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Review Article

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Phytochemical composition, ethnomedicinal uses, and pharmacological properties of *Achillea ligustica* All.: A review

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ABSTRACT: *Achillea ligustica* All., (Ligurian yarrow) is an aromatic herbaceous plant belonging to the Asteraceae family. This plant is widely distributed in the Mediterranean region and has been used in traditional medicine of some countries; it is used especially as a remedy to treat hemorrhage, stomach disorders, and skin diseases. Many chemical constituents were identified from different parts of the species; the most important are lactone sesquiterpenes, flavonoids, and essential oils (EOs). This chemical diversity is the main cause of its large spectrum of pharmacological properties. *A. ligustica* was reported to possess antioxidant, antimicrobial, anti-diabetic, and anti-psoriasis properties. Several studies have focused on the chemical composition and pharmacological properties of its EO suggesting using the EO in oral hygiene products. The present literature review draws up the importance of this medicinal plant by reviewing its recent ethnomedicinal uses, chemical constituents, and pharmacological activities.

KEYWORDS: *Achillea ligustica*; phytoconstituents; essential oil; pharmacological properties; antibacterial activity.

1. INTRODUCTION

The *Achillea* genus is historically related to some ancient civilizations (Chinese, Persian, Indian, Turkish, etc) which used differents species to cure several ailments. Its importance is also related to the Odyssey of Achilles in the Trojan War, from which the name of the genus is originated [1]. Consequently, numerous species of this genus remained used until today in the traditional medicine of different countries. The most widely medicinal species *A. millefolium* L. (Yarrow) is used particularly for wounds healing, digestive diseases, respiratory infections, and skin disorders [2]. This species is now recognized by World Health Organisation (WHO) [3] and the European Medicines Agency (EMA) [4] for the treatment of various therapeutic purposes. Some other species are also used for the treatment of several illnesses in traditional medicine in different regions all over the world.

Achillea ligustica All. (Asteraceae, Anthemideae) is a pungent herb with a bitter taste, commonly found in the Mediterranean region [5]. It is used in the folk medicine of Italy, Corsica (France), and Algeria particularly for hemostatic, stomach disorders, and skin diseases. Also, it seems that the plant is not toxic since it is added to cakes and soups [6-8]. Previous phytochemical investigations had confirmed that the plant is rich in sesquiterpenes especially guaianolide type, flavonoids substituted in position 6, and essential oils (EOs) that mainly contain oxygenated monoterpenes and oxygenated sesquiterpenes. Furthermore, biological studies had demonstrated wide biological activities such as antioxidant, antimicrobial, and antipsoriatic [6, 9-12].

To realize this review about *A. ligustica*, bibliographic research has been carried out until the end of 2022, through scientific databases, Google Scholar, PubMed, Science Direct, Scopus, WOS, and ACS. Ethnobotanical information in Algeria was collected by the authors in Mila and Jijel regions (Northeast of Algeria) from rural people. Some *Achillea* species are mentioned by researchers for their potential applications in food preservatives, pharmaceuticals, and cosmetic preparations but not *A. ligustica* [13-19].

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This literature review is the first about *A. ligustica* which covers all knowledge about it by focusing on its phytochemical, ethnomedicinal as well as pharmacological properties. The aim is to provide sufficient information to interested researchers in this medicinal species and to encourage them to further investigate its chemical composition and pharmacological properties.

2. BOTANICAL DESCRIPTION

A. ligustica All., is one of more than 120 species belonging to the *Achillea* genus of the Asteraceae family [20]. Species of this genus are characterized by numerous small flowers with different colors (white, yellow, orange, pink, or red). The genus can be described as perennial herbs of 6–80 cm tall with alternate leaves and finely pinnatisect. The capitula in corymbs are heterogamous with seriate phyllaries. The achenes are strongly flattened and not ribbed and the fruits are shiny, with no pappus [13]. The species *A. ligustica* is a perennial pubescent plant of 30-100 cm, with angular stem, and basilar leaves with 5-7 segments on each side. The flowers are small, white, and arranged in flat-topped clusters. The species is quite uniform, it can be found from Crete and Western Balkans to Italy, Sicily, and the Tyrrhenian islands to Northwest of Africa, where it grows in clear forest and stream edges and gives flowers during the period May-August [5, 10]. The most obvious characteristics of *A. ligustica* compared to other species is its high which cans reach 100 cm, with angular stems and white flowers (20-40 cm high and round stems for *A. odorata* which is the most closely related species for *A. ligustica*) [5]. While the species *A. nobilis* and *A. millefolium* have yellow and white to pink flowers respectively. In Italy the species is known as millefoglio ligure, in Corsica (France) *Erba santa* (Holy herb), while in Algeria it is called *Benkisson*.

3. ETHNOMEDICINAL USES

Several studies reported the use of *A. ligustica* in the Italian and Corsican folk medicine. The fresh leaves of *A. ligustica* are used in Sicily (Italy) as an antimicrobial, hemostatic, or swallowed as pellets against stomachache [21]. In Sardinia (Italy), the infusion of the species is used against gastric pains, and as an anti-inflammatory for skin diseases [8]. In the Urzulei region (Sardinia, Italy), the infusion of leaf is used against stomach pains. In addition, boiled leaves as cataplasm for rheumatic diseases. While hot leaf infusion is used against headache and cold pains [22]. In central Italy (Latium region), the species is used in folk veterinary medicine to cure sheep scabies [23].

In Corsica (France), *A. ligustica* is mainly used for the treatment of gastrointestinal disorders and as cataplasms to relieve sprains and insect bites, and against hemorrhages [7, 10]. In Algeria, *A. ligustica* was used during the Algerian revolutionary war in Mila and Jijel located in the northeast of Algeria for healing battle wounds. In addition, the fresh leaves of *A. ligustica* are used by the local population for stopping hemorrhage and for stomach pains.

