Hemi-synthesis of Thione and Thiol Derivatives from *Artemisia judaica* L. Essential Oil, and Antimicrobial Tests

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1 Introduction

Natural products and their structural analogues have historically made a major contribution to pharmacotherapy, especially for cancer and infectious diseases.1

Recently, an increase in demand for new drugs and dietary supplements derived from natural products has been observed, especially organosulfur compounds, such as thioketones due to their medicinal and biological properties2–3 as they can be used as precursors for the synthesis of new bioactive molecules.4

Many attempts have been made to study the thiol-thione tautomerism of thio carbonyl compounds in solution, especially thio ketones.5 The possibilities of these forms in solution and the influence of the nature of the solvent have been studied by nuclear magnetic resonance, Fourier transform infrared (FTIR), ultra violet-visible (UV-Vis)6, mass spectroscopy (MS)7, and liquid chromatography-mass spectrometry methods (LCMS).8

*Artemisia judaica* L. is a small perennial shrub of the Asteraceae family, which grows widely in arid regions and deserts. Its essential oil is known for different biological activities, such as antibacterial9,10, insecticidal11,12, and antioxidant activity13. Piperitone has been revealed as the major component of *Artemisia judaica* L. essential oil from Algeria14, Egypt15, Jordan16, and Saudi Arabia17 with very high levels. This essential oil was used as a raw material for the hemi-synthesis of new carbazones and thiosemicarbazones.18 In the present work, the extraction and the thionylation of the essential oil of *Artemisia judaica* L. were conducted. The native and thionylated essential oils were characterized by different spectroscopic methods and their antimicrobial activity was estimated against bacterial and fungal strains.

2 Experimental

2.1 Materials and methods

*Artemisia judaica* L., aerial part, was collected from Oued Talanteneche (at 6 km northeast of Tamanrasset) in the south of Algeria during November 2016, and authenticated at the Department of Botany of the National Institute of Agronomy (INA, Elharach, Algeria). A reference specimen (BENMANSOUR 2016 # AJN22016) was deposited in the Process Engineering Laboratory of the Process Engineering Department, University of Blida 1, Blida (Algeria).

The essential oil was extracted by hydrodistillation using a Clevenger type apparatus. The extracted essential oil was dried over sodium sulphate anhydrous, and stored at 4 °C. Phosphorus pentasulphide (98 %), sodium bicarbonate (98 %), carbon disulphide (98 %), and dimethyl sulphoxide – DMSO (98 %) were purchased from Sigma–Aldrich (Germany), Biochem (France), Riedel-de Haën (Germany), and Panreac (France), respectively.

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Abstract

The applications of organosulfur compounds, especially thioketones are varied which concerns both synthetic and biological chemistry. In this context, from the essential oil of *Artemisia judaica* L., extracted by hydrodistillation, and consisting mainly of piperitone (91.77 %), thione, and thiol derivatives were hemi-synthesized via a thionylation reaction by phosphorus pentasulphide (P2S5). The possible thione-thiol tautomerism in solution followed spectrophotometrically revealed the predominance of thiol form in solution. The antimicrobial activity of essential oils showed that the thionylation of essential oil caused a considerable increase in antimicrobial activity in particular against *Escherichia coli*.

Keywords

*Artemisia judaica* L., essential oil, thionylation, tautomerism, antimicrobial activity
2.2 General experimental procedure and gas chromatography

The essential oils were analysed by Jasco UltraViolet-Visible (France) apparatus after dissolving the essential oils in hexane and carbon disulphide. The infrared spectra were obtained by Bruker Fourier Transform Infrared (FTIR) spectrometer (Germany). The gas chromatography–mass spectrometry (GC-MS) analyses were carried out using Agilent HP 6890 gas chromatograph (USA) equipped with a quadrupole selective mass detector and capillary column: RTX-5Ms (30 m long and 0.25 mm i.d.) with 0.25 μm film thickness.

Essential oils were diluted with methanol (1:100 ratios) and ultra-pure helium was used as a carrier gas at a flow rate of 1 ml min⁻¹. The volume injected was 1 μl. The temperature programming was from 60 to 250 °C; with 2 °C min⁻¹ increases and isotherm for 10 min. Splitless injection mode was employed. The ionization was carried out with electronic impact and a filament intensity of 70 eV. The mass spectrometry analyses were performed in Scan mode from 40 to 550 UMA.

