Evaluation of the use *Citrullus colocynthis* against common isolated Fungi from leather bookbinding from the Mamluk's period

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Abstract:

This paper aims to evaluate the use of *Citrullus colocynthis* against common fungi isolated from leather bookbinding from the Mamluk's period. Analytical techniques such as scanning electron microscopy with EDX (SEM.EDX), optical microscopy, pH measurement, Fourier transform infrared spectroscopy FTIR and mechanical properties were used to evaluate the fungal effect on leather binding and to assess the effectiveness of colocynth fruit in treating leather bindings from fungal influence. Microbiological examination showed that *Aspergillus niger* and *Penicillium* are the most common microbes that infect antique leather bindings. *Citrullus colocynthis* has been used to treat leather bindings from fungal influence. The study proved that *Citrullus colocynthis* was effective in inhibiting fungi and treating historical leather bookbinding.

الملخص:

يهدف هذا البحث إلى تقييم استخدام نبات الحنظل ضد الفطريات الشائعة المعزولة من غلاف جلدي من العصر المملوكي. استُخدمت تقنيات تحليلية، مثل المجهر الإلكتروني الماسح باستخدام (SEM.EDX– EDX)، والمجهر الضوئي، وقياس الرقم الهيدروجيني، ومطيافية الأشعة تحت الحمراء بتقنية تحويل فورييه (FTIR)، والخصائص الميكانيكية، لتقييم تأثير الفطريات على الأغلفة الجلدية الأثرية، ولتقييم فعالية ثمرة الحنظل في حماية الأغلفة الجلدية الأثرية من التأثيرات الفطرية. أظهر الفحص الميكروبيولوجي أن فطر الأسبراجلس نيجر، فطر البنسليوم هما أكثر الميكروبات شيوعًا التي تُصيب الأغلفة الجلدية الأثرية. وقد استُخدم نبات الحنظل لعلاج الأغلفة الجلدية الأثرية من التأثيرات الفطرية. أثبتت الدراسة أن نبات الحنظل فعال في تثبيط الفطريات وعلاج الأغلفة الجلدية الأثرية.

Keywords

Aspergillus niger, Citrullus colocynthis, leather, Manuscript from the Mamluk's period, Penicillium.

الكلمات الدالة:

جلد ، فطر الأسبراجلس نيجر ، فطر البنسليوم ، مخطوطة من العصر المملوكي، نبات الحنظل.

1. Introduction

Historical Arabic manuscripts with leather bookbindings are one of the most important national treasures in the holdings of Arab libraries (1). The historical manuscript is composed of carbohydrate materials (cellulose) represented in the paper and protein materials represented in leather, which is a type of protein known as collagen. The chemical material

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composition of the manuscripts also consists of inks, which are mineral or organic chemical dyes [(2)]. There are different materials that have been used through the ages in the manufacturing of bookbinding. The most common material was leather. Goats and sheep's leather are the most common leathers used in bookbinding [(3)]. Leather is a protein primarily composed of collagen fibers and nonfibrous components [(4)]. The leather contains many nutrients for the growth of microorganisms [(5)]. Historical leather is an organic material and is very sensitive to unsuitable environmental conditions in museums and libraries, whether during storage or display [(6)]. Therefore, most manuscripts with leather bookbinding are exposed to many physical and chemical deterioration factors, which cause various physical and chemical changes that ultimately lead to a group of different manifestations of damage, such as loss of tensile strength and durability of the archaeological manuscript. The most common manifestations of leather bookbinding damage are hardness, loss of flexibility, yellowing, and fragility [(7)] [(8)]. The most dominant species of fungi found on the leather bookbinding are *Cladosporium, Cladosporioides, Aspergillus tamarii, Eurotium chevalieri, Aspergillus fumigatus, Wallemia sebi and Fusarium poae* [(9)]. Different fungal strains such as *Alternaria alternata, Aspergillus oryzae, A. niger, A. versicolor, A. wentii, Chaetomium globosum, Cladosporium sphaerospermum, Cladosporium herbarum, Fusarium chlamydosporum, Paecilomyces variotii, Penicillium commune, P. glabrum, P. funiculosum, P. verrucosum, P. ochrochloron, P. rubrum, Trichoderma viride, and Verticillium tenerum* are effective in deteriorating and distorting leather as the fungal hyphae adhere to the leather surface causing weakness, loss of structural integrity, reduction of tensile strength, increase of leather stiffness and shrinkage due to removal of oils and grease from the leather [(10)]. Acids produced by fungi are considered a major factor in the destruction of historical manuscripts with leather bookbindings[(11)]. Therefore, some aspects of deterioration can be found in historical manuscripts with leather bookbindings such as wear, shrinkage, cracks, distortion, microbial stains, discoloration, corrosion, insect holes, dust and dirt accumulation [(12)].

One of the most important factors that conservators must consider when determining the conditions for preserving paper manuscripts and leather bookbinding in libraries and archives is the study of fungi and their efficiency in producing enzymes that decompose the components of historical leather [(13)(14)]. Fungi play an important role in the production of proteases, and have the ability to degrade a wide range of proteins, especially collagen, the main component of leather [(15)(16)].

Therefore, many studies have addressed the use of natural materials in sterilizing and treating manuscripts with leather bookbinding from the effects of biological or microbiological damage.

Hence, the importance of this study in applying and evaluating the use of *Citrullus colocynthis* extract in protecting historical leather bookbinding from microbiological damage. The effectiveness of *Citrullus colocynthis* has been proven as a natural color additive to paper used in the restoration and completion of historical manuscripts or as a sterilizing material to protect manuscripts from biological and microbiological damage. The reason for the high ability of *Citrullus colocynthis* extract to inhibit fungi is due to the alkaline chemical nature of the materials extracted from the *Citrullus colocynthis* and the direct interaction of alkaline materials with microorganisms and the destruction of their plasma membrane and the fats and proteins it contains [(17)]. The *Citrullus colocynthis* plants were highly adaptable to unfavorable temperatures by regulating the plants' thermal homeostatic ability through their photosynthetic and biochemical processes [(18)].

Citrullus colocynthis gave satisfactory results in inhibiting *Fusarium oxysporum f.* sp. by 27.02% [(19)]. It also gave satisfactory results in inhibiting *Aspergillus fumigatus* and *Aspergillus niger* [(20)].

Therefore, this research aims to evaluate the use of *Citrullus colocynthis* in treating historical leather bookbindings from fungal damage through analytical techniques.

2. Materials and Methods.

2.1. Materials.

2.1.1 . The historical leather bookbinding studied.

The leather bookbinding of this study is a leather bookbinding of historical manuscript on Sufism by Sheikh Abdel Rahim Al-Qanawi, preserved at the Manuscripts and Papyrus Center at Minya University. The manuscript narrates the Sufi virtues and miracles of Sheikh Abdel Rahim Al-Qanawi. This manuscript was written on Sunday, Shawwal 11, 1314 AH, and dates back to the Mamluk era. The leather bookbinding is made of goat leather, and its dimensions are approximately (21 cm long, 16 cm wide and 1 cm thick). All damage to the leather bookbinding was documented with a digital camera (Samsung 16.1 megapixel camera, lens diameter 4.7-23.5 mm).

The photos [Figure 1 (A, B, C, D, E, F, G, H, I] showed that the historical leather bookbinding had many signs of deterioration, represented by erosion and loss in different areas of the leather bookbinding from all sides, with hardening and cracking. This is because the leather bookbinding lost the fatty content responsible for its flexibility, which helps in bending and straightening the leather bookbinding.

