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Research Article

Evaluation of the *in-vitro* anti-inflammatory activity of *Malva sylvestris* leaves extract

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Abstract: The primary aim of this research endeavor was to thoroughly evaluate and explore the potential anti-inflammatory properties exhibited by leaf extracts obtained from the *Malva sylvestris* plant species. In order to investigate the anti-inflammatory capabilities, a comprehensive set of *in vitro* experimental procedures was carried out to meticulously examine and gauge the extract's efficacy to stabilize human erythrocyte cell membranes under various stressful conditions, including exposure to varying concentrations of sodium chloride (NaCl), elevated temperatures, and oxidation induced by hypochlorous acid (HOCl). Additionally, the extract's potential to inhibit the denaturation of albumin, a process linked to inflammation, was evaluated. The findings revealed that the aqueous *Malva sylvestris* leaf extract exhibited notable anti-inflammatory properties by protecting red blood cell membranes from disruption caused by hypotonic NaCl solutions, heat stress, and oxidative damage from HOCl. These results suggest that the extract possesses significant anti-inflammatory potential and could be utilized as a natural remedy to mitigate inflammatory processes within the body. In summary, the aqueous extract derived from *Malva sylvestris* leaves demonstrated remarkable anti-inflammatory activity in vitro, making it a promising candidate for further exploration and potential therapeutic applications in the management of inflammatory conditions.

1. INTRODUCTION

The exploration of medicinal flora for their potential bioactive constituents has a rich legacy in traditional medicine practices, providing a fertile ground for modern medical advancements (Hill, 2022). Natural compounds have garnered increasing attention due to their emerging role as alternative antioxidant sources to synthetic counterparts. Radicals generated from external factors and metabolic processes pose a threat to biological molecules, ranging from minor tissue damage to cellular demise. The body employs antioxidant molecules, notably polyphenols, to combat this oxidative stress (Akbari *et al*., 2022).

Polyphenolic compounds, with their unique chemical architectures, act as effective electron donors, neutralizing free radicals and reactive oxygen species, thereby exhibiting antioxidant and anti-inflammatory properties (Mucha *et al*., 2021). In this context, *Malva sylvestris*,

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commonly referred to as mallow, is a perennial plant belonging to the Malvaceae family. Despite its historical use in traditional medicine (Panchenko, 2022), the anti-inflammatory capacities of *Malva sylvestris* leaves remain relatively underexplored. This study aims to investigate the anti-inflammatory potential of the aqueous extract of *Malva sylvestris* leaves.

2. MATERIAL and METHODS

The leaves used for extraction were collected from *Malva sylvestris* plants in the Tizi-Ouzou region of Algeria during the month of December 2022. The plant specimens were properly identified and authenticated by Professor SAADOUN-SMAIL Noria, a Botanist with the Department of Plant Biology at Mouloud Mammeri University of Tizi-Ouzou. A voucher sample of the identified plant material was deposited and preserved at the university's facilities (Voucher specimen: FSBSA/MK/2122).

The harvested leaves were air-dried and subsequently ground into a fine powder. This powdered material was stored away from light exposure at room temperature until the extraction process began. To prepare the extract, 20 grams of the powder was soaked in 200 milliliters of distilled water and allowed to macerate for a 24-hours at room temperature. Following this maceration step, the liquid portion was isolated by filtration and then lyophilized to obtain the dried extract.

2.1. Erythrocyte Suspension

Blood samples were obtained from healthy volunteers (ethical clearance certificate: CHU-UMMTO-N°0104/2024) and centrifuged for 10 minutes at 2,000 rpm and 4°C. The resulting red blood cells (RBCs) were washed three times with a saline buffer (PBS, 0.9% NaCl).

2.2. *In-vitro* Anti-inflammatory Studies

2.2.1. *Hypotonic induced hemolysis*

To assess the extract's ability to stabilize erythrocyte membranes, an experimental methodology involving hypotonic solution-induced hemolysis was employed. This approach followed the procedural guidelines previously described by de Freitas *et al*. (2008). Fourty microliter (40 µl) of the previously washed erythrocyte solution was introduced into different tubes containing a buffered hypotonic solution (PBS, pH 7.4) with varying NaCl concentrations (0.1%, 0.3%, 0.5%, 0.7% and 0.9%) and extract concentrations (18,75, 37,5,75, 150, 300, 600 and 1200 µg/mL). After 30 minutes of slow homogenization at 37°C, the tubes were centrifuged for 10 minutes at 2,000 rpm, and the absorbance was determined at 540 nm (UV-visible spectrophotometer MEDLINE MD2000).

