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Antimicrobial and antioxidant activities of Moroccan *Thymus satureioides* essential oil

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Abstract

In order to increase the Moroccan *T. satureioides* value, the antimicrobial and antioxidant activities of its essential oil were evaluated. The antimicrobial activity was carried out against frequent pathogenic microorganisms which cause problems in the medical and food fields. The study of antibacterial activity was performed on Gram positive bacteria *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Micrococcus luteus* and Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella thyphimirium* while the antifungal activity was performed against two yeasts *Candida albicans* and *Candida tropicalis* by the microdilution method. According to the results of the minimal inhibitory concentration (MIC) we can conclude that the essential oil has antimicrobial potential against all microorganisms studied. *Candida tropicalis* was the most sensitive strain against the studied essential oil while *Pseudomonas aeruginosa* was the most resistant one. The evaluation of the antioxidant activity by using DPPH radical scavenging assay showed that this essential oil has a strong antioxidant power with an IC50 of 0.23 mg/ml.

1. Introduction

Development of resistance for many bacteria to antibiotics and detergents used in various procedures, including sterilization, equipment disinfection in food and medical domains, food preservation [1] and nosocomial infections related to implants has become a major public health problem [2].

In recent years, there has been a growing interest in researching and developing new antimicrobial agents from various sources to combat microbial resistance. The research tracks are numerous but the exploitation of natural resources seems more promising especially medicinal and aromatics plants which are the source of high-value added products such as essential oils. These products show diverse chemical structures of great importance both in the medical field and in the food industry especially regarding the antioxidant properties.

Among these plants, thyme is the most popular plant throughout all of the Mediterranean civilizations [3, 4]. Thyme species are still widely used in traditional therapeutic and food preservation practices in Morocco especially the strictly Moroccan one *T. satureoides*. This shrub is widely distributed in the arid and semi-arid parts of the Moroccan mountains. It is believed that part of these biological activities is due to its essential oils which have been shown to have very high antimicrobial effects compared to those of other plants [5-8]. Indeed, Chraibi et al [9] have reported that the essential oil of *T. satureioides* showed an important antimycobacterial activity against *Mycobacterium smegmatis* and *Mycobacterium aurum*, and Lindeman et al. [10] repported that *T. satureioides* essential oil would make an excellent candidate for an alternative treatment of chancroid that would be relatively risk free and cheaply produced. Furthermore, other previous studies showed that this essential oil showed high nematicidal, antioxidant, anticandidal and insecticidal activities [11, 12, 13].

In this present paper, the main goal was to determine the components of *Thymus satureioides* (*T. satureioides)* essential oil and to evaluate *in vitro* its antioxidant activity and antimicrobial properties against nine strains.

2. **Experimental**

2.1 *Plant material*

The aerial part (leaves and stems) of *T. satureioides* was freshly harvested and collected from Ljoukak (30°59'50.365" N, 8°9'45.13" E, altitude 1 210 m). The botanical authentication was confirmed at the National Institute of Medicinal and Aromatic Plants, Morocco; and specimens were deposited in its herbarium.

2.2 *Bacterial strains*

The microbial strains used in this work were: *Escherichia* coli ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Micrococcus luteus* ATCC 14452, *Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* ATCC 6633, *Salmonella typhimurium*, *Bacillus cereus*, *Candida albicans* and *Candida tropicalis.* All of these strains were taken from 20% glycerol stock at -20°C, rejuvenated on Luria-Bertani-Agar medium (LB) and sub-cultured before use.

2.3. *Extraction of essential oil*

Extraction of *T. satureioides* essential oil (E.O.) was performed by hydro-distillation method using clevenger-type appartus [14] to recover the essential oil for 3h. Essential oil obtained was stored in opaque glass bottle at 4°C.

