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ANTIOXIDANT ACTIVITY OF BIOACTIVE COMPOUNDS IN *MALCOLMIA AEGYPTIACA* AND *MATTHIOLA LIVIDA*

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Abstract: Many plants contain bioactive compounds such as polyphenols, flavonoids and tannins, which have antioxidant properties. Antioxidants help protect cells from damage caused by free radicals. The objective of this study was to investigate the antioxidant activity of plant extracts from *Malcolmia aegyptiaca* and *Matthiola livida* species. Aerial parts of the plants were collected and dried, and methanolic extracts were prepared from the dried plants. The total phenolic, flavonoid and tannin content of the extracts was determined and the antioxidant activity of the extracts was evaluated using various *in vitro* and *in vivo* assays. *M. livida* had a higher total phenolic and flavonoid content than *M. aegyptiaca*. Both extracts showed antioxidant activity in the assays, with M. livida generally exhibiting higher antioxidant activity than *M. aegyptiaca*. *M. livida* and *M. aegyptiaca* are both good sources of bioactive compounds with antioxidant activity. Therefore, these plants have potential for use in the development of natural antioxidants for the prevention and treatment of oxidative stress-associated diseases.

***Keywords*:** *Malcolmia aegyptiaca*; *Matthiola livida*; Bioactive compounds; Antioxidant activity; Oxidative stress

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Introduction

Throughout history, humanity has turned to nature for remedies, harnessing the therapeutic potential of plants in traditional medicine.[1] Among the myriad plant species known for their medicinal properties, over 35,000 have been identified,[2] emphasizing the rich diversity that nature offers in combating various ailments.[3] Of particular interest to contemporary researchers are plants harboring bioactive compounds, notably antioxidants, which play a crucial role in protecting cells from oxidative stress induced by metabolic processes and environmental factors.[4]

Antioxidants are molecules that serve as defenders against harmful free radicals, which, when left unchecked, can inflict damage on cells and contribute to the development of chronic diseases such as cancer, heart disease, and neurodegenerative conditions like Alzheimer.[5] In this context, the exploration of natural antioxidants with anti-inflammatory and antioxidant activities has become a pivotal area of scientific inquiry.

The focus of this study is to delve into and illuminate the antioxidant potential of two plant extract derived from two species, namely *Malcolmia aegyptiaca* (Egyptian Brassica) and *Matthiola livida* (Wild Stock). These plants hold promise as valuable sources of bioactive compounds with the capacity to protect cells from oxidative stress and combat diseases caused by free radicals. *M. aegyptiaca*, native to North Africa, the Middle East, and parts of Europe,[6] and *M. livida*, widely distributed across Europe and Asia with a history of medicinal use,[7] have been selected for their potential antioxidant properties.

Building on our research in which we examined the presence of bioactive compounds such as polyphenols, flavonoids and tannins in these plants, known for their free radical scavenging abilities,[8] this study seeks to

use a comprehensive methodology to uncover the full range of bioactive compounds and their antioxidant capacity. Different antioxidant tests were used, each targeting different aspects of antioxidant activity.[9]

By amalgamating the historical use of plants in traditional medicine, the significance of antioxidants in mitigating cellular damage, and the specific focus on *M. aegyptiaca* and *M. livida*, this introduction sets the stage for a thorough scientific investigation into the antioxidant properties of these plant species. The subsequent sections detail the methodology and findings, contributing to the growing body of knowledge in the field of natural antioxidants and their potential applications in health and disease prevention.

Results and Discussion

Quantification of phytochemical compounds

M. livida exhibits a slightly higher total phenolic content (TPC) of 3.82 ± 0.06 mg E GA/g Ex, surpassing that of *M. aegyptiaca*, which stands at 3.63 ± 0.11 mg E GA/g Ex. Correspondingly, *M. livida* also demonstrated a similar total flavonoid content (TFC) with a measurement of 1.97 ± 0.08 mg E Qu/g Ex, in contrast to the TFC value of *M. aegyptiaca* of 1.86 ± 0.09 mg E Qu/g Ex. This indicated that *M. livida* might serves as an alternative source of flavonoids, which are recognized as another group of antioxidant compounds with potential health benefits. While the total tannin content (TTC) was slightly lower in *M. livida* of 1.52 ± 0.05 mg E TAE/g Ex, compared to that of *M. aegyptiaca* of 1.67 ± 0.08 mg E TAE/g Ex, the difference was still relatively small (Figure 1).