This could be due to internal damage factors represented by harmful inks and chemicals (alum, chlorine, and rosin), in addition to the loss of the color layer and a change in the color of the leather surface in many places to a darker color compared to the original color of the leather, with its fragility, especially at the upper and lower ends and the heel area. These signs of damage could be the result of a change and deterioration of the composition of the leather, in addition to its sensitivity to environmental conditions represented by air pollutants, light, humidity, and temperature. The deterioration of the leather may eventually lead to the disintegration and damage of the manuscript, according to (Vichi *et al.,* 2018)[(21)]. In addition, there are white spots over the color Darkening of the leather spreading over the lower and upper parts of the outer leather surface may be the result of microbial damage according to (Saada *et al.,* 2021)[22].

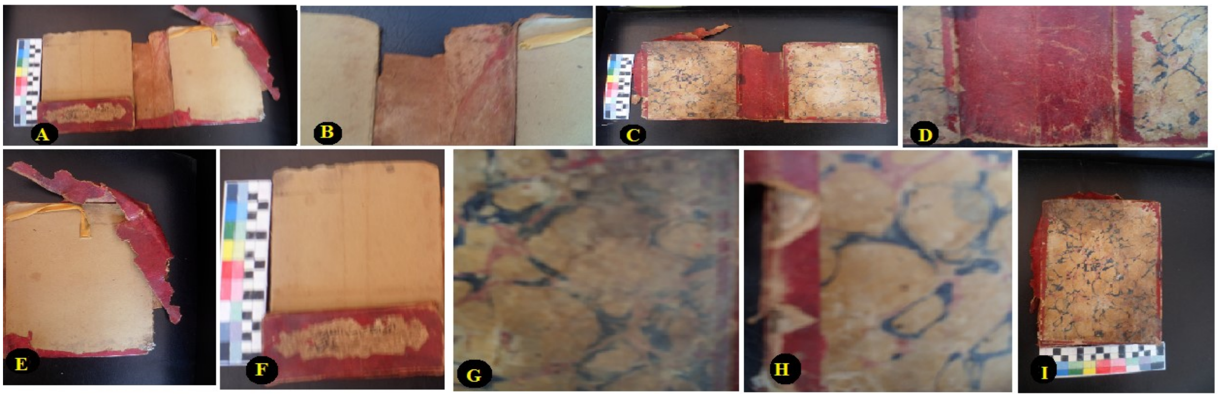


Figure (1) Manifestations of the deterioration of the leather bookbinding of the historical manuscript: (A) Erosion and loss in different areas of the lining of the front and back leather bookbinding, (B) Erosion of the upper part of the heel of the leather bookbinding, (C) Hardening, fragility and loss of the edges of the front and back leather bookbinding, (D) Loss of the colour layer in the lower part of the heel of the leather bookbinding, (E) Erosion and loss of the edges of the lining of the front leather bookbinding, (F) Loss of all edges of the back leather bookbinding, (G) Microbial spots resulting from microbial growth on the leather bookbinding, (H) Erosion and loss of the edges of the leather bookbinding, (I) Dirt and black spots on the surface of the leather bookbinding.

2.1.2. New Vegetable-Tanned Leather Samples (experimental samples):

Vegetable-tanned (mimosa) goat leather samples were used as an experimental sample because it was used in the leather bookbinding of the studied manuscript and prepared according to (Abdel-Maksoud, 2006)[(23)]. Thermal aging was performed by placing the prepared leather samples in an oven at 100°C for two weeks to accurately simulate the actual historical deterioration. This was done as reported by (Bansa 2002)[(24)]. Accelerated aging induces changes in the thermal stability of collagen, increases heterogeneity, alters its structural composition, and causes loss of its fibrous structure [(25)]. This allows us to produce modern leather samples that are similar to those of ancient leather in terms of aging within a short period of time [Figure 2(A, B)].

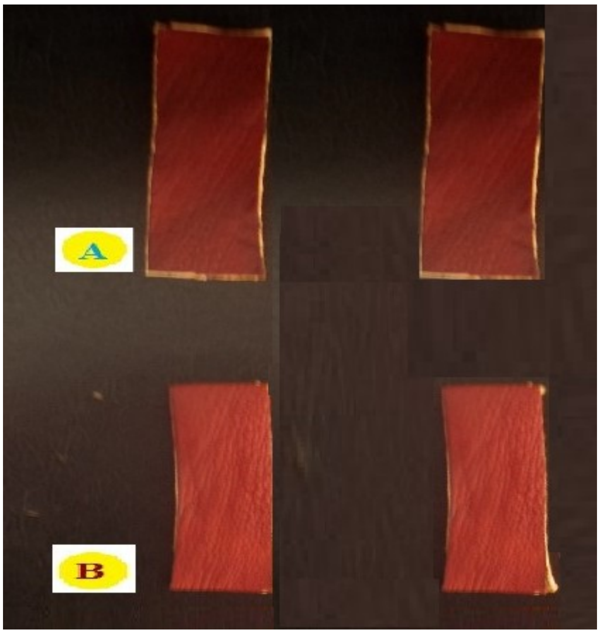


Figure (2) shows experimental leather samples: (A) before aged, (B) thermally aged leather samples.

2.1.3. *Citrullus colocynthis.*

The *Citrullus colocynthis* has many common names including Abu Jahl watermelon (native name in Türkiye), *Citrullus colocynthis*, Bitter apple, bitter cucumber, vine of Sodom, or wild gourd, is a poisonous desert vine native to the Mediterranean basin and western Asia, particularly the Levant, Turkey (especially in areas such as Izmir), and Nubia [26]. (Marouf, 2023) [27] pointed out that the *Citrullus colocynthis* contains resinous materials, alkaline materials, saponins, pectin, organic acids (butyric acid), and two substances, namely Colocyntine and Colocynthine, which are a mixture of alkaline materials, glucosides, and an alcoholic substance known as Citrollol.

2.2. Methods.

2.2.1. Measurement of pH Value of the Leather Samples.

The pH values of two measurements for new and historical leather bookbinding samples were measured according to (Abdel-Maksoud, 2011b)[28] with some modifications. Mechanically, a sample (0.025 g) of the leather taken from the backbone of the leather bookbinding was removed from as close to the damaged location as possible in the form of loose fibers. To allow the ions to transfer into the solution, the leather samples were soaked in deionized water for about 6 h, three for each sample. The pH value was determined using Adwa brand pH meter, at 21-22°C and the measurement was achieved at the Faculty of Fine Arts, Restoration Department, Minia University.

2.2.2. Isolation and identification of fungi on experimental leather samples.

- **Isolation of fungi.**

Fungal isolation was carried out using an indirect method by taking sterile swabs from the surface of the historical leather bookbinding.

- **Swab culture:** Media were prepared.
- **Czapek-Dox medium.**

It includes the following ingredients (sodium nitrate 2 g, potassium dihydrogen phosphate 1 g, potassium chloride 0.5 g, magnesium sulfate 0.5 g, sucrose 20 g) .These ingredients are dissolved in 1 liter of distilled water. This culture medium was sterilized after pouring it into Petri dishes at a temperature of 121°C (5 atmospheres pressure), and left to solidify. After that, fungal swabs were planted in the dishes and incubated at a temperature of (28-30°C) for a period ranging from 3-21 days [(29)].

- **Isolation and Purification**.

In this step, the growths that appeared in the previous plates after the incubation period were taken separately and cultured in the same media and incubated under the same conditions. This process was repeated until the organism was obtained in a pure form, enabling us to carry out the identification steps.

- **Fungal Identification.**

Pure fungal growths were identified through microscopic examination to determine the morphological characteristics of each organism by comparing them with standard morphological characteristics. The examination showed that the isolated fungal genus was *Aspergillus niger, Penicillium* fungus [Figure 3 (A, B, C, D)].

