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Antifungal and antibacterial potential of essential oil of *Boswellia sacra* resin in the biological control

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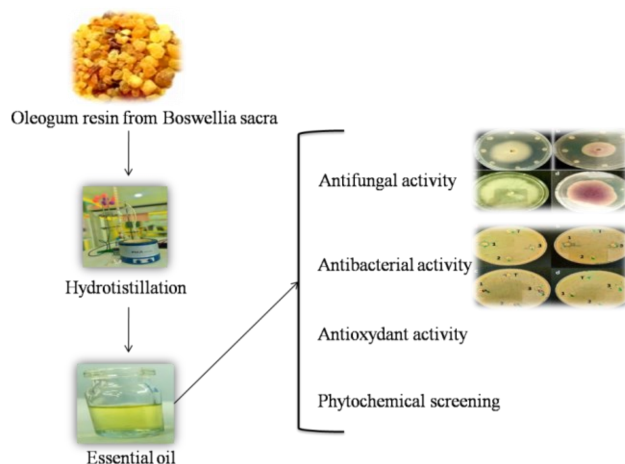
Graphical abstract



Abstract

This study focuses on extraction of *Boswellia sacra* resin essential oil that was performed using the hydrodistillation method, resulting in a yield of 2.45%. Phytochemical screening of the essential oil provided information about its major compounds, which include terpenes and anthocyanins. To assess the antioxidant and antimicrobial effects of the essential oil, multiple methods were employed. Positive results were obtained from tests such as DPPH and ABTS free radical scavenging, inhibition of bovine serum albumin protein denaturation, and antifungal and antibacterial assays. These tests confirmed the presence of antifungal, antibacterial and antioxidant activities and suggest numerous biotechnological applications of the essential oil.

Keywords: Boswellia sacra, antifungal activity, antibacterial activity, essential oil and biological control.

1. Introduction

As resinous substance Frankincense (Gum olibanum) (Boswellia sacra) belong to the botanical family Burseraceae, it is widespread both in Southern Arabian Peninsula and southeast Africa (Elmoslemany *et al.* 2024). Frankincense is known for having antifungal, antibacterial, and several pharmacological properties (Khalifa *et al.* 2023).

Aromatic and medicinal plants provide significant advantages through the discovery of their nutritional values, essential oils and their diverse applications across various domains (Ait Bouzid *et al.* 2024).

Within the field of medicinal plants research, this study focuses on the essential oil extracted from the resin of frankincense trees (Di Stefano *et al.* 2020). Frankincense is utilized in several industries, including cosmetics, pharmaceuticals, beverages, food, detergents, and perfumes (Al-Saidi *et al.* 2012). The essential oil derived from this kind of trees offers numerous benefits for human well-being, such as analgesic, anticoagulant, antioxidant, anti-inflammatory and a potential role in skin disorders (Khan *et al.* 2018). It serves as a significant source of bioactive compounds, notably terpenoids that exhibit a wide range of biological activities (El-Araby *et al.* 2021).

Given these factors, it is compelling to evaluate the biological activities of Frankincense essential oil with the goal of harnessing its qualities in the field of biotechnology. This effort is motivated by the desire to increase the value of the by-product through its incorporation into different biotechnological processes thereby promoting the development of natural, effective, and high-quality products.

2. Materials and methods

2.1. Vegetal material

Frankincense, the oleogum resin from *Boswellia sacra* of the Burseraceae family and imported from Yemen, was acquired from an herbalist in Guelma 36° 28′ 00″ North, 7° 26′ 00″ East, Algeria. Upon obtaining it, all visible impurities such as wood scrapings and debris were carefully removed. The frankincense then underwent a process of crushing and sieving to produce a finely homogeneous powder.

2.2. Extraction of Frankincense Essential Oil

Hydrodistillation is a conventional method used for extracting essential oils from plant samples and is endorsed by numerous pharmacopoeias (Al-Harrasi and Al-Saidi 2008). To begin this process, 140 g of finely ground frankincense is placed in a 1L bicol flask containing 350 mL

of distilled water. The flask is then heated to boiling using a mantle heater for duration of 7 to 13 hours. After boiling, the resulting liquid contains a delicate layer of the essential oil (EO) on its surface. The mixture is stirred and transferred into a separatory funnel. Tests are performed in triplicate.

