



Comparative study of the chemical composition and biological activities of the essential oils of *Senecio gallicus* from Tunisia.

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ABSTRACT

The essential oils of flowers and remaining parts of the plant *Senecio gallicus* (Asteraceae), growing wild in Sfax (Tunisia), were obtained by hydrodistillation over a period of two years (2012 and 2013). Their analysis by Gas Chromatography-Mass Spectrometry (GC-MS), led to a total number of 36 components, belonging to different classes of chemical compounds. Oils compositions were characterized by the abundance of monoterpenes hydrocarbons, the major compounds present in flowers for the two years of study were respectively the sabinene (49.45% and 28.86%), the α -pinene (9.67% and 9.1%), and the β -myrcene (9.88% and 10.97%). These compounds were also dominant in the essential oils of the plant without flowers where they represent (65.34% and 55%) for the sabinene, (4.14% and 7.3%) for α -pinene, and (6.86% and 0%) for β -myrcene. Obtained essential oils were tested for many biological activities and showed a moderate effect against the fungus *Trichoderma reesei* and bacteria such as *Bacillus* sp and *Staphylococcus aureus*. This study of the *Senecio gallicus* essential oils represents the first one in Tunisia.

Keywords

Senecio gallicus; Essential Oils; chemical composition; Sabinene; antifungal activity.

Academic Discipline And Sub-Disciplines

Sciences; Chemistry; Biochemistry.

SUBJECT CLASSIFICATION

Chemistry of fragrances and essential oils.

TYPE (METHOD/APPROACH)

Comparison of obtained experimental researches.

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INTRODUCTION

Senecio gallicus is a plant that belongs to the Asteraceae family [1,2]. The genus *Senecio* comprises about 1300 species [3]. Alkaloids and sesquiterpenes are the major components in this genus [4-6], especially pyrrolizidine alkaloids [7]. In folk medicine, *Senecio* species have been used for the treatment of wounds as well as anti-inflammatory and vasodilator preparations [8-10]. It was also reported that *Senecio graveolens* (Compositae) can be used for the treatment of mountain sickness [11]. These authors have isolated from molecules endowed of a strong antihypertensive activity through inhibition of the angio-tensin converting enzyme (ACE) [11]. Few reports about essential oil contents of members of the genus *Senecioneae* have been reported [12-18].

In Tunisia, *Senecio gallicus* species account for 5% of all Asteraceae localized in the south of the country [12], growing up during the period of spring when nutritional conditions are favorable [19]. Generally, this plant is found in altitudes between 15 and 1500 meters; its life cycle is very short and usually does not exceed few weeks [12]. Several previous works showed the richness of this plant in flavonoids, alkaloids [20], terpenes [21] and minerals [22]. In Tunisian traditional medicine, *Senecio gallicus* has been used to regulate blood circulation and as a uterine sedative [23,24]. Akrouf et al studied eight annual species growing wild in the southern of Tunisia (*Diplotaxis simplex*, *Chrysanthemum coronarium*, *Matthiola longipetala*, *Erodium glaucophyllum*, *Reseda alba*, *Diplotaxis harra*, *Senecio gallicus* and *Papaver rhoeas*) by evaluating their mineral contents and phytochemical screening [24]. They mentioned that *Senecio gallicus* and *Chrysanthemum coronarium* were the only species that contained essential oils but they didn't determine their exact composition and the nature of essential oils [24].

The richness of *S. gallicus* in mineral, oils and other secondary metabolites stimulated our interest to study the chemical composition of its essential oils, for the different compartments of the plant (flowers, and remaining parts) and to evaluate some of their biological activities.

EXPERIMENTAL PART

Plant Material

Senecio gallicus, in full bloom, were collected during the spring (month of March) over the two years 2012 and 2013 at the same place in the city of Sfax (Tunisia) (Table 1.). Voucher specimens (LCSN 116) were authenticated by Pr. Mohamed Chaieb and have been deposited in the "Laboratoire de Chimie des Substances Naturelles" of the Faculty of Sciences of Sfax. Fresh aerial parts were cut in small pieces and subjected to hydrodistillation for 3 hours. The volatiles were extracted using diethyl ether as solvent; the organic phases were subsequently dried over anhydrous sodium sulphate and then evaporated under vacuum. Obtained yellowish essential oils, with a pleasant scent, were stored under N₂ atmosphere in amber vials at 4 °C until they were analyzed.