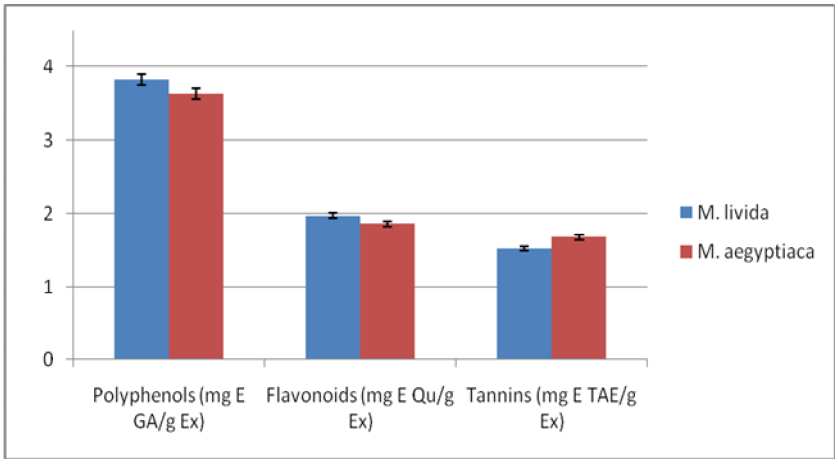

Figure 1. Quantification of phytochemical compounds.

Polyphenols, flavonoids and tannins are emerging as plant-derived phytochemicals of great interest due to their diverse pharmacological properties. These compounds exhibit remarkable properties, including antioxidant effects.[10] These compounds contain many hydroxyl groups on their aromatic rings, which act as magnets for harmful free radicals.[11] By donating electrons or hydrogen atoms, they neutralize reactive oxygen species (ROS) before they can damage cells and tissues.[12]

It is noteworthy that the quantity of polyphenols in both extracts surpasses the content observed in *M. africana*, a plant belonging to the same botanical family. In *M. africana*, the polyphenol percentage was determined to be approximately 1.282 (mg GAE/100 mL) according to the research conducted by Castañeda-Loaiza et al.[13]

Zeghoud et al.[14] reported a significant variation in the content of polyphenols and flavonoids between *M. aegyptiaca* and *M. livida*. *M. aegyptiaca* showed greater abundance with polyphenol concentrations ranging from 4.82 to 9.24 mg/g of dry weight, compared to 4.16 to

4.88 mg/g found in *M. livida*. Likewise, the flavonoid content was similar in *M. aegyptiaca* (0.75 to 2.93 mg/g) compared to *M. livida* (1.24 to 2.78 mg/g).

Evaluation of antioxidant activity in vitro

In vitro results from the DPPH free radical scavenging assay, hydrogen peroxide capacity assay, hydroxyl radical (HO-) scavenging capacity assay and hemolysis assay provide insight into the antioxidant potential of methanol extracts from *M. aegyptiaca* and *M. livida*, as well as the antioxidant ascorbic acid. In the DPPH free radical scavenging test, hydrogen peroxide capacity test, and hydroxyl radical (HO•) scavenging capacity test, ascorbic acid exhibited a higher level of efficacy compared to the methanol extract of *M. livida*, which, in turn, demonstrated greater effectiveness than the methanol extract of *M. aegyptiaca* (Figure 2, 3, 4). However, in the hemolysis test, while the same superiority was observed at lower concentrations, at a higher concentration of 813.46 mg/mL, the methanol extract of *M. aegyptiaca* surpassed the efficacy of the methanol extract of *M. livida* (Figure 5).

