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Comparative study of chemical compositions and antimicrobial effect of different genius of Thymus harvested during two period of development

Aïcha Touhami[1,2 *], Azzedine Chefrour[3,4], Abbes Boukhari[1,5], Fadhel Ismail[1,6]

[1]Laboratory of Organic Synthesis-Modeling and Optimization of Chemical Processes, Badji Mokhtar University, P.O. Box 12, 23000 Annaba, Algeria. [2]EPST-Annaba, P.O. Box 218, 23000 Annaba, Algeria. [3]Faculty of natural science and life, University of Mohamed Cherif Messaadia, P.O. Box 12, 41000 Souk-Ahras, Algeria. [4]Laboratory Development and Control of Hospital Pharmaceutical Preparations. Medical Science Faculty, University of Badji Mokhtar- Annaba, LP 205 Ezzafrania, Annaba (23000) Algeria. [5]Department of Chemistry, Faculty of Science, Badji Mokhtar University, P.O. Box 12, 23000 Annaba, Algeria. [6]Department of Process Engineering, Faculty of Engineering, Badji Mokhtar University, P.O. Box 12, 23000 Annaba, Algeria.

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ABSTRACT

The essential oils obtained by steam distillation of the aerial parts of *Thymus* taxa collected before and during the flowering period in different region of eastern Algeria, were analyzed by gas chromatography coupled with mass spectrometry (GC-MS). The extraction yields of essential oils of *T. numidicus* species are 1.28 % during pre-flowering. This yield is better during the flowering period with 2.85%. *T. algeriensis* gave extraction yields 1.156 % and 1.79 % in the two periods respectively. *T. ciliatus* produced oil with yields of 1.002 % before flowering and 1.79 % during flowering. Chromatographic analysis (GC-MS) showed that these essential oils are rich with oxygenated monoterpene compounds with 54.07 % and 80.37% of the composition for *T. ciliatis*, 61.86 % and 73.02 % for *T. algeriensis*, 54.48 % and 77.56 % for *T. numidicus*, corresponding to the two periods mentioned above respectively. The antimicrobial activity was tested against 3 gram positive and gram negative bacterial by the use of the disc diffusion method. The thymus taxa have shown great sensitivity. The three essential oils are effective against *Escherichia. coli 22*, while essential oil of *T. ciliatus* obtained before flowering and essential oil of *T. numidicus* in flowering period are most effective against *Staphyllococcus 23.*

INTRODUCTION

Essential oils, called usually essences are volatile substances contained in vegetation. Their volatility opposes them from fixed oils which are lipids. Essential oils are mixtures of numerous constituents called chemotype. The *Thymus* species being a part of the botanical family of *Lamiaceae*, include several developing around the Mediterranean sea, especially in North Africa it consist in 300-400 species (Evans, 1989; Pedersen, *2000).* All species of the *Thymus* genus produce essential oils (Marouki *et al.*, 2007) and are characterized by high chemical variability (Dob, 2006). This genus thymus has been always considered as spice (Tainter, 1993) and is usually used in savory formulation, sauces and liquors.

They are aromatic plants very used in galenic pharmacy and beauty care. This is due to their wide biological activity: antibacterian (Essawi *et al*., 2000; Marina, 1999), anti tumoral (Jaafar *et al*., 2007), anti oxydant (Miura *et al*., 2002) and antifungal (Giordani *et al*., 2008). Our work consists on the study of the chemical composition of the essential oil extracted from species of *Thymus* (*T. nimidicus*, *T. ciliatus* and *T. algeriensis*) collected before and during flowering periods from different regions in eastern Algeria. Antimicrobial activity of *Thymus* essential oils in two phase of growth were determined by agar disc diffusion method (Malabadi *et al*., 2012).