Table 1. Collection data and oils yields of investigated *Senecio gallicus*.

Species	Voucher number	Collection Period	Region	Essential oil yield (% v/ weight)	
				Flowers	Plant w/o flowers
<i>Senecio gallicus</i>	LCSN 116	Mars 2012	Tunis Road km 6, Sfax (Tunisia)	0,14	0,037
	LCSN 116	Mars 2013		0,14	0,038

Microorganism and Media

The test organisms used in this study were *Staphylococcus sp*, *Bacillus sp*, *Trichoderma reesei* and *Aspergillus niger* obtained from the Microbial Type Culture Collection, CBS, Sfax (Tunisia).

Essential oils isolation

The fresh plant was divided into two parts, flowers and the remaining part of the plant without flowers (named here plant w/o flowers). Extraction of the essential oils from each part was performed using the method of steam distillation. Each organ was hydrodistilled alone. 1082g and 700g of fresh flowers collected over the period of two years have been contacted with water in a conventional hydrodistillator for a period of 3 hours. After distillation, the oil was collected and dried with anhydrous sodium sulfate Na₂SO₄, and was kept away from light and heat at a temperature of 4 °C until analyzed. The same procedure was made with 1110g and 700g of plant devoid of flowers.

Analysis of the essential oils

Analyzes of the essential oils were performed using an Agilent 6890 gas chromatograph equipped with a fused silica capillary column type HP-5MS (5% phenyl-methyl-siloxane, 30m x 0.25 mm film, thickness 0.25 microns, Agilent Technologies, USA). The temperature of the interface and of the injector was operated at 280 °C and 250 °C, respectively.



The oven temperature arose from 35 °C to 325 °C with a heating rate of 5 °C/min. Helium was used as the cooling gas at a rate of 1 mL/min.

The essential oils were identified by determining their Kovats indices [25], calculated using the retention times of injected alkane series with a number of carbon atoms ranging from C₈ to C₂₈ with the same chromatographic conditions, in addition to the Kovats indices obtained from the literature, we have considered in this study only the compounds that possess similar Mass spectra to those obtained from the WILEY 7n.l library with a quality greater than 90%. The percentage of the identified compounds is deduced from their peak areas.

Biological Screening

The microbial inoculums were uniformly spread using sterile spreader on a sterile Petri contain microbial media agar (LB (Luria-Bertani) for bacteria and PDA (potato dextrose agar) for fungi). 100 µL of each Essential oil were loaded in wells dug in agar plates (7 mm diameter holes). at concentrations of 15 and 10 mg/mL in DMSO. Products were added to each well. The bacterial systems were incubated for 24 h at 37 °C and fungal system was incubated for 72 h at 30°C, under aerobic conditions. After incubation, confluent microbial (bacteria or fungi) growth was observed. Inhibition of the microbial growth was measured in mm.

The antibacterial and antifungal activities of the essential oils samples in terms of minimum inhibitory concentrations (MIC) and diameters of inhibition zones are reported in the table 4.

RESULTS AND DISCUSSION

Chemical composition of the essential oils

The chemical composition of essential oils extracted from *Senecio gallicus* species and identified by GC-MS is shown in Table 2. The quantitative and qualitative analyzes allowed us to identify a total number of 36 components belonging to different classes of chemical compounds. For those collected in the year 2012, 22 volatile compounds were found, among them 18 components were common to both compartments (flowers and plant w/o flowers). We can cite: α-Thujene, α-Pinene, Sabinene, β-Myrcene, 1-Phellandrene, α-Terpinene, Orthocymene, β-Phellandrene, γ-Terpinene, α-Terpinolene, 4-Terpineol, Trans Caryophyllene, α-Humulene and Germacrene D. Concerning the year 2013 collect, 32 compounds were identified among them only 4 components were common to both compartments (which are α-Pinene, Sabinene, 4-Terpineol and Germacrene D). This would be caused by the decrease in the number of components in the plant w/o flowers essential oil on the 2013 campaign. It is also apparent that the extraction yield of essential oils was more important in the second year than in the first one.

