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Phytochemical Characterization and Pharmacological Potential of *Moringa oleifera* Extract

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ABSTRACT

In the current study, the antioxidant potential of the extracts was evaluated by their ability to inhibit the autooxidation of epinephrine hydrochloride. The extracts derived from different parts of *Moringa oleifera* showed the highest antioxidant activity when extracted using a 40% alcohol-water mixture. The dry herb's alcohol extract contains a range of bioactive compounds, including tannins (up to 30%), flavonoids (up to 5%), coumarins (up to 3.5%), phenolic acids (up to 6%), amino acids (up to 2%), ascorbic acid, chlorophylls, carotenoids, and 61 other trace elements, which may contribute to its antioxidant effects. The antioxidant properties are primarily attributed to phenolic compounds (due to their double bonds), ascorbic acid, and metals with variable oxidation states, along with carotenoids and chlorophylls that can bind and neutralize reactive oxygen species. In terms of anti-inflammatory properties, both acute and chronic inflammation tests showed that the dry herb extract exhibited a more pronounced antiproliferative and anti-inflammatory effect than acetylsalicylic acid, the standard drug. A correlation was identified between the antioxidant and anti-inflammatory activities of *Moringa oleifera*'s dry herb extract.

Keywords: Anti-inflammatory activity, *Moringa oleifera*, Bioelement complex, Dry extract, Antioxidant activity

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Introduction

Numerous diseases are fundamentally influenced by oxidative stress and inflammation [1, 2]. As a result, drugs with both antioxidant and anti-inflammatory properties are often recommended for the comprehensive management and prevention of these conditions [3]. From this perspective, herbal preparations containing natural antioxidants and anti-inflammatory compounds have gained attention. *Moringa oleifera* stands out as a promising plant, known for its biologically active substances, exhibiting antiradical, antioxidant, and anti-inflammatory effects [4].

M. oleifera is a perennial plant that has been used for centuries in traditional medicine due to its anti-cancer, anti-inflammatory, antioxidant, and antidiabetic effects [5]. The anticancer properties of *M. oleifera* are largely attributed to the polyphenol agrimonine, which has been shown to inhibit tumor growth in vivo [6, 7]. In addition, *M. oleifera* extracts are used to combat fatigue and weakness, and they help reduce inflammation associated with allergic conditions [8, 9]. The plant's polyphenols and triterpenoids have been found to reduce oxidative stress and regulate blood sugar levels in type 2 diabetes patients [10, 11]. Furthermore, the dry aqueous extract derived from the plant's aerial parts has proven beneficial in regulating circadian rhythm disturbances [12]. The extensive range of biological activities of *M. oleifera* can be attributed to its wide variety of active compounds, many of which possess potent antioxidant and anti-inflammatory effects [13-15].

While traditional methods are still utilized in phytotherapy to assess the quality of herbal medicines, more advanced techniques like high-performance liquid chromatography, ultraviolet/visible spectrophotometry, gas

chromatography, and atomic absorption spectroscopy are increasingly employed for species identification, evaluating bacteriological contamination, determining effectiveness, and certifying material analysis [16, 17]. Phytotherapy is distinct from homeopathy and anthroposophical medicine, as it avoids the combination of plant and synthetic bioactive substances [18]. Nonetheless, it is crucial to standardize the bioactive components of herbs to align with their therapeutic benefits [19, 20]. Despite these advancements, there remains a lack of research on the correlation between the chemical composition, antioxidant activity, and anti-inflammatory properties of *M. oleifera* extracts. Hence, the objective of this study is to explore the association between the chemical composition and the antioxidant and anti-inflammatory effects of *M. oleifera* extracts.

Materials and Methods

The study utilized both dry extract (DE) and liquid extracts (LE) derived from *M. oleifera*. The LE was prepared using a triple extraction method under heating (60–90 °C) with a mixture of water or water-ethanol solutions containing either 40% or 90% ethanol. The production of DE involved three rounds of extraction of the above-ground parts of *M. oleifera* using 40% ethyl alcohol at 90 °C. The extract was then subjected to ethanol distillation, evaporation, and freeze-drying to obtain the final product [21].

