Antioxidant, mutagenic, and antimutagenic activities of *Tragopogon longirostis* var. *longirostis*, an edible wild plant in Turkey

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**ABSTRACT**

**Objectives:** The ethanolic extract of *Tragopogon longirostis* var. *longirostis*, a wild edible plant in Anatolia was isolated, and its antioxidant, mutagenic, and antimutagenic properties were investigated.

**Materials and Methods:** The antioxidant activity (AA) was determined by the inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, total AA, and phenolic compounds. The mutagenic and antimutagenic activities were investigated by Ames *Salmonella*/microsome mutagenicity test.

**Results:** The IC<sub>50</sub> value for DPPH radicals was 7.84 ± 0.603 mg/mL. The total AA increased with an increase in the concentration of the extracts (1, 5, 10, 20, and 30 mg/mL), containing linoleic acid emulsion. The total phenolic content was 284.71 ± 5.6 mg gallic acid equivalent/g extract. The results showed that the ethanolic extract can be considered safe, because it does not have any mutagenic effect at the tested concentrations. As a result, the ethanolic extract of the leaves exhibited antimutagenic effects at 2.5, 0.25, and 0.025 mg/plate concentrations.

**Conclusions:** To our knowledge, this is the first study of the antioxidant, mutagenic, and antimutagenic activities of *T. longirostis* var. *longirostis*. These activities are an important topic in the food industry, as well as in the medical field.

**KEY WORDS:** Ames, radical scavenging, *Salmonella typhimurium*

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Like natural antioxidants, natural antimutagens from edible and medicinal plants are of particular importance because they may be useful for human cancer prevention and have no undesirable xenobiotic effects on living organisms.[9] Herbal remedies and phytotherapy drugs, containing active principles are currently developed to protect against electrophile (e.g., free radical) attack on DNA and its widespread outcomes such as aging and cancer.[8]

Recently, there has been considerable interest in the antioxidant activity (AA),[9] mutagenicity[10] and antimutagenicity[10] of medicinal and edible plants.

As far as we know, no literatures on the mutagenic, and antimutagenic effects of *T. longirostis* var. *longirostis* have been published. Thus, this is the first study of *T. longirostris* var. *longirostris* to evaluate mutagenic, and antimutagenic activity in order to use in phytomedicine.

**Materials and Methods**

**Plant Material**

The young samples of *T. longirostis* var. *longirostis* naturally growing plants belonging to the Asteraceae family were collected from Mugla region, Turkey. The plant samples were air-dried at room temperature for later analysis.

**Preparation of the Ethanolic Extract**

The air dried and powdered plant samples were extracted with ethanol (Merck) using the Soxhlet apparatus. The extract was evaporated and then extracted in ethanol/water (1:1, v/v), and then kept in small sterile opac bottles under refrigerated conditions until used.

**Bacterial Strains**

*S. typhimurium* TA98 and TA100 were used for the mutagenity and antimutagenity tests. The strains were analyzed for their histidine requirement, biotin requirement, the combination of both, rfa mutation, excision repair capability, the presence of the plasmid pKM101, and spontaneous mutation rate according to Mortelmans and Zeiger.[11] Working cultures were prepared by inoculating nutrient broth with the frozen cultures, followed by an overnight incubation at 37°C with gentle agitation.[12]

**Antioxidant Activity**

**Determination of 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity**

Antioxidant activity of the extract was determined based on its ability to react with the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical.[13] Fifty microliter of the extract (1, 2.5, 5, and 10 mg/mL in ethanol/water (1:1, v/v)) was added to 5 mL DPPH solution (0.004%) in ethanol. After incubation at room temperature for 30 min, the absorbance of each solution was determined at 517 nm. Percentage of inhibition and the concentration of sample required for 50% scavenging of the DPPH free radical (IC₅₀) were determined. Butylated hydroxytoluene (BHT) and ascorbic acid were used as a control.

**Total antioxidant activity by the β-carotene-linoleic acid method**

The total AA of the ethanolic extract of *T. longirostis* var. *longirostis* was evaluated by the β-carotene-linoleic acid model.[14] About 0.5 mg of the β-carotene in 1 mL of chloroform, 25 μL of linoleic acid, and 200 mg of Tween-40 (polyoxyethylene sorbitan monopalmitate) were mixed together. The chloroform was completely evaporated using a vacuum evaporator and the resulting solution was diluted with 100 mL of oxygenated water. A volume of 2.5 mL aliquots of this mixture were transferred into different tubes containing 0.5 mL of samples at 1, 5, 10, 20, and 30 mg/mL concentrations in ethanol/water (1:1, v/v). The same procedure was repeated with the positive control BHT, ascorbic acid, and a blank. The emulsion system was incubated for up to 2 h at 50°C. The measurement of absorbance was continued until the color of β-carotene disappeared in the control. After this incubation period, the absorbance of the mixtures was measured at 490 nm. All determinations were performed in triplicate.

