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ANTIOXYDANT AND ANTIMICROBIAL ACTIVITIES OF ESSENTIAL OIL AND ETHANOL EXTRACT OF *SANTOLINA CHAMAECYPARISSUS* L.

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Abstract

Description of the subject: Essential oils and phenolic compounds extracted from the aromatic plants are widely used as antioxidants and antimicrobials agents.
Objective: Consists to evaluate the antioxidant and antimicrobial activities of essential oil and ethanol extract of *Santolina chamaecyparissus* L.
Methods: Essential oil and ethanol extract were tested for their antioxidant activity by DPPH free radical-scavenging and ferric reducing power. The antimicrobial activity is evaluated by the agar disc diffusion method.
Results: Twenty eight components were characterized with the major compound is Artemisia ketone 42%. The polyphenols and flavonoids are abundant in ethanol extract. The essential oil showed a very weak antioxidant capacity for the two essays. However the ethanol extract was found to be effective toward DPPH inhibition, and for reducing power test it exhibited high activity in comparison with the chemical standard butylated hydroxytoluene BHT. The antimicrobial activity indicated that essential oil possessed the highest activity. However, the ethanol extract showed low antimicrobial activity.
Conclusion: The studied plant might indeed be potential sources of natural antioxidant and antimicrobial agents.
Keywords : *Santolina chamaecyparissus* L.; essential oil; ethanol extract; antioxidant and antimicrobial activities.

ACTIVITE ANTIOXYDANTE ET ANTIMICROBIENNE DE L'HUILE ESSENTIELLE ET L'EXTRAIT ETHANOLIQUE DE *SANTOLINA CHAMAECYPARISSUS* L.

Résumé

Description du sujet : Les huiles essentielles et les composés phénoliques extraient des plantes aromatiques sont utilisés comme des agents antioxydants et antimicrobiens.
Objectifs : Consiste à évaluer l'activité antioxydante et antimicrobienne de l'huile essentielle et l'extrait phénolique de *Santolina chamaecyparissus* L.
Méthodes : L'huile essentielle et l'extrait éthanolique sont testés pour leur activité antioxydante par le test de piégeage du radical DPPH et le pouvoir réducteur. L'activité antimicrobienne est évaluée par la méthode de diffusion sur l'agar.
Résultats : Vingt huit composés ont était identifiés où l'Artemisia ketone est le composé majoritaire 42%. Les polyphénols et les flavonoïdes sont abondants dans l'extrait éthanolique. L'huile essentielle montre une très faible capacité antioxydante pour les deux tests. Toutefois l'extrait éthanolique s'est avéré efficace contre l'inhibition du radical DPPH et pour le test du pouvoir réducteur on observe une bonne activité en comparant avec le standard chimique butylated hydroxytoluene BHT. Cependant l'huile essentielle possède une forte activité antibactérienne. Par contre l'extrait éthanolique a montré une faible activité antimicrobienne.
Conclusion : La plante étudiée est une source naturel d'agent antioxydant et antimicrobien très potentielle.
Mots clés : *Santolina chamaecyparissus* L.; huile essentielle; extrait éthanolique; activités antioxydante etantimicrobienne.

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INTRODUCTION

Aromatic plants are frequently used in traditional medicine and essential oils extracted from them are widely used as antioxidants and antimicrobial agents as well as for the prevention and treatment of different human diseases, many aromatic and medicinal plants have become attractive to scientists as natural sources of natural agents that could be safer than synthetic sources.

Santolina chamaecyparissus L. commonly known as cotton lavender belonging to the family Asteraceae is a hardly aromatic dwarf fragrant, dense mound with attractive grayish-silver foliage, evergreen shrub native to the Mediterranean area [1]. The inflorescence are widely used in Mediterranean folk medicine for their analgesic, anti-inflammatory, antiseptic, antispasmodic, bactericidal, fungicidal digestive and vulnerary properties, and is also used in phytotherapy for different kinds of dermatitis [2]. Several products (acetylenes, essential oils, flavonoids, sesquiterpenes) obtained from *Santolina* spp. have been investigated for their biological activities [3] The oil has been found to be efficacious in treating eye infections, a stimulator in scar-tissue formation and an insect repellant [4].

Oxidation mediated by free radical reaction is also responsible for the rancidity of unpreserved food rich in unsaturated fatty acids and many synthetic antioxidant components butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA) have showed toxic and/or mutagenic effect [5]. In the other hand, the development of bacterial resistance to presently available antibiotics has necessitated the search for new antimicrobial agents [6]. In order to minimize or eliminate the use of synthetic additives in food; essential oils or plant extracts as natural agents have been used against the proliferation of microorganisms or protect food from oxidation [7].

