-cinnamyl acetate (7.44%), limonene (4.42%), and eugenol (2.77%) [17]. Notably, this study of the essential oil highlighted its antimicrobial and cytotoxic properties, indicating its potential as both an antibacterial and anticancer agent [20,21].

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Our Ames test results indicated the antimutagenic activity of the EO at all concentrations. The findings are consistent with previous research on the antimutagenic properties of natural plant extracts, including cinnamon derivatives, which have been shown to reduce the mutagenicity of various compounds. In particular, the study observed a dose-dependent antimutagenic activity of the essential oil, particularly at concentrations ranging from 8 to 64 µg/mL, which is consistent with previous reports indicating the ability of phenolic compounds in cinnamon to neutralise mutagens such as sodium azide and other genotoxic agents [22]. The anti-mutagenic effect observed in the present study is probably due to the high phenolic content of the essential oil, as similar results have been reported with other phenolic-rich plant extracts [23]. The essential oil is known to contain several bioactive compounds, including cinnamaldehyde, eugenol and linalool, which contribute to its medicinal properties [24]. In particular, cinnamaldehyde has been identified as the primary compound responsible for the biological activities of the oil, including its anticancer properties [25]. The antimutagenic activity is likely attributed to the presence of phenolic compounds in the essential oil, which are recognized for their antioxidant properties. These compounds can neutralise free radicals and reactive intermediates that contribute to mutagenesis [19]. Consequently, studies show that mutagenicity and carcinogenicity are qualitatively related, but quantifying the relationship is still difficult and controversial. Early research indicated a strong association between a chemical's capacity to induce mutagenesis and cancer; however, more recent analyses have uncovered inconsistencies and highlighted the impact of biological factors, such as metabolic activation and DNA repair mechanisms, on cancer risk [13]. The direct link between mutagenicity and cancer incidence may be complicated by several factors that can alter the way mutagens damage DNA and contribute to cancer development. In addition, different mutagens - such as chemical, physical and biological agents - can cause different types of mutations that lead to different types of cancer. Given the complexity of understanding how different mutagens contribute to cancer, it is essential to explore the molecular mechanisms of these agents, as their specific roles in mutagenesis and carcinogenesis are not yet fully understood. This highlights the importance of comprehensive studies at the molecular level to develop effective strategies for cancer prevention and treatment [13,26].

The antimutagenic effects observed in this study suggest that the EO from *C. zeylanicum* may contain compounds capable of interfering with the mutagenic process, possibly by preventing mutagen formation or by enhancing the detoxification mechanisms of the body [19]. This finding is important as it highlights the potential of the EO from *C. zeylanicum* as a natural antimutagenic agent for various medical applications.

Further research is needed to elucidate the precise mechanisms by which the EO from *C. zeylanicum* exerts its antimutagenic effects and to explore its potential clinical applications. In addition, in vivo studies and clinical trials are essential to assess the efficacy and safety of this essential oil in humans.

Conclusion

The results support the use of the EO of *C. zeylanicum* as a natural source of antimutagenic compounds and warrant further investigation into its mechanisms of action and potential clinical applications. The study provides valuable insights into the antimutagenic properties of the EO from *C. zeylanicum*, supporting its potential as a therapeutic agent. In addition, the EO from *C. zeylanicum* may act through multiple antimutagenic mechanisms, such as reducing alkylated DNA damage and decreasing the frequency of base substitution mutations. The findings highlight the importance of *C. zeylanicum* as a natural source of antimutagenic compounds and warrant further investigation into its mechanisms of action and potential clinical applications.

Conflict of Interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
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Ethical Approval
This article does not contain any studies with human participants performed by any of the authors.

