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# Applications of natural essential oils as antibacterial for archaeological organic materials

**Ali, M. O.[1], Taha, A. S.[2*],AboElgat, W. A. A.[3], Fatma, E .L.[4]**

[1] Microbiology Department, Conservation Center, Grand Egyptian Museum, Egypt
[2]Conservation Department, Faculty of Archaeology, Aswan University, Aswan 81528, Egypt;
[3]Restoration Department, High Institute of Tourism, Hotel Management and Restoration, Abukir, Alexandria 21526, Egypt;
[4]National Museum of Civilization, Ministry of Torism and Antiquities, Egypt.
*** Corresponding auther:** aymansalahtaha82@yahoo.com

## Abstract

Cultural heritage materials degrades over time, however conservation slows down the speed of its deterioration. Microbiological contamination with bacteria can pose a big destroy to old manuscripts and mummy or hazard to those working in archives or library.Most of the biological damage is started in poor environmental conditions for storage and display.The gas chromatography/mass spectrometry (GC/MS) analysis of the oils revealed the main constituents of the essential oils *Eucalyptus camaldulensis*, spathulenol (20.84%), eucalyptol (12.01%), sabinene (9.73%), α-phellandrene (8.18%), crypton (7.69%), terpinen-4-ol (3.69%), phellandral (3.54%) and *D*-limonene (2.28%), and *Citrus*sinensis, D-limonene (73.4%) and -terpinene (22.6%).
This work aimed to access the presence of microorganisms and their effect on the old manuscripts, mummy deterioration; it also studies their treatment methods, such as fumigation natural oils.The causes of the various efficacy of the treatments are observed, also because the potential risks of recolonization by viable cells left behind after treatment. The results further showed that the test oils were able to inhibit the mycelia growth by usingthe fumigation method.The potent properties of *E. camaldulensis*, the possibility of using it as eco-friendly, safe, and cost-effective antibacterials for bacterial that could cause discoloration of the archaeological organic materials.

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**Keywords:**Essentialoils, Fumigation, Deterioration, Microbiological, Antibacterial, Treatment.

## 1. Introduction

Antibiotics are one of our most significant to bacterial and fungal decay and have greatly benefited the health-related quality of human life since their introduction. The problem of microbial resistance is increasing for the use of antimicrobial medication in the future is still uncertain. Therefore, actions must resolve this problem, for example, to control the use of antibiotic, develop research to better understand the original mechanisms of resistance, and to continue studies to develop novel soporific, either artificial or natural. The extreme aim is to offer suitable and active antimicrobial real estate to the patient. [1,2]. For a long period of time, plants have been a precious source of natural products for use in the medical domain, principally in the last time, with more strong studies for natural treatment. The use of plant extracts for curative objective has progressively increased in Brazil.

According to World Health Organization [3] curative plants would be the better source to procure a assortment of soporific Plants are wealthy in a broad variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides, etc., which have been found in vitro to have antimicrobial characteristic [4,5].The World Health Organization estimates that plant extracts are used as people medicine in conventional treatment of 80% of the world's population [6].The felled microorganisms can be prevented with realty and these results in the germination of various medicament - renitent bacteria and it has created terrible clinical condition in the controlling of contamination[7,8].The performance of civilization of Ancient Egypt was indebted to the Nile River. Moreover, watery plants confer with a spacious set of capitalize to human being and form one of the wealthy ecological units in Egypt[9]. There are 25 families of watery plants subdivided into 45 genera and 87 species of effloresce plants in the Nile River[10-14]. The antimicrobial properties of water plants are owed to a variety of

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secondary metabolites such as alkaloids which have been found in firm floating leaved species, whereas submerged species contain both simple phenols and flavonoids [15,16].

The antimicrobial properties of plants have been inspected, by a number of researchers, especially in Latin America.In Argentina, a search tested 122 best-known plant species used for therapeutic treatments[17].

