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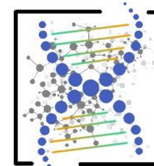

PHYTOCHEMICAL ASSESSMENT AND POTENTIAL PHARMACOLOGICAL ACTIVITY OF MORINGA OLEIFERA EXTRACT

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ABSTRACT

The antioxidant activity of the extracts was determined by their ability to protect epinephrine hydrochloride from autooxidation. Extracts obtained from the herb and morphological parts of *Moringa oleifera* showed the greatest activity when extracted with an aqueous alcohol mixture with an alcohol content of 40%. Dry alcohol extract from the herb *Moringa oleifera* contains a bio-element complex, potentially possessing antioxidant activity: tannins (up to 30%), flavonoids (up to 5%), coumarins (up to 3.5%), phenol carbonic and oxycoric acids (up to 6%), amino acids (up to 2%), ascorbic acid, chlorophylls, carotenodes and 61 elements. The antioxidant activity of extracts (inactivation of radicals) is provided by biologically active substances of a phenolic nature (due to the presence of double bonds), ascorbic acid, and elements with variable valence, carotenoids, chlorophylls bind reactive oxygen species. Studies of anti-inflammatory activity (in acute and chronic inflammation) have determined the antiproliferative and phlogogenic effect of the dry herb extract, which is more pronounced than that of the comparison drug, acetylsalicylic acid. The dependence between the manifestation of antioxidant activity and the anti-inflammatory effect of *Moringa oleifera* extract of dry grass was revealed.

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Introduction

It should be noted that the pathogenesis of a significant number of diseases is based on oxidative stress and the inflammatory process [1, 2]. Therefore, drugs with antioxidant and anti-inflammatory activity are recommended for complex therapy and prevention of most diseases [3]. From this point of view, phytopreparations containing both natural antioxidants and anti-

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inflammatory substances attract attention. A promising source of biologically active substances and phytopreparations with antioxidant, antiradical, and anti-inflammatory activity is *Moringa oleifera* [4].

M. oleifera is a perennial herbaceous plant with a wide range, which has long been used in folk and traditional medicine as an anti-cancer, antioxidant, anti-inflammatory, and antidiabetic agent [5]. The anticancer activity of *M. oleifera* is associated with the activity of agrimonine (polyphenol), which inhibits tumor growth *in vivo* [6, 7]. Extracts of *M. oleifera* reduce the feeling of fatigue and fatigue, and are used for asthenia and to relieve inflammation in allergic diseases [8, 9]. Polyphenols and triterpenoids of *M. oleifera* inhibit oxidative stress and hyperglycemia in type 2 diabetes mellitus [10, 11]. The dry aqueous extract of the aboveground part of *M. oleifera* is effective for correcting desynchronosis of the sleep-wake rhythm [12]. The wide range of biological activity manifested by *M. oleifera* is explained by the presence of a variety of biologically active substances (BAS) in the raw material of the plant, most of which have antioxidant and anti-inflammatory activity [13-15].

In modern phytotherapy, traditional methods can be used to assess the quality of herbal medicines, although modern processes such as high-performance liquid chromatography, gas chromatography, ultraviolet/visible spectrophotometry, or atomic absorption spectroscopy are more often used for species identification, measurement of bacteriological contamination, evaluate the effectiveness and create certificates of material analysis [16, 17]. Phytotherapy differs from homeopathy and anthroposophical medicine and avoids mixing plant and synthetic bioactive substances [18]. However, it is critically important to standardize the bioactive components of herbs and systematize them corresponding to therapeutic activity [19, 20]. At the same time, currently, there is a lack of studies on the relationship between chemical composition, antioxidant activity, and anti-inflammatory effects of *M. oleifera* extracts. Thus, this study aimed to identify the relationship between the chemical composition, antioxidant and anti-inflammatory activity of *M. oleifera* extracts.

