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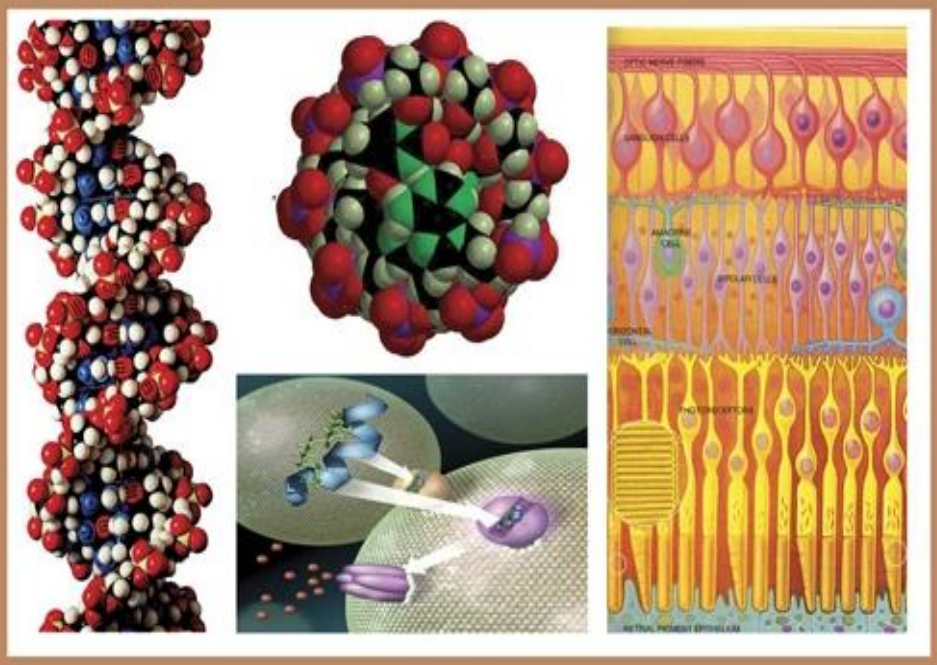

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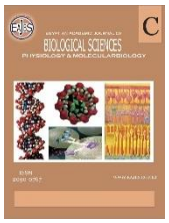

Anti-inflammatory and Antibacterial Activity of the Methanol Extract of *Artemisia Herba-Alba.*

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ABSTRACT

The use of medicinal plants from the traditional African pharmacopoeia in the treatment of various diseases has been known for a long time, and the positive effects of this phytotherapy are no longer in question. However, these practices are based on empiricism. The methanolic extract of the leaves of *Artemisia herba-alba* has analgesic and anti-inflammatory properties that justify its traditional use. This study evaluates the anti-inflammatory and antibacterial properties of the methanolic leaf extract of *Artemisia herba-alba*. The negative control and test groups received 40 mg/kg of the extract. The anti-hematogenic effects of the extract showed a significant decrease in carrageenan-induced paw edema at 1 and 3 hours, as well as dextran-induced inflammation, compared to the control. In addition, there was inhibition of xylene-induced ear edema with a significant reduction compared to the control ($p<0.05$). The bacterial activity was determined on five bacterial strains*: Klebsiella pneumoniae ATCC700603, Citrobacter freindii ATCC8090, Salmonella typhii ATCC13311, Enterobacter coloacae ATCC13047* and *Staphylococcus aureus ATCC25923,* according to the in vitro agar diffusion method (aromatogram). Our extract would therefore be an advantageous source of improved traditional medicine that is accessible and less expensive for people.

INTRODUCTION

Plants have populated the planet long before humans, and they were initially used to feed humans through gathering and cultivation (Lorrain, 2013). Their use quickly evolved with the recognition of their therapeutic properties to treat injuries and diseases. The use of aromas was also known to ancient civilizations for religious, cosmetic, and therapeutic purposes (Lardry and Haberkorn, 2007). It was during the Pharaonic era in Egypt, from 3150-1085 BC, when people first used aromatic plants to embalm the dead, especially with a mixture of essential oils such as cedar and basil oil (Franchomme *et al.*, 1990; Abrassart, 1997).

They also used plants with known antiseptic properties, such as Himalayan spikenard, cinnamon, and cistus, as well as aromatic secretion products like frankincense or myrrh (Couic-Marinier and Lobstein, 2013). In ancient Greece, Hippocrates indicated the use of aromatic baths in the treatment of women's diseases (Lardry and Haberkorn, 2007).