It **have been** documented **that a lot of** the compounds extracted from those plants, twelve **stifled** presence of Staphylococus aureus, **ten** stopped **Escherichia coli**and **4** inhibited Aspergillus niger and **moreover confirmed** that the **preliminary effective** compound **become** one extracted from Tabebuiaimpetiginosa [18].The chemical compositions of the extracts could be analyzed and examination by wherewithal of gas chromatographye mass spectrometry (GC/MS) technique[ 19]. Orange fruits are **typically** refers to Citrus sinensis (belongs to Rutaceae family) or Sweet Orange Group.The EO extracted from orange peels can be used as a green insecticide and have potential effects against microbes[20 - 24].Therefore, the aim of this study was to evaluate the antibacterial activity of the EOs from *E. camaldulensis* and *Citrus sinensis* against the growth of bacterial strains isolated from organic materials.

## 2. Materials and Methods

### 2.1. Collection of Swabs:

Swabs were obtained from degraded mummy at the grand Egyptian museum and rare books for the parliament of the Arab Republic of Egypt. We make examination for a similar books under (Description DeI'Egypte- Tome 1- 5 – Antiquities Descriptions) from the examination we found that most of books suffer from severe microbial infections, which are manifested in the presence of clear microbial lesions in the form of brown spots, which have been combined in many pages of books to cover the entire pages in brown color,To change the color of some pages to brown colormicrobial swabs were cultured on (Cellulose , Protein and Nutrient agar media).

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### 2.2. Isolation of Microorganisms and Growth Media:

The antibacterial contaminants wereisolated from the infected archaeological objects which characterized according to molecular approach by using sequencing of rRNA and ITS region of rDNA gene at Solgent Company, South Korea.

The following genera were identified: *Micrococcus luteus* (MH450098), *Microbacteriumschleiferi* (NR044936T), *Bacillus subtilis* (NR112629T).Other microorganisms isolated from degraded mummy. The following genera were identified: based on ribosomal RNA analysis, to bacteria found in the human skin microbiome (*Bacillus jeotgali* (NR025060T), *Kocuriaturfanensis* (NR043899T), *Microbacteriumimperial* (NR026161T), &*Bacillus megaterium*( NR112636T). The bacterial stock cultures were incubated for 24 hours at 37 °C on nutrient agar medium. The stock cultures were maintained at 4°C.

### 2.3. Essential Oils Extraction:

Fresh leaves of *Cimumbasilicum*and fresh peels of *Citrus sinensis*were collected during January 2020, from Alexandria, Egypt. These raw materials were cut to small pieces and were hydro-distillated for 3 h. in a Clevenger apparatus[25- 28]. The essential oils (EOs) were dried over anhydrous $Na_2SO_4$. The collected EOs were stored in brown glass bottles in a refrigerator at 4ºC.

### 2.4. Preparation of essential oils:

The extracted EOs were prepared at the concentrations of1000, 2000, 4000 and 5000 ppm . The respective amount of oil was diluted in a dimethyl sulfoxide (DMSO 10%), SDW (1:1 *v/v*) and 0.5 ml of Tween 40 was added.

### 2.5. GC/MS analysis of Essential Oils:

The chemical **components** of the EOs from *E. camaldulensis* **fresh** leaves and *C. sinensis* **fresh** peels **clean peels have been carried out using** GC-TSQ Quantum mass spectrometer (Thermo Scientific, Austin, TX, USA) with **an immediate** capillary column TG-5MS (30 m × 0.25 mm × 0.25 µm **film** thickness). The conditions of the separation and identification of the EOs can be found in the previous works [19, 26-30].

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### 2.6. Antibacterial activity:

#### 2.6.1. Determination of inhibition zones method:

Antibacterial activities of natural oils (*E. camaldulensis*, and *C. sinensis*) against the isolated microorganisms were tested using the agar diffusion method. The bacterial cultures were incubated for 24 hours at 37°C on nutrient agar medium. The zones of development inhibition around the wells have been measured after 18 to 24 hours of incubation at 37°C. The sensitivities of the microorganism species to the natural oils had been determined through the sizes of inhibition zones (inclusive of the diameter of the well) on the agar surface around the wells. Values <15 millimeters have been taken into consideration as no longer active in opposition to microorganisms [31,32].combination of 10% DMSO and SDW (1:1 v/v) became used as an alternative within the control pattern.

