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Laboratory testing of the antimicrobial effects of *kyphi* on a variety of micro-organisms

Alicia Maravelia – Elsa Faviou – Emmanuel Magiorkinis – Markos Filianos

ABSTRACT

Kyphi (*k3pt/k3pw*) was used as both incense and medicament during a very long period in ancient Egypt, as is attested in *Papyrus Ebers* and other sources. The present work is a continuation of recent papers and a book concerning the comparative study of the ancient Egyptian *kyphi* and the Orthodox Holy Chrism, with freshly confected *kyphi* containing all its 16 ingredients, at the premises of the Oenological Chemical Laboratory of the Agricultural University of Athens. Based on the recent results obtained from two independent Laboratories, this study attempts, in a third independent Laboratory, both to replicate a part of the previous experiments and to extend the scientific investigation, comparing the effectiveness of ancient Egyptian *kyphi* and pure smoke on other micro-organisms. The possible antimicrobial effects of pure smoke and of *kyphi*-smoke are investigated by performing fumigation experiments on bacterial and fungal cultures. Thus, the antifungal and antimicrobial activity of *kyphi* is studied by performing fumigation experiments to cultivations of *Morganella morganii* W. ssp *morganii* [Gram (–) bacterium], *Escherichia coli* M. [Gram (–) bacterium], *Staphylococcus hominis* K.&S. ssp *hominis* [Gram (+) coccus], *Staphylococcus epidermidis* W.&W. [Gram (+) coccus], *Klebsiella pneumoniæ* S.&T. ssp *pneumoniae* [Gram (–) bacterium], and *Candida albicans* (R.) B. [Gram (+) fungus] in different conditions. The results show that the action of fumigation (pure smoke and *kyphi*) on selective and differential culture media has almost no effect on the growth of micro-organisms. However, there is a clear direct effect of smoke (pure smoke and *kyphi*) on the micro-organisms that appears stronger with *kyphi* in all dilutions (and mostly in 1/1000). The experimental data reinforce the evidence from previous studies that the impact of *kyphi* on yeasts is mostly greater than that on bacteria and cocci, with indeed notable results at the 1/1000 dilution. The addition of all components of *kyphi* did not negate its antimicrobial activity but enhanced it, mainly against Gram (–) bacteria and less against Gram (+) cocci. Thus, the antimicrobial action of *kyphi* should be considered collectively, together with all its ingredients.

KEYWORDS

Kyphi (*k3pt*) – incense – medicines – censing – fumigation – *Escherichia coli* – *Candida albicans* – *Staphylococcus hominis* –*Staphylococcus epidermidis* – *Morganella morganii* – *Klebsiella pneumoniæ* – microbiology – experiments

الاختبارات المعملية للتأثيرات الميكروبية الاختبارات المعملية للتأثيرات الميكروبية لبخور *Kyphi* على مجموعة متنوعة من الكائنات الحية الدقيقة

أليسيا مارافيليا – إلسا فافيو – إيمانويل ماجيوركينيس – ماركوس فيليانوس

خلاصة

تم استخدام *kyphi* (*k3pt/k3pw*) كبخور ودواء خلال فترة طويلة جدًا فى مصر القديمة، كما هو مذكور فى بردية إيبرس ومصادر أخرى. تمثل تلك المقالة استمرار للأبحاث الحديثة وكتاب يتعلق بالدراسة المقارنة لـ *kyphi* المصرى القديم والزيت الأرثوذكسى المقدس «الميرون»، بالإضافة إلى بخور *kyphi* المحضر حديثًا، والذى يحتوى على جميع مكوناته الستة عشر، وذلك بمقر المختبر الكيميائى لعلم الخمور بكلية الزراعة بجامعة أثينا. واستناداً إلى النتائج الحديثة التى تم الحصول عليها من مختبرين مستقلين، تحاول هذه الدراسة، وذلك فى مختبر مستقل ثالث، تكرار جزء من التجارب السابقة وتوسيع نطاق البحث

العلمى، وأيضاً المقارنة الفعالية لبخور ***kyphi*** المصرى القديم ودخانه النقى على الكائنات الحية الدقيقة الأخرى. كما يتم التحقيق من التأثيرات المضادة للميكروبات المحتملة للدخان النقى للبخور ودخان ***kyphi*** من خلال إجراء تجارب التبخير على عناصر جرثومية وفطرية. وهكذا، تتم دراسة النشاط المضاد للفطريات ومضادات الميكروبات لبخور ***kyphi*** عن طريق إجراء تجارب التبخير على مزارع عينات من بكتيريا، مورغانيلة مورغانية (بكتيريا سالبة الجرام)، وأخرى إشريكية قولونية (بكتيريا سلبية الجرام)، وكذلك بكتيريا عنقودية بشرية (بكتيريا إيجابية الجرام)، وبكتيريا عنقودية جلدية (بكتيريا إيجابية الجرام)، وبكتيريا كلبسيلة رئوية (بكتيريا سالبة الجرام)، وبكتيريا مبيضة بيضاء (فطر ايجابى الجرام)، وذلك فى ظروف مختلفة.
أظهرت النتائج أن عملية التبخير (الدخان النقى بالإضافة إلى ***kyphi***) على وسائط الاستزراع الانتقائية والتفاضلية ليس لها أيه تأثير تقريبًا على نمو الكائنات الحية الدقيقة. ومع ذلك، هناك تأثير مباشر واضح للدخان (الدخان النقى والـ ***kyphi***) على الكائنات الحية الدقيقة التى تبدو أقوى مع بخور الـ ***kyphi*** فى جميع التخفيفات (ومعظمها في ***1/1000***). تعزز البيانات التجريبية الأدلة المستمدة من الدراسات السابقة والتى تشير إلى أن تأثير بخور ***kyphi*** على الخمائر أكبر فى الغالب من تأثيره على البكتيريا والمكورات، مع نتائج ملحوظة بالفعل عند التخفيف لـ ***1/1000***. كما أن إضافة جميع مكونات ***kyphi*** لم تلغى نشاطه المضاد للميكروبات ولكنها عززته، هذا بشكل رئيسي ضد البكتيريا سلبية الجرام وبدرجة أقل ضد المكورات إيجابية الجرام. وبالتالى ينبغي النظر في عمل مضاد الميكروبات من بخور ***kyphi*** بشكل جماعى، جنبًا إلى جنب مع جميع مكوناته.

الكلمات الدالة
kyphi (*k3pt*) – البخور – الأدوية – التبخير – إشريكية قولونية – المبيضات البيضاء – عنقودية جلدية – عنقودية بشرية – مورغانيلة مورغانية – الكلبسيلة الرئوية – علم الأحياء الدقيقة – التجارب

INTRODUCTION

The ancient Egyptian *kyphi*/*k3pt* (Otto – Helck –Westendorf 1980: col. 902–903; Loret 1887: 76–132; Tatomir 2008: 169–173) was a special sacred mixture of 16 aromatic ingredients, used both as burning incense and as a medicine during a very long period of the ancient Egyptian civilization. Its use and properties have been described already during Antiquity and there were special temple laboratories for its confection in several famous Egyptian temples, especially those of the Ptolemaic Period (Vadas 2020: 93–132).[1]

In a recent paper (Maravelia – Faviou – Filianos 2022: 7–41),[2] in order to check the possible effectiveness of *kyphi* as a medication, following *Papyrus Ebers* (see e.g. Wreszinski 1913; most recently Lalanne – Métra 2017: 200–201)[3] and other sources,[4] we endeavoured to investigate the possible antimicrobial effect of pure smoke and of *kyphi* by performing fumigation exper-

1 For more details on the *kyphi*, see Maravelia – Filianos (2020).

2 The basic studies on *kyphi* and its comparison to the Orthodox Holy Chrism include Maravelia – Filianos (2020); Maravelia – Faviou – Filianos (2023).