2.2.2. *Heat-induced hemolysis*

Following the method described by Sakat *et al*. (2010), a 2% erythrocyte solution was added to a buffered phosphate saline solution at a pH of 7.4, 1 milliliter of extract (18,75, 37,5,75, 150, 300, 600 and 1200 µg/mL) and were incubated for 30 minutes at 56°C. After cooling, the tubes were subjected to centrifugation for 10 minutes at 2000 rpm. Following this step, the supernatant from each tube was carefully collected, and its absorbance was measured spectrophotometrically at a wavelength of 560 nanometers to quantify the extent of hemolysis that had occurred.

2.2.3. *Oxidant-induced hemolysis*

Following the technique detailed by Suwalsky *et al*. (2007) and Chandler *et al*. (2013). Various quantities of the extract (200, 400, 600, 800 and 1000 µg/mL) were added to 1 mL of a 5% erythrocyte solution (PBS, pH 7.4). The mixture was incubated for 15 minutes at 37°C. The resulting red blood cells were collected by centrifugation (2000 rpm, 10 min, 4°C) and then exposed to 0.5 mM HOCl. The absorbance was determined spectrophotometrically at 540 nm.

2.2.4. *Albumin denaturation inhibition*

A buffered stock solution (PB, pH 6.4) containing 0.2% egg albumin was prepared. For each concentration level being tested, 50 µl of the aqueous plant extract (or the standard reference compound) was introduced into 5 mL of the hypotonic solution. These sample mixtures were then heated at 72°C for 5 minutes to induce erythrocyte lysis. After allowing the heated samples to cool down to room temperature, the absorbance of the samples was measured spectrophotometrically at a wavelength of 660 nanometers to quantify the degree of hemolysis that occurred in each sample. The inhibition of protein denaturation was inversely proportional to the sample absorbance, as described by Karthik *et al*. (2013).

3. FINDINGS

3.1. Heat-Induced Hemolysis

Figure 1 shows that the extract plays a preventive role in protecting the erythrocyte membrane against heat-induced lysis compared to aspirin. The maximum protection recorded at 1200 µg/mL was 62.97 ± 2.1% for aspirin, followed by the studied sample for which the percentage of protection was found to be 91%.

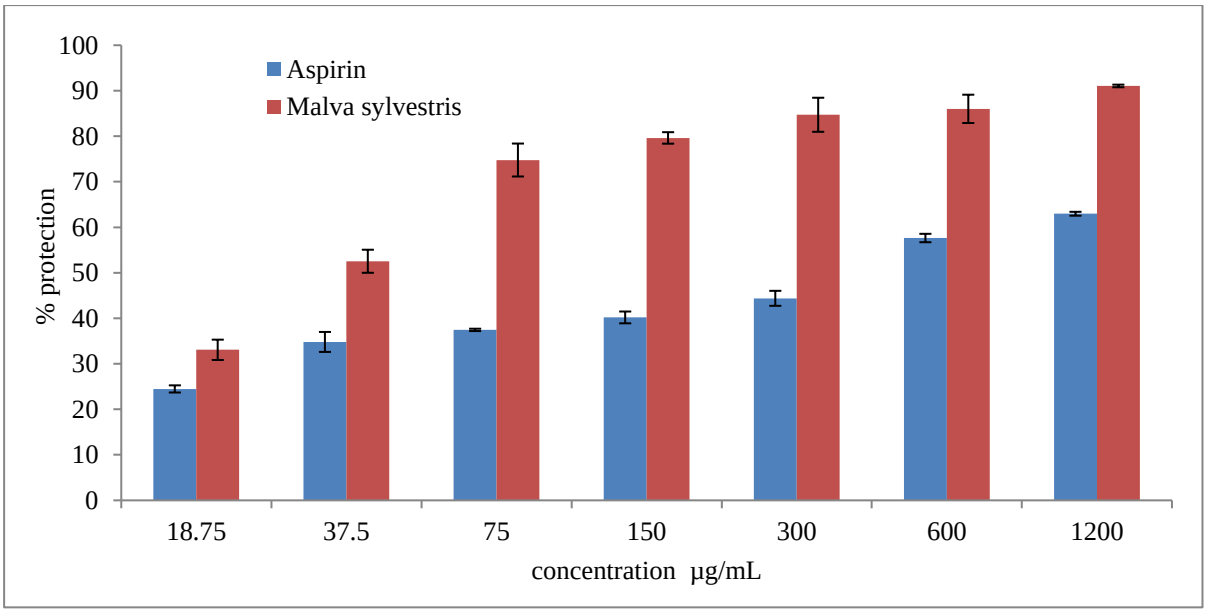

Figure 1. Effect of *Malva Sylvestris* aqueous extract on heat-induced hemolysis.