#### 2.6.2. Determination of minimal inhibitory concentration (MIC) of natural oils:

A stock solution of each microcide of natural oils were prepared by dissolving 1.0 ml of each natural oils in one letter of 10% DMSO .Gradient concentrations of natural oils(1000, 2000, 4000 and 5000 ppm ) were prepared by using 10% DMSO+ drops of Twen40. 1 ml of bacterial spore suspension was spread on a nutrient agar plate. The plates were allowed to dry,then a cork purer was used to make three pores (about 15 mm in diameter) to make three replecates. On one plate, 100µl of each concentration (1000, 2000, 4000 and 5000 ppm) of the tested oil were placed. The plates were incubated at 30° C for 24 h compared with control plates (10% DMSO).

The MIC was determined by measuring the sizes of inhibitory zones (including the diameter of the well) on the agar surface around the wells; values <15 mm were considered as not active against microorganisms. The MIC was determined by measuring the inhibition zone according toprevious papers [37,38]. MIC test is the gold standards for deciding the susceptibility of organisms to a specific antimicrobial

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substance so accustomed decide the performance of all different strategies of susceptibility testing[39].

**Determination of the effectiveness of oils in the elimination of bacterial infections by using the fumigation method.**

Treatment of the infected books was applied by a fumigation method with natural oils (Eucalyptus camaldulensis) at (5000 ppm/L) was prepared by dissolving 5 ml/L in 10% DMSOto prevent microbial growth for 6 months. Microbial growth was examined by taking of swabs from each treated specimen after 48 hours, 3 months and 6 months.

## 3. Results and discussion:

### 3.1. Bacterial activity

All tested bacterial isolates, *Micrococcus luteus* , *Microbacterium schleiferi* , *Bacillus subtilis,Bacillus jeotgali* ,*Kocuria turfanensis*, *Microbacterium imperial* and *Bacillus megaterium* were isolated from archaeological objects. Antimicrobial activities of natural oils were assessed in terms of zone inhibition of microorganism growth. The results are presented in table.1 showing that *Citrus sinensis* E.Oil has good sensitive to the some tested microorganisms, but*Eucalyptus camaldulensis*E.Oilhas good sensitive against all tested microorganisms .

Table 1.Diameter of inhibition zones in (mm) due to natural oils.

| Microbial strains | Natural oils | |
|---|---|---|
| | *Eucalyptus camaldulensis* | *Citrus* sinensis |
| *Micrococcus luteus* | 30mm | 25 mm |
| *Kocuria turfanensis* | 25mm | 20 mm |
| *Bacillus megaterium* | 20mm | 18 mm |
| *Microbacterium schleiferi* | 27mm | - ve |
| *Bacillus subtilis* | 25mm | - ve |
| *Bacillus jeotgali* | 30mm | - ve |
| *Microbacterium imperial* | 24mm | - ve |

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### 3.2.**Determination of minimal inhibitory concentration (MIC) of natural oils:**

In this experiment, the prepared natural oils applied to the tested bacteria to determine the MIC that inhibited each bacterial species proved, good response could be detected at concentration 5000 ppm gave the diameter of a clear zone ranged (18-25 mm) afterwards 24 h at 37 °C. but the other concentrations that gave no response in all isolates. But in case of *Citrus sinensis* natural oils that gave no response in all isolates at in all concentrations.

