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Introduction:

Algeria possesses a rich historical and cultural heritage, rooted in the succession of various civilizations, each leaving behind diverse material remains. Among these are human and animal bones, which constitute an essential component of many archaeological sites and were historically used in the manufacture of tools, weapons, and other artifacts.

The abundance of bone remains at archaeological sites has enabled anthropologists to analyze and study them, providing insights into the development of life over the ages, as well as the environmental factors that shaped past ecosystems. These studies also contribute to understanding the behavior of living organisms, developing hypotheses about their history, and identifying the animal species that existed during those periods.

Archaeological bones are organic materials known for their sensitivity to various forms of deterioration, including climatic and biological factors. Microorganisms can adapt to the organic components of bones, using them as a favorable environment for growth and reproduction.

Bacteria are unicellular microorganisms capable of rapid multiplication and adaptation to changing environmental conditions, inhabiting diverse ecological niches.

Bacterial infections of archaeological bone materials represent a major challenge for archaeologists and heritage conservation specialists due to the complexity of bacterial behavior and the structure of bones. Given the historical and scientific importance of bone remains, it is crucial to preserve and treat them using materials that are compatible with their properties and composition. Therefore, research is needed to identify suitable materials for the conservation of archaeological bones. Conventional conservation materials may lose some of their properties over time, potentially causing adverse effects on bone artifacts, highlighting the need for alternative substances that are sustainable and stable.

This study aims to explore materials with improved properties for the treatment and protection of archaeological bones, focusing on the potential use of essential oils as an effective solution for safeguarding cultural heritage.

In this context, a collection of bone samples from Oued El-Jebana, Bir El-Ater, Tebessa was analyzed to identify the bacterial strains present, followed by an evaluation of the efficacy of a set of essential oils against these bacterial strains.

1. Definition of Essential Oils:

Essential oils are among the most important secondary metabolites naturally produced by aromatic plants. They are aromatic, volatile compounds (Dubaie AS E. A., 2005, p. 53), present in very small amounts compared to the total weight of the plant, in the form of tiny droplets located in specialized structures and in various plant organs (fruits, leaves, flowers, roots, peels, stems, etc.). They have the ability to evaporate and volatilize under normal conditions and are responsible for the characteristic and fragrant aroma released when parts of the plant are removed (Vigan, 2010). The proportion of essential oils varies according to the plant species and the extraction method, ranging from relatively high (16–18%) to very low (0.02%) (Dubaie AS, 2005, p. 53). Essential oils can be easily separated from the plant parts containing them through hydrodistillation and other extraction methods. They differ significantly from fixed oils or vegetable fats in terms of chemical composition and physical properties (Bakali, 2008).

2. Efficacy of Essential Oils Against Bacterial Activity:

Recent studies have shown that essential oils possess significant therapeutic and antiseptic properties (Kaloustian (J), 2004). Most essential oils containing terpenes exhibit strong antibacterial activity (Rubin, 2004, p. 28), which is largely attributed to their chemical composition (Bouaoun (D), 2007).

_ Dorman et al. (2000) and Amarti (2008) investigated the effects of a wide range of pure essential oil compounds (after isolation) on 25 bacterial strains. The study revealed that thymol exhibited the highest antibacterial activity, followed by carvacrol and α -terpineol. Cosentino et al. (1999) also reported that phenolic compounds possess strong antibacterial effects, causing degradation and disruption of the bacterial cell wall. This leads to increased membrane permeability to protons and potassium ions, reduction of intracellular ATP levels, and damage to cellular proteins. (Amarti (F), 2008)

_ Erturk (2006) examined the activity of 11 essential oils against five bacterial strains using the agar dilution method. The results showed varying antibacterial effects, but each essential oil affected at least one bacterial strain (Erturk, 2006).

_ In 2013, Smaili T. and Zellagui A. studied the efficacy of essential oils and phenolic compounds from the Apiaceae family. Their biological activity was tested against three bacterial strains using the diffusion method in nutrient agar

and measuring the diameters of inhibition zones. The study showed that essential oils may be effective against some bacterial strains while being less effective against others. Notably, *D. sahariensis* Murb oil exhibited antibacterial activity against *Klebsiella pneumoniae* strain 22 and, to a lesser extent, against *E. coli* ATCC 25922 (Ismaili, 2014)

3. Materials and Methods :

3.1. Historical Overview of Oued El-Jebana Site:

The bone samples used in this study were collected from the archaeological excavation of Oued El-Jebana site, Bir el-Ater, Tébessa. The samples consist of small fragments of varying sizes—short, flat, and irregular—carefully extracted to ensure their preservation and stored in special bags.

