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TOPIC

Biological Activities of the Hydroethanolic Extract of an Algerian Medicinal Plant: Gastroprotective, Analgesic, Anti-inflammatory, and Antioxidant Effects

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Introduction

Nowadays, many health disorders are due to the increasing levels of stress. Oxidative stress (OS) has been linked to a wide range of diseases, including cardiovascular disease, lung disease, chronic kidney disease **(Hajam *et al.,* 2022)**, it can also affect gastrointestinal motility **(Vona *et al.,* 2021)**.

Gastric ulcer disease is characterized by an imbalance between the defensive mechanisms of the gastric mucosa and the aggressive factors that act upon it. These aggressive factors include gastric acid secretion, which can damage the mucosal lining and lead to tissue injury, as well as nonsteroidal anti-inflammatory drugs (NSAIDs), *Helicobacter pylori* infection, and oxidative stress. When exposure to these factors is prolonged or recurrent, the integrity of the gastric mucosa is compromised, ultimately resulting in the development of gastric ulcers **(Karoui *et al*., 2014; Mazdour *et al*., 2017)**. Conversely, the gastric mucosa relies on a complex network of defensive factors to maintain its structural and functional integrity. These factors include epithelial cells, which form a continuous physical barrier against luminal aggressors **(Ontsira, 2016)**; mucosal blood flow, which ensures an adequate supply of oxygen and nutrients while facilitating the removal of toxic metabolites; prostaglandins, which stimulate mucus and bicarbonate secretion and promote mucosal repair **(Wallace, 2008)**; and mucus itself, which forms a protective gel layer that shields the epithelium from direct exposure to gastric acid and digestive enzymes **(Allen & Flemström, 2005)**.

Pain is a cardinal clinical manifestation of gastric ulcer disease, resulting from mucosal injury and the subsequent activation of nociceptive pathways by gastric acid **(Lanas & Chan, 2017)**. According to the International Association for the Study of Pain (IASP), pain is defined as an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage **(Sidibé, 2003)**. Pain arises from a complex interaction of physiological, tissue-related, emotional, and cognitive factors **(Warne, 2014)**. Its perception and intensity may vary considerably among individuals and can be influenced by both physiological and psychological factors. Therefore, each patient should be considered as a unique individual in whom physiological, emotional, and cognitive dimensions collectively shape the pain experience **(Yvay, 2005)**.

Pain is not merely a symptomatic experience; it is deeply intertwined with the inflammatory process at the molecular level. Inflammation is a natural body response to some harmful stimuli such as irritants, damaged cells, pathogens, and thermal or mechanical injury in order to restore the body's homeostasis. It is divided into an acute response and chronic inflammation **(Medzhitov, 2008)**. The inflammatory process is resolved when the tissue is repaired or cicatrized, named acute inflammation. However, in some diseases, the inflammatory process could be mild but persistent in time, causing chronic low-grade inflammation, which

involves the participation of immune cells but also parenchymal cells in the periphery and the brain **(Furman *et al.,* 2019)**. The inflammatory activity can be distinguished into two interconnected components: peripheral inflammation, which occurs at the site of tissue injury and involves the local release of inflammatory mediators and immune cell infiltration, and central inflammation, which refers to neuroinflammatory processes occurring within the central nervous system **(Ji *et al.*, 2018).**

Gastric ulceration, pain, and inflammation are all linked to oxidative stress, which results from an imbalance between the production of reactive oxygen and nitrogen species (ROS/RNS) and antioxidant defences **(Di Carlo & Sorrentino, 2024).** Antioxidant defences consist of endogenous antioxidants, such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), as well as exogenous antioxidants obtained from the diet, including vitamins C and E and polyphenolic compounds **(Wang *et al.,* 2020)**. These antioxidants protect biological systems against the toxic effects of free radicals and reactive species. The primary risk posed by free radicals lies in the damage they can inflict upon essential cellular components, including DNA, lipids through peroxidation, and proteins **(Hadj Salem, 2009)**.

Polyphenols are organic particles that are largely water-soluble and represent secondary metabolites produced by plants as a means of protection against dehydration, ultraviolet radiation, and oxidative stress owing to their antioxidant properties **(Weaver, 2014).** They are divided into several classes based on the number of phenolic rings they contain and the chemical functions associated with those rings, namely: phenolic acids, flavonoids, and tannins **(Pérez *et al*., 2013)**.

Medicinal plants, which are generally considered safer, more affordable, and more accessible than synthetic medicines, have historically served as useful therapeutic agents in ethnomedicine. According to the World Health Organization **(WHO),** more than 80% of the world's population still relies on traditional medicines obtained from plants to meet their basic medical needs. Over the past few decades, there has been a surge in global interest in medicinal plants as alternatives to synthetic medicines. Unlike the latter, which is based on a single chemical entity, medicines based on plant extracts contain various phytoconstituents (e.g., flavonoids, alkaloids, polyphenols, and terpenoids) **(Veiga *et al.,* 2020)**.