Beside its use as remedy and to cure some diseases, *A. ligustica* is added to foods. In Corsica, the plant is added to cakes and fritters during the Good Friday festival [7]. In Ligurian Alpes (North-Western of Italy), it is mixed with *Urtica*, *Beta vulgaris*, garlic, and eggs to obtain a reinvigorating soup recommended for people who do strenuous work [24]. All mentioned uses in foods indicated that *A. ligustica* is not toxic.

4. PHYTOCHEMISTRY

The species *A. ligustica* has been the object of several phytochemical investigations in which more than fifty-four compounds were isolated from different extracts using chromatographic methods and identified by spectroscopic techniques, among them nineteen sesquiterpenes, nineteen flavonoids, five monoterpenes, two phenolic acids, one piperidine derivative, and eight other compounds. In addition, some volatile components were identified in the EO obtained using the hydrodistillation technique (HD) and identified by the gas chromatography-mass spectroscopy (GC-MS) method; the composition of the EOs varies according to the location of collect of the plant.

4.1. Flavonoids

Nineteen flavonoids (Table 1, Figure 1) have been reported from the aerial parts of *A. ligustica*, among them nine aglycones: apigenin **1**, genkwanin (7-methoxyapigenin) **2**, nevadensin (6,8,4′-trimethoxyapigenin) **3**, luteolin **4**, galetin-3,6-dimethylether **5**, santin **6**, 6-hydoxykaempferol-3,6,7,4′-tetramethylether **7**, quercetin **8**, quercetagetin-3,6,7-trimethylether **9** and ten flavonoid glycosides: apigenin 7-*O*-glycoside **10**, apigenin 7-*O*-glucuronide **11**, apigenin 7-*O*-rutinoside **12**, apigenin 6,8-di-*C*-glucoside (vicenin-2) **13**, apigenin 6-*C*-

arabinosyl-8-*C*-glucoside (isoschaftoside) **14**, apigenin 6-*C*-glucosyl-8-*C*-arabinoside (schaftoside) **15**, luteolin 7-*O*-glucoside **16**, luteolin 7-*O*-glucuronide **17**, quercetin 3-*O*-glucuronide **18**, quercetin 3-*O*-rhamnoglucoside (rutin) **19** [12, 20, 25-27]. Namely, flavonoids **7**, **8**, **12-16**, and **19** are characterized using only HPLC-ES-MS [12]. Among all reported flavonoids, santin **6** is probably the most abundant flavonoid in *A. ligustica*.

Table 1. Flavonoids identified from *A. ligustica*

Type	Comp.	Name	Reference
Flavone	**1**	Apigenin	[12, 27]
	2	Genkwanin	[20]
	3	Nevadensin	[26]
	4	Luteolin	[12, 20, 27]
Flavonol	**5**	6-hydroxykaempferol 3,6-dimethyl ether	[12, 20, 27]
	6	Santin	[12, 20, 26, 27]
	7	6-Hydroxykaempferol 3,6,7,4′-tetramethyl ether	[12]
	8	Quercetin	[12]
	9	Quercetagetin 3,6,7-trimethyl ether	[26]
Flavone glycoside	**10**	Apigenin 7-*O*-glucoside	[12, 27]
	11	Apigenin 7-*O*-glucuronide	[20]
	12	Apigenin 7-*O*-rutinoside	[12]
	13	Apigenin 6,8-di-*C*-glucoside	[12]
	14	Apigenin 6-*C*-arabinosyl-8-*C*-glucoside	[12]
	15	Apigenin 6-*C*- glucosyl -8-*C*- arabinoside	[12]
	16	Luteolin 7-*O*-glucoside	[12]
	17	Luteolin 7-*O*-glucuronide	[20]
Flavonol glycoside	**18**	Quercetin 3-*O*-glucuronide	[20]
	19	Rutin	[12]

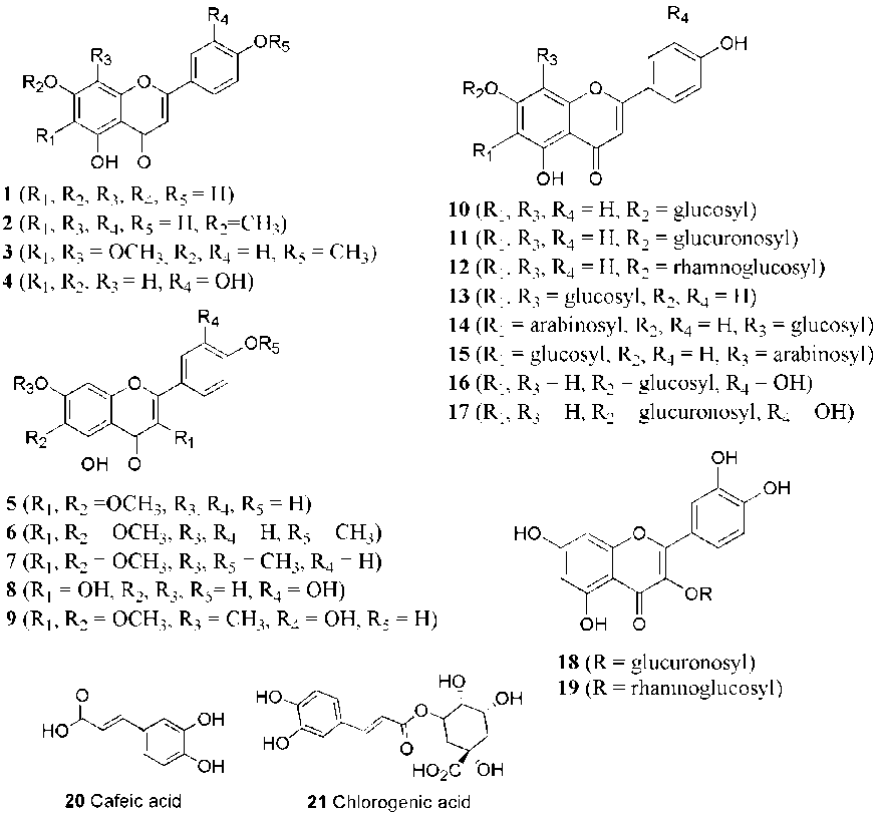

Figure 1. Structure of isolated flavonoids (**1-19**) and phenolic acids (**20**, **21**) from *A. Ligustica*