3 See more specifically *P. Ebers* (852: 98, 12–98,14b); *P. Ebers* (853: 98, 14b–98,18b); *cf.* also *P. Ebers* (793: 94, 5–7), treating a gynaecological case with fumigation, although in the relevant title, to which *kt* refers, *p*ḫ*rt* is given. Fumigation was a known method of medical treatment and is mentioned more frequently in the *Berlin Medical Papyrus* (see Recipes 66, 67, 69, 70, 72, 74, 76 and 175). For the various medical terms, see Hannig (^{5}2009).

4 According to Dioskoridēs (Sprengel 1829; Wellmann 1906–1914; Gunther 1934; *cf.* Theophrastus 1644; Plinius Presb. 1936), the *kyphi* was a perfume and the composition of a perfume welcome to the gods, used abundantly by the ancient Egyptian priests. According to Galēnos (*Περὶ Ἀντιδότων* II: 2, 900–901; see *e.g.* in Hopfner 1922: 783), the *kyphi* was also a medicinal substance. The most

No.	Ingredients of the *Kyphi*
1	***Acorus calamus* L.**
2	***Cymbopogon schœnanthus* L.**
3	***Cinnamomum cassia* J. Presl**
4	***Cinnamomum zeylanicum* Nees**
5	***Mentha piperita* L.**
6	***Calycotome villosa* (Poir.) Link**
7	***Juniperus phœnicea* L.**
8	***Acacia nilotica* (L.) Wild.**
9	***Lawsonia inermis* L.**
10	***Cyperus longus* L.**
11	***Pistacia terebinthus* L.**
12	***Pistacia lentiscus* L. var. *chia***
13	**Pulp of Dry White Raisins (*Vitis vinifera* L.)**
14	**Rosé (⅔) and Red (⅓) Wine (*Vitis vinifera* L.)**
15	**Honey**
16	**Myrrh (*Commiphora myrrha* Engl.)**

Tab. 1 All 16 ingredients of the *kyphi* used in the 2nd Experiment for its confection *in vitro* are shown

iments on bacterial and fungal cultures in two independent Laboratories. Both the antifungal and antimicrobial activity of *kyphi* have been studied by performing fumigation experiments on cultivations of *Escherichia coli* M. [Gram (–) bacterium] and *Candida parapsilosis* L.&T. (fungus) in two separate Laboratories, independently and in different conditions. Cultivations of two Gram (+) cocci, *Staphylococcus aureus* R. and *Staphylococcus capitis* K.&S., were also tested only in the 2nd Laboratory. Preliminary results showed that the action of fumigation (pure smoke and *kyphi*) on selective and differential culture media had almost no effect on the growth of micro-organisms. However, there was a clear direct effect of pure smoke and *kyphi*-smoke on the micro-organisms appearing much stronger with *kyphi* and mainly in 1/1000 dilutions. The experimental data of that study demonstrated that the effect of *kyphi* on yeasts was more powerful than that on bacteria. It was suggested to further proceed with more experiments, in order to confirm the above and to investigate the potentially «active» component(s) of *kyphi*, discovering the pathophysiological mechanisms through which this particular antimicrobial action of *kyphi* could be explained (table 1).

important description of the *kyphi* is that of Ploutarchos (*Περὶ Ἴσιδος καὶ Ὀσίριδος*: 80, 383e–384c; see *e.g.* Didot 1868: 429–469; *cf.* Griffiths 1970; Φιλιππίδης ¹1975: 3–231; Bernardakis 1889: II, 471–557).

Thus, with the current paper this has started to be performed with much more confidence, after the preparation of the *kyphi* with the 2nd Experiment gave us a confected substance with a synthesis much closer, or even identical, to that of the ancient Egyptian recipes. Interestingly, the fresh *kyphi* produced (2023) exhibits stronger tones in its very pleasant aroma compared to that of the 1st Experiment (2017).

AIMS, MATERIALS, METHODS AND PROCEDURES

The confection of *kyphi* reenacted *in vitro* was repeated with all its 16 ingredients (tab. 1), starting on Friday 13th October 2023 and ending about 5 weeks later, at the premises of the Oenological Chemical Laboratory of the Agricultural University of Athens (Director Dr Niki Proxenia), with the contribution of the Chemist Dr Angeliki Kouki (fig. 2). All Members of our Team were present during various phases of the experiment and particular care was taken to mimic the conditions of an ancient Egyptian temple laboratory at Autumn, keeping low humidity (< 35%) and constant temperature (25° C). All 16 ingredients of the *kyphi* were used (fig. 1), some of them that were not available in the 1st Experiment were acquired from the modern Egyptian market in Cairo (*i.e.*, *Cymbopogon schœnanthus* L., *Lawsonia inermis* L., *Cyperus longus* L., *Pistacia terebinthus* L., and *Acacia nilotica* (L.) Wild.). Due to the fact that — notwithstanding our restless efforts, even with the help of Egyptian colleagues— it was impossible to acquire henna-flowers, so finally we managed only obtaining henna-leaves, only leaves were used for the confection, having in mind that their smell is rather neutral and in any case not unpleasant at all, in order to definitely include this important ingredient in the 2nd Experiment. Modern facilities, apparatus, thermo-mix devices and assorted mortars were used (fig. 2), in order to perform the preparation.

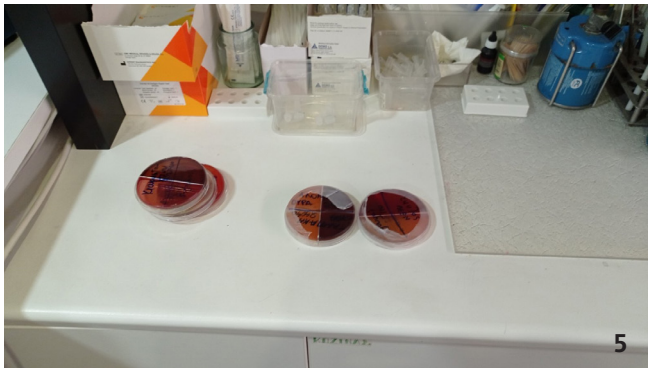
The confection experiment was performed with the highest possible care; due attention was paid in order to follow as close as possible the ancient Egyptian recipes of Philæ and Edfū, as discussed by Loret (1887), though after some necessary corrections. During the 2nd Experiment, an adequate quantity of *kyphi* (~ 1.10 kg) was produced, using all 16 ingredients for its confection (fig. 3). Approximately 1/10 of the quantities proposed by Loret was used, to obtain a satisfactory, but not huge, quantity of the product; this is also due to the high costs of several ingredients (*e.g.*, myrrh) and the difficulty of acquiring them.

Fig. 1 Samples of all 16 ingredients of the *kyphi* (some acquired from the Egyptian market in Cairo) are exhibited here, before the confection, in their special containers

Fig. 2 Aspect of the initial stages of the experimental preparation of *kyphi*, during which some of its herbal ingredients were finely ground, using a modern electric mortar. A characteristic view of the well-equipped Oenological Chemical Laboratory of the Agricultural University of Athens with many facilities can be easily seen. The contribution and voluntary participation of chemist Angeliki Kouki (figuring here, together with pharmacist Markos Filianos on the right) is to be acknowledged (Athens, Friday 13th October 2023)

Fig. 3 Total amount of the recently confected *kyphi* (*kꜣpt*) with all 16 ingredients (result of the 2nd Experiment), following ancient recipes of the Temples at Philæ and Edfu (after Loret 1887: 76–132), and its hieroglyphic name. No matter how "ugly" this substance might look, its fragrance is extremely sweet and particularly pleasant; curiously, the smoke of burned *kyphi* gives a less pleasant odour