**Corresponding Author*
Touhami Aicha, 1 Laboratory of Organic Synthesis-Modeling and Optimization of Chemical Processes, Badji Mokhtar University, P.O. Box 12, 23000 Annaba, Algeria 2 EPST-Annaba, P.O. Box 218, 23000 Annaba, Algeria. Email:a.touhami23@yahoo.fr

EXPERIMENTAL

Plant material

The different species of *Thymus* were collected: *T. numidicus* from Berrahal a western region of the department of Annaba, *Thymus algeriensis* from the region of Selaoua Anouna and the *Thymus ciliatus* from Ain Makhlouf in the southwest of the city of Guelma. The gathered *Thymus* was dried until the stabilization of their weight.

The extraction of the essential oil was made by hydrodistallation (Clevenger-type apparatus). The distillations were realized by boiling, during 3 hours, 50 g of the plant dried with 500 ml of water in a ball of 1L overcome a column 60 cm of length and connected with a refrigerant. The yields in essential oil were determined with a regard to the dried material. These oils were stored and maintained optimally at 4° C protected from light according to literature protocol (Moumni *et al*., 2013a).

Analysis of the essential oils

Essential oils analysis

The qualifications of the constituents of essential oil was realized by a chromatogram type SHIMATZU QP2010, provided with an FID type detector, equipped with a capillary column OV 17, of 25 m length and 0.25 mm diameter, with a thickness of the movie of 0.25 µm. The temperature of the oven was maintained at 60°C during 1 minute, and then increased at 200°C with a speed of 3°C/min. Later we had maintained in isotherm during 16 min. The temperature of injections is 250°C, the volume of each of the samples 0.1µl. After 1/10 dilution in the ethanol grad chromatography each sample, was injected in Split Less mode. The fragmentation is made by electronic impact in 70 Ev, the carrier gas is the helium with a constant pressure of 25.6 kPa. The identification of the compounds was made by comparison of the mass spectrum of with those contained in the NIST bookshop.

Antimicrobial activity

The antimicrobial activity of essential oils of *Thymus* taxa was qualitatively determined using the method aromatogram solid environment. This technique involves depositing sterile discs, impregnated with essential oils of the *Thymus* taxa to the surface of Petri dishes in a solid environment carrier " Muller Hinton agar and nutritive blood" of the bacteria studied. DMSO aprotic organic solvent chosen for its inactivity bacteria. A range of 6 dilutions 1/4, 1/8, 1/16, 1/32, 1/64, 1/128 was prepared. The bacterial strains used are gram-positive: *Escherichia coli* 22 and gram-negative: *Staphyllococcus* 23, *Pseudomonas* 53, they have been obtained from microbiological laboratory, Faculty of Medicinal Science, University of Badji Mokhtar-Annaba. Algeria.

RESULTS AND DISCUSSION

The yields of essential oils of the various species of *Thymus* of several regions in eastern Algeria were calculated with regard to the dray vegetable material for two periods: pre and during flowering. Table 1 summarizes the obtained results.

Chemical composition of the essential oils

The chromatographic analysis of the essential oil has allowed us to identify their constituents. The obtained results are mentioned in (table 2).

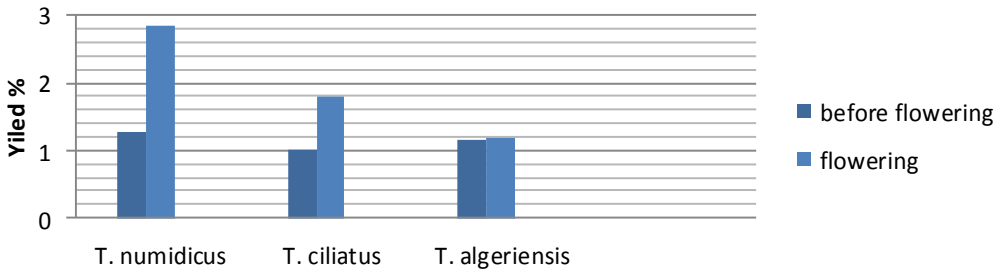

Fig1: Yields of essential oils during the two periods.

Table 1: Yields in essential oil of various studied species.