The oil yield is evaluated by comparing the mass of the obtained EO to the initial mass of the plant material used. This calculation is expressed as a percentage and is determined as follows

$R= (M_{EO} / M_{mv})\times100$

A: Yield of essential oil (%)

M_{EO}: Mass of essential oil (g)

M_{pm}: Mass of plant material (g)

2.3. Organoleptic examination

Organoleptic examination involves the utilization of human senses such as sight, smell, taste, and touch. This test is commonly used as an initial quality control measure before conducting any further tests (Rusdi *et al.* 2021).

2.4. Phytochemical screening

Phytochemical analysis of the plant's essential oil (HEO) was conducted through solubility tests, precipitation tests, and characteristic color reactions. These methods were employed to preliminarily identify the primary chemical groups present in the HEO (EL-Haoud *et al.* 2018).

2.4.1. Tannin detection

Tannins are detected through their reaction with iron chloride. In a test tube containing 1 mL of the extract, a few drops of 1% iron chloride solution in methanol ($FeCl_3$) are added. Upon agitation, the presence of gallic tannins is indicated by a blue-black color, while the presence of catechin tannins is indicated by a greenish-brown color (Karumi *et al.*, 2004).

2.4.2. Anthocyanins detection

Anthocyanins can be revealed by combining 5 mL of the extract with 5 mL of sulfuric acid (H_2SO_4), followed by the addition of 5 mL of ammonium hydroxide (NH_4OH). In the presence of anthocyanins, the color shifts to red in an acidic medium and purplish-blue in a basic medium (Mibindzou Mouellet 2004).

2.4.3. Coumarins detection

To detect coumarins, 5 mL of the extract is boiled until the volume reduces to 1 mL, and this is then mixed with 1 mL of hot water. After stirring, the resulting solution is divided into two portions. One portion serves as the control, while the other is combined with 0.5 mL of 10% ammonium hydroxide (NH_4OH) before being examined under a UV lamp at 366 nm. The presence of coumarins is indicated by fluorescence emission (Bruneton 2016).

2.4.4. Alkaloids detection

Alkaloids are detected using Mayer's or Wagner's reagents. First, 1.5 mL of 2% hydrochloric acid (HCl) is added to 10 mL of the extract after evaporation to a volume of 0.2 mL. After stirring the acidic solution, one to two drops of Mayer's or Wagner's reagent are added. The presence of alkaloids results in the formation of a yellowish-white or brown precipitate (Mojab *et al.* 2003).

2.4.5. Terpens detection

By adding 1 ml of extract to 0.5 ml of acetic anhydride ($C_4H_6O_3$) and 0.5 ml of chloroform ($CHCl_3$), terpenes become visible. The mixture is then transferred to a test tube, and once it has dissolved, 1 ml of strong sulfuric acid is added. The reaction takes place at room temperature. When sterols and triterpenes are present, a purple or brownish-red ring forms, and the supernatant layer assumes a green or purple hue (Bruneton 2016).

2.4.6. Saponins detection

Saponins dissolve in water, forming a foaming solution. To detect them, a test tube containing 10 mL of extract is shaken for 15 seconds and then allowed to stand for 15 minutes. If a persistent foam height greater than 1 cm is observed, it indicates the presence of saponins (Jiménez *et al.* 2021).

2.5. Antioxidant activity

2.5.1. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test

The DPPH radical scavenging test assesses the antioxidant's ability to participate in hydrogen transfer, thereby reducing the chemical radical DPPH° (2,2-diphenyl-1-picrylhydrazyl).The production of DPPH-H depends on both the concentration of the antioxidant and its specific properties, which govern the reaction rate (Baliyan *et al.* 2022).