And during major epidemics, lavender, savory, rosemary, and hyssop were burned (Franchomme *et al.,* 1990). In India, during the golden age of Ayurvedic medicine, which coincided with the peak of Buddhism from 327 BC to 750 AD, medicinal and aromatic plants were commonly recommended for various indications, such as massages, baths, hygiene, health, and dietetics (Lardry and Haberkorn, 2007; Roulier, 1990). In the 1st century AD, the treatise entitled "De materiamedica" written by Dioscorides, a physician and great traveler, appeared, listing 519 species of plants and serving as a reference in Roman and Arab society. The Arabs continued to research medicinal plants and were the first to develop the distillation of plants to extract essential oils, more than a thousand years ago (Azzi, 2013). Since ancient times, plants have been part of people's daily lives, as they have used them for food, medicine, and sometimes in their superstitious and religious traditions. The fragrant and therapeutic properties of plants were already known in ancient Egypt and China (Fellah *et al.,* 2006). According to the World Health Organization (WHO), 75-95% of rural populations (particularly in developing countries) use traditional medicine made largely from plants (WHO, 2003). Currently, it is proven that about 20% of the plant species growing worldwide have therapeutic or cosmetic virtues as they contain molecules or active principles with different biological properties. These properties find their application in various fields such as medicine, pharmacy, cosmetology, and agriculture, among others (Suffredini *et al.,* 2004).

MATERIALS AND METHODS

Plant Material and Extraction:

The plant *Artemisia Herba-Alba* was collected from the region of Stiten Bayadh (Algeria) in January 2022. The plant was identified by Prof. Mohamed Terras, who teaches in the Department of Biology at the University of Saida, Algeria. The aerial part of the plant was air-dried and then ground into a powder. Five hundred (500) grams of the powder were cold macerated in 2.5 L of methanol for 72 hours and shaken regularly. The solution was then decanted, and the extract was filtered and evaporated to dryness at 40°C.

Determination of Total Phenolic Content:

Wend Li *et al.* (2007) used the Folin-Ciocalteu method to determine the total polyphenol content. The samples (0.2 mL) were mixed with 1 mL of Folin-Ciocalteu reagent, which was produced with 10 mL of deionized water. After letting the solutions rest for 4 minutes at 25°C, 0.2 mL of saturated sodium carbonate solution (75 mg/mL) was added. The mixed solutions were left to rest for 120 minutes before the absorbance was measured at 765 nm. Gallic acid was used as a standard for the calibration curve, and the total phenolic content was expressed in mg gallic acid equivalent per gram of extract (mg EAG/GE).

Determination of Total Flavonoids Contents:

According to the method described by Bahorun *et al.* (1996), 1 mL of the methanol extract solution was added to 1 mL of 2% AlCl3 in methanol. The absorbance was measured at 430 nm after 10 minutes. Quercetin was used as a standard, and the results were expressed in mg quercetin equivalent per gram of extract (mg QE/GE).

Activity Anti-Inflammatory:

Estimation of in vitro anti-inflammatory activity was carried out using the human red blood cell (HRBC) membrane stabilization method, as described by Sadique *et al.* (1989), to assess the in vivo anti-inflammatory effect of the hexane

extract. The principle involved is the stabilization of the membrane of human red blood cells, which is achieved by preventing membrane lysis induced by hypotonicity.

To prepare the HRBC suspension, fresh human blood (10 mL) was collected and transferred into centrifuge tubes. The tubes were then centrifuged at 3000 rpm for 10 minutes and washed with an equal volume of normal saline each time. The volume of blood was measured, and it was reconstituted as a 10% v/v suspension with normal saline.

The principle involved here was the stabilization of the human red blood cell membrane by hypotonicity-induced membrane lysis. The mixture contained 1 mL of phosphate buffer (pH 7.4, 0.15 M), 2 mL of hyposaline (0.36%), 0.5 mL of HRBC suspension (10% v/v), and 0.5 mL of plant extract or standard drug (diclofenac sodium) at various concentrations (10, 50, 100, 250, 500 µg/mL). The control was distilled water instead of hyposaline to produce 100% hemolysis.

The mixtures were incubated at 37°C for 30 minutes and centrifuged at 2500 rpm for 5 minutes. The absorbance of the hemoglobin content in the suspensions was estimated at 560 nm. The percentage of hemolysis of the HRBC membrane can be calculated as follows:

Haemolysis (%) = (Optical density of the test sample / Optical density of control) × 100.

The Microorganisms tested for Antibacterial Activity:

The antibacterial activity of the extract was evaluated against different types of bacteria (Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus, and Bacillus subtilis) using the method presented by Mokhtar (2020). 0.5 mL of cultures were diluted in 20 mL of Muller Hinton liquid. The latter, which is apparently solid, is melted by heating and then cooled before being put in contact with the microbial suspension. The mixture was placed in Petri dishes of 90 mm. Using a Pasteur pipette, wells of 7 mm diameter were created in the Muller Hinton agar poured into sterile Petri plates. 30 mg of the extract was placed in the wells. The antibacterial activity was determined by measuring the inhibition zones of each sample around the spots at regular intervals after 24 hours of incubation at 37°C, visually.

