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Evaluation of antibacterial and antioxidant activities of three types of benzoin resin

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ABSTRACT

The benzoin resin is used extensively in traditional medicine for its many reported therapeutic properties. The essential oils of three different types of benzoin resin were extracted using the traditional method in this study. The yield of essential oils of the white, red and gray types of resin was 1.01, 0.92 and 0.54%, respectively. The obtained extracts were tested against two types of pathogenic bacteria, *Staphylococcus aureus* and *Escherichia coli*. The tests showed that essential oil of gray type resin is effective against both *Escherichia coli* (14 mm) and *Staphylococcus aureus* (11 mm). The antioxidant activity has been also evaluated to compare the efficiency of different type of resin with DPPH· assay. In the DPPH· system, the antioxidant activity of the red resin extract (0.01 μg/mL) was superior to that of the white (27.32 μg/mL) and gray (42.90 μg/mL) extracts, with IC_{50} values, respectively.

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1. Introduction

The discovery of many therapeutic agents from the natural sources has raised the international interest in the medicinal plant at the national and international levels. The recommendations of the medical and pharmaceutical conferences held in recent years, call for the need to reduce the consumption of synthetic drugs, and to return to medicinal plants and interest in them as a safe source for the manufacture of medicines [1,2]. Several studies on the use of these biologically active compounds from natural sources (plats, microbial cultures, etc) as substitutes for many synthetic materials have been considered due to both antioxidants and antimicrobial activities [3]

Due to the importance of medicinal plants, we have chosen the benzoin resin as a subject of this study because of its widely popular uses and past scientific studies of its therapeutic properties. An investigation was conducted to test the antibacterial and antioxidant efficacies of some resin plants with the ultimate aim to develop plant based drugs for the management of diseases caused by pathogenic bacteria and free radicals.

2. Experimental

2.1. Samples

Benzoin is the resin of various species of *Styrax* trees (*Styracaceae*). *Styrax* (*Styrax tonkinensis*, *Styrax benzoides*) is a species of tree native to Asian. Common names for the tree include, gum benjamin tree, loban, luban Jawi, Al-Jawi, kemenyan, onycha, and Sumatra benzoin tree. Three types (Red, gray and white) of resin of *Styrax* was obtained from the herbal shop of the city of Touggourt, Algeria (March 2017).

2.2. Extraction of essential oils

Essential oil extracts can be obtained by several methods such as water distillation, water vapor distillation, cold pressing extraction or extraction by organic solvents. In this study, we extracted essential oils of resin in a conventional way to obtain high quality oil at the same time inexpensive. The essential oils were extracted using the traditional method by placing 510 g of resin in a pot made of clay. Pot was closed with a special lid filled with cold water, set on fire and left for more than four hours, taking into account the change of water each time.

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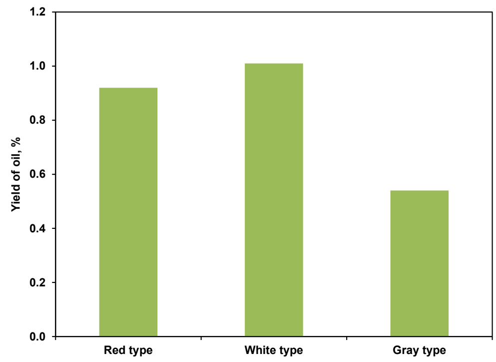

Figure 1. The yield of essential oils extracted from three types of resin.

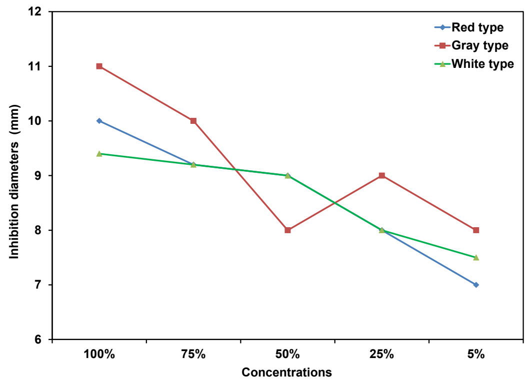

Figure 2. Inhibition diameters of *E. coli* in terms of concentrations of essential oils of resins.