The bleaching rate (R) of β-carotene was calculated using the following formula. R = ln (a/b)/t where, In = natural log, a = absorbance at time 0, b = absorbance at time t (120 min). The AA was calculated in terms of percent inhibition relative to the control using the formula AA = | (Rcontrol − Rsample) / Rcontrol | × 100. Antioxidative activities of the extracts were compared with those of BHT and ascorbic acid at 0.5 mg/mL.

**Determination of total phenolic compounds**

The phenolic constituent of the extract was determined by the method involving the Folin-Ciocalteu reagent and gallic acid as standard.[15,16] Two hundred microliter of extract solution containing 0.1 mg extract was added to a test tube. Then, 100 μL of Folin-Ciocalteu reagent was added, and the tube was shaken vigorously. After 3 min, a 2 mL solution of Na₂CO₃ (0.5%) was added, and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm.

Content of phenolic compounds was determined as mg gallic acid equivalents per gram of extract (mg/g GAE extract) using the following linear equation based on the calibration curve: A = 0.0265C, R² = 0.993 where A is the absorbance and C gallic acid equivalents.

**Mutagenic and Antimutagenic Activity**

**Viability assays and determination of test concentrations**

Cytotoxic doses of the *T. longirostis* var. *longirostis* ethanolic extract was determined by the method of Mortelmans and Zeiger.[11] The toxicity of ethanolic extract toward *S. typhimurium* TA98 and TA100 was determined as described in detail elsewhere.[17,18] These tests confirmed that there was the normal growth of the background lawn, spontaneous colony counts within the regular range, and no significant reduction in cell survival. Thus, for the concentrations and conditions reported here, no toxicity or other adverse effects were observed.

**Mutagenicity and Antimutagenicity Tests**

In this study, the plate incorporation method was used to assess the results of mutagenicity and antimutagenicity assays.[19] The known mutants 4-nitro-o-phenylenediamine (4-NPD) (3 μg/plate) *S. typhimurium* TA98 and sodium azide (Na₃N) (8 μg/plate) for *S. typhimurium* TA100 were used as positive controls and ethanol/water (1:1, v/v) was used as negative control in mutagenicity and antimutagenicity tests.

In the mutagenicity test performed with TA98 and TA100 strains of *S. typhimurium*, 100 μL of the overnight bacterial culture, 100 μL of test compounds at different concentrations (2.5, 0.25, and 0.025 mg/plate), and 500 μL of phosphate,...
buffer were added to 2 mL of the top agar containing 0.5 mM histidine/biotin. The mixture was poured onto minimal glucose plates. Histidine independent revertant colonies and viable cells were scored on plates after incubation at 37°C for 48 or 72 h.

In the antimutagenicity test performed with the same strains, 100 μL of the overnight bacterial culture, 100 μL of mutagen, 100 μL of test compounds at different concentrations (2.5, 0.25, and 0.025 mg/plate), and 500 μL of phosphate buffer were added to 2 mL of the top agar containing 0.5 mM histidine/biotin. The mixture was poured onto minimal glucose plates. Histidine independent revertant colonies and viable cells were scored on plates after incubation at 37°C for 48 or 72 h.

The plate incorporation method was used to assess the results of mutagenicity and antimutagenicity assays. For the mutagenicity assays, the mutagenic index was calculated for each concentration, which is the average number of revertants per plate divided by the average number of revertants per plate with the negative control.

For the antimutagenicity assays, the % inhibition was calculated according the formula given below.

\[
\text{% Inhibition} = [1 - (T/M)] \times 100
\]

Where T is the number of revertants per plate in the presence of mutagen, and the test sample, and M is the number of revertants per plate in the positive control. The antimutagenic effect (% inhibition) between 25–40% was defined as moderate antimutagenicity, 40% or more as strong antimutagenicity, and 25% or less inhibition as no antimutagenicity.

### Results

The free radical-scavenging capacity of the corresponding extract measured by DPPH assay, and the IC₅₀ values of the extract, BHT, and ascorbic acid are shown in Table 1. Lower IC₅₀ value indicates higher AA.

