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Research Article

Effect of Harvest Period on Yield, Chemical Composition and Antibacterial Activity of the Essential Oils of Thymus Satureioides

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ABSTRACT
This study was performed to examine the yield, the chemical composition and the antibacterial activity of essential oils of *thymus satureioides*, grown in two different periods (flowering and after flowering) in the south of Morocco. These two essential oils are obtained by hydrodistillation along with using Clevenger and then they are tested on pathogenic bacteria: *Escherichia coli, Mycobacterium smegmatis* and *Bacillus subtilis*, responsible of food borne disease in the region of Fez. The extraction of the essential oil of *Thymus satuerioides* in the Laboratory of National Institute of Aromatic and Medicinal Plants (NIAMP), Taounate, Morocco for extraction has provided a yield important enough to 0.75 % in Period of flowering compared to gasoline extract of the aerial part after flowering season which is 0.62 %. The chromatographic analyzes and spectrometric have helped to determine the chemical composition of essential oils of approximately 23 constituents of these plants that are different by the harvest period for each one.

Keywords: *Thymus satureioides*, Essential oils, yield, Chemical composition, Antibacterial activity.

INTRODUCTION

In Morocco the sector of aromatic and medicinal plants is in full evolution despite his seniority, because our country is a traditional producer of essential oils and aromatic extracts, for effective and sustainable development of exponential plant biotechnologies. The sector of aromatic and medicinal plants must provide the requested products on the national and international market according to the criteria of quality required and respondents to the standards of the markets recipients. In addition to the market of the aromatic and medicinal plants is marked by a difficult competition and large sensitivity to the commercial hazards of the international trade. In order to market them, it is necessary to know the bioactive substances contained in the aromatic and medicinal plants that are used as active principle in the Pharmaceutical products, Cosmetics and Food. For this, it is necessary to determine some factors such as their yields, chemical compositions, and their activities[1]. In this frame work, the objective of this study is to determine the influence of the harvest period on the essential part of the secondary metabolite of aromatic plants such as the volatile part. In Morocco, the genus *Thymus* is represented by 21 species of which 12 are endemic[2]. The species of the thyme are the most exploited in Morocco. Hence the interest of this study which aims the recovery of this species by the search for bioactive substances. *Thymus Satureioides* known in Morocco under the common name "Zaetra" in Arabic and "Azoukeni" in Berber is one of the most plants that are used as spices and extracts for their high antibacterial and anti-inflammatory activity in the traditional pharmacopoeia[3]. It is encountered in the clearings of forests, matorral of low and medium sized mountains up to 2200 m, on calcareous and siliceous rocky soils or more at least earthy but well drains. In the form of small shrub, which can reach 60 cm, very boughs, sheets spatulas, inflorescences in glomerulus loose, pink corolla pink or very blade[4]**.** This plant occupies the High Atlas Mountains and Anti-Atlas, under dry bioclimat and sub-humid to warm and fresh variants, also at stages of infra Mediterranean and meso Mediterranean vegetation[4].

Essential oil of thymus

The majority of essential oils of thymus are characterized by their rich content of monoterpenes, in particular the thymol of phenolic compounds and its isomer carvacrol, accompanied by a range more or less biologically active, including the eugenol, p-cymene, the terpinene, linalol, geraniol and borneol[5]**.**

An essential oil often contains 50 to 100 different biochemical molecules different. The gas chromatography coupled to mass spectrometry is used to identify and quantify each of these molecules and thus to obtain the precise composition of the essential oils.

According to the studies conducted to the chemical composition of the species *T.satureioides*[6]. (Bouhdid et *al.* 2006) have found borneol (26.40%), thymol (11.48%) and carvacrol (8.76%) are the predominant compounds.

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Essential oil of thymus satureioides depends on factors that are directly related to the conditions of the plant specific lives to know: the climate, the soil, the exposure of plants and the harvest period, which can directly influence on the yeild and the chemical composition of essential oils[7]**.**

Essential oils of thymus are classified among the antimicrobial agents that have the best effectiveness, according to their strong activities, particularly against the pathogenic micro-organisms resistant to antibiotics[8].