The additional ingredients (most significantly *Pistacia terebinthus* L., *Cymbopogon schœnanthus* L. and *Cyperus longus* L.) change the texture of the final product, making of it less malleable than that of the 1st Experiment. Thus, after the final product was left to rest, it was cut off in several small pieces, just like in the process of confecting incense nowadays [of course without adding any additional substances (*e.g.* $MgCO_3$), as is the practice today to avoid conglomeration and sticking together of the incense-pellets], and then it was left to dry. Furthermore, not withstanding the ancient Egyptian recipe, in some phases of the confection, certain ingredients were not boiled (as also discussed by Loret 1887: 104–107), because already dried plants were used by the present authors and not fresh ones, as was the case in ancient Egyptian temple laboratories. It was considered that by boiling the already dried plants, evaporation of some of the more volatile substances of the ingredients could happen, hence losing an important part of the quality and quantity of the aroma of the final product, occasionally making its



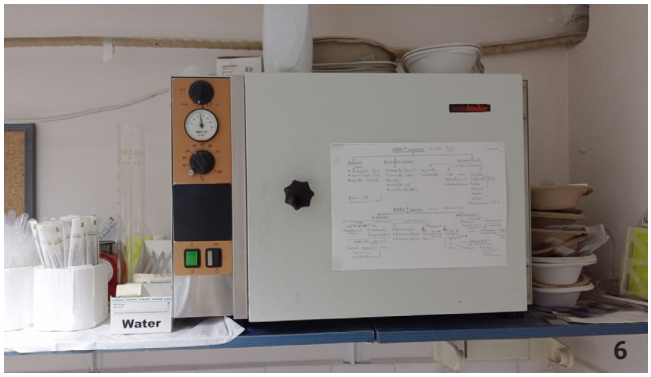


Fig. 4 Experimental setting for fumigating the testing plates and cultures in the Private Hospital Laboratory. The placement of the Petri plates before the initiation of the smoking process (fumigation under a 5000 ml beaker) is clearly visible.
Fig. 5 Samples of the strains isolated from outpatient cultures (Petri plates)
Fig. 6 Laboratory equipment in the Private Hospital Laboratory (37 °C Incubator)

fragrance "heavier". Therefore, the quantity of wine was slightly increased, in order to further "liquify" the final product, making of it more malleable for kneading, though without boiling. Finally, the fragrance of the new product is somehow different than that of the *kyphi* of the 1st Experiment, due to the addition of all ingredients that were not included at that time (*Cymbopogon schœnanthus* L., *Lawsonia inermis* L., *Cyperus longus* L., *Acacia nilotica* (L.) Wild., *Pistacia terebinthus* L.).

According to the results of the 1st Experiment, the properties (antiseptic and odoriferous) of the product were preserved almost intact, due to the fact that the *kyphi* was kept hermetically closed in glass recipients and stored in a dark place (out of a refrigerator, as there were no such devices in Antiquity). Under these circumstances, this shows that *kyphi* does not seem to be a sensitive substance. The freshly produced *kyphi* from the 2nd Experiment is being stored in the same manner. Additionally, it was noticed that the final product of the 2nd Experiment hardens a bit more easily than that of the 1st Experiment, which was the reason to cut it off in many small pieces for easier handling, especially for the forthcoming fumigation experiments. Comparative experiments using the old *kyphi* from the 1st Experiment (2017) and the new one from the 2nd Experiment (2023) are also currently being performed and the results are going to be thoroughly discussed later.

Based on the recent results obtained from two independent Laboratories (see *supra*), the present study attempted, in a third independent Laboratory, both to replicate a part of the previous experiments and to extend the scientific investigation, comparing the effectiveness of ancient Egyptian *kyphi* and of pure smoke[5] (provening from coconut hookah charcoal) on other micro-organisms.

The basic aim of the present study is to investigate and compare the possible antimicrobial effect of pure smoke and of the *kyphi* by performing fumigation experiments on known micro-organisms (bacteria, fungi and cocci). For the fumigation procedure, an improvised construction was used made of two clay containers (where the smoking materials were placed), an iron tripod and a Berzelius glass beaker with a liquid capacity of 5000 ml (fig. 4).

Given the observations from the two prior experiments (Maravelia – Faviou – Filianos 2022: 7–41), indicating that fumigation (using pure smoke or *kyphi*) on culture materials had almost no impact on the growth of micro-organisms, it was decided to limit the comparison of the effectiveness of *kyphi* and pure smoke in experiments where the inoculation of micro-organisms on suitable nutrient materials (see *infra*) preceded the smoking process.

The tested micro-organisms have been selected as representative of several classes of characteristic microbes, in order to include typical species of each one; thus, these were *Morganella morganii* W. ssp *morganii* [Gram (–) bacterium], *Escherichia coli* M. [Gram (–) bacterium], *Staphylococcus hominis* K.&S. ssp *hominis* [Gram (+) coccus], *Staphylococcus epidermidis* W.&W. [Gram (+) coccus], *Klebsiella pneumoniæ* S.&T. ssp *pneumoniae* [Gram (–) bacterium] and *Candida albicans* (R.) B. [Gram (+) fungus]. It should be noted that the collection of micro-organisms originated from cultures of daily laboratory routines. The strains were isolated from a private laboratory working on an outpatient basis and from a private hospital (fig. 5). Specifically, the strain of *Klebsiella pneumoniæ* used in our experiments was a multidrug-resistant strain (KPC carbapenemase producing strain) derived from a patient's bronchial secretions, hospitalized for an extended period, and the strain of *Staphylococcus epidermidis* was MRSE (+). It is important to note that both these multi-drug resistant strains were not existent in ancient Egypt, thus in non resistant strains the fumigation might possibly give more effective results.

Micro-organism identification was performed using the automatic identification and susceptibility Test System VITEK® 2 Compact (fig. 6), ensuring maximum assurance of result quality and high discrimination between species. Susceptibility tests to a wide range of antibiotics were conducted using the Kirby-Bauer Method, and the results were interpreted using the CLSI Guidelines for antimicrobial susceptibility testing (CLSI 2023. M100 Performance Standards for Antimicrobial Susceptibility Testing), confirmed by the VITEK® 2 Compact system using MIC. All cultivations were performed at a constant temperature of 37 °C during the first 24 hours, with the culture of all Gram (+) and Gram (–) bacteria remaining at the same temperature on the second day, while the temperature of the culture of *Candida albicans* was reduced to 25 °C, because it is the optimal temperature for its growth (as is the case for sev-

5 Usually, different species of wood have different components (see *e.g.* Toledo 2008: 55–61). For wood–smoke in general, see Helmenstine (2021). For the current experiments small «church» incense–burner's charcoal pieces (Toledo 2008: 55, 57, tab. 3) were used, provening from coconut hookah. The chemical composition of smoke is in fact extremely complex.

eral other fungi). The colonies for every microbe were counted in one quartenary of the Petri dishes and, assuming an even spread of colonies, they were multiplied by four.

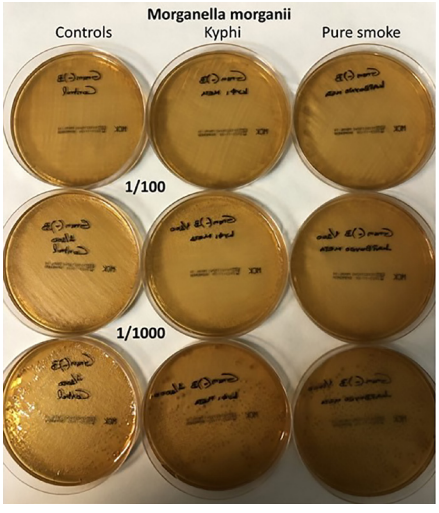
Process A – Controls: Cultivation with inoculum of Gram (+) cocci, Gram (–) bacteria, and fungi at dilutions of 1/100 and 1/1000 in sterile distilled water on the following Biomerieux SA or Bioprepare SA Petri Plates: Columbia Agar + 5% sheep blood (COS) or Blood Agar (BA), Mac Conkey Agar (MCK or MC2), Sabouraud Agar (SGC2 or SAB) Petri Plates. The initial bacterial suspension (0.5 McFarland) in distilled water was serially diluted until a 1/100 and a 1/1000 dilution were achieved, with inoculation done using a 1 µl stylus. The tested micro--organisms were *Morganella morganii* ssp *morganii*, *Escherichia coli*, *Staphylococcus hominis* ssp *hominis*, *Staphylococcus epidermidis*, *Klebsiella pneumoniæ* ssp *pneumoniæ* and *Candida albicans*.

