Evaluation of the mutagenic and antimutagenic potentials of plant raw materials for functional and food purposes

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Abstract. This study is devoted to the study of the mutagenic and antimutagenic potentials of extracts of Elaeágnus angustifólia L. using the Ames bacterial test system. The experiments used a strain of the bacterium Salmonella t. TA100. As a result, it was found that plant extracts in the range of the studied concentrations (from 1 to 0.01 mg / ml) do not have a mutagenic potential. Extracts of flowers and leaves of the plant in the range of the studied concentrations had a pronounced antimutagenic potential. The fruit pulp extract had a moderate antimutagenic potential.

1. Introduction
As a result of exposure to mutagens, the genome of living organisms becomes unstable, which leads to premature aging, cell death, reduced life expectancy, and the appearance of cancerous tumors [1]. The search for plant components that resist the action of mutagens leads to the need to test their antimutagenic potential.

Currently, there is an active search for substances with antimutagenic properties in nature. More than 300 natural antimutagenic compounds are known: amino acids, vitamins, and natural metabolites [2].

Antimutagens can be functional foods that, in addition to their nutritional value, have a positive effect on human health and contribute to the prevention of various diseases. Most functional foods have a plant-based composition. The plant body is unique in that it contains a large amount of biologically active substances.

2. Ames test system
The Ames bacterial test system, created in 1973 by the American scientist Bruce Ames, is currently actively used in genetic toxicology to assess the mutagenic and antimutagenic potentials of the tested substances [3]. Almost all new pharmaceutical substances and chemicals used in industry are tested by the Ames test [4].

The study uses strains of the bacterium Salmonella typhimurium, which are mutants that are auxotrophic for histidine. The essence of the method is to assess the ability of the tested substances to cause reverse mutations in test strains, leading to the transition of bacteria from auxotrophy (his-) to
prototrophy by histidine (his+) [5]. If the mutagenic potential of the test substance is absent, its antimutagenic potential can be evaluated using the Ames test system [6].

3. General characteristics Elaeagnum angustifolia L.

E. angustifolia L. is a plant represented in nature by a woody or shrubby form, superficially resembling an olive tree. The plant is able to tolerate a wide range of environmental conditions, such as severe drought, soil salinity, and flooding [7].

E. angustifolia L. it is known for its wound healing, muscle relaxant, antioxidant, cardioprotective, antinociceptive, anti-inflammatory, antimicrobial, antitumor and antimutagenic effects [8].

Some parts of the plant are used to treat sore throats, coughs, flu, colds, fever, nausea, vomiting, jaundice, asthma, diarrhea, various disorders of the gastrointestinal tract. Fruits of E. angustifolia L. they are widely used in Iranian folk medicine to relieve the pain of rheumatoid arthritis and to speed up the healing process of wounds. The wide range of beneficial effects on the human body is explained by the rich biochemical and mineral composition of the plant [9].

There are known data on the introduction of 1, 2 and 3% of aqueous extracts of the leaves of E. angustifolia L. as a component of feed for common carp. The results of the study demonstrate the stimulation of immune and antioxidant parameters in fish when adding 1% of an aqueous leaf extract to the diet. 2 and 3% plant leaf extracts can cause oxidative stress and be hepatotoxic [10].

E. angustifolia L. contains a large amount of condensed tannins, which have anti-inflammatory, wound-healing, anti-cancer, and cardioprotective effects [9]. The content of flavonoids and antioxidants in the plant explains its wound-healing effect. Flavonoids are known for their antimicrobial activity, they help to increase the rate of wound healing and regeneration of the skin's epidermis. Antioxidants play an important role in protecting tissues from oxidative damage and significantly promote DNA synthesis during wound healing [9].

A study of the antimicrobial properties of water-alcohol extracts of E. angustifolia L. showed that these extracts exhibit high antimicrobial activity against gram-negative bacteria and yeast [11].

Muscle relaxant activity is also explained by the presence of certain flavonoids or flavones in the plant, such as chrysin (which has a partially agonistic effect on benzodiazepine receptors) [9].

