Chemical composition, antibacterial, antifungal and antioxidant activities of *Taxus baccata* essential oil from Algeria

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**Abstract.** Benlembarek K, Lograda T, Ramdani M, Figueredo G, Chalard P. 2021. Chemical composition, antibacterial, antifungal and antioxidant activities of *Taxus baccata* essential oil from Algeria. Biodiversitas 22: 5475-5483. This work aims to study the chemical composition, the antibacterial, antifungal and antioxidant activities of *Taxus baccata* essential oil of Algeria. The essential oil was obtained by hydro distillation; its chemical composition was determined by GC and GC-MS analysis. The antibacterial and antifungal activities are evaluated by the disk diffusion method against three-gram negative bacteria, four-gram positive bacteria and four phytopathogenic fungi. The evaluation of antioxidant activity is carried out by the DPPH scavenging method. Twenty-seven compounds have been identified in the essential oil, where the major product was undecanone-2, followed by nonanone-2, tridecanone-2 and the methyl dehydroabietic. The *T. baccata* essential oil showed moderate antibacterial and antifungal activities against the strains tested, with the exception of *E. faecalis*, MRSA and *Fusarium graminum*, which have shown significant resistance. *T. baccata* essential oil has a moderate antioxidant activity.

**Keywords:** Algeria, biological activities, chemical composition, essential oil, *Taxus baccata*

**INTRODUCTION**

The genus *Taxus*, family of Taxaceae, contains about twenty species (Wahab et al. 2016). The majority of plants in the genus *Taxus* are endangered evergreen trees or shrubs that often grow slowly (Liu et al. 2016). In Algeria, *Taxus* is represented by only one species *Taxus baccata* L., known as the common yew (Quezel and Santa 1962). *Taxus baccata* is a dioecious tree ranging from small to medium-size, distributed in Europe, Asia and Africa (Dizdarević et al. 2019). The leaves of *T. baccata* are linear, scattered and spread in two opposite rows. The fruit is fleshy-succulent, reddish with a bony ovoid seed (Quezel and Santa 1962).

*Taxus baccata* is considered a valuable woody species with a significant medicinal value due to the presence of paclitaxel (Taxol), which is found in the leaves and considered one of the most promising natural anticancer agents (Jiang et al. 2011; Fenjuan et al. 2021).

The chemical composition of *T. baccata* has not been much studied. In Serbia, essential oils from needles are rich in oct-1-en-3-ol, (3Z)-hex-3-en-1-ol and myrtenol (Stefanović et al. 2016). Radulović et al. (2011) in a study on aerial parts of *T. baccata*, showed that the essential oil is rich in hexahydrofarnesyl acetone, myrtenol, (Z)-3-hexenol, 3-methyl-2-butenic acid and tricosan. The oils from the fresh leaves of *T. baccata* collected from the Aegean and Mediterranean region of Turkey are dominated by 1-octen-3-ol, (E)-2-hexen-1-ol, caryophyllene oxide, hexahydrofarnesyl acetone and 1-hexanol (Yasar 2013). The essential oils of *T. wallichiana* leaves contain 3-hexen-1-ol, 2-hexenal, 1-octen-3-ol, 3-octanone and hexadecanoic acid as the main products (Jian-chun et al. 2012).

The composition of *T. chinensis* essential oils in Vietnam has shown the dominance of α-pinene and caryophyllene oxide (Houng et al. 2020). The study by Zhao et al. (2016) showed that the cis-vaccenic acid and (E)-palmitoleic acid are major components in the *T. chinensis* essential oil, on the other hand, the Chinese populations of *T. chinensis* are rich in n-hexadecanoic acid and in Phthalic acid mono-2-ethylhexyl ester (Wei and Yin 2019).