Kovats retention indices (KI) of the identified compounds were determined using alkanes (C₈–C₃₂) as standard with the Eq. (1):

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KI = 100\frac{t_{r(p)} - t_{r(o)}}{t_{r(n+1)} - t_{r(o)}} + 100n
\]

where \(t_{r(p)}\) is retention time of respective compound; \(t_{r(o)}\) is retention time of the smaller alkane, and \(t_{r(n+1)}\) is retention time of larger alkane.²⁰

2.3 General procedure for the thionation of essential oil

In a round-bottom flask (1.43 g), dried essential oil was dissolved in 30 ml of carbon disulphide; 2.1 g (4.7 mmol) of phosphorus pentasulphide and 0.42 g (5.2 mmol) of sodium bicarbonate were then added to the mixture. The reaction mixture was heated under reflux, employing a thermostated water bath (50 °C) under continuous stirring, and monitored through thin layer chromatography (TLC) analysis. After 5 h of heating, the phosphorus pentasulphide was filtered, the solvent was evaporated, and the thionylated essential oil was stored at 4 °C until use.³

2.4 Antimicrobial activity

2.4.1 Disc diffusion method

The antimicrobial activity of the essential oils was evaluated against two bacterial strains and one fungal strain: Escherichia coli (ATCC 10536), Staphylococcus aureus (ATCC 6538), and Candida albicans (ATCC 10231) by the disk diffusion method (Aromatogram) following the Clinical and Laboratory Standards Institute guidelines (CLSI VET01-A4, 2013) with a few modifications for essential oils. The latter involved depositing sterilized disks (Whatman filter paper) of 6 mm diameter impregnated with 10 μl of pure and diluted essential oils in DMSO on the surface of the agar plates with microorganisms suspension adjusted to 0.5 McFarland (1.5 · 10⁶ CFU/ml), and measuring the inhibition diameters after incubation at 37 °C, 24 h for bacteria and 48 h for the Candida fungus. Negative control with DMSO and a positive control using antibiotic disks of Ce- phalexin and Metronidazole were released. The tests were repeated three times and the mean values given.

2.4.2 Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined by the macro-dilution method. Serial dilutions of essential oils were carried out in test tubes containing Muller-Hinton medium with 13 μl of bacterial inoculum and (1 %; v/v) of Tween 80, in which the turbidity of microorganisms was standardized at 0.5 McFarland (10⁶ CFU/ml) to obtain a concentration range between 20 and 0.3 mg ml⁻¹. MIC values were found at the lowest concentration, which inhibits the visible growth of microorganisms in the liquid medium. The antimicrobial activity tests were carried out in the laboratory safety and chemical hygiene of Blida (Algeria).

3 Results and discussion

3.1 Extraction and chemical composition of the essential oil

The hydrodistillation of the dry aerial part of Artemisia judaica L. provided yellow oil with an average yield of 2 %. Chromatographic analysis of Artemisia judaica L. essential oil (Fig. 1), presented in Table 1, revealed the predominance of cyclic ketones, mainly pipertone (91.77 %) followed by davanone (5.20 %). The presence of terpene esters, such as ethyl isovalerate (0.43 %) and ethyl cinnamate (1.63 %) was also observed. Other compounds were detected in low levels, such as p-cymene (0.66 %) and davana ether (0.31 %).

These results are in agreement with the literature, where the identification of Algerian Artemisia judaica L. essential oil collected at Tamanrasset (Algeria) revealed pipertone as the major component with higher levels in the vegetative growth stage (72.68 %)²¹ than at the flowering stage (65.88 % and 66.17 %).²²,²³

3.2 Chemistry

The thionylation of the essential oil of Artemisia judaica L. was confirmed by the different spectroscopic methods. The superposition of the infrared spectra of the native and thionylated essential oils in the solid phase in Fig. 2 shows the disappearance of the characteristic peak of the (C=O) bond of cyclic ketones located at 1670 cm⁻¹, and the appearance of a new peak at 964 cm⁻¹ responsible for the vibration of the (C=S) bond, while retaining the characteristic peaks of the CH bonds at 2959, 2925, and 2869 cm⁻¹, and (C=O) of esters at 1767 and 1732 cm⁻¹.

At the end of the thionation reaction, a blue-violet solution which discoloured after 10 h was recovered. The solvent
effect on the proton transfer reaction was investigated using ultra violet-visible spectroscopy by measuring its absorbance every 2 h (Figs. 3 and 4). The tautomerism is one of the characteristics of the carbonyl compounds having hydrogen atoms α- to carbonyl groups. Similarly for the thiocarbonyl compounds, this property has been observed and widely studied by authors. Hypsochromic shift in the wavelength by 3 nm of the absorption band situated at 375 nm attributed to the electronic transition $n \rightarrow \pi^*$ corresponding of thiocarbonyl group (C=S) occurred after 2 h. A decrease in this band as a function of time and then a total disappearance was observed after 12 h, with an increase in the band corresponding to the enthiol electronic transition $n \rightarrow \sigma^*$. These results confirm that the thionylated piperitone(6-isopropyl-3-methyl-cyclohex-2-enethione) can exist under two tautomeric forms (thione and enthiol), and that the thione group (C=S) is relatively unstable and it stabilizes by forming a stable single bond (CS).
These results were confirmed by the chemical identification of this oil by GC-MS (Fig. 5) summarized in Table 1, where the thionylation of piperitone present in high levels (91.77 %) gave thione and thiol forms (Figs. 6 and 7), identified by their mass spectra, with contents of 6.16, 48.81, and 24.47 %, respectively.