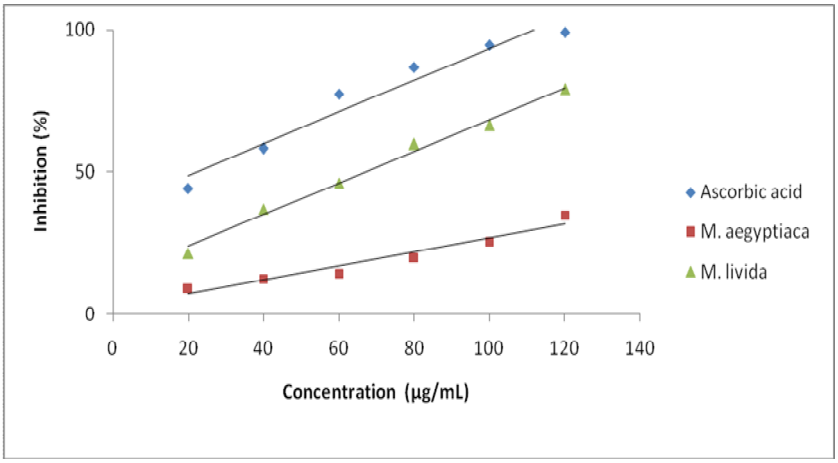

Figure 2. DPPH radical scavenging activity of *M. aegyptiaca*, *M. livida*, and ascorbic acid as a function of concentration.

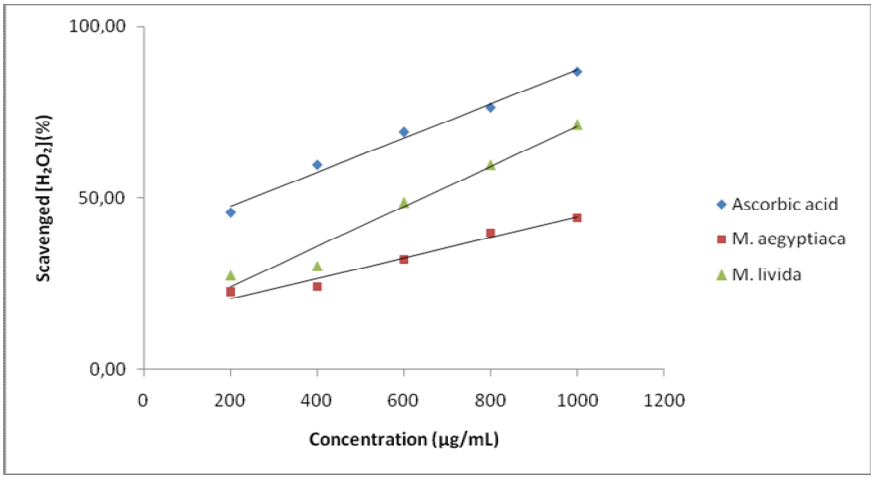

Figure 3. Hydrogen peroxide (H_2O_2) Scavenged of *M. aegyptiaca, M. livida*, and ascorbic acid as a function of concentration.

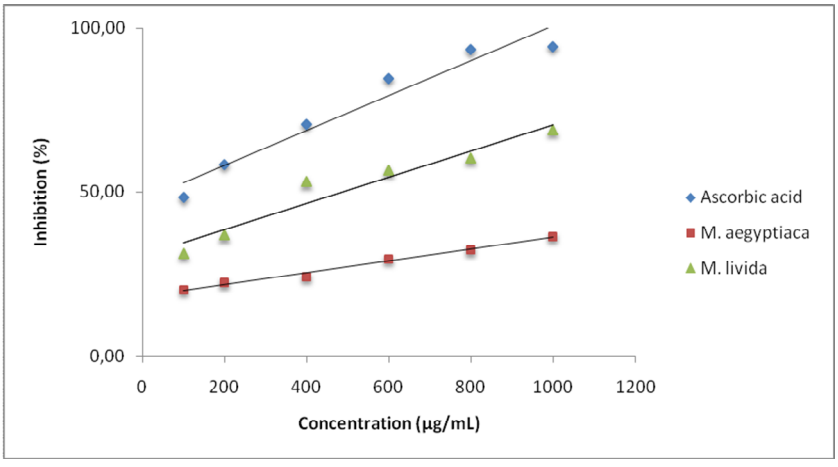

Figure 4. Hydroxyl radical (HO•) scavenging capacity of *M. aegyptiaca, M. livida*, and ascorbic acid as a function of concentration.