From a chemotaxonomic point of view, it is clear that *A. ligustica* contains flavonoids characteristic of the *nobilis* group species (Millefolium section) especially flavones, flavonols with their 6-methoxy derivatives, and *C*-glycosides [28]. Also, the presence of glucuronide derivatives of flavonoids is characteristic of *A. ligustica* from Lipari (Aeolian Islands, Italy) which represents a distinction from the other *A. ligustica* species growing elsewhere. This difference is possibly due to the geographical isolation of the Aeolian Islands [20].

4.2. Phenolic acids

Caffeic acid **20** and chlorogenic acid **21** are the only phenolic acids (Figure 1) reported from the aerial parts of *A. ligustica* [20].

4.3. Sesquiterpene lactones

Guaianolide sesquiterpene lactones types are the main sesquiterpenes identified from *A. ligustica* collected from different countries. Thus, ten guaianolides **22-31** were identified, with four iso-seco-tanpartholides **32-35**, two sesquiterpenes lactones with 5/6/5 skeleton (**36** and **37**), one eudesmane sesquiterpene lactone **38**, one isodaucane sesquiterpene **39**, and 5-hydroxy-5,6-seco-caryophellen-6-one **40** (Table 2, Figure 2) [20, 25, 26, 29-31]. The species is characterized by the presence of chlorinated guaianolide sesquiterpenes, three of them chlorinated in position 3 (compounds **22**, **27**, and **28**) and one in position 2 (compound **25**), and matricarin **23** was the most guaianolide sesquiterpene cited by the studies.

Table 2. Sesquiterpene identified from *A. ligustica*

Type	Comp.	Name	Reference
Guaianolide	**22**	3β-Cloro- 4α,10α-dihydroxy-1β,2β-epoxy-5α,7α-H-guia-11(13)-en-12,6α-olide	[29]
	23	Matricarin	[20, 26, 29-31]
	24	Desacetylmatricarin	[29, 30]
	25	2α-Chloro-4α-acetoxy-1β,10α-dihydroxy-3β,4β-epoxy-H-guaia-11(13)-en-12,6α-olide	[25]
	26	4α, 10α-Dihydroxy-1α,2α-epoxy-5α,7α-H-guaia-11(13)-en-12,6α-olide	[30]
	27	3α-Chloro-4β,10β-dihydroxy-1β,2β-epoxy-5α,7α-H-guai-11(13)-en-12,6α-olide	[31]
	28	Chlorinated guaianolide	[20]
	29	Chrysartemin A	[26]
	30	Chrysartemin B (Artecanin)	[26]
	31	Isoapressin	[26]
Iso-secotana-partholide	**32**	3-Deshydroxy-iso-seco-tanapartholide	[29]
	33	8-Hydroxy-3-methoxy-iso-seco-tanapartholide	[29]
	34	2α-Chloro-iso-seco-tanapartholide	[29]
	35	Iso-seco-tanapartholide	[20]
Iso-sesquiterpene	**36**	Ligustolide-A	[29]
	37	Ligustolide-B	[29]
Eudesmane	**38**	Artecalin	[29]
Isodaucane	**39**	2-Oxo-isodauc-5-en-12-al	[25]
Other	**40**	5-Hydroxy-5,6-seco-caryophellen-6-one	[29, 31]

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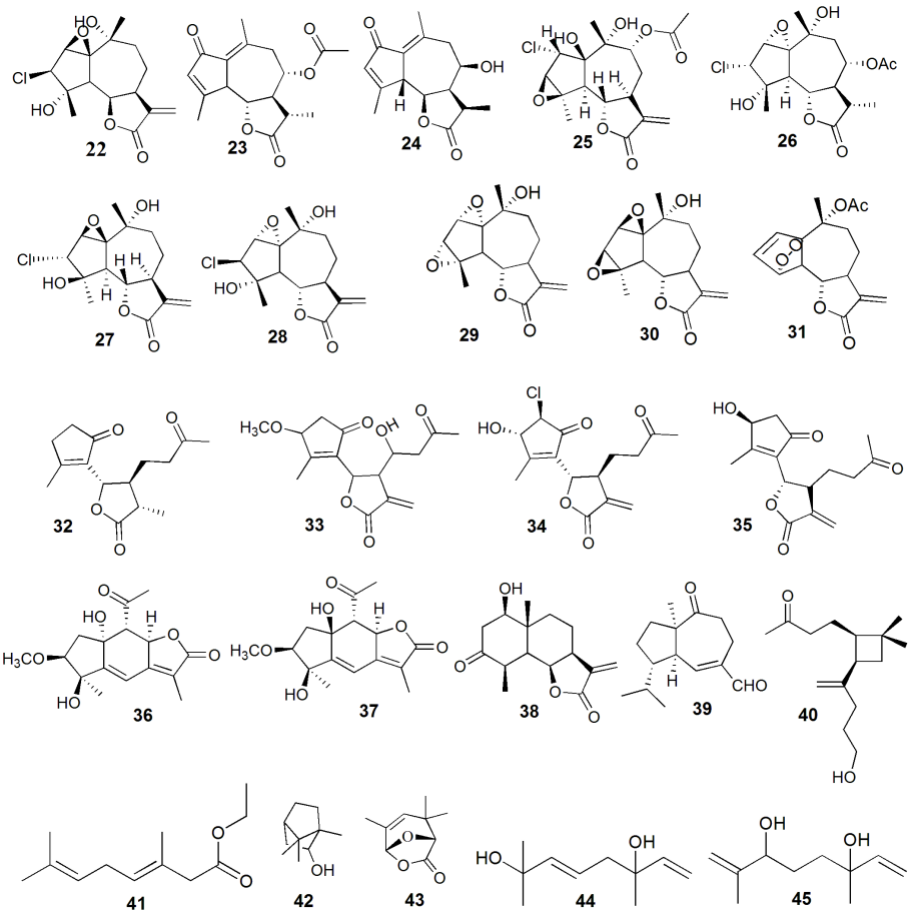