The present study is aimed mainly to: (1) analyze the chemical composition of the essential oil extracted from the aerial part and to determine the phenolic compounds from the ethanol extract; (2) Investigate the antioxidant and antimicrobial activities for to determine if these essential oil and ethanol extract could be used as natural preservatives.

MATERIALS END METHODS

1. Plant material

The aerial part of *Santolina chamaecyparissus* L. was collected in national experimental garden, El Hamma Algiers (Algeria). The taxonomic identity of the plant was confirmed by comparing the specimen with that of known identity already deposited in the herbarium of the National Superior School of Agronomy (ENSA), with the assistance of Professor Abdelkrim Hassen. The plant was dried in the shade at room temperature.

2. Essential oil extraction

Essential oil was isolated from the dried aerial part by hydrodistillation for 3 hours using a Clevenger-type apparatus according to the European Pharmacopoeia method. The essential oil was stored at +4°C in the dark until use.

3. Preparation of the ethanol extract

The aerial part was finely ground and 15 g was extracted in soxhlet with ethanol 95% for 6 hours, which was concentrated by using a rotary evaporator. Extract is kept in the dark at +4 °C until tested.

4. Analysis of the essential oils

The composition of the essential oil was performed by gas chromatography and accomplished with CP chrompack 9002 GC-FID system using a fused-silica capillary column with apolar stationary phase HP5 (30 m x 0.25 mm x 0.25 µm film thickness), the column temperature program was 50°C for 3 min, increasing at 2°C/min toward 250°C and held at 250°C for 10 min. 0.2 µL were injected by splitting and the split ratio was 1:50; nitrogen was used as carrier gas at a flow rate 1 mL/min. Flame ionization detection was performed at 320°C.

The essential oil was analysed by gaz chromatography coupled to mass spectrometry with apparatus Hewlett Packard 289 using the apolar column HP5 (30 m x 0.25 mm x 0.25 µm film thickness), the column temperature program was 60°C for 8 min, increasing at 2°C/min toward 280°C and held at 280°C for 15 min.

Oil constituents were identified by comparison of their GC Kovats retention indices (R.I.) and determined with reference to a homologous series of C8–C22 *n*-alkanes with those of literature and with those of available authentic standards. Confirmation of such identification was done by comparing their mass spectral fragmentation patterns with those stored in the MS database (NIST and Wiley) and with mass spectra literature data [8].

5. Total phenolic and total flavonoids contents

Total phenolic compounds from ethanol extract was determined by the Folin-Ciocalteu method with Galic acid as standard [9]. Thus 0.25 mL of extract dissolved in ethanol was mixed with 1.25 mL of Folin-Ciocalteu solution. After 3 min of reaction, 1 mL of solution of sodium carbonate (Na_2CO_3) at a concentration of 75 g/L was added. After 30 min of incubation in the dark at room temperature, the absorbance was measured at 765 nm. The test was performed in triplicate and average value recorded.

Total flavonoids contents of the sample was determined by the trichloride aluminium ($AlCl_3$) method with quercetin as standard [10]. Thus 1 mL of extract dissolved in ethanol was added to 1 mL of solution of trichloride aluminium. After 1 h of incubation at room temperature, the absorbance was measured at 420 nm. The experiment was carried out in triplicate and average value recorded.

6. Antioxidant Activity

The essential oil and ethanol extract were made at various concentration (100, 200, 400, 600, 800, 1000 mg/L) and (5, 10, 20, 50, 100, 200, 400 mg/L) respectively.

6.1. Free Radical Scavenging Activity (DPPH)

The DPPH (2,2-diphenyl-2-picrylhydrazine) free radical scavenging assay is one of the most extensively used antioxidant assays for plant samples. DPPH is a stable free radical that reacts with compounds that can donate a hydrogen atom. This method is based on the scavenging of DPPH through the addition of a radical species or an antioxidant that decolorizes the DPPH solution, its violet color is transformed into yellow as its reduction [11]. Each sample (25µL) at different concentrations was added to 975µL of ethanolic solution of DPPH and placed in dark and at room temperature. The antioxidant activity is then measured by the decrease in absorption at 517 nm after 30 min of reaction [12]. The percentage inhibition of the DPPH radical by samples was calculated according to the formula:

$$\%Inhibition = \left[\frac{(A_c - A_t)}{A_c}\right] \times 100$$

Where A_c is the absorption of the blank sample (t= 0 min) and A_t is the absorption of the tested oil or extract (t= 30 min). Tests were carried out in triplicate and BHT was used as positive control.