Botanical species Taxa	Botanical family	Region	Harvested period		Yields	
			Before flowering	Flowering	Before flowering	Flowering
T. numidicus	Lamiaceae	Berrahel (Annaba)	10/03/2010	29/05/2010	1.280 %	2.850 %
T. ciliatus		Ain Makhlouf (Guelma)	31/03/2010	06/05/2010	1.002 %	1.790 %
T. algeriensis		Selaoua Anouna (Guelma)	11/04/2010	06/05/2010	1.156 %	1.176 %

Table 2: Chemical compositions of the *Thymus* essential oils in different parts of Eastern Algeria before and during flowering periods.

components %	***T. numidicus***		***T. ciliatus***		***T. algeriensis***	
	B -flowering	**flowering**	**B -flowering**	**Flowering**	**B -flowering**	**Flowering**
Monoterpenes hydrocarbons	**38.23 %**	**16.9%**	**35.8 %**	**15.52%**	**25.36 %**	**16.46%**
α thujene	-	0.18	-	0.14	-	-
α pinene	0.56	0.57	-	0.46	4.15	3.08
Camphene	-	00.4	-	-	3.49	-
β pinene	1.02	-	-	-	0.20	-
myrcene	-	-	0.58	-	-	-
β myrcene	-	1.28	-	1.55	-	-
α phyllandrene	-	0.26	0.49	-	-	-
Limonene	-	0.10	-	0.48	5.13	-
Isolimonene	-	-	-	-	5.5	-
Ocimene	0.37	-	-	0.04	0.53	-
trans β ocimene	8.80	0.26	-	0.41	-	-
Cis β ocimene	-	0.40	-	0.65	-	-
O.Cymene	22.35	-	11.88	-	6.36	-
β cymene	-	6.54		5.96	-	7.74
α terpinene	0.28	-	-	-	-	-
γ terpinene	0.16	6.12	22.34	4.75	-	5.64
Terpinolene	4.33	0.17	-	0.20	-	-
2-carene	-	0.65	-	0.60		-
4-carene	0.36	0.01	0.51	-	-	-
Dimethyl styrene	-	0.32	-	0.28	-	-
Oxygen-containing monoterpenes	**54.48 %**	**76.96%**	**54.07 %**	**80.37%**	**61.86 %**	**77.56%**
Eucalyptol	-	1.88	-	2.34	5.31	2.01
Linalool	31.14	7.47	23.58	8.55	4.68	2.60
pinocarveol	-	0.05	-	-	-	2.67
Camphor	-	1.29	-	3.28	33.30	3.64
Borneol	0.38	0.94	-	0.75	4.05	-
Isoborneol	-	-	-	-	-	2.28
Terpinene-4-ol	-	1.90	-	0.96	-	-
γ terpineol	-	0.10	-	0.23	-	-
P-Cymene-7-ol (cumique alcool)	-	-	-	-	-	26.98
Thymol	20.28	39.66	25.08	54.04	-	-
Pinene-10-ol	-	-	-	0.23	-	-
Cis pinene-3-ol	-	-	-	0.58	-	-
Pinene-3-one (pinocarvone)	-	-	-	0.14	-	-
Trans-3-carene-2-ol	-	-	-	0.42	-	-
Cis Carveol	-	-	-	-	1.59	-
Carvacrol	-	2.52		7.09		-
Verbenone	--	0.05	-	0.13	-	13.18
Cis Verbenol trans	-	-	-	-	1.57	-
Thymol methyl ether	0.60	-	5.41	-	-	-
thujenal	-	-	-	0.14	-	-
isothujol	-	-	-	0.09	-	1.08
carveol	-	-	-	0.14	-	-
Thymol methyl ether	-	-	-	1.00	-	-
Thujen-3-ol acetate	-	-	-	-	0.69	-
Isothujol	-	-	-	-	3.06	-
Bergamol	1.81	-	-	-	-	1.7
Cis terpineol acetate	-	-	-	-	1.40	-
Trans-3- carene-2-ol	-	-	-	-	-	-
Trans linalool oxyde	-	0.07	-	-	-	-
Cis linalool oxyde	-	-	-	0.19	-	-
Gamma terpineol acetate	-	-	-	0.05	-	-
Cis sabinene hydrate	-	-	-	-	0.3	-
Sabinyl acetate	-	-	-	-	1.74	-
Isobornyl acetate	-	-	-	-	2.86	-
citroviol	0.27	-	-	-	-	-
Lignyl acetate	-	-	-	-	0.44	-
Camphénol	-	-	-	-	0.87	-
Caprylique alcool	-	0.03	-	-	-	-
Caproique aldehyde	-	-	-	0.02	-	-
Caprique aldehyde	-	-	-	-	-	0.96
Carveol dihydro	-	-	-	-	-	0.83
Methy tert butyl ether	-	-	-	-	-	19.63
Hydrocarbons sesquiterpene	**6.09 %**	**2.44%**	**10.13 %**	**1.6%**	**10.68 %**	**0%**
Bioallethrin	0.72	-	-	-	1.06	-
Myrtenyl acetate	-	0.04	-	0.04	-	2.59
α Trans bisabolene epoxyde	-	0.03	-	0.05		1.44
Valeral	-	-	-	-	-	-
α Tetradecyne	-	-	-	0.06	-	-
α Dodecynlen	-	-	-	0.07	-	-