Figure 2. Structure of isolated sesquiterpene lactones (**22**-**40**) and monoterpenes (**41**-**45**) from *A. ligustica*

4.4. Monoterpenes

Five monoterpenes namely (E)-ethyl-3,7-dimethyl-3,6-octadienoate **41**; borneol **42**; filifolide A **43**; (+)-E-2,6-dihydroxy-2,6-dimethylocta-3,7-diene **44**; (3*RS*,6*RS*)-2,6-dimethyl-1,7-octadiene-3,6-diol **45** (Figure 2) were identified from non-polar fractions of the aerial parts of *A. ligustica* collected from Algeria, Italy, and Greece [20, 25, 29].

4.5. Essential oil

Eleven previous phytochemical studies about *A. ligustica* were focused on the study of the chemical composition of EOs of the species most in Italy with eight papers, two in France, and one for Greece. The EOs were obtained using the hydrodistillation (HD) technique and the components of the EO were identified using the GC-MS technique. All the studies confirm that the EO has a pleasant smell with a blue color except that of Lipari (Italy), which gives a yellow color [9].

The EOs contents of *A. ligustica* vary according to used parts of the plant in hydrodistillation and the site of collect. The EOs are characterized by the presence of oxygenated monoterpenes and oxygenated sesquiterpenes as major components (Table 3, Figure 3). The maximum yield of oil was obtained from flowers (3.0%) followed by leaves (1.5%) in *A. ligustica* collected from Central Italy [32]. While, the minimum yield from flowers (0.31%), and leaves (0.21%) was found in Lipari (Italy) and Corsica (France), respectively [8, 9].

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Table 3. Characteristics of essential oils of *A. ligustica* from different countries

N°	Organ [a]	Ext. meth. (*t*) [b]	Yield (w/w)	Num. comps.	Area (Country)	Major constituents (%)	Ref.
1	FL	HD [c] (4 h)	-	83	Camerino (Italy)	Linalool (24.8), viridiflorol (9.6), β-pinene (6.4), 1,8-cineole (5.8), 4-terpineol (5.3)	[35]
2	AP	HD -	-	132	Camerino (Italy)	Viridiflorol (12.6), germacrene D (11.8), linalool (10.8), 4-terpineol (6.0), β-pinene (6.0)	[34]
3	FL	HD (3 h)	3.0	111	Camerino (Italy)	Linalool (25.9), virdiflorol (8.6), β-pinene (7.0), 4-terpineol (7.6), 1,8-cineole (6.8)	[32]
	FAP	HD (3 h)	0.6	101		β-Pinene (11.7), virdiflorol (9.8), 4-terpineol (8.0), linalool (7.7), germacrene D (7.0), 1,8-cineole (5.4)	
	L	HD (3 h)	1.5	107		β-Pinene (16.5), 4-terpineol (13.8), virdiflorol (8.7), 1,8-cineole (7.0), germacrene D (6.0)	
4	FL	HD (4 h)	0.91	84	Camerino (Italy)	Linalool (24.79), virdiflorol (9.59), β-pinene (6.39), 1,8-cineole (5.77), 4-terpineol (5.30)	[11]
	VP	HD (4 h)	0.18	89		Virdiflorol (14.54), 4-terpineol (12.95), β-pinene (9.55), γ-terpinene (4.26), 1,8-cineole (3.43)	
5	FL	HD (2 h)	0.48	37	Sicily (Italy)	Linalool (20.4), 4-terpineol (12.0), carvone (10.0), β-phellandrene (5.4), cedrol (4.3)	[21]
	L	HD (2 h)	0.38	42		4-Terpineol (19.3), carvone (8.9), γ-terpinene (7.2), β-phellandrene (6.8), α-terpinene (4.5)	
6	FL	HD	0.60	42	Greece	Linalool (70.84), 1,8-cineole (6.98), piperitone (2.01), α-terpineol (1.48), 4-terpineol (1.06),	[33]
	L	HD	0.31	40		Linalool (28.15), 1,8-cineole (4.57), piperitone (2.83), viridifloral (1.42), β-pinene (1.36)	
7	FAP	HD	-	68	Liguria (Italy)	Artemisia ketone (43.92), 2,7-dimethyl-4, 6-octa-dien-2-ol (16.15), linalool (9.58), germacrene (3.78)	[36]
8	FL	HD (3 h)	0.31	39	Lipari (Italy)	*Z*-Chrysanthenyl acetate (27.8), viridiflorol (21.6), bornyl acetate (11.6), 1,8-cineole (9.3)	[9]
	AP	HD (3 h)	0.25	43		*Z*-Chrysanthenyl acetate (29.6), viridiflorol (16.8), bornyl acetate (8.7), 1,8-cineole (7.4)	
9	FAP [d]	HD (2 h)	0.43-0.88 [e]	96	Sardinia (Italy)	Santolina alcohol (13.7), borneol (8.5), trans-sabinol (8.0), trans-sabinyl acetate (7.5), α-thujone (6.0)	[37]
10	FL	HD [c] (5h)	0.76-0.89	-	Sardinia (Italy)	trans-Sabinyl acetate (3.3-28.1), trans-sabinol (5.9-20.4), santolina alcohol (3.2-14.9), artemisia ketone (0.7-11.4)	[8]
	L	HD (5 h)	0.16-0.21	-		trans-Sabinyl acetate (4.2-26.6), trans-sabinol (3.1-13.2), santolina alcohol (4.1-13.6), artemisia ketone (0.6-13.4)	
	Coll.oil	-	-	72		trans-Sabinyl acetate (18.3), trans-sabinol (14.7), santolina alcohol (9.4), artemisia ketone (5.0), borneol (3.7)	
	FL	HD (5 h)	0.79-0.90	-	Corsica (France)	Camphor (8.5-26.2), santolina alcohol (3.3-30.1), viridiflorol (4.4-14.4), borneol (2.1-8.5), artemisia ketone (5.9-13.7)	
	L	HD (5 h)	0.13-0.21	-		Camphor (3.9-22.0), santolina alcohol (2.9-22.7), artemisia ketone (3.2-17.7), viridiflorol (5.1-15.0)	
	Coll.oil	-	-	76		Camphor (21.3), santolina alcohol (19.3), borneol (6.2), artemisia ketone (5.9), bornyl acetate (3.5)	
	FL	HD (5 h)	0.81	50	Corsica (France)	Camphor (17.2), linalool (11.6), trans-sabinyl acetate (10.2), viridiflorol (8.2), artemisia ketone (5.9)	