The predominance of the thiol forms is due to the instability of thioketones because of the high energy level of the valence atomic orbital of the sulphur atom, which is close to that of the valence atomic orbital of the carbon atom, due to the lower electronegativity of the sulphur atom and the lower polarity of the C=S band. Ethyl isovalerate and ethyl cinnamate were not converted due to their stability.

![Fig. 5 – Chromatogram of thionylated Artemisia judaica L. essential oil (S-EO$_{AJ}$)](image)

![Fig. 6 – Thionylation and tautomeric thione and enethiol transformation of piperitone](image)

![Fig. 7 – Tautomeric form of 6-isopropyl-3-methyl-cyclohex-2-enthione](image)

| No. | Compounds                        | RT / min | KI$^*$ | KI$^{**}$ | Concentration / % | EO$_{AJ}$ | S-EO$_{AJ}$ |
|-----|----------------------------------|----------|--------|-----------|-------------------|-----------|-------------|
| 1   | Ethyle isovalerate               | 7.2      | 0848   | 0849      | 0.43              | 0.43      |             |
| 2   | p-Cymene                         | 17.9     | 1089   | 1089      | 0.66              | 0.66      |             |
| 3   | Piperitone                       | 34.8     | 1249   | 1249      | 91.77             | 12.33     |             |
| 4   | 6-Isopropyl-3-methyl-cyclohex-2-enthione (b) | 36.3 | –       | –         | –                  | 6.16      |             |
| 5   | 6-Isopropylidene-3-methyl-cyclohex-2-enethiol (b2) | 38.9 | –       | –         | –                  | 24.47     |             |
| 6   | 2-Isopropyl-5-methyl-cyclohexa-1,5-dienethiol (b1) | 43.9 | –       | –         | –                  | 48.81     |             |
| 7   | Danava ether                     | 47.1     | 1450   | 1450      | 0.31              | 0.31      |             |
| 8   | Ethyl cinnamate (E)              | 48.8     | 1465   | 1465      | 1.53              | 1.53      |             |
| 9   | Danavanone                       | 55.7     | 1587   | 1587      | 4.20              | –         |             |

Table 1 – Chemical composition of Artemisia judaica L. essential oil (EO$_{AJ}$) and thionylated Artemisia judaica L. essential oil (S-EO$_{AJ}$)

RT: retention time index; KI$: Kovats retention index reported in the investigation; KI$^{**}$: Kovats retention index from the literature (R. P. Adams, 2007, Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th Ed.).
3.3 Antibacterial activity

The results of the antibacterial activity given in Table 2 show the effectiveness of the pure *Artemisia judaica* essential oil as an antimicrobial agent with an inhibition diameter of 18 mm against *Escherichia coli* and 20 mm against *Staphylococcus aureus*. In parallel, the diluted essential oil in DMSO was inactive against *Escherichia coli*, but it gave an average activity starting from 5% with *Staphylococcus aureus*. It has been reported that *Candida albicans* was the most sensitive strain, where the largest inhibition diameters have been noted even at low concentrations. This was due to the predominance of piperitone known by its antimicrobial activity already studied and the synergistic effect between essential oil components. It was demonstrated that biological activities of pure active ingredient were lower compared to the essential oils.

The introduction of sulphur in place of oxygen in the *Artemisia judaica* L. essential oil matrix caused an improvement in its antibacterial activity from 7 to 10% with the microbial strains and a remarkable improvement of the antifungal activity against *Candida albicans*, represented in Table 2, where a diameter of 25 and 30 mm was reported against *Escherichia coli* and *Staphylococcus aureus*, respectively. Also noticed was the effectiveness of this oil even at low doses from 1 to 10% with a diameter of 19 to 22 mm against *Escherichia coli* and 12 to 23 mm against *Staphylococcus aureus*; the essential oil became more active especially against the strain Gram negative *Escherichia coli*, and exhibited very strong antifungal activity against *Candida albicans*. Thioketones are known by their significant antifungal activity against highly aggressive fungi.