All these significant differences in the chemical composition of these different oils led us to conclude that the essential oils composition seems to be influenced by the geographical locations, seasons, climatological conditions and a possible existence of chemotypes or ecotypes.

Table 2. Chemical compositions (%) of *Senecio gallicus* essential oils.

RI ^a	RI ^b	RI ^c	RI ^d	RI ^e	Volatile compounds ^f	Relative peak area (%), year 2012		Relative peak area (%), year 2013	
						Flowers	Plant w/o flowers	Flowers	Plant w/o flowers
792	-	-	789	-	1-octene	-	-	0.07	-
805	-	-	796	-	Hexanal	-	-	0.03	-
892	874	-	868	-	1-Nonene	0.54	-	1.48	-
908	886	-	-	-	Santolina triene	0.04	-	-	-
923	-	-	892	-	Tricyclene	-	-	0.01	-
926	907	906	-	-	α-Thujene	0.18	0.1	-	-
936	917	913	911	905	α-Pinene	9.67	4.14	9.1	7.3
953	-	-	923	-	Camphene	0.02	-	0.06	-
973	971	963	971	952	Sabinene	49.45	65.34	28.86	55
991	988	983	986	-	β-Myrcene	9.88	6.86	10.97	-
1005	1000	994	999	-	1-Phellandrene	4.26	0.99	5.38	-
1018	1012	1009	1007	-	α-Terpinene	0.57	0.49	1.22	-
1020	1026	1019	1027	-	Orthocymene	15.98	2.83	18	-



1053	1029	1024	1030	-	β -Phellandrene	3.39	4.27	2.45	-	
1040	-	-	1033	-	Cis Ocimene	-	-	0.19	-	
1059	1036	1058	1056	-	γ -Terpinene	0.79	0.82	2	-	
1043	-	1047	1043	-	β -Ocimene	-	0.45	0.57	-	
1101	-	1058	1064	-	Cis Sabinene	-	0.82	0.51	-	
1084	1094	1089	1089	-	α -Terpinolene	1.15	3.06	2.99	-	
1068	-	1105	-	-	Isoterpinolene	-	0.24	-	-	
1198	-	-	1099	-	Terpineol	-	-	0.54	-	
1207	1153	-	1148	-	1-Terpineol	0.02	-	0.55	-	
1179	1196	1195	1195	1196	Terpinene-4-ol	2.3	3.02	7.27	21.36	
1288	-	-	1306	-	Lavandulyl Acetate	-	-	0.21	-	
1380	-	-	1344	-	Decanoic Acid	-	-	0.29	-	
1393	-	-	1369	-	β -Elmene	-	-	0.07	-	
1410	1405	1405	1396	-	Trans Caryophyllene	0.08	0.32	0.5	-	
1485	-	-	1449	-	β -Selinene	-	-	0.03	-	
1459	1450	1404	1437	-	α -Humulene	0.03	0.17	0.14	-	
1499	1484	1484	-	1473	Germacrene D	0.2	0.31	-	5.43	
1389	-	-	1473	-	β -Cubenene	-	-	0.03	-	
1494	1503	-	1490	-	Bicyclogermacrene	0.06	-	0.84	-	
1519	-	-	1523	-	δ -Cadinene	-	-	0.32	-	
1640	-	-	1690	-	Tau murolol	-	0.11	-	-	
1605	-	-	1624	1624	Oplopenone	-	-	0.11	0.35	
1658	-	-	1677	-	α -Cadinol	-	-	3.27	-	
Total identified						37	98.59	94.02	98.06	89.44
Monoterpenes						21				
Hydrocarbons						17				
Oxygenated						4				
Sesquiterpenes						11				
Hydrocarbons						8				
Oxygenated						3				
Others						5				

^a RI: Retention indices on HP-5 MS column relative to C9-C28 n-alkanes.

^b RI: Retention indices of volatile compounds of flowers, analyzed on 2012.

^c RI: Retention indices of volatile compounds of the plant devoid of its flowers, analyzed on 2012.