	L		0.21	52		Camphor (17.0), santolina alcohol (10.1), viridiflorol (9.5), trans-sabinyl acetate (7.6), β-pinene (5.2)	
	AP		0.58	58		Camphor (17.4), santolina alcohol (9.1), artemisia ketone (7.5), viridiflorol (7.2)	
	FL	HS-SPME[f]	-	-	Corsica (France)	Camphor (29.8), camphene (9.0), trans sabinyl acetate (5.5), β-pinene (4.3), viridiflorol (3.0)	
	L		-	-		Artemisia ketone (26.7), camphor (14.2), santolina alcohol (9.4), camphene (3.0), β-pinene (2.6)	
	AP		-	-		Camphor (22.8), artemisia ketone (20.4), camphene (4.8), santolina alcohol (3.8), β-pinene (3.6)	
11	AP	HD (5h)	0.4	82	Corsica (France)	Camphor (21.3), santolina alcohol (19.3), borneol (6.2), artemisia ketone (5.9), bornyl acetate (3.5)	[7]

FL, flowers; L, leaves; AP, aerial parts; VP, vegetative parts; FAP, flowering aerial parts; Coll. Oil, collective oil; [b] time of hydrodistillation; [c] hydrodistillation; [d] eight samples from Sardinia were studied; [e] the yield was calculated as volume / dry weight (v/w); [f] HS-SPME, headspace-solid phase micro-extraction technique; Ext. meth., extraction method; Num. comps., number of compounds.

Hence, EOs of *A. ligustica* from Greece, Sicily, and Central Italy showed similar profiles concerning the major components. Thus, linalool was the main component of flowers (>70%) and leaves (>28%) followed by 1,8-cineole and piperitone from the plant collected in Greece [33]. Also, linalool was the major constituent (>20%) of EO collected from flowers of the plant growing in Central Italy followed by viridiflorol and β-pinene. While β-pinene, viridiflorol, germacrene D, and 4-terpineol were the major components of oils from leaves and vegetative parts [11, 32, 34, 35]. In Sicily, linalool was also the major constituent (>20%) of the EO from flowers, followed by 4-terpineol (12.0%) and carvone (10.0%), whilst 4-terpineol (19.3%), carvone (8.9%), γ-terpinene (7.2%), and β-phellandrene (6.8%) were the main components of EO of leaves [21].

For the plant growing in Corsica, camphor was the main component (>20%) of its EO with santolina alcohol and viridiflorol [7, 8]. Furthermore, (*Z*)-chrysanthenyl acetate was the main constituent (>27%) of the EOs of flowers, leaves, and shoots of the species collected in Lipari (Italy) with viridiflorol (>17%), bornyl acetate (>8%), and 1,8-cineole (>7%) [9]. In the north of Italy, artemisia ketone was the main constituent (>43%) of flowering aerial parts EO, with 2,7-dimethyl-4,6-octadien-2-ol (16%) and linalool (9.6%) [36], while in Sardinia (Italy), trans-sabinyl acetate, santolina alcohol, trans-sabinol, borneol, and artemisia ketone were the main constituents of EOs of flowers, leaves, and flowering aerial parts [8, 37].

4.6. Others compounds

One piperidine derivative named (2E,4E,6E,10E)-1-(1-Piperidinyl)-2,4,6,10-tetradecatetraen-8-yn-1-one, was reported from the underground parts of *A. ligustica*, collected in Greece [38]. Also, two non-polar compounds (*β*-sitosterol and 1-tricontanol) were reported from the aerial parts of *A. ligustica*, collected from Algeria [25].

5. BIOLOGICAL ACTIVITIES OF *A. LIGUSTICA*

A. ligustica has attracted the attention of researchers for its ethnomedicinal uses and biological properties. Consequently, the extracts of the species, especially EO extract were tested in vitro for their antioxidant, antibacterial, antifungal, antidiabetic, antiproliferative, antipsoriatic, and insecticide activities.