3.2. Evaluation of Hypotonic Induced Hemolysis Protection

The data depicted in Figure 2 provides compelling evidence of the protective influence exerted by *Malva sylvestris* extract on erythrocytes subjected to osmotic stress conditions. When exposed to varying sodium chloride (NaCl) concentrations of 0.3%, 0.5%, 0.7%, and 0.9%, the extract (18,75, 37,5,75, 150, 300, 600 and 1200 µg/mL) demonstrated remarkable shielding capabilities for red blood cells. Specifically, *Malva sylvestris* exhibited maximum protective capacities of 79.57%, 86.79%, 60.3%, and 79.48%, respectively, against the hemolytic effects induced by these hypotonic NaCl solutions. These findings underscore the extract's potential to fortify erythrocyte membranes against osmotic stress-induced damage.

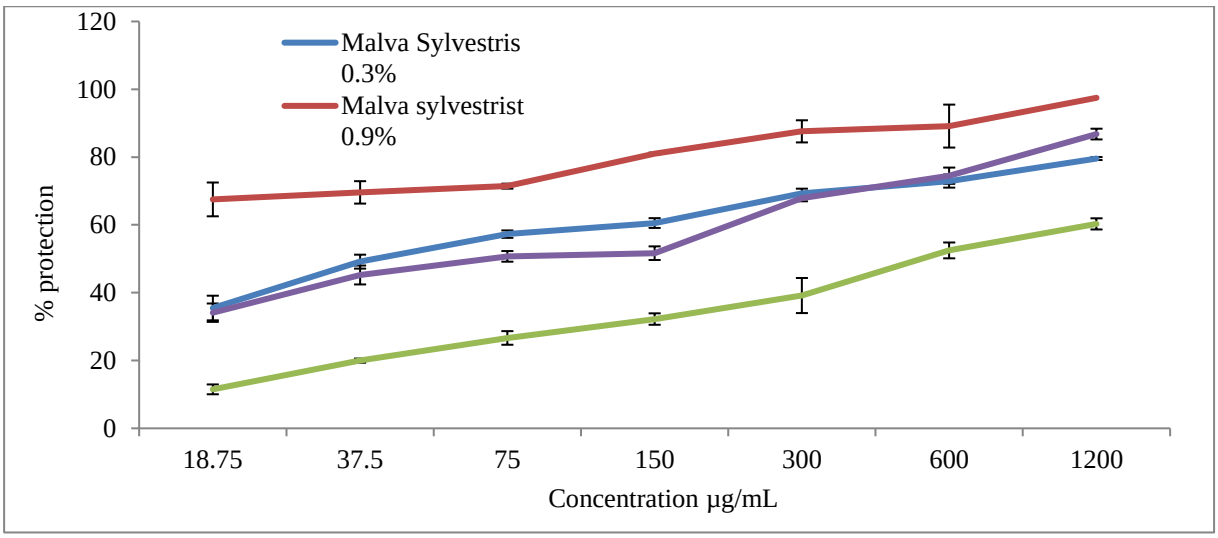

Figure 2. Effect of *Malva Sylvestris* aqueous extract on hypotonicity-induced hemolysis.

3.3. Evaluation of Oxidative Stress Protection

The data presented in Figure 3 showed the ability of the *Malva sylvestris* extract to safeguard against oxidative stress-induced damage. The findings reveal that the extract possesses a notable protective capacity, approximately 63.10%, in mitigating the deleterious effects of oxidative stress on cellular components. This observation highlights the extract's antioxidant potential and its capacity to confer protection against oxidative stress, which can have far-reaching implications for maintaining cellular integrity and function.