The leaves extract of *T. baccata* from India, tested against *Escherichia coli*, *Yersinia pestis*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Listeria monocytogenes* and *Staphylococcus aureus* showed moderate antibacterial activity (Prakash et al. 2018). In Ukrain, alcoholic extracts of *T. baccata* have low antimicrobial activity against *Listeria monocytogenes*, *Corynebacterium xerosis* and *Candida albicans* (Zazharskyi et al. 2019). Ethanolic extracts from the leaves of *T. baccata* has given a broad-spectrum antibacterial activity against multi-resistant bacterial strains (MDR) (Bernaitis et al. 2013). The essential oil from the leaves of *T. cuspidata* showed moderate antimicrobial action against *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhimurium* and *Escherichia coli* (Bajpai et al. 2013).
Extracts of various species of the genus *Taxus* possess an important antifungal activity. Methanolic extracts of *T. baccata* has a potential antifungal activity against *A. brasiliensis* and *C. albicans* (Dizdarević et al. 2019). Methanolic extracts from the leaves of *T. baccata* from Iran were very effective against *Candida albicans* (Fazeli-Nasab et al. 2021). Biflavones isolated from the leaves of *T. cuspidata* have moderate antifungal activity against phytopathogenic fungi (Tachibana et al. 2005).

The antioxidant activity is due to the richness of *T. baccata* in polyphenolic compounds (Milutinovic et al. 2015). In Algeria, Bekhouche et al. (2021) showed that the methanolic extract of *T. baccata* needles exhibits a greater antioxidant activity more than the synthetic antioxidant BHT. The essential oil from the stems of *T. cuspidata* has shown a strong antioxidant capacity against the DPPH radical (Bajapai and Baek 2016). In India, the study of the antioxidant activity of *T. wallichiana* raw extracts from the leaves, showed a very remarkable antioxidant activity (Ganie et al. 2015).

This study aims to identify the chemical composition of the Algerian *Taxus baccata* essential oil and to study its antibacterial, antifungal and antioxidant activity.

**MATERIALS AND METHODS**

**Plant material**

*T. baccata* was collected during October 2020 from the Babor forest in Algeria (Figure 1). The population of *T. baccata* is found at an altitude of 1940m with geographic coordinates of 36° 30' 08”N and 5° 27' 17”E.

**The essential oils extraction**

The aerial parts (100 g of dried stems and leaves), were hydro distilled for 3 h using a Clevenger-type apparatus according to the method recommended by the European Pharmacopoeia. Voucher specimens were deposited in the herbarium of the Department of Biology and Ecology, Setif-1 University, Algeria. The distilled essential oil was dried over anhydrous sodium sulfate filtered and stored in sealed vials at 4-5°C, prior to further analyses. The Essential oil yield was calculated by the following formula:

\[
\text{Essential oil yield} = \frac{\text{Essential oil mass}}{\text{plant matter mass}} \times 100
\]

**Essential oils analysis**

The essential oils were analyzed using a Hewlett Packard gas chromatograph (CG/FID 7890), coupled to a gas chromatograph (CG/MS 7890/5975C), equipped with a Column A-polar: DB5 MS: 40 m 0.18 mm 0.18 µm, programmed from 50°C for 5min at rate of 5°C/min up to 300°C. Helium was used as the carrier gas (1.0 mL/min); injected in the split mode (1:30), injector and detector temperature of 280°C with split 1/100. The mass spectrometer worked in the EI mode at 70 eV; electron multiplier, 2500 V; ion source temperature, 180°C; the MS data were acquired in the scan mode in the m/z range of 334-50. The identification of the components was based on the comparison of their mass spectra with those of NIST mass spectral library (Masada 1976; NIST 2002) and those described by Adams, and the comparison of their retention indices either with those of authentic compounds or with literature values (Adams 2007).

![Sampling station](image_url)
Antibacterial activity assessment

The in-vitro evaluation of antimicrobial activity of T. baccata essential oils was performed by the disc diffusion method. Bacterial species were tested individually with the essential oil including three Gram-negative bacteria (Escherichia coli ATCC 25922, Proteus mirabilis ATCC 35659 and Pseudomonas aeruginosa ATCC 27853), and four Gram-positive bacteria (Staphylococcus aureus ATCC 25923, Enterococcus faecalis ATCC 51299, Bacillus cereus ATCC 11778 and MRSA ATCC 43300). These bacteria were obtained from the Pasteur Institute of Algiers and Msila, the applied microbiology laboratory of Setif University and Setif Hospital. The bacterial inoculums were prepared from overnight broth culture in physiological saline (0.8% NaCl) to obtain an optical density ranging from 0.08 to 0.1 at 625 nm. Mueller Hinton agar (MHA) was poured in Petri dishes, solidified, and surface dried before bacteria inoculation. Sterile discs (6 mm) were placed on inoculated MHA, filled with 10 µl of stock solution of essential oils (1/1 v/v) or diluted essential oils (1/2 and 1/3 v; v of DMSO). DMSO was used as a negative control and the antibiotics Colistin, Cefotaxime, Gentamicin and Imipenem were used as positive control. The bacterial growth inhibition was determined as the diameter of the inhibition zones around the discs. The Petri dishes were incubated at 37°C for 18 to 24h aerobically. All the tests were performed in triplicate, and the means were calculated as results. The sensitivity to essential oils was classified by diameter of inhibition halos as follows: not sensitive (-) for diameter less than 8 mm; sensitive (+) for diameter 9-14 mm; very sensitive (+++) for diameter 1519 mm and extremely sensitive (++++) for diameter larger than 20 mm (Ponce et al. 2003).