2.5.2. 2,2'-azinobis-3ethylbenzothiazoline-6-acid radical scavenging test sulfonic acid (ABTS)

One of the most used methods for assessing the antioxidant activity of substances is the ABTS radical method (Torres-Martínez *et al.* 2018). It is based on reaction between potassium persulfate ($K_2S_2O_8$) and ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)) for generating the green ABTS radical. Antioxidants prevent further association by neutralizing this free radical form, The quantity of antioxidants present is inversely proportional to the absorbance of the radical, which is measured using spectrophotometry at 734 nm (Sánchez-Moreno 2002).

2.6. Antimicrobial activity

Among the most common and fundamental techniques for evaluating antibacterial action (antibiogram) are the disk diffusion methods in agar medium (Rahal 2006). This method is employed to assess the antibacterial and antifungal properties of the essential Oil.

2.6.1. Antibacterial activity

The antibacterial activity of the essential oil produced was tested on four strains of bacteria inoculated on Muller Hinton medium. Gram (+): *Staphylococcus aureus* and *Bacillus cereus* Gram (-): *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The method employed for this evaluation follows the description by (Murray 1999). Diameter measurements are taken after 24 hours of incubation at 37°C.

2.6.2. Anti fungal activity

The antifungal assessment is carried out in accordance with the approach outlined by (Nazzaro *et al.* 2017) with slight modifications. This technique enables the identification of

inhibitory effects of the essential Oil within the solid PDA culture medium against two fungal strains: *Aspergillus niger* and *Fusarium oxysporum*.

Furthermore, the antifungal activity of the essential oil against *Candida albicans* was examined utilizing the disc diffusion method on Sabouraud medium. The measurement of diameters is performed after 48 hours of incubation at 28°C.

3. Results and discussion

3.1. Frankincense Essential Oil exraction and organoleptic examination

After hydrodistillation two distinct phases have been formed, an aqueous phase, at the bottom, representing the hydrosol, and the top one, the organic phase, which is the sought-after essential oil. The essential oil obtained at the end is frozen to eliminate the rest of the hydrosol, and then it is stored at +4°C in a dark bottle. The organoleptic characteristics of olibanum essential oil obtained by hydrodistillation are shown in Table 1.

The results obtained corroborate those of Mikhaeil *et al.* (2003) who found that the oil is pale yellow with a pleasant, slightly spicy balsamic smell. The appearance of the extracted oil is illustrated by Figure 1.

Table 1. Organoleptic characteristics of the Frankincense essential oil extracted

Organoleptic properties	Essential oil extracted
Color	light yellow
Appearance	Clear liquid
Odor	Balsamic and characteristic of Resin *Boswellia*
Solubility	Miscible with alcohol

Table 2. Phytochemical composition of extracted essential oil

Constituent	Observation	Conclusion
Terpenes	Reddish-brown ring turning green to purpleWith a violet supernatant layer	Presence of terpenes
Tannins	Absence of greenish-brown color	Absence of catechic tannins
Coumarins	Medium fluorescence	Presence of coumarins
Flavonoids	Absence of red-orange color	Absence of flavonoid aglycones
Alkaloids	Absence of white-yellow or brown precipitate	Absence of alcaloids
Anthocyanins	Presence of red color	Presence of anthocyanins
Saponins	Absence of persistent foam	Absence of saponins

The yield of the extracted oil was estimated with respect to the mass of the EO and the mass of fresh plant material used. It is expressed as a percentage. After 7 hours of hydrodistillation the yield was 1.87%, whereas the 13-hour hydrodistillation was estimated at 2.45%. Yields of essential oil olibanum from *Boswellia sacra* obtained by Al-

Saidi *et al.* (2012) were 5% and 8.5% after 7 and 13 hours of hydrodistillation respectively. This difference can be explained by the difference in the variety of frankincense used, the harvest season and geographical location, etc. (Hayashi *et al.* 1998) and Di Stefano *et al.* (2020) for their part, obtained better yields of around 11.12 and 14.48% after the same extraction periods respectively.