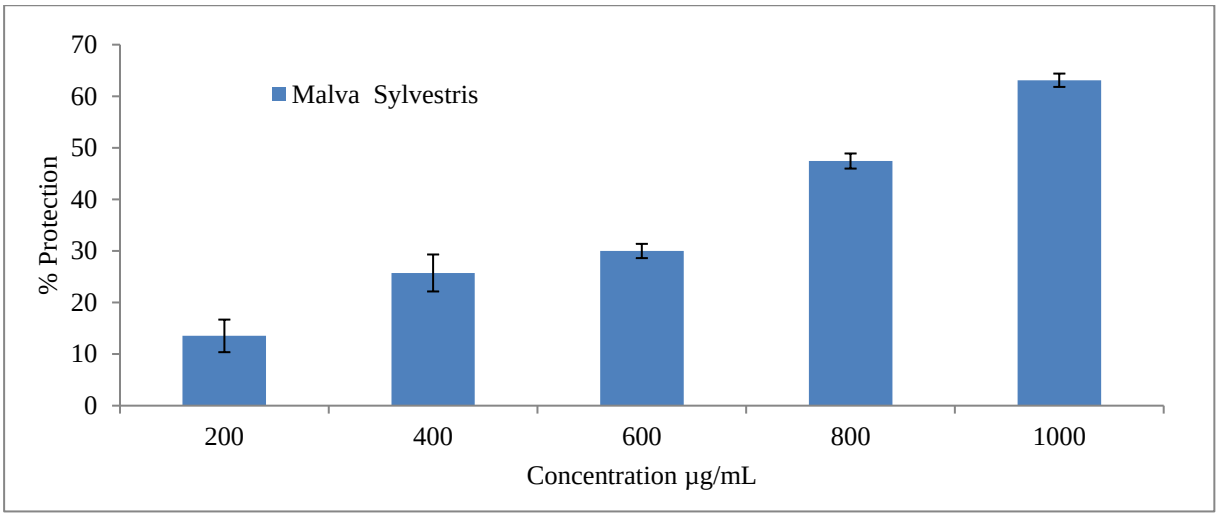

Figure 3. Effect of *Malva Sylvestris* aqueous extract on HOCl induced hemolysis.

3.4. Evaluation of Anti-Denaturation and Anti-Inflammatory Properties

Protein denaturation plays a pivotal role in the propagation of inflammatory processes, and plant extract that demonstrate the ability to inhibit denaturation are commonly evaluated for their anti-inflammatory potential. The study investigated the extract's capacity to prevent the thermal denaturation of albumin, a critical indicator of anti-inflammatory activity.

Malva sylvestris extract exhibited a remarkable effect in suppressing albumin denaturation across various concentrations (Figure 4). Notably, at a concentration of 500 mg/mL, the extract (50, 100, 200, 300, 400 and 500 µg/mL) achieved a maximum inhibition rate of 91.38±1.19%. Interestingly, this inhibitory effect was comparable to that observed with aspirin, a standard anti-inflammatory agent, which exhibited a similar inhibition rate of 92.23 ± 0.32% at the same concentration.

These findings suggest that the *Malva sylvestris* extract possesses potent anti-denaturation properties, which could contribute to its potential anti-inflammatory effects. The ability to prevent protein denaturation, a hallmark of inflammatory conditions, further underscores the therapeutic potential of this extract in mitigating inflammatory processes.

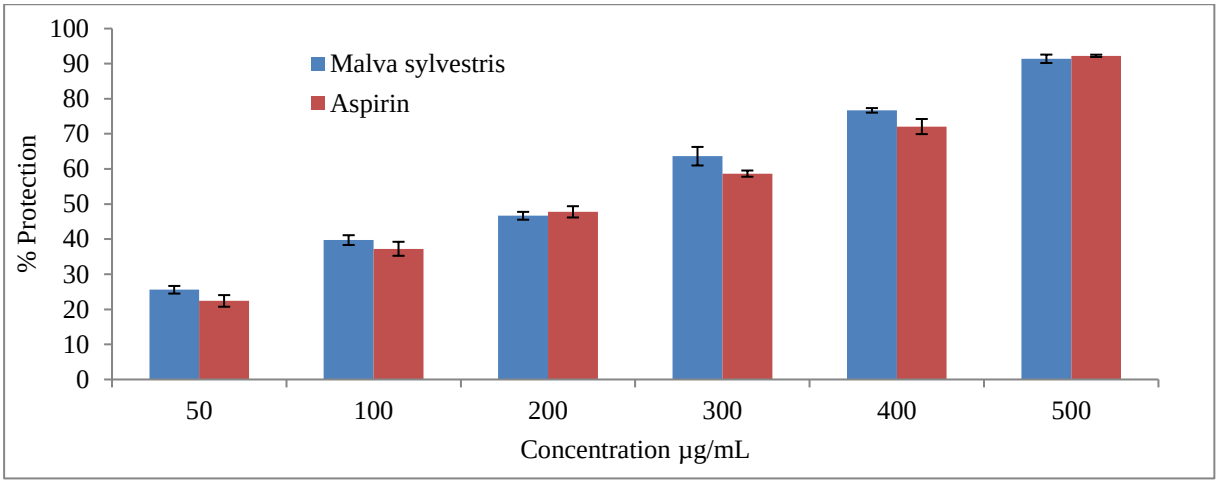

Figure 4. Effect of *Malva Sylvestris* aqueous extract on albumin denaturation.