2.4. *Determination of the Minimum Inhibitory Concentration (MIC)*

The MIC values represent the lowest essential oil concentration that completely inhibits the growth of microorganisms. Due to the immiscibility of essential oils to the water and thus the culture media, the emulsification was carried out with an Agar-agar solution at 0.2%. It provides homogeneous distribution of the essential oil in the culture medium and maximizes the contact germ / compound according to Remmal [15] and Satrani [16].

The MIC values were performed in 96 well-microplate using the micro-dilution assay according to the protocol previously described by Balouiri et al. [17] with slight modifications.

The final concentration of the essential oil was between 4 % and 0.003% (v/v). The 12^{th} well was considered as growth control (it contained only the culture medium and strain). Then, 50 µL of microbial inoculum was added to each well at a final concentration of 10^8 CFU/mL for bacteria and 10^3 CFU/ml for yeast inoculum. After incubation at 37°C for 24 h for bacteria and 30°C for 48 h for yeast, 10 µL of resazurin were added to each well as microbial growth indicator. After further incubation at 37 °C for 2 h, the microbial growth was revealed by the coloration change from purple to pink [18]. Experiments were carried out in triplicates.

2.5. *Antioxidant activity: Diphenyl-1-picrylhydrazyl (DPPH +) Method*

The ability of *T. satureioides* essential oil to scavenge free radicals was assayed with the use of a synthetic free radical compound 1,1-diphenyl-2-picrylhydrazyl (DPPH), according to the method described by Mighri et al [19]. Briefly, essentials oils were serially diluted (0.031, 0.625, 0.125, 0.25 and 0.5 mg/mL (w/v)) in methanol. A solution of DPPH (0.004% (w/v)) was prepared in the same solvent. Then, 3 mL of each dilution were mixed with 3 mL of DPPH solution. The mixtures were kept in the dark for 30 minutes and the optical density was measured at 517 nm. Butylhydroxytoluene (BHT) was used as standard. Each test was performed in triplicate.

The antioxidant activity was calculated as follows:

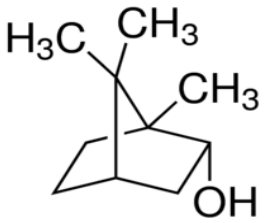
$$AA\% = \frac{Abscontrol - Abssample}{Abscontrol} \times 100$$

AA: antioxidant activity
Abs: absorbance

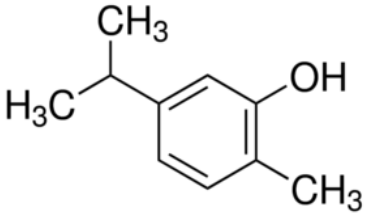
3. **Results and discussion**

3.1. *Essential oil composition*

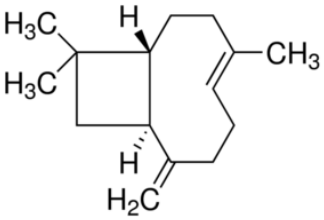
The essential oil was previously analyzed using GC-MS (Polaris Q ion trap MS). The results showed that 20 compounds were identified in the essential oil of *T. satureioides* representing 96.17% of its composition. The major constituents were borneol (34.26%), carvacrol (31.21%) and E-caryophyllene (6.32%) [9].This chemical composition of *T. satureioides* E.O. is broadly similar to that of *T. satureioides* from Ourika mainly composed by carvacrol (26.5%), borneol (20.1%), E-caryophyllene (5.7%) and α-pinene (4.6%) [20].



borneol (34.26%)



carvacrol (31.21%)



E-caryophyllene (6.32%)

3.2. *Antimicrobial effect of T. satureioides essential oil*

The results of the antibacterial activity of *T. satureioides* E. O. are represented in Table 1.

As can be noted in this finding, the essential oil tested has shown a remarkable antimicrobial effect towards all studied microorganisms; especially *Candida tropicalis* which showed a high sensitivity and was inhibited from a very low concentration of 0.007% followed by *Bacillus cereus* with a MIC of 0.015%. Moreover, the concentration of 0.03% (v/v) was sufficient to stop the growth of *B. subtilis*, *M. luteus*, *Candida albicans* and *Staphylococcus aureus*. While, *E. coli*, *S. typhimirium* and *P. aeruginosa* were inhibited by higher concentrations: 0.125 %, 0.25% and 1% respectively.