^d RI: Retention indices of volatile compounds of flowers, analyzed on 2013.

^e RI: Retention indices of volatile compounds of the plant devoid of its flowers, analyzed on 2013.

^f Volatile compounds: constituents listed in order of elution from a HP-5 MS column.

The essential oils composition of the different organs of *Senecio gallicus* was quite distinct as shown in table 2 and also figures 1, 2 and 3. The volatile oils extracted from different parts of *Senecio gallicus* species varied not only quantitatively but also qualitatively. It is easily distinguishable from the first sight, that Sabinene was the most abundant component in all plant organs during the two years. The plant w/o flowers posses the major concentration of Sabinene (65.34%) for the year 2012. This value decreased to 55% on the second year. This compound was also present in the plant w/o flowers

with a concentration ranging from 49.45% to 28.86% respectively to the two years. Thus, in *Senecio gallicus* species, the volatile oils extracted from plant w/o flowers had a higher concentration of Sabinene. For the Iranian essential oil already reported [26], the concentration of Sabinene was 7.2%. All these results could confirm the estimations of Lawrence [27], who had demonstrated that the concentration of Sabinene in essential oils ranges from 0.4% to 64.5%.

Regarding the variation on the chemical composition of obtained volatile oils, we can conclude that the percentage and the nature of components depend on the analyzed organ. This variation can also be seen in the same organ, in two different periods of time; like the case of flowers when some minor components appeared on the second year, such as (1-Octene 0.07%, Hexanal 0.03%, Tricyclene 0.01%, Camphene 0.06%, Cis Ocimene 0.19%, Terpineol 0.54%, Lavandulyl acetate 0.21%, Decanoic acide 0.29%, β -Elmene 0.07%, β -Selinene 0.03%, β -Cubenene 0.03%, Γ -Cadinene 0.32%, α -Cadinol 3.27%).

Concerning the plant w/o flowers, some of the components that were present the first year like (α -Thujene 0.1%, β -Myrcene 6.86%, 1-Phellandrene 0.99%, α -Terpinene 0.49%, Orthocymene 2.83%, β -Phellandrene 4.27%, γ -Terpinene 0.82%, β -Ocimene 0.45%, Cis Sabinene 0.82%, α -Terpinolene 3.06%, Isoterpinolene 0.24%, Trans Caryophyllene 0.32%, α -Humulene 0.17%, Tau murolol 0.11%) were absent in the second year. The only present compounds, in the second year were (α -Pinene 7.3%, Sabinene 55%, Terpinene-4-ol 21.36%, Germacrene D 5.43% and Oplophenone 0.35%). There are only 4 common compounds shared between the two compartments we mention: (α -Pinene, Sabinene, Terpinene-4-ol and Germacrene D).

The chemical composition of flowers and plant w/o flowers obtained in the first year (figure 1) shared some components, with some relative difference in the concentrations. The common compounds are: (α -Thujene, α -Pinene, Sabinene, β -Myrcene, 1-Phellandrene, α -Terpinene, Orthocymene, β -Phellandrene, γ -Terpinene, α -Terpinolene, Terpinene-4-ol, Trans Caryophyllene, α -Humulene and Germacrene D). The difference between these compartments consists of the minor compounds that are present only in flowers. We can respectively mention 1-Nonene 0.54%, santolinatriene 0.04%, 1-Terpineol 0.02%, Bicyclogermacrene 0.06%. For those which are present only in the plant w/o flowers we can name β -Ocimene 0.45%, Cis Sabinene 0.82%, Isoterpinolene 0.24% and Tau murolol 0.11%.