- **Extracellular Enzyme Activities.**

Each purified fungal strain was inoculated into mineral salt agar media (MSA) (containing g L–1: KCl, 5; $NaNO_3$, 6; $MgSO_4.7H_2O$, 0.5; KH_2PO_4, 1.5; $ZnSO_4$, 0.01; $FeSO_4$, 0.01; agar, 15, Dis. H_2O, 1 L) supplemented with a specific substrate. The activities of amylase, cellulase, pectinase, and gelatinase enzymes were estimated by inoculating the fungal strain on the MSA media supplemented with 1% soluble starch, carboxymethyl cellulose, pectin, and gelatin, respectively, and incubated for 96 h at 25 ± 2 °C.
The results were recorded as a diameter of a clear zone (mm) which is calculated by subtracting the diameter of fungal growth from the diameter of all clear zones.
The results were recorded after flooding the inoculated plates with an iodine solution to examine the activity of amylase, cellulase, and pectinase, whereas the acidic mercuric chloride was used to examine the gelatinase activity[(30)]. The experiment was performed in triplicate.
Hydrolytic Enzyme Activities reported that *Aspergillus niger* and *Penicillium* isolated Fungi from historical leather bookbinding have the potential to produce cellulase, amylase, gelatinase, and pectinase to various degrees.

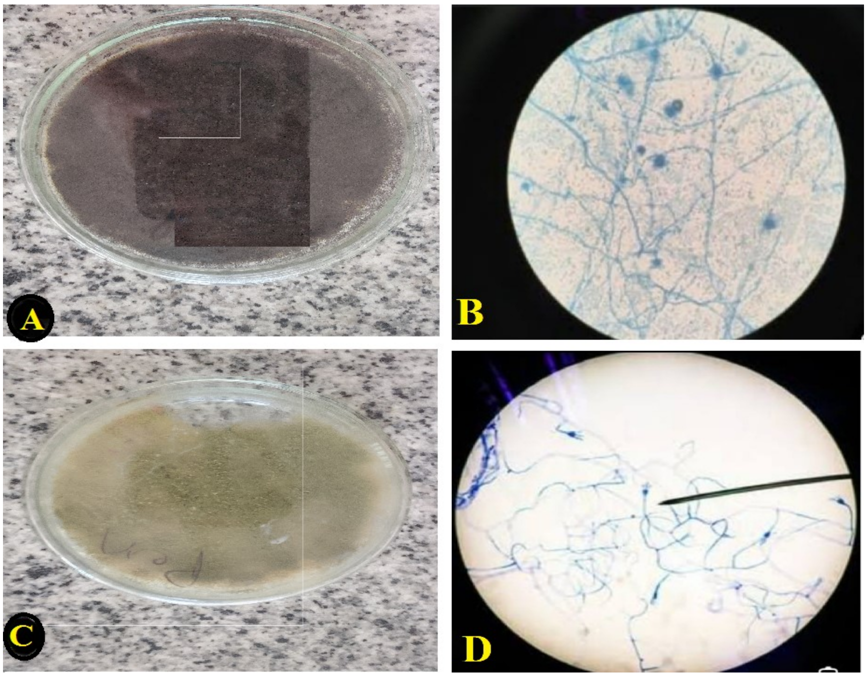


Figure (3) shows the fungi isolated from the surface of the historical leather bookbinding.: (A) *Aspergillus niger* fungus in a petri dish, (B) *Aspergillus niger* fungus under a light microscope, (C) *Penicillium* fungus in a petri dish, (D) *Penicillium* fungus under a light microscope.

Fungal inoculation on experimental leather samples.

The solid fungal growth medium Czapek (dox) ager (Sodium Nitrate $NaNO_3$ (2 g), Potassium dihydrogen phosphate KH_2PO_4 (1 g), Magnesium Sulphate $MgSO_4.7H_2O$ (0.5 g), Potassium Chloride KCl (0.5 g), Ager agar (20 g).

(Atlas, 2005)[31] prepared that without adding the carbon source, which is sucrose, so that the leather becomes an alternative carbon source for the fungus and then begins to grow on it. The pH value of the medium was pH 7.3 ± 2.0. After completing the preparation of this medium and pouring it into Petri dishes with a diameter of 14 cm, the experimental leather samples were placed inside the dishes in which the growth medium was poured according to what was mentioned by (Abdel-Maksoud, 2002)[32]. The dishes were placed after being tightly closed with plastic Parafilm rubber was placed inside the incubator at 28°C for two weeks, where newly prepared samples were placed in each dish, in order to study the properties used in this study as shown in [Figure 4,5].

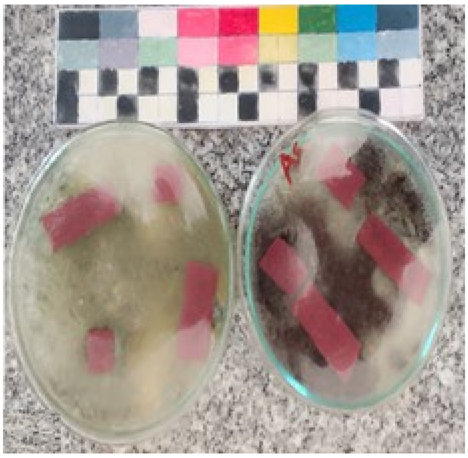


Figure (4) Experimental leather samples inside the dishes infected with fungi.

Figure (5) Experimental leather samples after fungal growth.

2.2.3. Preparation of *Citrullus colocynthis* Extract.

Weigh 200 g of dried *Citrullus colocynthis* powder into a 1000 ml glass beaker. Add 200 ml of ethyl alcohol to the powder for 48 hours, shaking occasionally. Filter the extract using Whatman No. 4 filter paper and vacuum. Concentrate the extract using a Rotary Vacuum Evaporator at 45°C [33][Figure 6 (A, B, C, D)].

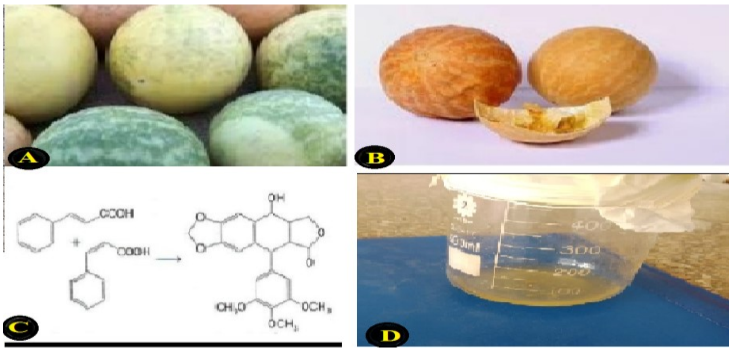


Figure (6) *Citrullus colocynthis*: (A) Green *Citrullus colocynthis*, (B) Dried *Citrullus colocynthis*, (C) Chemical composition of *Citrullus colocynthis*, (D) *Citrullus colocynthis* extract.

A- Evaluation of *Citrullus colocynthis* extract as an antifungal.

A clear zones experiment was conducted to evaluate the effect of *Citrullus colocynthis* extract on inhibiting the growth of fungi isolated from the historical leather bookbinding. This was done by measuring the diameter of the clear zones, as described by (Wafaa et *al*.,2019)[(34)], according to the following steps :

1. A crosslinking medium (Czapek's medium) was prepared.

2. The media was poured into dishes to a thickness of approximately 20 mm and left to solidify.

3. Four discs (each 1 cm in diameter) were emptied into each Petri dish using Cork Poral..

4. A spore suspension was prepared, consisting of 5 mm of distilled water placed inside the test tube containing the fungus.

5. 0.5 mm of the spore suspension was placed in each Petri dish, with more than one replicate for each fungal species. The spore suspension was distributed using a sterile inoculating needle.

6. Two concentrations of *Citrullus colocynthis* extract (50%-100%) were prepared.

7. Each concentration of *Citrullus colocynthis* extract was placed in each disc circle of a Petri dish using a micropipette.