Total antioxidant activities of the ethanolic extract of T. longirostis var. longirostis, according to the β-carotene-linoleic acid method, are shown in Table 2. When screened for its radical scavenging and total antioxidants properties, the ethanolic extract of T. longirostis var. longirostis provided dose-dependent results in different assays. The results indicate that the radical-scavenging activity of BHT and ascorbic acid were higher than that of the extract.

In this study, the phenolic content of the ethanolic extract of T. longirostis var. longirostis was found to be 284.71 ± 5.6 mg GAE/g extract. The data obtained from this part show a correlation with those obtained from the β-carotene-linoleic acid test system.

The ethanolic extract of T. longirostis var. longirostis, which was tested at three different concentrations including 0.025, 0.25, and 2.5 mg/plate, did not exhibit any mutagenic effect in the mutagenicity assays performed with S. typhimurium TA98 and TA100 (data not shown).

The possible antimutagenic potential of the extract was examined against 4-NPd and NaN₃ in S. typhimurium TA98 and TA100, respectively. The results were evaluated by using standard plate incorporation method and summarized in Table 3.

In the antimutagenicity assays performed with TA98 and TA100 strains, the extract exhibited antimutagenic effects at 2.5, 0.25, and 0.025 mg/plate concentrations. The strongest antimutagenic activity was observed at 2.5 mg/plate concentration against S. typhimurium TA 98 strain. The results showed that only one concentration (0.025 mg/plate) of the extract did not have any antimutagenic effect against S. typhimurium TA 98. The antimutagenic activity of the extract was determined as being dose dependent.

### Discussion

The DPPH-scavenging capacity of the extract may be mostly related to its phenolic content. The DPPH radical is a model that is widely used to evaluate the antioxidant property of plant extracts. DPPH is a stable nitrogen-centered free radical, the color of which changes from violet to yellow on reduction by the process of hydrogen or electron donation. Substances that are able to perform this reaction can be considered antioxidants, and, therefore, radical scavengers.

Phenolic compounds in plant extracts significantly contribute to their antioxidant potential because of their unique structure. Phenolics are composed of one or more aromatic rings bearing single or multiple hydroxyl groups and are, therefore, potentially able to quench free radicals by forming resonance stabilized phenoxyl radicals.

In recent years, antioxidants derived from natural resources, mainly from plants have been intensively used to prevent oxidative damages. These have some advantages over synthetic ones; for example, they can be obtained easily and economically, and have slight or negligible side effects.

Cancer is considered as one of the main causes of mortality throughout the industrial world in the present century. Scientists believe that damage to the genetic material changes in DNA sequence and continuity, mutation in genes and other genetic changes in chromosomal structures play important roles in carcinogenesis. The use of antimutagens and anticarcinogens in everyday life is the most effective procedure for preventing human cancer and genetic disease.

Antimutagenic properties elicited by plant species have a full range of prospective applications in human health. Herbal remedies and phytotherapy drugs, containing active principles are currently being developed to protect against an electrophile (e.g., free radical) attack on DNA and its widespread outcomes such as aging and cancer. The occurrence rate of cancer is increasing worldwide, and the determination of chemopreventive or chemophylaxis compounds is important.

### Table 1:

Free radical scavenging capacities of the ethanolic extract of T. longirostis var. longirostis and standards measured in DPPH assay

| Sample                       | IC₅₀ value (mg/ml) |
|------------------------------|-------------------|
| T. longirostis var. longirostis | 7.84±0.603*       |
| BHT                          | 0.95±0.014        |
| Ascorbic acid                | 0.48±0.019        |

*Values expressed are mean±SD of three parallel measurements. The IC₅₀ values were obtained by the linear regression analysis. DPPH: 2, 2-diphenyl-1-picrylhydrazyl, SD: Standard deviation, BHT: Butylated hydroxytoluene
in the effort to reduce the risk of cancer. A plant extract indicating antimutagenicity is not necessarily an anticarcinogen; however, it is an indication of possible candidates for such purposes.iii

Conclusions

The results of this study indicated that the ethanolic extract of *T. longirostis* var. *longirostis*, an edible plant in Anatolia, possessed high AA in vitro and can be an easy, accessible source of natural antioxidants. The ethanolic extract, which was investigated in the present study, can be considered safe at the tested concentrations, and the extract exhibited important antimutagenic properties.

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Nil.

Conflicts of Interest

There are no conflicts of interest.

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