Materials and Methods

Research material: dry extract (DE) and liquid extract (LE) of *Moringa oleifera*. LE was obtained by triple extraction under heating (60– 90°C) in distilled water or water-alcohol mixtures with ethyl alcohol content of 40% and 90%. The method for DE production consisted of three-fold extraction of the aboveground part of *M. oleifera* with 40% ethyl alcohol under heating (90°C) followed by distillation of ethyl alcohol, evaporation, and drying in a freeze dryer [21].

The obtained LE samples were examined for antioxidant activity (AOA) by their ability to protect epinephrine hydrochloride in solution from autooxidation [15]. This method is rapid, does not require complex equipment, is simple in execution, and gives unambiguous results, which is its advantage over other methods for determining AOA. The essence of the technique is as follows: 0.2 mL of a solution of 0.1% epinephrine hydrochloride in 3 mL of carbonate bicarbonate buffer (pH = 10.65) after exposure under normal lighting and at room temperature (for 10 minutes), spectrophotometry is performed on an SF-56 spectrophotometer at 347 nm against the background of carbonate bicarbonate buffer (Experimental group 1). For experimental group 2, 0.02 mL of LE was added to 0.2 mL of solution of 0.1% epinephrine hydrochloride in 3 mL of carbonate bicarbonate buffer. Spectrophotometry was performed under the same conditions. The AOA value of more than 10% indicated the presence of antioxidant activity in the LE.

Anti-inflammatory activity (in acute and chronic inflammation) was studied on white male rats weighing 180-200 g. The animals were kept in standard vivarium conditions (in cages of 6-7 animals, with wood shavings on the bottom, and free access to water and food) following Lyashenko *et al*. [22]. During the experiments, the principles set out in the directives of the European Community (86/609/EEC) and the Helsinki Declaration were followed. To study the anti-inflammatory activity of DE, all animals were divided into 6 groups of 6-7 animals each. In 3 groups of animals, a study was conducted on the model of acute aseptic inflammation, in 3 groups – on the model of chronic inflammation, of which: 2 groups of animals were control, 2 groups were experimental, and 2 groups were used for comparison.

The studied DE was dissolved in heated distilled water and injected through a probe into the stomachs of animals of experimental groups daily (7 days) at the same time of day (in the morning) at a dose of 100 mg/kg. Distilled water was injected into the control group of animals under the same conditions. Acetylsalicylic acid (ASA) was used as a comparison drug. The choice of the comparison drug is due to several factors: firstly, ASA is a derivative of salicylic acid (phenolic acid), which brings it closer to phenolic compounds in DE, and secondly, the physiological mechanisms of the anti-inflammatory effect of ASA have now been studied in detail [23]. The manifestation of anti-inflammatory and analgesic effects was observed in the range of high and standard doses [24]. ASA solution was administered to animals through a probe into the stomach at a dose of 20 mg/kg for 7 days (comparison group).

In the carrageenan edema model, when carrageenan was administered (0.1 mL of 1% aqueous solution) under plantar aponephrosis of the hind right paw of the animal, acute aseptic inflammation developed expressed in redness, increase in paw volume, fever and soreness at the injection site. At the peak of inflammation (after 3.5 hours), the amount of edema was determined by the volume of water displaced by the foot (in mL) [25].

For the cotton granuloma model, a sterile cotton swab (10 mg) was implanted under the animal's skin, which caused proliferative chronic inflammation [26]. The formed granulomas on the 8th day of the experiment were isolated and weighed, then dried at 60 ^{o}C, brought to a constant mass, and weighed again. The proliferative activity of the studied DE was estimated by the difference between the initial mass of a cotton swab (10 mg) and the mass of the dried granuloma. The effect on the exudative part of the inflammatory process was assessed by the difference between the masses of raw and dried granuloma to a constant mass.

The composition and content of elements in the DE were analyzed by inductively coupled plasma mass spectrometry (MS-ICP) using the ELAN DRC-e ICP-MS mass spectrometer and the Agilent 715 ICP-OES optical emission spectrometer. Sample preparation for element analysis included treatment of the DE samples with nitric acid, followed by the use of Speedwave TM MWS-3+ and BERGHOF microwave decomposition systems. The control was carried out by the additive method [27].