Antifungal activity assessment

The evaluation of the antifungal activity of T. baccata essential oil is carried out in vitro by the technique of diffusion on discs. Four phytopathogenic fungal strains of clinical origin, obtained from the Laboratory of Microbiology (Faculty of Nature and Life Sciences, Ferhat Abbas University of Setif) were used: Alternaria alternata, Fusarium oxysporum, Fusarium granirum and Puccinia sp. Agar disc of the fungal culture is applied to the Petri dish based on Sabouraud Dextrose Agar (SDA) culture media. Sterile discs of 6 mm in diameter are placed on the agar surface, after having been loaded with 10 µL of pure essential oil (1/1 v/v) or essential oil diluted with DMSO (1/2, 1/3 v/v). A negative control consisted of fungal agar disks in the absence of essential oil was carried out. Incubation was performed at room temperature of 25°C for seven days. Mycelia growth was assessed by measuring the zone of inhibition (mm).

Antioxidant activity assessment

The antioxidant activity of T. baccata essential oil was evaluated using the DPPH method described by Gülçin et al. (2012). 400 µL of the essential oil is mixed with 400 µL of methanol at different concentrations (1/1; 1/2; 1/4; 1/8; 1/16; 1/32 and 1/64). From each concentration, 100 µL of the mixture (methanol- essential oil) was incorporated with 2900 µL of methanolic solution of DPPH (4 mg / 100 mL of methanol equivalent to 0.004%). After vortexing, the mixture was left in the dark for 30 min at room temperature then the absorbance was measured using a spectrophotometer at 517 nm. DPPH was used as a negative control and BHT (Butylate Hydroxy Toluene) as a positive control. All the tests were carried out in triplicate. The antioxidant activity is estimated in percentage, according to the following formula:

\[ \text{Antioxidant Activity (\%)} = \frac{A_{\text{negative control}} - A_{\text{sample}}}{A_{\text{negative control}}} \times 100 \]

Statistical analysis

All data were expressed as means ± standard deviation of triplicate measurements. The significant results of biological activities were analyzed by ANOVA two-way using a CoStat v 6.4 software package. Differences with P < 0.05 were considered significant.

RESULTS AND DISCUSSION

Chemical composition

The hydro distillation of the aerial parts (leaves and stems) of T. baccata has given a yellow liquid. The essential oil yield was 0.06%. The essential oil composition analysis was carried out by gas chromatography coupled with mass spectrometry (GC-MS). The analysis of the essential oil has allowed to identify 27 compounds representing 98% of the total oil. The components identified and their relative abundances are listed in Table 1. Undecanone-2 with a rate of 76.96% represents the major component of T. baccata essential oil, followed by nonanone-2 (7.57 %), tridecanone-2 (4.43%), decanone-2 (3.92%) and methyl dehydroabietate (1.91%). T. baccata essential oil is very rich in ketone (90.97%), on the other hand the remaining chemical classes are represented in small percentage ranging from 0.18 to 2.66% in the essential oil.

