UNIVERSITÀ DEGLI STUDI DI PALERMO
Dottorato in Scienze Chimiche
Dipartimento di Fisica e Chimica
Settore Scientifico Disciplinare CHIM06

Metabolites from Mediterranean plants:

characterization and transformation.

Chemotaxonomic assessment and biological activity.

IL DOTTORE
Luana Riccobono

IL COORDINATORE
Paolo Giuseppe Maria Lo Meo

IL TUTOR
Sergio Rosselli

CICLO XXVI
ANNO CONSEGUIMENTO TITOLO 2016

UNIVERSITÀ DEGLI STUDI DI PALERMO

Dottorato in Scienze Chimiche
Dipartimento di Fisica e Chimica
Settore Scientifico Disciplinare CHIM06

Metabolites from Mediterranean plants:

characterization and transformation.

Chemotaxonomic assessment and biological activity.

IL DOTTORE
Luana Riccobono

IL COORDINATORE
Paolo Giuseppe Maria Lo Meo

IL TUTOR
Sergio Rosselli

CICLO XXVI
ANNO CONSEGUIMENTO TITOLO 2016

INDEX

1. INTRODUCTION

1.1. ABSTRACT IN ITALIANO

Questo progetto di ricerca ha lo scopo di ampliare le conoscenze di alcune piante appartenenti alla flora Mediterranea. Le piante da studiare sono state scelte in seguito ad un'attenta analisi bibliografica tra specie poco sudiate. La scelta è quindi ricaduta su nove specie appartenenti al genere *Anthemis*, due specie appartenenti al genere *Pulicaria* e sulle specie di *Tetraclinis articulata*, *Salvia argentea*, *Ajuga tenorei*, *Ballota hispanica, Moluccella Spinosa* e *Thapsia garganica*. Di esse sono stati preparati ed identificati i componenti degli oli essenziali e successivamente effettuati su di essi i saggi antibatterici. Dagli estratti delle specie di *Tetraclinis articulata* e *Ajuga tenorei* sono invece stati isolati e caratterizzati i metaboliti secondari non volatili. Di alcune delle molecole identificate da questi estratti è stata determinata la loro potenziale attività antitumorale attraverso l'utilizzo di metodi computazionali.

Le specie appartenenti al genere *Anthemis* collezionate in Sicilia rappresentano una grande fonte di dati per la classificazione chemotassonomica e per considerazioni di biodiversità. I dati di composizione di tre di loro, riconosciute appartenenti alla sezione *Hiorthia*, quindi strettamente collegate tra loro, sono stati comparati con i dati disponibili in letteratura di tutte le specie appartenenti al genere *Anhemis* usando l'analisi statistica dei cluster. I risultati ottenuti mostrano che questi taxa appartengono alla stessa sezione sulla base delle classi di composti in esse contenuti, soprattutto sesquiterpeni. Una estensione di questo lavoro, già programmata ed in corso, include tutti i dati di composizione collezionati per le alter specie di *Anthemis* analizzate. Inoltre, i loro oli essenziali hanno mostrato una moderata attività antibatterica contro batteri gram+ e gram-.

Di due specie siciliane di *Pulicaria* (*P. vulgaris* var. *graeca* and *P. sicula*) è stata identificata la composizione dell' olio essenziale. L'analisi della componente principale (PCA)

di *P.sicula,* rispetto alle altre specie di *Pulicaria* fino ad ora studiate, mostra una peculiare biodiversità di questa pianta siciliana. Il confronto, tra i dati di composizione di *P. vulgaris* var. *graeca* e i dati relativi a *P. vulgaris* Gaertner, mette in evidenza un profilo chimico completamente differente. Quindi i due taxa potrebbero essere considerati due entità distinte. L'olio essenziale di *P. vulgaris* mostra una media attività antimicrobica contro i batteri *Bacillus cereus* e *B. subtilis*.

L'analisi della composizione dell'olio essenziale di *Salvia argentea*, collezionata in Sicilia, mostra un profilo chimico differente rispetto a quello di *Salvia argentea* collezionate in altri paesi. inoltre è stata studiata la composizione degli estratti ottenuti con solventi a bassa polarità.

Anche la composizione dell'olio essenziale di *Ballota hispanica* mostra un profilo caratteristico, comparato a quello degli altri taxa. Inoltre il suo olio essenziale mostra una bassa attività antibatterica mentre l'attività antiossidante è veramente alta, tale che si potrebbe supporre l'uso di *B. hispanica* come fitoterapico.

Lo studio dell'olio essenziale di *Moluccella spinosa*, una pianta mai investigata in precedenza, ha mostrato differenti marker nella sua composizione rispetto all'olio essenziale di altre specie collezionate in Turchia, tuttavia si è riscontrata una buona correlazione con l'olio di *M. laevis*. Anche in questo caso si è osservata una moderata attività antibatterica dell'olio essenziale.

Infine i risultati ottenuti per l'olio essenziale di *Thapsia garganica*, indicano un profilo chimico completamente differente rispetto a quelli delle altre specie di *Thapsia* studiate fino ad oggi, indipendentemente dal metodo di estrazione utilizzato (SPME o idrodistillazione).

Lo studio dei metaboliti non volatili riguarda *Tetraclinis articulata* e *Ajuga tenorei*.

Solo un articolo sull'investigazione di *T. articulata* collezionata in Marocco è stato pubblicato. *T. ariculata* analizzata in questo lavoro è stata collezionata in Tunisia e ha mostrato un profilo metabolico simile a quello della specie marocchina. Infatti sono stati isolati derivati Δ^{15}-pimaranici dall'estratto esanico di *T. articulata*; quasi tutti i composti isolati da questa pianta erano già stati in precedenza isolati ad eccezione del composto ***5***.

I tre estratti (esano, diclorometano e metanolo) di *T. articulata* mostrano una buona attività antiproliferativa contro cellule tumorali. Gli estratti in diclorometano e metanolo sono stati cromatografati, ma non sono state ottenute frazioni pure. L'ottenimento di composti puri rappresenta il passo successivo della ricerca.

La possibile attività antitumorale delle molecole **3**, **4** e **5** verso differenti target è stata valutata attraverso metodi computazionali, utilizzando l' Inverse Virtual Screeneing (IVS). Da un'accurata analisi delle interazioni, le tre molecole sembrano altamente correlate con la proteina fxr (Farnesoid X receptor, Cod. PDB: 1OSV).

La purificazione dell'estratto metanolico di *Ajuga tenorei* ha portato all'isolamento di due iridoidi (arpagide e 8-*O*-acetilarpagide) e un fitosteroide (ajugalattone), prodotti di cui erano note le loro proprietà biologiche (antibatterica, anti-infiammatoria e antivirale). Per esplorare nuovi target per l'attività antitumorale, questi composti erano soggetti a IVS. Le molecole **6**, **7**, **9** e **10** mostravano la migliore correlazione con target differenti, ciò può essere spiegato dal cambiamento dei pattern di sostituzione nella struttura che porta a una differente interazione con la proteina.

Sfortunatamente non sono state possibili modificazioni chimiche dei composti isolati a cause delle basse quantità del materiale puro isolato.

1.2. AIM OF RESEARCH

This research project aims to broaden the knowledge on Mediterranean plants as the genus *Anthemis* and *Pulicaria* and as species *Tetraclinis articulata*, *Salvia argentea*, *Ajuga tenorei*, *Ballota hispanica, Moluccella Spinosa* and *Thapsia garganica*. These plants are widely used in folk medicine and their extracts will be analyzed for the isolation of metabolites. Appropriate biological target will be selected by computational methods. The biological activity of these molecules and chemically modified derivatives will be evaluated in order to obtain structure-activity relationships.

1.3. PLANTS STUDIED

1.3.1. *Anthemis*

The genus *Anthemis* (family Asteraceae, tribe Anthemideae) includes about 130 species, in Europe there are 62 species, of which 26 in Sicily.[1] The genus *Anthemis* is divided into three subgenera according to the botanical classification,[2] subgenus *Anthemis* includes four sections *Hiorthia*, *Anthemis*, *Maruta* and *Chia*, subgenus *Cota* involves sections *Anthemaria* and *Cota*, while the species from subgenus *Ammanthus* are not separated into sections.

In *figure 1* the most important organs of *Anthemis arvensis* can be seen.

[1] http://luirig.altervista.org/flora/taxa/floraindice.php S. Pignatti, *Flora d'Italia*, **1982**, 3, 770-771.

[2] Fernandes R., **1976**. Genus Anthemis L. In: Tutin T. G., Heywood V. H., Burges N. A., Moore D. M., Valentine D. H., Walters S. M., Webb A. (Eds.), Flora Europaea, vol. 4. Cambridge University Press, Cambridge, London, New York, Melbourne, 145–159.

Figure 1: *Anthemis arvensis*. Realistic draw of leaves, blossoms and cones of *A. arvensis*

In Sicily, as reported in the flora of Italy,[Errore. Il segnalibro non è definito.] are present 26 species (*table*) of *Anthemis*, 9 of which are endemic (*A. aetnensis, A. asperula*, *A. cupaniana*, *A. ismelia*, *A. lopadusana*, *A. muricata*, *A. rigida*, *A. secundiramea*, *A. urvilleana*) and only 2 are studied until today (*A. aetnensis*[3] and *A. cupaniana*[4]).

Table 1: species of *Anthemis* present in Sicily

	Scientific name		**Scientific name**
1	*A. aetnensis* Schouw	**14**	*A. mixta* L.
2	*A. arvensis* L.	**15**	*A. montana* L.
3	*A. arvensis* L. subsp. *arvensis*	**16**	*A. montana* L. subsp. *montana*
4	*A. arvensis* L. subsp. *incrassata* (Loisel.) Nyman	**17**	*A. muricata* (DC.) Guss.
5	*A. arvensis* L. subsp. *sphacelata* (Presl) Fernandes	**18**	*A. praecox* Link
6	*A. asperula* Bertol.	**19**	*A. rigida* Heldr.
7	*A. chia* L.	**20**	*A. secundiramea* Biv.
8	*A. cotula* L.	**21**	*A. secundiramea* Biv. subsp. *intermedia* (Guss.) Fernandes
9	*A. cretica* L. sinonimi: *A. montana* L.	**22**	*A. secundiramea* Biv. subsp. *secundiramea*
10	*A. cupaniana* Tod.	**23**	*A. tinctoria* L.
11	*A. ismelia* Lojac.	**24**	*A. tomentosa* L.
12	*A. lopadusana* Lojac.	**25**	*A. triumfetti* All.
13	*A. maritima* L.	**26**	*A. urvilleana* (DC.) Somm. et Car.-G.

[3] Bruno M., Bondì M. L., Vassallo N., Gedris T.E., Herz W., *Phytochemistry*, **1997**, *45,* 375-377.
[4] Bruno M., Diaz J. G., Herz W., *Phytochemistry*, **1991**, *30*, 3458-60.

1.3.2. *Salvia argentea*

The genus *Salvia* is one of the largest members of the family Lamiaceae (subfamily Nepetoideae), comprising more than 500 species. It is widely distributed in various regions including the temperate and warmer zones of the world such as the Mediterranean, where it is represented by 36 species,[5] Central Asia, the Pacific Islands, tropical Africa, and America.[6]

Several *Salvia* species are economically important since they have been used in therapy as antihydrotic, spasmolytic, antiseptic, anti-inflammatory and in the treatment of mental and nervous conditions[7] and furthermore as spices and flavouring agents in perfumery and cosmetics. Members of this genus have been shown to possess a significant array of pharmacological properties such as antimicrobial, antioxidant, cytotoxic, anti-HIV, etc..[8,9,10,11] The occurrence of the non-volatile secondary metabolites and the biological properties of all the studied species of *Salvia* have been recently reviewed[6]. The essential oils of *Salvia* species are also applied in the treatment of a range of diseases and it has been shown to possess antimicrobial, viricidal, cytotoxic, anti-mutagenic and antifungal activities.[12]

Salvia argentea L., (syn: *S. tmolea* Boiss.) is a perennial herb native to the Mediterranean region, in northwest Africa (Morocco, northern Algeria, Tunisia), southern Europe (Spain, Portugal, South Italy, Sicily, Malta, Albania, Bulgaria, Slovenia, Croatia, Bosnia, Kosovo, Montenegro, Serbia, Macedonia, and Greece), and the far west of Asia (Turkey). It occurs

[5] Hedge I. C., Salvia L. In Flora Europaea, vol 3, Tutin TG, et al. (eds). Cambridge University Press: Cambridge, 188.
[6] Wu Y. B., Ni Z. Y., Shi Q. W., Dong M., Kiyota H., Gu Y. C., Cong B., *Chemical Reviews*, **2012**, *112*, 5967-6026.
[7] Baricevic D., Bartol T., Sage: the genus Salvia. In: Kintzios, S.E. (Ed.), Pharmacology: The biological/pharmacological activity of the Salvia genus. Harwood Academic Publishers, The Netherlands, **2000**, 143–184.
[8] Blumenthal M., The complete German commission E monographs. American Botanical Council: Texas, **1998**, 198.
[9] Weiss R. F., Herbal Medicine, Beaconsfield Publishers: Beacons field, **1998**, 228.
[10] Bisset N. G., Herbal drugs and phytopharmaceuticals. CRC press: Stuttgart, **1994**, 440.
[11] Newall C. A., Anderson L. A., Phillipson J. D., Herbal Medicines: A guide for healthcare professionals. Pharmaceutical Press: London, **1996**, 231.
[12] Jalsenjak V., Peljnajk S., Kustrak D., *Pharmacology*, **1987**, *42*, 419-420.

primarily on stony hillside meadows, basalt, volcanic soils and rocky bluffs. Usually it is not found very near the sea or ocean, or at low altitudes, but it has often been found on highlands not far from the sea.[13] *S. argentea* has a large spread of basal leaves that measure 1 m wide and 30 to 60 cm high. The individual leaves are 20 to 30 cm long and 15 cm wide (***Figure 2***). Both leaf surfaces are heavily covered with silky hairs that give it a wooly appearance. The leaves are soft to the touch, first emerging as a distinctive silvery white and then turning to grey-green after flowering. Cool weather in the fall turns the leaves silvery again.[14] The flowers are white (***Figure 3***).

Figure 2: Leaves of *S. argentea*

Figure 3: Flowers of *S. argentea*

In Lucania (Italy), where it is known as "l'erva du tagliè", the young leaves of *S. argentea* were topically used as haemostatic[15] whereas the basal leaves, peeled and stewed, were consumed as food in Spain ("gordolobo").[16]

Several biological properties have been reported for this species. In fact, a good antioxidant activity has been shown from the aqueous and methanolic extracts[17] and from the

[13] http://www.bgbm.org/euroPlusMed/
[14] Clebsch B., Barner C. D., The new book of Salvias: sages for every garden. Portlan: Timber Press, **2003**, 36–37.
[15] Pieroni A., Quavec C. L., Santoro R. F., *Journal of Ethnopharmacology*, **2004**, *95*, 373–384.
[16] Tardío J., Pardo-De-Santayana M., Morales R., *Botanical Journalof the Linnean Society*, **2006**, *152*, 27-71.

methanolic extract.[18,19,20] Furthermore, good acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activity for the CH_2Cl_2 and methanolic extracts,[21] antibacterial activity on *S. aureus* and *S. epidermidis* for the ethanolic extract [22] and larvicidal activity, against the mosquito *Culex pipiens*[23] for the hexane extract, were determined.

Previous phytochemical studies of the plant indicated the presence of abietane diterpenoids in the roots[24] whereas several flavones, from the exudates of *S. argentea* collected in Bulgaria[25,26] and from the acetone extract of plants cultivated in Poland,[27] and oleanane and ursane derivatives[28 ,29] were identified in the aerial parts.

Some investigations have been published on the composition of the essential oil of *S. argentea* growing in Morocco,[30] Serbia,[31] Macedonia[32] and Tunisia[19] but nothing has been reported on Italian plants.

---

[17] Stagos D., Portesis N., Spanou C., Mossialos D., Aligiannis N., Chaita E., Panagoulis C., Reri E., Skaltsounis L., Tsatsakis A. M., Kouretas D., *Food and Chemical Toxicology*, **2012**, *50*, 4115-4124.

[18] Salah K. B. H., Mahjoub M. A., Ammar S., Michel L., Millet-Clerc J., Chaumont J. P., Mighri Z., Aouni M., *Natural Product Research*, **2006**, *20*, 1110-1120.

[19] Ben Farhat M., Landoulsi A., Chaouch-Hamada R., Sotomayor J. A., Jordan M. J., *Industrial Crops and Products*, **2013a**, *47*, 106-112.

[20] Ben Farhat M., Landoulsi A., Chaouch-Hamada R., Sotomayor J. A., Jordan M. J., *Industrial Crops and Products*, **2013b**, *49*, 904-914.

[21] Erdogan Orhan I., Sezer Senol F., Ercetin T., Kahraman A., Celep F., Akaydin G., Sener B., Dogan M., *Industrial Crops and Products*, **2013**, *41*, 21-30.

[22] Sarac N., Ugur A., *EurAsia Journal of BioSciences*, **2007**, *4*, 28-37.

[23] Şeref Gün S., Çinbilgel İ., Öz E., Çetin H., Kafkas Univ Vet Fak Derg., **2011**, *17* (Suppl A), S61-S65.

[24] Michavilla A., De La Torre M. C., Rodriguez B., *Phytochemistry*, **1986**, *25*, 1935-1937.

[25] Yang M. H., Blunden G., Xu Y. X., Nagy G., Mathe I., *Pharmaceutical Sciences*, **1996**, *2*, 69-71.

[26] Nikolova M. T., Grayer R. J., Genova E., Porter E. A., *Biochemical Systematics Ecology*, **2006**, *34*, 360-364.

[27] Sajewicz M., Staszek D., Wròbel M. S., Waksmundzka-Hajnos M., Kowalska T., *Chromatography Research International*., **2012**, Article ID 230903:1-8.

[28] Bruno M., Savona G., Hueso-Rodriguez J. A., Pascual C., Rodriguez B., *Phytochemistry*, **1987**, *26*, 497-501.

[29] Janicsàk G., Veres K., Zoltan Kakàsy A., Màthé I., *Biochemical Systematics Ecology*, **2006**, *34*, 392-396.

[30] Holeman M. A., Berrada M., Bellakhdar J., Ilidrissi A., Pinel R., *Fitoterapia*, **1984**, *55*, 143-148.

[31] Couladis M., Tzakou O., Stojanovic D., Mimica-Dukic N., Jancic R., *Flavour and Fragrance Journal*, **2001**, *16*, 227-229.

[32] Veličković D. T., Ristić M. S., Milosavljević N. P., Davidović D. N., Bogdanović S. Z., *Agro Food Industry Hi Tech*, **2014**, *25*, 70-72.

In this study, as a continuation of previous researches on Mediterranean plants,[33,34,35,36] we report the chemical composition of the essential oil and of the non-polar extracts from aerial parts of *Salvia argentea* L. growing wild in Sicily, a population not previously investigated.

1.3.3. *Pulicaria*

Pulicaria Gaertn. genus, belonging to the tribe *Inulae* of the family *Asteraceae,* comprises approximately 80 species which are widely distributed from Europe into North Africa and Asia.[37] It is represented in the flora of Italy by four species.[**Errore. Il segnalibro non è definito.**] The hemical investigation of the genus showed the presence of sesquiterpenes (germacranes, xanthanes, pseudoguaianes, guaianes, eudesmanes, caryophyllanes, bisabolanes), diterpenes (clerodanes, kauranes, abietanes) and flavonoids and their occurrence has been reviewed some years ago.[38]

Several *Pulicaria* species are used in popular medicine for the treatment of a variety of illnesses such as flu, intestinal disorders[38] and inflammation.[38,39] Furthermore, different biological properties such as cytotoxic,[40,41] antibacterial,[40,42] anti-inflammatory,[43] antihistaminic,[44] antifungal,[45] and insecticide[46] have been reported for species of this genus.

---

[33] Zito P., Sajeva M., Bruno M., Rosselli S., Maggio A., Senatore F., *Natural Product Research*, **2013**, *27*, 1305-1314.
[34] Rosselli S., Maggio A. M., Canzoneri M., Simmonds M. S. J., Bruno M., *Natural Product Communications*, **2012**, *7*, 1131-1132.
[35] Maggio A., Bruno M., Formisano C., Rigano D., Senatore F., *Natural Product Communicaions*, **2013**, *8*, 841-844.
[36] Maggio A., Riccobono L., Bancheva S., Bruno M., Senatore F., *Natural Product Communications.*, **2014**, *9*, 1373-1376.
[37] Pottier-Alapetite G., *Flore de la Tunisie,* **1981**, 236.
[38] Liu L. L., Yang J. L., Shi Y. P., *Chemistry & Biodiverity*, **2010**, *7*, 327-349.
[39] Ravandeh M., Valizadeh J., Noroozifar M., Khorasani-Motlagh M., *Journal of Medicinal Plants Research*, **2011**, *5*, 2035-2040.
[40] Kuete V., Wiench B., Alsaid M. S., Alyahya M. A., Fankam A. G., Shahat A. A., Efferth T., *BMC Complementary and Alternative Medicine*, **2013**, 13354.

Previous investigations on the roots of *Pulicaria sicula* (L.) Moris (***Figure 4***) indicated the presence of several polyacetylenes[47] whereas from the aerial parts, collected in Qatar, several xanthanolides and guaianolides were isolated.[48] *P. sicula* cultivated in the Botanic Garden of the Technical University Darmstadt (Germany) was analyzed for its exudate flavonoids. An array of quercetagetin derivatives and the new 2'-hydroxy-3,5,6,7,4',5'-hexamethoxyflavone were identified.[49] Further investigations on the surface and vacuolar constituents of *P. sicula* cultivated in the School of Plant Sciences, University of Reading (UK) showed a complex flavonoid profile with 6-hydroxyluteolin 5,6,7,3',4'-pentamethyl ether, as major component.[50] On the other hand, no report has been published on the composition of the essential oil of *P. sicula.*

Pulicaria vulgaris var*. graeca* (Sch.-Bip.) Fiori [Syn. *P. dentata* Guss. (1844) non DC.; *P. clausonis* Pomel] is native to the Mediterranean region, in northwest Africa (northern Algeria) and southern Europe (Italy, Sicily, Sardinia, Greece).[51] It is a herbaceous annual plant, erect, more than 30 cm high, much branched at least above, puberulous. The leaves are linear-oblong, subobtuse or subacute, mucronate, sessile, cordate semiamplexicaul, entire or

---

[41] El-Seedi H. R., Burman R., Mansour A., Turki Z., Boulos L., Gullbo J., Goransson U., *Journal of Ethnopharmacoogy,* **2013**, *145*, 746-57

[42] Nickavar B., Mojab F., *Fitoterapia* **2003**, *74*, 390-393.

[43] Alghaithy A. A., El-Beshbishy H. A., AbdelNaim A. B., Nagy A. A., Abdel-Sattar E. M., *Toxicology and Industrial Health*, **2011**, *27*, 899-910.

[44] Mahfouz M., Ghazal A., El-Dakhakhny M., Ghoneim M. T., *Journal Drug Research*, **1973**, *5*, 151-172.

[45] Znini M., Cristofari G., Majidi L., Paolini J., Desjobert J. M., Costa J., *LWT-Food Science and Technology*, **2013**, *54*, 564-569.

[46] Khani A., Asghari J., *Journal of Insect Science (Madison, WI, United States)* **2012**, *12*, 73.

[47] Schulte K. E., Reisch J., Hopmann J., *Archiv der Pharmazie und Berichte der Deuschen Pharmazeutischen Gesellschaft,* **1963**, *296*, 353-364.

[48] Zdero C., Bohlmann F., Rozk A. M., *Phytochemistry*, **1988**, *27*, 1206-1208.

[49] Wollenweber E., Christ M., Dunstan R. H., Roitman J. N., Stevens J. F., Naturforsch Z*., C: Journal of Bioscience*, **2005**, *60*, 671-678.

[50] Williams C. A., Harborne J. B., Greenham J. R., Grayer R. J., Kite G. C., Eagles J., *Phytochemistry*, **2003**, *64*, 275-283.

[51] http://ww2.bgbm.org/euroPlusMed/ The Euro+Med PlantBase- the information resource for Euro-Mediterranean plant diversity.

denticulate. The capitula are hemispherical with many-flowered. It grows in seasonal wet localities, maritime sands, hollows and wet grazing (***Figure 4***).

Figure 4*:* a) *P. sicula* b) *P. vulgaris*

No previous phytochemical research has been reported on *Pulicaria vulgaris* var. *graeca* whereas *P. vulgaris* Gaertner has been analysed for their surface and vacuolar constituents and several flavonoid derivatives were identified showing a pattern similar to *P. dysenterica*.[52] Recentely, the chemical composition of the essential oil of *P. vulgaris* collected in Iran, a taxa botanically closely related to *P. vulgaris* var. *graeca*, and its biological activities have been published.[53]

One of the main factors affecting historical art crafts material is the biodeterioration performed by bacteria and fungi, in archives, museums or private collections. Several microorganisms cause degradation to the natural organic material such as fibers, woods, dyes, etc. as well as to stone objects. These alterations produce deterioration of physical, chemical, mechanical and aesthetic properties. In order to fight against these

[52] Williams C. A., Harborne J. B., Greenham J. R., Grayer R. J., Kite G. C., Eagles J., *Phytochemistry*, **2003**, *64*, 275-283.
[53] Sharifi-Rad J., Miri A., Hoseini-Alfatemi S. M., Sharifi-Rad M., Setzer W. N., Hadjiakhoondi A., *Natural Product Communications*, **2014**, *9*, 1633-1666.

microorganisms, with alternative natural tools, the biological properties of essential oils derived from certain species of plants have been investigated.[54,55,56,57,58,59]

Consequently, in this study, as a continuation of previous researches on Sicilian species of Asteraceae,[60,61,62] we report the chemical composition and the antibacterial activity against several microorganisms, including *Bacillus cereus*, *B. subtilis*, and *Staphylococcus* ssp., species infesting historical art craft,[63] of the essential oils from aerial parts of of *Pulicaria vulgaris* var*. graeca* (Sch.-Bip.) Fiori, growing wild in Sicily.

1.3.4. *Ballota hispanica*

Ballota L. (Lamiaceae) is a genus belonging to the tribe *Stachydeae*, sub-tribe *Ballotae*. It consists of about 45 taxa, divided in ten sections[64] native to Macaronesia, Europe, Mediterranean to W. Asia, Mauritania, Chad and S. Africa. *Ballota* species are perennial herbs characterized by flowers held in verticillasters and by an unpleasant aromatic foliage.[65] *Ballota* species have been used in folk medicine as an antiulcer, antispasmodic, diuretic,

[54] El-Seedi H. R., Burman R., Mansour A., Turki Z., Boulos L., Gullbo J., Goransson U., The traditional medical uses and cytotoxic activities of sixty-one Egyptian plants: discovery of an active cardiac glycoside from *Urginea maritime*, **2013**.
[55] Rakotonirainy M. S., Lavèdrine B., *International Biodeterioration & Biodegradation*, **2005**, *55*, 141–147.
[56] Stupar M., Grbić M. Lj, Džamić A., Unković N., Ristić M., Jelikić A., Vukojević J., *South Africa Journal of Botany*, **2014**, *93*, 118-124.
[57] Casiglia S., Bruno M., Senatore F., *Natural Product Research*, **2014a**, *28,* 1739-1746.
[58] Casiglia S., Bruno M., Senatore F., *Natural Product Communications*, **2014b**, *9*, 1637-1639.
[59] Casiglia S., Ben Jemia M., Riccobono L., Bruno M., Scandolera E., Senatore F., *Natural Product Research*, **2015**, *29*, 1201-1206.
[60] Formisano C., Rigano D., Senatore F., Raimondo F. M., Maggio A., Bruno M., *Natural Product Communications*, **2012**, *7*, 1379-1382.
[61] Maggio A., Riccobono L., Spadaro V., Scialaba A., Bruno M., Senatore F., *Chemistry & Biodiversity*, **2014**, *11*, 652-672.
[62] Maggio A., Venditti A., Senatore F., Bruno M., Formisano C., *Natural Product Research*, **2015**, *29*, 857-863.
[63] Kamel F. H., Ismael H. M., Mohammadamin S. A., *Online International Interdisciplinary Research Journal*, **2014**, *4*, 10-17.
[64] Seidel V., Bailleul F., Tillequin F., Terpenoids and phenolics in the genus *Ballota* L. (Lamiaceae). *Recent Research Develpmens in Phytochem*istry, **1999**, *3*, 27-39.
[65] http://apps.kew.org/wcsp/qsearch.do.

choleretic, antihaemorrhoidal, and sedative agents.[66] The antimicrobial activities[67,68] and the antioxidant activities[69] of *Ballota* species were recently reported as well as the antifungal activities of some flavonoids isolated from some species.[70,71] Water extracts have been reported to have antinociceptive, antiinflammatory and hepatoprotective activities.[72] In Europe, the polar extracts of the flowered aerial parts of *Ballota* are commonly used due to their neurosedative activity.[73,74] More recently, the general antioxidant activity,[75] the *in vitro* inhibition of LDL (low-density lipoprotein) peroxidation,[76] and the antibacterial activity[77,78] of these plants have been published. The application of *Ballota* species in Italian folk traditions has been reviewed.[79] Phytochemical investigations showed that labdane diterpenoids, flavonoids and phenylpropanoids are the characteristic features of the genus.[70,71,80,81,82,83,84,85,86,87]

Ballota hispanica (L.) Benth. (***Figure 5***) is endemic of the Central Mediterranean region (Albania, Croatia, Bosnia and Hertzegovina, Montenegro, Italy, Sicily)[65] and is used in the

[66] Çitoğlu G., Tanker M., Sever B., Englert J., Anton R., Altanlar N., *Planta Medica*, **1998,** *64*, 484-485.
[67] Çitoğlu G. S., Yilmaz B. S., Altanlar N., *Journal of Faculty of Pharmacy of Ankara*, **2003a**, *32*, 93-97.
[68] Dulger B., Dulger G., *Turkish Journal of Pharmaceutical Science*, **2012**, *9*, 257-262.
[69] Çitoğlu G. S., Çoban T., Sever B., İşcan M., *Journal of Ethnopharmacology*, **2004a**, *92*, 275-280.
[70] Çitoğlu G. S., Sever B., Antus S., Baitz-Gacs E., *Pharmaceutical Biology*, **2003b**, *41*, 483-486.
[71] Çitoğlu G. S., Sever B., Antus S., Baitz-Gacs E., Altanlar N., *Pharmaceutical Biology*, **2004b**, *42*, 659-663.
[72] Özbek H., Çitoğlu G. S., Dülger H., Uğraş S., Sever B., *Journal of Ethnopharmacology*, **2004**, *95*, 143-149.
[73] Racz-Kotilla G., Racz G., Jozsa J., *Herba Hungarica*, **1980**, *19*, 49-53.
[74] Vural K., Ezer N., Erol K., Samin F. P., *Journal of Faculty of Pharmacy of Gazi University*, **1996**, *13*, 29-32.
[75] Couladis M., Tzakou O., Verykokidou E., Harvala C., *Phytotherapy Res*earch, **2003**, *17*, 194-195.
[76] Seidel V., Verholle M., Malard Y., Tillequin F., Fruchart J. C., Duriez P., Bailleul F., Teissier E., *Phytotherapy Research*, **2000**, *14*, 93-98.
[77] Didry N., Seidel V., Dubreuil L., Tillequin F., Bailleul F., *Journal of Ethnopharmacology*, **1999**, *67*, 197-202.
[78] Dulger B., Kilcik M. A., *Asian Journal of Chemistry*, **2011**, *23*, 416-418.
[79] Pieroni A., *Journal of Ethnopharmacology,* **2000,** *70*, 235-273.
[80] Siciliano T., Bader A., Vassallo A., Braca A., Morelli I., Pizza C., De Tommasi N., *Biochemical Systematics and Ecology*, **2005**, *33*, 341-351.
[81] Gray C. A. , Rivett D. E. A., Davies-Coleman M. T., *Phytochemistry*, **2003**, *63*, 409-413.
[82] Riaz M., Krohn K., Malik A., Flörke U., *Chemistry & Biodiversity*, **2004**, *1*, 458-462.
[83] Ahmad V. U., Farooq U., Hussain J., Ullah F., Nawaz S. A., Choudhary M. I., *Chemical and Pharmaceutical Bulletin*, **2004**, *52*, 441-443.
[84] Hussein A. A., Himeno M. L., Rodriguez B., *Magnetic Resonance in Chemistry*, **2007**, *45*, 899-901.
[85] Tòth E., Tòth G., Màthè I., Blunder G., *Biochememical Systemaics and Ecology*, **2007**, *35*, 984-897.
[86] Hennebelle T., Saphaz S., Ezer N., Bailleul F., *Biochememical Systematics and Ecology*, **2008**, *36*, 441-443.
[87] Farooq U., Khan A., Khan A. F., Khan S. S., Sarwar R., Ahmad V. U., Waseem A., *Natural Product Communicaions*, **2012**, *7*, 149-150.

popular medicine of western Sicily for treating skin illnesses[88] and for its anti-diabetic properties.[89]

Figura 5: Flowers of *B. hispanica*

The plant is widely used in herbal medicine and it is sold in herbalist shops for its sedative and antispasmodic properties. Previous phytochemical investigations of this species allowed to isolate some very interesting compounds belonging to the very small group of natural diterpenoids that exhibit a hispanane hydrocarbon skeleton.[90,91,92] Recently such diterpenoids have been reported to induce apoptosis in different tumor cell lines by activating caspase-8 with subsequent participation of mitochondrial signaling.[93] On the other hand no papers have been published on the composition or biological activity of its essential oil.