Process B – Fumigation: Cultivation with inoculum of the micro-organisms at dilutions of 1/100 and 1/1000 on Columbia Agar + 5% sheep blood (COS) or Blood Agar (BA), Mac Conkey Agar (MCK or MC2), Sabouraud Agar (SGC2 or SAB) Petri Plates. The Petri dishes, after inoculating the micro-organisms, underwent a smoking process with *kyphi* and pure smoke for approximately 30 min, with inoculation performed using a 1 µl stylus.

RESULTS

Regarding the dishes from Process B, the following observations were made:

1. When compared to the controls of *Morganella morganii* (Process A), the plates exposed to *kyphi* showed a growth of 90% at the 1/100 dilution and 65% at the 1/1000 dilution, while those exposed to pure smoke demonstrated a growth of 100% at 1/100 and 85% at 1/1000 dilutions (figs. 7a–7b).



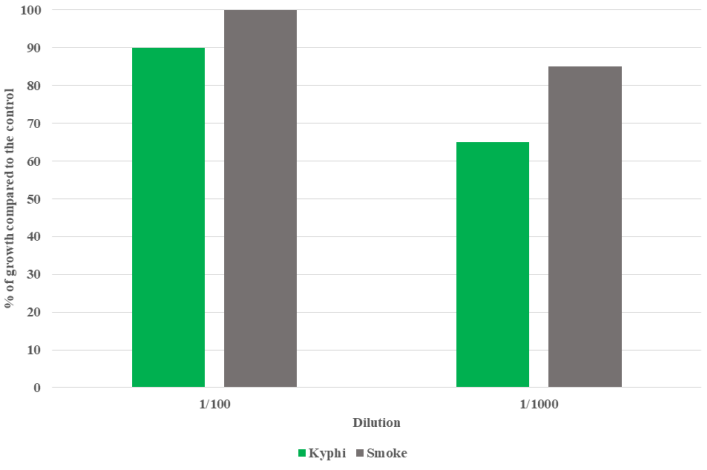
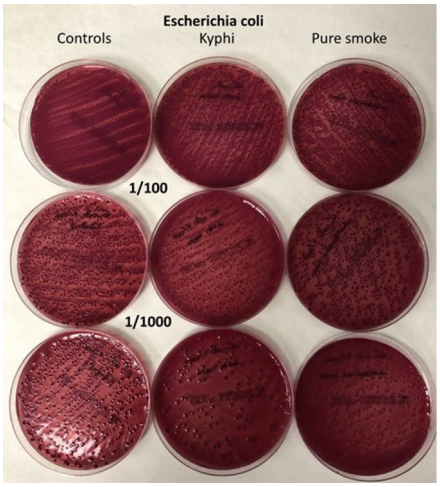


Fig. 7a (left) Effect of different smoking agents on the Mac Conkey Agar Petri plates of Process B on *Morganella morganii W.* (between them and in relation to the controls)
Fig. 7b (right) Bar chart of the effect of *kyphi* (green) and pure smoke (grey) on the growth of *Morganella morganii W.* in different dilutions

2. Compared to the controls of *Escherichia coli* (Process A), the plates exposed to *kyphi* exhibited a growth of 70% at 1/100 dilution and 30% at 1/1000, while the plates exposed to pure smoke showed a growth of 85% at both dilutions (figs. 8a–8b).



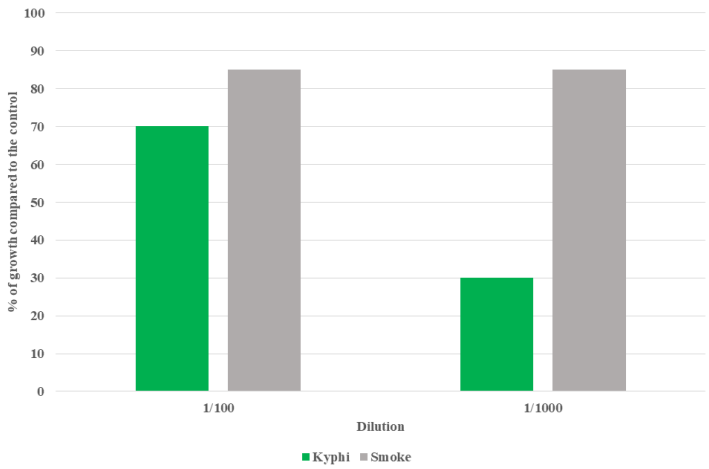
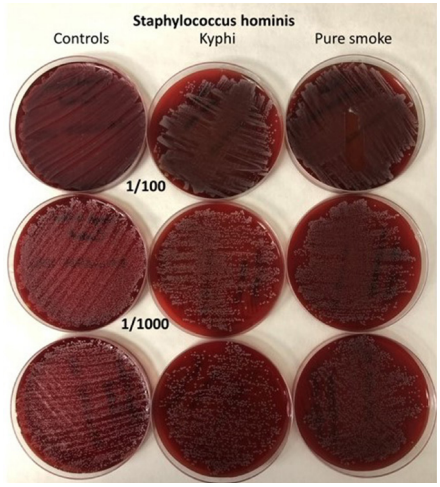


Fig. 8a (left) Effect of different smoking agents on the Mac Conkey Agar Petri plates of Process B on *Escherichia coli M.* (between them and in relation to the controls).
Fig. 8b (right) Bar chart of the effect of *kyphi* (green) and pure smoke (grey) on the growth of *Escherichia coli M.* in different dilutions

3. Compared to the controls of *Staphylococcus hominis*, the plates exposed to *kyphi* displayed a growth of 70% at 1/100 dilution and 55% at 1/1000, while those exposed to pure smoke showed a growth of 95% at 1/100 and 90% at 1/1000 dilutions (figs. 9a–9b).



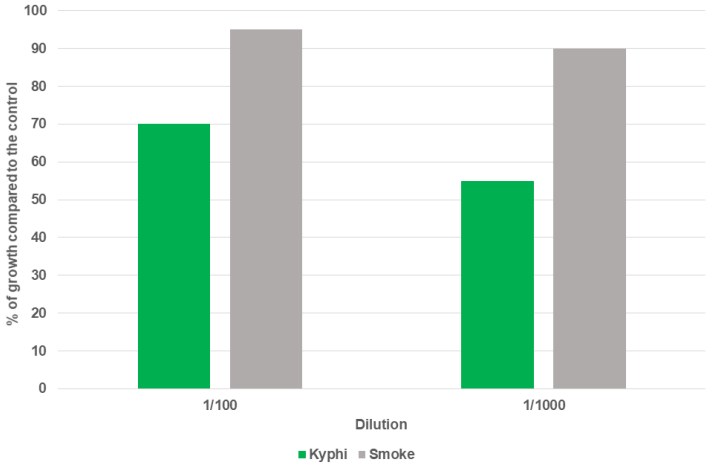
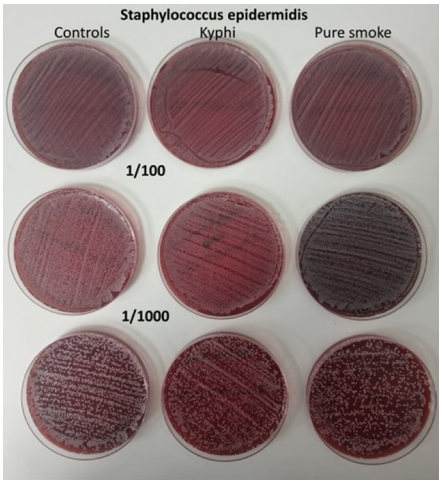


Fig. 9a (left) Effect of different smoking agents on the Columbia Agar + 5% Sheep Blood Plates or Blood Agar Petri plates of Process B on *Staphylococcus hominis* K.&S. (between them and in relation to the controls)
Fig. 9b (right) Bar chart of the effect of *kyphi* (green) and pure smoke (grey) on the growth of *Staphylococcus hominis* K.&S. in different dilutions

4. There was no difference in growth for plates exposed to *kyphi* or pure smoke at 1/100 dilutions for *Staphylococcus epidermidis*. At 1/1000 dilution, plates exposed to *kyphi* had a growth of 70%, while those exposed to pure smoke had a growth of 90% (figs. 10a–10b).