Table 2.Means of inhibition zones (mm) of essential oils (*E. camaldulensis* and *C. sinensis* ) at different concentrations (ppm).

| Microbial strains | concentration of natural oils (ppm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Eucalyptus camaldulensis* | | | | | *Citrus sinensis* | | | | |
| | control | 1000 | 2000 | 4000 | 5000 | control | 1000 | 2000 | 4000 | 5000 |
| *Micrococcus luteus* | 0.00 | 0.00 | 0.00 | 0.00 | 20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Kocuriaturfanensis* | 0.00 | 0.00 | 0.00 | 0.00 | 25 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Bacillus megaterium* | 0.00 | 0.00 | 0.00 | 0.00 | 19 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Microbacteriums chleiferi* | 0.00 | 0.00 | 0.00 | 0.00 | 20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Bacillus subtilis* | 0.00 | 0.00 | 0.00 | 0.00 | 23 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Bacillus jeotgali* | 0.00 | 0.00 | 0.00 | 0.00 | 18 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Microbacterium imperial* | 0.00 | 0.00 | 0.00 | 0.00 | 21 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

### 3.3. Chemical composition of the natural oils:

As mentiond in our previous study [27] Fig. (1), the major chemical compounds in *E. camaldulensis* EO, spathulenol (20.84%), eucalyptol (12.01%), sabinene (9.73%), α-phellandrene (8.18%), crypton (7.69%), terpinen-4-ol (3.69%), phellandral (3.54%).Table

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3 and *D*-limonene (2.28%).*D*-limonene (73.4%) and γ-terpinene (22.6%) were the abundant compounds in the EO from *C. sinensis*peels. Table 4.

**Table 3.**Phytochemical composition of *E. camaldulensis* essential oil by GC/MS.

| RT (min.) | Compound | Percentage in the Oil (%) | RSI-SI * |
|---|---|---|---|
| 6.68 | 2-Thujene | 1.12 | (944–905) |
| 6.98 | ά-Pinene | 1.14 | (938–919) |
| 8.63 | ά -pinene | 0.77 | (931–897) |
| 9.44 | ά -Phellandrene | 8.18 | (939–938) |
| 9.70 | 4-Terpinenyl acetate | 0.38 | (913–882) |
| 10.10 | D-Limonene | 2.28 | (924–916) |
| 10.39 | Sabinene | 9.73 | (940-936) |
| 10.53 | p–Cymene | 15.16 | (923-906) |
| 10.68 | Eucalyptol | 12.01 | (921–902) |
| 11.16 | γ -Terpinene | 1.09 | (919–879) |
| 12.06 | (E) - ά -Ocimene | 0.7 | (871–860) |
| 14.11 | cis-B-Terpineol | 0.64 | (918–885) |
| 14.87 | cis-para-2-menthen-1-ol | 0.38 | (913–863) |
| 16.11 | Terpinen-4-ol | 3.69 | (928–923) |
| 16.83 | ά -Terpineol | 0.42 | (869–853) |
| 17.85 | Crypton | 7.69 | (952–932) |
| 19.36 | Cuminaldehyde | 1.81 | (949–855) |
| 20.19 | Phellandral | 3.54 | (952–884) |
| 20.94 | 2-ethylidene-6-methyl-3,5-Heptadienal | 1.54 | (823–807) |
| 23.61 | Aromadendrene | 1.71 | (932–847) |
| 24.85 | Nerolidyl acetate | 0.41 | (797–787) |
| 27.59 | Spathulenol | 20.84 | (947–922) |
| 28.26 | 2-Methylene-5_-cholestan-3_-ol | 0.41 | (843–781) |
| 28.54 | Linoleic acid ethyl ester | 1.65 | (743–735) |
| 28.82 | Oleic acid | 0.27 | (808–792) |
| 29.06 | ά -Vetivol | 1.12 | (772–759) |
| 29.23 | ά -Sinensal | 0.21 | (793–759) |
| 29.54 | (Z,Z)-1,3-Dioctadecenoyl glycerol | 0.17 | (836–816) |
| 29.77 | (11Z)-12-(2-Oxiranyl)-11-dodecenyl acetate | 0.18 | (805–766) |

RT: Retention time (min.); * (RSI: Reverse Standard index; SI: Standard Index).