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References

1. Gheorghe-Irimia RA, Tăpăloagă D, Tăpăloagă PR, et al. Spicing up meat preservation: cinnamomum zeylanicum essential oil in meat-based functional foods-a five-year review. Foods. 2024;13:2479.
2. Espina L, Somolinos M, Lorán S, et al. Chemical composition of commercial citrus fruit essential oils and evaluation of their antimicrobial activity acting alone or in combined processes. Food Control. 2011;22:896-902.
3. Perricone M, Arace E, Corbo MR, et al. Bioactivity of essential oils: a review on their interaction with food components. Front Microbiol. 2015;6:76.
4. Sanchez-Tamayo M, Ruiz-Llacsahuanga B, Raad R, et al. Inactivation of foodborne pathogens on gala apples by application of antimicrobial waxes. Food Control. 2024;155:110049.
5. Maron DM, Ames BN. Revised methods for the Salmonella mutagenicity test. Mutat Res. 1983;113:173-215.
6. Marnett LJ. Adventures with Bruce Ames and the Ames test. Mutat Res Genet Toxicol Environ Mutagen. 2019 Oct;846:403070.
7. Forman D. Ames, the Ames test, and the causes of cancer. BMJ. 1991;303:428-9.
8. Mortelmans K. A perspective on the development of the Ames Salmonella/mammalian-microsome mutagenicity assay. Mutat Res Genet Toxicol Environ Mutagen. 2019;841:14-6.
9. DNA Repair and Mutagenesis, Chapter 11. In: Snyder L. Champness W. Molecular genetics of bacteria. 3rd ed. Washington, D.C.: ASM Press, 2007;375-405.
10. Barros B, Oliveira M, Morais S. Unveiling urinary mutagenicity by the ames test for occupational risk assessment: a systematic review. Int J Environ Res Public Health. 2022;19:13074.

DOI: 10.5455/medscience.2024.10.121

11. Bhalla Y, Gupta VK, Jaitak V. Anticancer activity of essential oils: a review. J Sci Food Agric. 2013;93:3643-53.

12. Ipek E, Sivas Zeytinoglu H, Okay S, et al. Genotoxicity and antigenotoxicity of origanum oil and carvacrol evaluated by Ames Salmonella/microsomal test. Food Chemistry, 2005;93:551-6.

13. Kumari S, Sharma S, Advani D, et al. Unboxing the molecular modalities of mutagens in cancer. Environ Sci Pollut Res Int. 202;29:62111-59.

14. Tejs S. The Ames test: a methodological short review. Environmental Biotechnology. 2008;4:7-14.

15. Dean BJ, Danford N. Assays for the detection of chemically-induced chromosome damage in cultured mammalian cells. Mutagenicity testing, A practical approach. Oxford: IRL Limited. 1984;187-232.

16. Rainer B, Pinter E, Prielinger L, et al. Direct comparison of the lowest effect concentrations of mutagenic reference substances in two ames test formats. Toxics. 2021;9:152.

17. Unlu M, Ergene E, Unlu GV, et al. Composition, antimicrobial activity and in vitro cytotoxicity of essential oil from Cinnamomum zeylanicum Blume (Lauraceae). Food Chem Toxicol. 2010;48:3274-80.

18. Zemheri Navruz F. The investigation of mutagenic effect of cinamon (Cinnamomum zeylanicum) by Ames test. Kocatepe Vet J. 2015;8:23-7.

19. Mohanty D, Padhee S, Sahoo C, et al. Integrating network pharmacology and experimental verification to decipher the multitarget pharmacological mechanism of Cinnamomum zeylanicum essential oil in treating inflammation. Heliyon. 2024;10:e24120.

20. Ranasinghe P, Pigera S, Premakumara GA, et al. Medicinal properties of 'true' cinnamon (Cinnamomum zeylanicum): a systematic review. BMC Complement Altern Med. 2013;13:275.

21. Çiçekli K. Cinnamomum zeylanicum - cinnamon. Acta Medica Ruha. 2023;1:154-7.

22. Jayaprakasha G, Negi P, Sankar Jena B, Rao LJM. Antioxidant and antimutagenic activities of Cinnamomum zeylanicum fruit extracts. Journal of Food Composition and Analysis. 2007;20:330-6.

23. Negi P, Jayaprakasha G. Antioxidant and antibacterial activities of Punica granatum peel extracts. Journal of Food Science. 2003;68:1473-7.

24. Rao PV, Gan SH. Cinnamon: a multifaceted medicinal plant. Evid Based Complement Alternat Med. 2014;2014:642942.

25. Peng J, Song X, Yu W, et al. The role and mechanism of cinnamaldehyde in cancer. J Food Drug Anal. 2024;32:140-54.

26. Mohn G. On the correlation between mutagenicity and carcinogenicity. In: Genetic Origins of Tumor Cells. Springer. 1980;11-24.