The values of IC_{50} were determined as reported above. Tests were carried out in triplicate and BHT was used as positive control.

6.2. Reducing Power Assay

The reducing antioxidant power of essential oil and ethanol extract made at different concentration was assayed by the method of Oyaizu [13]. The 0.125 mL of sample was added with 2.5 mL of a sodium phosphate buffer (0.2 M) and 2.5 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$]; the mixed was incubated in a water bath at 50 ◦C for 20 min. Afterwards 2.5 mL of 10% trichloroacetic acid was added to the mixture that was centrifuged for 10 min at 1500 rpm. Finaly 2.5 mL of the supernatant was then mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$. The absorbance was measured at 700 nm; high absorbance indicates high reducing power. Tests were carried out in triplicate and BHT was used as positive control.

7. Antimicrobial Activity

Susceptibility of the microbial strains to the essential oil and ethanol extract were investigated by using the agar diffusion method [14]. Sterile Whatman discs (9 mm diameter) were impregnated with 10 µL of the pure essential oil or ethanol extract and placed on the center surfaces of the inoculated (10^7-10^8 CFU/mL) Mueller-Hinton agar (for bacteria) and Sabouraud agar (for fungi). After the plates were incubated at 37 °C during 24 h for bacteria and at 25°C during 48 h for fungi, the diameters of the distinctly clear zones were measured in millimeters. All the tests were performed in triplicate and the antimicrobial activity was expressed as the mean of inhibition diameters produced.

In this study the standard microbial strains were obtained from the Biotic SAIDAL Algiers, three bacteria strains were used: Gram-positive *Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* ATCC 6633, Gram-negative *Escherichia coli* ATCC 8739. Two fungi strains *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC 9763.

8. Statistical Analysis

Statistical comparisons were made with ANOVA followed by Tukey's test; the level of significance was set at $p < 0.05$.

RESULTS

1. Essential oil yield

The yield of essential oil extracted from the dried aerial part of *Santolina chamaecyparissus* L. is 0.85% (v/w) collected after 110 min; the volatile oil is a mobile liquid of pale yellow color having an aromatic harsh smell.

2. Chemical composition

Component identification along with their percent composition and RI values are summarized in Table 1, the chromatographic analysis lead to identification of 28 components corresponding to 85.32% of the total essential oil. The most abundant of them were Artemisia ketone (42%) and other compounds present are as follow: Vulgarone B (8.44%), β-phellandrene (8.32%), β-Myrcene (7.67%), Cubenol (3.42%), Sabinene (2.87%), β-Pinene (2.51%), Camphor (2.51%) and Camphene (1.31%). Oxygenated monoterpenes are the most dominant with 47.51% of the total composition. The proportion of the oxygenated fraction, the most aromatic and therefore the most recyclable of the essential oil in the olfactory level represent 60.49%.

Table 1**.** Composition of essential oil of *Santolina chamaecyparissus* L.

N°	Compounds	RI	RI^{Adams}	Area (%)
1	Santolina Triene	914	908	0.26
2	Tricyclene	931	926	0.05
3	α-Pinene	939	939	0.26
4	Camphene	953	953	1.31
5	Sabinene	980	976	2.87
6	β-Pinene	983	980	2.51
7	β-Myrcene	1000	991	7.67
8	Yomogi alcool	1010	998	0.46
9	p-Cymene	1031	1026	0.17
10	β-Phellandrene	1036	1031	8.32
11	Artemisia ketone	1073	1062	**42.00**
12	cis-Sabinene hydrate	1076	1068	0.11
13	Artemisia alcool	1096	1083	1.04
14	Camphor	1151	1143	2.51
15	Borneol	1169	1165	0.97
16	4-Terpineol	1179	1177	0.10
17	p-Cymen-8-Ol	1197	1183	0.49
18	Myrtenal	1203	1193	0.19
19	Myrtenol	1205	1194	0.07
21	α-Longipinene	1363	1351	0.50
22	Patchoulene alpha	1445	1456	0.05
24	Germacrene-D	1474	1480	0.04
25	α-Curcumene	1492	1483	0.28
26	Caryophyllene oxyde	1584	1581	0.16
27	Cubenol	1616	1614	3.42
28	Vulgarone B	1671	1647	8.44
29	α-Bisabolol	1702	1683	0.69
30	Aristolone	1767	1756	0.24
Yield (% v/w)				0.85
Identified components (%)				85.32
Monoterpene hydrocarbons				23.92
Oxygenated monoterpenes				47.51
Sesquiterpene hydrocarbons				0.89
Oxygenated sesquiterpenes				12.98

IR^{Adams} [15], retention indices relative to C_9- C_{25} n-alkanes on the HP 5MS column.