The antioxidant capacity of the LE was assessed by determining its ability to prevent the autooxidation of epinephrine hydrochloride in solution [15]. This method, which is straightforward and rapid, involves exposing a 0.1% epinephrine hydrochloride solution to normal lighting for 10 minutes, followed by spectrophotometric measurement at 347 nm. The addition of LE to the epinephrine solution results in an antioxidant activity greater than 10%, which confirms the presence of its antioxidant properties.

For testing the anti-inflammatory effects, white male rats (180–200 g) were used in both acute and chronic inflammation models. The animals were housed in standard vivarium conditions, ensuring proper care according to Lyashenko *et al*. [22]. Ethical standards were maintained throughout the study, adhering to the guidelines of the European Community (86/609/EEC) and the Helsinki Declaration. DE was dissolved in warm distilled water and administered via probe to the experimental groups at 100 mg/kg daily for 7 days. Control groups received only distilled water, and acetylsalicylic acid (ASA) was included as a comparison drug due to its known anti-inflammatory effects and structural similarity to phenolic compounds found in DE [23].

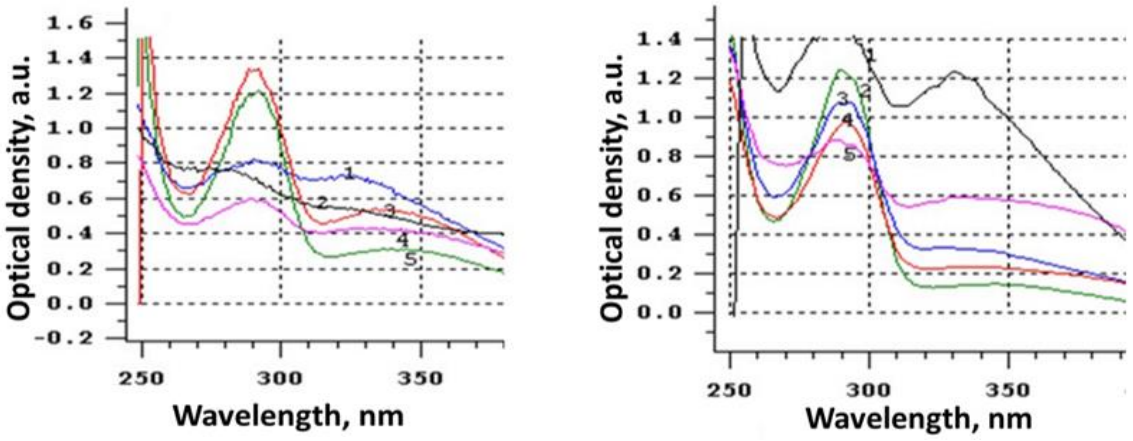
In the acute inflammation model, carrageenan (0.1 mL of a 1% aqueous solution) was injected into the plantar aponeurosis of the right hind paw, causing a classic inflammatory response of redness, swelling, and heat. After 3.5 hours, edema was quantified by measuring the displaced volume of water [24, 25]. For chronic inflammation, a sterile cotton swab (10 mg) was inserted under the skin to induce granuloma formation, and the granulomas were excised on day 8. The weight of the granulomas was measured both before and after drying at 60 °C to evaluate the proliferative and exudative effects of DE.

The elemental composition of DE was analyzed using inductively coupled plasma mass spectrometry (ICP-MS) with an ELAN DRC-e ICP-MS and an Agilent 715 ICP-OES optical emission spectrometer. Sample preparation included treatment with nitric acid and subsequent microwave digestion using Speedwave TM MWS-3+ and BERGHOF systems. The control analysis followed the additive method [26, 27].

Statistical analysis of the experimental results was conducted using Statistica 12.0 software. Differences were considered significant when the P-value was ≤ 0.05, determined through Student's t-test.