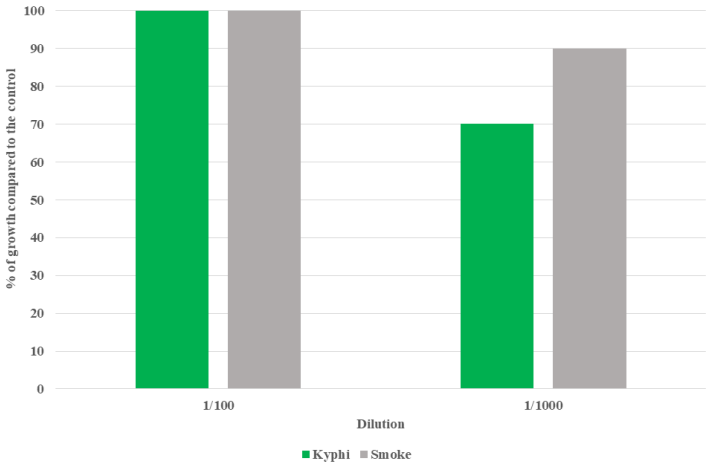
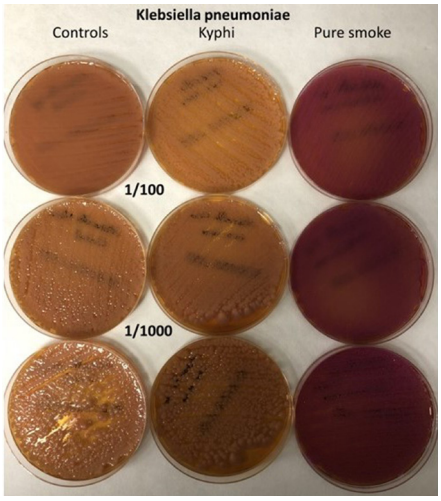


Fig. 10a (left) Effect of different smoking agents on the Columbia Agar + 5% Sheep Blood Plates or Blood Agar Petri plates of Process B on *Staphylococcus epidermidis* W.&W. (between them and in relation to the controls)
Fig. 10b (right) Bar chart of the effect of *kyphi* (green) and pure smoke (grey) on the growth of *Staphylococcus epidermidis* W.&W. in different dilutions

5. At 1/100 dilution, there was no difference between plates exposed to *kyphi* or pure smoke for *Klebsiella pneumoniæ*. At 1/1000, both showed a growth of approximately 95–98% compared to controls (figs. 11a–11b). However, as already noted *supra*, this strain is multi-drug resistant.



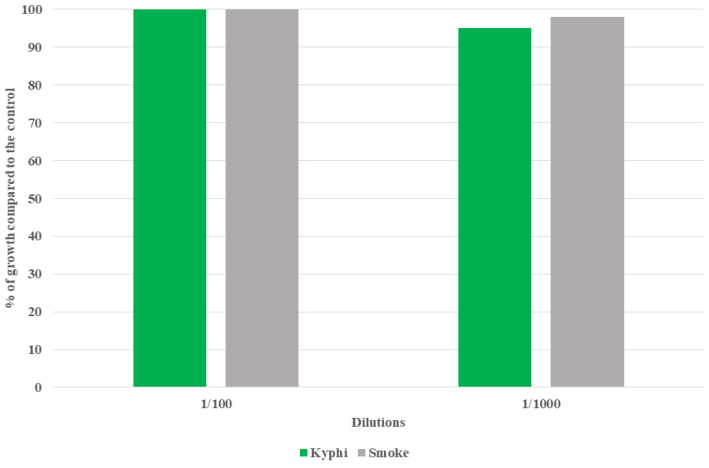
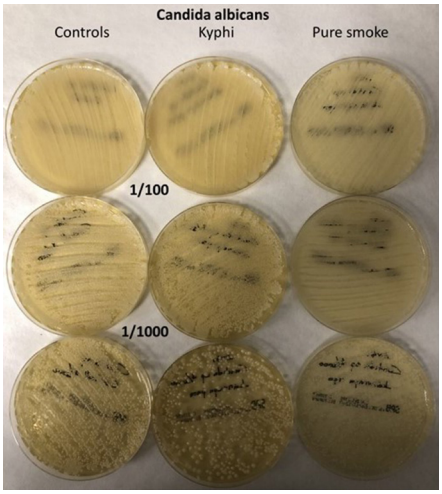


Fig. 11a (left) Effect of different smoking agents on the Mac Conkey Agar Petri Plates of Process B on *Klebsiella pneumoniæ* S.&T. (between them and in relation to the controls).
Fig. 11b (right) Bar chart of the effect of *kyphi* (green) and pure smoke (grey) on the growth of *Klebsiella pneumoniæ* S.&T. in different dilutions

6. Compared to the controls of *Candida albicans*, plates exposed to *kyphi* showed a growth of 90% at 1/100 and 35% at 1/1000, while those exposed to pure smoke displayed a growth of 100% at 1/100 and 85% at 1/1000 dilutions (figs. 12a–12b).



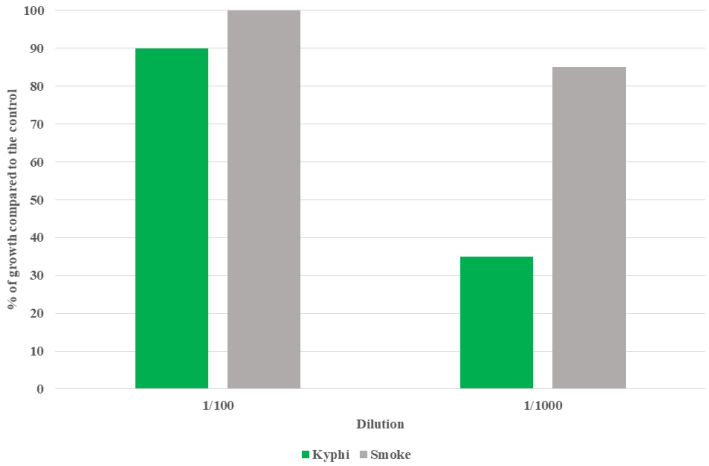


Fig. 12a (left) Effect of different smoking agents on the Sabouraud Agar (SGC2 or SAB) Petri Plates of Process B on *Candida albicans* (R.) B. (between them and in relation to the controls).
Fig. 12b (right) Bar chart of the effect of *kyphi* (green) and pure smoke (grey) on the growth of *Candida albicans* (R.) B. in different dilutions

DISCUSSION

The medicinal and pharmaceutical properties of the *kyphi*'s ingredients (Maravelia – Filianos 2020: 266–283; Maravelia – Faviou – Filianos 2022: 10–12; most recently Maravelia – Faviou – Filianos 2023: 10–29) were, to a more or lesser extent, expected to justify the above action of fumigation. From plants of the same species used in the *kyphi* recipes or from plants of different species but of the same *genus* effective results of the action of each plant's smoke produced during its burning have been reported (Pennacchio *et al.* 2010; Moursi 1992; Germer 1993: 69–80; van Wyk – Wink 2004; Turner 2004; Peter 2006; Kaliora – Kountouri 2012: 261–284):