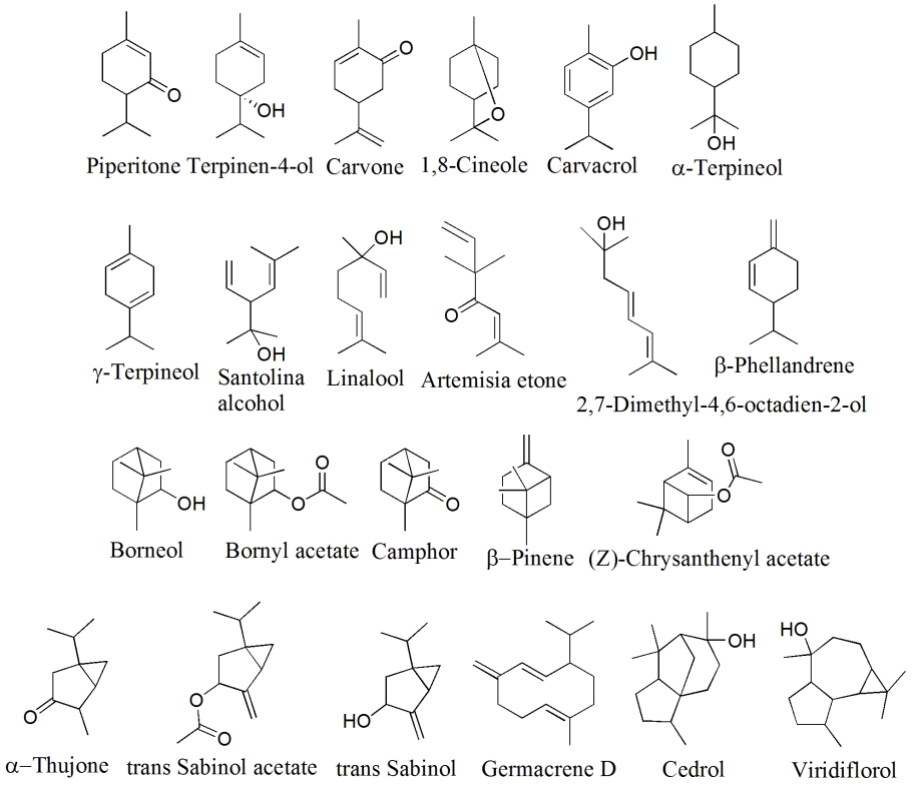

Figure 3. Structure of the main components of EOs of *A. ligustica*

5.1. Antioxidant activity

The antioxidant activity of *A. ligustica* EOs and extracts has been investigated using different methods such as DPPH, ABTS, lipid peroxidation of liposomes, β-carotene, and linolic acid oxidation assays (Table 4). The EO of flowering tops of eight samples from Sardinia (Italy) showed interesting antioxidant activity against free radical DPPH, with values of Trolox equivalent antioxidant capacity (TEAC) ranging between 0.40 - 0.88 mmol/L. The investigated EO was rich in Santolina alcohol, borneol, trans-sabinol. According to the authors, the *A. ligustica* investigated EO could be used as a natural additive in the food industry [37]. In addition, the antioxidant activity of the EOs of flowers and vegetable parts of *A. ligustica* collected from Central Italy was assessed using DPPH, ABTS, and β-carotene bleaching assays. Both EOs exhibited moderate antioxidant activity against DPPH and ABTS. The main components of the studies EOs were linalool, viridiflorol, and β-pinene. The flowers EO (IC_{50}= 47.2 and 35.4 µg/mL for DPPH and ABTS respectively) was more active than EO of the vegetative parts (IC_{50}= 55.1 and 68.2 µg/mL for DPPH and ABTS respectively). Similar results were reported for the β-carotene assay, with flowers EO (PI=27.6%) was more than the vegetative parts EO (12.3%). However, both EOs were less active than butyl hydroxyl toluene (BHT) and butyl hydroxyl anisole (BHA) used as positive standards with PI = 64.6 and 62.6% respectively [11].

Table 4. Antioxidant activity of *A. ligustica* extracts

Organ	Extract	Method	Results	Reference
FAP	EO	DPPH	TEAC = 0.40-0.80 mmol/L	[37]
FL, VP	EO	DPPH	IC_{50} = 47.2, 55.1 µg/mL	[11]
		ABTS	IC_{50} = 35.4, 68.2 µg/ mL	
		β-Carotene	PI = 27.6, 12.3%	
AP	EO	DPPH	PI = 24% (at 10 mg)	[9]
		ABTS	IP = 26.6% (at 10 mg)	
FAP	Methanolic	DPPH	IC_{50} = 20 µg/ mL	[10]
	Phenolic		IC_{50} = 50 µg/ mL	
	n-Hexane		Inactive	

	Methanolic	Lipid peroxidation of liposomes	IC_{50} = 416 µg/ mL	
	Phenolic		IC_{50} = 27 µg/ mL	
	n-Hexane		Inactive	
FAP	Hydroalcoholic	DPPH	TEAC = 4.18-12.53 mmol/L	[12]
FAP	Hydroalcoholic	Linolic acid autoxidation	All extracts had protective effect at 50 and 100 µg	
L	Methanolic	ABTS	IC_{50} = 71-83 µg/ mL	[6]

FL, flowers; L, leaves; AP, aerial parts; VP, vegetative parts; FAP, flowering aerial parts; PI, percentage of inhibition; TEAC, Trolox equivalent antioxidant capacity.

The EO of the aerial parts of *A. ligustica* from Sicily (Italy) which was rich in *Z*-chrysanthenyl acetate, viridiflorol, and bornyl and showed a lower capacity to inhibit DPPH and ABTS when compared to the standard antioxidant BHT. At a concentration of 10 mg/mL, the EO inhibited 24% of DPPH and 26.6% of ABTS [9].

The methanolic, phenolic, and n-hexane extracts of flowering parts of *A. ligustica* collected from Calabria (Italy) were assessed using DPPH and lipid peroxidation of liposome assays. The study demonstrated that the methanolic and phenolic extracts exhibited antioxidant activity while the n-hexane extract was inactive [10]. The hydroalcoholic extracts from eight samples of *A. ligustica* collected in different regions of Sardinia (Italy) were also evaluated using DPPH and linoleic acid assays. Regarding DPPH, the TEAC values of the tested extracts varied between 4.18-12.53 mmol/L, whereas for the linoleic acid assay, all extracts inhibit the oxidative process to the highest concentrations (50 and 100 µg), but only five extracts exerted a significant protective effect at a low concentration of 5 µg [12]. Furthermore, modest antioxidant activity of the methanolic extract of *A. ligustica* collected in Sicily (Italy) was reported [6].