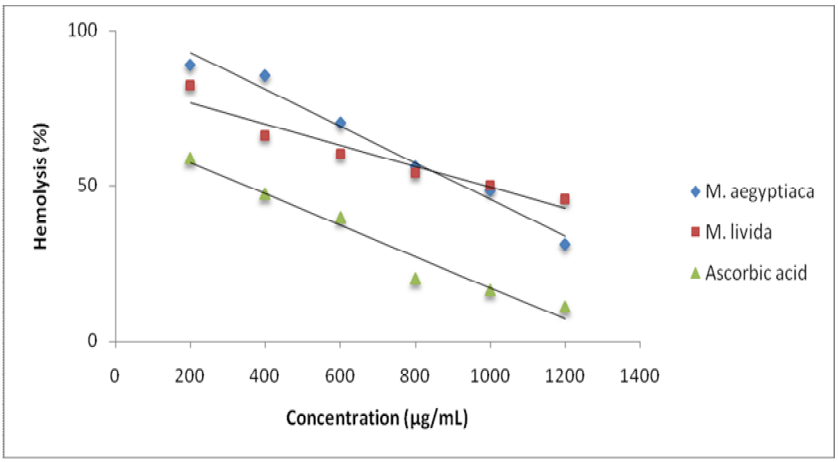

Figure 5. Hemolysis activity of *M. aegyptiaca, M. livida*, and ascorbic acid as a function of concentration.

Lower IC_{50} values indicate higher antioxidant activity. Ascorbic acid consistently showed the lowest IC_{50} values, demonstrating its strong antioxidant capacity in all tests (Table 1). In all tests except the hemolysis test, *M. livida* showed lower IC_{50} values compared to *M. aegyptiaca* in most tests, indicating a possible stronger antioxidant effect of *M. livida*. Hydroxyl radical scavenging ability test showed the greatest difference in IC_{50} values between *M. aegyptiaca* and *M. livida*.

Table 1. *In vitro* antioxidant activities of *M. aegyptiaca, M. livida* and ascorbic acid.

Antioxidant activities (IC_{50} µg/mL)	***M. aegyptiaca***	***M. livida***	**Ascorbic acid**
DPPH$^{\bullet}$ free radical scavenging	195.99±3.06	67.21±1.17	22.47±0.74
Hydrogen peroxide (H_2O_2) scavenging capacity	1194.67±8.09	643.74±1.56	245.32±0.9
Hydroxyl radical (HO$^{\bullet}$) scavenging capacity	1759.61±6.05	486.26±4.11	45.71±1.06
Hemolysis test	923.78±13.59	1010.6±14.9	288.14±4.09

The evaluation of DPPH free radical scavenging, the alcoholic extract derived from *Matthiola fruticulosa* and *Matthiola longipetala*, two species of the same genera to *M. livida*, showed lower efficacy compared to

the investigated plant samples. This is demonstrated by an IC_{50} value of 1.25 ± 0.02 mg/mL as documented by Taviano et al.,[15] and 492 µg/mL as documented by Abdelshafeek et al.,[16] respectively.

In evaluations of hydrogen peroxide (H_2O_2) scavenging capacity and hydroxyl radical ($HO^{\bullet}$) scavenging capacity, the methanol extract was contrasted with that of the *Helianthemum Lippii* (L.) plant, which thrived in the same geographical region. The latter demonstrated superior performance in both tests, with an IC_{50} estimated at 464.48 ± 45.50 µg/mL for hydrogen peroxide scavenging and 401.32 ± 51.51 µg/mL for hydroxyl radical scavenging, as reported by Chouikh et al.[17]

In the hemolysis test, the efficacy of both extracts was compared with extracts from various plants thriving in the same geographical region. In contrast to the *Calligonum comosum* L'Hér extract, which exhibited a hemolysis degree of 15.01 % at 1 mg/mL according to Chouikh et al.,[18] both tested extracts demonstrated lower effectiveness, surpassing 40 percent at the same concentration. Notably, the methanol extract of *M. livida* displayed diminished activity compared to the methanol extract of *Genista saharae*, which ranged from 31.26 to 34.81 percent at a concentration of 1.2 mg/mL, depending on different plant stages, as reported by Chouikh et al.[19] Additionally, the methanol extract of *M. aegyptiaca* exhibited an effectiveness of 33.7 percent at the same concentration.