Antibacterial activity

The antibacterial activity is estimated by measuring the diameters of inhibition of the bacterial strains (Table 2). Antibacterial test of T. baccata essential oil against several bacterial species have shown that P. aeruginosa, B. cereus, P. mirabilis, E. coli and S. aureus, are sensitive to the essential oil. The bacterial strain MRSA was the most resistant to T. baccata oil with an average inhibition diameter of 8.33 ± 0.57mm, in contrast P. aeruginosa was the most sensitive with an average inhibition diameter of 15.33 ± 0.5mm. The pure essential oil was the most effective against E. faecalis, S. aureus, E. coli, P. aeruginosa and B. cereus. The essential oil diluted to ½ was more effective than pure oil in inhibiting the growth of B. cereus, with an average diameter of inhibition of 14.66 ± 0.6mm. The essential oil diluted to 1/3 did not affect the strains tested.
The diameter of inhibition of T. baccata pure essential oil was greater than that of the antibiotics cefotaxime and imipenem, on the other hand, the action of this oil is less than the action of the two antibiotics cefotaxime and gentamicin, which are classified in the group "a" (Figure 2.A). The essential oil dilution with 1/3 showed the lowest activity with an inhibition diameter of 3.83 mm; statistically these results are significant in inhibiting the growth of bacteria. The study of the sensitivity of the bacteria to the essential oil of T. baccata showed that S. aureus, classified in the group "a", is the most sensitive with an average diameter of inhibition of 15.67 mm (Figure 2.B). On the other hand, the MRSA bacteria classified in the category "f" is the most resistant to essential oil with an average diameter of inhibition of 7.71 mm.

The desirability profile of the essential oil of T. baccata against the bacteria tested had a predictive value of 0.47323 (Figure 3). The value of the essential oil is low (less than the predicted value) with the exception of pure essential oil, which indicates a value very close to the predicted value. The essential oil dilutions (1/2 and 1/3) have low activity against the bacteria tested. Desirability test shows that the bacterial strains used in this study are not sensitive to the essential oil of T. baccata.

Antifungal activity

The disk diffusion method has allowed evaluating the antifungal activity of the essential oil of T. baccata against four phytopathogenic fungal strains (Table 4). Pure essential oil was the most effective against the four fungal strains tested. With an inhibition diameter of 15.07 ± 1.75 mm, Puccinia sp. is the most sensitive to essential oil, whereas the fungi Fusarium oxysporum, Alternaria alternata and Fusarium graminum are less sensitive.

Statistical analysis shows that the effects of doses and fungi as well as their interactions are highly significant (P < 0.001) (Table 5).

Pure essential oil of T. baccata showed the highest effect relative to its dilutions, with a diameter of 13.60 mm. On the other hand, its action on the fungi tested is weak than the negative control (Figure 4.A. The effect of T. baccata essential oil on the growth of the fungi tested show that Fusarium oxysporum and Puccinia sp., with inhibition diameters of 12.77 mm and 12.57 mm, are the most sensitive, these two fungi are classified in group "a". While Fusarium graminum classified in the group "c", with an inhibition diameter of 10.28 mm (Figure 4.B), was the most resistant to essential oil.

Table 2. Inhibition diameter (mm) of Taxis baccata essential oil

| Dilution | E. coli | S. aureus | E. faecalis | P. aeruginosa | P. mirabilis | B. cereus | MRSA |
|----------|---------|-----------|------------|--------------|--------------|----------|------|
| 1        | 10.3±0.6| 11.3±0.6  | 10.3±0.6   | 15.3±0.6     | 11.3±1.2     | 11.7±0.6 | 6    |
| 1/2      | 7.7±0.6 | 8.7±0.6   | 0          | 10.3±0.6     | 8.7±0.6      | 14.7±0.6 | 8.3±0.6 |
| 1/3      | 0       | 4±0       | 0          | 7.7±0.5      | 0            | 0        | 8±0  |
| Colistin | 15.7±0.6| 17.3±0.6  | 6.3±0.6    | 14.3±0.6     | 0            | 10.3±0.6 | 0    |
| Gentamicin| 31.7±0.6| 29.3±0.6  | 19.7±0.6   | 24.5±0.5     | 29.3±0.6     | 24±1     | 17.3±0.6 |
| Cefotaxim| 29.7±0.6| 35±1      | 12.7±0.6   | 29.7±0.6     | 30±1         | 29.3±0.6 | 20.3±0.6 |

Note: Statistical analysis showed that the dose and bacteria effects are highly significant (P < 0.001) (Table 3)