8. It was found that a 100% concentration of *Citrullus colocynthis* extract had an antifungal effect.

9. *Citrullus colocynthis* extract was placed in each circle (disk) of the Petri dish using a micropipette.

10. The dishes were placed in an incubator for 7 days at 28°C.

11. The diameter of the clear zone resulting from the *Citrullus colocynthis* extract was measured for each fungus using a ruler and the average of its replicates was taken to evaluate the efficiency of *Citrullus colocynthis* in inhibiting the fungi isolated from the historical leather bookbinding (*Aspergillus niger, Penicillium* fungus). *Citrullus colocynthis* was found to be successful in inhibiting *Aspergillus niger, Penicillium* fungus, as shown in [Figure 7 (A, B)].

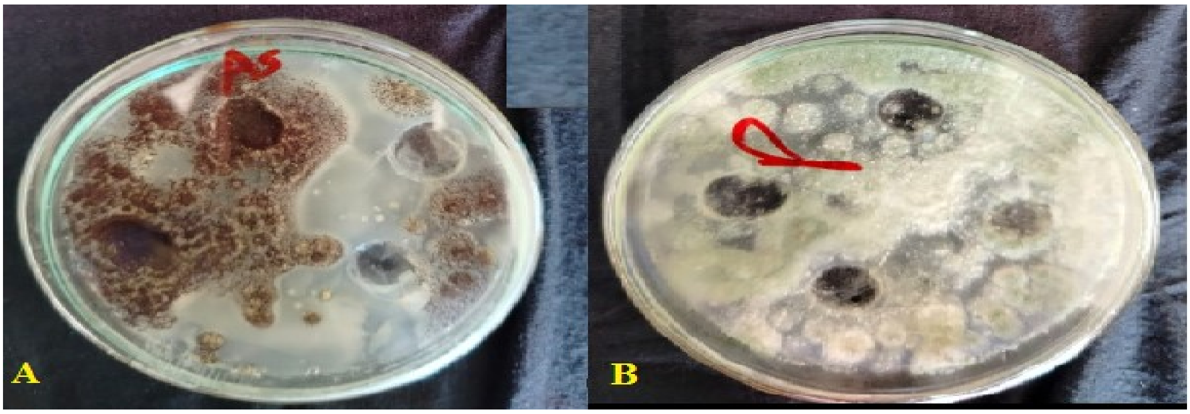


Figure 7 : Shows the clarizone experiment to inhibit the growth of fungi by extract of *Citrullus colocynthis*:(A) *Aspergillus niger, (B) Penicillium* in a petri dish.

Based on the previous results, experimental leather samples inoculated with *Aspergillus niger and Penicillium* fungi were treated with 100% *Citrullus colocynthis* extract.

B- Applying *Citrullus colocynthis* extract to the experimental leather samples.

The treatment with *Citrullus colocynthis* extract was applied using compresses and local cleaning. A cotton compress wrapped in a piece of cloth soaked in *Citrullus colocynthis* extract was applied. Local cleaning was performed by wrapping a piece of cotton around a cotton ball and dipping it in *Citrullus colocynthis* extract. The affected area was then painted with the fungi spots formed on the experimental leather samples. The application method for cleaning the spots was maintained to ensure no discrepancies in the spot areas occurred, according to (Nadia *et al.,* 2016)(35), No chemical residues of the *Citrullus colocynthis* extract were observed on the treated leather after use, and it did not affect the long-term preservation of the leather. The high fungal inhibition capacity of the *Citrullus colocynthis* extract is due to the alkaline chemical nature of the *Citrullus colocynthis* and the direct interaction with alkaline materials. This inhibition result is largely consistent with the result obtained by (Khudair, 2010)(36) in a previous study using *Citrullus colocynthis* extract at different concentrations to inhibit the growth of the fungus *Alternaria alternata* as shown in [Figure 8 (A,B)].

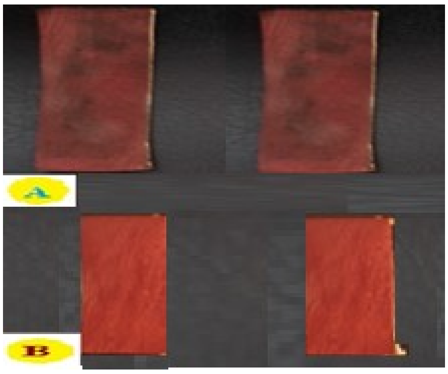


Figure (8) shows the experimental leather samples A: before treatment, B: After treatment.

2.2.4 Scanning Electron Microscopy with Energy-Dispersive X-ray Analysis (SEM - EDX).

To investigate the surface morphology of the historical leather bookbinding, changes in the surface morphology of experimental leather samples (modern, standard, and heat-aged, as well as those inoculated with fungi (*Aspergillus niger and Penicillium*)), and samples treated with colocynth fruit, were analyzed. 10 mm leather samples were placed on aluminum stubs. These samples were then coated with gold using a Fine Coating Sputter (JFC-11000) to create a thin gold-carbon film. These samples were examined using a JSM-IT200 scanning electron microscope at the Microanalysis Center at Minya University.

2.2.5. ATR-FTIR Analysis.

ATR-FTIR analysis was used to study the chemical changes that occur in experimental leather samples due to heat aging, the effects of fungi (*Aspergillus niger and Penicillium),* and the extent of improvement after treatment with *Citrullus colocynthis*. This was done by assessing the differences in the density values of structural functional groups. FTIR spectra of leather samples (new vegetable-tanned leather, thermally aged vegetable tanned leather and infected with fungi (*Aspergillus niger and Penicillium*)) and *Citrullus colocynthis* treated samples were analyzed using an IRXross device in the range of 400-4000 cm^{-1} and at a resolution of 4 cm-1. The analysis was conducted at the Faculty of Science, Department of Physics, Minia University.

2.2.6. **Tensile Strength and Elongation**.

The mechanical properties were measured to assess the effectiveness of *Citrullus colocynthis* in treating leather samples infected with fungi. To measure this property, leather samples were prepared and cut according to (Thomson, 1995[37]; Hanacziwski, *et al.,* 1991[38]). Measurements were carried out at the Metrology and Calibration Center in Cairo, using a Textiles Tensile - Properties of Fabrics ISO 13934 device using the strip method.

3. Results and Discussion

3.1. Measurement of pH Value.

The pH value of the new vegetable tanned leather sample was 4.3, while the pH value of the historical leather sample was 3.2. (Vyskočilová *et al.*, 2022)(39) reported that acid degradation of historical leather may be a result of the leather manufacturing process. Increased use of acid may accelerate acid degradation and cause rapid degradation of historical leather. Furthermore, the acidity of leather is due to fungi, as acids produced by fungi are a major factor in the destruction of historical manuscripts.

3.2. Scanning electron microscope with energy dispersive X-ray.

3.2.1. SEM investigation for the surface of historical leather bookbinding compared with the experimental samples.

The photos [Figure 9(A)] from the experimental sample show that the surface of goat leather was smoothed and glossed. (Jawahar *et al.,* 2016)(40), who found that goat leather is distinguished by trios configurations of large pores surrounded by small pores in a cluster, confirmed this finding. The historical sample results [Figure 9(B)] indicate that it was similar to goat leather through its distinctive grain surface pattern. However, it was seen that the surface was rough and damaged, which may have been caused by the impact of the surrounding environmental conditions' pollution and dust. It might also be the result of repeatedly handling the manuscript incorrectly. Some fine lines were also observed, which may be due to the effect of insects. Furthermore, the leather deterioration could be attributed to the colonization of fungi.