Statistical processing of the results of pharmacological studies was carried out using Statistica 12.0 software. The statistical significance of the differences was assessed using the Student's criterion. The differences in the compared values were considered significant at $p \leq 0.05$

Results and Discussion

When analyzing the electronic absorption spectra of the initial 0.1% solution of epinephrine hydrochloride and a mixture of 0.1% solution of epinephrine hydrochloride with LE of *M. oleifera* in a carbonate bicarbonate buffer, it was noted that all LE samples prevent the oxidation of epinephrine, but to varying degrees **(Figure 1)**.

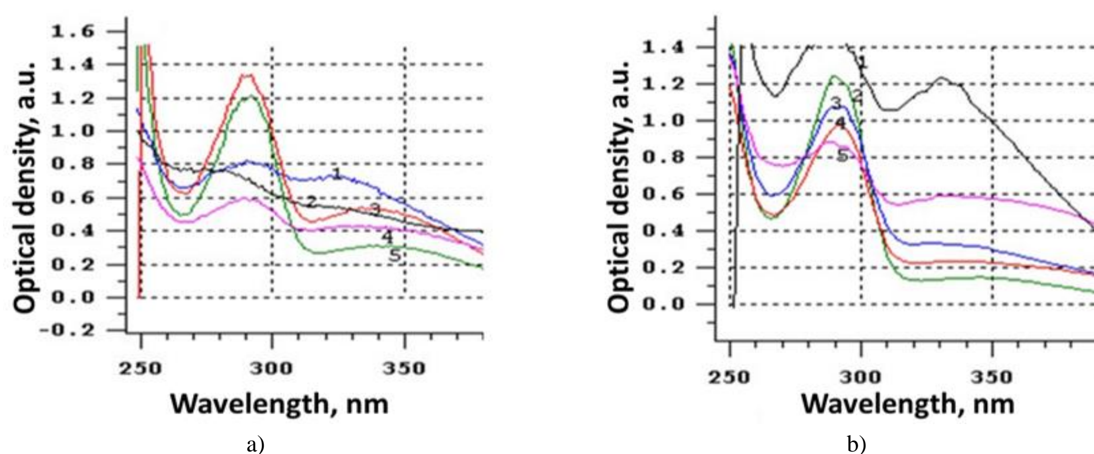

Figure 1. Absorption spectra of 0.1% epinephrine hydrochloride solution in carbonate bicarbonate buffer (1) and products of interaction of 0.1% epinephrine hydrochloride solution with *M. oleifera* LE in carbonate bicarbonate buffer. Absorption spectra: a) aqueous LE (2 – from grass, 3 – from stems, 4 – from leaves, 5 – from inflorescences); b) alcoholic LE (2 – from inflorescences, 3 – from grass, 4 – from leaves, 5 – from stems)

The bioelement complex (biologically active substances and elements with variable valence) contained in the LE largely prevents the autoxidation of adrenaline hydrochloride, which is reflected in a decrease in the absorption rate of their interaction products in the series: LE from inflorescences, LE from leaves, LE from grass and LE from stems **(Figure 1)**.

The results of calculations of the AOA indicators of the LE are shown in **Figure 2**. The highest AOA indicators were found in LE obtained from inflorescences and leaves, and the lowest – in LE obtained from stems. This pattern is observed for all those obtained using different extractants.