3. Total phenolic and flavonoids contents

Total phenolic and flavonoids contents from ethanol extract was determined by the Folin-Ciocalteu method and the trichloride aluminium ($AlCl_3$) method respectively. They are summarized in table 2.

Table 2**.** Polyphenols and flavonoids contents of ethanol extract (EE).

	Yeld (%)	Polyphenols (a)	Flavonoids (b)
EE	18.44	43.22	27.41

(a)mg equivalent of gallic acid per gram of extract, (b) mg equivalent of quercetin per gram of extract.

4. Antioxidant activity

Antioxidant activity of the essential oil and ethanol extract from *Santolina chamaecyparissus* L. has been determined by two test namely DPPH and reducing power. They are summarized in table 3 and 4 respectively.

5. ***Antimicrobial activity.***

The antimicrobial activity of the essential oil and ethanol extract from *Santolina chamaecyparissus* L. was recorded in Table 5.

Table 3. Scavenging activity of DPPH (%) free radical and reducing power of essential oil (EO) and BHT

Concentration (mg/L)	DPPH (%)		*Reducing power	
	EO	BHT	EO	BHT
100	2.17±0.35	73.15 ± 0.85	0.007 ±0.005	0.863 ± 0.054
200	2.03±0.20	80.63 ± 0.32	0.045 ±0.009	1.063 ± 0.023
400	2.25±0.46	84.75 ± 0.52	0.085 ±0.011	1.161 ± 0.031
600	2.23±0.19	86.35 ± 0.59	0.164 ±0.009	1.178 ± 0.030
800	2.13±0.14	88.81 ± 0.27	0.179 ±0.008	1.218 ± 0.015
1000	3.47±0.25	91.07 ± 0.13	0.116 ±0.010	1.303 ± 0.055
IC_{50} mg/L	ND	ND	/	/

Table 4. Scavenging activity of DPPH (%) free radical and reducing power of ethanol extract (EE) and BHT.

Concentration (mg/L)	DPPH (%)		*Reducing power	
	EE	BHT	EE	BHT
5	9.65±0.39	ND	0.128 ±0.010	ND
10	11.19±0.61	31.40 ± 0.70	0.136 ±0.011	ND
20	14.52±0.51	42.14 ± 0.48	0.200 ±0.016	0.311 ± 0.006
50	28.28±0.91	62.49 ± 0.69	0.443 ±0.038	0.576 ± 0.001
100	29.47±1.34	73.15 ± 0.85	0.465 ±0.028	0.863 ± 0.054
200	48.52±0.57	80.63 ± 0.32	0.804 ±0.072	1.063 ± 0.023
400	63.65±0.95	84.75 ± 0.52	1.129 ±0.016	1.161 ± 0.031
IC_{50} mg/L	257.76±0.75	32.84±0.59	/	/

*The results are expressed as the average absorbance at 700 nm of three repetitions ±S; ND : not determined.

Table 5. Diameter of inhibition zone Diameter (mm) of the essential oil (EO) and ethanol extract (EE) of *Santolina chamaecyparissus* L.

	Diameter of inhibition zone (mm)				
	S. aureus ATCC 6538	*B. subtilis ATCC 6633*	*E.coli ATCC8739*	*C. albicans ATCC 10231*	*S. cerevisiae ATCC 9763*
EO	37 ±0.4	36 ±0.3	9	17 ±0.2	15 ±0.1
EE	14 ±0.4	11 ±0.2	9	9	11 ±0.5

Inhibition zone includes the diameter of the disk (9 mm). Each result is the mean ± SD of three replicates.

DISCUSSION

Many studies are reported about the chemical composition of essential oil extracted from *Santolina chamaecyparissus* L. Comparing the results it can be divided the components of the oil of the various existing studies into two groups. The first group which includes Artemisia Ketone as a major component [16, 17, 18, 19]. The second group is that possesses other key compounds [20, 21, 22, 23]. Table 6 showed the major components. This great variability and diversity observed in the chemical composition of the EOs of the same aromatic herb species and subspecies can be attributed to several factors, including local climatic and environmental conditions, season, geographical location, geology, postharvest drying and storage, availability of water, height above sea level, presence of fungal diseases and insects, part of the plant and the method used to obtain the EO [24].

Table 6. Major components of different studies.