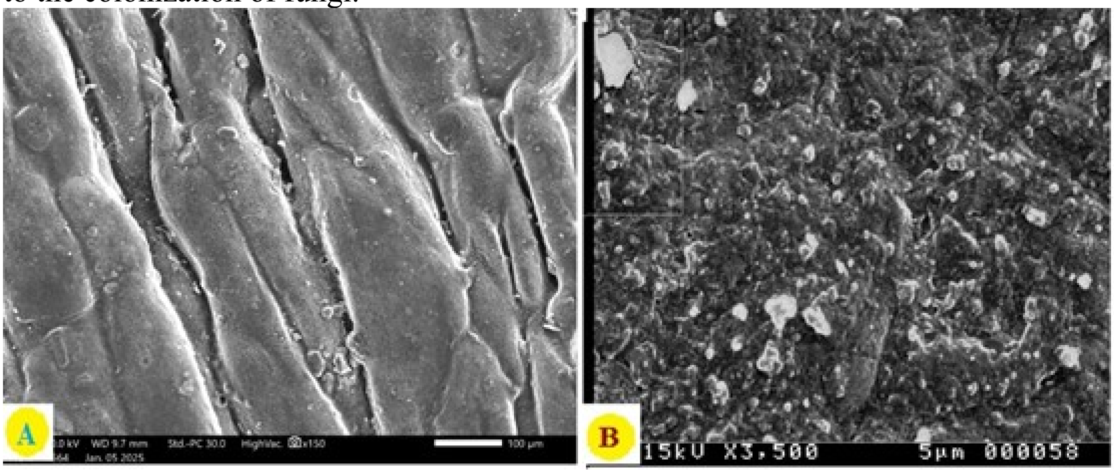


Figure (9) SEM analysis for the surface of historical leather bookbinding compared with the control showing the deterioration aspects: (A) SEM image of SEM image of new goat vegetable-tanned leather sample (experimental samples), (B) SEM image of historical vegetable tanned leather sample.

Results of EDX analysis results for historical leather bookbinding sample.

The data obtained from EDX analysis (Table 1) of the historical leather bookbinding sample showed the presence of the trace elements, N (8.88), C (31.84), O (51.70), Al (1.34), Hg

(0.02), Si (0.52), S (0.05), Ca (5.65), and Nitrogen content (N) (8.88) indicates that the leather used to make the historical leather-binding manuscript was goat leather. Ca content (5.65) indicates the presence of calcium carbonate. Both (Vichi *et al.,* 2018)[(41)] reported that calcium carbonate is commonly found in historical leather as a result of the reaction of calcium hydroxide residues from lime baths added during manufacturing with atmospheric carbon dioxide.

Table 1 EDX analysis results of the historical leather bookbinding sample.

Elements	Historical binding sample (Mass %).
C	31.84
N	8.88
O	51.70
Al	1.34
Hg	0.02
Si	0.52
S	0.05
Ca	5.65

3.2.2. SEM investigation for the surface of experimental leather samples.

Image results [Figure 10 (A)] of a new vegetable-tanned leather sample show that the surface of goat leather was smoothed and glossed. As for thermally aged vegetable tanned leather sample for two weeks [Figure 10 (B)], the surface granular layer became rough and there are some cracks as a result of the thermal aging. As for the experimental samples infected with *Aspergillus niger* [Figure 10 (C)], its surface appearance shows severe damage to the fibers, and the lack of awareness of the surface granular layer that is characteristic of goat leather, in addition to the presence of mycelium, conidiophores, and completely clear fungal spores, with erosion in the leather and loss in some its parts, and it can be said that the fungus played a very large role in destroying the leather fibers, leaving the leather behind in the form of severe damage.

The experimental samples infected with *Penicillium* [Figure 10(D)] showed the presence of fungal hyphae on the surface of the leather sample and the superficial layer of the leather was hidden due to the formation of fungal hyphae. The result of the samples treated with *Citrullus colocynthis* extract [Figure 10 (E)] showed the shape of the surface granular layer characteristic of goat leather, and cracks and signs of microbial biological deterioration disappeared compared to the aforementioned leather samples inoculated with fungi. We conclude from the results of scanning electron microscopy (SEM) examination of leather samples that *Citrullus colocynthis* is successful in eliminating microbial spoilage and no chemical residues of the *Citrullus colocynthis* extract were observed on the treated leather after use.

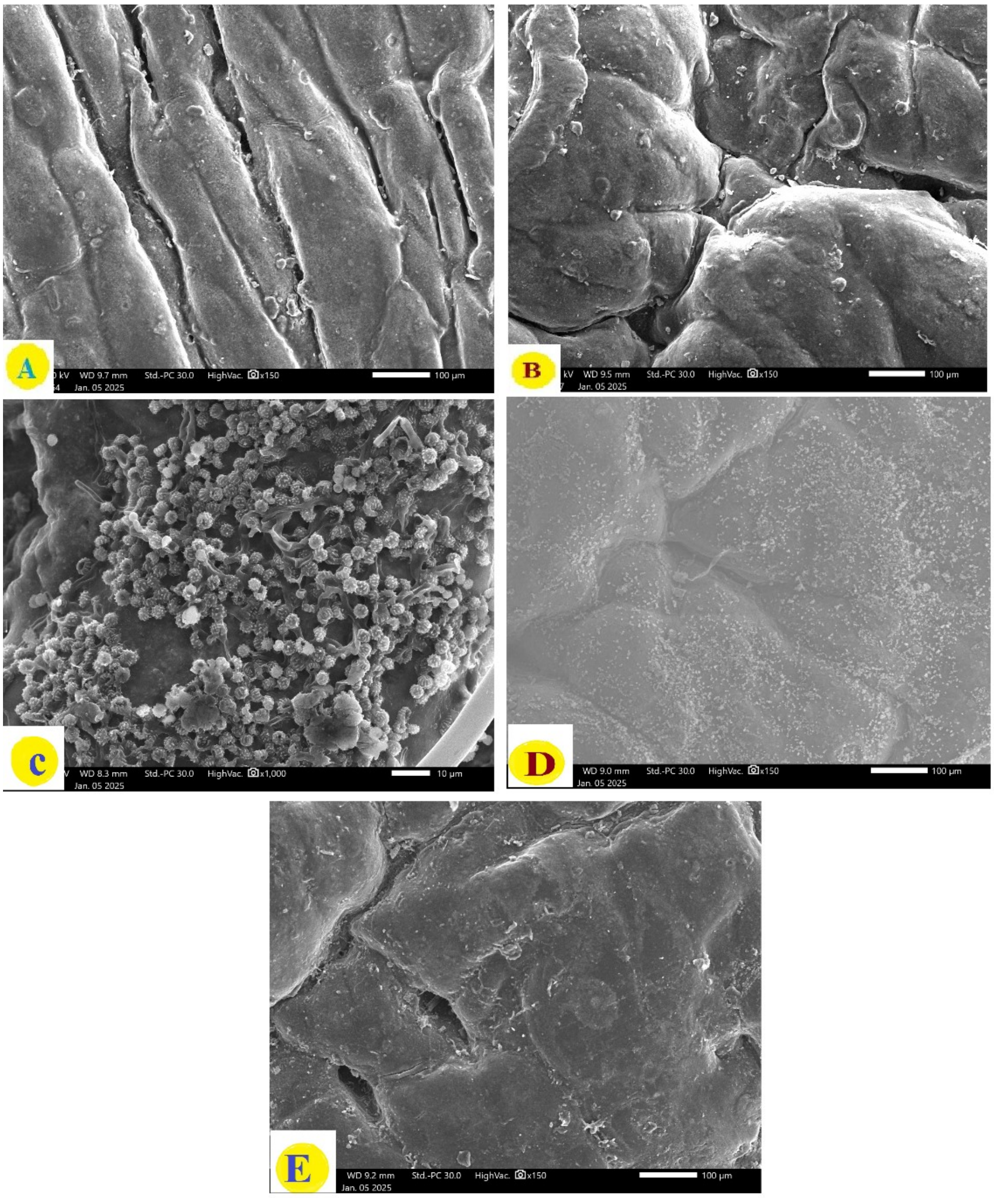


Figure (10) SEM analysis for the surface of experimental leather samples: (A) new vegetable-tanned leather sample, (B) thermally aged samples, (C) samples infected with *Aspergillus niger*, (D) samples infected with *Penicillium*, (E) samples treated with *Citrullus colocynthis* extract.