Table 3. Main effects and interactions of essential oils of Taxis baccata

| Source      | DF | F    | P    |
|-------------|----|------|------|
| Main effects|    |      |      |
| Doses       | 6  | 66.459997 | .0000 *** |
| Bacteria    | 6  | 70.28952  | .0000 *** |
| Interaction | 36 | 257.03619 | .0000 *** |

Table 1. Chemical composition of Taxis baccata essential oil from Babor, Algeria

| Components                      | RT (min) | Kovats retention index | %  |
|----------------------------------|----------|------------------------|----|
| Isopropyl-2-methyl butyrate      | 5.091    | 883                    | 0.03 |
| Heptanone-2                      | 5.175    | 987                    | 0.05 |
| α-pinene                         | 6.168    | 933                    | 1.13 |
| Benzaldehyde                     | 6.876    | 964                    | 0.03 |
| Sabine 6                         | 7.085    | 973                    | 0.37 |
| β-pinene                         | 7.192    | 1077                   | 0.07 |
| Octane-2                         | 7.490    | 1190                   | 0.05 |
| Δ2-carene                        | 8.082    | 1117                   | 0.03 |
| Limonene                         | 8.354    | 1230                   | 0.29 |
| β-phellandrene                   | 8.385    | 1231                   | 0.05 |
| β-octimene-(E)                   | 8.728    | 1247                   | 0.03 |
| γ-terpinene                      | 8.982    | 1259                   | 0.04 |
| Nonanone-2                       | 9.699    | 1292                   | 5.57 |
| Linalool                         | 8.863    | 1299                   | 0.07 |
| Sabine hydrate-trans             | 9.908    | 1202                   | 0.07 |
| n-nonanal                        | 9.970    | 1205                   | 0.15 |
| Mentha-2,8-dien-1-ol             | 10.322   | 1323                   | 0.01 |
| Terpin-en-4-ol                   | 11.484   | 1382                   | 1.11 |
| Decane-2                         | 11.689   | 1393                   | 3.92 |
| Myrtenol                         | 13.274   | 1380                   | 0.57 |
| Undecane-2                       | 13.795   | 1310                   | 76.96 |
| Tridecane-2                      | 13.821   | 1311                   | 4.43 |
| γ-undeca lactone                 | 18.171   | 1383                   | 0.29 |
| Caryophyllene oxide              | 18.242   | 1388                   | 0.65 |
| Humulene-14-hydroxy              | 18.429   | 1301                   | 0.09 |
| Bergapten-iso                    | 24.101   | 1434                   | 0.03 |
| Methyl dehydro-abietate          | 25.741   | 1476                   | 1.91 |
| Chemical classes (%)             |          |                       |     |
| Hydrocarbon monoterpenes         |          | 2.66                   |     |
| Hydrocarbon sesquiterpenes       |          | 0.75                   |     |
| Ketones                          |          | 90.97                  |     |
| Aldehyde                         |          | 0.18                   |     |
| Ester                            |          | 1.94                   |     |
| Alcohol                          |          | 1.18                   |     |
| Other                            |          | 0.32                   |     |
The desirability profile of the essential oil of *T. baccata* against the fungal strains tested shows a predictive value of 0.33673 (Figure 5). The pure essential oil and the oil diluted to 1/2 have an important activity on the tested fungus. They have presented a greater value more than the predicted value, while the diluted concentrations showed a value close to the prediction value. The positive control BHT has marked a higher value more than the essential oil and the predicted value. The desirability test shows that the different concentrations of essential oil do not have effective antioxidant activity compared to BHT.

### Discussion

The Algerian population of *T. baccata* from the Babor region is rich in ketone, with high concentrations of undecanone-2 (76.96%), nonanone-2 (5.57%), tridecanone-2 (4.43%) and decanone-2 (3.92%). *T. baccata* chemical composition is very different from the composition of the same species studied elsewhere. The populations of Turkey present as major components 1-octene-3-ol, caryophyllene oxide, and hexahydro farnesyl acetone (Yasar 2013)

In Serbia, the essential oil of *T. baccata* is rich in 1-octene-3-ol, (3Z)-hexa-3-en-1-ol and myrtenol (Stefanovic et al. 2016). Another study carried out in Serbia by Radulovic et al. (2011) showed that the essential oil of *T. baccata* exhibits high concentrations of hexahydrofarnesyl acetone, myrenol, (Z)-3-hexenol, senecioic acid and tricosane.