Oued El-Jebana is the first archaeological site where the Aterian industry was discovered in a stratified context, making it one of the most important sites in Africa. It was discovered in 1917 following the erosion of an archaeological layer rich in stone tools, charcoal, and animal bones. The site has been extensively studied by researchers including Rygasse, Balout, Tixier, and especially Jean Morel, whose most recent studies estimated the probable age of Oued El-Jebana using radiometric dating (Guelmaoui, 2003)



3.2. Bacterial Identification:

_ Sampling Process

To isolate different bacterial species from bone samples, a solution known as the mother solution was prepared using Brain Heart Infusion (BHI) broth. Small fragments of bone samples were placed into the solution with a measured volume; for instance, 9 mL of BHI broth was used for each bone fragment. The mixture was thoroughly homogenized to ensure an even distribution of bacteria.

This solution was subsequently used to perform a series of serial dilutions ranging from 10^{-1} to 10^{-4} , allowing the isolation of individual bacterial colonies and preparation for further analyses.

3.3. Antibacterial Activity Testing of Selected Essential Oils:

In this study, four types of essential oils were tested against bacterial strains isolated from archaeological bone samples: eucalyptus (E), lavender (L), geranium (G), and oregano (O).

The antibacterial activity of these essential oils was evaluated using both the direct contact method and the disc diffusion technique on solid media. This technique, commonly used for antibiotic susceptibility testing, involves placing discs impregnated with the active substance—here replaced with essential oils—onto agar media previously

inoculated with bacterial cultures. The antibacterial activity was assessed by measuring the diameter of the inhibition zones surrounding the discs. The presence of a clear inhibition zone indicates antibacterial effectiveness, whereas the absence or minimal size of the zone indicates weak or no activity.

_ Tested Bacterial Isolates:

Mueller-Hinton agar was used as the culture medium. A volume of 20 mL of the medium was poured into sterile Petri dishes with a diameter of 9 cm and a depth of approximately 4 mm, then allowed to solidify before inoculation.

_ Preparation of the Bacterial Suspension:

The bacterial suspension was prepared from a fresh bacterial culture aged between 18 and 24 hours in sterile physiological saline solution. The turbidity was adjusted to a density equivalent to 0.5 McFarland standard using a densitometer, corresponding to approximately 1×10^8 CFU/mL. The suspension was mixed thoroughly to ensure uniform distribution and was used within 15 minutes of preparation to prevent excessive bacterial growth.

_ Inoculation of Agar Plates:

A sterile cotton swab was dipped into the bacterial suspension and evenly streaked over the entire surface of the agar plate in overlapping lines. This procedure was repeated three times, rotating the plate by 60° each time to ensure uniform inoculation. All steps were performed under sterile conditions to ensure accurate and reliable results (Jiménez-Esquilin, 2005).

_ Impregnation of Discs with Essential Oils:

A very small amount of each essential oil was applied to sterile discs placed on the inoculated agar plates. The plates were then incubated at 37 °C for 18-24 hours.

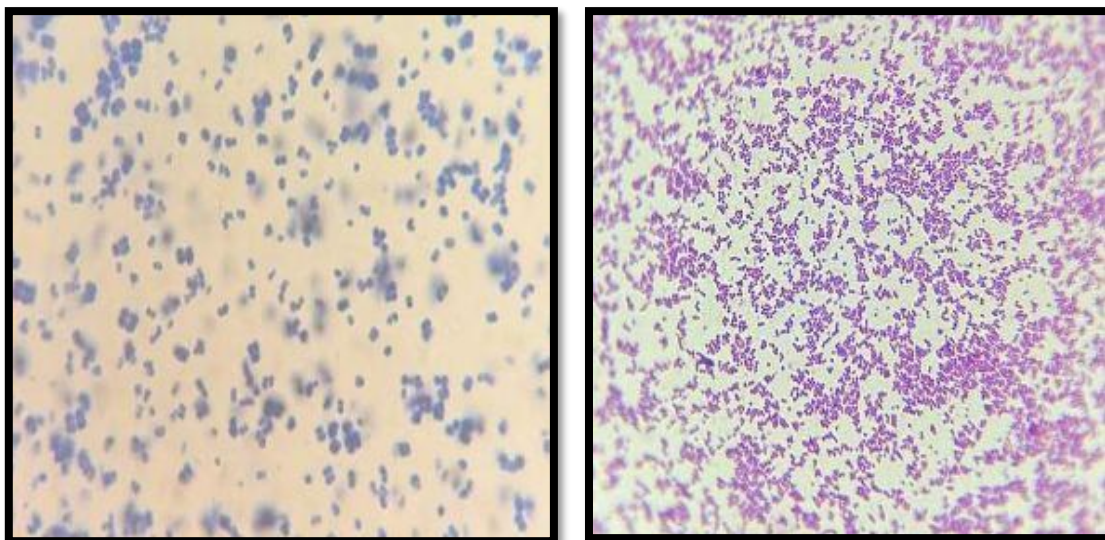
_ Measurement of Inhibition Zones and Interpretation of Results:

The antibacterial effectiveness of the essential oils was evaluated by measuring the diameters of the inhibition zones surrounding the discs. A large inhibition zone indicates strong antibacterial activity, whereas a very small or absent inhibition zone indicates weak or no antibacterial effect.