δcadinol	-	-	-		0.50	-
Longipinene epoxyde	0.17	-	-	0.03	-	-
α bisabolol	-	-	-		0.54	-
Vinyl amyl carbinol	-	3.37		2.03	-	1.12
Citroviol	-	0.26	-	-	-	-
isovaleral	-	-	-	-	-	0.83
Isosativene	0.31	-	-	-	-	-
Ascaridol epoxyde	-	-	-	0.07	-	-
Myrcnyl acetate	-	-	-	0.03	-	-
3-decen-2-ol	-	-	-	0.02	-	-
Eugol acetate	-	-	-	0.11	-	-

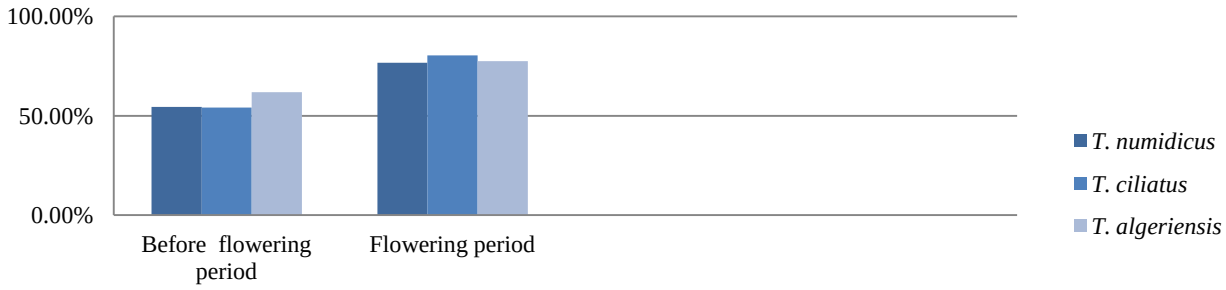

Fig 2: Yield of oxygenated monoterpens of the Thymus during two period of development.

T. numidicus

The essential oil yield in before flowering period was (1.28%) and became (2.85%) during the flowering period, this increase may explain the change of the rate of the components. the result obtained during flowering is better compared to (Zeghib *et al*., 2013), which was (2.00%). In period before flowering, we found 24 components; that number climbed to 43 components during the flowering period. The major component is linalool in before flowering period (31.14%) and thymol (39.66%) during flowering period. The percentage of thymol increased (20.28%) before flowering period to (39.66%) during flowering, the same remark is attributed to γ terpinene, the rate increased from (0.16%) to (6.12%). We also note the appearance of new components during the flowering period: carvacrol (23.52%), eucalyptol (1.88%) and α phyllandrene (0.26%). The essential oil of *T. numidicus* was dominated by oxygenated monoterpenes in the two periods with 54.48% and 76.96% (Figure 2). The essential oil composition of Thymus numidicus obtained in flowering period is in agreement with the result found by (Zeghib *et al*., 2013) where thymol is the most important compound (23.92%) followed by linalool (17%).