2.3. Antibacterial activity

After extraction of essential oils of resin, the obtained extracts was tested for the two types of pathogenic bacteria *Staphylococcus aureus* (Gram (+), ATCC 25922) and *Escherichia coli* (Gram (-), ATCC 25923). Tests of antimicrobial activity of essential oils were conducted in the Laboratory of Microbiology at Sliman Armart Hospital Touggourt, Algeria. We prepared five concentrates (100, 75, 25, 50 and 5%) for each extract using ethanol as solvent. The antibacterial activity test for the essential oil of three types of resin was followed by the direct method proposed by National Committee for Clinical Laboratory Standards (NCCLS) for the testing of antibiotics with the replacement of antibiotics with essential oils [4]

2.4. Antioxidant activity

The antioxidant activity of the resin extracts and the standard was evaluated on the basis of the radical scavenging effect of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH)-free radical activity by the method of Blois [5]. The stock solution of crude extracts (2 mg/mL) was prepared by dissolving a known amount of dry extract in 10% DMSO of ethanol. The working solution (10, 20, 30, 40 and 50 µg/mL) of the extracts were prepared from the stock solution using suitable dilution. Ascorbic acid was used as standard in 1-100 µg/mL solution. 0.1 mM of DPPH was prepared in ethanol and 1 mL of this solution was mixed with 3 mL of sample solution and standard solution in test tubes separately in triplicates. These solution mixtures shanked vigorously, then were allowed to stand at dark for 30 min and optical density was measured at 517 nm using UV-VIS Spectrophotometer. Ethanol (3 mL) with DPPH solution (0.1 mM, 1 mL) was used as blank. Ethanol was used for base line corrections in absorbance of sample.

The effective concentration of sample required to scavenge DPPH· radical by 50% (IC_{50} value) was obtained by linear regression analysis of dose-response curve plotting between % inhibition and concentrations. The optical density was recorded and % inhibition was calculated by the formula given below:

$$\% \text{ Inhib. of DPPH activity} = ((Abs_{blank} - Abs_{sample})/Abs_{blank}) \times 100 \quad (1)$$

3. Results and discussion

3.1. Extraction of essential oils

The yields of extractions were calculated according to weight basis. The highest yield was obtained with white type (1.01%), followed by red type (0.92%) and gray type (0.54%). The essential oil yields for three types resin are shown in Figure 1. This difference in yield can be due to the environment, heat, moisture, vegetable time of reaping, the age of the plant and the development of growth [6,7].

3.2. Antibacterial activity

The antibacterial activity of resin extracts was evaluated against Gram-positive *S. aureus* and Gram-negative *E. coli*. The diameter of the zone of inhibition around the well was measured in millimeter, and the obtained results are presented in Figure 2 and 3. As can be seen from the Figure 2 and 3, extracts showed different antimicrobial activity against the test microorganisms.

The results revealed that the essential oil showed antibacterial activity with varying magnitudes, depending on the size of inoculums and the concentration of essential oil [8-19].

Table 1. DPPH· IC_{50} (μg/mL) and DPPH· free radical inhibiting activity (%) of red, white and gray type resins and ascorbic acid.

Compound/Resin type	Ascorbic acid	Gray type	White type	Red type
Concentration (μg/mL)	10.00	50.00	50.00	50.00
DPPH· free radical inhibiting activity (%)	86.00	31.51	56.87	72.27
IC_{50} (μg/mL)	6.739	42.90	27.32	0.008

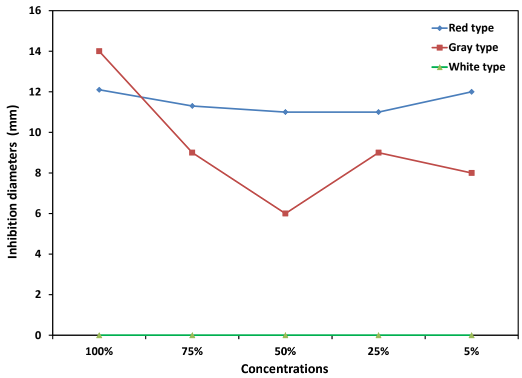

Figure 3. Inhibition diameters of *S. aureus* in terms of concentrations of essential oils of resins.