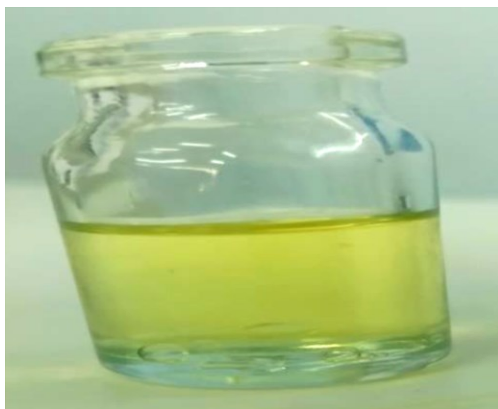


Figure 1. The appearance and the color of the extracted essential oil.

3.2. Phytochemical screening

The chemical screening of the essential oil extracted from frankincense made it possible to have the results mentioned in Table 2.

According to the results obtained and mentioned in table 2, it appears that the extracted essential oil is rich in terpenes, with a significant presence in the case of anthocyanins and low in that of coumarins. However, the tests came back negative for flavonoids, saponosides, tannins and alkaloids. The phytochemical analysis of essential oil carried out by Al-Harrasi and Al-Saidi (2008) shows that monoterpenes and sesquiterpenes are the major constituents of the studied essential oil. A recent review focuses on the bioactive components of frankincense essential oil, indicating its richness in terpene hydrocarbons such as pinene and limonene, in addition to oxygenated acid and anthocyanins (Abbood *et al.* 2022)

3.3. Antioxidant activity

The results of the anti-free radical power of the extracted essential oil and the acid ascorbic acid expressed as a percentage of inhibition of the DPPH radical are represented on the Figure 2.

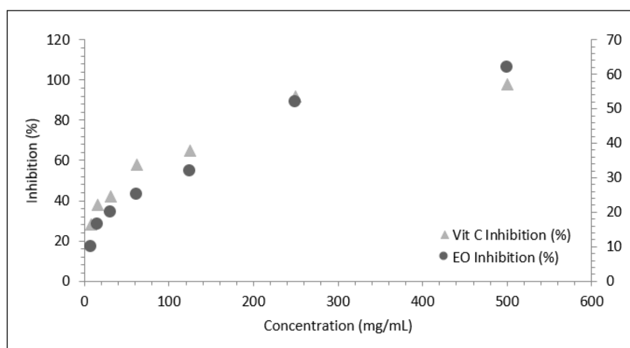


Figure 2. The DPPH free radical scavenging activity of green mint essential oil and vitamin C

The IC50 is inversely related to the antioxidant capacity of a compound, the higher the value the lower the IC50, the stronger the antioxidant activity of a compound. According to the results obtained, the extracted olibanum essential oil has a radical scavenging capacity DPPH lower than that of vitamin C. The antioxidant activity of the oil is due to the presence of large varieties of terpenes which could be donor electrons, and can therefore react with free radicals to convert them into more stable products and put an end to the radical chain reaction (Prakash *et al.* 2014). The results obtained indicate that the increase in the anti-free radical power is correlated with the increase in the concentration of essential oil and vitamin C. The oil has been shown to essential *B. sacra* gum resin has a strong antioxidant effect, however, its antioxidant activity is weaker than the antioxidant property of ascorbic acid (Al-Harrasi and Al-Saidi 2008).

The calculation of the percentages of inhibition of the 2,2'-azinobis-3-acid radical ethylbenzothiazoline-6-sulfonic acid (ABTS) was used to determine the value of IC50 and draw the graph shown in figure 3.

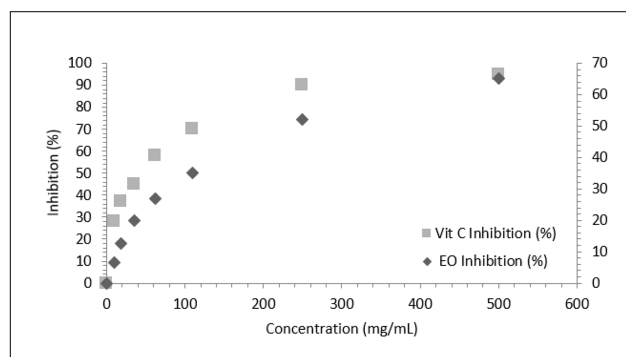


Figure 3. ABTS antiradical activity of frankincense essential oil and vitamin C.