Some sitosterols, flavonoids, and terpenoids that are part of E. angustifolia L. provide antinociceptive and anti-inflammatory effects. Triterpenoids, flavonoids, lignoids, and benzenoids provide antitumor effect [9].

3.1. Biochemical composition of Elaeagnum angustifolia L.

Numerous phytochemical studies of extracts of various parts of the plant E. angustifolia L. they indicate the presence of flavonoid compounds, polysaccharides, sitosterols, cardiac glycosides, terpenoids, coumarins, phenol-carboxylic acids, amino acids, and carotenoids [9]. Alkaloids, saponins, and tannins were also found in plant extracts [12].

Results of the study of extracts of leaves and flowers of E. angustifolia L. indicate the content of phenolic and flavonoid compounds with antioxidant properties. At the same time, it was found that there are more flavonoid compounds in the leaves than in the flower of the plant [9].

The most common phenolic compounds of the plant were determined by HPLC: 4-hydroxybenzoic acid (45.8 mg/100 g dry matter) and caffeic acid (32 mg/100 g dry matter) [13].

A group of flavonoids was also found in the plant, such as catechin, epicatechin, gallocatechin, epigallocatechin, kaempferol, quercetin, luteolin, 4-0-β-D-glucoside, isorhamnetin-3-0-β-D-galactopyranoside [9].

By general spectral analysis, a macrocyclic flavonoid glycoside was isolated from the flowers of E. angustifolia L., which was named Angustifolinoid A. It has a 13-membered heterocyclic ring fragment [14].

From the flowers of E. angustifolia L. 7 acylated flavonol glycosides were also isolated, called elaegnosides A, B, C, D, E, F, G. Two compounds had a significant antioxidant effect, and one of them showed weak inhibitory activity against tyrosinase [15].
The following fatty acids were identified in the fruits of E. angustifolia L.: lauric, tridecanoic, myristic, pentadecanoic, palmitic, lignocerinic, palmitoleic, heptadecanoic, linolenic, oleic, stearic, eicosan and docosan. The predominant percentage of fatty acid was palmitic acid (34.31%), followed by oleic acid (26.23%) and lignocerinic acid (17.47%) [16].

Results of the study of the fruit peel and seeds of E. angustifólia L. the content of fatty acids is indicated by the presence of palmitoleic acid in the peel, linoleic and palmitic acids in the seeds [9]. In the peel and pulp of the fruit of E. angustifólia L. a high content of gallic acid was also found (1179 and 820.85 mg/100g of fresh sample, respectively) [17]. The fruits of the plant are rich in vitamins: tocopherol, carotene, ascorbic acid, thiamine [18].

As a result of the study of soluble sugars of the plant using GC (gas chromatography) and HPLC, it was found that fructose (27.1% dry weight) and glucose (22.3% dry weight) are the main monosaccharides of E. angustifólia L. Sucrose was not detected in the plant [13]. E. angustifólia L. contains tannins, while the bark contains more of them than the annual branches and leaves of the plant [9].

3.2. Mineral composition of Elaeágnus angustifólia L.
The roots, branches, leaves, and stem bark of E. angustifolia L. were found to contain iron (Fe), lead (Pb), boron (B), copper (Cu), cadmium (Cd), zinc (Zn), chromium (Cr), nickel (Ni), and cobalt (Co) in various concentrations.

Potassium is the most common mineral found in plant fruits (8504 mg/kg), followed by sodium (1731 mg/kg) and phosphorus (635 mg/kg) [19].

In the fruits of E. angustifólia L. calcium (Ca), magnesium (Mg), iron (Fe), and manganese (Mn) were also found. It is known that the content of zinc (Zn) and copper (Cu) in the fruits of the plant does not exceed the toxicity threshold [18].

4. Materials and methods
4.1. Preparation of plant extracts
For the study, four water extracts of E. angustifolia L. were prepared: an extract of seeds, fruit pulp, flowers and leaves.