T. ciliatus

The distillation of this oil gave a yield (1.002%) in before flowering period, and (1.790%) during flowering. The number of components increased for 13 components before flowering period to 54 components during flowering period. A net decrease in the production of certain compounds was observed in the flowering period such as γ terpinene (22.34%, 4.75%), linalool (23.58%, 8.55%) and caryophyllene (1.71%, 0.38%). Thymol is the major component in the two periods (25.08%) before flowering period and (54.04%) during flowering. The same result was found by (Ghorab *et al*., 2014; Amarti *et al*., 2010; Heni *et al*., 2015), where the major component was thymol (79.1%, 44.2%, 67.78%) respectively, but the majority component of *T. ciliatus* collected in Tlemcen (Khadir *et al*., 2013) was carvacrol with (80.1%).

Other components are present only in the flowering period with a low rate, for example: carvacrol (7.09%), β cymene (5.96%), camphor (3.28%), eucalyptol (2.34 %), β myrcene (1.55%) and ether methyl thymol (1.00%). *T. ciliatus* is rich in oxygenated monoterpenes their percentage (61.86%) in the period before flowering (77.56%) during flowering.

T. algeriensis

The essential oil yields of *T. algeriensis* obtained in two periods are slightly different: (1.155%) in period before flowering and (1.176%) during flowering period. The number of components fell for 30 to 19 during flowering period. Camphor is the predominant compound in before flowering period (33.30%), but it completely fells during the flowering period where we found only (3.30%).

During the flowering period, we have the P-cymen-7-ol with a important percentage (26.18%) while this compound did not exist in before flowering period. The major components obtained during the flowering period found by (Amarti *et al*., 2010) and (Chemat *et al*., 2012) were respectively: camphor (27.70%) and thymol (71.45%).

The isolimonene and limonene were (5.5%) and (5.13%) in before the flowering period have completely disappeared in flowering period. We also noticed the appearance of new components during flowering period such as Methyl tert-butyl ether (19.29%), verbenone (12.84%), and γ terpinene (5.30%). The oxygenated monoterpenes represent a significant rate: (61.86%) before the flowering period and (77.56%) during the flowering period.

Table 3: Antimicrobial activity of the different genius of *Thymus* collected before and during the flowering period in different regions of Eastern Algeria.

		T. numidicus		***T. ciliatus***		***T. algeriensis***	
		Before flowering	**Flowering**	**Before flowering**	**Flowering**	**Before flowering**	**Flowering**
E. coli 22	1/4	34.25	35	33	27.7	9.8	42.2
	1/8	22.95	14.2	30.2	18.8	9.5	16.2
	1/16	12.3	14.4	17.5	11.7	7.7	13.1
	1/32	11.55	13.6	11.7	9.2	-	11.1
	1/64	10.1	-	9.1	-	-	-
Staphyllococcus 23	1/4	26.4	43.4	50.8	21.5	12.9	10.1
	1/8	24.8	23.9	36.2	14.0	11.2	-
	1/16	12.2	13.1	25	12.5	7.5	-
	1/32	11.2	12.7	24.3	10.4	-	-
	1/64	10.3	12.1	7.1	08.0	-	-
	1/124	-	10.7	-	06.0	-	-
Pseudomonas 53	1/4	22.15	16.2	11.5	-	8.3	-
	1/8	13.9	-	10.1	-	7.3	-
	1/16	-	-	-	-	-	-

Microbial activity

The discs diffusions tested with 10 µl of the essential oils of different taxa of *Thymus* collected before and during flowering period have proved the good results with reference bacteria microorganisms with (7.3 to 50.8 mm) inhibition zones. The results obtained are mentioned in table 3. The tests of antibacterial activities on the three referenced strains have shown that the majority of essential oils of three taxa (*T. numidicus, T. ciliatus. and T. algeriensis*) have high inhibition values in particular for dilutions 1 / 4, 1/8 and 1/16.