In this context, Algeria constitutes an important reservoir of plant genetic resources owing to its remarkable floristic diversity, which places it among the most botanically rich countries of the Mediterranean basin. This region has long been renowned for its rich heritage of traditional medicine and the ancestral use of medicinal plants for the treatment of a wide range of diseases. Among these medicinal plants, members of the Caprifoliaceae family, belonging to the order Dipsacales and commonly known as the honeysuckle family, have attracted considerable interest **(Belhouala & Benarba 2021).** Caprifoliaceae is a family of dicotyledonous flowering plants

comprising approximately 860 species distributed among about 42 genera. It has a nearly cosmopolitan distribution, with major centers of diversity located in eastern North America, Africa, and eastern Asia. Species of this family are predominantly shrubs and climbing vines, while herbaceous forms are relatively uncommon **(Xu & Chang, 2017)**.

Lomelosia (Caprifoliaceae: Dipsacoideae) is a genus of perennial herbs and subshrubs comprising approximately 30–35 species distributed throughout the Mediterranean Basin and extending into the Irano-Turanian region, from Morocco and the Iberian Peninsula in the West to the Caucasus in the East. Species of this genus typically inhabit rocky, alpine, and steppe environments. *Lomelosia* was reinstated as a distinct genus following its separation from Scabiosa and is typified by *Lomelosia stellata* (Raf.). The work of **Greuter & Raus, (1986)** formally established the generic name and clarified its taxonomic status relative to earlier classifications.

The genus includes over 50-63, perennial and annual species (*Lomelosia albocincta*, *Lomelosia crenata, Lomelosia lycia*, *Lomelosia rotata…*), diffused around the Mediterranean Sea, with the greatest diversity of species concentrated in the Eastern Mediterranean and the Middle East (including the Arabian Peninsula), and also has a few species reaching as far East as China **(De Castro & Caputo, 1999).**

Based on these findings, this study aims to investigate the gastroprotective, analgesic, anti-inflammatory, and antioxidant activities of the hydroethanolic extract of one plant of this family. This thesis is structured in two parts:

- The first part presents an introduction to gastric ulcers, pain, inflammation, oxidative stress, antioxidants and Caprifoliaceae family.
- The second part addresses the experimental study, including the extraction of bioactive compounds, the quantification of polyphenols, flavonoids, and tannins, the investigation of gastroprotective, analgesic, and anti-inflammatory activities, and the evaluation of the antioxidant activity of the hydroethanolic extract.

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Abstract

Caprifoliaceae is a family of flowering plants comprising shrubs, trees, lianas, and herbs. The family includes approximately 860 species distributed among 42 genera and is widely distributed worldwide. Several species belonging to this family are traditionally used for therapeutic and preventive purposes. The present study aimed to determine the phenolic compound content and evaluate the gastroprotective, analgesic, anti-inflammatory, and antioxidant activities of the hydroethanolic extract of a species belonging to this family. Extraction was carried out by maceration of plant powder in 50% ethanol, yielding 5.72%. Quantitative analysis revealed that the extract contained significant amounts of polyphenols (138.29 ± 3.39 µg GAE/mg of dry extract), flavonoids (37.09 ± 0.09 µg QE/mg of dry extract), and tannins (107.24 ± 0.65 µg TAE/mg of dry extract). The gastroprotective activity study demonstrated that the hydroethanolic extract protected the gastric mucosa against damage induced by absolute ethanol in mice. The percentages of protection were 84.38 ± 4.00%, 83.24 ± 5.93%, and 64.08 ± 13.81% for omeprazole and the extract doses of 200 and 400 mg/kg, respectively. Evaluation of analgesic activity using the acetic acid-induced writhing test showed that the extract reduced the number of abdominal writhes with inhibition percentages of 58.83 ± 5.18% and 55.63 ± 10.52% for extract doses of 200 and 400 mg/kg, respectively, compared with 63.00 ± 11.91% for the positive control (sodium diclofenac 10 mg/kg). Anti-inflammatory activity assessment revealed that the hydroethanolic extract significantly inhibited xylene-induced ear edema, with inhibition percentages of 73.07 ± 3.84%, 63.46 ± 2.81%, and 61.53 ± 2.90% for extract concentrations of 2.5, 5, and 10 mg/ear, respectively, compared with 73.07 ± 2.51% for the positive control. Antioxidant activity, evaluated using the 2,2-diphenyl-1-picrylhydrazyl assay, demonstrated the extract's ability to scavenge free radicals, with an IC_{50} value of 0.09 ± 0.006 mg/mL. This activity was lower than that of the standard antioxidant, butylated hydroxytoluene (IC_{50} = 0.02 ± 0.0003 mg/mL). In the iron chelation assay, the extract exhibited concentration-dependent chelating activity by sequestering ferrous ions prior to their complexation with ferrozine, with an IC_{50} value of 0.18 ± 0.013 mg/mL. However, this activity was markedly lower than that of ethylenediaminetetraacetic acid (IC_{50} = 0.011 ± 0.0002 mg/mL). The observed biological activities of the extract may be associated with the presence of polyphenolic compounds, especially flavonoids and tannins, which are known for their antioxidant, anti-inflammatory, analgesic, and gastroprotective properties.