The dry substance obtained as a result of 60% alcohol extraction from plant raw materials was diluted with sterile distilled water in 3 working concentrations: 1 mg/ml, 0.1 mg/ml, 0.01 mg/ml.

4.2. Description of the bacterial strain Salmonella t. TA100
The present study used the Salmonella typhimurium TA100 strain, which is derived from the laboratory strain LT-2. The bacterium is a mutant, auxotrophic to histidine.

In order to increase the sensitivity of the test strain, additional mutations were carried out in it:

- gal bio deletion (captures the biotin and part of the galactose operon);
- UvrB deletion (violation of the DNA repair system);
- rfa mutation (increases the permeability of the bacterial cell by disrupting its structure).

The sensitivity of Salmonella t. TA100 is also increased due to the introduction of the pCM 101 plasmid (Ampr).

Thus, under laboratory conditions, the following highly sensitive test strain was obtained: TA 100 hisG46 gal bio UvrB rfa pKM 101 Amrg, through which mutations such as substitution of nitrogenous base pairs and reading frame shift can be recorded.

For each test strain, there is a diagnostic mutagen-a substance whose action will cause an active response in the form of the growth of his+ revertant colonies that have passed to prototrophy by histidine. For Salmonella t. TA100, the diagnostic mutagen is sodium azide (NaN₃). The substance was used in the study as a positive control when studying the mutagenic potential of plant extracts, and was also
added together with plant extracts to the composition of the upper agar when studying their antimutagenic potential. The substance was diluted in a concentration of 1.5 micrograms/Petri dish.

4.3. Preparation of bacterial suspension
The bacterial suspension was prepared on the basis of the bacterium S. typhimurium TA100, incubated for 48 hours at a temperature of 37 ºC, by washing in saline solution. The bacterial suspension met the 10ME turbidity standard. For the study, 2 test tubes with a bacterial suspension of 10 ml were prepared.

4.4. Method for assessing the mutagenic potential of plant extracts
Plant extracts in the appropriate concentration and bacterial suspension were added to test tubes with an upper agar containing a minimum amount of histidine. The upper agar was poured into Petri dishes with the lower agar. For each concentration of plant extracts, 3 repetitions were performed.

The diagnostic mutagen sodium azide (NaN₃) was a positive control. The negative control was distilled water (H₂O).

Then the Petri dishes were incubated in a thermostat for 48 hours at a temperature of 37 ºC. The mutagenic potential was evaluated by calculating the mutagenic index (MI) for each experimental concentration of substances, which shows how much the number of revertant colonies exceeds the negative control.

\[
MI = \frac{\text{number of his + revertant colonies of the test substance}}{\text{number of his + revertant colonies of the negative control}}
\]

The mutagenic potential of plant extracts was evaluated according to the following criteria:

- MI < 2.5 - no mutagenic potential;
- MI = 2.5-10 - weak mutagenic potential;
- MI = 1-2 orders of magnitude-average mutagenic potential;
- MI > 100 - strong mutagenic potential.

4.5. Method for assessing the antimutagenic potential of plant extracts
Plant extracts in the appropriate concentration, the diagnostic mutagen sodium azide (NaN₃) and bacterial suspension were added to test tubes with top agar and poured into Petri dishes with a nutrient medium. For each concentration of plant extracts, 3 repetitions were performed.

The Petri dishes were then incubated in a thermostat for 48 hours at 37 ºC. The antimutagenic potential was evaluated by calculating the reduction factor (RF) for each concentration of plant extracts, which shows how much the plant extract reduces the frequency of induction of reverse mutations in the bacterium.

\[
RF = 100 - \frac{\text{number of his + revertant colonies of the test substance}}{\text{number of his + revertant colonies of the positive control}} \times 100\%.
\]

The antimutagenic potential of plant extracts was evaluated according to the following criteria:

- RF < 25% - no antimutagenic potential;
- RF = 25-40% - moderate antimutagenic potential;
- RF > 40% - pronounced antimutagenic potential.