Diameter of inhibition zone of essential oil of resins leaves varied from 6 to 14 mm. The largest zone of inhibition was obtained for *S. aureus* with 100 % concentration of essential oil of gray type resin and the lowest for *S. aureus* with 50 % concentration of essential oil. The results showed that the gray type was more sensitive to *S. aureus* than *E. coli*. This finding was supported by Smith Palmer *et al.* [20], who found that some Gram positive bacteria is more sensitive to aromatic oils than Gram negative bacteria.

Gram-negative bacteria are surrounded by a thin peptidoglycan cell wall, which itself is surrounded by an outer membrane containing lipopolysaccharide. Gram-positive bacteria lack an outer membrane but are surrounded by layers of peptidoglycan many times thicker than is found in the Gram-negatives [21-23]. The water-like nature of aromatic oils is very important and allows them to assemble on the cytoplasmic membrane of the bacterial cell, producing effects where the cytoplasmic cell irritation leads to toxicity [5-18].

Belaiche also noted that some essential oils contain objects that are responsible for the antibacterial properties such as alcohols, phenols and ketones [24]. We also know previous reports that the resin oils contain the ammonium and benzoic acid, a phenolic type that is antioxidant and oxidative. So far, there is no study that can give us a clear and accurate idea of the work of essential oils, so it is very likely that each component of essential oils have a special mechanism of action, and takes its work in three stages: (i) Attack essential bacterial wall oils, causing increased permeability and loss of cellular components, (ii) Acidification within the cell, disrupting cellular energy production and (iii) Destruction of genetic material, resulting in the death of bacteria [8-27].

3.3. Antioxidant activity

The antioxidant activity of the obtained essential oils was evaluated using DPPH· radical scavenging and the obtained results are presented in Table 1. The percent DPPH· scavenging activity of aqueous extract ranged from 31.51 to 72.27%. For comparison, ascorbic acid was used as an oxidative oxidation. In the DPPH· test system, free radical-scavenging activity of the red type resin, with DPPH· inhibition value of 72.27%, was superior to those of all tested samples and positive control ascorbic acid (86.00 %). The essential oil of the red type resin showed the strongest antioxidant activity with its significantly smaller IC_{50} values in comparison with gray and white type resin, being 0.008, 42.90 and 27.32 μg/mL, respectively.

Antioxidant activity can be explained by the hydrogen atom's transport of the hydroxyl group to the phenolic compounds present in the primary oil. With the free radicals of DPPH·, the hydrogen atom moves to the latter making it stable. This reduces the concentration of these free radicals and decreases the optical density during the reaction time until the antioxidant capacity of the hydrogen atoms ends. The main role of compounds as inhibitors of free radicals is mentioned in many reports [28-30]. The most effective essential oils in antioxidant activity are phenols and alcohols. Phenolic compounds can give a hydrogen atom to the free radicals DPPH·, which causes a decrease in concentration of free radicals, and also absorption over time. Many researchers have shown that the inhibitory capacity of plant compounds on the root of DPPH· has a significant relationship to the chemical structure [28-31]. The antioxidant efficacy of these extracts can be correlated with phenolic compounds. The efficiency of these phenolic compounds as antioxidants depends on the number of hydroxyl groups associated with the aromatic ring, as well as their flavonoids content [28-31].

4. Conclusion

Due to the importance of medicinal and aromatic plants in their biological and therapeutic properties, they have been used in many applications in various fields in medicine, pharmacy, cosmetics and agriculture. The aim of this work was the evaluation of biological activity (antibacterial and antioxidant activity) of the three types of benzoin resin. We extracted the essential oils of resins (red, white and gray) by a traditional method. The antibacterial activity results indicate that the essential oils have antimicrobial activity on all tested strains, except for the white type which showed no effect of *S. aureus*. The obtained results from this study suggest that red resin have stronger antioxidant properties than other resins, which can be attributed to its high content of total phenolics and flavonoids. As a result, it can be said that some of investigated resins can be used as a good natural source of antioxidants and antibacterial agent.

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Disclosure statement

Conflict of interests: The authors declare that they have no conflict of interest.
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Ethical approval: All ethical guidelines have been adhered.
Sample availability: Samples of the compounds are available from the author.

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