[88] Lentini F., Aleo M., Amenta R., *Acta Phytoterapeutica*, **1997**, *4*, 88-94.
[89] Lentini F., Amenta R., *Giornale Botanico Italiano,* **1992**, *126*, 371.
[90] Savona G., Piozzi F., Rodriguez B., *Heterocycles*, **1978**, *9*, 257-261.
[91] Rodriguez B., Savona G., Piozzi F., *Journal of Organic Chemistry*, **1979**, *44*, 2219-2221.
[92] Lopez de Lerma J., Garcia-Blanco S., Rodriguez J. G., *Tetrahedron Letters,* **1980**, *21*, 1273-1274.
[93] Través P. G., López-Fontal R., Cuadrado I., Luque A., Boscá L., De Las Heras B., Hortelano S., *Oncogene*, **2013**, *32*, 259-268.

1.3.5. *Moluccella Spinosa*

Moluccella L. (Lamiaceae) is a genus of eight species of annual and short-lived perennial plants native to Asia and the Mediterranean.[65] They are tall, upright, branched plants to 1 meter or more with toothed leaves and small white fragrant flowers. Leaves are palmately crenate or incised. Calyx is large, with obliquely campanulate tube, membranous or rigid; corolla is bilabiate, upper lip hooded, hairy, lower 3-lobed. *M. otostegioides* Prain and *M. aucheri* (Boiss.) Scheen, known also as *Otostegia aucheri* (Boiss) are endemic of N. Pakistan and Iran, respectively and the latter one is used as hair tonic, strengthening gums, dental cleaning and brightness, prevention of hair loss.[94] The recently described *M. bucharica* (B. Fedtsch.) Rydingis, *M. fedtschenkoana* (Kudr.) Ryding, *M. olgae* (Regel) Ryding and *Moluccella sogdiana* (Kudr.) Ryding are reported for Central Asia.[65] *M diacanthophyllum* Pall. and *M. mongholica* from China are, instead, synonyms of *Lagochilus diacanthophyllus* (Pall.) Benth. and. *Lagopsis eriostachys* (Benth.) Ikonn.-Gal., respectively.[65,95] Only two species are present in the Mediterranean area: the most popular species widely cultivated as an ornamental, *M. laevis* L. (Syria, Turkey, Ukraine, etc.), commonly referred to as Bell of Ireland on account of its green colour and bell-shaped leaf bracts, not because it is native to Ireland and *M. spinosa* L. (***Figure 6***), a quite rare plant present in North Africa, Middle East, Spain and Italy, where is located only in Apulia, Calabria and Sicily.[96,97]

[94] Sadeghi Z., Kuhestani K., Abdollahi V., Mahmood A., *Journal of Ethnopharmacology*, **2014**, *153*, 111–118.
[95] http://www.catalogueoflife.org/col/search/all/key/moluccella/match/1
[96] Quintanar A., Cabezas F., Pujadas A. J. & Cirujano S., *Flora Iberica. Vascular plants of the Iberian Peninsula and Balearic Islands* Vol. 12, Ed. R. Morales, Madrid **2010**, 295-298.
[97] Pignatti S., *Flora d'Italia*, vol II., Edagricole: Bologna, **1982**, 458.

Only one previous communication has been reported on the composition of the essential oil of *M. spinosa*, collected in Turkey,[98] whereas no papers have been published on the composition of the essential oils of the other taxa of this Genus.

***Figura 6**: M. spinosa*

1.3.6. *Thapsia garganica*

Thapsia L. genus belongs to the Laserpitiae tribe of the Apiaceae family and comprises nine species distributed in the Mediterranean area on the Iberian peninsula and North Africa[51] although, based on the most recent phylogenetic analysis of *Thapsia*, the genus has been reported to include 14 species.[99] It is represented in Sicily by only one taxa, *Thapsia garganica* L. although *Elaeoselinum asclepium* (L.) Bertol. and *Elaeoselinum meoides* (Desf.) W. D. J. Koch ex DC. have been previously known with the synonymous of *Thapsia asclepium*

[98] Güvenç A., Özek G., Hürkul M. M., Özek T., K.H.C.,The 2nd International Symposium of Modern Medicine, Traditional Chinese Medicine and Uygur Medicine. Urumqi, Xinjiang, China. September 14-20, **2012**, A-116.
[99] Weitzel C., Ronsted N., Simonsen H. T., *Botanical Journal of the Linnean Society*, **2014**, *174*, 620–636.

L. and *Thapsia meoides* Guss., respectively.[100] The chemical investigation of the genus showed the presence of sesquiterpenes (germacranes, thapsanes, guaianes, etc). Their occurrence and the biological activities of thapsigargins have been reviewed.[101,102,103] Species of *Thapsia* are herbaceous perennials, growing 50 to 200 cm high. The inflorescences are large, regularly distributed umbels. The fruits have two membranous wings very peculiare. The name *Thapsia* derives from the ancient Sicilian village Thapsos from which the Greeks believed it to have originated. It has been largely used in ancient traditional medicine. In fact, Algerians used it as a pain-reliever though they recognized that the plant was deadly to camels. The Greek colony of Cyrene exported a medicinal plant known as *silphion*, used as a purgative and emetic, although its exact identity remains controversial, some historians believe that the plant might have been *Thapsia garganica*.[104]

Several different biological properties such as antioxidant,[105] antifungal, anti-inflammatory,[106] cytotoxic[107] and anticancer[108] have been reported for species of this genus and some of them are still used in folk medicine.[109,110]

Thapsia garganica L. is native to the Mediterranean region, in northwest Africa (Libya, Tunisia, Algeria), southern Europe (Italy, Sicily, Sardinia, Greece, Baleares) and Turkey.[51] It is an herbaceous perennial plant growing up to 200 cm. It is in flower from July to August. The

[100] Giardina G., Raimondo F. M., Spadaro V., *A catalogue of plants growing in Sicily. Bocconea*, **2007**, *20*, 5-583.
[101] Christensen S. B., Andersen A., Smitt U. W., *Progress in the Chemistry of Organic Natural Products*, **1997**, *71*, 129-167.
[102] Drew D. P., Krichau N., Reichwald K., Simonsen H. T., *Phytochem Rev.*, **2009**, *8*, 581-599.
[103] Andersen T. B., Lopez C. Q., Manczak T., Martinez K., Simonsen H. T., *Molecules*, **2015**, *20*, 6113-6127.
[104] Greive M., A Modern Herbal. http://botanical.com/botanical/mgmh/mgmh.html, **1996**.
[105] Djeridane A., Yousfi M., Nadjemi B., Boutassouna D., Stocker P., Vidal N., *Food Chemistry*, **2006**, *97*, 654-660.
[106] Goncalves M. J., Cruz M. T., Tavares A. C., Cavaleiro C., Lopes M. C., Canhoto J., Salgueiro L., *Industrial Crops and Products*, **2012**, *35*, 166-171.
[107] Liu H., Jensen K. G., Tran L. M., Chen M., Zhai L., Olsen C. E., Sohoel H., Denmeade S. R., Isaacs J. T., Christensen S. B., *Phytochemistry*, **2006**, *67*, 2651-2658.
[108] Jakobsen C. M., Denmeade S. R., Isaacs J. T., Gady A., Olsen C. E., Christensen S. B., *Journal of Medicinal Chemistry*, **2001**, *44*, 4696-4703.
[109] Abderrahim O., Martin G. J., Abdelaziz A., *Journal of Medicinal Plants Research*, **2013**, *7*, 2156-2169.
[110] Ouarghidi A., Powell B., Martin G., de Boer H., Abbad A., *Economic Botany*, **2012**, *66*, 370–382.

flowers are hermaphrodite and are pollinated by insects (***Figure 7***). The plant is self-fertile. It cannot grow in the shade and prefers dry or moist soil.

Figure 7*:* Flowers of *T. garganica*

For centuries preparations containing resin from the root of *Thapsia garganica* L. have been used in Arabian and European medicine for treatment of pulmonary diseases, catarrh and as counterirritants for relief of rheumatic pains. The properties of the resin were described already by Theophrastos (372-287 B.C.), Dioscorides (approximately A.D. 50), and Plinius (A.D. 24-79). *Radix Thapsiae* and *Resina Thapsiae* have been included in several pharmacopoeias, the latest in the French pharmacopoeia from 1937. The two major active principles were found to be the sesquiterpene lactones thapsigargin and thapsigargicin.[101] In particular, the bioactivity of the sesquiterpenoid thapsigargin has been deeply investigated. In 1978 it was shown that thapsigargin functions as a potent histamine liberator when tested

on rat mast cells.[111] In addition, the treatment of mammalian cells with thapsigargin was shown to result in raised calcium levels in the cytoplasm and in 1990 thapsigargin was established as an inhibitor of the sarco-endoplasmic reticulum Ca2+-ATPase (SERCA).[112] Recently, an innovative procedure coupling high pressure automatized extraction with centrifugal partition chromatography, allowing a fast and safe large-scale isolation of highly pure thapsigargin has been developed.[113] Previous phytochemical researches on *T. garganica* reported, as previously highlighted, the presence of numerous sesquiterpenoids with very interesting biological properties.[102,103] Furthermore, antioxidant flavonoids were detected in the aerial parts of plants collected in Algeria,[114] cytotoxic phenylpropanoids[107] and tethered lipids[115] in the fruits collected in Ibiza, and coumarins in the roots of a population collected in Lybia.[116] With regard the composition of the essential oil from different organs of *T. garganica* several papers have been published[117,118,119,120,121,122] but no one of them concerns populations collected in Sicily. The alterations produced by several microorganisms (bacteria and fungi) can cause degradation to the natural organic material such as fibers, woods, dyes, etc. as well as to stone objects kept in archives, museums or private collections, with consequent deterioration of physical, chemical, mechanical and aesthetic properties. In order to fight against these microorganisms, with alternative natural

[111] Rasmussen U., Christensen S. B., Sandberg F., *Acta Pharmaceutica Suecica,* **1978**, *15*, 133–140.
[112] Thastrup O., Cullen P. J., Drobak B. K., Hanley M. R., Dawson A. P., *Proceedings of the National Academy Sciences USA,* **1990**, *87*, 2466–2470.
[113] Ollivier A., Grougnet R., Cachet X., Meriane D., Ardisson J., Boutefnouchet S., Deguin B., *Journal of Chromatography B*, **2013**, *926*, 16-20.
[114] Chibani S., Al-Dabbas M., Abuhamdah S., Aburjai T., Bencheraiet R., Kabouche A., Jay M., Kabouche Z., *Chemistry of Natural Compounds*, **2014**, *50*, 357-359.
[115] Liu H., Olsen C. E., Christensen S. B., *Journal of Natural Products*, **2004**, *67*, 1439-1440.
[116] Larsen P. K., Sandberg F., *Acta Chemica Scandinavica*, **1970**, *24*, 1113-1114.
[117] Avato P., *Planta Medica*, **1991**, *57*, 585-586.
[118] Avato P., Rosito I., *Journal of Essential Oil Research*, **2002**, *14*, 20-22.
[119] Ladjel S., Zellagui A., Gherraf N., *Rev. Sci. Fond. App.*, **2011**, *3*, 30-34.
[120] Drew D. P., Rasmussen S. K., Avato P., Simonsen H. T., *Phytochemical Analysis*, **2012**, *23*, 44-51.
[121] Evergetis E., Haroutounian S. A., *Industrial Crop and Products*, **2014**, *54*, 70-77.
[122] Hassen I., M'Rabet Y., Belgacem C., Kesraoui O., Casabianca H., Hosni K., *Chemistry & Biodiversity*, **2015**, *12*, 637-651.

tools, the biological properties of essential oils derived from certain species of plants have been investigated.[55,56,57,58,59,123]

Consequently, in this study, as a continuation of researches on Sicilian species of Apiaceae,[35,124,125] we report the chemical composition and the antibacterial activity against several microorganisms, including *Bacillus subtilis*, *Staphylococcus* ssp., *Fusarium oxysporum* and *Aspergillus niger* species infesting historical art craft,[63] of the essential oils from flowers and leaves of *T. garganica* L., growing wild in Sicily.

1.3.7. *Tetraclinis articulata*

Tetraclinis articulata (Vahl) Mast. (Sandarac tree) belongs to the Cupressaceae family and has two synonyms: *Thuya articulata* Desf. and *Callitris quadrivalvis* Rich. It has been known since ancient times for its resistance to adverse environmental conditions, including fire and drought, which makes it a useful tree for infertile and nonarable lands. The wood and its veneer are also highly prized in the handicraft industry. It is native to North Africa where is used in traditional and veterinary medicine, to treat diabetes, hypertension, intestinal and respiratory ailments as well as skin conditions,[126,127,128] and in less spread populations in the north-east of Tunisia, Spain and Malta.[129,130,131]

---

[123] Mansour M. M., *Journal of Applied Sciences Research*, **2013**, *9*, 1917-1930.
[124] Khaoukha G., Ben Jemia M., Amira S., Laouer H., Bruno M., Scandolera E., Senatore F., *Natural Product Research*, **2014**, *28*, 1152-1158.
[125] Autore G., Marzocco S., Formisano C., Bruno M., Rosselli S., Ben Jemia M., Senatore F., *Molecules*, **2015**, *20*, 1571-1578.
[126] Le Floc'h E., Contribution to the ethnobotanical study of Tunisian vegetation and flora program. Tunisian Scientific Publications, Official Printing of Republic of Tunisia, **1983**, 36–37.
[127] Buhagiar J., Camilleri Podesta M. T., Cioni P. L., Flamini G., Morelli L., *Journal of Essential Oil Research*, **2000**, *12*, 29-32.
[128] Boudy P., In Guide Foristier en Afrique du Nord; Lamaison Roustique: Paris, **1952**, 273.
[129] Tekaya-karoui A., Jannet H. B. et. al., *Pakistan Journal of Biological Sciences,* **2007**, *10* (15), 2495-2499
[130] Tekaya-karoui A., Boughalleb N. et. al., *African Journal Plant Sci*ence, **2011**, *5* (2), 115-122

In *figure 8* the most important organs of *T. articulata* can be seen. Characteristic for this species is the evergreen and erect tree of a maximum height of 15.2 m, containing both male and female cones (monoecious). The scaly leaves of 1–2 mm diameter are medium to dark green and the cones differ in size (from 3 to 13 mm) and color (from yellow or bright brown to bluish) according to the sex.[131]

Figure 8: *Tetraclinis articulata*.[132] Realistic draw of leaves, blossoms and cones of *T. articulata*

The organs of *T. articulata* had been analyzed for oil composition, in fact there are researches on antifungal activity of volatile components from woody terminal branches and roots[130] and antibacterial activity of essential oil extracted from leaves of *Tetraclinis articulata* (Vahl).[133] Also of interest was the plain research for "Essential Oil Composition of Terminal Branches, Cones and Roots of *Tetraclinis articulata* […]"[129] which could be of high relevance in case of chemotaxonomic issues.

[131] Schulz C., Differential diagnose und Evolution der Cupressaceae s. l.(Zypressengewachse). Dissertation – Ruhr-Universitat Bochum, **2005**, 218
[132] Kohler F. E., Kohler's Medizinal-Pflanzen. Gera-Untermhaus – Verlag von Franz Eugen Kohler, **1897**, *1*, 270
[133] ABI-Ayad F. Z., ABI-Ayad M. et. al., *Journal of Microbiology and Biotechnology Research*, **2011**, *1* (1), 1-6

In terms of previous reports on the chemical composition of this plant, a previous investigation reported the presence of 8 new pimarane diterpenoids, a new aromatic menthane dimer and a new totaratriol, together with a number of known compounds, from the leaves and wood of *T. articulata* collected in Morocco.[134]

1.3.8. *Ajuga tenorei*

Ajuga is described as a genus with about 40 annuals and perennials from the mint family, occurring in the cooler parts of Europe, Asia, Africa and Australia or (*Ajuga*) plants are annual, biennial or perennial, herbaceous, rarely shrubs with about 40–50 species: (distributed over) Asia, Europe, especially in the Near East. In Europe the genus is represented by 10 species (*orientalis, genevensis, pyramidalis, reptans, tenorii, salicifolia, laxmannii, piskoi, iva, chamaepitys*) and four subspecies (*A. chamaepitys* (L.) Schreber subsp. *chamaepitys* and subsp. *chia*, and *A. salicifolia* subsp. *salicifolia* and subsp. *bassarabica*).[135] The genus *Ajuga* (Labiatae) has attracted attention since the report in 1976 that *A. remota* plants, grown in Kenya, were not attacked by African armyworms.[136] Thereafter, the isolation of neo-clerodane diterpenes as the allelochemicals responsible of antifeedant activity from this genus has been reviewed.[137,138]

[134] Barrero A. F., Quílez del Moral J. F., Lucas R., Payá M., Akssira M., *Journal of Natural Products*, **2003**, *66*, 844-850.
[135] Ball P. W., Ajuga L. In: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA (eds) Flora Europaea III. Diapensiaceae to Myoporaceae. Cambridge University Press, Cambridge, **1972**, 128–129.
[136] Kubo I., Lee Y. W., Balogh-Nair V., Nakanishi K., Chapya A., *Journal of the Chemical Society, Chemical Communication*, **1976**, 949-50.
[137] Camps F., Coll J., *Phytochemistry*, **1993**, *32*, 1361-70.
[138] Coll J., Tandrón Y., *Phytochemistry Reviews*, **2008**, *7*, 25-49.

Ajuga tenorei is a perennial herb reported to grow in Herbaceous flora of Dehra Dun,[139] Flora of Kumaun[140] and Flora Yunnanica.[141]

The herb *Ajuga tenorei* (***Figure 9***) is used in folk medicine to alleviate fever and remove phlegm.[141] It is also reported to be used medicinally in nephritis.[142] The extract is active against *Pyricularia oryzae*[143] and also shows cell cycle inhibitory activity against tsFT 210 cell line.[144] Several neo-clerodane diterpenoids,[145,146] and the triterpenes betulinic and 3-epi-betulinic acids were reported as constituents of *Ajuga macrosperma* Wall.[147]

Figure 9: Flowers of *A. tenorei*

[139] Babu C. R., Herbaceous flora of Dehra Dun. Publication and information directorate CSIR. New Delhi, **1977**, 405.
[140] Strachey R., Flora of Kumaun, Garhwal and adjoining areas of Tibet. Dehra Dun: Bishen Singh and Sons, **1974**.
[141] Wu C. Y., Flora Yunnanica. In: Botany YIs, Peking (China): Tomus Wall Science Press **1977**.
[142] Flora of China, [accessed: September 2007]; available from www.efloras.org **1994**.
[143] Hu K., Dong A., Liu H., Feng H., Sun Q., Yao X., *Pharmaceutical Biology*, **1999**, *37*, 225-30.
[144] Qingchun Z., Chengbin C., Bing C., Feng Q., Tao G., Xinsheng Y., *Pharmaceutical Biology*, **2005**, *43*, 135-9.
[145] Shen X., Isogai A., Furihata K., Sun H., Suzuki A., *Phytochemistry*, **1993**, *33*, 887-9.
[146] Shen X., Isogai A., Furihata K., Sun H., Suzuki A., *Phytochemistry*, **1993**, *34*, 1091-1094.
[147] Dhinda B., Banerjee J., Guha S., *Journal of the Indian Chemical Society*, **1997**, 74, 424.

2. RESULTS AND DISCUSSIONS

2.1. STUDY OF ESSENTIAL OILS OF *ANTHEMIS*

2.1.1. Characterization of essential oils of *Anthemis*

Hydrodistillation of several species of *Anthemis* gave green or yellow oils. The components of *A. montana* flower (***A1f***) and leaves (***A1l***), *A. cupanina* aerial parts (***A2a***) and flower (***A2f***), *A. arvensis* subsp. *sphacelata* aerial parts (***A3a***), *A. affine cupaniana* flower (***A4f***) and leaves (***A4l***), *A. aetnensis* flower (***A5f***) and leaves (***A5l***), *A. species* collected at Cavagrande aerial parts (***A6a***), *A. messanensis* aereal parts on the rocks (***A7a***), *A. messanensis* aereal parts in the greenhouse (***A8a***), *A. pignattorum* aerial parts (***A9a***) and *A. ismelia* (***A10a***) aerial parts are listed in Table 2 according to their retention indices on a HP 5MS column and are classified on the basis of their chemical structures into ten classes.

Hydrodistillation of ***A1f*** and ***A1l*** gave a pale yellow oil. Overall, 70 and 76 compounds respectively were identified, representing 93.7% (w/w) and 92.2% (w/w) respectively of the total components.

The four main classes of secondary metabolites were all representative of both flowers and leaves oils of ***A1***, monoterpene hydrocarbons (10.4% w/w and 10.3% w/w resp.), sesquiterpene hydrocarbons (29.5% w/w and 31.1% w/w resp.), oxygenated monoterpenes (24.3% w/w and 19.6% w/w resp.) and oxygenated sesquiterpenes (10.3% w/w and 27.8% w/w resp.). 1,8-Cineole (13.3% w/wand 12.2% w/w resp.), δ-cadinene (9% w/w and 8.2% w/w resp.) and (*E*)-caryophyllene (8.3% w/w and 8.2% w/w resp.) were recognized as the main constituents of ***A1f*** and of ***A1l*** together with torreyol (4.6% w/w and 5.0% w/w resp.), α-terpineol (4.0% w/w and 3.6% w/w resp.) and α-pinene (3.1% w/w in both). 14-Hydroxy-α-humulene (7.2% w/w) and epi-α-bisabolol (5.3% w/w) were present in good amount, only, in ***A1l***. Moreover ***A1f*** was characterized by a high amount of carbonylic compound (10.9%

w/w), with hexahydrofarnesylacetone as main component, monoterpene hydrocarbons (10.4% w/w) and hydrocarbons (6.3% w/w).

In the oil of ***A2a*** (70 compounds) the main class is represented by oxygenated monoterpenes (41.1% w/w) being artemisyl acetate (12.7% w/w), β-thujone (11.8% w/w) and yomogi alcohol (8.2% w/w) the main components. The most abundant compound of the oil is α-pinene (18.4% w/w) that together with sabinene (5.0% w/w) are the principal components of the monoterpene hydrocarbons class (26.8% w/w). These two compounds are also present in good amount (13.2% w/w and 7.6% w/w, respectively) in the oil from the flower of ***A2f***, where the former is the principal component of the oil. By the way ***A2f*** is characterized by a lower amount of hydrocarbons (23.4% w/w), where tricosane (9.8% w/w) and pentacosane (6.4% w/w) are the main ones[148], by the large quantity of oxygenated monoterpenes (17.8% w/w) in which *cis*-piperitone oxide (6.7% w/w), absent in the aerial part, is the main compound.

From hydrodistillation of ***A3a*** aerial parts 17 compounds were identified, representing 91.9% (w/w) of the total oil composition. The ***A3a*** oil consist almost exclusively of oxygenated sesquiterpenes (87.2% w/w), with torreyol (85.4% w/w) as the main constituent.

Forty-two and thirty-nine compounds were identified from flower and leaves of ***A4***, representing 89.6% (w/w) and 92.9% (w/w) respectively. Torreyol (63.0% w/w and 63.5% w/w resp.) was recognized as the main constituent of ***A4f*** and ***A4l***, making of oxygenated sesquiterpenes (65.1% w/w and 64.7% w/w) the most abundant class. The second and third major classes were monoterpene hydrocarbons (6.3% w/w and 16.1% w/w resp.) and

[148] Maggio A., Riccobono L., Spadaro V., Scialabba A., Bruno M., Senatore F., *Chemistry & Biodiversity*, **2014**, *11* (4), 652-672.

sesquiterpene hydrocarbons (7.5% w/w and 6.9% w/w resp.). Carbonylic compounds and oxygenated monoterpenes were present in lesser amount, whereas hydrocarbons practically absent.

Geranyl acetate (22.7% w/w and 18.2% w/w resp.), (*Z*)-chrysanthenyl acetate (8.6% w/w and 5.7% w/w resp.), 14-Hydroxy-α-humulene (16.5% w/w and 18.5% w/w resp.), torreyol (8.0% w/w and 6.2% w/w resp.) and α-pinene (3.9% w/w and 10.6% w/w resp.) were recognized as the main compounds of ***A5*** flower and leaves. Generally, ***A5f*** and ***A5l*** consisted mainly of oxygenated monoterpenes (48.5% w/w and 36.5% w/w resp.), oxygenated sesquiterpenes (30.4% w/w and 26.9% w/w) and monoterpene hydrocarbons (5.8% w/w and 16.7% w/w resp.). Hydrocarbons (3.3% w/w and 0.6% w/w resp.) and sesquiterpene hydrocarbons (2.6% w/w and 0.5% w/w resp.) were presented in lesser amount.

From hydrodistillation of ***A6a*** 37 compounds were identified, representing 91.2% (w/w) of the total component. 14-Hydroxy-α-humulene (39.9% w/w) was recognized as the main compound, together with geranyl acetate (10.5% w/w) and τ-muurolol (7.3% w/w). Generally ***A6a*** consisted mainly of oxygenated sesquiterpenes (52.3% w/w) and oxygenated monoterpenes (20.6% w/w). Sesquiterpene hydrocarbons were present for the 11.1% (w/w) with α-cedrene as main component, whereas monoterpene hydrocarbons (4.6% w/w) and hydrocarbons (1.8% w/w) were present in lesser amounts.

In the oils of *Anthemis* collected on the rocks (***A7a***) and in the greenhouse (***A8a***) 49 compounds in both were identified, representing 90.8% (w/w) of the total components in the two cases. (*E*)-Chrysanthenyl acetate (28.8% w/w and 24.2% w/w resp.) was the major component of the oils making of oxygenated monoertpenes (36.1% w/w and 40.9% w/w resp.) the main classe. The second and third major classes were oxygenated sesquiterpenes

(21.1% w/w and 20% w/w resp.) with 14-Hydroxy-α-humulene (8.1% w/w and 5.3% w/w resp.) as major component, and monoterpene hydrocarbons (19.5% w/w and 20.9% w/w resp.) with santolina triene (8% w/w and 5.8% w/w resp.), α-pinene (6.7% w/w and 5.4% w/w resp.) and β-pinene (3.6% w/w and 5.0% w/w) as majors compounds of the class. Other most abundant classes of the oils of ***A7a*** and ***A8a*** were sesquiterpene hydrocarbons (13.8% w/w and 8.1% w/w resp.), represented by δ-cadinene (5.0% w/w and 3.7% w/w resp.).

Hydrodistillation of ***A9a*** gave a pale green oil. Overall, 67 compounds were identified, representing 98.3% (w/w) of the total components. (Z)-Muurola-4(14),5-diene (27.3% w/w) was recognized as the main constituent of ***A9a***, together with isospathulenol (10.6% w/w), sabinene (7.7% w/w), artemisyl acetate (6.8% w/w), caryophyllene oxide (3.8% w/w), δ-muurolene (3.5% w/w) and neryl acetate (3.5% w/w). Generally, ***A9a*** consisted mainly of sesquiterpene hydrocarbons (37.4% w/w) and oxygenated sesquiterpenes (30.2% w/w), whereas oxygenated monoterpenes (14.1% w/w) and monoterpene hydrocarbons (12.2% w/w) were the other most abundant classes. Hydrocarbons, carbonylic compounds and phenolic compounds practically absent.[148]

Forty-eight compounds were identified in the oil of the ***A10a*** accounting 91.1% (w/w) of the total components. Oxygenated monoterpenes was by far the main class (46.8% w/w) with geranyl propionate (8.8% w/w), the main product of the oil, bornyl acetate (7.9% w/w), β-thujone (7.8% w/w) and neryl propionate (6.5% w/w) present in similar quantity. Among the oxygenated sesquiterpenes (24.3% w/w), the second class of the oil, only τ-muurolol (6.5% w/w) and isospathulenol (4.4% w/w) are worthy of mention.[148]

The composition of the essential oils of the *Anhemis* species is quite different.

A3a, ***A4f***, ***A4l*** and ***A6a*** have a higher content in oxygenated sesquiterpenes compared to the other, while ***A2a***, ***A5f***, ***A5l***, ***A7a***, ***A8a*** and ***A10a*** have a higher content in oxygenated monoterpenes compared to the others.

A1l, **A2f** and ***A9a*** show a comparable amounts of both monoterpenes and sesquiterpenes whereas ***A4f*** and ***A4l*** have almost exclusively oxygenated sesquiterpenes.