Results and Discussion

Upon examining the electronic absorption spectra of the original 0.1% epinephrine hydrochloride solution and its combination with LE of *M. oleifera* in a carbonate-bicarbonate buffer, it was observed that oxidation of epinephrine was inhibited by all LE samples, albeit to different extents **(Figure 1)**.



a) b)

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; bsorption spectra: a) aqueous LE (2: from grass, 3: from stems, 4: from leaves, and 5: from inflorescences); and b) alcoholic LE (2: from inflorescences, 3: from grass, 4: from leaves, and 5: from stems)

The bioelement complex, comprising biologically active compounds and variable-valence elements, significantly hinders the autoxidation of adrenaline hydrochloride. This effect manifests in a decline in the absorption rate of reaction products, following the order: LE from inflorescences, LE from leaves, LE from grass, and LE from stems **(Figure 1)**.

Figure 2 illustrates the AOA values calculated for the LE samples. The strongest antioxidant properties were identified in LE derived from inflorescences and leaves, whereas the weakest were found in LE extracted from stems. This trend was consistent regardless of the type of extractant applied.

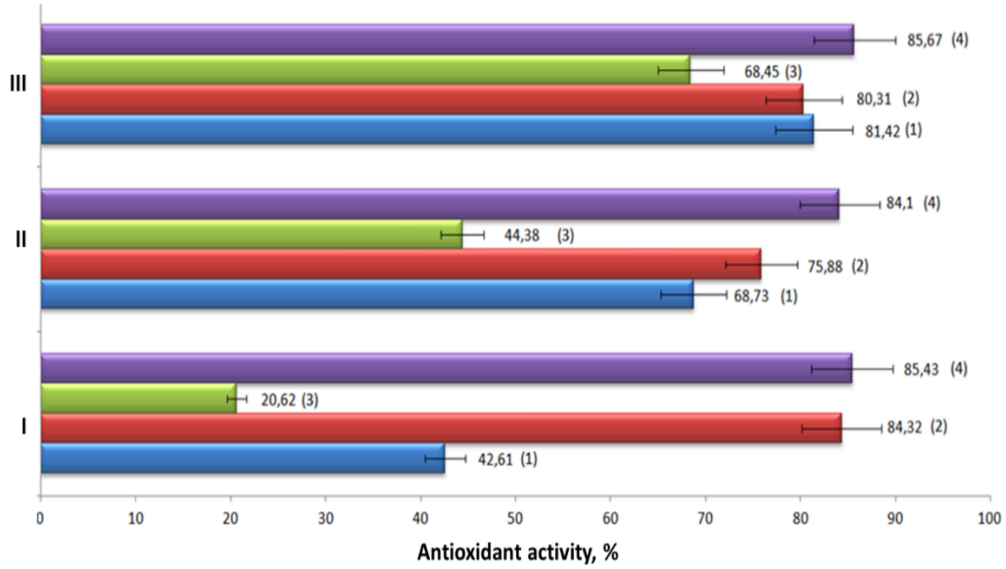


Figure 2. Dependence of the antioxidant activity of *M. oleifera* LE on the extractant (I: distilled water, II: 90% ethanol, and III: 40% ethanol) and morphological part of the plant (1: grass, 2: leaves, 3: stem, and 4: inflorescences)

A prior investigation into the distribution of key biologically active substances (BAS) in *M. oleifera* revealed that leaves and inflorescences contain the highest concentrations of antioxidants, including flavonoids, tannins, ascorbic acid, carotenoids, chlorophylls, oxycoric acids, and phenol carboxylic acids [28-30]. The observed antioxidant activity (AOA) in liquid extracts (LE) obtained from these plant parts further supports a strong correlation between bioelement content and AOA expression [31, 32]. The choice of extraction solvent

significantly influences AOA, with 40% ethyl alcohol demonstrating superior efficacy in isolating bioactive compounds compared to distilled water or 90% ethyl alcohol [33, 34]. Despite this, extracts from inflorescences consistently exhibited the highest AOA values across all extraction conditions **(Figure 2)**. Notably, when extracted with 40% ethyl alcohol, LE from whole plant material (including all morphological parts) displayed greater AOA than LE obtained solely from leaves.

To further explore these properties, a dry extract (DE) was prepared using 40% ethyl alcohol. The resulting substance was a finely textured, light brown powder with a mild herbal aroma, a bitter taste, and an astringent mouthfeel. It exhibited good solubility in 40% ethyl alcohol at ambient temperature and dissolved in heated distilled water. Phytochemical evaluation identified a substantial presence of polyphenolic oxidizable compounds, including up to 30% tannins, 5% flavonoids, 3.5% coumarins, 6% phenol carboxylic and oxycoric acids, 2% amino acids, ascorbic acid (14 mg), chlorophylls (71 mg), and carotenoids, consistent with previous studies [35-37].