Evaluation of antioxidant activity in vivo

In vivo and in the measurement of thiobarbituric acid reactive substances (TBARS) test, ascorbic acid, as usual, showed the greatest effectiveness in suppressing MDA, followed by the methanol extract of *M. aegyptiaca* and then the methanol extract of *M. livida* (Figure 6).

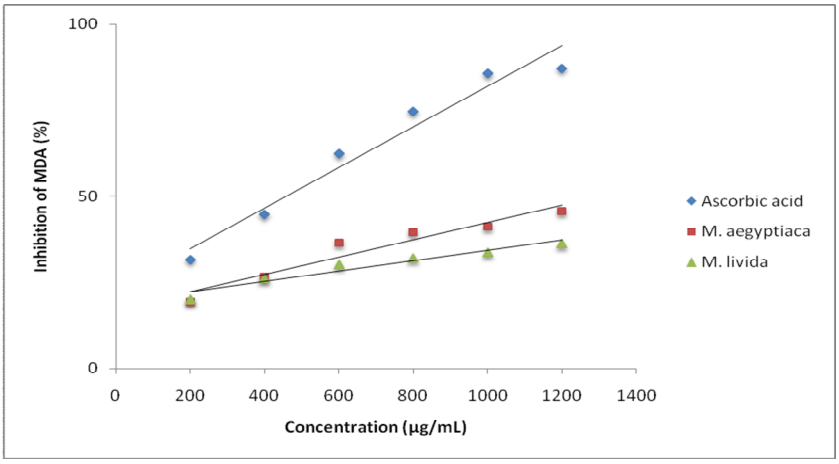

Figure 6. Inhibition of MDA activity of *M. aegyptiaca, M. livida*, and ascorbic acid, as a function of concentration.

TBARS serves as an indirect indicator of lipid peroxidation, a phenomenon in which free radicals adversely affect fatty acids within cellular membranes. The assessment of TBARS stands as a prevalent method for gauging oxidative stress in diverse biological specimens, encompassing food items, tissues, and blood. This assay hinges on the interaction between malondialdehyde (MDA), a principal derivative of lipid peroxidation, and thiobarbituric acid (TBA).[20]

In the TBARS assay, owing to the absence of investigations on certain species closely related botanically and geographically to the plants under examination, the two extracts were juxtaposed with the *Pitanga cherry* extract. The *Pitanga cherry* extract exhibited an MDA inhibition percentage exceeding 30% at a concentration of 0.5 mg/mL According to the results of Kade et al.,[21] whereas both tested extracts demonstrated lower values, *M. aegyptiaca* being estimated at 29.61% and *M. livida* extract at 26.73% at identical concentration.

Experimental

Plant materials

The aerial parts of *M. aegyptiaca* and *M. livida* were harvested in March 2021 from El Oued Province, situated in south-eastern Algeria. Post-harvest, the plant samples underwent a meticulous process to ensure their quality.

To eliminate any extraneous matter, the plant samples were thoroughly washed using cold running water. Subsequently, they were carefully dried in a dark environment to preserve their integrity. Once dried, the plants were finely ground to obtain a powder. The resulting powder was stored for future use. The systematic method used to collect and process plant materials guarantees the dependability and uniformity of the samples for future analyses and investigations.