5.2. Antimicrobial activity

Antimicrobial and antifungal activities of *A. ligustica* have been evaluated on several bacterial and fungal strains. According to several authors, both activities are related to the EO composition. So, the EOs of *A. ligustica* collected from different regions located in central Italy were tested using in vitro broth micro-dilution method against six microbial strains *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 13706, *Streptococcus mutans* DSM 20523, and *Candida albicans* ATCC 14053 [11]. The tested EOs which were rich in linalool, viridiflorol, and β-pinene, exhibited interesting activity against *S. mutans* and *B. subtilis* and moderate activity against *C. albicans* (Table 5). In addition, the antimicrobial activity of the EOs of different parts of the species from Camerino (Italy) was also evaluated [32] using the above method on oral pathogens: *Lactobacillus acidophilus* IMC101, *Bacillus cereus* IMC 102, *Enterococcus avium* IMC103, *Streptococcus salivarius* IMC104, *Streptococcus pygenes* IMC105, *Streptococcus dysgalactiae* IMC106, *Streptococcus mutans* DSM20523, *Staphylococcus aureus* ATCC25923, and *Candida albicans* ATCC14053. The results indicated that the tested EOs were more active on *B. cereus*, *S. pyogenes*, and *C. albicans* (Table 6) suggesting the use of the EOs of *A. ligustica* in oral hygienic products such as mouthwash, toothpaste, and other pharmaceutical preparations [32, 39].

Table 5. Antibacterial activity (MIC values, µg/mL) of EOs of *A. ligustica* from Central Italy

Organ	*Strep. mutans*	*Staph. aureus*	*C. albicans*	*B. subtilis*	*Enter. faecalis*	*Esch. coli*	Reference
FL	155	1250	625	78	2500	310	[11]
	(310)	(2500)	(625)	(78)	(5000)	(625)	
AP	39	1250	625	39	1250	625	
	(39)	(2500)	(625)	(39)	(5000)	(2500)	

Values between parentheses are for minimum lethal concentration (MLC, in µg/mL).

Table 6. Antibacterial activity (MIC values, µg/mL) of EOs of different parts of *A. ligustica* from Camerino (Italy)

Organ	*Strep. mutans*	*Staph. aureus*	*C. albicans*	*Strep. salivarius*	*Strep. pyogenes*	*Strep. dysgalactiae*	*B. cereus*	*L. acidophilus*	*Enter. avium*	Ref.

FL	155	310	155	1250	78	625	78	310	5000	[32]
L	155	625	78	1250	155	1250	310	2500	2500	
AP	38	625	155	625	78	1250	155	1250	2500	

Moreover, the antifungal activity of the EO of flowering tops was evaluated (Table 7) against five phytopathogenic fungi *Fusarium avenaceum*, *Fusarium graminearum*, *Fusarium semitectum*, *Alternaria solani*, and *Phytopthoracry ptogea* using in vitro agar disc diffusion (ADD) and agar vapor assays [35]. A good antifungal activity was found suggesting the use of this EO as antifungal preparation against plant pathogens.

Table 7. Antifungal activity of EO of flowers against some phytopathogenic fungi

Organism	MIC (µg/mL)	MFC (µg/mL)	Reference
F. graminearum	750	1200	[35]
F. semitectum	750	1200	
F. avenaceum	750	1200	
P. cryptogea	270	300	
A. solani	270	300	

The antimicrobial activity of the EOs of flowers atablend leaves from *A. ligustica* growing in Sicily (Italy) with linalool, 4-terpineol, and carvone as the major components were also evaluated using ADD method [21]. Both EOs were active against all tested microorganisms *Bacillus cereus*, *Escherichia coli, Hafnia alvei*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus,* and *Candida albicans*. Among all tested microorganisms, *C. albicans* was the most sensitive (Table 8). While the EO of aerial parts of *A. ligustica* collected from Corsica (France) was found to be rich in camphor, santolina alcohol, and borneol considered as the main components. The tested EO exhibited moderate antibacterial activity against some Gram-positive bacteria strains *Enterococcus faecalis* 14C1104, *Staphylococcus aureus* 18C2204, *Nocardia asteroides* ATCC 19247, *Corynebacterium jeikeium* ATCC 43734 (Table 8). Also, the EO was found to possess moderate activity against Gram-negative bacteria strains *Streptomyces coelicolor* M15, *Streptomyces avidinii* ATCC 31267, *Streptomyces albus*, and *Pseudomonas aeruginosa* 13C3104 with the highest activity against *Streptomyces* species [7].

Table 8. Antibacterial activity (ADD values, mm) of EOs of different parts of *A. ligustica*.

Organ	*Staph. aureus*	*Esch. coli*	*Pseud. aeruginosa*	*C. albicans*	*B. cereus*	*H. alvei*	*L. monocytogenes*	Reference
FL	10 (> 900)	12 (> 900)	0 (> 900)	20 (> 900)	-	-	-	[37]
FL	10.7	11.3	0	23.7	12.7	13.3	11.7	[21]
L	10.3	11.3	0	24.3	11.2	13.0	12.0	
AP	9	8	9	-	-	-	-	[7]

Values between parentheses are for MIC in µg/mL

On the contrary, Tuberoso et al. [37] have investigated the EO of flowering tops of *A. ligustica* collected from Sardinia (Italy) against *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, *Candida albicans* ATCC 14053, *Fusarium oxysporum*, *Rhizoctonia solani*, *Penicillium commune*, and *Aspergillus flavus* using ADD method (Table 8) and reported a law antibacterial activity and no activity against fungal strains. The main components of the EO were santolina alcohol, borneol, and trans-sabinol. It may be concluded that EOs rich in linalool, viridiflorol, β-pinene, and 4-terpineol are more active against microbial strains than the other EOs rich in santolina alcohol, borneol, camphor, and trans-sabinol.