In the present study, the hydrophobization of the essential oil of *Artemisia judaica* L. caused an improvement in antibacterial activity, observed in the increase in inhibition diameter, and a diminution of MIC values against all tested strains.

Table 3 – Minimum inhibitory concentrations of essential oil (EO<sub>AJ</sub>) and thionylated *Artemisia judaica* L. essential oil (S-EO<sub>AJ</sub>)

| Microbial strains | Bacteria | Fungus |
|-------------------|----------|--------|
|                   | Concentration /% | Escherichia coli /mm | Staphylococcus aureus /mm | Candida albicans /mm |
| EO<sub>AJ</sub>   | 1        | –      | –      | 13       |
|                   | 5        | –      | 12     | 19       |
|                   | 10       | –      | 15     | 27       |
|                   | 100      | 18     | 20     | >40      |
| S-EO<sub>AJ</sub> | 1        | 19     | 12     | 15       |
|                   | 5        | 20     | 15     | 23       |
|                   | 10       | 22     | 23     | 30       |
|                   | 100      | 25     | 30     | >40      |
| Cephalexin        | 21       | 19     | 21     |
| Metronidazole     | 20       | 22     | 25     |

*Tests with pure solvent (DMSO) were negative.

These results were confirmed by the minimal inhibitory concentration study shown in Table 3, where the thionylated essential oil of *Artemisia judaica* was found to be more active against *Staphylococcus aureus* and *Escherichia coli* with MIC values of 0.5 and 1 mg ml<sup>-1</sup>, respectively, lower than those obtained from the native essential oil (1 and 2.5 mg ml<sup>-1</sup>). The antifungal activity of the thionylated essential oil against the fungus *Candida albicans* appeared the most significant with MIC of 0.031 mg ml<sup>-1</sup>.

Bacteria have a barrier system, biofilm formation, which inhibits the entry of disinfectants, antibiotics, and host immune molecules into bacterial cells, and is the major cause of drug-resistance of bacteria. The inhibition of biofilm formation has been studied in various scientific and technological fields, and it has been reported that hydrophobic products, especially natural sulphur compounds, provide effective resources for the inhibition of biofilm formation. These results confirm the results of this study, where the hydrophobization of the essential oil of *Artemisia judaica* L. caused an improvement in antibacterial activity, observed in the increase in inhibition diameter, and a diminution of MIC values against all tested strains.

4 Conclusion

In the present study, the hydrophobization of the essential oil of *Artemisia judaica* L. consisting mainly of piperitone (91.77%) by thionylation reaction caused a considerable improvement in its antimicrobial activity against the three microbial strains. The replacement of oxygen atom with sulphur atom rendered the oil more volatile and more reactive, which explains the increase in the sensitivity of the tested strains.

The thione-thiol tautomerism, monitored by UV-Vis and confirmed by GC-MS analysis, showed the predominance of the thiol form in solution.

ACKNOWLEDGEMENTS

This work was carried out with the support of the Directorate General for Scientific Research and Technological Development (DGRSDT) Ministry of Higher Education and Scientific Research of Algeria (MESRS).

The authors and in particular Prof. Hocine BOUTOUIMI would like to thank our dear Prof. Hussein KHALAF for his personal and scientific skills.
List of abbreviations and symbols

DMSO – dimethyl sulphoxide
EOAI – essential oil of Artemisia judaica L.
FTIR – Fourier-transform infrared spectroscopy
GC-MS – gas chromatography–mass spectrometry
Kl – Kovats retention index
MIC – minimal inhibitory concentration
RT – retention time, min
S-EOAI – silylated essential oil of Artemisia judaica L.
UV-Vis – ultraviolet–visible

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SAŽETAK

Hemisinteza derivata tiona i tiola iz eteričnog ulja biljke Artemisia judaica L. i antimikrobni testovi

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Iz eteričnog ulja biljke Artemisia judaica L., koje se uglavnom sastoji od piperitona (91,77 %), a dobiveno je hidrodestilacijom, reakcijom tionilacije pomoću fosforova pentasulfida (P2S5) hemi-sintetizirani su tion i tiol derivati. Moguća tion-tiolna tautomerija praćena je spektrofotometrijski.

Analiza je pokazala da u otopini dominira tiolni oblik. Također, pokazalo se da tionilacija eteričnog ulja uzrokuje znatno povećanje antimikrobne aktivnosti, osobito kad je u pitanju bakterija Escherichia coli.

Ključne riječi

Artemisia judaica L., eterično ulje, tionilacija, tautomerija, antimikrobna aktivnost

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Izvorni znanstveni rad

Prispjelo 20. rujna 2021.

Prihvaćeno 11. studenoga 2021.