Hydrocarbons occur in lesser quantity in all species except that ***A2f***. Carbonylic compounds are present in lesser quantity in all species except that ***A1f*** and ***A10a***.

The composition of the essential oils of ***A1f*** and ***A1l*** are comparable to those of ***A4f***, ***A5f*** and ***A4l***, ***A5l*** respectively.

2.1.2. Statistical analysis of the essential oils composition of all *Anthemis* taxa

As stated above, Table 3 reports the main compounds identified in all the taxa of *Anthemis* studied so far. For this compilation, the following points were considered: i) the contents of the compounds in the oils of *A. marittima* L. (***mari1*** and ***mari2***),[149] *A. ruthenica* M. Bieb (***rut1*** and ***rut2***),[150] *A. tinctoria* L. collected in Serbia-Montenegro (**tin1**),[150] *A. cotula* L. growing in Serbia-Montenegro (***cot4***),[150] *A. austriaca* Jacq. (***aus***)[150] and *Chamaemelum nobile* (L.) All. (***cno1***)[151] were reported as average values of the contents of the compounds detected in the different populations, in accordance with the conclusions of the authors of the original studies; ii) the compositions of the oils of the different populations of *A. melanolepis* Boiss. (***mel1*** and ***mel2***)[152] *A. tomentosa* L. (***tom1***, ***tom2***, and ***tom3***),[152,60] and *A. auriculata* Boiss. (***aur1*** and ***aur2***),[152] were reported as separate line items; iii) the oils of three of the four

[149] Darriet F., Desjobert J. M., Costa J., Muselli A., *Phytochemical Analysis*, **2009**, *20*, 279.
[150] Pavlovic´ M., Lakusˇic´ D., Kovacˇevic´ N., Tzakou O., Couladis M., *Chemistry & Biodiversity*, **2010**, *7*, 1231.
[151] Hethelyi E., Palinkas G., Palinkas J., *Olaj, Szappan, Kozmetika*, **1999**, *48*, 116.
[152] Saroglou V., Dorizas N., Kypriotakis Z., Skaltsa H. D., *Journal of Chromatography, A* **2006**, *1104*, 313.

studied populations of *A. chia* L.[152] (collected in Nomos Magnisias, Nomos Korinthias, and Nomos Attikis) appeared similar to each other, but different from the oil of the fourth population (collected in Nomos Achaia), hence, in Table 3, the average of the oil compositions of the first three populations (***chia1***) was reported, whereas the fourth one (***chia2***) was considered separately; iv) the same considerations were made for the four populations of *A. triumfetti* collected in Serbia-Montenegro,[150] consequently, three oil compositions were joined and averages were reported (***tri2***), while the forth one (***tri3***) was left as separate line item.

Furthermore, it is important to evidence that the data reported in the literature are quite heterogeneous. In fact, in some cases, the composition of the essential oils of flowers and leaves was analyzed separately, whereas in others, the composition of the oils of the aerial parts was reported.

On the basis of all the mentioned points, a comparison of the literature data is possible only by a statistical analysis of presence-absence (cluster analysis, CA). A metabolite was considered present if its oil content was higher than 5%. A preliminary analysis (data not shown) was performed considering all the metabolites present with contents > 5%. Unfortunately, the obtained graph gave very poor information, and no clear relationships between taxa could be observed.

In a previous paper,[153] a statistical study of the composition of the essential oils of Turkish *Anthemis* was performed. The authors indicated the existence of four chemotypes, i.e., a 1,8-cineole, a β-caryophyllene, an α-pinene, and a sabinene/germacrene D chemotype. Consequently, to check whether this classification could be extended to all *Anthemis* taxa, we decided to carry out a statistical analysis by considering the presence/absence (> 5%) of

[153] Kilic O., Kocak A., Bagci E., *Naturforsch. Z.*, *C* **2011**, *66*, 535.

the following compounds: 1,8-Cineole, β-caryophyllene, α-pinene, β-pinene, sabinene, and germacrene D (***Figure 10***).

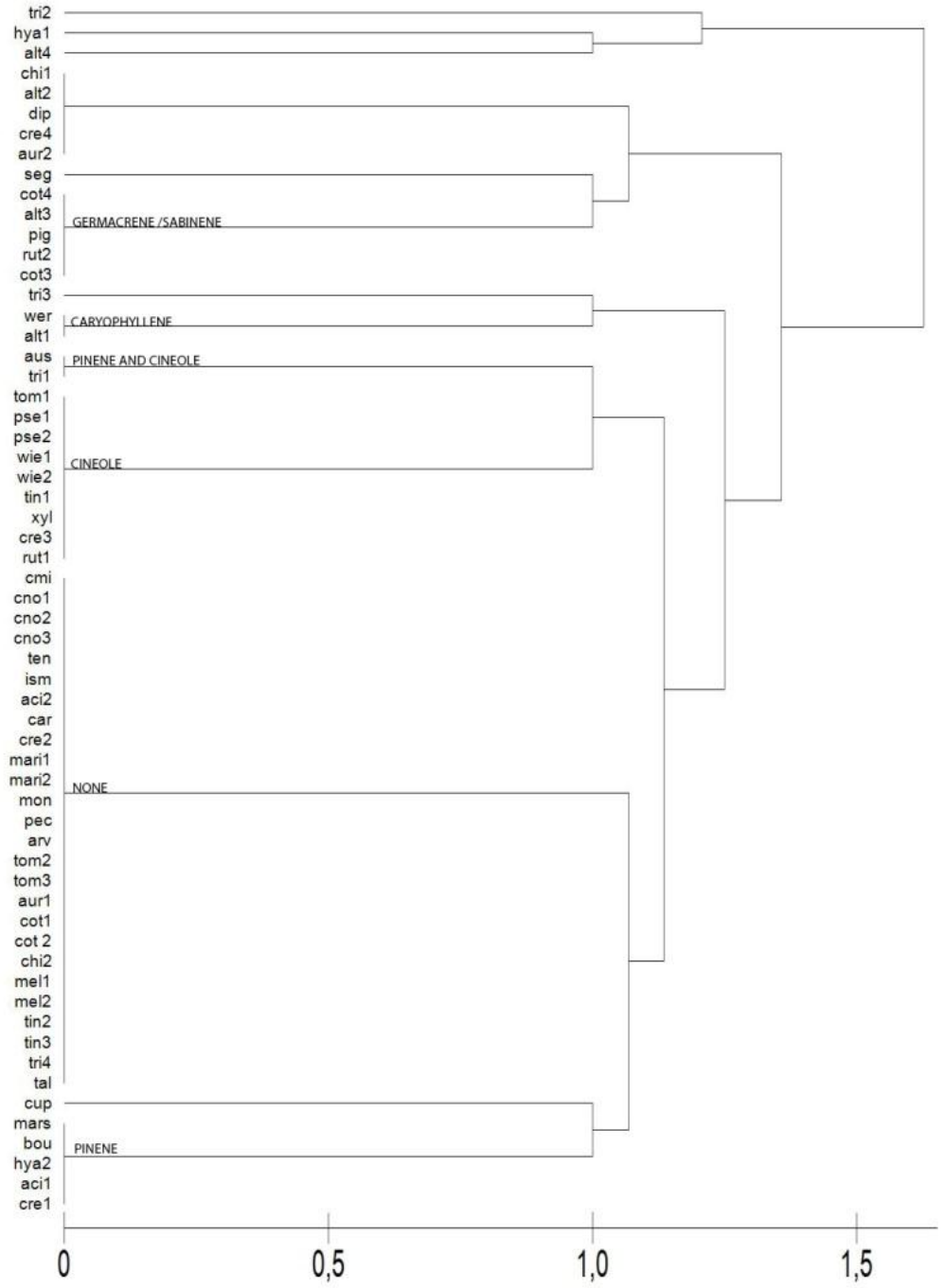


Figure 10*:* Dendrogram based on the linkage distance obtained by cluster analysis (CA) of the essential oil contents (>5%) of 1,8-cineole, β-caryophyllene, α-pinene, β-pinene sabinene and germacrene for the 60 *Anthemis taxa* listed in Table 3.

The dendrogram shown in figure 10 clearly evidences that 26 populations of *Anthemis* did not fit into any of these chemotypes (None). The remaining 34 were distributed as follows. Populations pse1, pse2, wie1, wie2, tin1, xyl, cre3, and rut1 belonged to the 1,8-cineole chemotype, as well as tom1, although tom2 and tom3 did not show the same classification. Population tin1 belonged to this first chemotype, but its oil composition was different from those of tin2 and tin3, since the oils of the latter ones contained predominantly sesquiterpenes. Moreover, the oil of population cre3 also showed this chemotype, although those of cre1 and cre4 had α-pinene/β-pinene and β-caryophyllene chemotypes, respectively. The rut1 oil contained mainly 1,8 cineol, but the rut2 oil belonged to the germacrene D/sabinene chemotype. Finally, *A. xylopoda* O. Schwarz (xyl), with an essential oil also belonging to this chemotype, has an unresolved botanical status. It should be noted that the populations who fit perfectly in this chemotype did belong neither to the same subgenera nor to the same section.

The α-pinene/β-pinene chemotype included five populations, i.e., aci1, cre1, mars, bou, and hya2. The oil of the latter was different with respect to that of hya1, which contained a-pinene, but also β-caryophyllene. It is noteworthy that all the species of this chemotype belong to the subgenus Anthemis, although they are positioned in different sections. This cluster is closely related to that constituted of the oil of *A. cupaniana* (cup), which contained α-pinene, but also small quantities of sabinene.

The only populations belonging to the β-caryophyllene chemotype were wer and alt1, which were closely related to tri3, having an oil that also contained a small quantity of 1,8-cineole. The germacrene D/sabinene chemotype comprised Populations cot3, cot4, pig, and rut2, belonging to the subgenus Anthemis, as well as alt3 (subgenus Cota).

Most (seven out of twelve listed in *Table 3*) of the species of the sect. Hiorthia of the subgenus *Anthemis* (***aci2***, ***cre2***, ***car***, ***mari1***, ***mari2***, ***pec***, and ***mon***) did not fit into this classification (None), but three of them (***aci1***, ***cre1***, and ***mars***) had oils with the α-pinene/β-pinene chemotype.

Since in this first CA not all populations were associated with a specific chemotype, further statistical analysis was performed, considering the significant compound classes, i.e., monoterpene hydrocarbons (***MH***), oxygenated monoterpenes (***MO***), sesquiterpene hydrocarbons (***SH***), and oxygenated sesquiterpenes (***SO***). A compound class was considered present if its oil content was higher than 0.1% (***Figure 11***).

Four main groups of taxa were distinguished. Those that contained exclusively (***chi2***, ***wie1***, ***wie2***, ***cno2***, ***car***, ***cre2***, ***cre3***, ***mari1***, ***mari2***, ***mon***, ***rut1***, ***pse1***, and ***xyl***) or mainly (***ism***, ***arv***, ***tom1***, ***mel2***, ***aus***, ***pse2***, and ***cup***) oxygenated monoterpenes (***MO***), those composed exclusively (***bou***, ***hya2***, ***mel1***, ***aci2***, and ***pec***) or predominantly (***tri1*** and ***aci1***) of monoterpene hydrocarbons (MH), those that contained exclusively (***cot1***, ***cot3***, ***alt2***, ***chi1***, ***dip***, ***tom2***, and ***cre4***) or predominantly (***cot1*** and ***cot4***) sesquiterpene hydrocarbons (***SH***), and, finally, those that comprised exclusively (***tin1***, ***tin2***, ***tin3***, ***tri4***, ***mars*** and ***alt3***) or mainly (***tin1***, ***tal***, ***cmi***, and ***aur1***) oxygenated sesquiterpenes (SO).

Moreover, the oils of aur2, hya1, and alt4 contained compounds belonging to all classes (All). Also the oil of *A. pignattiorum* (***pig***) belonged to this group. Three populations (***cno1***, ***cno3***, and ***tom3***) did contain any of the above groups of compounds (None), although the oil compositions of *Chamaemelum nobile* (***cno1***, ***cno2***, ***cno3***, and ***cno4***) looked quite homogeneous and quite different from the other species belonging to this genus, i.e., *Chamaemelum mixtum* (cmi), with an oil that was attributed to the MO+SO cluster.

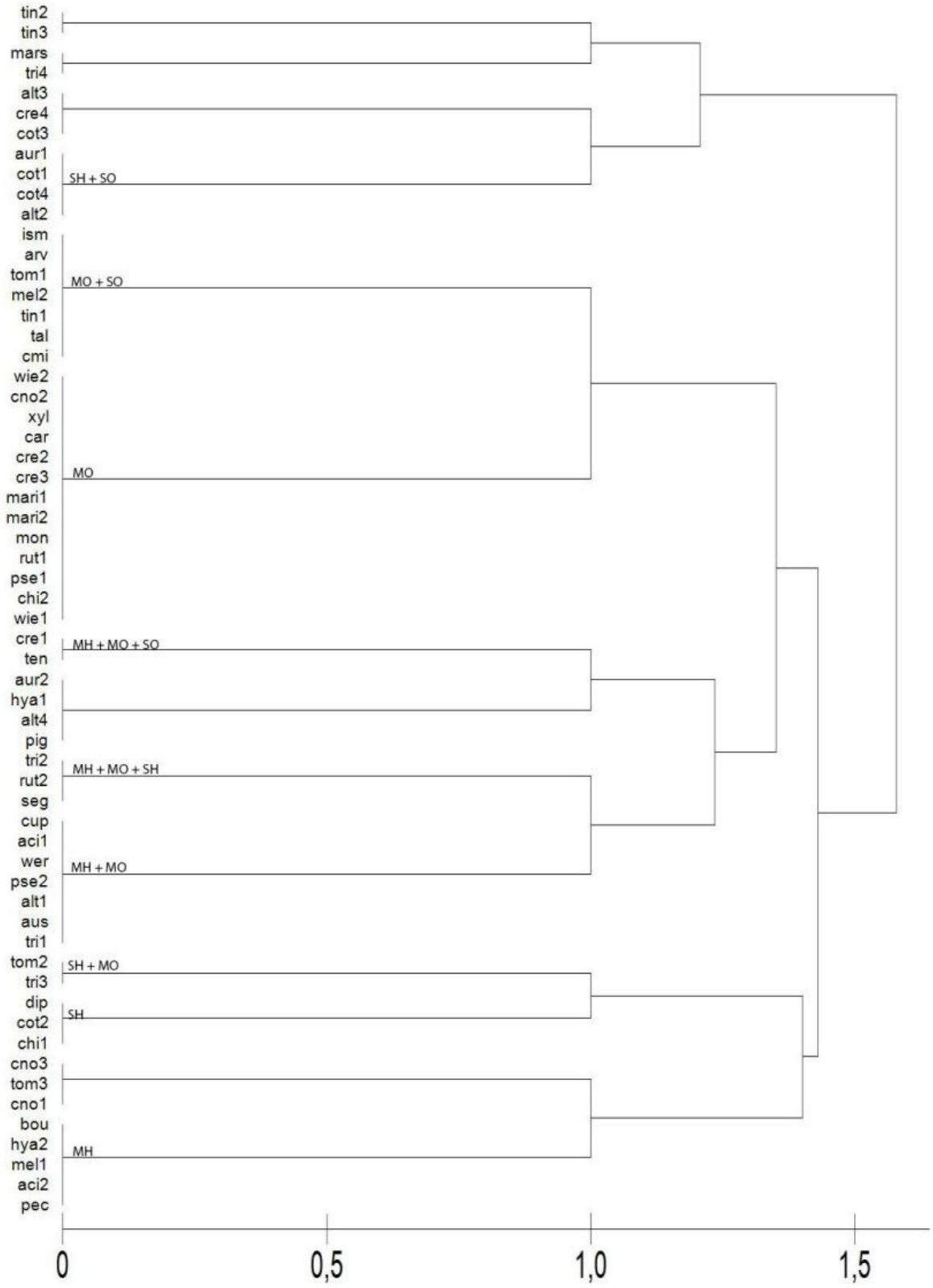


Figure 11: Dendogram based on the linkage distance obteined by cluser analysis (CA) of the essential oil contents of the four main compound classes for the 60 Anhemis taxa listed in Table 3. MH: Monoterpene hydrocarbons, MO: oxygenated monoterpenes, SH: Sesquiterpene hydrocarbons, SO: oxygenated sesquiterpenes.

2.1.3. Biological activity of the essential oil of *Anthemis* species

The oil showed a quite good antibacterial activity (***Table 4***) towards *Staphylococcus aureus* and a moderate activity toward *Bacillus cereus* and *Straphylococcus faecalis*. About the only gram- *Escherichia coli* show a moderate activity.

2.2. STUDY OF ESSENTIAL OILS OF *SALVIA ARGENTEA*

2.2.1. Characterization of essential oil and extracts of *Salvia argentea*

Hydrodistillation of *Salvia argentea* aereal parts (***Si***) gave a yellow oil. Overall, 35 compounds were identified, representing 93.8% w/w of the total oil composition. The components, listed in Table 5 according to their retention indices (*RI*) on a HP 5 MS column, were divided into ten classes on the basis of their chemical structures. 14-Hydroxy-α-humulene (40.1% w/w) was recognized as the main constituent of the essential oil of *S. argentea*, together with 1,3,8-*p*-menthatriene (12.1% w/w), globulol (7.4% w/w) and β-sesquiphellandrene (5.8% w/w). Generally, the oil consisted mainly of oxygenated sesquiterpenes (58.6% w/w) and monoterpene hydrocarbons (21.4% w/w), whereas sesquiterpene hydrocarbons (13.6% w/w) were present in lower amounts and hydrocarbons and carbonylic compounds were almost absent.

Four previous studies reported the chemical composition of *S. argentea* oils from plants collected in different regions[19,30,31,32] and their results have been inserted in Table 5. Caryophyllene oxide was reported as the main component of the oil from Macedonia (***Ma***) (37.6% w/w), followed by α-copaene (8.5% w/w), humulene epoxyde II (6.3% w/w) and β-caryophyllene (6.1% w/w)[32], while the major components of Serbian oil (**S**) were viridiflorol (32.4% w/w), manool (14.6% w/w), α-humulene (10.7% w/w) and β-thujone (7.3% w/w).[31]

The profile of the two Tunisian populations (***T1*** and ***T2***)[19] is quite similar to the Serbian one. In fact, although they were richer in monoterpene hydrocarbons (14.5% w/w and 13.5% w/w) with respect to ***S*** (0.5% w/w) the main constituents were viridiflorol (26.9% w/w and 18.7% w/w), manool (6.1% w/w and 13.6% w/w), *α*-thujone (7.3% w/w and 8.1% w/w) and *α*-humulene (4.1% w/w and 5.3% w/w). On the other hand, the oil sample obtained from the Moroccan *S. argentea* (***Mo***)[30] was characterized by camphor (45.1% w/w), camphene (19.4% w/w), *α*-pinene (9.3% w/w) and borneol (9.0% w/w). The composition of the essential oil of *S. argentea* collected in Sicily (***Si***) was found to be quite different from the composition of the oils of the other populations studied so far. In fact, although it had a high content in oxygenated sesquiterpenes such as Ma, *S*, T1 and T2, 14-hydroxy-*α*-humulene, 1,3,8-*p*-menthatriene, globulol and *β*-sesquiphellandrene, the main components of Si, were totally absent in the other populations. Furthermore, viridiflorol, manool, caryophyllene oxide, *α*-humulene, thujone, camphor and camphene, major compounds of the other oils were not present in the Sicilian population.

With regard to *S. argentea* essential oil, the results presented here in indicate a quite different chemical profile of the Sicilian population with respect to the other ones studied so far and show that environmental conditions such as soil composition, climate can drastically influence the composition of the secondary metabolites. The previously reported larvicidal activity of the hexane extract against the mosquito *C. pipiens*, whose chemical composition was not reported,[23] could be explained by the huge presence of free fatty acids (63.7% w/w), which have been already proved to be very active against several mosquito species.[154]

[154] Rahuman A. A., Venkatesan P., Gopalakrishnan G., *Parasitology Research*, **2008**, *103*, 1383–1390. doi:10.1007/s00436-008-1146-6.

Aerial parts *S. argentea* were extracted with petroleum ether and dichloromethane at room temperature for one week to give two residues: ETP1 and DCM1, respectively. In order to identify the free fatty acids, a portion of these extracts was successively treated with a solution of diazomethane in Et_2O to afford ETP2 and DCM2.

The analysis of the petroleum ether (***ETP1***) and dichloromethane (***DCM1***) extracts allowed the identification of 26 and 15 compounds, representing 90.2% w/w and 93.2% w/w respectively of the total composition, whereas in ETP2 and DCM2 21 and 26 compounds were identified, representing 90.5% w/w and 90.1% w/w respectively of the total composition. The components, listed in Table 6 according to their retention indices (*RI*) on a HP 5 MS column, were divided into seven classes on the basis of their chemical structures.

Tritriacontane (9.9% w/w and 14.1% w/w), heptacosane (8.4% w/w and 10.5% w/w), hentriacontane (8.3% w/w and 10.9% w/w), methyldotriacontane (7.9% w/w and 7.6% w/w) and tetradecanal (8.4% w/w and 10.2% w/w) were recognized as the main constituents of the extracts ETP1 and DCM1. Generally, ETP1 and DCM1 consisted mainly of hydrocarbons (60.1% w/w and 63.1% w/w), carbonylic compounds (18.3% w/w and 17.5% w/w) and monoterpene hydrocarbons (40% w/w and 5.5% w/w) whereas other classes of compounds were absent.

Methyl ester was, by far, the main class of ETP2 and DCM2 (63.7% w/w and 50.4% w/w) with methyl linolenate (36.6% w/w and 13.5% w/w) and methyl myristoleate (10.5% w/w and 18.5% w/w) as the major compounds together with methyl palmitate (8.0% w/w and 1.9% w/w).

Among the hydrocarbons (17.0% w/w and 26.8% w/w), the second most abundant class, only tritriacontane (4.1% w/w and 5.0% w/w), heptacosane (2.9% w/w and 4.6% w/w) and hentriacontane (3.2% w/w and 4.4% w/w) are worthy of mention, whereas carbonylic

compounds (8.9% w/w and 11.4% w/w) and other classes of compounds were present in lower amount.

The compositions of petroleum ether and dichloromethane extracts were found to be quite similar. In fact, both ETP1 and DCM1 had a high content in hydrocarbons (60.1% w/w and 63.1% w/w) and the distribution of monoterpene hydrocarbons and carbonylic compounds appeared to be similar.

The profile of ETP2 and DCM2 was also analogue. Both had a high amount of methyl esters (63.7% w/w and 50.4% w/w) and hydrocarbons and carbonylic compounds were present in comparable quantity.[155]

2.3. STUDY OF ESSENTIAL OILS OF *PULICARIA SICULA* AND *PULICARIA VULGARIS*

2.3.1. Characterization of essential oils of *Pulicaria sicula* and *Pulicaria vulgaris*

Hydrodistillation of *P. sicula* aerial parts (***S***) gave a pale yellow oil. Overall, sixty-six compounds were identified in the oil, representing 91.8% (w/w) of the total components. The components are listed in Table 7 according to their retention indices on a HP 5MS column and are classified on the basis of their chemical structures into seven classes.

The oil of *S* is particularly rich in oxygenated terpenoids (78.9% w/w). Oxygenated monoterpenes (16 compounds, 43.2% w/w) is the main class and among these borneol (23.7% w/w) is the major compound followed by bornyl acetate (6.5% w/w) and isothymol isobutyrate (6.2% w/w). Oxygenated sesquiterpenes (17 compounds, 35.7% w/w) are present in similar amount with respect to oxygenated monoterpenes with caryophyllene derivatives accounting for 23.4% w/w. The main products of this class are: caryophyllene

[155] Riccobono L., Maggio A., Rosselli S., Ilardi V., Senatore F., Bruno M., *Natural Product Research*, (in press) DOI: 10.1080/14786419.2015.1030742

oxide (10.2% w/w), the second major component of the oil, caryophylladienol I (4.3% w/w) and caryophylla-3,8(13)-dien-5β-ol (4.3% w/w). Monoterpene hydrocarbons and hydrocarbons are practically absent whereas among the sesquiterpene hydrocarbons (4.7% w/w) only β-caryophyllene (2.9% w/w) is worthy of mention. Among oxygenated monoterpenes the phenolics were represented by only two compounds: isothymol isobutyrate (6.2% w/w) and thymohydroquinone dimethyl ether (1.0% w/w).[156]

Hydrodistillation of the aerial parts of *Pulicaria vulgaris* var. *graeca*, collected at Capo Zafferano (***P.v.g.***), gave a yellow oil. Overall, fifty-two compounds were identified in the oil, representing 93.6% (w/w) of the total components. The components are listed in Table 7 according to their retention indices on a HP 5MS column and are classified on the basis of their chemical structures into nine classes.

The oil of *P.v.g*. is quite rich in sesquiterpenoids (39.1% w/w). Sesquiterpene hydrocarbons (12 compounds, 31.9% w/w) is the main class and among these β-caryophyllene (14.3% w/w) is the major compound followed by γ-curcumene (4.6% w/w), *ar*-curcumene (3.8%) and 1,7-di-*epi*-β-cedrene (3.5% w/w). Fatty acids are quite abundant (27.2% w/w) although they are only represented by hexadecanoic acid (21.7% w/w), the main compound of the oil, and (*Z,Z*)-9,12-octadecadienoic acid (5.5% w/w).[157]

Among the oxygenated monoterpenes (8 compounds, 9.2% w/w) the only compound present in significant quantity is geranyl propionate (8.2% w/w) whereas monoterpene hydrocarbons are completely absent. Six hydrocarbons were recorded, forming 7.2% of the total, with pentacosane (3.3% w/w) as the most abundant one, and among the carbonylic compounds (3 compounds, 2.8% w/w) only hexahydrofarnesyl acetone (2.3% w/w) is worth

[156] Maggio A., Riccobono L., Spadaro V., Campisi P., Bruno M., Senatore F., *Chemistry & Biodiversity*, **2015**, *12* (5), 781-799.
[157] Casiglia S., Riccobono L., Bruno M., Senatore F., Senatore F., *Natural Product Research,* DOI: 10.1080/14786419.2015.1055267

of mention. Finally, it has to be highlighted the good quantity of manoyl oxide (5.7% w/w) which represents three quarters of the diterpenoidic components of the present oil.

As stated before no previous communications reported on the composition of the essential oil of *P. vulgaris* var. *graeca*, but the comparison of our results with those reported for the composition of the essential oil of *P. vulgaris* Gaertner (***P.v.***)[158] and with all the other taxa of *Pulicaria* studied so far, recently reviewed,[156] shows some interesting points.

The oil of *P.v.* is characterized by the huge amount of oxygenated monoterpenes (90.6% w/w) with thymol (50.2% w/w), *p*-menth-1(6)-en-2-one (carvotanacetone, 20.2% w/w) and thymol isobutyrate (16.9% w/w) as main components. The total absence of these compounds in *P.v.g*., as well as the absence of fatty acids in *P.v.* (accounting in ***P.v.g.*** for 21.7% w/w), shows a complete different chemical profile of the two taxa. Although the huge presence of thymol derivatives as in *P.v.*, with the exception of *P. arabica* collected in Tunisia,[159] is not a common feature in *Pulicaria* genus,[156] the occurrence of carvotanacetone has been reported in several taxa such as *P. jaubertii* collected in S. Arabia (98.6% w/w)[160] and Yemen (64.0% w/w),[161] *P. mauritanica* collected in Morocco (87.3% w/w),[162] *P. undulata* collected in Yemen (91.4% w/w)[163] and collected in Sudan (55.9% w/w)[164] and *P. inuloides* collected in Yemen (47.3% w/w)[165]. On the other hand, the main compound of *P. vulgaris* var. *graeca*, hexadecanoic

[158] Sharifi-Rad J., Miri A., Hoseini-Alfatemi S. M., Sharifi-Rad M., Setzer W. N., Hadjiakhoondi A., *Natural Product Communications*, **2014**, *9*, 1633-1666.
[159] Abed N. E., Harzallah-Skhiri F., Boughalleb N., *Agric Segment*., **2010**, *1*, 1530-1534.
[160] Fawzy G. A., Al Ati H. Y., El Gamal A. A., *Pharmacognosy Magazine*, **2013**, *9*, 28-32.
[161] Algabr M. N., Ameddah S., Menad A., Mekkiou R., Chalchat J. C., Benayache S., Benayache F., *Journal of Medicinal and Aromatic Plants*, **2012**, *2*, 688-690.
[162] Znini M., Cristofari G., Majidi L., Paolini J., Desjobert J. M., Costa J., *LWT-Food Science and Technology*, **2013**, *54*, 564-569.
[163] Ali N. A. A., Sharopov F. S., Alhaj M., Hill G. M., Porzel A., Arnold N., Setzer W. N., Schmidt J., Wessjohann L., *Natural Product Communications*, **2012**, *7*, 257-260.
[164] El-Kamali H. H., Yousif M. O., Osama A. I., Sabir S. S., *Ethnobotanical Leaflets.*, **2009**, *13*, 467-471.
[165] Al-Hajj N. Q. M., Ma C., Thabit R., Al-alfarga A., Gasmalla M. A. A., Musa A., Aboshora W., Wang H., *Journal of Academia and Industrial Research*, **2014**, *2*, 675-678.

acid, has been detected only in *P. inuloides* (12.8% w/w),[166] *P. jaubertii* collected in Yemen (4.0% w/w)[161] and *P. arabica* collected in Tunisia (3.5% w/w),[167] wheras β-caryophyllene, the second most abundant compound of *P.v.g.* is present in good quantity in *P. dysenterica* collected in Greece[168] and *P. stephanocarpa* collected in Soqotra.[169] Finally, geranyl propionate (8.2% w/w in *P.v.g.*) was detected, in small amount (1.5% w/w), only in *P. inuloides* collected in Yemen[166] and manoyl oxide (5.7% w/w in *P.v.g.*) has never been found in any *Pulicaria* taxa.

2.3.2. Statistical analysis of the essential oils composition of all *Pulicaria* taxa

Table 8 reports the main compounds of the essential oils of the different taxa of *Pulicaria* studied so far and for its compilation the following points were considered:

1) Investigations on root oils were omitted (*P. odora* (L.) Rchb.). [170]

2) The investigations on *P. orientalis* Jaub. & Spach,[171] *P. somalensis* O. Hoffm.[171] and *P. crispa* Sch. Pip. (syn. *P. undulata* (L.) C. A. Mey.)[172] were not inserted since authors do not report the percentages of the compounds.

3) The composition given for the oil of *P. paludosa* Link[173] is extremely poor, devoid of any statistical meaning and consequently it was omitted.