Elemental composition analysis identified 61 elements in DE, except organogen elements (C, H, N, O), which are not detectable via MS-ICP analysis. All vital macro- and microelements were present [38, 39]. Ranking the elements by concentration (above 1 µg/g) produced the following sequence: K > Mg > Ca > P > Si > Na > Al > Br > Fe > B > Zn > Mn > Rb > Sr > La > Ti > Cu > Ni > As > Cr > Cs > Sb > V > Co. The presence of elements with variable valence states plays a key role in the antioxidant properties of DE **(Figure 3)** [40].

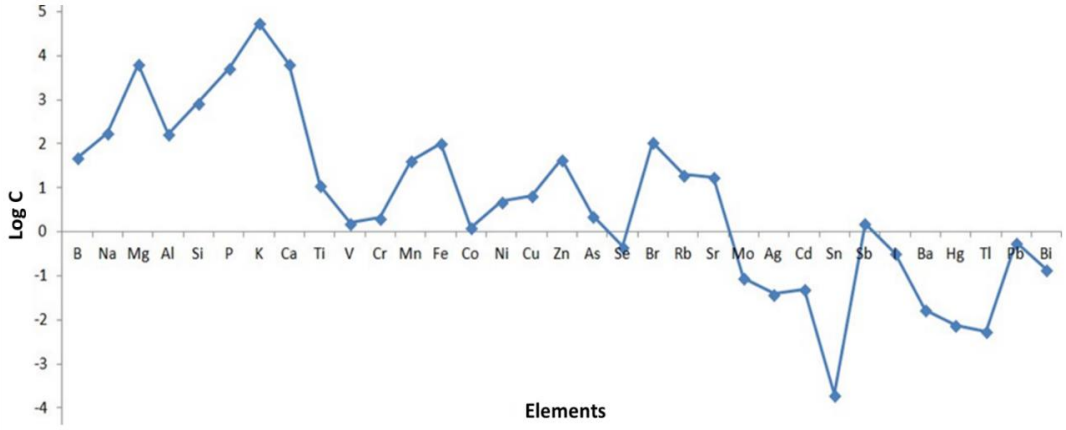
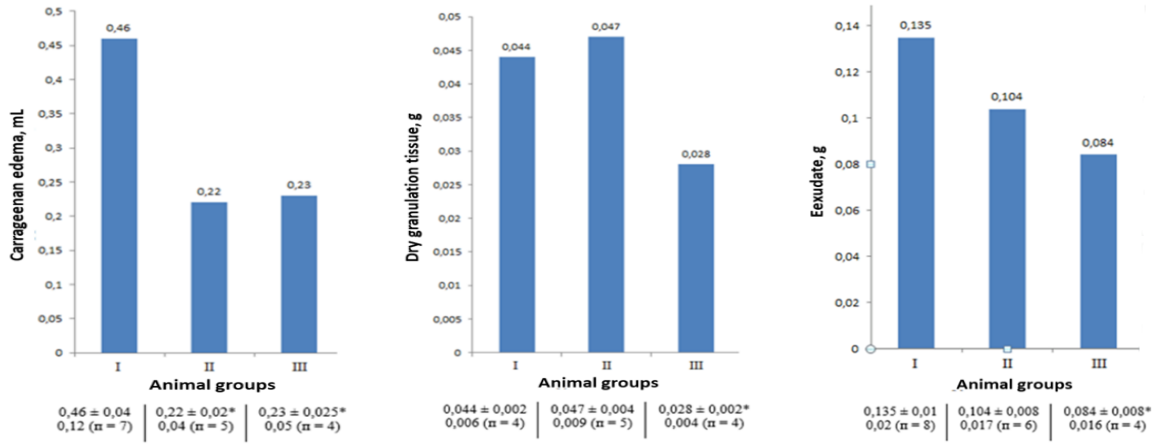


Figure 3. Composition and content of elements in *M. oleifera* dry extract (in logarithmic scale log10)

Analysis of the dry extract (DE) confirmed that the levels of toxic elements remain within the maximum permissible limits for beverages [41]. The presence of diverse antioxidant compounds from multiple chemical classes in plant extracts is known to enhance antioxidant activity (AOA) through synergistic interactions [42]. It is possible that the high AOA observed in *M. oleifera* DE from grass results from the combined effects of bioactive substances (BAS), particularly phenolic compounds and elements with variable valence states, contributing to this synergistic effect [43, 44].