4.6. Statistical data processing
Statistical data processing was performed using the Microsoft Office Excel 2007 program. The data is presented as an average value with a standard deviation. To assess the reliability of the differences between the results in the variants of the experiment, the Student's criterion was used. The critical level of significance when testing statistical hypotheses is assumed to be p≤0.05.
5. Results and discussion

5.1. Mutagenic potential of extracts of Elaeagnus angustifolia L.
The MI of the seed extract was 1; 2 and 1.3 for the three concentrations, respectively. The MI of the flower extract was 0.5; 0.4 and 0.8 for the three concentrations, respectively. The MI of the fruit pulp extract was 0.6; 1 and 0.8 for the three concentrations, respectively. The MI of the leaf extract was 0.6; 1 and 1.2 for the three concentrations, respectively.

The MI of the studied plant extracts did not exceed 2.5, respectively, the studied plant extracts do not have a mutagenic potential. Plant extracts are of interest for studying their antimutagenic potential.

5.2. Antimutagenic potential of extracts of Elaeagnus angustifolia L.
Seed extract at concentrations of 1 and 0.01 mg / ml has no antimutagenic potential (RF 17 and 15%, respectively), and at concentrations of 0.1 mg/ml has moderate antimutagenic activity (RF 26%).

Flower extract at concentrations of 0.1 and 0.01 mg/ml has moderate antimutagenic activity (RF 31 and 24%, respectively), and at concentrations of 1 mg/ml - pronounced antimutagenic activity (RF 60%).

Fruit pulp extract in the range of the studied concentrations has moderate antimutagenic activity (RF 38, 38 and 27%, respectively).

Leaf extract at a concentration of 1 mg / ml has a pronounced antimutagenic activity (RF 52%), at a concentration of 0.1 mg/ml - moderate antimutagenic activity (RF 26%), and at a concentration of 0.01 mg/ml does not have an antimutagenic potential (RF 19%).

| Table 1. Antimutagenic potential of Elaeagnus angustifolia L. extracts. |
|---------------------------------------------------------------|
| Test extract       | Concentration, mg / ml | The number of his+ revertant colonies | RF/reduction of the frequency of induction of reverse mutations, % | Antimutagenic activity |
| Seed of E. angustifolia L. | 1              | 174±20.1                  | 17                      | absent                   |
|                     | 0.1           | 155±3                    | 26                      | moderate                 |
|                     | 0.01          | 178±23.7                 | 15                      | absent                   |
| Flowers of E. angustifolia L. | 1             | 85±36.3                  | 60                      | pronounced               |
|                     | 0.1           | 146±35.7                 | 31                      | moderate                 |
|                     | 0.01          | 160±19.2                 | 24                      | absent                   |
| Fruit pulp of E. angustifolia L. | 1            | 130±5.7                  | 38                      | moderate                 |
|                     | 0.1           | 130±24.4                 | 38                      | moderate                 |
|                     | 0.01          | 153±12.8                 | 27                      | moderate                 |
| Leafs of E. angustifolia L. | 1             | 101±21.6                 | 52                      | pronounced               |
|                     | 0.1           | 156±4.7                  | 26                      | moderate                 |
|                     | 0.01          | 170±8.1                  | 19                      | absent                   |
| NaN3 (+ control) 1.5 mcg/ petri dish |              | 209±21                  |                          |                         |
| H2O (- control) | -             | 24±13                   |                          |                         |

6. Conclusions
Based on the results obtained in the Ames bacterial test system, extracts of seeds, flowers, fruit pulp and leaves of E. angustifolia L. can be characterized as non-mutagenic in the concentration range of 1-0.01 mg/ml.

The studied plant extracts showed an antimutagenic effect, most pronounced in flower and leaf extracts. The antimutagenic potential of E. angustifolia L. extracts can be explained by the fact that the plant contains many amino acids, antioxidant vitamins, flavonoids, saponins, and terpenoids. The studied plant extracts can be proposed for development as antigenotoxic and genoprotective drugs.
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