---

[166] Al-Hajj N. Q. M., Wang H., Gasmalla M. A. A., Ma C., Thabit R., Rahman M. T. R., Tang Y., *Journal of Food and Nutrition Research*, **2014**, *2*, 221-227.
[167] Abed N. E., Harzallah-Skhiri F., Boughalleb N., *Agriculture Segment*, **2010**, *1*, 1530-1534.
[168] Basta A., Tzakou O., Couladis M., Pavlović M., *Journal of Essential Oil Research*, **2007**, *19*, 333-335.
[169] Ali N. A. A., Crouch R. A., Al-Fatimi M. A., Arnold N., Teichert A., Setzer W. N., Wessjohann L., *Natural Product Communcations,* **2012**, *7*, 113-116.
[170] Hanbali F. E., Akssira M., Ezoubeiri A., Gadhi C. E., Mellouki F., Benherraf A., Blazquez A. M., Boira H., *Journal of Ethnopharmacology*, **2005**, *99*, 399-401.
[171] Alkhathlan H. Z., Al-Hazimi H. M. G., *Journal of the Chemical Society of Pakistan*, **1996**, *18*, 309-312.
[172] Al-Yahya M. A., El-Sayed A. M., Hassan M. M. A., El-Meshal I., *Arab Gulf J.Sci. Res. B-Agricul. Biol. Sci*, **1989**, *7*, 1-6.
[173] Diaz N., Ortega T., Pardo M. P., *Anales de la Real Academia Naciolal de Farmacia*, **1988**, *54*, 526-31.

The statistical analysis was carried out on the principal classes of compound (***PCA***) that were significant according to the loadings plot: Monoterpenes hydrocarbons (***MH***), Oxygenated monoterpenes (***MO***), Sesquiterpenes hydrocarbons (***SH***), Oxygenated sesquiterpenes (***SO***) (***Figure 12***).

The score plot of the *Pulicaria* taxa shows the presence of some clusters (***Figure 12***).

The first group is characterized by the presence of sesquiterpenes. Monoterpenes are completely absent or constitute less than 20% w/w of the total composition. It includes: *P. arabica* (L.) Cass. collected in Saudi Arabia, Najd (***A***), *P. arabica* collected in Tunisia (***B***), *P. dysenterica* (L.) Bernh. collected in Iran, Malayer (***C***), *P. dysenterica* collected in Greece, Katara (***D***), *P. dysenterica* collected in Greece, Arahova (***E***), *P. gnaphalodes* (Vent.) Boiss. collected in Iran, Mashhad (***I***), *P. glutinosa* Jaub. & Spach collected in UAE (***K***), *P. stephanocarpa* Balf. f. collected in Soqotra (***M***).

It is important to point out that although for *P. arabica* collected in Tunisia the composition of leaves (***Bl***), flower (***Bf***) and steam (***Bs***) was studied separately and for *P. gnaphalodes* collected in Iran, Mashhad (***I***) only the leaves composition is reported, their profile is comparable with those of the other taxa of this group, for which the composition of the essential oils of the aerial parts has been determined. The distribution between sesquiterpene hydrocarbons and oxygenated sesquiterpenes within this group is variable. In fact, *P. arabica* collected in Saudi Arabia, Najd (***A***), *P. dysenterica* collected in Greece, Katara (***D***), *P. dysenterica* collected in Greece, Arahova (***E***), *P. gnaphalodes* collected in Iran, Mashhad (***I***) and *P. stephanocarpa* collected in Soqotra (***M***) contain mainly oxygenated sesquiterpenes whereas in the other taxa of this cluster sesquiterpene hydrocarbons predominate.

A very well-defined second cluster includes *P. gnaphalodes* collected in Iran, Birjand (***G***), *P. undulata* (L.) C. A. Mey. collected in Saudi Arabia, Medinah (***N***) and *P. undulata* collected in Iran, Saravan (***Q***). Distinct from these, but for a variation on PC2 is *P. undulata* collected in Iran, Ramsar (***O***).

The third group that includes *P. jaubertii* E. Gamal-Eldin collected in S. Arabia (***J***) *P. mauritanica* Coss. collected in Morocco (L), *P. undulata* collected in Yemen, Zingibar (***P***) and *P. undulata* collected in Sudan, El-Fiteehab (***R***) is characterized by the huge presence of oxygenated monoterpenes with carvotanacetone as the almost exclusive metabolite.

The last cluster that includes *P. sicula* collected in Sicily (***S***) and *P. gnaphalodes* collected in Iran, Qom (***H***) monoterpenes and sesquiterpenes are both present. An isolate position has been observed for *P. gnaphalodes* collected in Iran, Elbrus Mountain (***F***).

This work represents a large effort and will be of interest to other research in this field. The conclusions based on the statistical analysis can be considered as starting points for a chemotaxonomic classification of the genus.

The comparison of our data on *P. sicula* with those reported in literature for the other taxa (***Table 8***) allows to point out some interesting considerations. The composition of *P. sicula* oil shows a complete different and peculiar profile with respect to all the other taxa of *Pulicaria*. In fact, the main compounds identified in *P. sicula*, borneol was practically absent in the other taxa. On the other hand carvotanacetone, the main compound of *P. undulata* from Sudan, Egypt, Yemen, *P. jaubertii* and *P. mauritanica*, is totaly absent in the *P. sicula* oil.

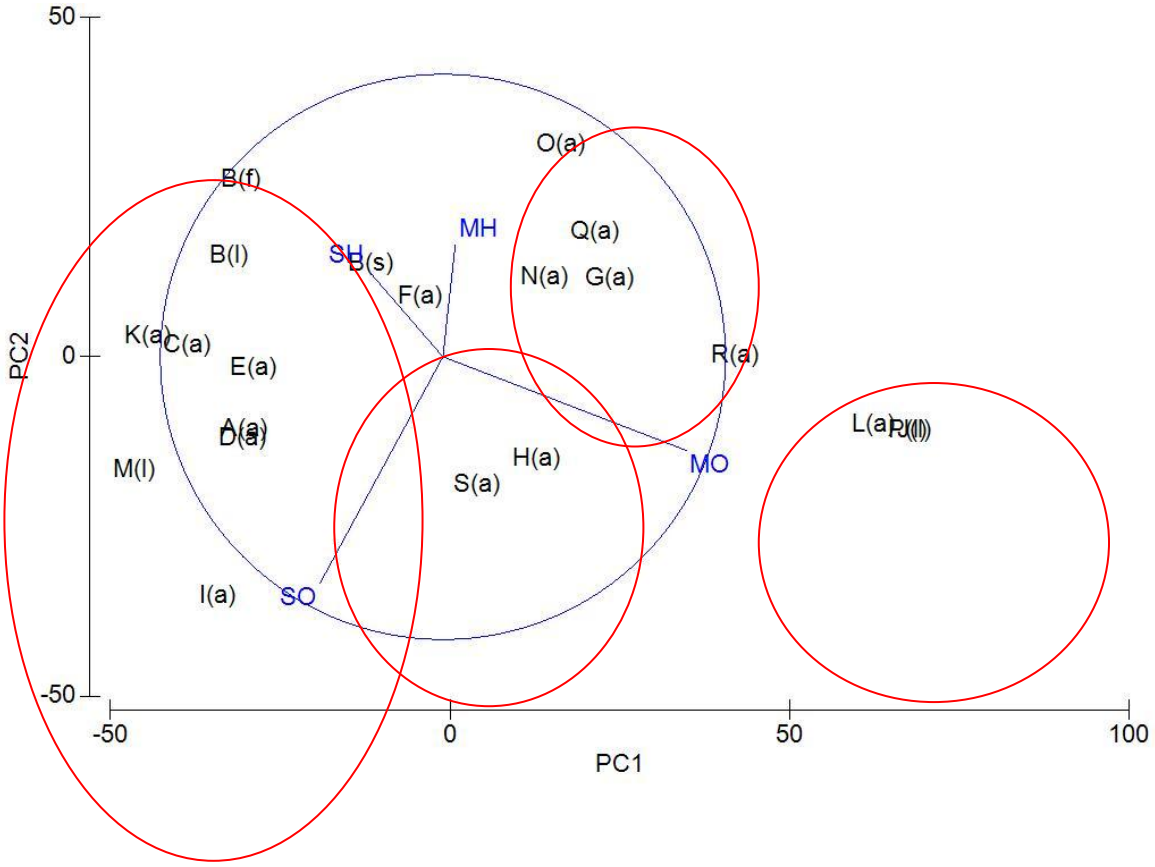


Figure 12: PCA of a multivariate Gaussian distribution of the Pulicaria taxa based on the principal classes of compound that were significant according to the loadings plot: Monoterpenes hydrocarbons (MH), Oxygenated monoterpenes (MO), Sesquiterpenes hydrocarbons (SH), Oxygenated sesquiterpenes (SO) . The vectors shown are the eigenvectors of the covariance matrix.

2.3.3. Biological activity of the essential oil of *Pulicaria vulgaris*

The oil showed a quite good antibacterial activity (***Table 9***) towards the two *Bacillus* species and a moderate one toward *Staphylococcus aureus* and *S. epidermidis*. It could be attributed to the presence of β-caryophyllene and palmitic acid, compounds for which a certain antibacterial activity has been previously described.[174,175,176]

[174] Goren A. C., Piozzi F., Akcicek E., Kılıç T., Çarıkçi S., Mozioglu E., Setzer W. N., *Phytochemistry Letters*, **2011**, *4*, 448-453.
[175] McGraw L. J., Jäger A. K., Van Staden J., *Fitoterapia*, **2002**, *73*, 431-433.
[176] Yff B. T. S., Lindsey K. L., Taylor M. B., Erasmus D. G., Jäger A. K., *Journal of Ethnopharmacology*, **2002**, *79*, 101-107.

2.4. STUDY OF ESSENTIAL OILS OF *BALLOTA HISPANICA*

2.4.1. Characterization of essential oil of *Ballota hispanica*

Hydrodistillation of *B. hispanica* aerial parts (***B.h.)*** gave a pale yellow oil. Overall, 64 compounds were identified in the oil, representing 90.1% (w/w) of the total components. The components are listed in Table 10 according to their retention indices on a HP 5MS column and are classified on the basis of their chemical structures into seven classes.

The oil of *B.h.* is quite rich in sesquiterpenoids (56.7% w/w). Oxygenated sesquiterpenes (18 compounds, 32.8% w/w) is the main class and among these α-elemol (10.9% w/w) is the major compound followed by γ-eudesmol (4.2% w/w) and β-eudesmol (3.7% w/w). Sesquiterpene hydrocarbons are quite abundant (24 compounds, 23.9% w/w) although the only compounds present in significant quantity are α-ylangene (8.5% w/w), the second major component of the oil, and germacrene D (3.5% w/w). Monoterpene hydrocarbons and oxygenated monoterpenes are practically absent whereas diterpenes accounted for 9.9% (w/w) with manoyl oxide (4.8% w/w) as the principal one. Nine hydrocarbons were recorded, forming 13.8% (w/w) of the total, with 1-pentadecene (3.7% w/w) and heptacosane (2.8% w/w) as the most abundant ones. Fatty acids were not recorded and among the other compounds it is noteworthy the presence of γ-dodecalactone (5.1% w/w). Among the carbonylic compounds (3.8% w/w) only hexahydrofarnesyl acetone (2.9% w/w) is worth mentioning.[177]

The comparison of the composition of the oil of *Ballota* with the oils of other *Ballota* taxa showed a peculiar profile of *B. hispanica*.

The oil showed a low antimicrobial activity on Gram-positive bacteria only. On the other hand, the free radical scavenging activity of the oil, determined by DPPH and ABTS methods,

[177] Riccobono L., Ben Jemia M., Bruno M., Senatore F., *Plant Biosystem* (in press)

showed an interesting activity. However, due to the complexity of the oil analyzed (more than 60 compounds) it seems difficult to explain which component of this complex mixture may be responsible for the expressed activity.

2.4.2. Biological activity of the essential oil of *Ballota hispanica*

Table 11 reports the Minimum Inhibitory Concentration (***MIC***) and the Minimum Bacterial Concentration (***MBC***) values of the essential oil against ten selected microorganisms representative of the Gram-positive and Gram-negative classes and known to cause gastrointestinal, respiratory, skin and urinary disorders in humans.

The values of MIC indicate a low activity for the oil. Furthermore, only some bacteria were affected by the oil and the strains more sensitive were the Gram +.

Bacillus cereus and *Staphylococcus epidermidis* appeared to be the more sensitive strains to the biocidal effect of the oil.

In the light of the differences among the wide number of test systems available, the results of a single-assay can give only a reductive indication of the antioxidant properties of extracts toward food matrices and must be interpreted with some caution. Moreover, the chemical complexity of extracts, often a mixture of dozens of compounds with different functional groups, polarity and chemical behavior, could lead to ambiguous results, depending on the test employed. Therefore, an approach with multiple assays in screening work is highly advisable. Among the plethora of methods that can be used for the evaluation of the antioxidant activity, very few of them are useful for determining the activity of both hydrophilic and lipophilic species, thus ensuring a better comparison of the results and

covering a wide range of possible applications.[178] Taking this into account, *B. h.* oil was individually assessed for its anti-oxidative activities by employing two complementary tests, the DPPH free radical scavenging and ABTS free radical scavenging assays. According to the results (***Table 12***) obtained with the DPPH[179] *B. h*. oil was found to be active with a PI value of 26% for 10 mg/mL of oil. The percentage inhibition of the synthetic antioxidant BHT was also determined. None of the samples, for concentrations ranging from 1.25 mg/mL to 10mg/mL, showed activity as strong as that of the standards. The potential of B.h. to scavenge free radicals was also assessed by its ability to quench $ABTS^{\cdot+}$. As shown in Table 13, the concentration-dependent decolorization of $ABTS^{\cdot+}$, expressed as PI values, of the essential oil in comparison with BHT indicates that the essential oil showed the highest activity at 10 mg/mL, with PI values of 26.61%. As for the DPPH scavenging test, these data indicate a lower capacity of the essential oil to quench $ABTS^{\cdot+}$ when compared with the synthetic antioxidant BHT.

2.5. STUDY OF ESSENTIAL OILS OF *MOLUCCELLA SPINOSA*

2.5.1. Characterization of essential oil of *Moluccella spinosa*

Hydrodistillation of *Moluccella spinosa* aerial parts (***Ms***) gave a pale yellow oil. Overall, 35 compounds were identified, representing 95.8% (w/w) of the total components. The components are listed in Table 14 according to their retention indices on a HP 5MS column and are classified on the basis of their chemical structures into eight classes. The oil is particularly rich in terpenoids (69.0% w/w). Monoterpene hydrocarbons (7 compounds,

[178] Sacchetti G., Maietti S., Muzzoli M., Scaglianti M., Manfredini S., Radice M., Bruni R., *Food Chemistry*, **2005**, *91*, 621-632.
[179] Soares J. R., Dins T. C. P., Cunha A. P., Ameida L. M., *Free Radical Research*, **1997**, *26*, 469-478.

39.6% w/w) is the main class and among these α-pinene (26.6% w/w) is the major compound followed by α-thujene (5.9% w/w). Oxygenated sesquiterpenes are quite abundant (18.1% w/w) although the only compound present in significant quantity is caryophyllene oxide (16.8% w/w), the second major component of the oil.

Sesquiterpene hydrocarbons (3 compounds, 9.3% w/w) are represented practically only by β-caryophyllene (8.6% w/w). Six hydrocarbons were recorded, forming 16.3% (w/w) of the total, with nonacosane (5.5% w/w) and heptacosane (5.3% w/w) as the most abundant ones. Fatty acids were not recorded and oxygenated monoterpenes (2.0% w/w) were present in quite few amount. Among the carbonylic compounds (8.6% w/w) only ethylbenzaldehyde (3.4% w/w) is worth of mention.[180]

2.5.2. Biological activity of the essential oil of *Moluccella spinosa*

In this study, we find slight activity of the oil on Gram + and Gram - strains except *Pseudomonas aeuriginosa* that results resistant. *Staphylococcus epidermidis* resulted slightly more sensitive.

Among the yeast and moulds only a moderate antifungal activity against *Aspergillus niger* has been determined (***Table 15***). Regarding to the contribution of pure components to the antimicrobial activity of the oil, α-pinene, caryophyllene oxide, β-caryophyllene and others present in minor concentrations play some role in the antimicrobial action. These components have been shown to posses antimicrobial activity as reported in previous work.[181]

[180] Casiglia S., Ben Jemia M., Riccobono L., Bruno M., Senatore F., *Natural Product Research*, **2015**, *29* (13), 1201-1206.
[181] Setzer W. N., Schmidt J. M., Noletto J. A., Vogler B., *Pharmacologyonline*, **2006**, *3*, 794-802.

2.6. STUDY OF ESSENTIAL OILS OF *THAPSIA GARGANICA*

2.6.1. Characterization of essential oils of *Thapsia garganica*

Hydrodistillation of the flowers (***T.f.***) and leaves (***T.l.***) of *Thapsia garganica* gave two blue oils. Overall, eight compounds were identified in the oil from ***T.f.***, representing 98.2% (w/w) of the total components. The components are listed in Table 16 according to their retention indices on a HP 5MS column and are classified on the basis of their chemical structures into five classes.

The oil of ***T.f.*** is quite rich in sesquiterpenoids (74.3% w/w). Sesquiterpene hydrocarbons (63.0% w/w) are mainly represented by chamazulene (58.3% w/w), by far the main component of the oil, whereas humulene oxide II (9.0% w/w) and curzerene (2.3% w/w) are the only representatives of the oxygenated sesquiterpenes. Hydrocarbons were also present in significant quantity (23.9% w/w). Also the oil of *T.l.* is characterized by a large quantity of chamazulene (49.2% w/w) and 1,4-dimethylazulene (18.5% w/w), present in *T.f.* in lesser amount (4.7% w/w). Oxygenated sesquiterpenes are present in lesser amount (5.3% w/w) with respect to *T.f.* with furanoeremophil-1-one (3.9% w/w), absent in *T.f.*, as the main component of the class. It is noteworthy the occurrence of two diterpenes: neophytadiene (5.1% w/w) and (*E*)-phytol (6.3% w/w), absent in *T.f.*

The comparison of the composition of the essential oil of *Thapsia garganica*, collected in Sicily with those reported for the essential oil of other populations of *Thapsia garganica* reported in literature (***Table 17***) and of all the other taxa of *Thapsia* studied so far (***Table 17***) shows several interesting points.

As regard to previous investigations on *Thapsia garganica* essential oil from flowers, it was shown that *p*-vinylguaiacol was the main product in the populations collected in Algeria[119] and Italy,[117] whereas in the population collected in France, the main components were 2-

ethylhexanol (18.3% w/w), β-myrcene (10.8% w/w) and geranyl acetate (10.1% w/w).[120] Finally an investigation on several populations from Tunisia indicated bicyclogermacrene and epicubenol as main metabolites.[122] It has to be pointed out that all these compounds were completely absent in *T.f.* and that chamazulene and humulene oxide II, main components of *T.f.* are not present in all the other populations studied so far.

Previous analysis on the essential oils from the leaves of *T. garganica* indicates *p*-vinylguaiacol (61.6% w/w), linalool (6.5% w/w) and 1,4-dimethylazulene (6.3% w/w) as main products in plants collected in Algeria[119], and bicyclogermacrene (30.8% w/w), linalool (10.9% w/w), hexadecanoic acid (5.8% w/w) and (*E*)-phytol (5.2% w/w) in a population from Tunisia.[122] Apart from (*E*)-phytol, present in *T.l.* in similar amount (6.3% w/w) and 1,4-dimethylazulene, all the other metabolites were not detected in the leaves of *T. garganica* collected in Sicily.

Although hydrodistillation is the most diffused method for obtaining essential oils and therefore it allows a wider comparison with the results reported in literature, it is likely to alter original composition of essential oil because hydrolytic or thermal reactions can occur for some components[182]. Chamazulene is an artifact produced during the distillation process and is responsible for the blue color of the distillate. It derives by one hydrolytic and three elimination steps from proazulenic guaiane lactones as matricin and analogues[183] (***Figure 13***).

[182] Riela S., Bruno M., Formisano C., Rigano D., Rosselli S., Saladino M. L., Senatore F., *Journal of Separation Science*, **2008**, *31*, 1110–1117.
[183] Ramadan M., Goeters S., B. Watzer B., Krause E., Lohmann K., Bauer R., Hempel B., Imming P., *Journal of Natural Products*, **2006**, *69*, 1041-1045.

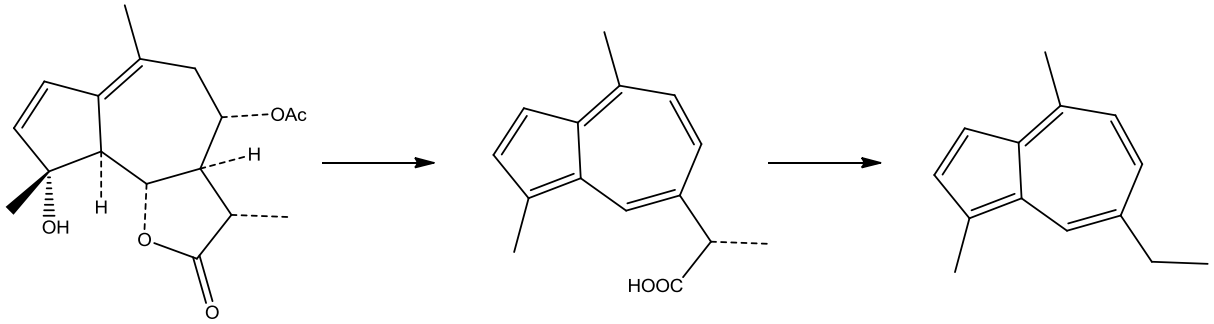


Figure 13: Synthesis of Chamazulene from Matricin

In the previous investigation[120] on different parts of *T. villosa* and *T. garganica*, the solid phase microextraction (SPME) method has been compared with classic hydrodistillation and some composition differences have been observed, but no azulenes derivatives have been found after a 4 hours hydrodistillation. This proves the absence of proazulenic sesquiterpene lactones. Traces and the 0.33% w/w of chamazulene have been found in the essential oils, from leaves and flowers,respectively, of *T. garganica* collected in Tunisia.[122] Although in this case a 3 hours long lasting hydrodistillation has been applied, no proazulenic compounds have been detected and the most abundant compound was bicyclogermacrene, a germacrane type sesquiterpene. Germacrenes are normally stable compounds, nevertheless they can be affected by thermal Cope rearrangement under peculiar conditions[184] to yield elemane sesquiterpenes. In the present study, only curzerene, detected in *T. garganica* flowers and leaves (2.3% w/w and 1.4% w/w respectively), could be arise from a Cope rearrangement of germacrene.

[184] Rosselli S., Maggio A., Raccuglia R. A., Bruno M., *European Journal of Organic Chemistry*, **2003**, 2690-2694.

Among the other taxa of *Thapsia* studied so far no one of them showed this massive presence of chamazulene, whereas humulene oxide was identified only in the oil of *T. villosa*.[185]

2.6.2. Biological activity of the essential oils of *Thapsia garganica*

The oil showed a quite good antimicrobial activity (***Table 18***) towards *Bacillus subtilis* and *Candida albicans* and a moderate one toward *Staphylococcus aureus* and *S. epidermidis*.

The antimicrobial activity of the oils could be explained by considering the high percentage, present in the oils, of chamazulene, in fact, it has been shown to possess strong antimicrobial activity[186,187] and to display antifungal properties against *Tricophyton mentagrophytes*, *T. rubrum* and *Candida albicans*.[186,188] Furthermore, its anti-inflammatory properties *in vivo*,[189] antioxidant and radical scavenging activities have been largely demonstrated.[190,191,192] Moreover the good antibacterial activity of (*E*)-phytol against *Staphylococcus* ssp., present in *T.l.* (6.3% w/w) has been already proved[193,194] and neophytadiene (5.1% w/w in ***T.l.***) was identified as strong bactericidal compound.[195] On the other hand, the two furanosesquiterpenes, curzerene and furanoeremophil-1-one, whose

[185] Avato P., Smitt U. W., *Journal of Essential Oil Research*, **2000**, *12*, 303-309.
[186] Kedzia B., *Herba Polonica*, **1991**, *37*, 29-38.
[187] Bozin B., Mimica-Dukic N., Bogavac M., Suvajdzic L., Simin N., Samojlik I., Couladis M., *Molecules*, **2008**, *13*, 2058-2869.
[188] Ahmed F. H., El Badri A. A., Ibrahim M. M. K., El Shahed A. S., El Khalafawy M. M., *Fats Oils.*, **1994**, *45*, 260–264.
[189] Safayhi H., Sabieraj J., Sailer E. R., Ammon H. P., *Planta Medica*, **1994**, *60*, 410–413.
[190] Sizova N. V., *Pharmaceutical Chemistry Journal*, **2012**, *46*, 369–371.
[191] Capuzzo A., Occhipinti A., Maffei M. E., *Natural Product Reseach*, **2014**, *28*, 2321-2323
[192] Ornano L., Venditti A., Ballero M., Sanna C., Quassinti L., Bramucci M., Lupidi G., Papa F., Vittori S., Maggi F., Bianco A., *Chemistry & Biodiversity*, **2013**, *10*, 1464–1474.
[193] Inoue Y., Hada T., Shiraishi A., Hirose K., Hamashima H., Kobayashi S., *Antimicrobial Agents and Chemotherapy*, **2005**, *49*, 1770-1774.
[194] Xiong L., Peng C., Zhou Q. M., Wan F., Xie X. F., Guo L., Li X. H., He C. J., Dai O., *Molecules*, **2013**, *18*, 963-973.
[195] Mendiola J. A., Santoyo S., Cifuentes A., Reglero G., Ibanez E., Senorans F. J., *Journal of Food Protection*, **2008**, *71*, 2138-2143

antiproliferative activity has been demonstrated, seemed to not possess a reliable antimicrobial activity.[196]

2.7. STUDY OF THE EXTRACTS OF *TETRACLINIS ARTICULATA*

2.7.1. Biological activity of the extracts of *Tetraclinis articulata*

In this study, we find a good in vitro antiproliferative activity of the hexane, dichloromethane and methanol extracts of *T. articulata* against tumor cell lines J774.A1 macrophages, A- 375 human melanoma cells and MCF-7 breast cancer cells, at 72 h (***Table 19***).

However the cytotoxic activity of the extracts is not comparable with the antiproliferative activity of the 6-mercaptopurine.

2.7.2. Characterization of the extracts of *Tetraclinis articulata*

Workup of the hexane extract from aerial parts led to isolation of five pimarane diterpenoids (***1***-***5***).

The following known substances were isolated from this plant: isopimaric acid[197] (***1***), sandaracopimaric acid[198] (***2***), 13-*epi*-pimar-16-ene-8α,18-diol[134] (***3***), 13-*epi*-pimar-16-ene-6α,8α,18-triol[134] (***4***), and 12β-acetoxy-sandaracopimaric acid (***5***)[199] (***Figure 14***).

[196] Quassinti L., Bramucci M., Lupidi G., Barboni L., Ricciutelli M., Sagratini G., Papa F., Caprioli G., Petrelli D., Vitali L. A., Vittori S., Maggi F., *Food Chemistry*, **2013**, *138*, 808-813.
[197] Antkowiak W., Apsimon J. W., Edwards O. E., *Journal of Organic Chemistry,* **1962**, *27*, 1930-1931.
[198] Fang J. M., Lee C. K., Cheng Y., *Phytochemistry,* **1993**, *33*, 1169-1172.
[199] Apsimon J. W., Edwards O. E., *Canadian Journal of Chemistry*, **1961**, *39*, 2543-2548.

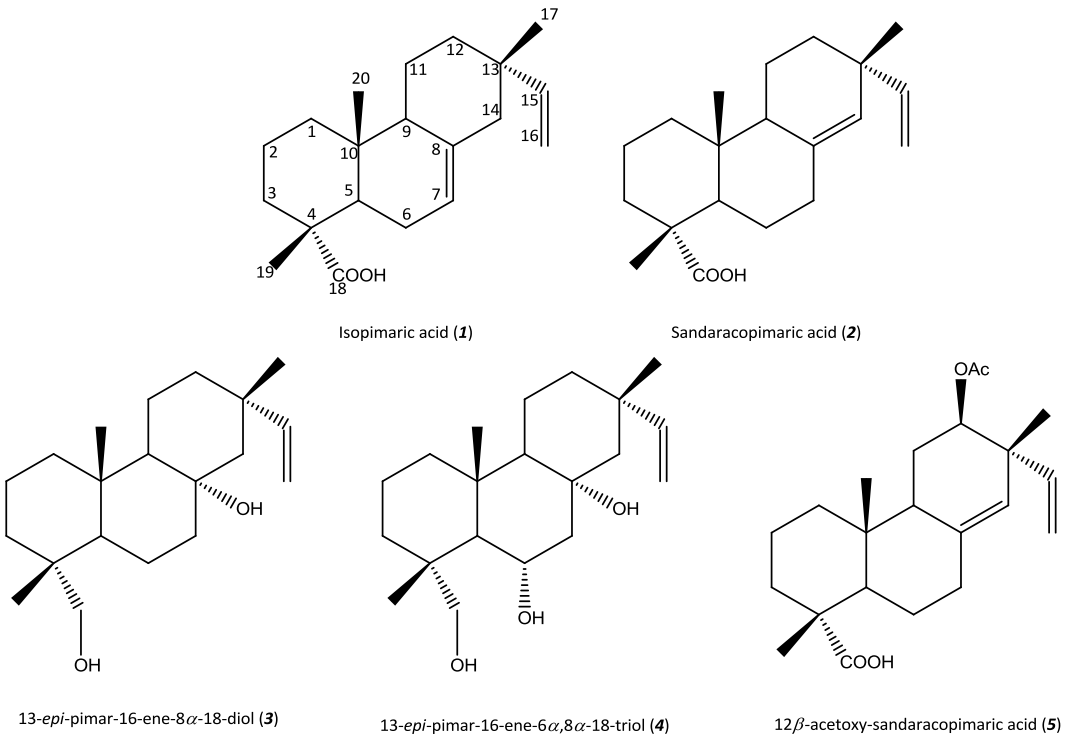


***Figure 14**:* Compound isolated from *T. articulata*

Although repeated isolation procedures, compounds ***1*** and ***2*** were isolated in a 8:1 mixture and for this reason it was possible to assign its signals at each molecules in the NMR spectra. The data collected for these compounds are in perfect agreement with literature for the isopimaric[197] (***1***) and sandaracopimaric[198] (***2***) acids.

The spectral data of compound ***3*** were consistent with the structure of a Δ^{15}-pimarene-type diterpenoid possessing only a primary (^{1}H-NMR: AB system, δ_A, 3.11, d, J = 10.7 Hz; δ_B 3.40, d, J = 10.7 Hz; ^{13}C-NMR: δ = 72.1) and a tertiary hydroxyl group (δ = 73.0). The location of the oxygenated functions at C-8 and C-18 was corroborated by the analysis of 2D-NMR techniques (COSY, HSQC and HMBC). The interannular junction of the B and C rings in **4** was carefully studied. This spatial disposition was determined to be *cis* on the basis of both the ROESY correlations observed between Me-20 and H-14β (***Figure 15***).