Statistical Analysis:

The data analysis was performed using Microsoft Office Excel 2007 for the classification of raw data and graph development, and StatBox version 6.0 for ANOVA analysis and the Newman-Keuls test.

RESULTS AND DISCUSSION

Results of Extraction:

The results of the extraction method showed a very low yield, containing 2.03 ± 0.19 mg EAG/GE of polyphenols and 0.533 ± 0.03 mg QG/GE of flavonoids

Results of Anti-Inflammatory Activity:

The anti-inflammatory activity of *Artemisia Herba-Alba* extract was confirmed by the erythrocyte membrane stabilization test. The results showed that the membranes of human erythrocytes were protected against lysis induced by hypotonic solutions at different concentrations of the extract, particularly at higher concentrations.

The protective effect was greater than that of sodium diclofenac at a concentration of 200 µg/mL and above. The membrane-stabilizing attributes were acknowledged for their ability to interfere with the release of phospholipases that activate the establishment of inflammatory intercessors (Aitadafouri *et al.*,1996).

During inflammation, lysosomal enzymes and hydrolytic components are released from the phagocytes into the extracellular space, causing damage to surrounding organelles and tissues and contributing to a variety of disorders (Ackerman and Beebe, 1994). Hence, the methanol extract of Cyclamen africanum acts as an anti-inflammatory agent

Results of the Antibacterial Activity of the Methanol Extract:

The principle of the aromatogram is inspired by the antibiogram. It consists of

testing the sensitivity of bacterial strains by diffusing the extract on a solid medium in a Petri dish. The appearance and size of the diameter of the inhibition zone reflect the impact of the EO/aqueous extract on the bacterial strains. The strains can be classified as sensitive, very sensitive, or resistant based on the results (Ouibrahim, 2015).

In this study, we tested the sensitivity of five bacterial strains (*Klebsiella pneumoniae, Citrobacter freundii, Salmonella typhii, Enterobacter cloacae, and Staphylococcus aureus*) to the *Artemisia herba-alba* Asso extract via the EM method. The results, represented by the diameter (mm) of the inhibition zones (R), are presented in the following table:

Table 1: Antibacterial test results

EA	*ArtemisiaHerba albaAsso*		
Klebsiella pneumoniae	12	11	11
Citrobacter freindii	09	10	11
Salmonella typhii	10	11	10
Enterobacter coloacae	08	08	09
Staphylococcus aureus	10	10	10

According to Al-Bakri and Afifi (2007), antimicrobial activity can be considered present if the diameter of the inhibition zone observed around the Whatman paper disc is greater than or equal to 9 mm. Generally, the diameters of the inhibition zones obtained with Gram-positive bacteria are larger than those obtained with Gram-negative bacteria.

To determine the sensitivity of each bacterial strain to the extracts, the average R value for each extract at each bacterial strain was calculated:

- If the R value is 9.00 mm or greater, the bacterial strain is considered sensitive to the extract.
- If the R value is less than 9.00 mm, the bacterial strain is considered resistant to the extract.

For *Klebsiella pneumoniae,* the value of R representing *Artemisia Herba-alba asso* is 11.33mm, indicating susceptibility to the extract. Similarly, *Citrobacter freindii* has an R value of 10.00mm, indicating susceptibility to the extract.

Salmonella typhii also has an R value of 10.33mm, indicating susceptibility to the extract. However, Enterobacter cloacae has an R value of 08.33mm, indicating resistance to the extract.

Finally, *Staphylococcus aureus* has an R value of 10.00mm, indicating susceptibility to the extract.

On the other hand, upon comparing the impact of *Artemisia Herba-alba asso,* it can be observed that it has varying degrees of effectiveness.

It can also be inferred that *Klebsiella pneumoniae* is the most susceptible strain among the tested bacteria with an average inhibition zone diameter of 10.5 mm, while *Enterobacter cloacae* is the most resistant strain with an average diameter of 7.83 mm.

CONCLUSION

The traditional use of plants has been recognized for various purposes, particularly in the medicinal field due to their important biological properties. Medicinal plants are considered an inexhaustible source of bioactive substances and are preferred over drugs due to the latter's potential side effects.

In this study, we aimed to evaluate the anti-inflammatory and antibacterial activities of the extract of *Artemisia herba-alba asso* (family Asteraceae), which was obtained through the cold maceration method. The antibacterial activity of the extract was tested on five bacterial strains, including *Klebsiella pneumoniae* ATCC700603, Citrobacter freundii ATCC8090, *Salmonella typhii* ATCC13311, *Enterobacter cloacae* ATCC13047, and *Staphylococcus aureus* ATCC25923, using the in vitro agar diffusion method (aromatogram).

The results showed remarkable antibacterial activity of the extract against all tested bacterial strains.

In conclusion, the biological extract of *Artemisia herba-alba asso* exhibited significant antibacterial activity, thus

validating its use in traditional medicine.

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