1. *Acorus calamus* L. (Pennacchio *et al.* 2010: 35; Germer 2008: 142; Sharma *et al.* 2014: 1454–1466; *cf.* Γεννάδιος 1914: 36): used against piles or hemorrhoids, epilepsy, hysteria, toothache, colds.
2. *Andropogon schœnanthus* L. (Germer 2008: 129, 246 – contra Loret ²1892; 'Al-Snafi 2016: 53–61; *cf.* also Γεννάδιος 1914: 115–116; Zahran – Willis 2008: 363–364, *Cymbopogon schœnanthus* L.): its essential oil is used as antispasmodic, diuretic and antihistaminic, as well as for flavouring; its crude oil is used as insecticide.
3. *Cinnamomum zeylanicum* Nees (Pennacchio *et al.* 2010: 69; Rao – Gan 2014: 1–12; Germer 2008: 151–152; *cf.* Γεννάδιος 1914: 507–508): used as antioxidant, antiinflammatory, antipyretic, antibacterial, antifungal and antidiabetic, in general against diseases, as incense, as perfume for clothes, etc.

4. *Laurus cassia* J. Presl. = *Cinnamomum cassia* J. Presl. (Pennacchio *et al.* 2010: 69; Rao – Gan 2014: 1–12; *cf.* also Γεννάδιος 1914: 508): used as antidiabetic, antispasmodic, against colds, infections and hernia, as incense, etc.
5. *Mentha piperita* L. (Pennacchio *et al.* 2010: 24, 122; Ait-Ouazzou – Lorán *et al.* 2012: 313–319; Akbari *et al.* 2015: 413–420; also Ahmed *et al.* 2018: 463–475; Long 1984: 145–159; *cf.* Γεννάδιος 1914: 409–410): used against mental diseases and cold, for menthol cigarettes, to repel mosquitoes, as insecticide, for cigarettes against asthma, against tuberculosis and for other respiration ailments, against IBS and indigestion, as an oxytocic.
6. *Calycotome villosa* (Poir.) Link (Pistelli *et al.* 2003: 417–419; Alhage *et al.* 2018: 851; Cherfia *et al.* 2020: 103535):[6] having a moderate antimicrobial potency and a good ability to scavenge DPPH, it has been used (in Sicilian folk medicine) for cutaneous abscesses, furuncles, chilblains, as an anti-tumour agent; and as an infusion of its flowers (in Palestine) for cardiovascular and nervous system problems; its extracts (methanol and dichloromethane stems) have moderate antimicrobial potency, but show no antiinflammatory, no hypoglycemic and no haemolytic effects.
7. *Juniperus phœnicea* L. (Pennacchio *et al.* 2010: 109; Ait-Ouazzou *et al.* 2012: 313–319; Germer 2008: 14: *Juniperus phœnicea* L., 46, 50–53, 276–278: *Juniperus oxycedrus* L. and *Juniperus phœnicea* L.; *cf.* also Γεννάδιος 1914: 584–587): Hippocrates used it against plague in Athens (430 BC); also used to purify/air-out hospital rooms, as digestive, against rheumatisms, for disinfections, against asthma and for colds (according to Avicenna, it is abortifacient); added to tobacco it enhances flavour; it is used for cooking to give pleasant aromas, while Shamans use it for childbirth, against colds and headache, after illnesses, to purify the air, against Eagle sickness and to cure insanity.
8. *Acacia farnesiana/nilotica* (L.) Wild. (Pennacchio *et al.* 2010: 33; Germer 2008: 15, 46, 135–139, 142, 176–178: *Acacia nilotica* (L.) Del.; Deshmukh – Bhajipale 2018: 24–34; *cf.* Γεννάδιος 1914: 27–29): used as antimicrobial, antihyperglycemic and antispasmodic, against diarrhea, to strengthen new born babies, to induce lactation, to promote good health for babies, against epilepsy, as a perfume, as a disinfectant, for post partum bleeding, to clean milk containers, for over-excited children, for deep and lengthy sleep, against migraines and as an insecticide.
9. *Lawsonia inermis* L. (Pennacchio *et al.* 2010: 115; Germer 2008: 96, 280–282, 151–152; Semwal *et al.* 2014: 80–103; Petrovics *et al.* 2022: 67–71; *cf.* Γεννάδιος 1914: 599): used to cure skin infections, to heal wounds and burns, for hair health and to flavour milk-gourds.
10. *Cyperus longus* L. (Ait-Ouazzou *et al.* 2012: 313–319; Lawal – Oyedeji 2009: 2909–2917; Tran 2014: 74–77; *cf.* Samra *et al.* 2013: 648–659; Aghassi *et al.* 2013: 382–386; Germer 2008: 18, 91, 146–148, 251–252; Γεννάδιος 1914: 587–588): used to fumigate the body during sickness, as incense, and for treating deep wounds.
11. *Pistacia terebinthus* L. (Pennacchio *et al.* 2010: 143, *idem* for *Pistacia lentiscus* L.; Loret 1949; Germer 2008: 45, 115, 117–122, 131, 212, 319–321, *Pistacia terebinthus* L. and *Pistacia lentiscus* L.;

6 Note that calythropsin is an hypoglycemic (90%, 0.3 mg/ml), most effective in DPPH scavenging assay (100%, 0.1 mg/ml) and cytotoxic assay on HeLa cells (99%, 0.1 mg/ml after 24^{H}; 90% after 48^{H}); the dichloromethane, ethanol and aqueous extracts had also inhibited HeLa cells proliferation at 2 mg/ml (*cf.* Γεννάδιος 1914: 151–152).

Γεννάδιος 1914: 783, 785, 946; Καββάδα 1964: VII, 3144): used as antiseptic, antispasmodic, cytostatic, expectorant and vulnerary; according to Avicenna, it is used for sore eyes.

12a. *Pistacia lentiscus* L. (Tassou – Nychas 1995: 411–420; Magiatis *et al.* 1999: 749–752; de Vartavan 2007: 63–92; Γεννάδιος 1914: 783): used to flavour meats, as incense, for open sores, coughing, etc.

12b. *Pistacia lentiscus* L. var. *chia*[7] (Chios mastic; Kaliora – Kountouri 2012: 261–284; Committee on Herbal Medicinal Products 2015; Pachi – Mikropoulou *et al.* 2020: 112485; Pachi – Mikropoulou *et al.* 2021: 418): it exhibits antiinflammatory, antidiabetic and healing properties, while it is also used against gastro-intestinal disorders and as *par excellence* effective mouth flavouring. According to studies *in vitro* against Gram (+) and Gram (–) bacteria and fungi, a limitation of rate or prohibition of the development and in cases extermination of the pathogens has been proved, corroborating the results of the current paper. Additionally, and in accordance to these results, mastic is very active against *Staphylococcus aureus* R., *Lactobacillus plantarum* B., *Pseudomonas fragi* E., *Salmonella enteritidis* (K.&E.), *Escherichia Coli* M., etc.

13 and 14. *Vitis vinifera* L. and raisins (Μανούσος – Λαδοπούλου 2003: 1–18; Murray *et al.* [5]2009: 577–608; Vadas 2020: 93–132; *cf.* Γεννάδιος 1914: 53–101)[8]: used as excipient in the *vina medicata*, against vascular and cardiac ailments, and (externally) as a mild antiseptic.

15. Honey (Zumla – Lulat 1989: 384–385; Lafont 2017: 97–121): used as antiseptic, antibacterial, antioxidant, against cough and as a basis for *electuaria*.