Preparation of the methanol crude extracts

A total of 50 grams of the dehydrated botanical substance were immersed in 500 mL of 99% methanol at ambient temperature, shielded from light, for duration of 24 hours. Afterwards, the solution underwent filtration, and the solvent was eliminated through evaporation using a rotary evaporator (specifically, the Buchi R-200 model) at a temperature of 50 °C. This process resulted in the production of the methanol crude extracts.[22]

Quantification of phytochemical compounds

Determination of the total polyphenols content (TPC)

The determination of the overall polyphenol content in the extract was conducted using the Folin-Ciocalteu method, as described by Singleton-Rossi,[23] with minor modifications. During this process, a volume of 0.4 mL of the sample solution was added to a test tube that already contained 2 mL of Folin-Ciocalteu reagent (10%) and 1.6 mL of Na_2CO_3 (7.5%). After being

incubated at room temperature for 30 minutes, the absorbance was determined at a wavelength of 765 nm using a Shimadzu type UV-Vis spectrophotometer. The overall phenolic content was subsequently calculated and represented as milligrams of gallic acid equivalents per gram of the extract (mg E GA/g Ex). This revised methodology guarantees precise assessment and documentation of the overall phenolic content in the extract.

Determination of total flavonoids content (TFC)

The quantification of flavonoids was conducted using the methodology established by Kherraz et al.[24] The process involves combining 0.5 mL of the initial methanol plant extract with 0.5 mL of a 2% $AlCl_3$ solution after a 15-minute waiting period. The absorbance at 430 nm was measured using a spectrophotometer to determine the flavonoid content in milligrams of quercetin equivalent per gram of extract (mg E Qu/g Ex).

Determination of total tannin content (TTC)

The quantification of tannin content was conducted using the Folin-Denis assay, as described by Suresh et al[25]. The procedure involved adding 0.5 mL of Folin reagent and 1 ml of Na_2CO_3 (35%) to 100 µL of the diluted sample. The volume was then adjusted to 100 mL with distilled water. The resulting mixture was left undisturbed for 30 minutes, after which the absorbance was quantified at a wavelength of 700 nm using a spectrophotometer. The measurement of tannins was represented as milligrams of tannic acid equivalents per gram of the extract (mg E TAE/g Ex).

Evaluation of antioxidant activity in Vitro

DPPH$^{\bullet}$ free radical scavenging

The extracts were evaluated for their DPPH$^{\bullet}$ scavenging activity using the methodology described by Ben Ali et al.[26] Concisely, 1 mL of

each extract at different concentrations was mixed with 1 mL of DPPH solution (10^{-4} mol) in methanol. After being left at room temperature for 15 minutes, the absorbance was quantified at a wavelength of 517 nm. The inhibition activity was assessed using the formula:

$$\text{Inhibition (\%)} = [\text{Abs Control - Abs Sample / Abs Control} \times 100]$$

The IC_{50}, which represents the concentration at which 50% of the free radicals were suppressed by the extract, was determined by performing linear regression analysis on the relationship between concentration and the percentage of inhibition. A lower IC_{50} value indicates a greater antioxidant capacity. This technique offers a dependable evaluation of the extracts' capacity to remove DPPH radicals, providing valuable information about their antioxidant capabilities.

Hydrogen peroxide (H_2O_2) scavenging capacity

The extracts' ability to scavenge hydrogen peroxide was evaluated using the methodology described by Keser et al.,[27] A solution of hydrogen peroxide with a concentration of 40 millimolar (mM) was prepared in a phosphate buffer solution with a pH of 7.4. The extracts were added to a hydrogen peroxide solution with a concentration of 0.6 mM, at a concentration of 100 µg/mL in distilled water. The absorbance of hydrogen peroxide at 230 nm was measured after a time interval of 10 minutes. A blank solution containing phosphate buffer without hydrogen peroxide was used as reference. The percentage of hydrogen peroxide scavenging was determined by calculating the ratio of the scavenging activity of the extracts and standard compounds to the total amount of hydrogen peroxide present, using the following formula:

$$\text{Scavenged } [H_2O_2] \text{ (\%)} = [\text{Abs Control - Abs Sample / Abs Control} \times 100]$$

Hydroxyl radical (HO·) scavenging capacity

The capacity of the samples to eliminate hydroxyl radicals was evaluated by utilizing the hydroxyl radical system produced through the Fenton reaction[28]. Concisely, the samples were dissolved in distilled water at concentrations ranging from 0 (control) to 1 mg/mL, with increments of 0.1 mg/mL. The reaction mixture consisted of 1 mL of brilliant green solution with a concentration of 0.435 mM, 0.5 mL of $FeSO_4$ solution with a concentration of 2 mM, 1.5 mL of H_2O_2 solution with a concentration of 3.0%, and 1 mL of samples with different concentrations.