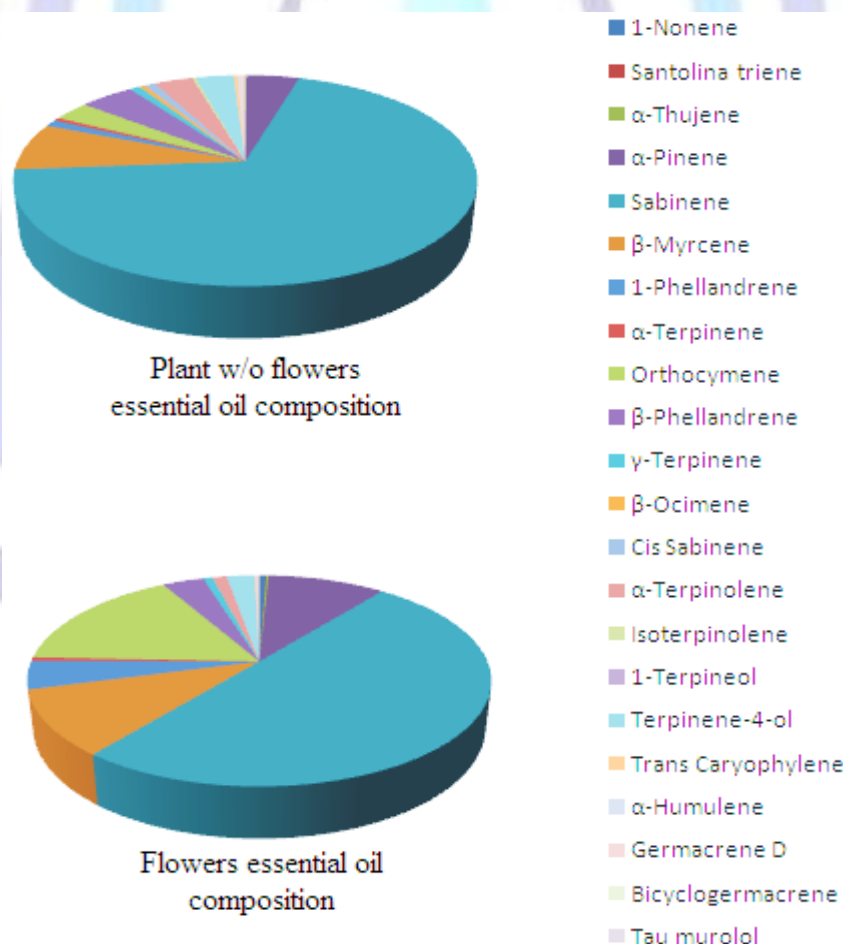


Figure 1. Essential oils composition of *Senecio gallicus* harvested in 2012.

For the second year, the plant w/o flowers showed a low countenance of terpenes and possessed only (α -Pinene 7.3%, Sabinene 55%, Terpinene-4-ol 21.36%, Germacrene D 5.43% and Oplophenone 0.35%). The Germacrene D was absent in the flowers, these last showed a total number of 27 specific different volatiles represented by: (1-Octene 0.07%, Hexanal 0.03%, 1-Nonene 1.48%, Tricyclene 0.01%, α -Pinene 9.1%, Camphene 0.06%, Sabinene 28.86%, β -Myrcene 10.97%, 1-

Phellandrene 5.38%, α -Terpinene 1.22%, Orthocymene 18%, β -Phellandrene 2.45%, Cis Ocimene 0.19%, γ -Terpinene 2%, β -Ocimene 0.57%, Cis Sabinene 0.51%, α -Terpinolene 2.99%, Terpeneol 0.54%, 1-Terpeneol 0.55%, Terpinene-4-ol 7.27%, Lavandulyl Acetate 0.21%, Decanoic Acid 0.29%, β -Elmene 0.07, Trans Caryophyllene 0.5%, β -Selinene 0.03%, α -Humulene 0.14%, β -Cubenene 0.03%, Bicyclogermacren 0.84%, δ -Cadinene 0.32%, Oploenone 0.11% and α -Cadinol 3.27%). Essential oils obtained from flowers on 2013, acquired a large number of components compared by those obtained on the first year.