In the control group, injection of carrageenan into the paw led to noticeable inflammation, characterized by swelling, redness, increased temperature, and pain at the injection site. Maximum edema formation occurred approximately 3.5 hours post-injection during a six-hour observation period. When carrageenan was administered alongside DE or ASA, inflammation-related symptoms such as paw swelling, redness, heat, and pain were also present but with reduced severity. Comparative evaluation of the anti-inflammatory effects of DE (100 mg/kg) and the reference drug ASA (20 mg/kg) in acute inflammation models demonstrated that both significantly mitigated paw edema, reducing swelling by 50% and 52.2%, respectively, compared to the control group **(Figure 4a)**. The observed reduction in edema volume is linked to decreased capillary permeability and improved microcirculation [45]. This effect is attributed to the high concentration of phenolic compounds, including flavonoids and coumarins, within the DE, while polyphenolic substances such as tannins may contribute to the stabilization of lysosomal cell membranes [46].



a) b) c)

Figure 4. Anti-inflammatory activity of *M. oleifera* dry extract (n = 6), (M ± m; P ≤ 0.05); a) acute inflammation ("carrageenan edema" model), and 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 about control

Chronic inflammation, if left untreated, can result in detrimental effects, such as the overgrowth of fibrous tissue that may replace healthy tissues, leading to deformities and loss of function [47]. In this regard, the antiproliferative effects of the dry extract (DE) under investigation present a notable advantage over other anti-inflammatory treatments, which typically only address the acute stage of inflammation. When rats were administered a 100 mg/kg dose of DE orally in the presence of chronic inflammation, there was a significant reduction (36.6%) in the weight of granulomatous tissue when compared to the control group. Conversely, when ASA (20 mg/kg), the comparison drug, was used in the same way, it exhibited significant anti-inflammatory effects during acute inflammation but failed to produce any effects during chronic inflammation and even led to a proliferative response **(Figure 4b)**.

Further comparison of the anti-exudative activity of DE (100 mg/kg) and ASA (20 mg/kg) in chronic inflammation revealed that DE performed more effectively. DE resulted in a 37.8% reduction in exudate mass, whereas ASA only showed a 23.1% reduction when compared to the control group **(Figure 4b)**.

Thus, the results obtained from this study concerning the antioxidant and anti-inflammatory properties of *M. oleifera* DE are in close agreement with previous research conducted using various in vitro and in vivo methods to assess these biological activities [48-50].

Conclusion

Extracts derived from *M. oleifera*'s grass and other plant parts exhibit considerable antioxidant activity, with the most potent effects seen in extracts from the leaves, inflorescences, and grass when using 40% ethyl alcohol. The DE from *M. oleifera* grass demonstrated substantial anti-inflammatory activity, reducing both exudate volume (50% in acute and 37.8% in chronic inflammation) and granulomatous fibrous tissue mass (36.6%), showing effects similar to acetylsalicylic acid (ASA).

The strong antioxidant and anti-inflammatory properties of *M. oleifera* DE are linked to its bioelement composition, which includes a variety of polyphenolic compounds like tannins (up to 30%), flavonoids (up to 5%), coumarins (up to 3.5%), phenolic and oxycoric acids (up to 6%), amino acids, ascorbic acid, chlorophylls, and elements such as Si, Br, Fe, Mn, Cu, Ni, Cr, and Co. This chemical composition directly correlates with the extract's observed biological activities, supporting its role in managing inflammation and oxidative stress.

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Conflict of Interest: None

Financial Support: None

Ethics Statement: All experiments with laboratory animals adhered to the standards set by the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes.

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