The bacterial strain *Escherichia coli 22* showed sensitivity to essential oils of three taxa. Essential oils of *Thymus algeriensis* obtained during flowering period with 1/4 dilution gave a very significant inhibition diameter (42.2mm), followed by the essential oil of *Thymus numidicus* in pre flowering and flowering periods with inhibition diameters (34.25mm) and (35mm). The results obtained during the flowering period is better than found by (Zeghib *et al*., 2013), which was (20.4 ± 0.5 mm). With the 1/8 dilution inhibition the important result was observed with the essential oil of *Thymus ciliatus* harvested before flowering period (30.2mm). The germ *Staphyllococcus 23* showed a high sensitivity to essential oil *Thymus ciliatus* harvested before flowering period with diameter between 24.3mm and 50 mm depending on the dilution, and the essential oil of *Thymus numidicus* obtained during flowering with (43.3 mm) for a dilution 1/4. *Pseudomonas 53* showed resistance for the full range of dillution of essential oils except for *Thymus numidicus* in two periods of development with dilutions 1/4 and 1/8.

The same result was found by (Zeghib *et al*., 2013) for the *T. numidicus* harvested during flowering with (16.4 ± 0.5 mm) of inhibition against *Pseudomonas* 53. (Kouch *et al*., 2014) found (38.5 mm) of inhibition with the essential oil obtained during flowering. The results obtained show that the three essential oils are effective against *Escherichia. coli* 22, while essential oil of *T. ciliatus* obtained before flowering and essential oil of *T. numidicus* in flowering period are most effective against *Staphyllococcus* 23. The importance of antimicrobial activity for the species *thymus* is related to their high content of phenolic compounds; in fact several authors (Pellecuer *et al*., 1980), (Gergis *et al*., 1990), (Panizzi *et al*., 1993), (Sivropoulou *et al*., 1996; Trombetta *et al*., 2002; Satrani *et al*., 2008; Chemat *et al*., 2012) showed that the essential oils rich in phenolic derivatives possess strong antimicrobial activity. (Dorman *et al*., 2000) showed that thymol is the compound that has the broadest spectrum of antibacterial activity, which explains our results found with the essential oils of *T. numidicus* and *T. cilatus* before and in flowering periods, where the percentage of thymol was between (20, 28% and 54, 04%), the latter is totally absent in the essential oil composition of *T. algeriensis*. Camphor is the main component for *T. algeriensis*, it is also known for his anti-microbial activity (Aligrannis *et al*., 2000) and (prudent *et al*., 1993).

CONCLUSION

In this work we studied the chemical composition of essential oils of different genius of Thymus (*T. numidicus, T. ciliatus and T. algeriensis*) collected before and during the flowering periods in different regions of Eastern Algeria. The chemical study of essential oil of the collected *Thymus*, revealed that essential oil yield of the three *Thymus* species in two stages were ranged from (1.002%) to (2.850%).

The percentage of essential oil increased in the flowering period. Analysis of essential oil by GC-MS indicated that the *T. numidicus* is constituted by linalool (31.14%), O- cymene (22.35%) and thymol (20.28%) in before flowering stage, and thymol (39.66%), linalool (7.47%) and beta- cymene (6.54%) in flowering stage. The chemotype of the *T. ciliatus* in two development period is thymol (25.08% and 54.0 4%). Linalool and γ terpinene presents respectively 23.58% and 22.34% before flowering period. In flowering period we have carvarcol with (7.09%) and β cymene (5.96%).

The chemotype of *T. algeriensis* is camphor before flowering stage with (33.30%) and p-cymen-7-ol in flowering period with (26.98%). We concluded that the chemical compositions of different geniuses of Thymus are mostly constituted by oxygenated monoterpens. The essential oils were tested by 3 bacterial strains and gave good results, we explain this by the presence of phenol derivatives such as thymol , linalool and γ terpineol.

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