Country	Reference	Components
Algeria	[16]	Artemisia ketone 40.33%, (Z)-Thujone 9.82%, (2Z,6E)-Farnesol 7.30%.
Spain	[17]	Artemisia ketone 27.19%, Dihydroaromadendrene 18.21%, b-Phellandrene 7.49%.
France	[18]	Artemisia ketone 45%, Myrcene 15%, Sabinene 5.5%.
Spain	[19]	Artemisia ketone (27.80-35.00) %, T-Cidanol (23.60-4.80) %.
Spain	[20]	1,8-cineole, camphor, borneol, terpinen-4-ol, terpinolene
Algeria	[21]	1,8-cineol (11.22%), spathulenol (7.59 %), terpinen-4-ol (6.13%).
Portugal	[22]	1,8 Cineol (25-30) %, Camphor (7-9) %, Borneol (7-8) %.
Tunisia	[23]	1,8-cineole (12.94 %), β-eudesmol (10.49 %) terpinene-4-ol (6.97 %), γ-cadinene (6.55 %).

The yield of the ethanol extract is 18.44 g per 100 g of plant material was higher than that reported by Benbrinis [25] who found 12 g per 100 g in the methanol extract. The results show that the polyphenols compounds and flavonoids are abundant in ethanol extract; these results are higher than that found in methanol extract 105.88 µg equivalent of gallic acid per mg of extract and 20.99 µg equivalent of quercetin par mg of extract respectively. The polyphenol content of the extract depends on the extraction method used and the nature of the solvent [26].

Essential oil exhibited very weak antioxidant abilities for reduce DPPH radicals when compared to BHT which remained below 3.47±0.25 % at concentration 1000 mg/L and the IC_{50} could not be calculated due to their low inhibition activity. This result was very lower than reported by Nouasri [16] (IC_{50} was determined as 43.010 mg/mL). In the case of reducing power assay, a similar activity was observed as given in the first assay. Essential oil has been found significantly less effective than this antioxidant agent BHT ($P< 0.05$).

The DPPH radical-scavenging activities show that the IC_{50} of ethanol extract was found to be

257.76±0.75 mg/L was less active than BHT IC_{50} 32.84±0.59 mg/L. For reducing power the ethanol extract showed potent activity compared to BHT at the concentration of 400 mg/L (1.129±0.016 and 1.161±0.031 respectively). This antioxidant power is attributed to its richness in phenolic compounds which have a great capacity to trap free radicals [27, 28]. The antioxidant potential of polyphenols is provided with an inhibitory effect of lipid peroxidation of foodstuffs [3]. While the ethanol extract have a significant antioxidant power compared to the synthetic antioxidant BHT ($P< 0.05$).

The determination of the zone of inhibition by the agar method shows that the EO has a strong inhibitory activity against *Staphylococcus aureus* and *Bacillus subtilis* with their respective diameter zones of inhibition 37 and 36 mm. While no activity showed against *Escherichia coli*. The essential oil of *S. chamaecyparissus* L. in this study showed a significant antibacterial activity ($P< 0.05$). Most of the researches were found that the *S. chamaecyparissus* EO showed the highest antibacterial activity [16, 17, 29]. The composition, structure as well as functional groups of the EO play an important role in determining their antimicrobial activity [30]. Considering the large number of different groups of chemical compounds present in EOs, it is most likely that their antibacterial activity is not attributable to one specific mechanism, but that there are several targets in the cell [31]. It is believed that the phenolic components of essential oil show strongest antimicrobial activity, followed by aldehyde, ketone and alcohol [32].

The antifungal activity against *Candida albicans* and *Saccharomyces cerevisiae* was moderate with a diameter zones of inhibition were respectively 17 and 15 mm. The agar disc diffusion method indicated that the EO showed the significant antifungal activity ($P< 0.05$). Surech [33] and Khubeiz [29] reached that the volatile oil of *S. chamaecyparissus* L. possessed potent antifungal properties against *Candida albicans*. The essential oil containing terpenes are also reported to possess antimicrobial activity [34], which are consistent with our present study. In addition, the components in lower amount may also contribute to antimicrobial activity of the essential oil, involving probably some type of synergism with other active compounds [35]. However the ethanol extract showed low inhibitory activity for all tested microorganisms.

CONCLUSION

The chemical composition show that essential oil of *Santolina chamaecyparissus* L was dominating by Artemisia ketone 42%,Vulgarone B 8.44%, β-phellandrene 8.32%, β-Myrcene 7.67%. The results showed that the essential oil was less effective than the synthetic antioxidant agent BHT. However the ethanol extract exhibited the strongest free radical scavenging and reducing power activity compared to essential oil and showed a moderate efficiency compared to BHT. Moreover the essential oil showed significant antimicrobial activity but the ethanol extract showed low antimicrobial activity. In conclusion, we can told that this medical aromatic plant has very interesting biological properties for subsequent use in various sectors.

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