### Table 6. Percent inhibition of essential oil of *Taxus baccata* and BHT

| Dilutions (mg/mL) | 1/1          | 1/2          | 1/4          | 1/8          | 1/16         | 1/32         | 1/64         |
|-------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Percent           | 35.18±0.4    | 21.76±0.2    | 16.07±0.6    | 11.64±0.4    | 9.50±0.2     | 8.31±0.4     | 7.1±0.3      |
| Inhibition        | 91.32±0.4    | 89.97±0.3    | 86.87±0.2    | 74.04±2.2    | 47.3±1.9     | 37.48±1.2    | 23.1±0.7     |

**Note:** LSD 0.05 = 33.3743779467
The populations of Serbia studied by Stefanović et al. (2016) and those from Turkey studied by Yasar (2013) are dominated by alcohol; while the population of Serbia studied by Radulovic et al. (2011) was found, dominated by monoterpenoids. A recent study on *T. baccata* red arils in Poland showed that alcohols, ketones, esters and aldehydes are dominated in their volatile profile (Tabaszewska et al. 2021). The intra-specific difference of the chemical composition can be justified by the diversity of environmental and geographic parameters as well by the harvest period (Aboukhalid et al. 2017; Yeddes et al. 2018).

The results of the antibacterial test are consistent with the study by Prakash et al. (2018) which showed that the methanolic extract of *T. baccata* leaves has significant antibacterial activity against gram-positive bacteria. The study by Bernaitis et al. (2013) confirms that methanolic extracts of *T. baccata* have antibacterial activity against multidrug-resistant bacterial strains. Fazeli-Nasab et al. (2021) has classified the bacteria *B. cereus* as moderately sensitive to plant extract of *T. baccata*.

Few studies on the antifungal activity of the essential oil of *T. baccata* have been carried out. Diwan et al. (2013) has shown that ethanolic extracts of *T. baccata* exhibit moderate antifungal activity against *Candida* species from clinical origin. A similar study has found that the extract of *T. baccata* leaves is very active against *Candida albicans* and *Aspergillus niger* (Kumar et al. 2006). Methanolic extracts from the leaves of *T. baccata* in Iran showed high efficacy against *Candida albicans* (Fazeli-Nasab et al. 2021). A study on the bioactive compounds contained in the arils of *T. baccata* has shown that taxol and taxinine may be responsible for the antifungal activity of *T. baccata* (Siegle and Pietsch. 2018). Nisar et al. (2008) in a study on the antifungal activity have found that *Trichophyton longifusus*, *Microsporum canis* and *Fusarium solani* are sensitive to methanolic extracts of aerial parts of *T. wallichiana*.

Figure 2. Effect of *Taxus baccata* essential oil on the bacteria tested. A. Effect of oil dilutions; B. Susceptibility group of bacteria

Figure 3. Profile of predicted values and desirability for the inhibition zones induced by *Taxus baccata* essential oils against bacteria
Figure 4. Effect of Taxus baccata essential oil on the fungi tested: A. Effect of oil dilutions; B. susceptibility group of fungi)

Figure 5. Profile of predicted values and desirability of essential oil of Taxus baccata

The essential oil of T. baccata from the Babor region exhibited a low antioxidant activity with an average IC50 of 56.50 ± 2.78 mg/mL. Senol et al. (2015) showed that T. baccata shoot extract exhibits potential antioxidant activity due to its richness in phenolic compounds. The methanolic extracts of leaves of T. baccata, showed strong antioxidant activity, greater than aqueous and acetone extracts (Prakash et al. 2018). The antioxidant test of methanolic extracts obtained from T. baccata in Serbia shows that the plant has significant DPPH scavenging activity (Milutinović et al. 2015). A study on the bioactive compounds found in red arils of T. baccata, shows that they have an important antioxidant capacity to scavenge DPPH (Tabaszewska et al. 2021). Taxus baccata can be considered as a source of natural antioxidants due to its strong antioxidant capacity (Guleria et al. 2013).

In conclusion, the Taxus baccata essential oil collected from the Babor region in Algeria has a significant yield of 0.6%. GC-GC / MS analysis led to the identification of 27 components in the essential oil, of which undecanone-2 is
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