MATERIAL AND METHODS

Equipment

Plant material

The plant material used is constituted of the sheets of the *thymus satureioides* harvested in two different periods, in a season of flowering which is in the spring and the other after flowering which is in the summer during the year 2014, in the region of Taroudant at the south of Morocco. After drying in the shade during a dozen days.

Bacterial equipment

In the present study, the micro-organisms listed below are chosen for their high frequencies to contaminate foodstuffs also for their pathogenicity. Gram-negative bacteria used: *Escherichia coli*, Gram-positive: *Bacillus subtilis* and *Mycobacterium smegmatis.*

Methods

Extraction of essential oils

The extraction of essential oils has been carried out by steam distillation (hydro-distillation) in a Clevenger apparatus in the Laboratory of National Institute of Aromatic and Medicinal Plants (NIAMP). Three distillations operations have been accomplished by boiling 100 g of the plant material with 400 ml of water in a flask of 1 liter surmounted in a column of 60cm in length connected to a refrigerant for 3h.

The yield of the essential oil has been determined in relation to the dry matter, evaluated from 3 samples of 30 g dried during 48 hours in the oven at 60°C. The essential oil has been stored at 4°C in the dark in the presence of sodium sulphate anhydrous. It is diluted in methanol (1/20, v/v) before proceeding analyze and CG CG/SM.

Chromatographic analysis

The chromatographic analyzes were performed on a gas chromatograph with electronic regulation of pressure of type Hewlett Packard (HP Series 6890), equipped with a capillary column HP-5 (30 m x 0.25 mm) with a thickness of 0.25 µm film, a Detector FID set at 260°C and is powered by a mixture of gases H_2/air and an injector splitsplitless set at 275°C. The mode of injection is split (leak report: 1/50). The gas used is the nitrogen with a flow rate of 1.7 $ml.min^{-1}$.

The temperature of the column is programmed 50 to 250°C to reason 4°C.min^{-1}. The device is controlled by a computer system of the type "HP Chemstation", managing the operation of the device and to monitor the evolution of the chromatographic analyzes.

The identification of the components has been carried out based on their indices of Kovats (IK), and on gas chromatography coupled with mass spectrometry (GC-MS). This last is performed on a gas chromatograph type Hewlett-Packard (HP Series 6890) coupled with a mass spectrometer (HP Series 5973).

The fragmentation is performed by electron impact to 70 ev. The column used is a capillary column HP-5ms (30 m x 0.25 mm), the thickness of the film is to 0.25 µm. The temperature of the column is programmed 50 to 250°C to reason 4°C.min-1. The carrier gas is helium whose flow is fixed at 1.5 $ml.min^{-1}$. The mode of injection is the split mode (Report of leak:1/70). The device is connected to a computer system managing a library of mass spectrum NIST 98.

Antibacterial activity

The antimicrobial activity is tested by using the method of dissemination on disc (Aromatogram), identical to that of sensitivity used to test the antibiotics. It is an in vitro measurement method of antibacterial activity that consist of depositing a sterile disc, soaked in essential oil, on the microbial carpet the very beginning of its growth and to measure the area where the bacteria have not been able to