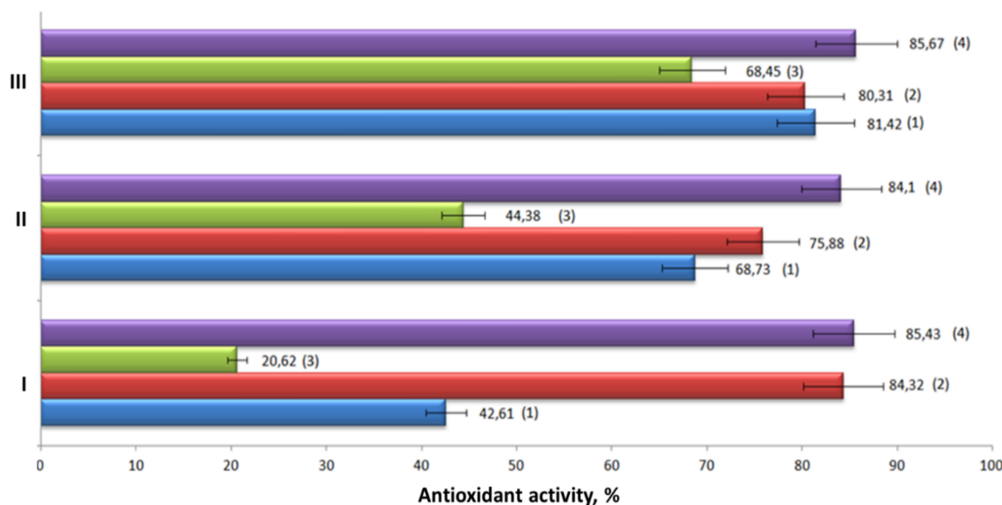

Figure 2. Dependence of the antioxidant activity of *M. oleifera* LE on the extractant (I – distilled water, II – 90% ethanol, III – 40% ethanol) and morphological part of the plant (1 –grass, 2 – leaves, 3 – stem, 4 – inflorescences)

Previously, when studying the accumulation of the main groups of BAS in the grass and certain morphological parts of *M. oleifera*, it was found that the highest concentration of antioxidants (flavonoids, tannins, carotenoids, ascorbic acid, chlorophylls, oxycoric, and phenol carboxylic acids) is observed in leaves and inflorescences [28-30]. The results obtained for the AOA manifested by the LE from the leaves and inflorescences of *M. oleifera* confirm the existence of a direct relationship between the content of the bio-element complex and the degree of manifestation of this activity [31, 32]. It should be noted that the manifested AOA depends on the extractant used to produce LE. Thus, LE obtained using 40% ethyl alcohol exhibits greater AOA than LE obtained by extraction with distilled water and 90% ethyl alcohol. This is due to the fact that 40% ethyl alcohol extracts biologically active substances and elements responsible for AOA from the grass and morphological parts of *M. oleifera* to a greater extent than water and 90% ethyl alcohol [33, 34]. From this pattern, all the LE from the inflorescences are knocked out, the values of the AOA indicators of which are the highest and closest to each other **(Figure 2)**. It should be noted that the LE from the grass (including all morphological parts) shows a greater AOA than the LE from the leaves (obtained by extraction with 40% ethyl alcohol).

For further research, DE (40% ethyl alcohol extractant) was obtained from *M. oleifera*. Organoleptic characteristics of DE: fine-grained powder of light brown color, faint herbal odor, bitter taste, astringent effect. DE is well soluble in 40% ethyl alcohol at 20 ºC and distilled water when heated. Phytochemical analysis of the DE showed a significant content of polyphenolic oxidizable compounds (tannins) (up to 30%), flavonoids (up to 5%), coumarins (up to 3.5%), phenol carbonic and oxycoric acids (up to 6%), amino acids (up to 2%), ascorbic acid (14 mg%), chlorophylls (71 mg%) and carotenoids, which is consistent with results of other researchers [35-37].

The elemental composition of the DE includes 61 elements, except for organogen elements (C, H, N, O), which are not determined by the MS-ICP method. All the vital macro- and microelements are present [38, 39]. When the elements were arranged in descending order of their concentrations (up to 1 µg/g), the following accumulative series was obtained: K > Mg > Ca > P > Si > Na > Al > Br > Fe > B > Zn > Mn > Rb > Sr > La > Ti > Cu > Ni > As > Cr > Cs > Sb > V > Co, confirming the presence and significant content of elements with variable valence in the DE, which is very important for the manifestation of antioxidant activity **(Figure 3)** [40].