4. Results:

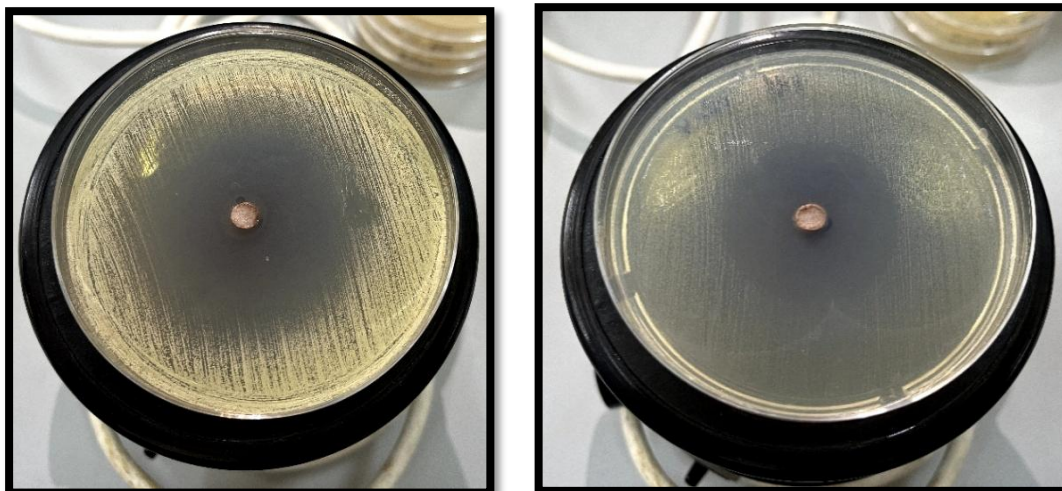
The isolation of bacteria from the different bone samples revealed the presence of 20 bacterial strains belonging to various genera. Based on morphological, physiological, and biochemical analyses, the isolates were classified as follows:

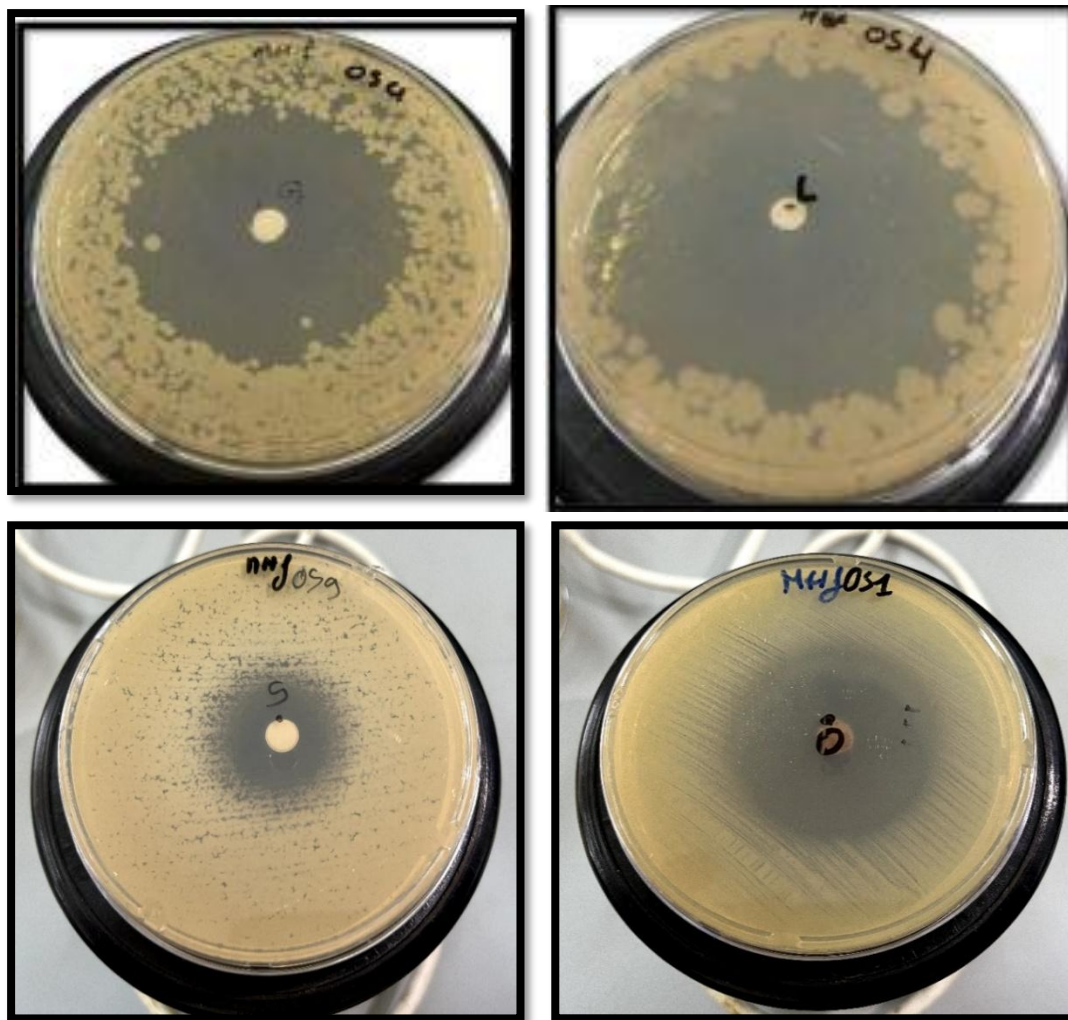
- _ 6 strains of *Bacillus* spp.
- _ 4 strains of *Staphylococcus* spp.
- _ 3 strains of *Enterococcus* spp.
- _ 3 strains of *Micrococcus* spp.
- _ 3 strains of *Planococcus* spp.
- _ 1 strain of *Serratia* sp.