After being incubated for 20 minutes at room temperature, the absorbance of the mixture was determined at a wavelength of 624 nm. The solution exhibited a vivid green hue due to the presence of hydroxyl radicals, and alterations in absorbance indicated the ability to eliminate hydroxyl radicals. The hydroxyl radical-scavenging rate was determined by applying the following formula:

$$\text{The hydroxyl radical scavenging (\%)} = [(1 - \text{Abs Sample} / \text{Abs Control}) \times 100]$$

Hemolysis test

This assay was used to evaluate the ability of plant extracts to protect erythrocyte blood cells from membrane damage or disruption caused by oxidative stress and free radicals. The assessment relies on quantifying the proportion of erythrocytes that have dissolved.[29]

As per the methodology outlined by Ben Ali and Chouikh,[30] 40 µL of human erythrocytes were mixed with 2 mL of plant extract and left to incubate for 5 minutes at a temperature of 37 °C. Afterwards, 40 µL of hydrogen peroxide (30×10^{-3} M), 40 µL of ferric chloride (80×10^{-3} M), and 40 µL of ascorbic acid solution (50×10^{-3} M) were added in sequence. After incubation for 1 hour at 37 °C, the mixture was centrifuged at 700 rpm for

10 minutes. The supernatant absorbance was quantified at 540 nm. The hemolysis percentage was calculated using the following formula:

$$\text{Inhibition (\%)} = [(\text{Abs Control} / \text{Abs Sample}) \times 100]$$

Evaluation of antioxidant activity in Vivo

Measurement of thiobarbituric acid reactive substances (TBARS)

Involved male rats weighing between 140 and 160 grams, procured from the central pharmacy farm of Tunis (SIPHAT). The experimental procedures adhered to ethical guidelines inherent to animal experimentation.

The experimental procedures outlined by Garcia et al[31] some modifications. The experimental drug was administered to rats orally at various doses, following a 24 - hour resting period post-treatment. Subsequently, euthanasia was performed, and the brains were promptly harvested. The harvested brains were immersed in a refrigerated 0.1 M sodium phosphate (NaPi) buffer (pH 7.2) for preservation.

A precise slicing of the brains into small fragments was followed by homogenization in NaPi buffer utilizing a mechanical homogenizer. The resultant homogenate was appropriately diluted to achieve the desired concentration. Prepared samples (350 µL) were combined with solutions containing 500 µL of thiobarbituric acid (TBA) and 250 µL of trichloroacetic acid (TCA).

The composite samples underwent a controlled incubation process in a water bath, followed by a cooling phase and subsequent centrifugation. Subsequently, absorbance readings at 532 nm were performed. The concentration of malondialdehyde (MDA) in the samples was determined by employing an MDA calibration curve. The inhibitory concentration was calculated using the following equation:

$$\text{Inhibition of MDA (\%)} = [(\text{MDA Control} - \text{MDA Treated}) / \text{MDA Control} \times 100]$$

Conclusions

This study underscores the significant presence of bioactive compounds, including polyphenols, flavonoids, and tannins, in *Matthiola livida* and *Malcolmia aegyptiaca*, both known for their antioxidant properties. The quantification revealed that *M. livida* has a slightly higher total phenolic and flavonoid content than *M. aegyptiaca*, indicating its potential as an alternative source of antioxidants. *In vitro* assays showed that *M. livida* generally has stronger antioxidant effects than *M. aegyptiaca*, except in the hemolysis test at higher concentrations. Comparisons with related species highlight the competitive antioxidant capacities of these extracts. *In vivo* TBARS assays confirmed the antioxidant effectiveness of both extracts, however ascorbic acid showed the highest activity. These findings suggest that *M. livida* and *M. aegyptiaca* could be valuable sources of natural antioxidants for dietary and medicinal use, warranting further research to explore their specific mechanisms and applications in health.

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