The spectral data of compound ***4*** were also consistent with the structure of a Δ^{15}-pimarene-type diterpenoid, possessing only a primary (^{1}H-NMR: AB system, δ_A, 3.05, d, J = 11.7 Hz; δ_B 3.45, d, J = 11. 7 Hz; ^{13}C-NMR: δ = 72.6) and two hydroxyl groups (δ = 67.4 and 75.0). The location of the oxygenated functions at C-6, C-8 and C-18 was corroborated by the analysis of 2D-NMR techniques (COSY, HSQC and HMBC). The values of the coupling constant measured for H-5 (d, J = 6.5 Hz) and H-7β (dd, J = 5.2 Hz, and J = 15.2 Hz) protons can be explained if the B ring adopts a half-chair conformation (***Figure 15***). To account for this ring conformation change with respect to compound ***3***, a decrease of steric strain due to the relative *syn* disposition of C-18 and the hydroxyl group at C-6, together with the possible existence of hydrogen bonding in the resulting conformation, was proposed. Finally, the selected ROESY correlations, shown in figure 15, agree with the conformational proposal.

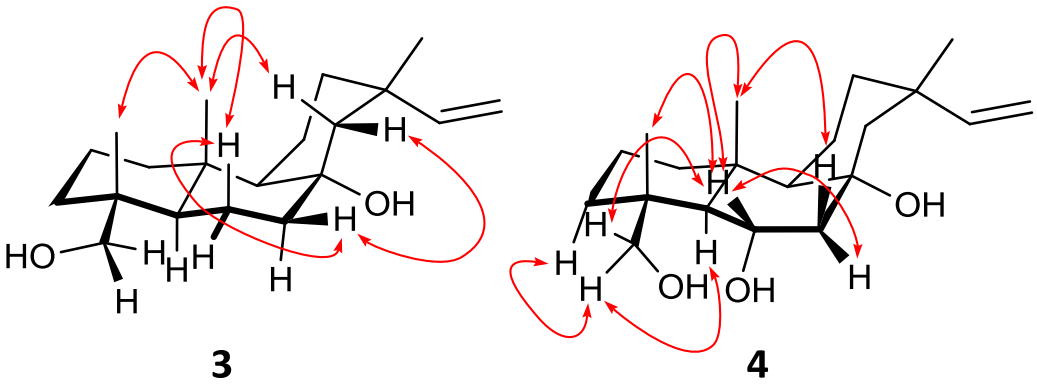


Figure 15: Selected ROEs for ***3*** and ***4***

Another product has been isolated from exane extract of *T. articulata*. Its ^{1}H-NMR spectrum showed the characteristics of a Δ^{15}-pimarene-type diterpenoid. Furthermore an olefinic proton signal at δ 5.14 as singlet and an oxygenated methine signal at δ 4.82 as double doublet appeared. The occurrence of acetoxy group at δ 2.02 was associated to the signal at δ 4.82 using 2D-NMR spectroscopy (COSY, HSQC and HMBC) that allowed us to assign the structure of 12β-acetoxy-sandaracopimaric acid (***5***).

An exhaustive conformational search was performed for compounds ***3, 4*** and ***5.*** (See Computational Detail). Two best conformations, on the bases of the energies (***Table 20***), were evaluated for compound ***4*** (chair-boat-chair conformation (*cbc*) and chair-chair-chair conformation (*ccc*)). About compounds **3** and **5,** ccc were evaluated as the best conformation (***Figure 16***).

Table 20**:** Energies of the best conformations of 3, 4 and 5

Molecules	E (HF)
13-*epi*-pimar-8,16-dien-6r,18-diol (*ccc*) (***3***)	-932.8768312
13-*epi*-pimar-16-ene-6α,8α,18-triol (*cbc*) (***4***)	-1008.0162809
13-*epi*-pimar-16-ene-6r,8r,18-triol (*ccc*) (***4***)	-1008.0145037
12β-acetoxy-sandarapimaric acid (***5***)	-1158.3019918

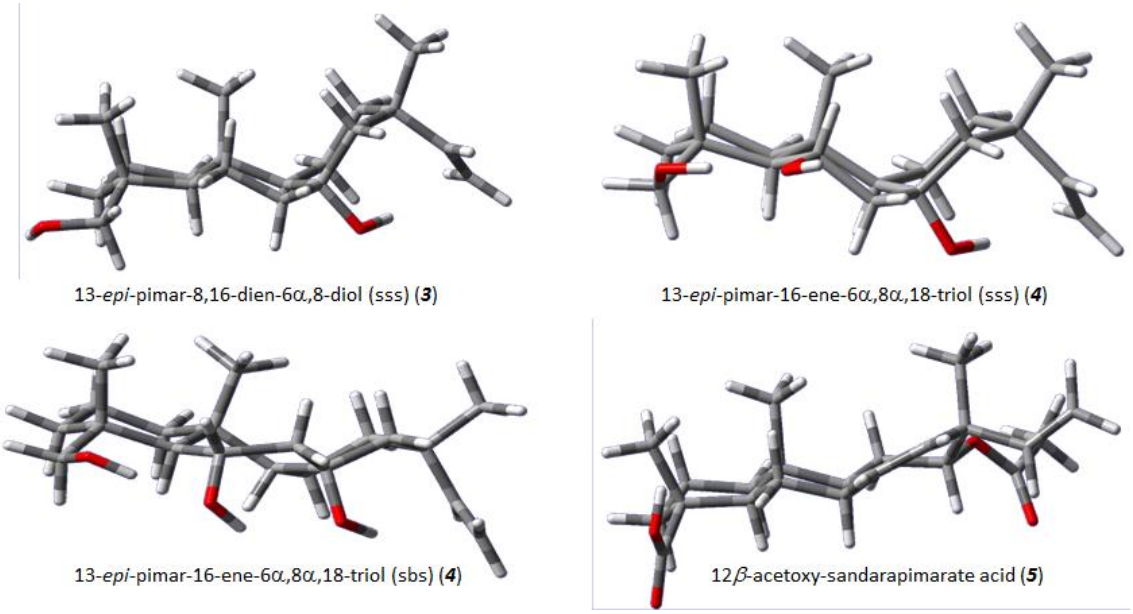


Figure 16: Best conformation of ***3***, ***4*** and ***5***

Compounds ***3, 4*** and ***5*** were screened against a panel of targets selected for their correlations in cancer, using the Inverse Virtual Screening computational method for the selection of the most promising ligand/target interactions.[200,201,202]

This innovative approach allows a prediction of activity and selectivity of a bioactive compound against a panel of targets by the evaluation and a subsequent normalization of the predicted binding energies, so it is possible to obtain a restricted group of proteins as promising candidates for the biological tests. In particular, Autodock_Vina[203] calculations were performed. This software has been shown to produce, together with an increased efficiency in predicting the experimental binding poses and energies, a 2 orders of magnitude speed-up compared with Autodock 4[204] and it has been designed for parallel computing. For the above reasons, it represents a particularly suitable tool for this study, for large virtual screening studies in general, and for the investigation of ligands presenting large numbers of active torsion angles, such as naturally occurring compounds.

Docking calculations were performed between three molecules against a panel of 303 protein targets involved in tumor processes.

The results of inverse virtual screening are collected in Table 21 with energies expressed in kcal/mol and the normalized values (V values) using the equation 1.

$$V = V_0/V_R$$

Equation 1

[200] Lauro G., Romano A., Riccio R., Bifulco G., *Journal of Natural Products*, **2011**, *74*, 1401-1407.
[201] Cheruku P., Plaza A., Lauro G., KefferJ. R., Bifulco G., Bewley C. A., *Journal of Medicinal Chemistry*, **2012**, *55*, 735-742.
[202] Lauro G., Masullo M., Piacente S., Riccio R., Bifulco G., *Bioorganic and Medicinal Chemistry*, **2012**, *20*, 3596-3602.
[203] Trott O., Olson A. J., *Journal of Compuational Chemistry*, **2010**, *31*, 455–461.
[204] Huey R., Morris G. M., Olson A. J., Goodsell D. S., *Journal of Computational Chem*istry, **2007**, *28*, 1145–1152.

Where V is the normalized value of binding energy, V_0 is the value of binding energy before the normalization, and V_R is the average value of binding energy for each targets.[201,205,206] In this way, it was possible to identify ligands with good affinity and selectivity by evaluation of the normalized predicted binding energies.

We observed that the best results highlighted the correlation between ***4*** with *sbs* conformation with kga (Cod. PDB: 3VOY), caspase7 (Cod. PDB: 1SHL) and fxr (Cod. PDB: 1OSV), while ***4*** with *sss* conformation with mdm2 (Cod. PDB: 3EQS), fxr (Cod. PDB: 1OSV) and pkct (Cod. PDB: 2JED). We observed that the best results highlighted the correlation between ***3*** with pkct (Cod. PDB: 2JED), tdp1 (Cod. PDB: 1RFF) and fxr (Cod. PDB: 1OSV) while ***5*** with caspase2 (Cod. PDB: 1PYO), fxr (Cod. PDB: 1OSV), and rxr (Cod. PDB: 4M8H).

An accurate analysis of the main interactions of the compounds (***3***, ***4*** and ***5***) with fxr (Farnesoid X receptor, Cod. PDB: 1OSV) target highlighted the good accommodation of the ligands in the protein binding site, prompting us to further evaluate the predicted biological activity. In details, molecular docking experiments showed the establishment of both hydrophobic/polar interactions with important residues (Leu284, Met287, Leu345, Tyr366, His291, Arg328, Ser329) in the FXR ligand binding site (LBS)[207] (***Figure 17***).

[205] Gong J., Sun P., Jiang N., Riccio R., Lauro G., Bifulco G., Zheng Q. F., Tang H., Li T. J., Gerwick W. H.,Zhang W., *Organic Letters,* **2014**, *16*, 2224-2227.

[206] Scrima M., Lauro G., Grimaldi M., Di Marino S., Tosco A., Picardi P., Gazzerro P., Riccio R., Novellino E., Bifulco M., Bifulco G., D'Ursi A. M., *Journal of Medicinal Chemistry*, **2014**, *57*, 7798-7803.

[207] Renga B., Mencarelli A.,D'Amore C., Cipriani S., D'Auria M. V., Sepe V.,Chini M. G., Monti M. C., Bifulco G., Zampella A., Fiorucci S., *Plos One*, **2012**, *7* (1), e30443.

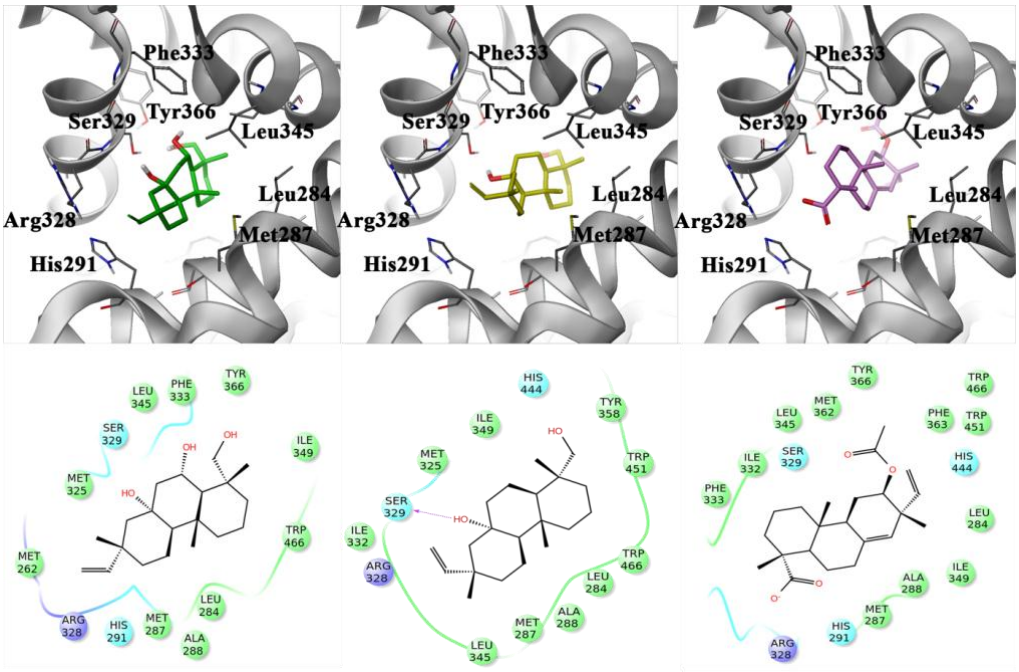


Figure 17: Interactions hydrophobic/polar with important residues in the FXR ligand binding site

Table 21: V_0 and V values in kcal/mol

Targets	Codice PDB	Classification	3	4(sbs)	4 (sss)	5	Target	Codice PDB	Classification	3	4 (sbs)	4 (sss)	5
dhfr	1PD8	Oxidoreductase		V_0 = -9.00 V = 1.193	V_0 = -8.90 V = 1.180		**bclw**	1ZY3	Apoptosis			V_0 = -8.40 V = 1.197	
pkct	2JED	Transferase	V_0 = -9.30 V = 1.270	V_0 = -8.90 V = 1.215	V_0 = -9.40 V = 1.284	V_0 = -9.80 V = 1.033	**lsd1**	2EJR	Oxidoreductase			V_0 = -8.30 V = 1.191	
pka	3L9L	Transferase / Transferase Inhibitor	V_0 = -8.90 V = 1.186	V_0 = -8.90 V = 1.186			**pkc_iota_2**	1ZRZ	Transferase			V_0 = -8.00 V = 1.172	
fxr	1OSV	Dna Binding Protein	V_0 = -8.70 V = 1.266	V_0 = -8.60 V = 1.252	V_0 = -8.90 V = 1.295	V_0 = -10.00 V = 1.257	**nqo1**	2F1O	Oxidoreductase / Inhibitor	V_0 = -7.80 V = 1.234		V_0 = -7.80 V = 1.234	
cdk6	2F2C	Cell Cycle / Transferase	V_0 = -8.30 V = 1.178	V_0 = -8.60 V = 1.221			**mdm2**	3EQS	Ligase			V_0 = -7.70 V = 1.331	
cdk7	1UA2	Cell Cycle Transferase		V_0 = -8.60 V = 1.189			**hspa1lA**	3GDQ	Chaperone			V_0 = -7.60 V = 1.184	
fgfr2	2PVF	Transferase		V_0 = -8.60 V = 1.185			**nek7**	2WQN	Transferase			V_0 = -7.30 V = 1.240	V_0 = -7.20 V = 1.040
clk3	2WU6	Transferase	V_0 = -8.60 V = 1.199	V_0 = -8.50 V = 1.185	V_0 = -8.50 V = 1.185	V_0 = -9.70 V = 1.060	**jak3**	1YVJ	Transferase	V_0 = -8.40 V = 1.230			
pkc_iota_apo	3A8X	Transferase		V_0 = -8.40 V = 1.217			**pkcbII**	2I0E	Transferase	V_0 = -8.40 V = 1.194			
gsk3	3F7Z	Transferase		V_0 = -8.40 V = 1.201			**gstp1**	2A2R	Transferase	V_0 = -7.70 V = 1.186			
kga	3VOY	Hydrolase		V_0 = -8.30	V_0 = -7.80		**egfr**	2J6M	Transferase	V_0 = -7.70			

				V = 1.259	V = 1.184					V = 1.174			
zap70	1U59	Transferase		V_0 = -8.30 V = 1.206			**ape1**	2ISI	Lyase	V_0 = -7.30 V = 1.197			
mek4_no_anp	3ALN	Transferase	V_0 = -8.30 V = 1.198	V_0 = -8.30 V = 1.198		V_0 = -9.40 V = 1.109	**plk1**	3FVH	Cell Cycle Peptide Binding Protein	V_0 = -7.30 V = 1.183			
mpges_1_no_gsh	4AL0	Isomerase		V_0 = -8.20 V = 1.208		V_0 = -7.80 V = 1.015	**nm23_h2**	3BBB	Transferase		V_0 = -7.40 V = 1.233	V_0 = -7.40 V = 1.233	
caspase7	1SHL	Hydrolase	V_0 = -7.80 V = 1.209	V_0 = -8.10 V = 1.256	V_0 = -7.70 V = 1.194		**hdac6**	Homology modeling					V_0 = -7.20 V = 1.015
rock1	3TWJ	Transferase / Transferase Inhibitor	V_0 = -8.30 V = 1.217	V_0 = -8.10 V = 1.188	V_0 = -8.70 V = 1.276	V_0 = -8.60 V = 1.031	**topII_atp**	1QZR	Isomerase			V_0 = -9.50 V = 1.196	V_0 = -9.90 V = 1.069
cdk9	3BLQ	Transcription		V_0 = -8.10 V = 1.185		V_0 = -8.40 V = 1.036	**aif**	1M6I	Oxidoreductase			V_0 = -8.90 V = 1.176	
ftase	1LD8	Transferase		V_0 = -7.90 V = 1.203	V_0 = -8.20 V = 1.249		**errB_ant**	Homology modeling				V_0 = -8.90 V = 1.174	
igf	3F5P	Transferase		V_0 = -7.80 V = 1.226			**rack1**	4AOW	Receptor			V_0 = -8.50 V = 1.223	
tdp1	1RFF	Hydrolase / Dna	V_0 = -7.60 V = 1.269	V_0 = -7.50 V = 1.251	V_0 = -7.10 V = 1.185	V_0 = -7.80 V = 1.052	**prp31**	2OZB	Rna Binding Protein / Rna				V_0 = -7.40 V = 1.032
a2a_ant	3EML	Membrane Protein Receptor				V_0 = -9.60 V = 1.061	**fgfr1**	1AGW	Protein Kinase				V_0 = -8.10 V = 1.027
mpges_1_with_gsh	4AL0	Isomerase				V = 1.018	**pyk2**	3FZS	Transferase				V_0 = -8.50 V = 1.025
14_3_3_sigma	1YWT	Signaling Protein / De Novo Protein				V_0 = -7.50 V = 1.035	**caspase2**	1PYO	Hydrolase / Hydrolase Inhibitor				V_0 = -7.10 V = 1.269
bubr1k	3SI5	Cell Cycle				V_0 = -7.10 V = 1.094	**rxr**	4M8H	Transcription				V_0 = -8.50 V = 1.215
pdk2	4MP2	Transferase /				V_0 = -7.50	**mtor**	3FAP	Cell Cycle				V_0 = -0.30

		Transferase Inhibitor				V = 1.044							V = 1.031
chk1	2QHN	Transferase				V_0 = -8.00 V = 1.039	**aman2**	3DDF	Hydrolase				V_0 = -7.70 V = 1.026
fak	3BZ3	Transferase				V_0 = -8.50 V = 1.035	**pgm**	1YFK	Isomerase Hydrolase				V_0 = -7.10 V = 1.023
Soyb_lox	1HU9	Oxidoreductase			V_0 = -7.80 V = 1.255								

2.8. STUDY OF THE EXTRACT OF *AJUGA TENOREI*

Reverse phase column chromatography of the methanolic extract from aerial parts of *Ajuga tenorei* led to isolation of three products (***Figure 18***). The ^{1}H-NMR spectrum of the first eluted product clearly displayed the presence of glucoside moiety as shown by the signal at δ 4.60 whose coupling constant (J = 8.0 Hz) indicated the β configuration. Signals for olefinic protons at δ 6.30 (d, J = 6.4 Hz) and δ 4.85 (dd, J = 6.4, 2.0 Hz) were compatible with iridoid structure. The occurrence of a signal for an acetyl group at δ 1.91 allowed to assign the structure of 8-*O*-acetylharpagide[208] (***6***) (a compound that has already been isolated before from *A. decumbens* and shown to be a potent antitumor promoter and chemopreventive agent in chemical carcinogenesis)[209] to the compound. Literature data confirmed the assignment.

The following eluted product showed a very similar ^{1}H-NMR spectrum with respect to the previous compound. The lack of acetyl group was the only difference. The comparison with literature data assigned to the product the structure of harpagide[210] (***7***).

Pharmacological studies have shown that 8-*O*-acetylharpagide and harpagide, which are abundant iridoid glycosides of *Ajuga decumbens* Thunb, have diverse biological activities, such as antibacterial, anti-inflammatory, and antiviral activities.[211,212,213,214,215]

The last identified compound showed a quite complex ^{1}H-NMR spectrum. It was possible to recognize five methyl terminations, one of these was a triplet indicating an ethyl termination.

---

[208] Dinan L., Whiting P., Bourne P., Coll J., *Insect Biochemistry and Molecular Biology*, **2001**, *31*, 1077-1082.
[209] Takasaki M., Tokuda H., Nishino H., Konoshima T*., Journal of Natural Products*, **1999**, *62*, 972-975.
[210] Li Y. M., Jiang S. H., Gao W. Y., Zhu D. Y., *Phytochemistry*, **1999**, *50*, 101-104
[211] Breschie M. C., Martmotti Catalanog S., Flamini G., Morelli I., Pagni A. M., *Journal of Natural Products*, **1992**, *55*, 1145–1148.
[212] Konoshima T., Takasakia M., Tokuda H., Nishino H., *Cancer Letters*, **2000**, *15*, 87–92
[213] Li W. W., Wu W. L., Liu S. J., Fang C. W., Liang Y. M., *Anhui Medical Pharmacy*, **2009**, *13*, 329–335.
[214] Xie Z. Y., Qin M. Z., Fang Y. L., *World Notes Plant Medica*, **2005**, *20*, 56–58.
[215] Zhang L. Q., Feng L., Jia Q., Xu J. W., Wang R., Wang Z. T., Wu Y. C., Li Y. M., *Bioorganic & Medicinal Chemistry*, **2011**, *19*, 4882–4886.

The presence of an olefinic proton at δ 6.43 along with several signals indicating oxygenated methines and the presence in ^{13}C spectrum of a ketone (δ 210.2), an α,β unsaturated ketone (δ 202.7)and an α,β unsaturated lactone (δ 162.1) allowed to suppose the structure of ajugalactone[216] (***8***), a chemotaxonomic marker of the genus, for the compound. It was confirmed by literature comparison.

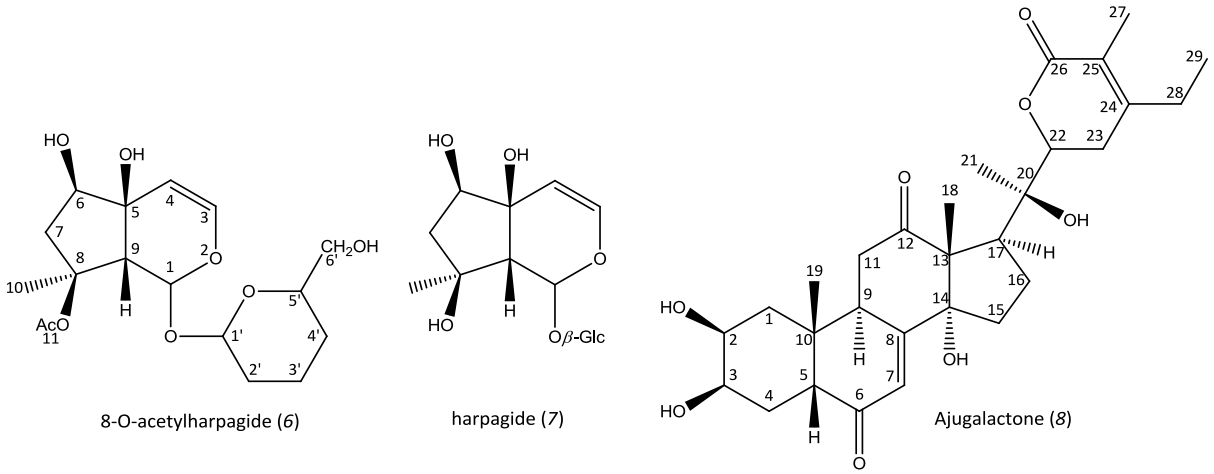


Figura 18: Compounds isolated from *A. tenorei*

An exhaustive conformational search was performed for compounds ***6***, ***7*** (See Computational Detail). The best conformations on the bases of the energies (***Table 22***) were evaluated for compounds ***6*** and ***7***. (***Figure 19***). We decided also to carry out a conformational search (***Table 22***) and to evaluate the best conformation on the bases of the energies of 8-*O*-Acetyl-harpagide (***9***) aglicon and Harpagide aglicon (***10***) (***Figure 19***).

Table 22: Energies of the best conformations of ***6***, ***7***, ***9*** and ***10***

Molecules	E (kj/mol)
8-O-Acetylharpagide (***6***)	+126.9692
Harpagide (***7***)	+221.6037
8-O-Acetylharpagide aglycon (***9***)	+84.80556
Harpagide aglycon (***10***)	+147.4504

[216] Calcagno M. P., Camps F., Coll J., Melé E., Sanchez-Baeza F., *Tetrahedron*, **1996**, *52* (30), 10137-10146.

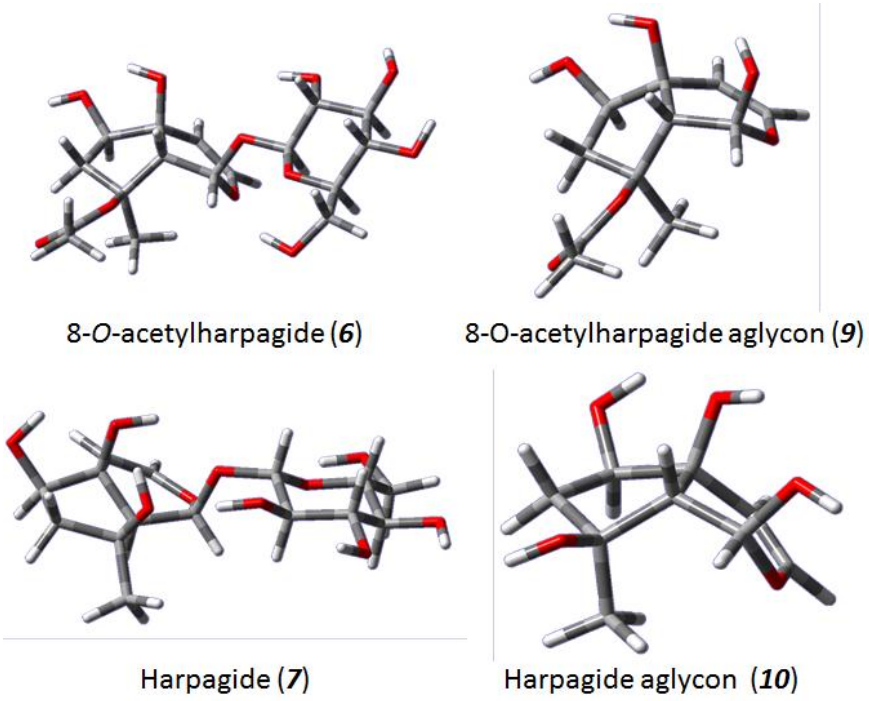


Figure 19: Best conformations of ***6, 7, 9*** and ***10***

Compounds ***6*** and ***7*** were screened against a panel of targets selected for their correlations in cancer, using the Inverse Virtual Screening computational method for the selection of the most promising ligand/target interactions.[200,201,202]

This innovative approach allows a prediction of activity and selectivity of a bioactive compound against a panel of targets by the evaluation and a subsequent normalization of the predicted binding energies, so it is possible to obtain a restricted group of proteins as promising candidates for the biological tests. In particular, Autodock_Vina[203] calculations were performed. This software has been shown to produce, together with an increased efficiency in predicting the experimental binding poses and energies, a 2 orders of magnitude speed-up compared with Autodock 4[204] and it has been designed for parallel computing. For the above reasons, it represents a particularly suitable tool for this study, for large virtual

screening studies in general, and for the investigation of ligands presenting large numbers of active torsion angles, such as naturally occurring compounds.

Docking calculations were performed between three molecules against a panel of 303 protein targets involved in tumor processes.

The results of inverse virtual screening are collected in Table 23 with energies expressed in kcal/mol and the normalized values (V values) using the equation 1.

In this way, it was possible to identify ligands with good affinity and selectivity by evaluation of the normalized predicted binding energies.

We observed that the best results highlighted the correlation between ***6*** with pgm (Cod. PDB: 1YFK), rxr (Cod. PDB: 4M8H) and fpps_no_ipp (Cod. PDB: 1ZW5) while ***9*** with rihb (Cod. PDB: 3B9X), aurikinB (Cod. PDB: 2VG0) and mpges_1_4al1 (Cod. PDB: 4AL1).

We observed that the best results highlighted the correlation between ***7*** with pgm (Cod. PDB: 1YFK), tp (Cod. PDB: 1UOU) and rac1 (Cod. PDB: 4GZL) while ***10*** with enolase3_gr1 (Cod. PDB: 2XSX), enolase3_gr2 (Cod. PDB: 2XSX) and aurikinB (Cod. PDB: 2VG0).

Table 23: V_0 and V values in kcal/mol

Target	Codice PDB	Classification	6	9	7	10	Target	Codice PDB	Classification	6	9	7	10
topII_atp	1QZR	Isomerase	V_0 = -9.50 V = 1.018		V_0 = -9.20 V = 0.994	V_0 = -7.80 V = 1.157	**errB_gsk**	homology_modeling		V_0 = -7.20 V =1.051		V_0 = -7.10 V =0.996	
fpps_no_ipp	1ZW5	Transferase	V_0 = -9.30 V = 1.097		V_0 = -8.50 V = 1.013		**aurkinB**	2VG0	Transferase		V_0 = -7.80 V = 1.266		V_0 = -7.20 V = 1.178
rack1	4AOW	receptor	V_0 = -9.20 V = 0.994	V_0 = -7.10 V =1.173	V_0 = -9.10 V = 1.017		**p300**	3BIY	Transferase		V_0 = -7.70 V = 1.189	V_0 = -7.20	V_0 = -7.20 V = 1.127
Pgm	1YFK	Isomerase hydrolase	V_0 = -8.50 V = 1.232		V_0 =-8.20 V = 1.181		**Rihb**	3B9X	Hydrolase		V_0 = -7.60 V = 1.340		
Upa	2VIP	Hydrolase	V_0 = -8.40 V = 1.086		V_0 = -7.50 V = 0.986		**mpges_1_4al1**	4AL1	Isomerase		V_0 = -7.60 V = 1.123		
Hras	2UZI	Signaling protein/immune system	V_0 = -8.40 V = 1.018				**mek4_no_anp**	3ALN	Transferase		V_0 = -7.20 V = 1.199		
erk2	2OJG	Trnsferase	V_0 = -8.40 V = 0.984				**mpges_1_no_gsh**	4AL0	Isomerase		V_0 = -7.10 V = 1.193		
cathepsinB	1GMY	Hydrolase/inhibitor	V_0 = -8.10 V = 1.012		V_0 = -7.90 V = 1.016		**pig3**	2J8Z	Oxidoreductase			V_0 = -8.60 V =1.028	
jmjd3_akg	2XXZ	oxidoreductase	V_0 = -8.00 V = 0.998				**jak1**	3EYG	Transferase			V_0 = -8.10 V = 0.997	

Tp	1UOU	Transferase	V_0 = -7.80 V = 1.056		V_0 = -8.80 V =1.163		**hsp90**	2WI6	Chaperone			V_0 = -7.90 V = 0.985	
bcl6	3LBZ	Transcription	V_0 = -7.70 V = 0.993				**rac1**	4GZL	Hydrolase			V_0 = -7.80 V = 1.0.82	
Rxr	4M8H	Transcription	V_0 = -7.60 V = 1.129				**hspa6B**	3FE1	Transcription			V_0 = -7.50 V = 1.001	
Mif	3B9S	Cytokine	V_0 = -7.60 V = 1.021				**cox_1**	3N8X	Oxidoreductase			V_0 = -7.30 V =0.997	
mek5_pb1_domain	1WI0	Glycoprotein	V_0 =-7.60 V = 0.997		V_0 = -7.30 V = 0.978		**ape1**	2ISI	Lyase			V_0 = -7.30 V = 0.994	
arf6	2W83	Protein transport	V_0 = -7.50 V = 1.006		V_0 = -7.30 V = 0.990		**CPU**	3D67	Hydrolase				V_0 = -7.30 V = 1.139
nr3c4	2Q7K	Hormone	V_0 =-7.30 V = 1.032		V_0 = -7.70 V = 1.073		**enolase3_gr1**	2XSX	Lyase			V_0 = -7.20	V_0 = -7.20 V = 1.305
akap13	2LG1	Protein Binding	V_0 = -7.20 V = 1.057				**enolase3_gr2**	2XSX	Lyase			V_0 = -7.10	V_0 = -7.10 V = 1.241

3. CONCLUSIONS

Fifteen plant species of the flora Mediterranea, have been analysed for the composition of their essential oils. In particular, nine species of the *Anthemis* genus collected in Sicily represent a big source of data for chemotaxonomic classification and for biodiversity considerations.