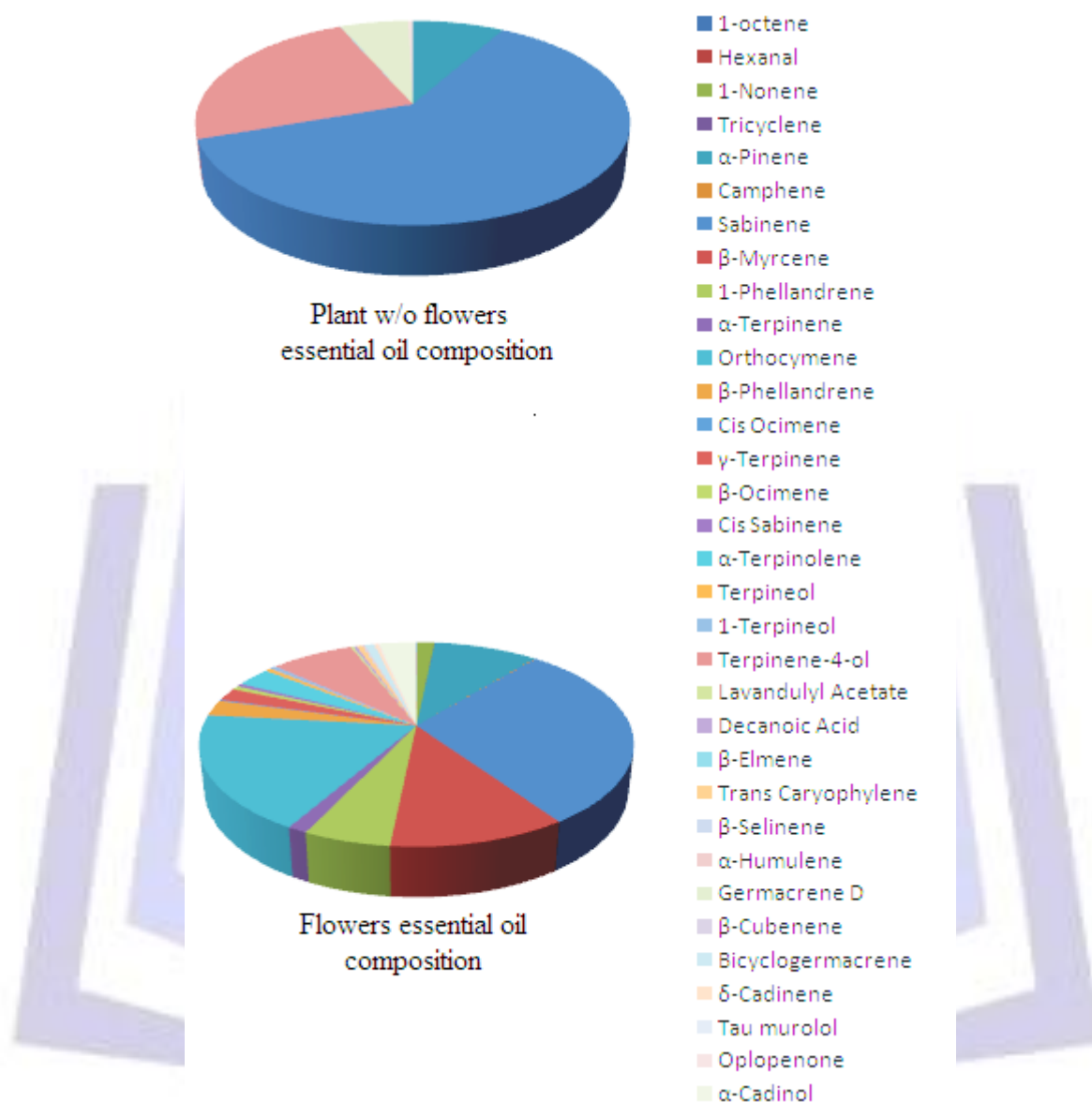


Figure 2. Essential oils composition of *Senecio gallicus* harvested in 2013.

We compared our results with the previously published ones by Mohammadhosseini et al for the same plant *Senecio gallicus* from Iran [26]. Some similarity and a large difference are observed between the compositions of all studied samples (our two years samples for the two compartments and the Iranian one for the whole plant). Oils reported in this current work were characterized by the dominance of Sabinene which is present as major compound in the two compartments during the two years with concentration varying between 49.45% and 28.86% in flowers, and 65.34% - 55% in the plant w/o flowers. The Iranian work [26] which studied oils obtained from of the whole plant (without separating its organs) has shown that β -Phellandrene is the most abundant component at a concentration of 12.2%, along with some other volatile compounds that we have already found in our samples (α -Pinene, Sabinene, Terpinolene, Terpinen-4-ol, α -Humulene, Germacrene-D, δ -Cadinene).