Table 1: Antimicrobial activity of *Thymus satureioides* essential oil

Microorganisms	**Concentrations v/v**											
	4 %	2%	1%	0.5%	0.25%	0.125%	0.062 %	0.03%	0.015%	0.007%	0.003%	Control
Candida tropicalis	-	-	-	-	-	-	-	-	-	-	+	+
Candida albicans	-	-	-	-	-	-	-	-	+	+	+	+
Bacillus cereus	-	-	-	-	-	-	-	-	-	+	+	+
Bacillus subtilis	-	-	-	-	-	-	-	-	+	+	+	+
Micrococcus luteus	-	-	-	-	-	-	-	-	+	+	+	+
S. aureus	-	-	-	-	-	-	-	-	+	+	+	+
Escherichia coli	-	-	-	-	-	-	+	+	+	+	+	+
Salmonella thyphimirium	-	-	-	-	-	+	+	+	+	+	+	+
Pseudomonas aeruginosa	-	-	-	+	+	+	+	+	+	+	+	+

This thyme's E.O. antimicrobial activity can be mainly attributed to its chemical composition rich in monoterpenes such as borneol and carvacrol [21]. Knoblock et al [22] have clearly shown that borneol possess rather good antimicrobial activity. A correlation of the E.O's antimicrobial activity and its chemical composition suggests that this activity could be attributed to the presence of high concentration of carvacrol. This compound, which is characterized by phenolic group, is indeed among the most efficient plant's antibacterial agents known to date [23, 24]. Thus, the Carvacrol can destabilize the cytoplasmic membrane and can act as a proton exchange agent, thereby reducing the pH gradient across the membrane. This conduces to cell death resulting from the collapse of the proton motive force and depletion of the ATP pool [25]. The major constituents are not necessarily responsible for the total antimicrobial activity; the contribution of less abundant components should be taken into consideration [26]. The lower susceptibility of Gram-negative bacteria to the essential oils may be explained by the structure of their cell wall which contains primarily lipopolysaccharides molecules and forms a hydrophilic barrier conferring protection against the effects of highly hydrophobic compounds [27].

3.3. *Antioxidant activity of Thymus satureioides essential oil*

The assessment of the antioxidant activity in this study was performed by using DPPH radical scavenging assay. The results were compared with the synthetic antioxidant BHT. The IC50defined as the amount of antioxidant required to reduce the free radical concentration of 50%, was determined for *T. satureioides* essential oil at 0.23

mg/ml (Figure1). While, the BHT exhibited a greater radical scavenging activity compared to the studied essential oil with an IC50 of 0.00682 mg/ml (Figure2). However, the synthetic antioxidants have fallen out of favor because of their carcinogenicity [28]. The antioxidant activity of essential oils is linked to the presence of phenolic compounds such as carvacrol. The main role of these compounds as reducing free radicals has been mentioned in several reports [29]. The major compounds of the essential oils are not the only ones responsible for the antioxidant activity. In general, it's the product of additive, synergistic and/or antagonistic effects, as they are complex mixtures of several classes of compounds. These findings agree those obtained by this natural plant showing that the use of *Thymus Satureioides* in the conservation process allows the preservation and the storage of plants with great hygienic and safety quality [30].