The composition data of three of them, recognized to belong to the section *Hiorthia* of genus *Anthemis*, therefore supposed to be strictly correlated, have been compared to the available literature data of all *Anthemis* genus, using the cluster statistical analysis.

The obtained results show that these taxa belong to the same section on the basis of the classes of compounds contained, predominantly sesquiterpenes and monoterpenes, in their essential oils.

An extension of this work, including all composition data collected for the other analysed *Anthemis* species is planned and in progress.

Moreover, the antibacterial activity of the essential oils of these *Anthemis* species has been tested against a panel of gram+ and gram- bacteria, showing in some case a moderate activity.

The composition of the essential oils of two Sicilian species of *Pulicaria* (*P. vulgaris* var. *graeca* and *P. sicula*) has been obtained. The PCA analysis of the oil components of *P. sicula* respect to the other *Pulicaria* species studied up to now, shows the peculiar biodiversity of this Sicilian plant. The comparison of the composition data of *P. vulgaris* var. *graeca* with the data for the botanically closely related *P. vulgaris* Gaertner results in a completely different chemical profile. Therefore the two taxa should be considered as two different identities. Furthermore the antimicrobial activity of the essential oil of *P. vulgaris* against the bacteria *Bacillus cereus* and *B. subtilis*, was measured showing a mild activity.

The analysis of the composition of the essential oil from *Salvia argentea*, collected in Sicily, shows a different chemical profile from the other species of *S. argentea* from other countries. Also the composition of the oil of *Ballota hispanica*, compared with the oils of other *Ballota* taxa, showed a peculiar profile. Although the essential oil of *B. hispanica* shows a low antibacterial activity, the antioxidant activity of this oil was very high and could support the use of *B. hispanica* as phytotherapic and as a good candidate for raw material phyto-preparations.

The study the oil of *Moluccella spinosa*, a plant not previously investigated, showed some marked differences of composition with respect of the oil of the same species collected in Turkey but a close relationship with the oil of *M. laevis*. Also in this case a moderate antibacterial activity was observed.

Finally the results obtained for the *Thapsia garganica* essential oil, indicate a completely different chemical profile with respect to the other *Thapsia* ssp. essential oils studied so far, independently from the extraction method used (SPME or hydrodistillation). In fact, chamazulene, the main component of the oil, was never detected in other *Thapsia* species at this high rate. The antimicrobial activity detected for this essential oil against some bacteria was good.

The study on not volatile metabolites regards *Tetraclinis articulata* and *Ajuga tenorei*. Only one paper on the phytochemical investigation of *T. articulata* from Morocco has been published[134]. The *T. articulata* analysed in this work was collected in Tunisia and showed a very similar metabolic profile with respect the previous investigation. In fact five Δ^{15}-pimarene derivatives were isolated from the hexane extract of *T. articulata*, almost all the compounds occurring in this plant have been previously isolated with the exception of compound ***5***.

Three different solvent extracts (hexane, dichloromethane, methanol) of *T. articulata* showed a good antiproliferative activity against tumor cell.

Dichloromethane and methanol extract of *T. articulata* was chromatographed but not pure fractions have been obtained, further efforts to obtain pure compounds represent the sequel of the research.

The possible activity of the molecules ***3***, ***4*** and ***5*** towards several antitumoral targets was evaluated by computational method using IVS. Molecules ***3***, ***4*** and ***5*** highligthed the best correlation with fxr (Farnesoid X receptor, Cod. PDB: 1OSV) by accurate analysis of the interactions.

The purification of the methanol extract of *Ajuga tenorei* yielded two iridois (harpagide and 8-*O*-acetyl-harpagide) and a phytosteroid (ajugalactone). These products are well known for their biological activity (antibacterial, anti-inflammatory and antiviral activities). To explore new target for antitumor activity, these compounds were subjected to IVS.

The possible activity of the molecules ***6***, ***7***, ***9*** and ***10*** towards several antitumoral target was evaluated by computational method using IVS. All molecules showed the best correlation with different targets, this fact can be explained by change of substitution pattern in the structures resulting in a different linkage with proteins.

Unfortunately no chemical modifications could be performed on isolated compounds because of low quantity of the purified material.

4. EXPERIMENTAL SECTION

4.1. GENERAL EXPERIMENTAL PROCEDURES

Optical rotations were determined on a Perkin-Elmer model 141 polarimeter, using MeOH as solvent.

NMR studies were performed on a (^{1}H 300 MHz / ^{13}C 75 MHz), on a Bruker ARX 400 (^{1}H 400 MHz / ^{13}C 100.4 MHz) spectrometer and on a Bruker (^{1}H 600 MHz / ^{13}C 150.9 MHz) spectrometer

4.2. PLANTS MATERIAL

Anthemis plant species have been collected in Sicily in November 2013.

Aerial parts of *S. argentea* L. were collected on the southern side of Monte delle Rose (Agrigento, Sicily, Italy) (37838018.1900 N, 1382506.6200 E, 1177ms/L), in July 2014, from plants at the full flowering stage. Typical specimens (PAL 14/63MB), identified by Prof. V. Ilardi, have been deposited in the Department STEBICEF, University of Palermo, Palermo, Italy.

The aerial parts of *Pulicaria sicula* were collected near Gela (Sicily, Italy), at Piana del Signore, on alluvial saline sediment, at 10-11 m asl. Typical specimens were identified by Prof. F. M. Raimondo, University of Palermo, and have been deposited in the Herbarium Mediterraneum of the Palermo University, Palermo, Italy (voucher numbers PAL, 15/13).

The aerial parts of *Pulicaria vulgarius* var. *graeca* were collected at Capo Zafferano (P.v.g.), 20 km east of Palermo (Sicily, Italy) on the rocky sea-coast (38°06’38” N; 13°31’47” E; 22 m s/l), in the middle of June 2014, from plants at the full flowering stage. Typical specimens (PAL 14/79), identified by Mr. E. Schimmenti, have been deposited in the Department STEBICEF, University of Palermo, Palermo, Italy.

The aerial parts of *Ballota hispanica* (L.) Benth. (B.h.) were collected, at full bloom, near Macari, Trapani, 100 km west of Palermo, Sicily (Italy), in June 2013. Typical specimens (PAL 13/7MB), identified by Prof. F. M. Raimondo, have been deposited in the Department STEBICEF, University of Palermo, Palermo, Italy.

The aerial parts of *Moluccella spinosa* L. (Ms) were collected near Alcamo, Trapani (38°08'37" N, 12°44'55" E, 81 m s/l), 80 km west of Palermo, Sicily (Italy), at the beginning of June 2014. Typical specimens (PAL 14/60 MB), identified by Mr. E. Schimmenti, have been deposited in the Department STEBICEF, University of Palermo, Palermo, Italy.

Aerial parts (leaves and flowers) of *Thapsia garganica* were collected at Capo Zafferano, 20 Km east of Palermo (Sicily, Italy) on the rocky sea-coast (38°06'38" N; 13°31'47" E; 22 m s/l), in the middle of May 2014, from plants at the full flowering stage. Typical specimens (PAL 14/92), identified by Mr. E. Schimmenti, have been deposited in the Department STEBICEF, University of Palermo, Palermo, Italy.

The aerial parts of *Tetraclinis articulata* was collected in February 2013 in the region of Tunisia.

Ajuga tenorei was collected in June 2013 at Monte Soro (Sicily).

4.3. ISOLATION OF THE ESSENTIAL OILS

For the isolation of the essential oils, the air-dried samples were ground in a Waring blender and then subjected to hydrodistillation for 3 h using *n*-pentane as a solvent, according to the standard procedure previously recommended in the European Pharmacopoeia.[217] The oils

[217] *European Pharmacopoeia* 5th ed., **2005**, Council of Europe, Strasbourg (EDQM)

were dried over anhydrous sodium sulphate and stored under N_2 at +4°C in the dark until tested and analyzed.

4.4. GAS CHROMATOGRAPHY-MASS SPECTROMETRY

Analytical gas chromatography was carried out on a Perkin-Elmer Sigma 115 gas chromatograph equipped with a HP-5MS capillary column (30m x 0.25 mm, 0.25 µm film thickness), a split–splitless injector heated at 250°C and a flame ionisation detector at 280°C. Column temperature was initially kept at 40°C for 5 min, then gradually increased to 250°C at 2°C min^{-1}, held for 15 min and finally raised to 270°C at 10°C min^{-1}. The injection volume was 1.0 µL (split ratio 1:20). A fused silica HP Innowax polyethylenglycol capillary column (50m x 0.20 mm, 0.25 µm film thickness) was also used for the analysis. In both cases, helium was the carrier gas (1mL min^{-1}). Gas chromatography–mass spectrometry analysis was performed on an Agilent 6850 Ser. II apparatus, fitted with a fused silica DB-5 capillary column (30m x 0.25 mm, 0.33 µm film thickness), coupled to an Agilent Mass Selective Detector MSD 5973; ionisation voltage 70 eV; electron multiplier energy 2000 V; source temperature 250°C. Mass spectra were scanned in the range 35–450 amu, scan time 5 scans s^{-1}. Gas chromatographic conditions were the same as those for GC; transfer line temperature, 295°C.

4.5. IDENTIFICATION OF COMPONENTS OF THE ESSENTIAL OILS

Most constituents were identified by GC by comparison of their retention indices (LRI) with either those of the literature[218,219,220] or with those of authentic compounds available in our laboratories. The linear retention indices were determined in relation to a homologous series of *n*-alkanes (C_8-C_{30}) under the same operating conditions. Further identification was achieved by comparison of their MS spectra, either with those stored in NIST 08 and Wiley 275 libraries or with MS from the literature[219,220] and our home-made library.

4.6. ESSENTIAL OIL DATA ANALYSIS

The essential-oil compound percentages that exceeded 5.0% of the total oil composition in at least one species were considered as original variables and subjected to cluster analysis (CA). The statistical analysis of the absence/presence was carried out using the cluster method by Primer 6.[221]

4.7. BIOLOGICAL ACTIVITY OF THE ESSENTIAL OILS

4.7.1. Antimicrobial screening

The antimicrobial activity was evaluated by determining the minimum inhibitory concentration (MIC) and the minimum microbicidal concentration (MMC), which includes

[218] Jennings W., Shibamoto T., *Qualitative Analysis of Flavour and Fragrance Volatiles by Glass Capillary Gas Chromatography*. **1980**, Academic Press, New York.
[219] Davies N. W., *Journal of Chromatography A*, **1990**, *503*, 1.
[220] Adams R. P., *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th Ed.*. **2007**, Allured Publishing Corp., Carol Stream, IL.
[221] Clarke K. R., Gorley R. N., *PRIMER v6: User Manual/Tutorial*., **2006**, PRIMER-E, Plymouth.

minimum bactericidal and minimum fungicidal concentrations, as previously described[222], using the broth dilution method.[223] Oil samples were tested in triplicate.

4.7.2. Microbial strains

The antimicrobial and antifungal activities of essential oil were tested against a panel which included eight bacteria species, selected as representative of the class of Gram positive and Gram negative, *Bacillus cereus* (ATCC 11778), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Streptococcus faecalis* (ATTC 29212), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 10031), *Proteus vulgaris* (ATCC 13315), *Pseudomonas aeruginosa* (ATCC 27853) one yeast, *Salmonella typhi Ty2* (ATCC 19430), *Candida albicans* (ATCC 10231); two moulds, *Fusarium oxysporum* (ATCC 695) and *Aspargillus niger* (ATCC 16401). The strains were grown on Tryptone soya Agar (Oxoid, Milan, Italy) for the bacteria, Saboureaud dextrose agar (SDA) with chloramphenicol for yeasts and SDA for moulds. For the antimicrobial tests, Tryptone soya broth (Oxoid, Milan, Italy) for bacteria and Sabouraud dextrose broth for yeasts and fungal strains were used.

4.7.3. DPPH free radical scavenging activity

The electron donation ability of essential oils was measured by bleaching of the purple-coloured 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) solution, according to standard

[222] Rigano D., Arnold Apostolides N., Conforti F., Menichini F., Formisano C., Piozzi F., Senatore F., *Natural Product Research*, **2011**, *25*, 614–626.
[223] Barry A., The antimicrobic susceptibility test: principles and practices. Philadelphia, **1976**, PA: Lea and Febiger.

methods.[224] Samples (*A1*, 1 mL) were added to 0.25 mL of 0.2 mM DPPH methanolic solution. Percentage inhibition of free radical DPPH (PI %) was calculated as follows:

$$PI\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

where A_{blank} is the absorbance of the control reaction and A_{sample} the absorbance in the presence of the test compound. BHT was used as a positive control (PI = 100%). Samples were analyzed 3 times. Four concentrations of essential oil (10 mg/mL, 5 mg/mL, 2.5 mg/mL, and 1.25 mg/mL) were tested.

4.7.4. ABTS free radical scavenging activity

The ABTS scavenging activity was measured using a photometric method.[225] Briefly, 0.662 mg of potassium persulfate ($K_2S_2O_8$) was added to 3.84 mg ABTS using water as solvent. The mixture was incubated for 12 h in the dark. The resulting blue solution (formation of the radical $ABTS^{\bullet+}$) was diluted with absolute ethanol to achieve an optical density of 0.7 ± 0.02 at 734 nm. Four concentrations of the essential oil (10 mg/mL, 5 mg/mL, 2.5 mg/mL, and 1.25 mg/mL) were tested. The absorbance corresponding to each concentration of the mixture was read against a blank containing only ethanol and the test sample. This was followed every 5 min spectrophotometrically at a wavelength of 734 nm. Results are expressed as a percentage of inhibition using the following formula:

$$PI = [(DO_i - DO_f)/DO_i] \times 100$$

where DO_i is the initial optical density and DO_f the final optical density.

[224] Hanato T., Kagawa H., Yasuhara X., Okuda T., *Chemical and Pharmaceutical Bulletin*, **1988**, *36*, 1090-1097.
[225] Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C., *Free Radical Biology & Medicine*, **1999**, *26*, 1231-1237.

4.8. EXTRACTION AND PURIFICATION OF *TETRACLINIS ARTICULATA*

The air-dried aerial part of *T.articulata* (1.453 Kg) were extracted in a Soxhlet apparatus with hexane, resulting in 44.9 g of crude extract. This extract was subjected to column chromatography over Si gel using mixtures of Etp/AcOEt of increasing polarity as eluents. Ten main fractions were collected, which were combined after monitoring by TLC. F_V (Etp/AcOEt 20%) consisted of a mixture that repurified by column chromatography over Si gel (Etp/AcOEt 5%) to afford ***1*** and ***2*** (isopimaric acid (40.0 mg)and sandaracopimaric acid (80.0 mg)). F_{VIII} and F_{IX} (Etp/AcOEt 60%) consisted of a mixture that repurified by column chromatography over Si gel (hexane/ethyl ether 50%) to afford ***3***and ***4*** (13-*epi*-pimar-16-ene-8α,18-diol (12.8 mg) and 13-*epi*-pimar-16-ene-6α,8α,18-triol (23.83 mg)).

The remaining residue was re-extracted in a Soxhlet apparatus with dichloromethane to give 16.7 g of dried extract. This extract was subjected to column chromatography over Si gel eluting with a DCM/MeOH gradient system to furnish seven main fractions, which were combined after monitoring by TLC.

The remaining residue was re-extracted in a Soxhlet apparatus with methanol to give 100 g of dried extract (***A***). 30 g of this extract were solubilized in 500 mL of water acidified with dry ice (pH ≈ 3-4) and extracted with ethyl acetate (100 mL x 4 times). The organic phase was dried with anhydrous sodium solphate and evaporated to give 1.147 g of dried extract (***B***). The aqueous phase was re-extracted with n-butanol (100 mL x 3 times). The organic phase was dried with anhydrous sodium solphate and evaporated to give 3.879 g of dried extract (***C***). The aqueous phase was frozen (***D***). **B** was subjected to column chromatography over Si gel eluting with a DCM/MeOH gradient system to furnish five main fractions, which were combined after monitoring by TLC. F_{IV} (DCM/MeOH 10%) consisted of a mixture that repurified by column chromatography over Si gel (Etp/AcOEt 5%).

C (1 g) was subjected to column chromatography over sephadex eluting with a MeOH to furnish nine main fractions, which were combined after monitoring by TLC.

Isopimaric acid (***1***): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.87 (3H, s, H-19), 0.92 (3H, s, H-20), 1.13 (1H, m), 1.26 (3H, s, H-17), 1.37 (2H, m), 1.55 (3H, m), 1.64 – 2.01 (9H, m), 4.88 (1H, dd, J = 2.5, H-16β), 4.93 (1H, dd, J =, H-16α), 5.33 (1H, d, J =, H-7), 5.81 (1H, dd, J =, H-15); ^{13}C-NMR (75MHz, $CDCl_3$) δ 15.9 (C-20), 17.4 (C-19), 17.8 (C-2) 19.3 (C-11), 26.7 (C-6), 30.4 (C-17), 35.2 (C-10), 36.8 (C-3), 37.2 (C-13), 37.4 (C-12), 38.0 (C-1) 46.8 (C-5) 47.0 (C-14), 48.0 (C-4) 51.3 (C-9), 109.9 (C-16), 121.7 (C-7), 136.2 (C-8), 150.8 (C-15), 181.0 (C-18).

Sandaracopimaric acid (***2***): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.84 (3H, s, H-20), 1.02 (3H, s, H-17), 1.21 (3H, s, H-19), 4.86-4.91 (1H, m, H-16β), 4.95 (1H, dd, J =, H-16α), 5.23 (1H, s, H-14), 5.80 (1H, dd, J =, H-15); ^{13}C-NMR (75MHz, $CDCl_3$) δ 14.7 (C-19), 18.9 (C-11), 20.7 (C-2), 25.8 (C-6), 30.0 (C-20) 30.1 (C-17), 35.7 (C-7), 36.2 (C-12), 37.7 (C-3), 38.4 (C-10), 39.0 (C-13), 39.5 (C-1), 45.7 (C-4), 49.5 (C-9), 52.7 (C-5), 110.8 (C-16), 129.8 (C-14), 137.2 (C-8), 149.4 (C-15), 186.0 (C-18).

13-*epi*-pimar-16-ene-8α,18-diol (***3***): ^{1}H-NMR (600 MHz, $CDCl_3$) and ^{13}C-NMR (150.9 MHz, $CDCl_3$): see Table 24, ROESY spectra see Figure 20.

13-*epi*-pimar-16-ene-6α,8α,18-triol (***4***): $[\alpha]_D^{24}$ = +4.31° (c = 1.4, MeOH), ^{1}H-NMR (600 MHz, $CDCl_3$) and ^{13}C-NMR (150.9 MHz, $CDCl_3$): see Table 24, ROESY spectra see Figure 21.

12β-Acetoxy-sandaracopimaric acid (***5***): ^{1}H-NMR (600 MHz, $CDCl_3$) and ^{13}C-NMR (150.9 MHz, $CDCl_3$): see Table 24.

4.9. EXTRACTION AND PURIFICATION OF *AJUGA TENOREI*

The air-dried aerial part of *A. tenorei* (66.203 g) were extracted with petroleum ether, resulting in 820 mg of crude extract.

The remaining residue was re-extracted with DCM to give 1.813 g of dried extract. This extract was subjected to column chromatography over Si gel eluting with a Etp/AcOEt gradient system to furnish six main fractions, which were combined after monitoring by TLC.

The remaining residue was re-extracted with methanol to give 10.532 g of dried extract. This extract was subjected to column chromatography over Si gel 100 C18 reversed phase using mixtures of MeOH/H_2O of decreasing polarity as eluents. Six main fractions were collected, which were combined after monitoring by TLC. F_{II} consisted of a mixture that repurified by column chromatography over Si gel 100 ^{18}C reversed phase to afford 8-*O*-acetylharpagide (***6*** 50.0 mg).F_{III} was identified by spectroscopic techniques as harpagide (***7*** 1.78 mg). F_{IV} consisted of a mixture that repurified by column chromatography over Si gel 100 ^{18}C reversed phase to afford ajugalactone (***8*** 5.91 mg).

8-O-Acethylharpagide (***6***): ^{1}H-NMR (400 MHz, D_2O) δ 1,29 (3H, s, H-10), 1.87 (1H, dd, J = 4.4, 16, H-7), 1.91 (3H, s, OCO***CH₃***), 2.01 (1H, d, J = 16, H-7), 2.71 (1H, s, H-9), 3.69 (1H, d, J = 4.4, H-6), 4.85 (1H, dd, J = 2, 6.4, H-4), 5.93 1H, d, J = 1.2, H-1), 6.30 (1H, d, J = 6.4, H-3); 3.16 (t, J = 8.4, H-2'), 3.26 (t, J = 9.2, H-4'), 3.33 (dd, J = 2, 6, H-5')3.36 (t, J = 8.8, H-3'), 3.60 (dd, J = 6, 12.2, H-6') 3.80 (dd, J = 2, 12.2, H-6'), 4.60 (d, J = 8, H-1'), ^{13}C-NMR (100.7 MHz, D_2O) δ 21.18 (C-10), 21.57 (OCO***CH₃***), 44.21 (C-7), 52.96 (C-9), 72.22 (C-5), 76.18 (C-6), 87.82 (C-8), 93.75 (C-1), 104.71 (C-4), 142.55 (C-3), 174.06 (O***C***OCH_3), 60.56 (C-6'), 69.50 (C-4'), 72.39(C-2'), 75.33 (C-3'), 76.06 (C-5'), 98.58 (C-1').

Harpagide (***7***): ^{1}H-NMR (400 MHz, D_2O) δ 1.66 (1H, dd, J = 4.4, 14, H-7), 1.85 (1H, dd, J = 4.4, 14, H-7), 1.09 (3H, s, H-10), 2.40 (1H, s, H-9), 3.66 (1H, t, J = 4.4, H-6), 4.89 (1H,

dd, J = 1.6, 6.4, H-4), 5.57 (1H, d, J = 1.6, H-1), 6.21 (1H, d, J = 6.4, H-3), 3.14 (t, J = 8.6, H-2'), 3.23 (t, J = 9.6, H-4'), 3.33 (m, H-5'), 3.34 (t, J = 9.2, H-3'), 3.56 (dd, J = 5.6, 12.4, H-6'), 3.76 (dd, J = 2,12.4, H-6'), 4.58 (d, J = 8, H-1'). ^{13}C-NMR (100.7 MHz, D_2O) δ 25.3 (C-10), 47.6 (C-7), 60.0 (C-9), 72.9 (C-5), 77.9 (C-6), 78.7 (C-8), 93.6 (C-1), 108.8 (C-4), 142.9 (C-3), 63.2 (C-6'), 72.2 (C-4'), 74.9 (C-2'), 78.6 (C-5'), 78.7 (C-3'), 99.8 (C-1').

Ajugalactone (***8***): ^{1}H-NMR (400 MHz, D_2O) δ 0.72 (t, H-29), 1.20 (s, H-19), 1.57 (s, H-18) 1.63 (H-4), 1.73 s, (H-21), 1.87 (H-28), 1.91 (s, H-27) , 1.97 (H-1), 2.02 (H-4), 2.06 (H-15), 2.09 (H-16), 2.20 (H-23), 2.40 (H-23), 2.48 (H-15), 2.63 (H-16), 2.89 (H-11), 3.11 (dd J = 3.72, 13.39, H-5), 3.54 (H-17), 4.06 (m, H-2), 4.06 (m, H-9) 4.22 (m, H-3), 4.49 (dd J = 3.14, 12.86, H-22), 6.43 (d J = 2.33, H-6); ^{13}C-NMR (100.7 MHz, D_2O) δ 11.5 (C-29), 12.2 (C-27), 17.4 (C-18), 21.0 (C-16), 22.1 (C-21), 23.7 (C-19), 27.0 (C-28), 30.1 (C-23), 31.9 (C-15), 32.1 (C-4), 36.8 (C-9), 36.9 (C-11), 37.8 (C-1), 39.8 (C-10), 43.8 (C-17), 50.8 (C-5), 61.5 (C-13), 67.7 (C-3), 67.9 (C-2), 74.9 (C-20), 83.1 (C-22), 89.1 (C-14), 121.2 (C-25), 123.3 (C-7), 154.2 (C-24), 162.1 (C-26), 166.8 (C-8), 202.7 (C-6), 210.2 (C-12).

4.10. INVERSE VIRTUAL SCREENING (*IVS*)

Maestro 9.6[226] was used to build the chemical structures of compounds ***3***, ***4*** and ***5***. Optimization of the 3D structures was performed with MacroModel 10.2[226] using the OPLS force field[227] and the Polak-Ribier conjugate gradient algorithm (PRCG, maximum derivative less than 0.001 kcal/mol). Starting from the obtained 3D structures, exhaustive conformational searches at the empirical molecular mechanics (MM) level with Monte Carlo Multiple Minimum (MCMM) method (50,000 steps) and Low mode Conformational Search

[226] Schrödinger, *LLC New York NY*, **2013**.
[227] Jorgensen W. L., Tiradorives J., *Journal of he American Chemical Society*, **1988**, *110*, 1657-1666.

(LMCS) method (50,000 steps) were performed, in order to allow a full exploration of the conformational space. Furthermore, molecular dynamic simulations were performed at 450, 600, 700, 750 K, with a time step of 2.0 fs, an equilibration time of 0.1 ns, and a simulation time of 10 ns.

For each compound, the conformations obtained from the previously mentioned conformational searches were minimized (PRCG, maximum derivative less than 0.001 kcal/mol) and compared. The "Redundant Conformer Elimination" module of Macromodel 10.2[226] was used to select non-redundant conformers, excluding the conformers differing more than 21.0 kJ/mol from the most energetically favoured conformation and setting a 0.5 Å RMSD (root-mean-square deviation) minimum cut-off for saving structures.

Next, the obtained conformers were optimized at quantum mechanical (QM) level by using the MPW1PW91 functional and the 6-31G(d) basis set. Prior to performing docking calculations, the selected conformers (See Results and Discussion) of each compound were then converted in the .pdbqt format.

Protein targets, known to be involved in tumor processes, were prepared by a search of crystallized structures in the Protein Data Bank database. In particular, crystallized water molecules were removed, all hydrogens were added, and bond orders were assigned. Protein .pdb file obtained was then converted in .pdbqt format, using Autodock Tools 1.5.7 software.[228] Molecular docking were performed using Autodock Vina software. For all investigated compounds, all open-chain bonds were treated as active torsional bond. Autodock Vina results were analyzed with Autodock Tools 1.5.7.

[228] Trott O., Olson A. J.*, Journal of Computational Chemistry,* **2010**, *31*, 455-461.

5. ACKNOWLEDGEMENTS

Inverse Virtual Screening were performed at the Pharmacy Department of the University of Salerno.

Purification and characterization of the compounds isolated from *Ajuga tenorei* were performed at CSIC of Bercelona.

The biological assays were performed at the University of Naples.

6. BIBLIOGRAPHY

[1] http://luirig.altervista.org/flora/taxa/floraindice.php S. Pignatti, *Flora d'Italia*, **1982**, 3, 770-771.

[2] Fernandes R., **1976**. Genus Anthemis L. In: Tutin T. G., Heywood V. H., Burges N. A., Moore D. M., Valentine D. H., Walters S. M., Webb A. (Eds.), Flora Europaea, vol. 4. Cambridge University Press, Cambridge, London, New York, Melbourne, 145–159.

[3] Bruno M., Bondì M. L., Vassallo N., Gedris T.E., Herz W., *Phytochemistry*, **1997**, *45,* 375-377.

[4] Bruno M., Diaz J. G., Herz W., *Phytochemistry*, **1991**, *30*, 3458-60.

[5] Hedge I. C., Salvia L. In Flora Europaea, vol 3, Tutin TG, et al. (eds). Cambridge University Press: Cambridge, 188.

[6] Wu Y. B., Ni Z. Y., Shi Q. W., Dong M., Kiyota H., Gu Y. C., Cong B., *Chemical Reviews*, **2012**, *112*, 5967-6026.

[7] Baricevic D., Bartol T., Sage: the genus Salvia. In: Kintzios, S.E. (Ed.), Pharmacology: The biological/pharmacological activity of the Salvia genus. Harwood Academic Publishers, The Netherlands, **2000**, 143–184.

[8] Blumenthal M., The complete German commission E monographs. American Botanical Council: Texas, **1998**, 198.

[9] Weiss R. F., Herbal Medicine, Beaconsfield Publishers: Beacons field, **1998**, 228.

[10] Bisset N. G., Herbal drugs and phytopharmaceuticals. CRC press: Stuttgart, **1994**, 440.

[11] Newall C. A., Anderson L. A., Phillipson J. D., Herbal Medicines: A guide for healthcare professionals. Pharmaceutical Press: London, **1996**, 231.

[12] Jalsenjak V., Peljnajk S., Kustrak D., *Pharmacology*, **1987**, *42*, 419-420.

[13] http://www.bgbm.org/euroPlusMed/.

[14] Clebsch B., Barner C. D., The new book of Salvias: sages for every garden. Portlan: Timber Press, **2003**, 36–37.

[15] Pieroni A., Quavec C. L., Santoro R. F., *Journal of Ethnopharmacology*, **2004**, *95*, 373–384.

[16] Tardío J., Pardo-De-Santayana M., Morales R., *Botanical Journalof the Linnean Sociey*, **2006**, *152*, 27-71.

[17] Stagos D., Portesis N., Spanou C., Mossialos D., Aligiannis N., Chaita E., Panagoulis C., Reri E., Skaltsounis L., Tsatsakis A. M., Kouretas D., *Food and Chemical Toxicol*ogy, **2012**, *50*, 4115-4124.