Microscopic images of some isolated strains under a light microscope.

The results demonstrated that the essential oils exhibited varying degrees of antibacterial activity, ranging from moderate to strong, against the tested bacterial strains. Oregano essential oil showed the highest antibacterial activity, with inhibition zone diameters ranging from 30 to 60 mm. Lavender oil also exhibited strong antibacterial effects against certain strains, with inhibition zones reaching up to 60 mm. Eucalyptus oil demonstrated high antibacterial activity against some strains, with inhibition zones up to 40 mm, while showing lower effectiveness against others. Geranium oil also produced positive inhibitory effects. The following images illustrate the antibacterial activity of essential oils against bacterial strains isolated from archaeological bone samples





5. Discussion:

The isolation of bacteria from archaeological bone samples revealed the presence of diverse bacterial species. Although the samples originated from the same archaeological site, a significant diversity of bacterial strains was observed. This diversity reflects historical environmental and health variations over time. Such findings are important as they provide insights into bacterial diversity during past historical periods and contribute to a better understanding of the environmental and health conditions that prevailed during those times.

Moreover, these studies offer valuable information on how environmental conditions influenced human health and community dynamics in the past, thereby contributing to a deeper understanding of human and environmental evolution. They also enhance knowledge regarding the different environmental conditions experienced by the studied region.

The antibacterial activity results indicated that all tested essential oils exhibited inhibitory effects against bacterial growth.

The variation in antibacterial activity among the essential oils can be attributed to the structural and physiological differences between Gram-positive and Gram-negative bacteria. Gram-negative bacteria, such as *Serratia* sp., possess a cell wall with low permeability, which limits the penetration of essential oils into the cell, resulting in weak or absent inhibition zones. In contrast, Gram-positive bacteria, such as *Bacillus* spp. and *Staphylococcus* spp., have higher membrane permeability, facilitating the entry of essential oils into the cell and leading to greater sensitivity and larger inhibition zones.

Furthermore, previous studies have demonstrated that the chemical composition of essential oils plays a crucial role in their antibacterial activity, particularly oils containing phenolic compounds such as thymol and ketonic compounds such as carvone, which exhibit strong antibacterial properties.

Conclusion:

These findings contribute to enhancing the understanding of the role of essential oils in the preservation of cultural heritage. The study emphasizes the importance of integrating knowledge from biological, chemical, and physical sciences with the humanities—particularly in the field of conservation and restoration—to develop innovative and effective strategies for protecting and preserving archaeological and cultural heritage.

Although this study represents an initial step, it opens new perspectives in the field of conservation and restoration and encourages further research to expand understanding of the use of essential oils as effective tools for safeguarding cultural heritage and ensuring its sustainability for future generations.

Ethical Considerations

This study was conducted in accordance with internationally accepted ethical standards for archaeological and microbiological research. All archaeological bone samples analyzed were collected with official authorization from the relevant cultural heritage and archaeological authorities in Algeria. The sampling process was designed to be minimally invasive, ensuring the preservation and integrity of the archaeological materials. No human or animal subjects were involved in this research. All laboratory procedures involving bacterial isolation and testing were carried out following standard biosafety and microbiological ethics protocols to prevent contamination and ensure researcher safety.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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