5.3. α-Amylase inhibitory activity

Conforti et al., [10] showed that *n*-hexane extract of the flowering parts of *A. ligustica* collected from Calabria (Italy) exerts an important α-amylase inhibitory with 89.30%, 85.03%, and 74.96% at 1, 0.5, and 0.25 mg/mL respectively. The methanolic extract was inactive at 0.5 mg/mL and exhibited 28.18% of inhibition at 1 mg/mL. While, the phenolic extract was inactive.

5.4. Neurotrophic activity

The sesquiterpene lactone 4α,10α-dihydroxy-1α,2α-epoxy-5α,7α H-guaia-11(13)-en-12,6α-olide (**26**) isolated from CH_2Cl_2: MeOH extract of the aerial parts of *A. ligustica* collected from Greece, was tested on PC12 cells. The compound was toxic against the tested cell at a concentration equal or higher than 10 µM [30].

5.5. Antiproliferative activity

The antiproliferative activity of EOs of flowers and vegetative parts of *A. ligustica* collected from central Italy was carried out by MTT assay against four tumor cell lines: T98G, B16-F1, A431, and PC-3. The EOs were active against all tested cell lines. The flowers EO were more active than the vegetative parts EO against the B16-F1 cell line with IC_{50} value of 0.22 and 0.459 mg/mL, respectively. The lowest activity was observed against A431 cell line [11].

5.6. Cytotoxicity and protective effect

The protective effect of hydroalcoholic extracts of samples of *A. ligustica* collected from different areas of Sardinia (Italy) was investigated against cell damage induced by tert-butyl hydroperoxide (TBH) on CaCo-2 intestinal cells at different concentrations (1-100 µg/mL). Initially, CaCo-2 intestinal cells were treated with two hydroalcoholic extracts with different concentrations. After 24 h, the cell viability remained unchanged even at concentrations of up to 100 µg/mL, indicating that *A. ligustica* extracts are not toxic for the tested cells. Then, CaCo-2 intestinal cells were exposed to 5 mM TBH with or without pre-treatment with *A. ligustica* extracts (1-100 µg/mL). The results demonstrated that the tested extracts exerted a good protection at 10 µg/mL and higher. The study suggests the use of *A. ligustica* as a basic component of dietary supplements [12].

5.7. Anti-psoriasis activity

Extracts of *A. ligustica* were tested against the psoriatic disease [6]. Potential effects on 5-, 12-, 15-LOX, and COX-1 enzymes and NF_KB activation intact cells were evaluated in vitro. The methanol extract of the species inhibited both 5-LOX and COX-1 activities at 200 µg/mL, but it was inactive on the 12-LOX activity, whereas NF_KB activation was prevented by the methanol extract. In addition, it was found that the species increased the biosynthesis of the anti-inflammatory eicosanoid named 15(S)-HETE. The IC_{50} = 49.5 µg/mL was superior to the used standard (IC_{50} = 147.8 µg/mL). The *n*-hexane, dichloromethane, and ethyl acetate extracts inhibited the LTB4 biosynthesis with IC_{50} values of 9.5, 20.3, and 68.0 µg/mL, respectively. The study supported that the non-polar extracts of *A. ligustica* can be used as herbal ingredients for future psoriasis drugs.

5.8. Insecticide activity

The EO of the aerial parts of *A. ligustica* collected from Camerino (central Italy) was reported to possess an insecticide activity against *Culex quinquefasciatus* (a filariasis mostuito, an important vector of St. Louis encephalitis and West Nile virus) and *Musca domestica* (a housefly insect transmitter of pathogens). The EO of the species showed a lethal dose (LC_{50} = 89.5 µl/L and 121 µg/adult) against 4th instar larvae of *C. quinquefasciatus* and adults of *M. domestica*, respectively [34].

5.9. Toxicology of *A. ligustica*

Only one study has evaluated the toxicity of the methanolic extract of the aerial parts of *A. ligustica* against CaCo-2 intestinal cells. The viability of the tested cells has not changed even at concentrations up to 100 µg/mL [12]. In the absence of suitable studies, the nontoxicity of the species is only confirmed also by its use in folk medicine as an intern drug and in food preparations in some regions.

6. CONCLUSION

The medicinal plant *A. ligustica* growing in the Mediterranean basin is rich in secondary metabolites where matricarin and santin are the major sesquiterpene and flavonoid compounds respectively. The composition of the EO varies according to the geographical area, but it is generally rich in oxygenated monoterpenes and oxygenated sesquiterpenes. Several studies on *A. ligustica* extracts were carried out to evaluate the antioxidant activity using different assays, and the antimicrobial activity with a large wide of

bacteria, and fungi strains. The antidiabetic, neurotrophic, antiproliferative, anti-psoriasis, and insecticide activities were also evaluated. Consequently, biological studies had demonstrated that the plant is a good protective and can be used as dietary suppliants, though the EO of the species can be used in mouthwash and dentifrice preparations. In addition, polar extracts of the plant can be exploited for a future psoriasis drug.

Insufficient studies about this medicinal species were the major limit during writing this manuscript. Further extensive phytochemical studies are needed to better determine its chemical constituents. In vitro and in vivo investigations of its biological potential will be also of great interest. A bio-guided approach can help to better understand the different mechanisms of action involved and to find out the biological active compounds. More, toxicological studies can prove the safe use of this species, which will lead to exploit its extracts in pharmaceutical preparation and the food industry.

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