3.3. ATR-FTIR Analysis.

- **ATR/FTIR Analysis Leather Samples .**

ATR/FTIR analysis revealed an indicator of the chemical changes that occurred in the leather samples due to the effect of heat aging and the effect of fungi (*Aspergillus niger and Penicillium*), and the extent of improvement after treatment with *Citrullus colocynthis*, by assessing the difference in the density values of structural functional groups.

The results of ATR/FTIR analysis [Fig. 11(A)] showed that there were changes in the intensity and positions of the bands for the new vegetable-tanned leather and thermally aged vegetable tanned leather samples. The (OH) stretch reading was in the range of 3392.85 cm^{-1} - 3658.06 cm^{-1} with respective intensities of 41.5 - 21.5 respectively for the new vegetable-tanned leather and thermally aged vegetable tanned leather samples. C-H stretching (alkene group) appeared at the ranges 2929.92 cm^{-1} - 2929.92 cm^{-1}, respectively, with respective intensities of 40.5 - 24.5 for the new vegetable-tanned leather and thermally aged vegetable tanned leather samples. Another C-H stretching (alkene group) appeared at the range 2855.66 cm^{-1}, with respective intensities of 27.5 for the thermally aged vegetable tanned leather sample. Amide I stretching (carbonyl group C=O) appeared at the ranges 1664.60 cm^{-1} - 1649.17 cm^{-1}, with respective intensities of 38.5 - 21.5, respectively for the new vegetable-tanned leather and thermally aged vegetable tanned leather samples.

(Abdel-Maksoud, 2011b)[42] indicated that the spectra of collagen-based materials are characterized by the presence of amide I at the range 3294 cm^{-1} with respective intensities of 0.042 for new vegetable-tanned leather. The stretching of amide II (CN stretching and NH bending groups) appeared at the ranges 1650.01 cm^{-1} - 1634.58 cm^{-1} with respective intensities of 39.5 - 22.5, respectively, for the new vegetable-tanned leather and thermally aged vegetable tanned leather samples. (Carşote *et al.,* 2014)[43] reported that the amide I and amide II ranges are related to the stretching of the N-H peptide groups involved in interchain hydrogen bonding. The C-O stretching (alkyl aryl ether group) appeared at the ranges 1235.43-1205.53 cm^{-1} with respective intensities of 44.5-29.5, respectively, for the new vegetable-tanned leather and thermally aged vegetable tanned leather samples. The C-O (ester group) reading was at the range 1169.58 cm^{-1} with intensities of 45.5 for the new vegetable-tanned leather sample, and disappeared in the thermally aged vegetable tanned leather sample due to thermal aging.

The C-O (vinyl ether group) reading was at the ranges 1035.79 cm^{-1} - 1034.83 cm^{-1} with respective intensities of 43.5-31.5, respectively, for the new vegetable-tanned leather and thermally aged vegetable tanned leather samples.

The (C-Cl) expansion (halo compound) appeared in the range 825.55 – 800 cm^{-1} with respective intensities of 47.5 – 34.5 cm^{-1}, respectively, for the new vegetable-tanned leather and thermally aged vegetable tanned leather samples. The (C-Br) expansion (halo compound) appeared in the range 576.73 cm^{-1} with intensities of 29.5 cm^{-1} for the thermally aged vegetable tanned leather samples.

It was also observed that the absorption intensity of a standard new vegetable-tanned leather sample was higher than the absorption intensity of thermally aged vegetable tanned leather sample, as heat aging caused a change in the protein structure. This change is considered normal since the aged leather sample was heat-aged at 100°C for two weeks, which led to a change in the protein structure, according to (Abdel-Wahab *et al.,* 2018)[44].

The results of ATR/FTIR analysis [Figure 11(B)] showed changes in the intensity and locations of the bands for vegetable tanned leather samples infected with *Aspergillus niger* and *Penicillium* and treated with *Citrullus colocynthis*. The (N-H) stretching (aliphatic primary group) appeared at the bands 3409.24 – 3400.56 cm^{-1} with respective intensities of 19 – 3.5, respectively, for the vegetable tanned leather samples infected with *Aspergillus niger* and *Penicillium*. C-H stretching (alkene group) appeared at the ranges 2927.03 cm^{-1} - 2927.01 cm^{-1} with respective intensities of 33.5 - 23.5 respectively for the vegetable tanned leather samples infected with *Aspergillus niger* and *Penicillium,* The appearance of these bands in vegetable-tanned leather samples infected with *Aspergillus niger and Penicillium* fungi is due to the fact that the fungi *(Aspergillus niger and Penicillium* fungi*)* led to a change in the protein composition of the main component of the leather. We also note that the absorption intensity of the leather samples infected with the fungi is weaker than that of the thermal-aged samples, and this is normal, as the sample infected with *Aspergillus niger* and *Penicillium* fungi was first thermally aged [(45)].

The amide I stretching reading was at the ranges 1643.38 cm^{-1} - 1657.85 cm^{-1} with respective intensities of 19 - 17.5 respectively for the vegetable tanned leather samples infected with *Aspergillus niger* and *Penicillium*. The amide II group appeared 1628.79 cm^{-1} - 1629.42 cm^{-1} with respective intensities of 19.5 - 18, respectively, for the vegetable tanned leather samples infected with *Aspergillus niger* and *Penicillium* fungi.

The N-O stretching (nitro compound) reading was at the range 1546.94 cm^{-1} - 1543.08 cm^{-1} with respective intensities of 23.5 - 21.5, respectively, for the vegetable tanned leather samples infected with *Aspergillus niger* and *Penicillium*. The OH stretching (carboxylic acid group) was at the range 1408.06 cm-1 - 1402.27 cm^{-1} with respective intensities of 25.5 - 24, respectively, for the vegetable tanned leather samples infected with *Aspergillus niger* and *Penicillium*. The C-O stretching (alkyl aryl ether group) was at the range 1232.53 cm^{-1} with intensities of 28.5, respectively, for the vegetable tanned leather sample infected with *Penicillium*.

C-O (aliphatic ether) appeared at the ranges 1081.12 cm^{-1} - 1078.23 cm^{-1} with respective intensities of 11.5 - 19, respectively, for the vegetable tanned leather samples infected with *Aspergillus niger* and *Penicillium*. C-Cl (halo compound) appeared at the range 796.61 cm^{-1} with intensities of 26.5 for the vegetable tanned leather sample infected with *Aspergillus niger*. C=C (alkene) stretching reading appeared at the ranges 692.46 cm^{-1} - 680.88 cm^{-1} with respective intensities of 30.5 - 29.5, respectively, for vegetable tanned leather samples infected with *Aspergillus niger* and *Penicillium*. C-Br (halo compound) stretching was observed at 611.45 cm^{-1} with intensities of 32.5 for the vegetable tanned leather sample infected with *Aspergillus niger*. The change in protein composition is due to the acids produced by the isolated fungi (*Aspergillus niger* and *Penicillium*) from the historical leather bookbinding under study.

This is due to a change in the protein composition of the main component of the leather as a result of acids secreted by the isolated fungi.

The fungi are characterized by their ability to produce acids that cause acidic degradation of historical manuscripts with leather bookbindings according to (Borrego, *et al.,* 2012). The fungal strains *A. niger, A. terreus, A. ustus, A. versicolor, P. commune, P. chrysogenum, P. citrinum* and *Cladosporium* sp. Acids are secreted, lowering pH levels by up to four and increasing acid hydrolysis, according to (Trovão *et al.,* 2013)[(46)]. Furthermore, a wide range of proteins, such as collagen used in leather, can be hydrolyzed into small subunits by the action of protease enzymes such as gelatinase, according to (Caneva *et al.,* 2003)[(47)].