4. DISCUSSION and CONCLUSION

This research investigated the anti-inflammatory properties of the aqueous extract derived from *Malva sylvestris* leaves. The evaluation encompassed assessing the extract's ability to stabilize erythrocyte membranes against various stressors and its capacity to inhibit thermal denaturation of albumin, a process intricately linked to inflammation.

Given the paucity of studies investigating the anti-inflammatory effects of the aqueous *Malva sylvestris* extract, direct comparison of our findings with existing literature remains challenging. However, our results agrees with the work of Belkhodja *et al*. (2024), who reported a protective effect of 91.97±2.87% against hypotonic solution-induced hemolysis at a concentration of 1000 µg/mL (re-cast). In our study, we achieved a comparable rate of protection (97.48±0.82%) at 1200 µg/mL. Interestingly, while the rate of protection against oxidant-induced hemolysis in the aforementioned study (93.42±3.45% at 50 µg/mL) surpassed our observations (63.1±1.29% at 1000 µg/mL), our extract demonstrated superior protection against erythrocyte lysis than *Murraya paniculata* (33.49 ± 0.51% at 2 mg/mL) reported by Laboni *et al*. (2015).

These collective findings provide compelling evidence supporting the anti-inflammatory power of the *Malva sylvestris* extract, which could be attributed to its ability to regulate calcium influx into erythrocytes, as proposed by Chopade *et al*. (2012). Moreover, given the structural similarities between erythrocyte and lysosomal membranes, the membrane-stabilizing effects observed in erythrocytes could potentially extend to lysosomal membranes, as suggested by Omale and Okafor (2008).

The inhibition of lysosomal content release at sites of inflammation, as postulated by Govindappa *et al*. (2011), could contribute to the anti-inflammatory activity exhibited by plant extract. Notably, our evaluation of albumin denaturation inhibition revealed that the *Malva sylvestris* extract displayed superior protection compared to the rates reported for *Erythrina indica* (65.21 ± 1.77%) at 800 mg/mL (Sakat *et al*., 2009).

Protein denaturation is widely recognized as a pivotal factor in the initiation and progression of inflammatory processes. The observed protection against albumin denaturation by the *Malva sylvestris* extract not only corroborates but also reinforces its anti-inflammatory potential. In conclusion, the collective evidence from this study strongly suggests that the aqueous extract of *Malva sylvestris* leaves possesses appreciable anti-inflammatory properties, rendering it a

promising candidate for further exploration and potential therapeutic applications in the management of inflammatory conditions.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors. **Ethics Committee Number**: CHU-UMMTO-No: 0104/2024.

Authorship Contribution Statement

Idir Moualek: Data Collection, Processing, Analysis, Interpretation, and Writing. **Karima Benarab**: Methodology and formal analysis. **Karim Houali**: Supervision and final approval.

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REFERENCES

Akbari, B., Baghaei-Yazdi, N., Bahmaie, M., & Mahdavi Abhari, F. (2022). The role of plant-derived natural antioxidants in reduction of oxidative stress. *BioFactors*, *48*(3), 611-633. https://doi.org/10.1002/biof.1831

Belkhodja, H., Bouhadi, D., Sedjrari, K., & Sehanine, S. (2024). Evaluation of the anti-inflammatory and anti-hemolytic potential of polyphenolic components of common mallow (Malva sylvestris). *Asian Journal of Dairy and Food Research*, *43*(2), 295-300. https://doi.org/10.18805/ajdfr.DRF - 321

Chandler, J.D., Min, E., Huang, J., Nichols, D.P., & Day, B.J. (2013). Nebulized thiocyanate improves lung infection outcomes in mice. *British Journal of Pharmacology*, *169*(5), 1166-1177. https://doi.org/10.1111/bph.12206

Chopade, A.R., Somade, P.M., & Sayyad, F.J. (2012). Membrane stabilizing activity and protein denaturation: A possible mechanism of action for the anti-inflammatory activity of *Phyllanthus amarus*. *Journal of Krishna Institute of Medical Sciences University*, *1*(1), 67-72.