After analysis, the IC50 of oil was 335.38 mg/mL compared to that of vitamin C which was equal to 66.69 mg/mL, knowing that the lower the IC50 value, the greater the antioxidant activity, strong, better activity compared to the DPPH test for vitamin C was observed, thus an antioxidant effect of olibanum essential oil less than DPPH, on their side (Zaki *et al.* 2014) also obtained a weak antioxidant activity. This effect is essentially due to the presence of the hydroxyl groups of the compounds phenolics present in the essential oil, transforming a hydrogen atom to the radical free (ABTS*) which will be stabilized thereafter causing a decrease in the concentration and therefore the absorbance until the exhaustion of the capacity of the hydrogen donors. There difference between the IC50 values found by the two DPPH and ABTS tests can be explained by the different properties and chemical compositions of free radicals, in other words the radicals used for each test have an influence on the result found, as well as the electron reduction potential is also different for the two radicals (Cakir *et al.* 2003).

3.4. Antimicrobial Activity

3.4.1. Antibacterial activity

Antimicrobial activity was estimated in terms of diameters of the zone of inhibition around the wells containing different concentrations of Olibanum EO with respect to the four bacterial strains: *S. aureus*, *B cereus*, *K.*

pneumoniae and *P. aeruginosa*. The results of the inhibition diameters of these strains are shown in Table 3.

The extracted essential oil exhibits antibacterial activity for the majority of strains studied. Gram positives are: *B. cereus, S. aureus*; and Gram negatives: *P. aeruginosa* and *K. pneumoniae* (Figure 4) knowing that *B. cereus* is the species most sensitive to EO of frankincense. It has been proven that Gram-positive bacteria are more sensitive to oils essential and plant extracts than Gram-negative bacteria, moreover this resistance gram-negative strains can be explained by the nature of their walls, so the periplasmic space is filled with enzymes which degrade the complex substances which can cross the cytoplasmic membrane (Cuvelier *et al.* 1996). According to certain studies, olibanum EO is endowed with numerous antibacterial effects due to the presence of monoterpenes or sesquiterpenes and their oxygenated derivatives, in addition this oil is rich in molecules gifted inhibitory effect on the growth of certain bacterial strains such as Staphylococci (Cakir *et al.* 2003). In addition, E-beta ocimene, Cembrene, Alpha Cubebene, Sabinene, Alpha Thujen and the Alpha pinene present in this essential oil are known, mainly, for their antimicrobial power (Karpiński 2020).

Table 3. Antibacterial activity of Oliban essential oil

Bacteria		Positive control	Negative control	Tests
		Diameters (cm)		
Gram (+)	*S. aureus*	1.7	0	0.975 ± 0.6
	B. cereus	1.9	0	1.75 ± 0.3
Gram (-)	*K. pneumoniae*	1.7±	0	1.3 ± 0.1
	P. aeruginosa	1.6	0	1.02 ± 0.1

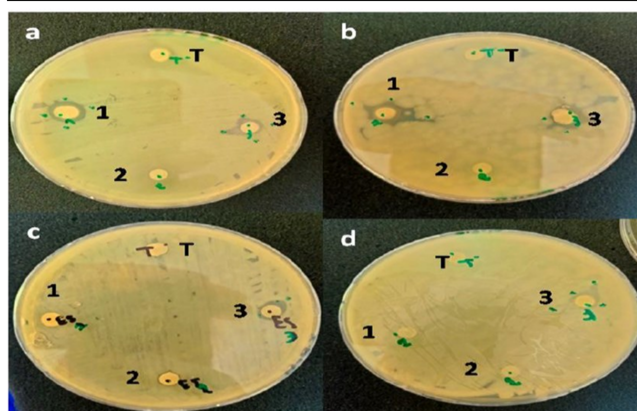


Figure 4. Antibacterial activity of essential oil against different bacterial species; a: *S. aureus*; b: *B. cereus*; c: *K. pneumonia*; d: *P. aeruginosa*; T: sterile physiological water; 1: Essential oil obtained after 7 hours of hydrodistillation; 2: Hydrosol obtained from the extraction of essential oil; 3: Essential oil obtained after 13 hours of hydrodistillation