[18] Salah K. B. H., Mahjoub M. A., Ammar S., Michel L., Millet-Clerc J., Chaumont J. P., Mighri Z., Aouni M., *Natural Product Research*, **2006**, *20*, 1110-1120.

[19] Ben Farhat M., Landoulsi A., Chaouch-Hamada R., Sotomayor J. A., Jordan M. J., *Industrial Crops and Products*, **2013a**, *47*:106-112.

[20] Ben Farhat M., Landoulsi A., Chaouch-Hamada R., Sotomayor J. A., Jordan M. J., *Industrial Crops and Products*, **2013b**, *49*, 904-914.

[21] Erdogan Orhan I., Sezer Senol F., Ercetin T., Kahraman A., Celep F., Akaydin G., Sener B., Dogan M., *Industrial Crops and Products*, **2013**, *41*, 21-30.

[22] Sarac N., Ugur A., *EurAsia Journal of BioSciences*, **2007**, *4*, 28-37.

[23] Şeref Gün S., Çinbilgel İ., Öz E., Çetin H., Kafkas Univ Vet Fak Derg., **2011**, *17* (Suppl A), S61-S65.

[24] Michavilla A., De La Torre M. C., Rodriguez B., *Phytochemistry*, **1986**, *25*, 1935-1937.

[25] Yang M. H., Blunden G., Xu Y. X., Nagy G., Mathe I., *Pharmaceutical Sciences*, **1996**, *2*, 69-71.

[26] Nikolova M. T., Grayer R. J., Genova E., Porter E. A., *Biochemical Systematics Ecology*, **2006**, *34*, 360-364.

[27] Sajewicz M., Staszek D., Wròbel M. S., Waksmundzka-Hajnos M., Kowalska T., *Chromatography Research International*., **2012**, Article ID 230903:1-8.

[28] Bruno M., Savona G., Hueso-Rodriguez J. A., Pascual C., Rodriguez B., *Phytochemistry*, **1987**, *26*, 497-501.

[29] Janicsàk G., Veres K., Zoltan Kakàsy A., Màthé I., *Biochememical Systematics Ecology*, **2006**, *34*, 392-396.

[30] Holeman M. A., Berrada M., Bellakhdar J., Ilidrissi A., Pinel R., *Fitoterapia*, **1984**, *55*, 143-148.

[31] Couladis M., Tzakou O., Stojanovic D., Mimica-Dukic N., Jancic R., *Flavour Fragrance Journal*, **2001**, *16*, 227-229.

[32] Veličković D. T., Ristić M. S., Milosavljević N. P., Davidović D. N., Bogdanović S. Z., *Agro Food Industry Hi Tech.*, **2014**, *25*, 70-72.

[33] Zito P., Sajeva M., Bruno M., Rosselli S., Maggio A., Senatore F., *Natural Product Research*, **2013**, *27*, 1305-1314.

[34] Rosselli S., Maggio A. M., Canzoneri M., Simmonds M. S. J., Bruno M., *Natural Product Communications*, **2012**, *7*, 1131-1132.

[35] Maggio A., Bruno M., Formisano C., Rigano D., Senatore F., *Natural Product Communications*, **2013**, *8*, 841-844.

[36] Maggio A., Riccobono L., Bancheva S., Bruno M., Senatore F., *Natural Product Communications.*, **2014**, *9*, 1373-1376.

[37] Pottier-Alapetite G., *Flore de la Tunisie,* **1981**, 236.

[38] Liu L. L., Yang J. L., Shi Y. P., *Chemistry & Biodiversity*, **2010**, *7*, 327-349.

[39] Ravandeh M., Valizadeh J., Noroozifar M., Khorasani-Motlagh M., *Journal of Medicinal Plants Research*, **2011**, *5*, 2035-2040.

[40] Kuete V., Wiench B., Alsaid M. S., Alyahya M. A., Fankam A. G., Shahat A. A., Efferth T., *BMC Complementary and Alternative Medicine* **2013**, 13354.

[41] El-Seedi H. R., Burman R., Mansour A., Turki Z., Boulos L., Gullbo J., Goransson U., *Journal of Ethnopharmacology*, **2013**, *145*, 746-57

[42] Nickavar B., Mojab F., *Fitoterapia* **2003**, *74*, 390-393.

[43] Alghaithy A. A., El-Beshbishy H. A., AbdelNaim A. B., Nagy A. A., Abdel-Sattar E. M., *Toxicology and Industrial Health*, **2011**, *27*, 899-910.

[44] Mahfouz M., Ghazal A., El-Dakhakhny M., Ghoneim M. T., *Journal Drug Research*, **1973**, *5*, 151-172.

[45] Znini M., Cristofari G., Majidi L., Paolini J., Desjobert J. M., Costa J., *LWT-Food Science and Technology*, **2013**, *54*, 564-569.

[46] Khani A., Asghari J., *Journal of Insect Science (Madison, WI, United States)* **2012**, *12*, 73.

[47] Schulte K. E., Reisch J., Hopmann J., *Archiv der Pharmazie und Berichte der Deuschen Pharmazeutischen Gesellschaft*, **1963**, *296*, 353-364.

[48] Zdero C., Bohlmann F., Rozk A. M., *Phytochemistry* **1988**, *27*, 1206-1208.

[49] Wollenweber E., Christ M., Dunstan R. H., Roitman J. N., Stevens J. F., Naturforsch Z*., C: Journal of Bioscience*, **2005**, *60*, 671-678.

[50] Williams C. A., Harborne J. B., Greenham J. R., Grayer R. J., Kite G. C., Eagles J., *Phytochemistry* **2003**, *64*, 275-283.

[51] http://ww2.bgbm.org/euroPlusMed/ The Euro+Med PlantBase- the information resource for Euro-Mediterranean plant diversity.

[52] Williams C. A., Harborne J. B., Greenham J. R., Grayer R. J., Kite G. C., Eagles J., *Phytochemistry*, **2003**, *64*, 275-283.

[53] Sharifi-Rad J., Miri A., Hoseini-Alfatemi S. M., Sharifi-Rad M., Setzer W. N., Hadjiakhoondi A., *Natural Product Communications*, **2014**, *9*, 1633-1666.

[54] El-Seedi H. R., Burman R., Mansour A., Turki Z., Boulos L., Gullbo J., Goransson U., The traditional medical uses and cytotoxic activities of sixty-one Egyptian plants: discovery of an active cardiac glycoside from *Urginea maritime*, **2013**.

[55] Rakotonirainy M. S., Lavèdrine B, *International Biodeterioration & Biodegradation*, **2005**, *55*, 141–147.

[56] Stupar M., Grbić M. Lj, Džamić A., Unković N., Ristić M., Jelikić A., Vukojević J., *South Africa Journal of Botany*, **2014**, *93*, 118-124.

[57] Casiglia S., Bruno M., Senatore F., *Natural Product Research*, **2014a**, *28,* 1739-1746.

[58] Casiglia S., Bruno M., Senatore F., *Natural Product Communications*, **2014b**, *9*, 1637-1639.

[59] Casiglia S., Ben Jemia M., Riccobono L., Bruno M., Scandolera E., Senatore F., *Natural Product Research*, **2015**, *29*, 1201-1206.

[60] Formisano C., Rigano D., Senatore F., Raimondo F. M., Maggio A., Bruno M., *Natural Product Communications*, **2012**, *7*, 1379-1382.

[61] Maggio A., Riccobono L., Spadaro V., Scialaba A., Bruno M., Senatore F., *Chemistry & Biodiversity*, **2014**, *11*, 652-672.

[62] Maggio A., Venditti A., Senatore F., Bruno M., Formisano C., *Natural Product Research*, **2015**, *29*, 857-863.

[63] Kamel F. H., Ismael H. M., Mohammadamin S. A., *Online International Interdisciplinary Research Journal*, **2014**, *4*, 10-17.

[64] Seidel V., Bailleul F., Tillequin F., Terpenoids and phenolics in the genus *Ballota* L. (Lamiaceae). *Recent Research Develpments in Phytochem*istry, **1999**, *3*, 27-39.

[65] http://apps.kew.org/wcsp/qsearch.do.

[66] Çitoğlu G., Tanker M., Sever B., Englert J., Anton R., Altanlar N., *Planta Medica*, **1998,** *64*, 484-485.

[67] Çitoğlu G. S., Yilmaz B. S., Altanlar N., *Journal of Faculty of Pharmacy of Ankara*, **2003a**, *32*, 93-97.

[68] Dulger B., Dulger G., *Turkish Journal of Pharmaceutica Sciences*, **2012**, *9*, 257-262.

[69] Çitoğlu G. S., Çoban T., Sever B., İşcan M., *Journal of Ethnopharmacology*, **2004a**, *92*, 275-280.

[70] Çitoğlu G. S., Sever B., Antus S., Baitz-Gacs E., *Pharmaceutical Biology*, **2003b**, *41*, 483-486.

[71] Çitoğlu G. S., Sever B., Antus S., Baitz-Gacs E., Altanlar N., *Pharmaceutica Biology*, **2004b**, *42*, 659-663.

[72] Özbek H., Çitoğlu G. S., Dülger H., Uğraş S., Sever B., *Journal of Ethnopharmacology*, **2004**, *95*, 143-149.

[73] Racz-Kotilla G., Racz G., Jozsa J., *Herba Hungarica*, **1980**, *19*, 49-53.

[74] Vural K., Ezer N., Erol K., Samin F. P., *Journal of Faculty of Pharmacy of Gazi University*, **1996**, *13,* 29-32.

[75] Couladis M., Tzakou O., Verykokidou E., Harvala C., *Phytotherapy Research*, **2003**, *17*, 194-195.

[76] Seidel V., Verholle M., Malard Y., Tillequin F., Fruchart J. C., Duriez P., Bailleul F., Teissier E., *Phytotherapy Research*, **2000**, *14*, 93-98.

[77] Didry N., Seidel V., Dubreuil L., Tillequin F., Bailleul F., *Journal of Ethnopharmacology*, **1999**, *67*, 197-202.

[78] Dulger B., Kilcik M. A., *Asian Journal of Chemistry*, **2011**, *23*, 416-418.

[79] Pieroni A., *Journal of Ethnopharmacoloy,* **2000,** *70*, 235-273.

[80] Siciliano T., Bader A., Vassallo A., Braca A., Morelli I., Pizza C., De Tommasi N., *Biochememical Systematics Ecology*, **2005**, *33*, 341-351.

[81] Gray C. A. , Rivett D. E. A., Davies-Coleman M. T., *Phytochemistry*, **2003**, *63*, 409-413.

[82] Riaz M., Krohn K., Malik A., Flörke U., *Chemistry & Biodiversity*, **2004**, *1*, 458-462.

[83] Ahmad V. U., Farooq U., Hussain J., Ullah F., Nawaz S. A., Choudhary M. I., *Chemical and Pharmaceutical Bulletin*, **2004**, *52*, 441-443.

[84] Hussein A. A., Himeno M. L., Rodriguez B., *Magnetic Resonance in Chemistry*, **2007**, *45*, 899-901.

[85] Tòth E., Tòth G., Màthè I., Blunder G., *Biochemical Systematics and Ecology*, **2007**, *35*, 984-897.

[86] Hennebelle T., Saphaz S., Ezer N., Bailleul F., *Biochememical Sysematics and Ecology*, **2008**, *36*, 441-443.

[87] Farooq U., Khan A., Khan A. F., Khan S. S., Sarwar R., Ahmad V. U., Waseem A., *Natural Product Communications*, **2012**, *7*, 149-150.

[88] Lentini F., Aleo M., Amenta R., *Acta Phytoterapeutica*, **1997**, *4*, 88-94.

[89] Lentini F., Amenta R., *Giornale Botanico Italiano,* **1992**, *126*, 371.

[90] Savona G., Piozzi F., Rodriguez B., *Heterocycles*, **1978**, *9*, 257-261.

[91] Rodriguez B., Savona G., Piozzi F., *Journal of Organic Chemistry*, **1979**, *44*, 2219-2221.

[92] Lopez de Lerma J., Garcia-Blanco S., Rodriguez J. G., *Tetrahedron Letters,* **1980**, *21*, 1273-1274.

[93] Través P. G., López-Fontal R., Cuadrado I., Luque A., Boscá L., De Las Heras B., Hortelano S., *Oncogene*, **2013**, *32*, 259-268.

[94] Sadeghi Z., Kuhestani K., Abdollahi V., Mahmood A., *Journal of Ethnopharmacology*, **2014**, *153*, 111–118.

[95] http://www.catalogueoflife.org/col/search/all/key/moluccella/match/1

[96] Quintanar A., Cabezas F., Pujadas A. J. & Cirujano S., *Flora Iberica. Vascular plants of the Iberian Peninsula and Balearic Islands* Vol. 12, Ed. R. Morales, Madrid **2010**, 295-298.

[97] Pignatti S., *Flora d'Italia*, vol II., Edagricole: Bologna, **1982**, 458.

[98] Güvenç A., Özek G., Hürkul M. M., Özek T., K.H.C.,The 2nd International Symposium of Modern Medicine, Traditional Chinese Medicine and Uygur Medicine. Urumqi, Xinjiang, China. September 14-20, **2012**, A-116.

[99] Weitzel C., Ronsted N., Simonsen H. T., *Bot. J. Linn. Soc.*, **2014**, *174*, 620–636.

[100] Giardina G., Raimondo F. M., Spadaro V., *A catalogue of plants growing in Sicily. Bocconea*, **2007**, *20*, 5-583.

[101] Christensen S. B., Andersen A., Smitt U. W., *Progress in the Chemistry of Organic Natural Products*, **1997**, *71*, 129-167.

[102] Drew D. P., Krichau N., Reichwald K., Simonsen H. T., *Phytochem Rev.*, **2009**, *8*, 581-599.

[103] Andersen T. B., Lopez C. Q., Manczak T., Martinez K., Simonsen H. T., *Molecules*, **2015**, *20*, 6113-6127.

[104] Greive M., A Modern Herbal. http://botanical.com/botanical/mgmh/mgmh.html, **1996**.

[105] Djeridane A., Yousfi M., Nadjemi B., Boutassouna D., Stocker P., Vidal N., *Food Chem.*, **2006**, *97*, 654-660.

[106] Goncalves M. J., Cruz M. T., Tavares A. C., Cavaleiro C., Lopes M. C., Canhoto J., Salgueiro L., *Industrial Crops and Product*, **2012**, *35*, 166-171.

[107] Liu H., Jensen K. G., Tran L. M., Chen M., Zhai L., Olsen C. E., Sohoel H., Denmeade S. R., Isaacs J. T., Christensen S. B., *Phytochemistry*, **2006**, *67*, 2651-2658.

[108] Jakobsen C. M., Denmeade S. R., Isaacs J. T., Gady A., Olsen C. E., Christensen S. B., *Journal of Medicinal Chemistry*, **2001**, *44*, 4696-4703.

[109] Abderrahim O., Martin G. J., Abdelaziz A., *Journal of Medicinal Plants Research*, **2013**, *7*, 2156-2169.

[110] Ouarghidi A., Powell B., Martin G., de Boer H., Abbad A., *Economic Botany*, **2012**, *66*, 370–382.

[111] Rasmussen U., Christensen S. B., Sandberg F., *Acta Pharmaceutica Suecica,***1978**, *15*, 133–140.

[112] Thastrup O., Cullen P. J., Drobak B. K., Hanley M. R., Dawson A. P., *Proceedings of the National Academy of Sciences USA,* **1990**, *87*, 2466–2470.

[113] Ollivier A., Grougnet R., Cachet X., Meriane D., Ardisson J., Boutefnouchet S., Deguin B., *Journal of Chromatography B.*, **2013**, *926*, 16-20.

[114] Chibani S., Al-Dabbas M., Abuhamdah S., Aburjai T., Bencheraiet R., Kabouche A., Jay M., Kabouche Z., *Chemistry of Natural Compounds*, **2014**, *50*, 357-359.

[115] Liu H., Olsen C. E., Christensen S. B., *Journal of Natural Products*, **2004**, *67*, 1439-1440.

[116] Larsen P. K., Sandberg F., *Acta Chemica Scandinavica*, **1970**, *24*, 1113-1114.

[117] Avato P., *Planta Medica*, **1991**, *57*, 585-586.

[118] Avato P., Rosito I., *Journal of Essential Oil Research*, **2002**, *14*, 20-22.

[119] Ladjel S., Zellagui A., Gherraf N., *Rev. Sci. Fond. App.*, **2011**, *3*, 30-34.

[120] Drew D. P., Rasmussen S. K., Avato P., Simonsen H. T., *Phytochemical Analysis*, **2012**, *23*, 44-51.

[121] Evergetis E., Haroutounian S. A., *Industrial Crop and Products*, **2014**, *54*, 70-77.

[122] Hassen I., M'Rabet Y., Belgacem C., Kesraoui O., Casabianca H., Hosni K., *Chemistry & Biodiversity*, **2015**, *12*, 637-651.

[123] Mansour M. M., *Journal of Applied Sciences Research*, **2013**, *9*, 1917-1930.

[124] Khaoukha G., Ben Jemia M., Amira S., Laouer H., Bruno M., Scandolera E., Senatore F., *Natural Product Research*, **2014**, *28*, 1152-1158.

[125] Autore G., Marzocco S., Formisano C., Bruno M., Rosselli S., Ben Jemia M., Senatore F., *Molecules*, **2015**, *20*, 1571-1578.

[126] Le Floc'h E., Contribution to the ethnobotanical study of Tunisian vegetation and flora program. Tunisian Scientific Publications, Official Printing of Republic of Tunisia, **1983**, 36–37.

[127] Buhagiar J., Camilleri Podesta M. T., Cioni P. L., Flamini G., Morelli L., *Journal of Essential Oil Research*, **2000**, *12*, 29-32.

[128] Boudy P., Guide Foristier en Afrique du Nord; Lamaison Roustique: Paris, **1952**, 273.

[129] Tekaya-karoui A., Jannet H. B. et. al., *Pakistan Journal of Biological Sciences,* **2007**, *10* (15), 2495-2499.

[130] Tekaya-karoui A., Boughalleb N. et. al., *African Journal Plant Sci*ence, **2011**, *5* (2), 115-122.

[131] Schulz C., Differential diagnose und Evolution der Cupressaceae s. l. (Zypressengewachse). Dissertation – Ruhr-Universitat Bochum, **2005**, 218.

[132] Kohler F. E., Kohler's Medizinal-Pflanzen. Gera-Untermhaus – Verlag von Franz Eugen Kohler, **1897**, *1*, 270.

[133] ABI-Ayad F. Z., ABI-Ayad M. et. al., *Journalof Microbiology and Biotechnology Research*, **2011**, *1* (1), 1-6.

[134] Barrero A. F., Quílez del Moral J. F., Lucas R., Payá M., Akssira M., *Journal of Natural Products*, **2003**, *66*, 844-850.

[135] Ball P. W., Ajuga L. In: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA (eds) Flora Europaea III. Diapensiaceae to Myoporaceae. Cambridge University Press, Cambridge, **1972**, 128–129.

[136] Kubo I, Lee YW, Balogh-Nair V, Nakanishi K, Chapya A., *Journal of the Chemical Society, Chemical Communicaion*, **1976**, 949-50.

[137] Camps F., Coll J., *Phytochemistry*, **1993**, *32*, 1361-70.

[138] Coll J., Tandrón Y., *Phytochemistry Reviews*, **2008**, *7*, 25-49.

[139] Babu CR., Herbaceous flora of Dehra Dun. Publication and information directorate CSIR. New Delhi, **1977**, 405.

[140] Strachey R., Flora of Kumaun, Garhwal and adjoining areas of Tibet. Dehra Dun: Bishen Singh and Sons, **1974**.

[141] Wu CY., Flora Yunnanica. In: Botany YIs, Peking (China): Tomus Wall Science Press **1977**.

[142] Flora of China, [accessed: September 2007]; available from www.efloras.org **1994**.

[143] Hu K., Dong A., Liu H., Feng H., Sun Q., Yao X., *Pharmaceutical Biology*, **1999**, *37*, 225-30.

[144] Qingchun Z., Chengbin C., Bing C., Feng Q., Tao G., Xinsheng Y., *Pharmaceutical Biology*, **2005**, *43*, 135-9.

[145] Shen X., Isogai A., Furihata K., Sun H., Suzuki A., *Phytochemistry*, **1993**, *33*, 887-9.

[146] Shen X., Isogai A., Furihata K., Sun H., Suzuki A., *Phytochemistry*, **1993**, *34*, 1091-1094.

[147] Dhinda B., Banerjee J., Guha S., *Journal of the Indian Chemical Society*, **1997**, *74*, 424.

[148] Maggio A., Riccobono L., Spadaro V., Scialabba A., Bruno M., Senatore F., *Chemistry & Biodiversity*, **2014**, *11* (4), 652-672.

[149] Darriet F., Desjobert J. M., Costa J., Muselli A., *Phytochemical Analysis*, **2009**, *20*, 279.

[150] Pavlovic´ M., Lakusˇic´ D., Kovacˇevic´ N., Tzakou O., Couladis M., *Chemistry & Biodiversity*, **2010**, *7*, 1231.

[151] Hethelyi E., Palinkas G., Palinkas J., *Olaj, Szappan, Kozmetika*, **1999**, *48*, 116.

[152] Saroglou V., Dorizas N., Kypriotakis Z., Skaltsa H. D., *Journal of Chromatography, A* **2006**, *1104*, 313.

[153] Kilic O., Kocak A., Bagci E., *Naturforsch. Z.*, *C* **2011**, *66*, 535.

[154] Rahuman A. A., Venkatesan P., Gopalakrishnan G., *Parasitology Research*, **2008**, *103*, 1383–1390. doi:10.1007/s00436-008-1146-6.

[155] Riccobono L., Maggio A., Rosselli S., Ilardi V., Senatore F., Bruno M., *Natural Product Research*, (in press) DOI: 10.1080/14786419.2015.1030742

[156] Maggio A., Riccobono L., Spadaro V., Campisi P., Bruno M., Senatore F., *Chemistry & Biodiversity*, **2015**, *12* (5), 781-799.

[157] Casiglia S., Riccobono L., Bruno M., Senatore F., Senatore F., *Natural Product Research,* DOI: 10.1080/14786419.2015.1055267

[158] Sharifi-Rad J., Miri A., Hoseini-Alfatemi S. M., Sharifi-Rad M., Setzer W. N., Hadjiakhoondi A., *Natural Product Communications*, **2014**, *9*, 1633-1666.

[159] Abed N. E., Harzallah-Skhiri F., Boughalleb N., *Agric Segment*., **2010**, *1*, 1530-1534.

[160] Fawzy G. A., Al Ati H. Y., El Gamal A. A., *Pharmacognosy Magazine*, **2013**, *9*, 28-32.

[161] Algabr M. N., Ameddah S., Menad A., Mekkiou R., Chalchat J. C., Benayache S., Benayache F., *Journal of Medicinal and Aromatic Plants*, **2012**, *2*, 688-690.

[162] Znini M., Cristofari G., Majidi L., Paolini J., Desjobert J. M., Costa J., *LWT-Food Science and Technology*, **2013**, *54*, 564-569.

[163] Ali N. A. A., Sharopov F. S., Alhaj M., Hill G. M., Porzel A., Arnold N., Setzer W. N., Schmidt J., Wessjohann L., *Natural Product Communications.*, **2012**, *7*, 257-260.

[164] El-Kamali H. H., Yousif M. O., Osama A. I., Sabir S. S., *Ethnobotanical Leaflets.*, **2009**, *13*, 467-471.

[165] Al-Hajj N. Q. M., Ma C., Thabit R., Al-alfarga A., Gasmalla M. A. A., Musa A., Aboshora W., Wang H., *Journal of Academia and Industrial Research*, **2014**, *2*, 675-678.

[166] Al-Hajj N. Q. M., Wang H., Gasmalla M. A. A., Ma C., Thabit R., Rahman M. T. R., Tang Y., *Journal of Food and Nutrition Research*, **2014**, *2*, 221-227.

[167] Abed N. E., Harzallah-Skhiri F., Boughalleb N., *Agriculture Segment*, **2010**, *1*, 1530-1534.

[168] Basta A., Tzakou O., Couladis M., Pavlović M., *Journal of Essential Oil Research*, **2007**, *19*, 333-335.

[169] Ali N. A. A., Crouch R. A., Al-Fatimi M. A., Arnold N., Teichert A., Setzer W. N., Wessjohann L., *Natural Product Communications,* **2012**, *7*, 113-116.

[170] Hanbali F. E., Akssira M., Ezoubeiri A., Gadhi C. E., Mellouki F., Benherraf A., Blazquez A. M., Boira H., *Journal of Ethnopharmacology*, **2005**, *99*, 399-401.

[171] Alkhathlan H. Z., Al-Hazimi H. M. G., *Journal of the Chemical Society of Pakistan*, **1996**, *18*, 309-312.

[172] Al-Yahya M. A., El-Sayed A. M., Hassan M. M. A., El-Meshal I., *Arab Gulf J.Sci. Res. B-Agricul. Biol. Sci*, **1989**, *7*, 1-6.

[173] Diaz N., Ortega T., Pardo M. P., *Anales de la Real Academia Naciolal de Farmacia*, **1988**, *54*, 526-31.

[174] Goren A. C., Piozzi F., Akcicek E., Kılıç T., Çarıkçi S., Mozioglu E., Setzer W. N., *Phytochemistry Letters*, **2011***, 4*, 448-453.

[175] McGraw L. J., Jäger A. K., Van Staden J., *Fitoterapia*, **2002**, *73*, 431-433.

[176] Yff B. T. S., Lindsey K. L., Taylor M. B., Erasmus D. G., Jäger A. K., *Journal of Ethnopharmacoogyl*, **2002**, *79*, 101-107.

[177] Riccobono L., Ben Jemia M., Bruno M., Senatore F., *Plant Biosystem* (in press)

[178] Sacchetti G., Maietti S., Muzzoli M., Scaglianti M., Manfredini S., Radice M., Bruni R., *Food Chemistry*, **2005**, *91*, 621-632.

[179] Soares J. R., Dins T. C. P., Cunha A. P., Ameida L. M., *Free Radical Research*, **1997**, *26*, 469-478.

[180] Casiglia S., Ben Jemia M., Riccobono L., Bruno M., Senatore F., *Natural Product Research*, **2015**, *29* (13), 1201-1206.

[181] Setzer W. N., Schmidt J. M., Noletto J. A., Vogler B., *Pharmacologyonline*, **2006**, *3*, 794-802.

[182] Riela S., Bruno M., Formisano C., Rigano D., Rosselli S., Saladino M. L., Senatore F., *Journal of Separation Science*, **2008**, *31*, 1110–1117.

[183] Ramadan M., Goeters S., B. Watzer B., Krause E., Lohmann K., Bauer R., Hempel B., Imming P., *Journal of Natural Products*, **2006**, *69*, 1041-1045.

[184] Rosselli S., Maggio A., Raccuglia R. A., Bruno M., *European Journal of Organic Chemistry*, **2003**, 2690-2694.

[185] Avato P., Smitt U. W., *Journal of Essential Oil Research*, **2000**, *12*, 303-309.

[186] Kedzia B., *Herba Polonica*, **1991**, *37*, 29-38.

[187] Bozin B., Mimica-Dukic N., Bogavac M., Suvajdzic L., Simin N., Samojlik I., Couladis M., *Molecules*, **2008**, *13*, 2058-2869.

[188] Ahmed F. H., El Badri A. A., Ibrahim M. M. K., El Shahed A. S., El Khalafawy M. M., *Fats Oils*, **1994**, *45*, 260–264.

[189] Safayhi H., Sabieraj J., Sailer E. R., Ammon H. P., *Planta Medica*, **1994**, *60*, 410–413.

[190] Sizova N. V., *Pharmaceutical Chemistry Journal*, **2012**, *46*, 369–371.

[191] Capuzzo A., Occhipinti A., Maffei M. E., *Natural Product Research*, **2014**, *28*, 2321-2323

[192] Ornano L., Venditti A., Ballero M., Sanna C., Quassinti L., Bramucci M., Lupidi G., Papa F., Vittori S., Maggi F., Bianco A., *Chemistry & Biodiversity*, **2013**, *10*, 1464–1474.

[193] Inoue Y., Hada T., Shiraishi A., Hirose K., Hamashima H., Kobayashi S., *Antimicrobial Agents and Chemotherapy*, **2005**, *49*, 1770-1774.

[194] Xiong L., Peng C., Zhou Q. M., Wan F., Xie X. F., Guo L., Li X. H., He C. J., Dai O., *Molecules*, **2013**, *18*, 963-973.

[195] Mendiola J. A., Santoyo S., Cifuentes A., Reglero G., Ibanez E., Senorans F. J., *Journal of Food Protection*, **2008**, *71*, 2138-2143

[196] Quassinti L., Bramucci M., Lupidi G., Barboni L., Ricciutelli M., Sagratini G., Papa F., Caprioli G., Petrelli D., Vitali L. A., Vittori S., Maggi F., *Food Chemistry*, **2013**, *138*, 808-813.

[197] Antkowiak W., Apsimon J. W., Edwards O. E., *Journal of Organic Chemistry,* **1962**, *27*, 1930-1931.

[198] Fang J. M., Lee C. K., Cheng Y., *Phytochemistry,* **1993**, *33*, 1169-1172.

[199] Apsimon J. W., Edwards O. E., *Canadian Journal of Chemistry*, **1961**, *39*, 2543-2548.

[200] Lauro G., Romano A., Riccio R., Bifulco G., *Journal of Natural Products*, **2011**, *74*, 1401-1407.

[201] Cheruku P., Plaza A., Lauro G., KefferJ. R., Bifulco G., Bewley C. A., *Journal of Medicinal Chemistry*, **2012**, *55*, 735-742.

[202] Lauro G., Masullo M., Piacente S., Riccio R., Bifulco G., *Bioorganic and Medicinal Chemistry*, **2012**, *20*, 3596-3602.

[203] Trott O., Olson A. J., *Journal of Computational Chemistry*, **2010**, *31*, 455–461.

[204] Huey R., Morris G. M., Olson A. J., Goodsell D. S., *Journal of Compuational Chemistry*, **2007**, *28*, 1145–1152.

[205] Gong J., Sun P., Jiang N., Riccio R., Lauro G., Bifulco G., Zheng Q. F., Tang H., Li T. J., Gerwick W. H.,Zhang W., *Organic Letters,* **2014**, *16*, 2224-2227.

[206] Scrima M., Lauro G., Grimaldi M., Di Marino S., Tosco A., Picardi P., Gazzerro P., Riccio R., Novellino E., Bifulco M., Bifulco G., D'Ursi A. M., *Journal of Medicinal Chemistry*, **2014**, *57*, 7798-7803.

[207] Renga B., Mencarelli A.,D'Amore C., Cipriani S., D'Auria M. V., Sepe V.,Chini M. G., Monti M. C., Bifulco G., Zampella A., Fiorucci S., *Plos One*, **2012**, *7* (1), e30443.

[208] Kuria K. A. M., Chepkwony H., Govaerts C., Roets E., Busson R.,De Witte P., Zupko I., Hoornaert G.,Quirynen L., Maes L., Janssens L., Hoogmartens J., Laekeman G., *Journal of Natural Products*, **2002**, *65*, 789-793.