de Freitas, M.V., Rita de Cássia, M.N., da Costa Huss, J.C., de Souza, T.M.T., Costa, J.O., Firmino, C.B., & Penha-Silva, N. (2008). Influence of aqueous crude extracts of medicinal plants on the osmotic stability of human erythrocytes. *Toxicology in vitro*, *22*(1), 219-224. https://doi.org/10.1016/j.tiv.2007.07.010

Govindappa, M., Naga Sravya, S., Poojashri, M.N., Sadananda, T.S., Chandrappa, C.P., Santoyo, G., Sharanappa P., & Anil Kumar, N.V. (2011). Antimicrobial, antioxidant and in vitro anti-inflammatory activity and phytochemical screening of water extract of *Wedelia trilobata* (L.) Hitchc. *Journal of medicinal plants research*, *5*(24), 5718-5729.

Gulcin, İ. (2020). Antioxidants and antioxidant methods: An updated overview. *Archives of Toxicology*, *94*(3), 651-715. https://doi.org/10.1007/s00204-020-02689-3

Hill, R.A. (2022). Making scientific sense of traditional medicine: Efficacy, bioprospecting, and the enduring hope of drug discovery in Ethiopia. *History of Pharmacy and Pharmaceuticals*, *63*(2), 270-301. https://doi.org/10.3368/hopp.63.2.270

Karthik, K., Kumar, B.R.P., Priya, V.R., Kumar, S.K., & Rathore, R.S.B. (2013). Evaluation of anti-inflammatory activity of *Canthium parviflorum* by *in-vitro* method. *Indian Journal of Research in Pharmacy and Biotechnology*, *1*(5), 729.

Laboni, F.R., Afsari, F., Howlader, M.S.I., Labu, Z.K., & Julie, A.S. (2015). Thrombolytic and membrane stabilizing activities of ethanolic extract of local medicinal plant *Murraya paniculata* (Family: Rutaceae). *Journal of Pharmacognosy and Phytochemistry*, *4*(2), 17-20.

Mhamdia, C., Seguenı, N., Hamdi, B., Hamdi, A.R.B., Bakı, A., & Hamdi, M.D.M. (2023). Ethnobotanical survey and *In vivo* study of the anti-inflammatory effect of *Malva sylvestris*

L. *Ethnobotany Research and Applications*, *26*, 1-13. http://dx.doi.org/10.32859/era.26.9.1-13

Mucha, P., Skoczyńska, A., Małecka, M., Hikisz, P., & Budzisz, E. (2021). Overview of the antioxidant and anti-inflammatory activities of selected plant compounds and their metal ions complexes. *Molecules*, *26*(16), 4886. https://doi.org/10.3390/molecules26164886

Omale, J., & Okafor, P.N. (2008). Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of *Cissus multistriata*. *African Journal of Biotechnology, 7*(17), 3129-3133.

Panchenko, K. (2022). Bioclimatic projection of the ecological niche of curly mallow (*Malva verticillata*) based on the forecast of the dynamics of the geographical range in the context of global climate change. *Regulatory Mechanisms in Biosystems*, *13*(4), 400-411. https://doi.org/10.15421/022253

Sakat, S., Juvekar, A.R., Gambhire, M.N. (2010). *In vitro* antioxidant and anti-infammatory activity of methanol extract of *Oxalis corniculata* Linn. *Int J Pharm Pharm Sci*, *2*(1), 146-155.

Sakat Sachin, S., Tupe Preeti, N., & Juvekar Archana, R. (2009). *In-vitro* anti-inflammatory activity of aqueous and methanol extracts of *Erythrina indica* Lam leaves. *Pharmacologyonline*, *3*, 221-229. https://doi.org/10.52711/0974-360X.2021.00637

Suwalsky, M., Orellana, P., Avello, M., & Villena, F. (2007). Protective effect of *Ugni molinae* Turcz against oxidative damage of human erythrocytes. *Food and Chemical Toxicology*, *45*(1), 130-135. https://doi.org/10.1016/j.fct.2006.08.010

Truong, V.L., & Jeong, W.S. (2021). Cellular defensive mechanisms of tea polyphenols: Structure-activity relationship. *International Journal of Molecular Sciences*, *22*(17), 9109. https://doi.org/10.3390/ijms22179109

Yan, Z., Zhong, Y., Duan, Y., Chen, Q., & Li, F. (2020). Antioxidant mechanism of tea polyphenols and its impact on health benefits. *Animal Nutrition*, *6*(2), 115-123. https://doi.org/10.1016/j.aninu.2020.01.001