3.4.2. Anti fungal activity

Like the antimicrobial activity, the antifungal activity of the extracted 4HEO was estimated in terms of diameters of the zone of inhibition around the wells containing different concentrations of olibanum essential oil against the two strains *Aspergillus niger* and Fusarium oxysporium. Appearance of cultures after placement of discs soaked in 10µl of EO extracted is represented by Figure 5.

Figure 5 reveals a growth inhibition in the case of the two strains after seven days of incubation in the presence of the extracted essential oil, nevertheless the growth of the Fusarium oxysporium strain seems more affected by this oil than that of A. niger. A study reports that the terpene compounds of EO and their functional groups phenols and aldehydes react with fungal membrane enzymes and degrade the plasma membrane (Al-Yasiry and Kiczorowska 2016). Other studies have shown that the activity antifungal agent of olibanum essential oil is due to the presence of terpenes and sesquiterpenes that cause fungal membranes to rupture and inhibit development of these fungi (Di Stefano *et al.* 2020).

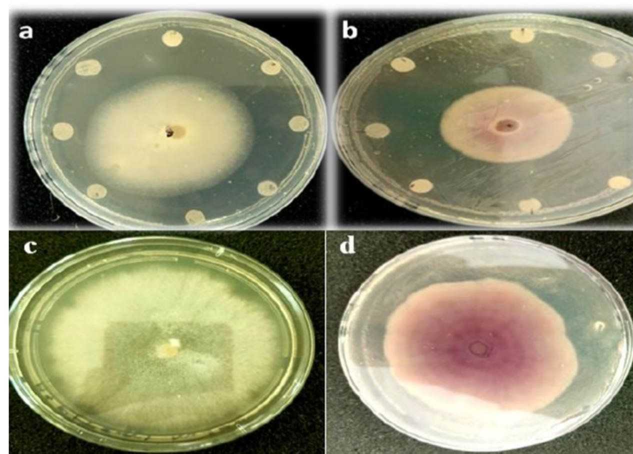


Figure 5. Antifungal activity of essential oil of Oliban againt two fungal strains; a: *A. niger*; b: *F. oxysporium*, c: control strain *A. niger*; d: control strain *F. oxysporium*

The results of the effect of Olibanum essential oil on the growth of the strain Candida albicans are shown in Figure 6.

Figure 6. Antifungal activity of essential oil against *Candida albicans strain*; T: control (distilled water); HN: Essential oil obtained after 13 hours of hydrodistillation; H: Essential oil obtained after 7 hours of hydrodistillation

According to Figure 6, the extracted olibanum essential oil has no inhibitory effect on the growth of the *Candida albicans* strain because no zone of inhibition was observed, in despite the interesting antifungal activity possessed by α-pinene (monoterpenes) one components of this oil (Giordani and Kaloustian 2006).

4. Conclusion

This work conducted on frankincense, the resin of an aromatic plant (*Boswellia sacra*), to confirm its biological properties. The study began with the extraction of frankincense essential oil, yielding a 2.45% return. Phytochemical screening provided information on the major compounds in the oil, which were identified as anthocyanins and terpenes. The antioxidant activity, measured by DPPH and ABTS assays, as well as the antimicrobial activity in vitro, yielded satisfactory results. The maximum antibacterial activity of the extracted essential oil was observed against *Bacillus cereus*, showing an inhibition diameter of 1.75 $\pm$ 0.3 cm. The results obtained appear promising for biotechnological applications and open up further exploration of this essential oil.

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