Keywords: Caprifoliaceae family, analgesic activity, anti-inflammatory activity, antioxidant activity, polyphenols, gastric ulcer.

تعد الفصيلة الشعرية احدى فصائل النباتات المزهرة وتضم الشجيرات والأشجار والمتسلقات والأعشاب. تشمل هذه الفصيلة حوالي 860 نوعًا موزعة على 42 جنسًا، وتنتشرعلى نطاق واسع في جميع أنحاء العالم. تُستخدم العديد من الأنواع التابعة لهذه الفصيلة تقليديًا لأغراض علاجية ووقائية. تهدف هذه الدراسة إلى تحديد محتوى المركبات الفينولية، وتقييم الانشطة الواقية للمعدة و المسكنة للالم والمضادة للالتهاب والمضادة للاكسدة للمستخلص المائي الايثانولي لأحد أنواع هذه الفصيلة. تم الاستخلاص عن طريق نقع مسحوق النبات في الايثانول بنسبة 50%، وكان مردود الاستخلاص 5.72%. أظهر التحليل الكمي أن المستخلص يحتوي على كميات معتبرة من عديدات الفينول (138.29 ± 3.39 ميكروغرام مكافئ لحمض الغاليك/مغ من المستخلص الجاف)، والفلافونويدات (37.09 ± 0.09 ميكروغرام مكافئ للكارسيتين/مغ من المستخلص الجاف)، والدباغ (107.24 ± 0.65 ميكروغرام مكافئ لحمض التانيك/مغ من المستخلص الجاف). أظهرت دراسة النشاط الواقي للمعدة أن المستخلص المائي الايثانولي يحمي مخاطية المعدة من التلف الناجم عن الإيثانول المطلق لدى الفئران. وبلغت نسب الحماية 84.38 ± 4.00%، و83.24 ± 5.93%، و64.08 ± 13.81% لكل من الاوميبرازول وجرعتي المستخلص 200 و400 مغ/كغ على التوالي. أظهر تقييم النشاط المسكن للالم باستخدام اختبار التقلصات البطنية المحدثة بحمض الخل أن المستخلص قلل من عدد التقلصات البطنية، حيث بلغت نسبة التثبيط 58.83 ± 5.18٪ و55.63 ± 10.52٪ عند جرعتي 200 و400 مغ/كغ من المستخلصعلى التوالي، مقارنة بنسبة 63.00 ± 11.91٪ المسجلة لدى الشاهد الايجابي (ديكلوفيناك الصديوم 10 مغ/كغ). أظهر تقييم النشاط المضاد للالتهاب أن المستخلص المائي الايثانولي ثبط بشكل ملحوظ وذمة الأذن الناجمة عن الزيلين، حيث بلغت نسبة التثبيط 73.07 ± 3.84% و63.46 ± 2.81% و61.53 ± 2.90% عند التراكيز2.5 و5 و10 مغ/أذن من المستخلص على التوالي، مقارنةً بنسبة 73.07 ± 2.51% المسجلة لدى الشاهد الايجابي. أظهرالنشاط المضاد للأكسدة باستخدام اختبار 2،2-ثنائي فينيل-1-بيكرايل هيدرازيل قدرة المستخلص على إقتناص الجذور الحرة، حيث بلغت قيمة التركيز المثبط ل50% من الجذر الحر0.09 ± 0.006 مغ/مل وكانت هذه الفعالية أقل من تلك المسجلة لمضاد الأكسدة المرجعي (بوتيل هيدروكسي تولوين) الذ بلغت قيمة التركيزالمثبط ل50% من الجذر الحر 0.02± 0.0003 مغ/مل. في اختبار استخلاب الحديد اظهرالمستخلص نشاطا يعتمد على التركيز، وذلك من خلال عزل ايونات الحديد الثنائية قبل ارتباطها بمركب الفيروزين حيث بلغت قيمة التركيزالمثبط ل50% من الجذر الحر0.18 ± 0.013 مغ/مل. مع ذلك، كان هذا النشاط أقل بكثير من نشاط حمض الإيثيلين ثنائي امين رباعي الأسيتيك الذي بلغت قيمة التركيز المثبط ل50% من الجذرالحر 0.011 ± 0.0002 مغ/مل. قد ترتبط الأنشطة البيولوجية الملحوظة للمستخلص الى الى احتوائه على مركبات عديدات الفينول، وخاصة الفلافونويدات والدباغ المعروفة بخصائصها المضادة للأكسدة والمضادة للالتهاب والمسكنة للألم والواقية للمعدة.

الكلمات المفتاحية: الفصيلة الشعرية ، النشاط المسكن للالم، النشاط المضاد للالتهاب، النشاط المضاد للأكسدة، عديدات الفينول، القرحة المعدية.