16. *Commiphora myrrha* Engl. (Pennacchio *et al.* 2010: 73; Van-Beak 1960: 70–95; Janzen *et al.* 1989; also Steuer 1933; Steuer 1943: 279–284; Groom 1981; Lecocq 2009: 107–130; Germer 2008: 32, 43–45, 88, 106, 117, 122, 230–232; *cf.* also Γεννάδιος 1914: 169–170): used as incense, as antiseptic, against migraine and headache, to perfume the clothes, to strengthen new born babies, to clean the body, against breathing difficulties, for chest colds, for swollen glands, to purify the air, against nasal catarrh, laryngitis, bronchitis, fever, against asthma, as a flavouring agent for foods and beverages, to keep snakes away, and (of course) as incense for religious ceremonies.

The antimicrobial effects of medicinal plants against several bacterial pathogens, including *Escherichia coli* and *Candida parapsilosis*, have been thoroughly discussed.[9] The same is true

7 On the general beneficial properties of the mastic see Fukazawa *et al.* (2018: 773–780). It is to be noted that mastic can also act as an antioxidant (Andrikopoulos *et al.* 2002: 279–289); it can also inhibit cancer generation and the proliferation of cancer cells (Λουτράρη *et al.* 2006: 1–4). On its biological activity and its essential oil, see Andrikopoulos *et al.* (2003: 501–507) and Koutsoudaki *et al.* (2005: 7681–7685).

8 For more on wine and vineyards, see Otto – Helck –Westendorf (1986: col. 1169–1182 s.v. Wein, col. 1190–1192 s.v. Weintrauben, *cf.* col. 1186–1190 s.v. Weinopfer). See also Germer (2008: 18–19, 32, 54–55 s.v. raisins, 362–363 s.v. *Vitis vinifera* L.).

9 For *E. coli*, see *e.g.* Peter (2006: 154–156) for enteric bacterial pathogens, including *E. coli*, with cinnamon oil and its constituents (cinnamaldehyde and eugenol) acting against it; *cf.* also Peter (2006*:* 352, 429, 449–450, *passim*). For *Candida albicans*, see Peter (2006: 361) and Samadi *et al.* (2019: 28–32), mainly for *C. albicans*, referring also to *C. parapsilosis*, highlighting the best inhibitory antifungal effects of *Lawsonia inermis* L. against these fungi. For the antifungal properties of *Cinnamomum verum* J. Presl = *Cinnamomum zeylanicum* Nees (ancient Egyptian *tišps*), see Hsu *et al.* (2021: 90–117).

for many other micro-organisms, such as *Staphylococcus aureus* and *Staphylococcus capitis*,[10] *Staphylococcus hominis* and *Staphylococcus epidermidis*.[11] The same holds also for *Morganella morganii*[12] and *Klebsiella pneumoniæ*[13]. In fact, there is a very rich literature on the action of pharmaceutical plants against pathogenic micro-organisms, with promising results.

Furthermore, comparison with our previous experiments (Maravelia – Faviou – Filianos 2022: 7–41; Faviou – Magiorkinis – Maravelia – Filianos 2023: 25–42) shows that the impact of *kyphi* on the common tested micro-organism in all three of them was stable (figs. 13a–13b). In the last figures, the consistent observations regarding the impact of *kyphi* and pure smoke on the micro-organism *Escherichia coli* M. across various dilutions are evident. Our findings indicate a notable antimicrobial effect of *kyphi* on this prevalent Gram(–) bacterium, which was slightly augmented with the application of the recently confected *kyphi* containing all its sixteen ingredients. Given the random selection of micro-organisms from routine laboratory cultures

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There is a rather rich literature on these topics, of which only the most important related works are hereby refered to (the same holds too for nos. 10–13, *infra*).

10 For *Staphylococcus aureus* and *Staphylococcus capitis*, see *e.g.* Newstead – Varjonen *et al.* (2020: 40) for staphylococcal-produced bacteriocins and antimicrobial peptides acting against them; Gishen – Tadesse *et al.* (2020: 101121) for the antimicrobial activity of 6 Ethiopian Medicinal Plants against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*, between which *Mentha piperita*, *Cymbopogon citratus*, etc. For *Staphylococcus capitis*, see Yu – Zheng – Xiao *et al.* (2020: 2017–2026), mainly for short-term clinical antibiotic use associated with resistance mutations, collateral sensitivity, and positive *in vivo* fitness advantages to this coccus during infection. For the anti-*Staphylococcus aureus* activity of methanol extracts of 12 plants used in Cameroonian Folk Medicine, see Fonkeng – Mouokeu *et al.* (2015: 710), whose results are interesting indeed, as they may be connected to the as of old African tradition of using (medicinal) plants against pathogenic organisms. Finally, it is worthwile noting that Limonoids (phytochemicals of the Triterpenoid Class), as well as dehydroabietic acid, can act against multi-drug resistant pathogens as *S. aureus* and *S. capitis* (see *e.g.* Subramani – Narayanasamy – Feussner 2017: 172), having also in mind that the latter acidic compound is one of the basic constituents of coniferous resins (used also in mummification; see *e.g.* ᶜAbd ʾel-Maksoud – ʾEl-Amin 2011: 129–150).

11 For *Staphylococcus hominis* and *Staphylococcus epidermidis*, see other literature (*e.g.* Parekh – Chanda 2008: 63–71; Silva – Fernandes Júnior 2010: 402–413); *cf.* Chovanová *et al.* (2013: 760969), studying the *in vitro* antibacterial and antibiotic resistance modifying effect of bioactive plant extracts on methicillin-resistant *Staphylococcus epidermidis*; Kačániová – Terentjeva *et al.* (2020: 765), referring to the strongest antimicrobial activity of the *Juniperus communis* essential oil found against *Staphylococcus hominis*; and also Wirasisya – Hamid *et al.* (2023: 1–8).

12 For *Morganella morganii*, see *e.g.* ʾAl-Defiery – Al-Terehi *et al.* (2019: 1403–1409) on the antimicrobial activity of some plant extracts, provening from plants different than the *kyphi* ingredients, against it and other pathogenic bacteria; also Abdallah – ᶜAbd ʾEl-Rahman *et al.* (2013: 115–124).

13 For *Klebsiella pneumoniæ*, see mainly Gowsiya – Sujatha – Santosh (2014: 135–147), who study many efficient medicinal plants as a source of antibacterial agents to counter *Klebsiella pneumoniæ*, interestingly including *Acacia nilotica* L. and *Cinnamomum zeylanicum* Nees. Bhatia – Sharma *et al.* (2021: e06310) discuss the antibacterial activity of medicinal plants against it and other micro-organisms, using also plants such as *Cinnamomum zeylanicum* Nees, *Mentha* sp., *Lawsonia inermis* L., *Acacia nilotica* L., and other relative species, like *Cinnamomum tamala* Nees, *Cymbopogon citratus* (DC.) Stapf, & c.: this paper is very important, because it discusses the efficiency of pharmaceutical plants against ESKAPE-pathogens, including *Klebsiella pneumoniæ*, *Staphylococcus aureus*, & c., presenting a rich bibliography too. See also Shahidi Bonjar (2004: 82–86) that offers evaluation of antibacterial properties of Iranian medicinal plants, including *Lawsonia inermis* L., against it and against other pathogenic strains; also Dostalova *et al.* (2014: 403–406).