For the sample treated with *Citrullus colocynthis*, ATR/FTIR results showed the appearance of amide I (carbonyl group C=O) at 1649.17 cm^{-1} with intensities of 10.5, while amide II (CN stretching and NH bending) appeared at 1634.58 cm^{-1} with intensities of 11.

Compared to the vegetable tanned leather samples infected with *Aspergillus niger* and *Penicillium* fungi, we find the amount of migration and change in the functional groups amide I and amide II, this is due to the result the effect of the *Citrullus colocynthis* treatment by replacing the leather 's specific groups with the groups of the treatment substance (*Citrullus colocynthis*). This was confirmed by (Abdel-Wahab, *et al.,* 2018) [(48)].

The OH (alcohol group) appeared at the range of 3524.01 cm^{-1} with intensities of 5.5, the N-H (primary amine group) appeared at the range of 3430.46 cm^{-1} with intensities of 1.5, the C-H stretch appeared at the range of 2929.92 cm^{-1} with an intensity of 23.5, the N-O (nitro compound group) appeared at the range of 1543.08 cm^{-1} with an intensity of 20.5, the C-O (vinyl ether group) appeared at the range of 1035.79 cm^{-1} with an intensity of 29.5, the C=C (alkene) appeared at the range of 995 cm^{-1} with an intensity of 39.5. Looking at the previous results, we note that the intensity of absorption of the treated samples was higher than the intensity of absorption of the vegetable tanned leather samples infected with *Aspergillus niger* and *Penicillium* fungi, and the disappearance of some groups specific to acids that were present in the samples infected with *Aspergillus niger* and *Penicillium* fungi.

The appearance of groups specific to the treatment substance, " *Citrullus colocynthis*," are likely due to the effect of the *Citrullus colocynthis* treatment by replacing the leather 's specific groups with the groups of the treatment substance (*Citrullus colocynthis*). This indicates the *Citrullus colocynthis* success in inhibiting fungi and its resistance to microbial damage. This result, which was obtained in terms of the degree of inhibition, is largely consistent with the result obtained by (Khudair, 2010) [(49)].

We conclude from the above that the *Citrullus colocynthis* is successful in treating leather samples from fungi isolated from the historical leather bookbinding that is the subject of the study, namely (*Aspergillus niger* and *Penicillium*).

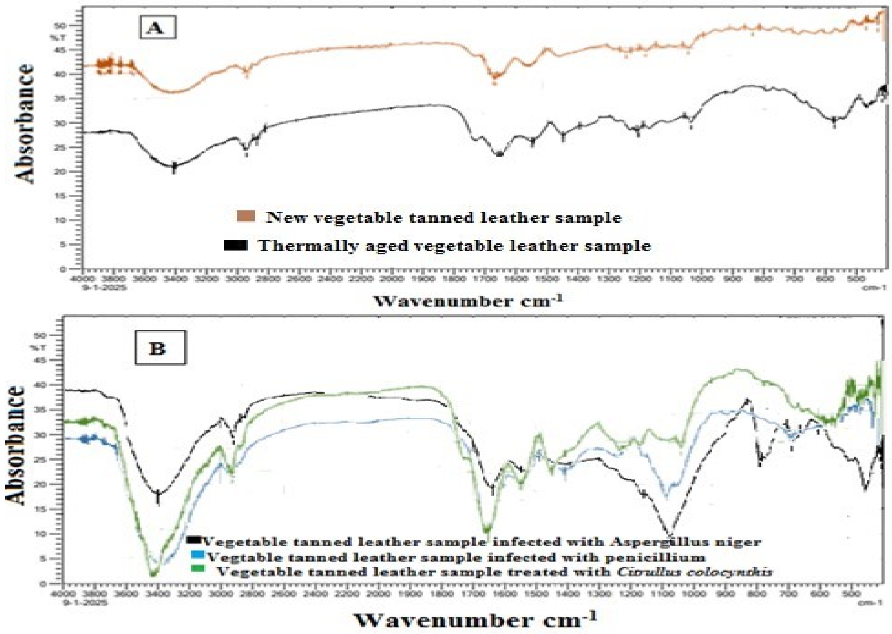
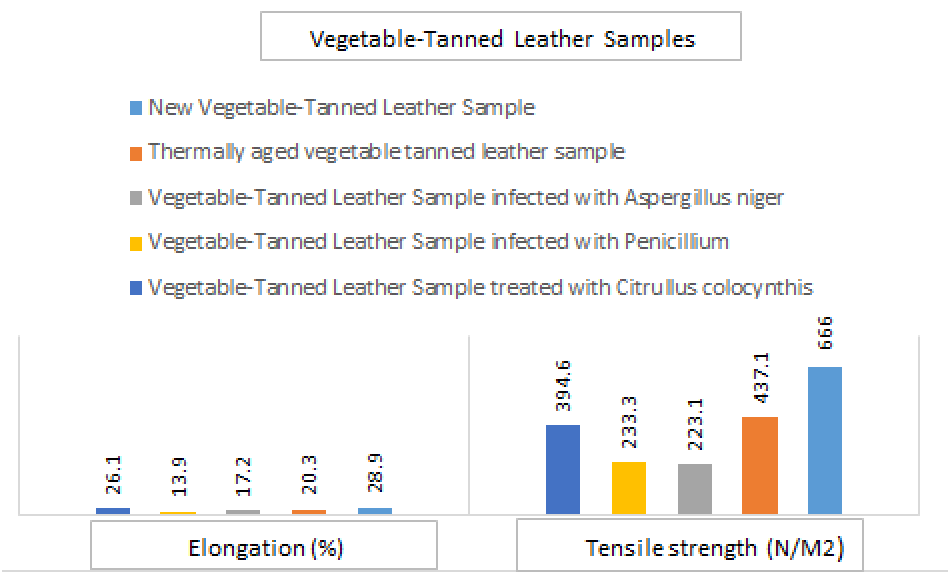


Figure (11) ATR/FTIR analysis of leather samples :(A) New vegetable tanned leather samples and thermally aged samples, (B) samples infected with *Aspergillus niger,* samples infected with *Penicillium* and vegetable tanned leather samples treated with *Citrullus colocynthis* extract.

3.4. Tensile Strength and Elongation.

The results of measuring mechanical properties [Figure 12] showed that thermal aging affected the mechanical properties (tensile strength and percentage elongation) of the leather samples, as the loss in tensile strength reached 34 N/m² after thermal aging, 67after infection with *Aspergillus niger*, and 65 after infection with *Penicillium*. The loss in percentage elongation was 30% after thermal aging, 40% after infection with *Aspergillus niger*, and 52% after infection with *Penicillium* compared to the new vegetable tanned leather sample. It can be said that treating vegetable-tanned leather with 100% *Citrullus colocynthis* improved tensile strength by 77 and percentage elongation by 52% compared to the leather sample infected with the fungus. The results showed that a 100% concentration of *Citrullus colocynthis* gave good results in the mechanical properties of the leather and in resistance to fungal damage.



[Figure (12)]: Results of measuring the mechanical properties of the leather samples.

4. Stages of treatment and restoration of the historical leather bookbinding.

4.1. Initial chemical sterilization.

Sterilization was used with *Citrullus colocynthis* at a concentration of 100% dissolved in ethyl alcohol. Sterilization was applied using compresses, where the compress saturated with *Citrullus colocynthis* was placed on the historical leather bookbinding as shown in [Figure 13 (A)] with local cleaning by wrapping a piece of cotton around a piece of gauze and dipping it in the sterilizing material (*Citrullus colocynthis*), then painting the affected area with the fungi spots formed on the historical manuscript and its leather-binding, taking into account the application method in cleaning the spots so that no difference occurs in the areas of the spots according to |(Nadia, *et al.,* 2016)[(50)].