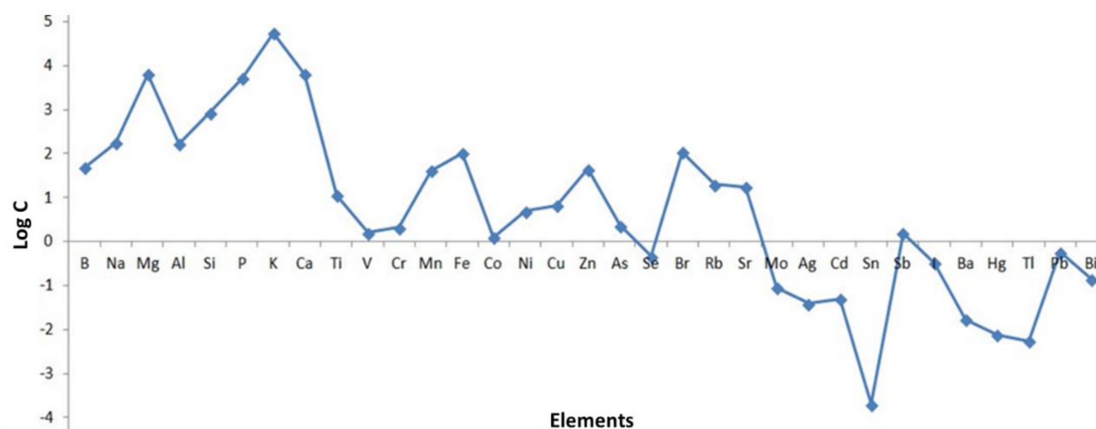

Figure 3. Composition and content of elements in *Moringa oleifera* dry extract (in logarithmic scale log10)

It is worth noting that the content of toxic elements in the DE does not exceed the maximum permissible concentration for beverages [41]. It is known that with the simultaneous presence of antioxidant compounds belonging to different chemical classes in plant extracts, synergism is observed in the manifestation of their AOA [42]. Perhaps, in the manifestation of the high AOA of *Moringa oleifera* DE of grass, the phenomenon of synergism of the activity of BAS, mainly substances of phenolic nature and elements with variable valence was observed [43, 44].

When carrageenan was injected into the paw of an animal of the control group, the development of swelling (edema), redness, fever, and soreness at the site of its administration was observed. When observing animals for 6 hours from the moment of administration of the phlogogenic agent, the maximum size of edema was observed after 3.5 hours. When carrageenan was administered against the background of DE and ASA, an increase in the volume of the animal's foot, the presence of redness, fever, and soreness at the injection site were also observed, but to a lesser extent. A comparative analysis of experimental data on the anti-inflammatory activity of CE (100 mg/kg) and the comparison drug - ASA (20 mg/kg) in acute inflammation revealed that DE and ASA significantly reduce (by 50% and 52.2%, respectively) the volume of edema of the animal's paw compared with the control group **(Figure 4a)**. A decrease in the volume of edema of the animal's foot is associated with a decrease in capillary permeability and normalization of microcirculation processes [45]. A decrease in capillary permeability and an improvement in microcirculation occur due to the high concentration of phenolic compounds (flavonoids and coumarins) in the DE, and polyphenolic compounds (tannins) can have a stabilizing effect on the cell walls of lysosomes [46].