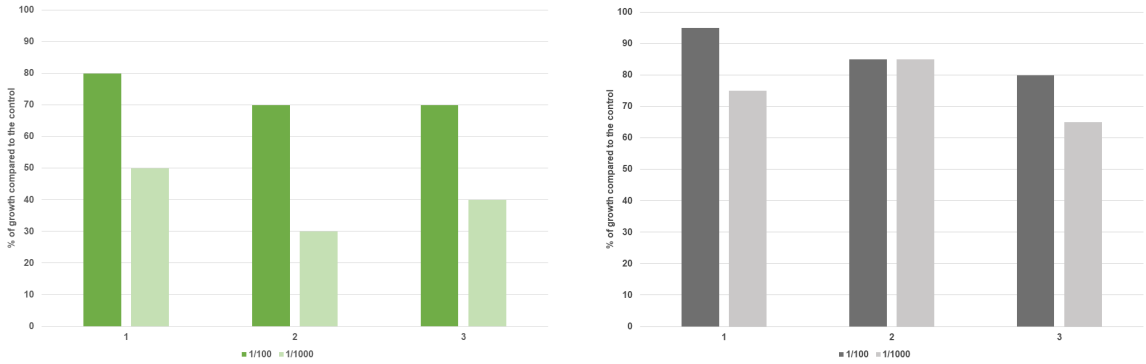


Fig. 13a (left) Effects of *kyphi* on Escherichia coli M. during all three experiments (previous and current), in different solutions (dark green = 1/100, light green = 1/1000)
Fig. 13b (right) Effects of pure smoke on E. coli during all three experiments, as above (dark grey = 1/100, light grey = 1/1000; for both, see 1: Maravelia et al. 2022: 17–19, 21–27; 2: Faviou et al. 2023: 32; 3: current paper)

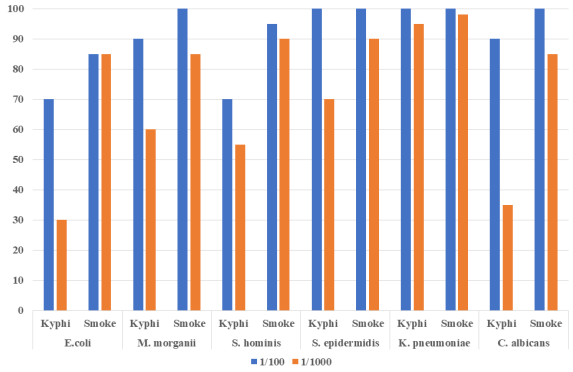
of each independent facility, *Escherichia coli* M. emerged as the only common micro-organism across all participating Laboratories, both in previous and current studies. However, in upcoming studies, we intend to replicate our experiments using the freshly confected *kyphi* and other micro-organisms, such as *Staphylococcus aureus* R., which have been previously examined.

After the current corroboration of the results of our previous study (see Conclusions), the current *Kyphi* Project shall continue with more experiments, applying statistical analysis, using more sophisticated methods, (ethanol- and other) extracts of *kyphi* and of its ingredients, antibiotic-discs (to present antibiograms and special graphs) and with the testing of many more microbes. Consequently, the most "active" component(s) of the *kyphi* shall be detected, as well as the pathophysiological mechanism through which answers can be provided concerning the antimicrobial action of *kyphi*. *In vivo*, the balsamic properties of all the aromatic ingredients of *kyphi* should also be taken into account, as well as their capacity to be absorbed, hence entering the circulation and excreted by the lungs, thus exercising an antiseptic action on the human organism from inside.

CONCLUSIONS

With the completion of this 3rd experimental study in several independent Microbiological Laboratories, it became evident that there is a clear direct effect of smoke (pure smoke and *kyphi*) on micro-organisms, which appears much more pronounced with *kyphi*, especially at the 1/1000 dilution (Process B) (fig. 14a). Additionally, our experimental data reinforce the evidence from previous studies that the impact of *kyphi* on yeasts is mostly greater than that on bacteria and cocci, with indeed notable results at the 1/1000 dilution [fig. 14b]. Moreover, according to the current results, an equally strong action of *kyphi* on Gram (–) bacteria was observed, leading to a growth of only around 30% at the 1/1000 dilution for a common strain of *Escherichia coli* and 65% for the corresponding dilution of another Gram (–) bacterium of the community, namely *Morganella morganii* (fig. 14b). The effect of fumigation on *Candida albicans* (35% at the 1/1000 dilution for a common strain) was also very strong. However, regarding Gram (–) bacteria, it became apparent that the effect of smoke (pure smoke and *kyphi*) on antibiotic-resistant strains (*i.e.*, strains formed after the irrational use of antibiotics over

time) is negligible to nonexistent (figs. 14a–14b). Similarly, the effectiveness of *kyphi* seemed reduced on Gram (+) Staphylococci when the strains were methicillin resistant. Our future experiments will test the *kyphi*-ingredients (organic and inorganic) separately for their corresponding antimicrobial properties.



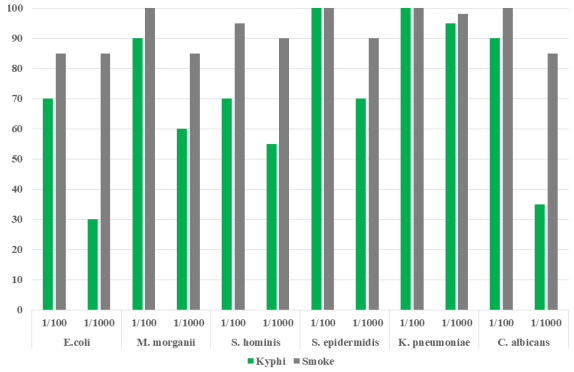


Fig. 14a (left) Composite bar chart of the effect of *kyphi* and pure smoke on all strains in different dilutions (blue = 1/100, orange = 1/1000). In both diagrams, the ordinate shows the percentage of growth compared to the control

Fig. 14b (right) Composite bar chart of the effect of *kyphi* (green) and pure smoke (grey) on the growths of all strains comparatively for each dilution

Hence, *kyphi* was indeed an ancient *ὀσφράδιον/ὀσφραντήριον* (Latin: *Olfactorium*)[14], both an incense *and* a pharmaceutical substance with quite noticeable anti-fungal and anti-microbial properties. Given that the ancient Egyptian culture was probably the highest in NE Africa and the Middle East, one may guess that *kyphi* was not randomly composed, but consisted the outcome of a proto-scientific process of trial-and-error, hence it was a laboratory-confected experimental concoction, a *μῖγμα* (Latin: *commixtio*, *mixtura cuncta*), whose many properties were such and beneficial in several aspects. Let it be noted that the properties of the Orthodox Holy Chrism[15] against various pathogenic micro-organisms shall also be studied in the immediate future (of course before consecration!). The Hellenic Institute of Egyptology, with its international Research Projects (between which *Kyphi* and Egypt/Herbal) is endeavouring to open new ways towards modern sophisticated methods and approaches for the interdisciplinary study of (Egyptian) Antiquity, ancient Medicine/Pharmacology and (Ethno)Botany.

14 See *e.g.* Liddell – Scott (1968: *s.vv.* ὀσφράδιον, ὀσφραντήριον, μῖγμα). The *kyphi* was a *cunctum* that reminds us (more or less) of the following classic definition: *Mixtura seu mistura, in Chemia, est quod provenit cum duæ aut plures materiæ chemicæ nulla reactione chemica intercedente miscentur. Ingredientes mixturæ, cum una miscentur, naturas chemicas suas adtinent. Tamen physicæ mixturæ proprietates, sicut punctum fusionis, possunt discrepare a proprietatibus componentum singulorum. Ob naturas componentum adtentas, mixturæ usitate in componentes originales modo facultatibus mechanicis dividi possunt. Compositum chemicum, quod sic dividi non potest, igitur exemplum mixturæ non est*. As the *in vitro* confected *kyphi*, for the purposes of this study, was prepared without any boiling (see Introduction, *supra*) or any other *ad hoc* chemical reaction, the definition of what a mixture is fits perfectly for this case.

15 As the fundamental study on the Holy Orthodox Chrism always remains Μενεβίσογλου ([1]1972); and other studies (Κωνσταντινίδης – Παλαβίδης 2000; Χαραμαντίδης 2012; additionally, see also Savvits *et al.* 1989: 1–9; Maravelia – Filianos 2020: 284–295; Maravelia – Faviou – Filianos 2023: 30–41).

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