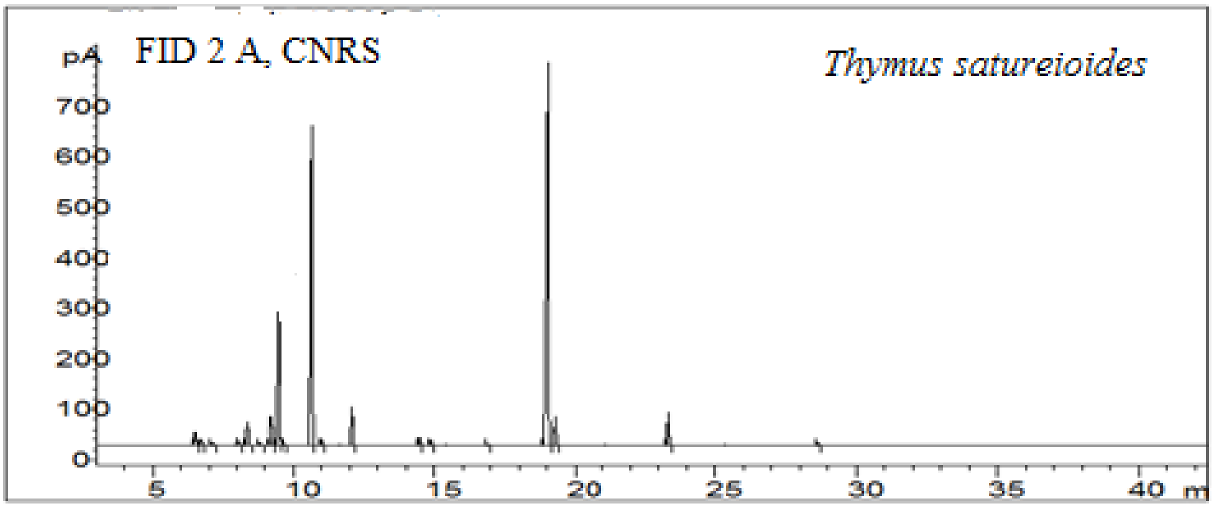
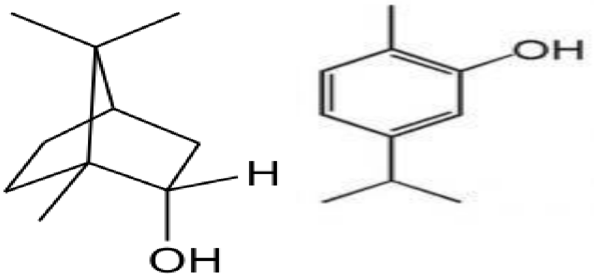


Figure 1: Chromatographic Profile of the essential oil T_1.

Table 1: Sampling and yield in essential oil.

Sample Code	Physiological stage	Yields
T_1	In Bloom	0.75±0.06%
T_2	After flowering	0.62±0.04%



Borneol Carvacrol

Figure 2: Chemical Structures of the constituent of the *T. Satureioides.*

develop. The diameter of inhibition which translated the antibacterial activity of the essential oil is thus determined. A bacterial suspension of equivalent density to the Standard 0.5 of Mac Farland (108 cfu.mL^{-1}) is prepared and then diluted to the 1/100. 20 mL of the agar medium Mueller Hinton are sunk by Petri dish. 2 ml of inoculum are deposited on each box. After an impregnation of 5 minutes, the surplus of inoculum is eliminated by suction. On the surface of each box, a commercial disc of sterile 6 mm diameter soaked with 10 of essential oil is field.

The boxes are left for 1 hour at ambient temperature and then returned and incubated at 37°C for 18 to 24 hours. After incubation, the diameter of the inhibition is measured in millimeters including the disc. Each test is conducted three times during the course of three successive experiences.

RESULT

Yield

The results of these samples extraction showed an average yield of essential oils with yellowish color extracted by hydro-distillation. In addition, they were calculated according to the dry plant material of the aerial part of the plant.

The best yield of the essential oil of *T. Satureioides* has been obtained by the samples of T_1: in Bloom (Flowering) who provided a rate of approximately 0.75% relatively higher than that obtained from the T2 samples: After bloom and which is 0.62 %. (Table1).

This difference is true similarly due to the vegetative stage of the plant at the harvest time of the sample. This is in agreement with the literature which shows a strong correlation between the yield in essential oil and the stage of development of the plant with a maximum at the stage of flowering[9]. We can conclude that the performance of essential oils reaches its maximum during the flowering phase in spring and its minimum during the period of dormancy in summer. Therefore, this difference in performance is attributed to a principle factor: the harvest period.

Chemical composition of essential oils

The composition of many essential oils has been described in the literature. Essential oils are among the mixtures that are more difficult to characterize, due to their complexity. The set of techniques of analyzes described namely the GC/FID and the coupling GC/SM, have enabled us to identify the majority of the compounds of these two essential oils (Table 2, Figure 1).

The essential oil of *T. satureioides* of Morocco is composed mainly of the borneol, carvacrol and thymol accompanied other constituents at low levels relatively: α-pinene and α-terpineol.

Indeed, qualitatively, for the two samples, a difference between the percentages has been detected. For petrol of the thyme in flowering (T_1) the results are the following: Borneol (34, 06%), thymol (1.71%), carvacrol (31, 12%).

In contrast for the essential oil of the plant after flowering (T_2) we obtained the following percentages: borneol (29.86%), thymol (2.71%), carvacrol (27.23%).