**Table 4.**Phytochemical composition of *C. sinensis*essential oil by GC/MS.

| RT (min.) | Compound | Percentage in the Oil (%) | RSI-SI * |
|---|---|---|---|

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| | | | |
|---|---|---|---|
| 8.57 | Myrcene | 1.13 | (952–944) |
| 10.13 | D-Limonene | 73.4 | (945–944) |
| 10.46 | p–Cymene | 1.02 | (923–840) |
| 11.13 | γ –Terpinene | 22.6 | (950–949) |
| 16.74 | ά –Terpineol | 0.81 | (931–923) |
| 29.42 | Ylangenal | 1.04 | (803–783) |

RT: Retention time (min.); * (RSI: Reverse Standard index; SI: Standard Index)

### 3.4. Determine the appropriate method for bacterial treatment of pathogenic organisms:

There are various methods that can be used for applying the microbial treatment to the books by using the MIC of natural oils *E. camaldulensis* at concentration 5000 ppm. These methods differ based on the condition of the books. The authors declare that there is no conflict of interest specific techniques were used for this purpose brushing method, sparing method and fumigation method. the first and second approach were carried out in a small scale, however the third technique was carried out to the book inside the sealed tight cabinet. After the examine, we found the suitable method to apply the treatment of oils is the approach of fumigation within the area of tightly sealed.these so-known as " fumigation " techniques require the use of sealed chambers, and are sometimes a great alternative to microcides. Eucalyptus camaldulensis vital oil had activity towards the bacteria. however, the antibacterial activity of crude vital oil varied among the test pathogens. The findings of the current study concur with reports from previous studies on different levels of antibacterial activity of vital oil of E. camaldulensis of varied chemical profiles against a various group of plant pathogenic bacteria[40].

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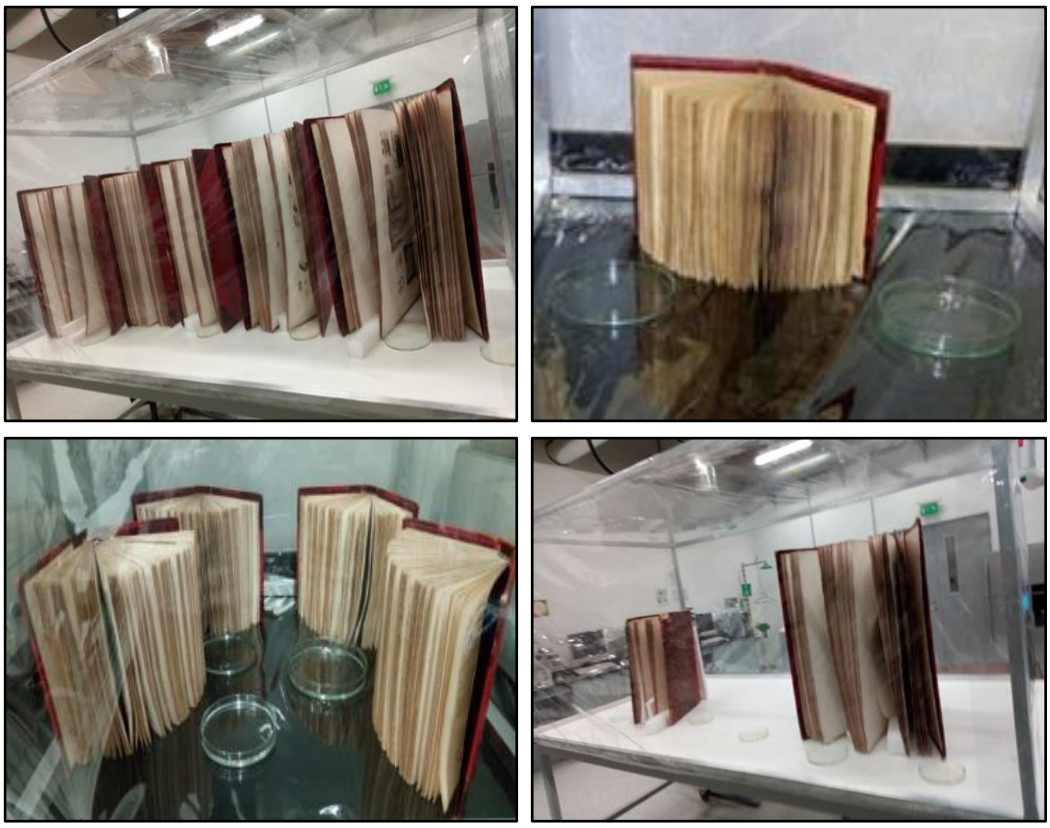