4.2. Cleaning.

• Mechanical cleaning.

Mechanical cleaning was used to remove surface dust on the historical leather bookbinding, which had darkened. Dry cleaning was used as a safe process. A very soft brush was used for dry cleaning, taking care to brush the outer edges, as shown in [Figure 13 (B)].

• Chemical cleaning.

Organic solvents were used for chemical cleaning that does not affect the integrity of the historical leather bookbinding. Ethyl alcohol was used in a ratio of 1:3 in distilled water, where dry cotton was wrapped in it and moistened with water and alcohol. Cleaning was carried out in a local manner, one part after another. Water and alcohol also give the treated historical leather bookbinding some of the lost flexibility, especially in the very dry parts on the edges, as shown in [Figure 13 (C)].

4.3. Completion.

• Completing the missing parts of the historical leather bookbinding.

Due to the loss of a large part of the edges, it was necessary to resort to completing this loss in order to reinforce the remaining parts of historical leather bookbinding, where the reinforcement was done with leather of the same type of leather, as goatleather was prepared using the same methods of preparation and old tanning (vegetable tanning using mimosa). Then the places of loss that would be reinforced were identified and their prints were taken on calk paper to be printed on modern leather. Then the part to be completed was cut from the modern leather to the same dimensions as the one signed on it. Then the edges where the glue was attached to the old leather were thinned to achieve the greatest degree of integration and overlap, with the help of acid-free cardboard to make a filler under the modern leather until the modern leather was at the same level as the old leather attached to it. After that, each part designated for completion was glued separately, until all the missing parts were completed, where PVA adhesive was used at a high concentration of 10%. Then the piece was placed under the press for 24 hours to achieve the greatest degree of integration, as shown in [Figure 13 (D)].

4. 4. Final chemical sterilization.

Sterilization was done with *Citrullus colocynthis* at a concentration of 100% dissolved in ethyl alcohol. Sterilization was applied using compresses, where the compress saturated with *Citrullus colocynthis* was placed on the historical leather bookbinding, as shown in [Figure 13 (E)], for fear of the possibility of fungal infection in the future.

4. 5. Binding the historical leather bookbinding.

The pages of the historical manuscript were sewn together with treated cotton thread, and were reattached to the outer historical leather bookbinding using 10% PVA adhesive, so that the final appearance of the manuscript after restoration was as shown in [Figure 13 (F-G)].

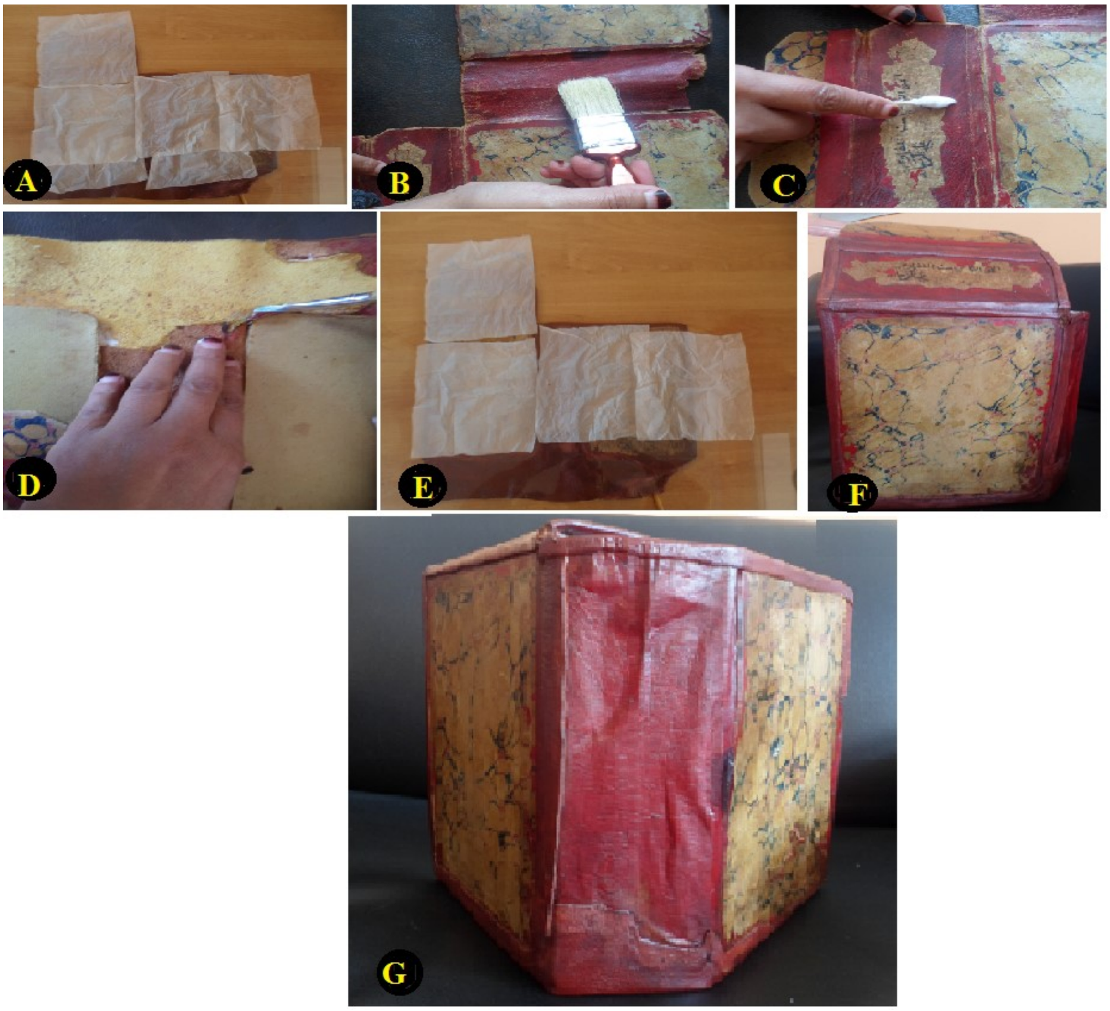


Figure 13: Stages of treatment and restoration of the historical leather bookbinding: (A) Initial chemical sterilization of the leather bookbinding, (B) Mechanical cleaning of the historical leather bookbinding, (C) Chemical cleaning of the leather bookbinding, (D) Completion of the missing part of the leather bookbinding, (E) Final chemical sterilization of the leather bookbinding of the historical manuscript, (F-G) The shape of the leather bookbinding after the stages of treatment and restoration.

5. Conclusion

This study focused on evaluating the use of *Citrullus colocynthis* extract against fungi isolated from leather bookbinding from the Mamluk's period. Electron microscopic examination revealed that the leather bookbinding was made of goat leather. It also clearly demonstrated the different manifestations of fiber damage and areas of fungal infection, and the success of *Citrullus colocynthis* extract in treating experimental leather samples from fungi isolated from the historical leather bookbinding. The study, using an EDX analyzer attached to a scanning electron microscope, revealed the elements present in the historical leather bookbinding, which are as follows: N, C, O, Al, Hg, Si, S, and Ca. FTIR analysis also demonstrated the efficiency of *Citrullus colocynthis* extract in treating experimental leather samples from fungi. Microbiological examination and light microscopy revealed that *Aspergillus niger* and *Penicillium* are the most common microbes infecting historical leather bookbinding and these fungi were inhibited by the *Citrullus colocynthis* extract. Other mechanical measurements used in the research, such as measuring tensile strength and elongation, demonstrated how well the *Citrullus colocynthis* extract treated experimental leather samples.

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