We mention here that α -Pinene that is common to all the samples, posses a relative stable concentration especially in both flowers and Iranian essential oils. This concentration decreases in the oils of Plant w/o flowers.

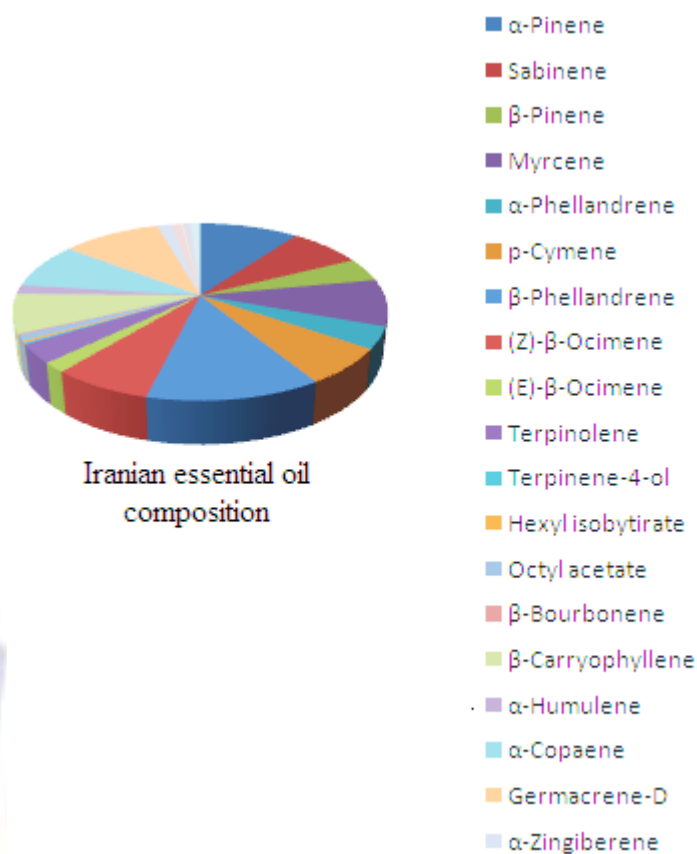


Figure 3. Composition of *Senecio gallicus* essential oils from Iran [26].

Common compounds and concentrations of our oils and those already reported are listed in table 3.

Table 3. Common compounds between different essential oils.

Common volatile compounds	Relative peak area(%), year 2012		Relative peak area(%), year 2013		Relative peak area for already reported essential oils
	Flowers	Plant w/o flowers	Flowers	Plant w/o flowers	
α-Pinene	9.67	4.14	9.1	7.3	9.8
Sabinene	49.45	65.34	28.86	55	7.2
β-Ocimene	-	0.45	0.57	-	(Z)-β-Ocimene: 7.0
					(E)-β-Ocimene: 1.7
Terpinene-4-ol	2.3	3.02	7.27	21.36	0.3
α-Humulene	0.03	0.17	0.14	-	1.6
Germacrene-D	0.2	0.31	-	5.43	9.8
δ-Cadinene	-	-	0.32	-	0.2

Biological activities of the essential oils

Total extracts of essential oils from *Senecio gallicus* were assayed for their biological activities such as the antioxidant activity that was evaluated by the DPPH method.

The essential oils were not able to capture free radicals and the antioxidant activity assays were very low. This result can be related to the low reactivity of the highly concentrated compound which is Sabinene, toward hydroxyl groups. This reaction is confirmed by Atkinson, Ashmann et al [28]. Some other few studies [28-30], expected that Sabinene can be oxidized by ozone or hydroxyl groups to give a new compound containing 9 carbons named sabinacetone; but there is no experimental test that verified this theory.



We have also tested their antimicrobial activity using the disc diffusion assay as a screening method against *Staphylococcus* sp, *Bacillus* sp, *Trichoderma reesei* and *Aspergillus niger*. We have also investigated the anti-apoptotic test on *Saccharomyces cerevisiae* over-expressing the p53 tumor suppressor gene [31]. No positive results were obtained but the assayed oils caused toxicity on this yeast. Because of the great number of constituents, essential oils seem to have no specific cellular targets [32].