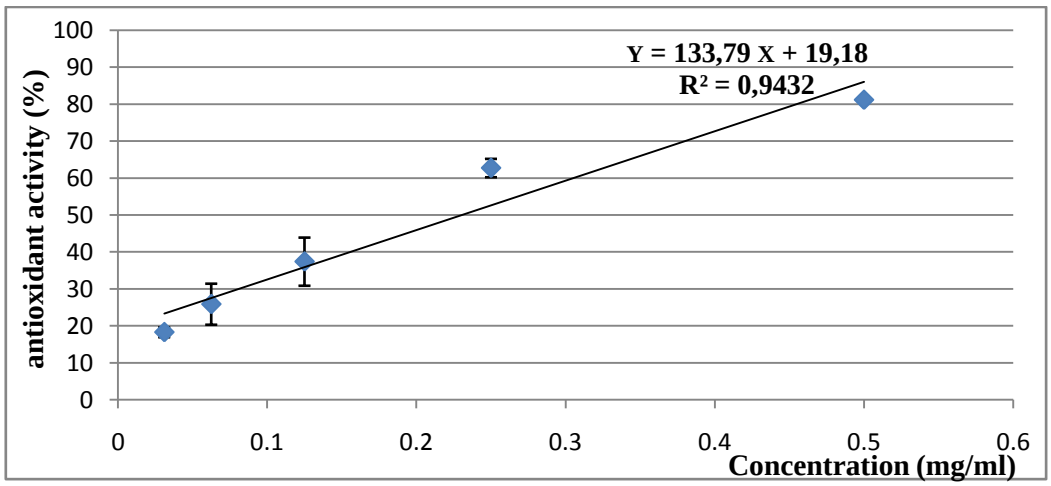


Figure 1: DPPH radical scavenging activity of *T. Satureioides* essential oil

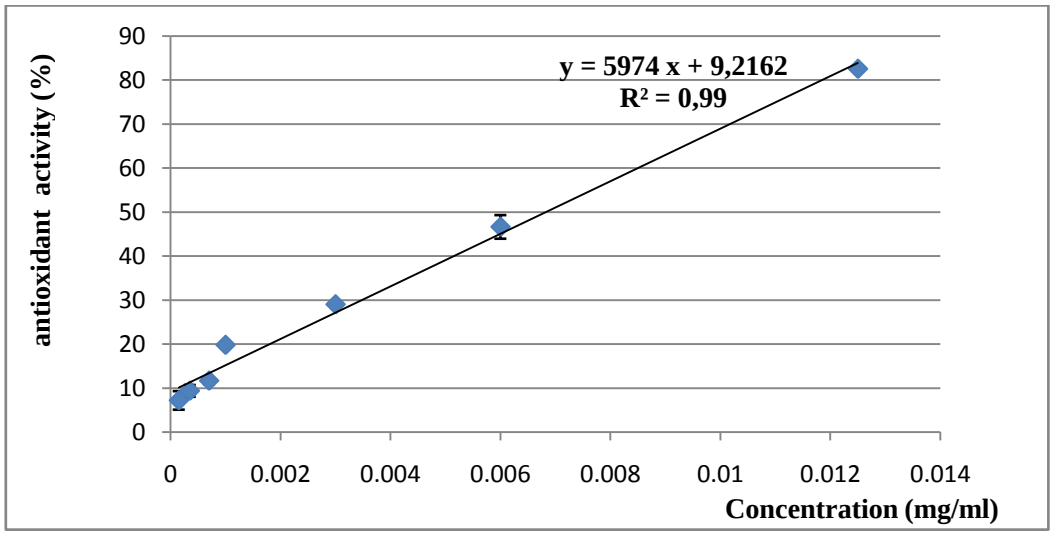


Figure 2: DPPH radical scavenging activity of BHT

Conclusion

The *T. satureioides* essential oil has shown an important inhibitory activity vis-a-vis all microorganisms studied: *Candida tropicalis*, *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Micrococcus luteus*, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella thyphimirium*. *C. tropicalis* was the most sensitive strain with a MIC value of 0.007% and *Pseudomonas aeruginosa* was the most resistant one with a MIC value of 1%. This remarkable activity is due to its major components particularly borneol (34.26%) and carvacrol (31.21%). Moreover, the studied essential oil has shown an important antioxidant activity, with an IC50value of 0.23 mg/ml. These results are very encouraging and indicate that this plant should be studied more extensively to explore its potential in the treatment of infectious diseases and in food preservation.

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