Quantitatively, the difference between all the compounds differ and change since it depends mainly on these factors: the stage of development of the plant, the removed organs (the presence of flowers), and the harvest period[10]. Therefore, the contribution of the state of the plant is very decisive in respect to the chemical quality of essential oils (Figure 1).

Antibacterial activity of essential oils of the t. Satureioides

The biological activity of an essential oil is linked necessarily to its chemical composition, to functional groups of compounds majority (alcohols, phenols, terpene and cetone) and their synergistic effects. It has been evaluated by observing the power inhibitor of our sample of essential oil of *T.Satureioides* on bacteria to test. The results are grouped in the Table 3.

The important bioactivity of the essential oil of *Thymus satureioides* is in relationship with its high content in borneol and carvacrol. According to the results obtained it can be concluded that the bacterium *Escherichia coli* which is of Gram-negative bacteria to manifest a resistance to the two essential oils tested to a diameter of 13.66 mm to extract gasoline during the period of flowering and 19.42 mm after flowering, compared to other bacterial species. The bacterium is the most sensitive to this essential oil: *M.smegmatis* to a diameter of inhibition 28.34 mm for T_1, and 24.3 mm for T_2. Concerning *A.subtilis* a small difference in area of inhibition between the two samples.

Moreover, the results obtained are consistent with those of Amarti[12] and Satrani[11] which has demonstrated the relationship that exists between the chemical composition and the antimicrobial activity.

The essential oil of Thymus after Bloom is more active compared to the essence of thymus in bloom against the Gram-negative bacteria. This difference may be due to the presence of the thymol (2.71%) after Bloom which exerts

Table 2: Chemical composition of essential oils extracted from samples of the *T. Satureioides*.

IK	Compounds	T1	T2
931	α –thujene	0.05	0.04
939	α –pinene	3.14	8.24
967	Verbenene	2.34	1.34
976	Sabinene	1.24	1.64
980	β –pinene	2.44	1.98
1011	Δ -3-carene	0.34	0.34
1018	α –terpinene	0.05	0.04
1026	P-cymene	2.29	4.22
1031	Limonene	0.38	0.31
1033	1,8-cineole	3.34	4.38
1062	Γ-terpinene	2.02	2 ,56
1087	Fenchone	2.54	4.54
1143	Camphor	0.43	1.02
1156	Isoborneol	0.08	0.02
1165	Borneol	34,06	29.86
1177	Terpin-4-ol	0.08	1.07
1189	α –terpineol	3.16	6.16
1290	Thymol	1.71	2.71
1298	Carvacrol	31,12	27.23
1404	Z-caryophyllene	0.02	0.02
1418	E-caryophyllene	2.02	1.02
1440	β –humulene	0.03	0.05
1480	germacrene D	0,05	0.03
	Total (%)	92,93	98.82

Table 3: Evaluation of the antibacterial activity of essential oils of the *Thymus satureoides*.

Cooperatives	Diameter of the zone of inhibition (mm)		
	E. coli	Subtilis	M. Smegmatis
T1	13.66 ± 0.43	$26{,}21 \pm 2.08$	$28{,}34 \pm 1{,}05$
T2	19.42 ± 1	$27{,}34 \pm 1.02$	24.32 ± 1.52

an inhibitory effect and lethal on different strains, Gram (-) among them, Escherichia coli and on which it causes leakage of potassium ions. The essential oil of the plant harvested season of flowering has demonstrated a significant inhibitory effect against microorganisms Gram positive studied.

CONCLUSION

Thymus satueioides has provided an essential oil with an important yield with a percentage of 0.75% in Period of flowering compared to gasoline extract of the aerial part in the presence of the flowers which is 0.62%. The chromatographic and spectrometric analyzes have made it possible to determine the chemical composition of essential oils approximately 98% of the constituents of these plants that are different by the harvest period for each one.

Thanks to their wealth in terpene alcohols: borneol and carvacrol the essences of T. Satureioides have proved their effectiveness against the three micro-organisms studied. These preliminary results have allowed us to determine that the best harvest period of the thymus satureioides is after flowering in the month.

The highlight of the yeild of these essential oils, on different bacterial strains, can lead to a thorough study and to the prospects for their application as the agent of phyto-medication and conservation of food.

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