Essential oils of flowers showed antifungal activity against *Trichoderma reesei* with inhibition zone diameter in range of 4 mm for the 2012-extract to 5 mm in 2013 at 15mg/mL, as well as on *Bacillus* sp with an inhibition zone in the range of 2 mm for both extracts of 2012 and 2013. Interestingly, the essential oils extracted from plants w/o flowers showed highest antimicrobial activity: Antifungal activity against *Trichoderma reesei* was pronounced at a dose of 10mg/mL for both two years 2012 and 2013. Similarly, stronger positive results were obtained with either *Bacillus* sp or *Staphylococcus aureus* at the dose of 10mg/mL.

Table 4. Concentrations and inhibitory zones of obtained essential oils.

Microorganisms	Flowers Essential oil				Plant w/o flowers essential oil			
	2012		2013		2012		2013	
	^a 10	15	10	15	10	15	10	15m
<i>Staphylococcus</i> sp	-	nt		nt	6	nt	4	nt
<i>Bacillus</i> sp	^b 2	nt	2	nt	5	nt	4	nt
<i>Trichoderma reesei</i>	-	5	-	4	4	5	2	7

^a concentrations in mg/mL.

^b inhibition diameter in mm. nt, not tested .

In conclusion, these observations show that the studied essential oils possess a low or relatively moderate antimicrobial activity. Tests performed show that essential oils from plant w/o flowers are more active than those obtained directly from flowers. Nevertheless, more studies are required to elucidate the structure of the unidentified components in the oils and evaluate more biological activities of purified and concentrated components.

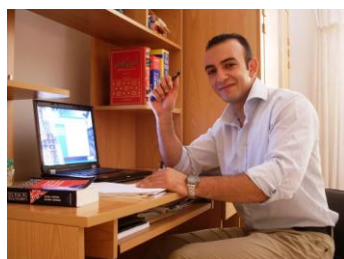
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Author' biography



Mohamed Mihoubi was born in Sakiet Sidi Youssef (Tunisia) on Sunday 20 March 1988. He is the youngest of four children, what some might call the "baby" of the family. There he cultivated his love of traveling, discovering and adventure through the clubs and associations that he had joined and also through the trips that he had made. After 18 years and after finishing the high school and obtaining the baccalaureate degree he moved to the south of Tunisia in the city of Sfax where his home base now resides. On 2007 he attended the University of Sfax, precisely the faculty of science of Sfax, that satisfied his ambitions. There, he joined an academic training on the field of general chemistry. In 2010, he received two bachelor degrees in respectively general chemistry and physics. He was a very active student, when he is not in school and when

he is not preoccupied by other local activities, Mohamed enjoys spending his time writing (Poetry, Sciences, Articles) as it is a dream of him to have his writings published. After that he had continued his studies in the field of organic chemistry to



get graduated on July 16, 2012 the master degree with honors under the supervision of Professor Raoudha Jarraya. After all these years of studies Mohamed decided to join the Tunisian national guard (militarian organisation) with the experience that he had made and with his love toward this domain, January 7, 2013 he was graded as a lieutenant and his dream became a reality, he was the youngest officer of his promotion. But since the research interests him more than any other domain he decided to quit the work and back again to the laboratory. On his return, conditions were encouraging. In fact he started a 6 months internship in the laboratory of synthesis of heterocyclic compounds directed Professor Giovanni Grassi under the supervision of professor Anna Piperno at the faculty of sciences of Messina, he used to synthesize molecules belonging to the family of alkaloids and possessing interesting biological activities wich is his Phd topic.

Mohamed speaks fluently arabic, french and english and in addition deutch and Italian. He visited a lot of countries such as (Egypt, Algeria, Libya, France, Belgium and Italy).

He is currently a junior researcher at the "Laboratoire de Chimie des Substances Naturelles" in the faculty of sciences of sfax, directed by professor Ridha Ben Salem, he is making researches on chemistry of plants and natural products in order to obtain his doctorate and achieve his new dream.