**Fig.1:**Showing Fumigation methods to applying the microbial treatments of natural oils.

## 4. Discussion

Testing and proving of biological activity of EOs is one of the main objectives of the study of natural materials. It is now popular through all the scientific community that these research must be aware of the variability of the biological material concerned. In reality, it is known that the composition of EOs can be distinctive due to the species, the agro-ecological components and the part of the plant that's being analysed [41].results of the study show the considerable values of antibacterial activity of essential oils (EOs) of *Eucalyptus camaldulensis* air-dried aerial parts, *Citrus aurantium* leaves, and *C. sinensis* peels against microbials singly and in combination together with equal volume. The occurred results are due to presence of components inclusive of spathulenol, p-cymene, eucalyptol, linalyl

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acetate, linalool, sabinene, phellandrene, crypton, terpinen-4-ol, D-limonene, terpinene, terpineol, longifolene, neryl acetate, p-cymene, phellandral cuminaldehyde, and alloaromadendrene in the Eos [42,43]. The EO from aerial parts of *E. camaldulensis* showed the presence of p-cymene (27.8–42.7%), 1,8-cineole (4.1–39.5%), spathulenol (2.1–15.5%), and cryptone (3.2–10.2%) as main compounds[44].The EOs with their chemical compositions of 1,8-cineole (60%), aromadendrene (_5%), and limonene( ≥4%) or p-cymene (10%), β-pinene (8%) and spathulenol (10%), were characterized some *E. camaldulensis*clones grown in Australia [45]. p-Cymene, cyptone, and spathulenol with 22.9%, 14.1%, and 16.5%, respectably, were found to be the abundant compounds of E. camaldulensis EO from Australia [46].1,8-Cineole (34.7%), β -pinene (7.7%), p-cymene (9.3%), and spathulenol (9.5%) were reported as main compounds in EO of *E. camaldulensis* from Greece [47]. *E. camaldulensis* leaf EO with its principal compounds eucalyptol, β -pinene, and terpinene carried out to wood showed accurate inhibition towards microbial[19]. For the usually common mechanisms of antimicrobial interaction that produce synergism, it was found that the combinations of EOs led to inhibition of the common biochemical pathway with inhibition of the protective enzymes, with subsequent use of cell wall-active agents to enhance theuptake of other antimicrobials [48, 49].which is related to the presence of phenolic compounds such as p-hydroxy benzoic acid, gallic acid, salicylic acid, and caffeine. *E. camaldulensis*books treated has shown antibacterial activities with a high content of quercetin, benzoic acid, naringenin, caffeine, o-coumaric acid, and kaempferol [50].

## 5. Conclusion

The findings have shown the potential of natural oil only with bacteria, but when we diluted with the solvent gave no response in all isolates. Therefore, it could be reported that using natural oils (*E. camaldulensis*) at (5000 ppm) was sufficient to

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completely prevent the growth of all microbial isolates. The result revealed that natural oils could be used to controlling the microbial deterioration of historical books applied with fumigation method. The use of natural oils products will, therefore, reduce over dependence on the use of synthetic chemicals in controlling microbial pathogens as well as reducing cost of management and environmental pollution.

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**Conflicts of interest**

The authors declare that there is no conflict of interest.

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