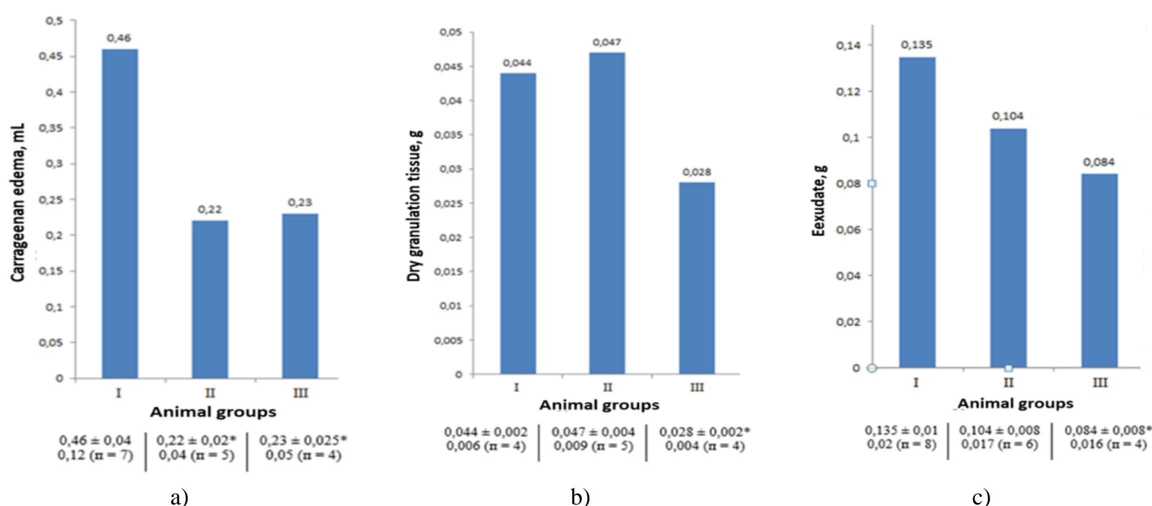

Figure 4. Anti-inflammatory activity of *M. oleifera* dry extract (n=6), (M ± m; p ≤ 0.05). a) acute inflammation ("carrageenan edema" model), b, c) chronic inflammation ("cotton granuloma" model). Animal groups: 1 – control (pathology model + purified water equivalent to experimental administration); 2 – comparisons (pathology model + ASA (20 mg/kg)); 3 – experimental (pathology model + DE (100 mg/kg)).
* – the difference is statistically significant in relation to control

Chronic, sluggish inflammatory processes often lead to undesirable consequences – the proliferation of granulomatous fibrous tissue and its replacement of normal tissues, which leads to deformation and loss of functions [47]. Therefore, the presence of an antiproliferative effect in the studied DE is its advantage over other anti-inflammatory drugs that have activity only in the acute phase of inflammation. With oral administration of DE solution to rats at a dose of 100 mg/kg against the background of chronic inflammation, a significant decrease in the mass of granulomatous fibrous tissue was observed compared with the control group (36.6%). The comparison drug (20 mg/kg ASA) with the same method of administration against the background of chronic inflammation, which showed a significant anti-inflammatory effect in the acute inflammatory process, did not show activity in this case, even had a proliferative effect **(Figure 4b)**.

A comparative analysis of the effect of DE (100 mg/kg) and ASA (20 mg /kg) when administered orally against a background of chronic inflammation on the mass of exudate revealed that DE has an anti-exudative effect to a greater extent than the comparison drug, since it reduced the mass of exudate by 37.8%, and ASA by 23.1% compared with the control group **(Figure 4b)**.

Thus, the experimental results on the study of the antioxidant and anti-inflammatory activity of *M. oleifera* DE obtained in this work are highly correlated with the results of experiments conducted using other methods for studying these types of biological activity *in vivo* and *in vitro* [48-50].

Conclusion

Extracts obtained from the herb and morphological parts of *M. oleifera* have pronounced antioxidant activity. Extracts obtained from herbs, inflorescences, and leaves using 40% ethyl alcohol as an extractant showed the greatest antioxidant activity. A decrease in the volume of exudate in acute and chronic inflammation (by 50% and 37.8%, respectively) and the mass of granulomatous fibrous tissue (by 36.6%) when using *Moringa oleifera* DE of grass indicates the presence of anti-inflammatory activity comparable to the anti-inflammatory activity of the comparison drug (acetylsalicylic acid).

The high antioxidant and anti-inflammatory activity of *Moringa oleifera* DE of grass is determined by its bioelement complex: it contains polyphenolic oxidizable compounds (tannins) (up to 30%), flavonoids (up to 5%), coumarins (up to 3.5%), phenol carbonic and oxycoric acids (up to 6%), amino acids (up to 2%), ascorbic acid, chlorophylls and elements with variable valence (Si, Br, Fe, Mn, Cu, Ni, Cr, Co). There is a pronounced relationship between the chemical composition and the manifestation of antioxidant activity and anti-inflammatory effects of total extracts from the herb and morphological parts of *Moringa oleifera*.

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Conflict of interest: None

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Ethics statement: The protocol for experiments with laboratory animals complied with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

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