[209] Takasaki M., Tokuda H., Nishino H., Konoshima T., *Journal of Natural Products*, **1999**, *62*, 972-975.

[210] Li Y. M., Jiang S. H., Gao W. Y., Zhu D. Y., *Phytochemistry*, **1999**, *50*, 101-104

[211] Breschie M. C., Martmotti Catalanog S., Flamini G., Morelli I., Pagni A. M., *Journal of Natural Products*, **1992**, *55*, 1145–1148.

[212] Konoshima T., Takasakia M., Tokuda H., Nishino H., *Cancer Letters*, **2000**, *15*, 87–92

[213] Li W. W., Wu W. L., Liu S. J., Fang C. W., Liang Y. M., *Anhui Medical Pharmacy*, **2009**, *13*, 329–335.

[214] Xie Z. Y., Qin M. Z., Fang Y. L., *World Notes Plant Medica*, **2005**, *20*, 56–58.

[215] Zhang L. Q., Feng L., Jia Q., Xu J. W., Wang R., Wang Z. T., Wu Y. C., Li Y. M., *Bioorganic & Medicinal Chemistry*, **2011**, *19*, 4882–4886.

[216] Calcagno M. P., Camps F., Coll J., Melé E., Sanchez-Baeza F., *Tetrahedron*, **1996**, *52* (30), 10137-10146.

[217] *European Pharmacopoeia* 5th ed., **2005**, Council of Europe, Strasbourg (EDQM)

[218] Jennings W., Shibamoto T., *Qualitative Analysis of Flavour and Fragrance Volatiles by Glass Capillary Gas Chromatography*, **1980**, Academic Press, New York.

[219] Davies N. W., *Journal of Chromatography A*, **1990**, *503*, 1.

[220] Adams R. P., *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th Ed.*, **2007**, Allured Publishing Corp., Carol Stream, IL.

[221] Clarke K. R., Gorley R. N., *PRIMER v6: User Manual/Tutorial*, **2006**, PRIMER-E, Plymouth.

[222] Rigano D., Arnold Apostolides N., Conforti F., Menichini F., Formisano C., Piozzi F., Senatore F., *Natural Product Research*, **2011**, *25*, 614–626.

[223] Barry A., The antimicrobic susceptibility test: principles and practices. Philadelphia, **1976**, PA: Lea and Febiger.

[224] Hanato T., Kagawa H., Yasuhara X., Okuda T., *Chemical and Pharmaceutical Bulletin*, **1988**, *36*, 1090-1097.

[225] Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C., *Free Radical Biology & Medicine*, **1999**, *26*, 1231-1237.

[226] Schrödinger, *LLC New York NY*, **2013**.

[227] Jorgensen W. L., Tiradorives J., *Journal of the American Chemical Society*, **1988**, *110*, 1657-1666.

[228] Trott O., Olson A. J., *Journal of Computational Chemistry*, **2010**, *31*, 455-461.

[229] Mossa J. S., Hifnawy M. S., Al-Yahya M. A., Al-Meshal I. A., Mekkawi A. G., *International Journal of Crude Drug Research*, **1987**, *25*, 113.

[230] Mumivand H., Rustaii A. R., Jahanbin K., Dastan D., *Journal of Essential Oil Bearing Plants,* **2010**, *13*, 717.

[231] Weyerstahl R., Marschall H., Christiansen C., Rustaiyan A., Mirdjalili F., *Flavour Fragrance Journal*, **1999**, *14*, 121.

[232] Khani A., Asghari J., *Journal of Insect Science* (Madison, WI, United States), **2012**, *12*, 73.

[233] Kazemi M., Nagafi G. R., Azad A., *Asian Journal of Chemistry*, **2013**, *25*, 3215.

[234] Bashi D. S., Ghani A., J. Asili, *Journal of Essential Oil Bearing Plants,* **2013**, *16*, 252.

[235] Yousuf M. A., Bashir A., Veres K., Dobos A., Nagy G., Mathe I., Blunden G., *Journal of Essential Oil Research*, **2001**, *13*, 454.

[236] Nematollahi F., Rustaiyan A., Larijani K., Nadimi M., Masoudi S., *Journal of Essential Oil Research*, **2006**, *18*, 339.

[237] Ravandeh M., Valizadeh J., Noroozifar M., Khorasani-Motlagh M., *Journal of Medicinal Plants Resarch*, **2011**, *5*, 2035.

[238] Avato P., Jacobsen N., Smitt U. W., *Journal of Essential Oil Research*, **1992**, *4*, 467-473.

[239] Goncalves M. J., Cruz M. T., Tavares A. C., Cavaleiro C., Lopes M. C., Canhoto J., Salgueiro L., *Industrial Crops and* Products, **2012**, *35*, 166-171.

[240] Avato P., Trabace G., Smitt U. W., *Phytochemistry*, **1996a**, *43*, 609-612.

[241] Avato P., Trabace G., Smitt U. W., *Journal of Essential Oil Research*, **1996b**, *8*, 123-128.

[242] Rufino A. T., Ferreira I., Judas F., Salgueiro L., Lopes M. C., Cavaleiro C., Mendes A. F., *Pharmaceutical Biology*, **2015**, *53*, 1220-1230.

7. TABLES OF THE COMPOSITION OF ESSENTIAL OILS, EXTRACTS AND OF THE BIOLOGICAL ACTIVITY

7.1. TABLES OF THE COMPOSITION AND OF THE BIOLOGICAL ACTIVITY OF ESSENTIAL OILS FROM *ANTHEMIS*

***Table 2*:** Chemical constituents of the essential oils from some species of *Anthemis* arranged by class.

KI[a]	KI[b]	COMPONENT[d]	*A*1f	*A*1l	*A*2a	*A*2f	*A*3a	*A*4f	*A*4l	*A*5f	*A*5l	*A*6a	*A*7a	*A*8a	*A*9a	*A*10a	Id.[c]
		Hydrocarbons	**6.3**	**2.2**	**0.5**	**23.4**	**3.2**	**5.2**	**0.5**	**3.3**	**0.6**	**1.8**	**0.1**	**0.7**	**1.2**	**5.7**	
1735	2434	Chamazulene													0.3		1,2
2100	2100	Heneicosane	0.2	0.2				0.1									1,2,3
2200		Docosane				t[d]											1,2,3
2300	2300	Tricosane	1.4	0.5		9.8	0.2	2.3	0.1	1.3		0.2				0.2	1,2,3
2400	2400	Tetracosane	0.2			0.2		0.1	t	t	0.1						1,2,3
2500	2500	Pentacosane	1.2	0.3		6.4	0.5	1.6	0.1	0.9		0.3			0.2	0.4	1,2,3
2700	2700	Heptacosane	1.9	0.5	0.2	4.5	1.1		0.1	0.7	0.2	0.7		0.4	0.4	2.1	1,2,3
2900	2900	Nonacosane	1.4	0.7	0.3	2.5	1.4	1.0	0.2	0.4	0.3	0.6	0.1	0.3	0.3	3.0	1,2,3
3100	3100	Hentiacontane						0.1									1,2
		Carbonylic Compounds	**10.9**	**0.3**	**1.1**	**1.9**	**0.0**	**0.5**	**0.1**	**0.3**	**0.1**	**0.0**	**0.2**	**0.0**	**1.5**	**7.1**	
963	1543	Benzaldehyde	0.4	0.1				0.1		0.1							1,2,3
987	1346	6-methyl-5-hepten-2-one (Prenylacetone)			t											3.9	1,2
1044	1663	Phenylacetaldehyde		0.1													1,2,3
1102	1616	Nonanal	0.4		0.2	t		0.1		0.1	0.1		0.2		t	0.1	1,2
1203	1510	Decanal	0.7														1,2
1315	1827	(*E*,*E*)-2,4-decadienal	t		t									t			1,2
1315	1827	(*E*,*E*)-2,4-heptadienal			t												1,2
1397	1959	*Cis*-jasmone	0.1	0.1													1,2
1484	1958	(*E*)-β-ionone													0.4		1,2
1715	2040	Pentadecanal	0.6		0.3	1.1										0.5	1,2
1760	2655	Benzyl benzoate	0.3					0.1		0.1							1,2
1815	2118	Hexadecanal	0.3														1,2
1845	2131	Hexahydrofarnesylacetone	7.9		0.6	0.8		0.2	0.1						1.1	2.4	1,2

2008	2165	Octadecenal	0.2													1,2
2221	2571	Eicosanal													0.2	1,2,3
		Monoterpene Hydrocarbons	**10.4**	**10.3**	**26.8**	**22.4**	**0.2**	**6.3**	**16.1**	**5.8**	**16.7**	**4.6**	**19.5**	**20.9**	**12.2**	**4.1**	
909	1032	Santolina triene	1.5	0.6							0.2		8.0	5.8			1,2
925	1073	Artemisia triene			0.1	0.1			t		0.1	3.5			0.2		1,2
930	1014	α-thujene		0.2	0.2	0.1			0.2	0.2	0.1	0.2			0.1		1,2
938		Verbenene									0.1						1,2
938	1032	α-pinene	3.1	3.1	18.4	13.2		0.8	2.6	3.9	10.6	0.5	6.7	5.4	2.5	1.4	1,2,3
952	1073	α-fenchene			0.5	t										1.2	1,2,3
953	1076	Camphene		0.1				0.2		0.5	1.2		0.3	0.8			1,2
973	1132	Sabinene	1.7	2.0	5.0	7.6		3.3	8.7	0.2	0.8				7.7	0.9	1,2
980	1118	β-pinene	0.4	0.5	1.0	1.2	0.1			0.7	2.2		3.6	5.0	0.5	0.3	1,2,3
993	1174	Myrcene	2.4	2.7				1.1	2.3	0.1	0.2		0.3	1.1			1,2,3
1012	1157	δ^3-carene	0.6														1,2
1013	1189	α-terpinene	0.1	0.2	t	t		0.1	0.4						0.1		1,2,3
1025	1278	*p*-cymene	0.3	0.2	1.0	0.1		0.2	0.5		0.2	0.4	0.2	0.7	0.7	0.3	1,2,3
1029		β-phellandrene									0.1						1,2
1030	1203	Limonene			0.3	t	0.1				0.8		0.4	1.6	0.1		1,2,3
1038	1243	(Z)-β-ocimene						0.2	0.3					0.3			1,2
1049	1262	(E)-β-ocimene		0.1				0.3	0.5	0.1				0.2			1,2
1057	1256	γ-terpinene	0.3	0.2	0.3	0.1		0.1	0.4	0.1					0.3		1,2,3
1086	1265	Terpinolene		0.1					0.1								1,2,3
1091	1492	*p*-cymenene		0.1													1,2
1114	1408	1,3,8-*p*-menthatriene		0.2					0.1		0.1						1,2
		Sesquiterpene Hydrocarbons	**29.5**	**31.1**	**8.6**	**9.7**	**1.0**	**7.5**	**6.9**	**2.6**	**0.5**	**11.1**	**13.8**	**8.1**	**37.4**	**2.3**	
1337	1468	δ-elemene		0.1											0.3		1,2
1352	1466	α-cubebene	0.4	0.2		0.2								0.1			1,2

1356	1579	α-longipinene	t												1.5	0.4	1,2
1373	1493	α-ylangene			0.3								0.2				1,2
1377	1497	α-copaene	0.7	0.6	0.6			0.2	0.1			0.1	0.5		t	0.1	1,2
1378	1600	β-elemene	0.5			t					t				t		1,2
1382	1502	1,7-di-epi- α-cedrene; α-funebrene	0.3									0.8					1,2
1385	1535	β-bourbonene			0.1	t								t	0.1	0.7	1,2
1398	1523	Cyperene		0.3													1,2
1403	1592	β-longipinene							0.3								1,2
1407	1538	α-gurjunene	t		0.1	0.1								t			1,2
1411	1568	α-cedrene										5.9	0.2		1.9		1,2
1418	1612	(*E*)-caryophyllene	8.3	5.6	1.7	2.9		1.2	0.4	0.5	0.2		0.5	1.1		0.6	1,2,3
1432	1612	β-cubebene	0.4	0.2								0.7					1,2
1437	1628	Aromadendrene	0.3		0.5							0.1					1,2
1438	1573	*trans*-α-bergamotene		1.2													1,2
1442	1498	epi-bicyclosesquiphellandrene		0.8					0.4					t			1,2
1449	1662	α-himachalene							0.6								1,2
1452	1672	(*E*)-β-farnesene	0.7		1.7	1.5				0.1					0.6	0.5	1,2
1454	1691	α-acoradiene							0.4								1,2
1455	1689	α-humulene	2.6	1.1				0.3		0.1	t			0.2	0.5		1,2
1456	1634	(*E*)-muurola-3,5-diene		0.4					1.6								1,2
1463	1661	*Allo*-aromadendrene	0.6	0.4			0.1										1,2
1467	1545	(*Z*)-muurola-4(14),5-diene	0.6	0.5											27.3		1,2
1474	1661	*trans*-cadina-1(6),4-diene							0.3								1,2
1475	1715	β-selinene			0.2								0.1				1,2
1477	1726	Germacrene D			0.6					1.4	0.3						1,2
1478	1704	γ-muurolene	0.5	1.0	t	4.1	0.5					0.2			3.5		1,2
1482	1692	γ-curcumene	0.2	1.2				0.5					0.5	0.2			1,2

1485	1675	Epizonarene						1.2					0.1				1,2
1486	1733	α-selinene											0.7	0.3			1,2
1487	1679	α-amorphene	0.4	0.6	1.7		0.1	1.1					1.2		0.1		1,2
1489	1729	(*Z*,E)-α-farnesene	0.5		0.3			0.9						0.2			1,2
1490	1694	*ar*-curcumene	0.9	0.7									1.2	0.4	0.1		1,2
1490	1612	β-guaiene										2.3					1,2
1491	1756	Bicyclogermacrene													0.4		1,2
1495	1740	Valencene							1.4	0.2		0.1					1,2
1495	1723	Zingiberene	0.9	0.2									1.0	0.2	0.5		1,2
1495	1712	β-himachalene											0.1				1,2
1496	1785	(*E*)-muurola-4(14),5-diene		1.5													1,2
1498	1725	δ-selinene	0.5	0.1		t		0.2				0.1					1,2
1503	1740	α-muurolene		2.2	0.3			0.1					1.3	0.4	t		1,2
1506	1760	(*E*,*E*)-α-farnesene		0.1					0.3	0.3		0.6	0.5	1.2			1,2
1506		α-chamigrene			t												1,2
1509	1746	(*Z*)-α-bisabolene													0.3		1,2
1510	1743	β-bisabolene		1.3									0.3				1,2
1515	1776	γ-cadinene		1.2													1,2
1520	1839	1-*S*-*cis*-calamenene	0.5	0.5			0.3										1,2
1526	1773	δ-cadinene	9.0	8.2	0.5	0.9		1.8	1.1				5.0	3.7	0.3		1,2
1533	1802	Cadina-1,4-diene = Cubenene	0.7	0.5									0.2				1,2
1541	1918	α-calacorene		0.1									0.2				1,2
1558	1818	Germacrene B		0.3								0.2		0.1			1,2
		Oxygenated Monoterpenes	**24.3**	**19.6**	**41.1**	**17.8**	**0.1**	**4.9**	**4.6**	**48.5**	**36.5**	**20.6**	**36.1**	**40.9**	**14.1**	**46.8**	
994	1405	Yomogi alcohol			8.2	1.5									1.6	0.7	1,2
1034	1213	1,8-cineole	13.3	12.2	0.2	0.5		2.9	3.9	1.9	1.2				0.2	0.1	1,2,3
1063	1555	(*Z*)-sabinene hydrate													0.2	0.7	1,2

1085	1512	Artemisia alcohol			1.9	0.7						2.7			0.2		1,2
1093	1474	*trans*-sabinene hydrate													0.1	t	1,2
1098	1553	Linalool													0.3		1,2,3
1105	1431	α-thujone		0.5	1.0	0.7							0.4	0.9		1.4	1,2
1115	1451	β-thujone			11.8	3.4										7.8	1,2
1115	1575	*endo*-fenchol			0.5												1,2
1124	1684	*trans*-chrysanthenol								2.7	1.0	0.7		5.2			1,2
1125	1540	Chrysanthenone									0.2		0.5	2.6			1,2
1128	1487	α-campholenal		0.1	0.6	0.1			0.1				0.2	0.4			1,2
1142	1721	*trans*-sabinol	2.0														1,2
1142	1639	*trans-p*-2,8-menthadien-1-ol			t												
1144	1663	*trans*-verbenol		0.1	0.4	t									t		1,2
1144	1663	*cis*-verbenol			0.3										t		1,2
1145	1532	Camphor		1.3		t		0.2	0.5	2.5	1.6	1.5	0.3	1.0			1,2,3
1145	1665	Umbellulone		0.2													1,2
1276	1658	Sabinyl acetate						0.2									1,2
1479	1835	Geranyl propionate								1.3	0.5						1,2
1156	1765	*cis*-chrysanthenol												2.3			1,2
1167	1718	Borneol	0.1	0.4		0.1		0.2	t							0.4	1,2,3
1167	1685	Lavandulol								0.1	t	0.2				4.5	1,2
1174	1565	*cis*-pinocamphone	0.2	0.1	0.2								0.1				1,2
1176	1611	Terpinen-4-ol	2.9	1.0	0.7	0.1		0.9		0.4	0.3		0.3		0.4	0.3	1,2,3
1185	1856	*p*-cymen-8-ol	0.1		t				0.1								1,2
1189	1706	α-terpineol	4.0	3.6	0.2	0.1	0.1	0.4						0.2			1,2,3
1196	1804	Myrtenol			0.1												1,2
1215	1449	Artemisyl acetate			12.7	3.6									6.8		1,2
1217	1845	*trans*-carveol			0.2												1,2,3

1218		*endo*-fenchyl acetate													t		1,2
1223	1584	α-campholenic acid methyl ester			0.3												1,2
1226	1809	Nerol			t										0.3		1,2,3
1235	1583	(*E*)-chrysanthenyl acetate	0.3	0.1						4.7	2.9	1.3	28.8	24.2			1,2
1235	1857	Geraniol			t										t		1,2,3
1241	1752	Carvone											0.2				1,2
1254	1983	*cis*-piperitone oxide				6.7											1,2
1257	1585	(*Z*)-chrysanthenyl acetate	0.5		1.2	0.2				8.6	5.7	1.9	1.5	2.7			1,2
1265	2113	Cumin alcohol	0.3		t												1,2
1284	1597	Bornyl acetate	0.2		0.4			0.1		3.3	4.8	1.8	0.4	1.1	0.1	7.9	1,2,3
1289	1619	Lavandulyl acetate								0.3			0.7			4.3	1,2
1329	1748	Piperitenone		t		t											1,2
1358	1734	Neryl acetate											0.2		3.5	2.4	1,2
1365	1983	Piperitenone oxide: *p*-menth-4(8)-en-3-one				0.1											
1377	1765	Geranyl acetate	0.4			t				22.7	18.2	10.5	2.5	0.3	0.4	0.3	1,2
1439	1684	Linalyl butyrate														0.7	1,2
1463	1789	Neryl propionate														6.5	1,2
1479	1835	Geranyl propionate			0.2											8.8	1,2
1602	1893	Geranyl isovalerate									0.1						1,2
		Oxygenated Sesquiterpenes	**10.3**	**27.8**	**10.1**	**12.6**	**87.2**	**65.1**	**64.7**	**30.4**	**26.9**	**52.3**	**21.1**	**20.0**	**30.2**	**24.3**	
1497	1805	Linalyl isovalerate								0.4							1,2
1553	2076	*cis*-α-copaen-8-ol				0.8						1.4					1,2
1564	2050	(*E*)-nerolidol		0.2													1,2
1578	2150	Spathulenol			0.8					0.1	0.1				2.4	1.6	1,2
1579	2208	Caryophyllene oxide		1.7	1.2		0.2	0.4						0.5	3.8	2.1	1,2,3
1585	2135	β-copaen-4-α-ol		0.2								1.1	0.3			0.2	1,2
1591	2104	Viridiflorol							t					t	0.3		1,2

1598	2107	Guaiol										0.2	0.4	t		1,2
1599	2120	α-cedrol													2.0	1,2
1602	2037	Salvial-4(14)-en-1-one (Mintketone)			0.3										0.6	1,2
1632	2371	Caryophylla-3,8(13)-dien-5-α-ol							0.1	0.2				0.2	1.3	1,2
1634	2089	1-*epi*-cubenol										0.5	5.4	2.7		1,2
1637		Muurola-4,10(14)-dien-1 β-ol	0.2												0.7	1,2
1638	2223	Isospathulenol			1.9										10.6	4.4	1,2
1640	2185	τ-cadinol	3.4				0.5	0.4				1.5	2.4	4.4	1.4	0.7	1,2
1643	2209	τ-muurolol	1.1		3.9	4.1	0.9	0.6		5.0	2.1	7.3	2.2	2.9	0.8	6.5	1,2
1645	2145	Torreyol	4.6	5.0			85.4	63.0	63.5	8.0	6.2	0.1	0.7	1.8			1,2
1649	2396	Caryophyllenol II														0.7	1,2
1650	2258	β-eudesmol	0.7		0.8	1.0										2.8	1,2
1652	2255	α-cadinol	0.3	4.2		1.6	0.2	0.7	0.9	0.2						1.8	1,2
1672	2357	(*Z*)-α-santalol													1.8	0.4	1,2
1681	2332	Khusinol													0.4		1,2
1682	2232	α-bisabolol		2.6									1.1	1.0			1,2
1687	2143	Valeranone		t		2.1			0.2								1,2
1689	2359	8-cedren-13-ol													2.1	2.4	1,2
1692	2342	(2*Z*,6*E*)-farnesol	t	0.9											0.5	0.7	1,2
1692	2245	*epi*-α-bisabolol		5.3								0.3	0.5	1.2			1,2
1716	2478	14-hydroxy-α-humulene		7.2						16.5	18.5	39.9	8.1	5.3	0.3		1,2
1751	2332	α-Sinensal		0.5													1,2
1771		α-muurolen-15-al													0.2		1,2
1780	2607	β-Costol			0.6	0.8											1,2
1797	2298	α-(*Z*)-bergamotyl acetate			0.3												1,2
1843	2274	(*E*,*E*)-farnesyl acetate			0.3	2.2									1.0		1,2
		Diterpenes	**1.7**	**0.4**	**0.2**	**0.0**	**0.2**	**0.1**	**0.0**	**0.0**	**0.0**	**0.8**	**0.0**	**0.2**	**1.7**	**0.8**	

1989	2393	Manoyl oxide						0.1								1,2
2135	2625	(*E*)-phytol	1.7	0.4	0.2		0.2					0.8		0.2	1.7	0.8	1,2
		Phenolic Compounds	**0.5**	**0.5**	**0.3**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	
1189	1789	Methyl salicylate	0.2							t							1,2,3
1294	2198	Thymol		0.1	0.3												1,2
1299	2239	Carvacrol			t												1,2,3
1867	2810	Benzyl salicylate	0.3	0.4													1,2,3
		Fatty Acids and Derivatives	**0.0**	**0.0**	**0.0**	**4.4**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	
1680	2396	γ-dodecalactone				1.1											1,2
2122	3157	(*Z,Z*)-9,12-octadecadienoic acid				3.3											1,2,3
		Others	**0.0**	**0.0**	**0.1**	**0.0**	**0.0**	**0.0**	**0.0**	**0.1**	**0.1**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	
977	1452	1-octen-3-ol			0.1												1,2
1490	2016	Isoamyl phenylacetate								0.1	0.1						1,2
		TOTAL	**93.7**	**92.2**	**88.8**	**92.2**	**91.9**	**89.6**	**92.9**	**91.0**	**81.4**	**91.2**	**90.8**	**90.8**	**98.3**	**91.1**	

[a]: retention index on a HP-5MS column; [b] : retention index on a HP-Innowax column; [c]: Identification, 1 = comparison of retention index; 2 = comparison of mass spectra with MS libraries identification; 3 = co-injection with authentic compounds; [d]: t = trace, less than 0.05 %.

a = aerial parts, l = leaves, f = flowers, A1: *A. Montana*, A2: *A. cupaniana*, A3: *A. arvensis* subsp. s*phacelata*, A4: *A. affine cupaniana*, A5: *A. aetnensis*, A6: *A.*collected in cava grande, A7: *A. messanensis* on the rocks, A8: *A. messanensis* in the greenhouse, A9: *A. pignattorum*, A10: *A. ismelia*

Table 3*:* Main constituents of previously investigated essential oils of the taxa belonging to the genera *Anthemis* L. and *Chamaemelum* P. Mill.

Genus Anthemis L.																				
Subgenus *Anthemis*																				
	Section *Hiorthia* (DC.) R. Fernandes														**Section *Anthemis***					
Component	***A*(a)**	***A*(l)**	***A*(f)**	***B*(a)**	***C*(a)**	***D*(a)**	***E*(a)**	***F*(a)**	***G*(a)**	***H*(a)**	***I*(a)**	***J*(a)**	***K*(a)**	***L*(a)**	***M*(a)**	***N*(a)**	***O*(f)**	***P*(f)**	***Q*(a)**	***R*(a)**
Carbonylic Compounds	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**15.6**	**6.2**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**8.3**	**0.0**	**0.0**
6-Methyl-5-hepten-2-one										15.6	6.2							8.3		
Monoterpene Hydrocarbons	**55.1**	**11.5**	**46.7**	**44.2**	**0.0**	**28.9**	**0.0**	**0.0**	**15.0**	**7.8**	**1.2**	**4.8**	**0.0**	**51.9**	**0.0**	**60.0**	**48.5**	**48.5**	**3.6**	**4.4**
Azulene									15.0											
Limonene	1.5	2.1	3.4																	

Myrcene										2.7										
Sabinene																	15.2			
Santolinatriene				44.2										49.5		14.8				
α-Pinene	49.4	9.4	39.0			14.3				3.4	1.2			2.4			27.0	28.1		
β-Myrcene																		4.8		
β-Pinene	4.2		4.3			14.6				2.7		4.8				45.2		15.6	3.6	4.4
Sesquiterpene Hydrocarbons	**0.0**	**0.0**	**0.0**	**3.3**	**0.0**	**0.0**	**0.0**	**0.0**	**26.1**	**3.7**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**3.2**	**9.6**	**5.8**	**0.0**	**9.8**
Bicyclogermacrene										3.7										
Germacrene																	9.6			
Germacrene D				3.3					5.8							3.2				9.8
α-Curcumene																		5.8		
β-Caryophyllene									20.3											
Oxygenated Monoterpenes	**21.8**	**27.0**	**32.1**	**4.1**	**81.7**	**10.6**	**46.1**	**36.8**	**0.0**	**0.0**	**9.6**	**0.0**	**62.9**	**0.0**	**5.4**	**6.8**	**6.8**	**0.0**	**16.6**	**10.2**
1,8-Cineole								7.2											6.8	
Borneol						10.6		4.5												3.4
Camphor								19.4			3.8									
Geraniol Formate																	6.8			
Methyl chavicol				4.1																
Myrtenal																3.5				
Myrtenol																3.3				
Pinocarvone															5.4					
Terpinen-4-ol	21.8	24.3	32.1		9.7			5.7											6.2	6.8
Thujone					13.3															
trans-Chrysantenol											5.8									
trans-Thujone							39.0													
trans-Verbenol		2.7																		
Yomogi Alcohol					18.5		7.1													
α-Terpineol																			3.6	
α-Thujone					40.2								46.9							
β-Thujone							39.0						16.0							
Oxygenated Sesquiterpenes	**0.0**	**5.2**	**0.0**	**3.0**	**0.0**	**6.5**	**0.0**	**4.3**	**6.0**	**27.2**	**54.4**	**32.2**	**11.3**	**0.0**	**4.6**	**3.0**	**14.5**	**0.0**	**0.0**	**0.0**
Caryophyllene oxide				3.0												3.0				
Cedren-13-ol																	14.5			

cis-Chrysanthenyl 2-methylbutyrate										7.9	4.3									
cis-Chrysanthenyl angelate										3.6										
cis-Chrysanthenyl isobutyrate										2.5	1.2									
cis-Chrysanthenyl isovalerate										7.9	1.8									
cis-Chrysanthenyl propionate										1.8	0.7									
cis-Chrysanthenyl tiglate										3.5	1.4									
Humulene Epoxide II												5.9								
Isofaurinone		5.2																		
Isospathulenol															4.6					
Spathulenol								4.3	6.0			21.7								
trans-Chrysanthenyl acetate											45.1		11.3							
α-Bisabolol												4.6								
β-Acorenol						6.5														
Diterpenes	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**5.4**	**0.0**
Isophyllocladene																			5.4	
Fatty Acid	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**5.8**	**27.7**	**0.0**	**0.0**	**0.0**	**9.9**	**0.0**
Dodecanoic acid														3.1						
Linoleic acid															6.5					
Palmitic acid														2.7	21.2				9.9	
TOTAL	**76.9**	**43.7**	**78.8**	**54.2**	**81.7**	**46.0**	**46.1**	**41.1**	**47.1**	**38.7**	**65.3**	**37**	**74.2**	**57.7**	**37.7**	**73**	**79.4**	**62.6**	**35.5**	**24.4**

a = aerial parts, l = leaves, f = flowers, A: *A. aciphylla* Boiss. var. *discoidea* Boiss. (syn. *Anthemis rouyana* Azn.) collected in Turkey, B: *A. aciphylla* Boiss. var. *aciphylla* Boiss. Collected in Turkey, C: *A. carpatica* Willd. Collected in Serbia, D: *A. cretica* L. subsp. *argaea* (Boiss.) Grierson collected in Turkey, E: *A. cretica* L. subsp. *carpatica* (Willd.) Grierson collected in Serbia Montenegro, F: *A. cretica* L. subsp. *leucanthemoides* (Boiss.) Grierson collected in Turkey, G: *A. cretica* L. subsp. *pontica* (Willd.) Grierson collected in Turkey, H: *A. maritima* L. collected in Corsica west Sardinia, I: *A. maritima* L. collected in Est Sardinia, J: *A. marschalliana* Wild ssp. *pectinata* (Boiss) Grierson collected in Turkey, K: *A. montana* Willd. (syn. *A. cretica* L. subsp. *cretica*) collected in Serbia, L: *A. pectinata* (Bory & Chaub.) Boiss. & Reut. (= *Anthemis pectinata* (Bory & Chaub.) collected in Turkey, M: *A. arvensis* L. collected in Serbia, N: *A. bourgaei* Boiss. & Reut. (=*Anthemis coelopoda* Boiss. var. *bourgaei* Boiss.) collected in Turkety, O : *A. mauritiana* Maire & Sennen collected in Morocco, P: *A. mauritiana* Maire & Sennen collected in Morocco, Q: *A. ruthenica* Bieb. Collected in Serbia, R: *A. ruthenica* Bieb. Collected in Serbia Montenegro

***Table 3**: continuation*

Genus *Anthemis* L.																				
Subgenus *Anthemis*																				
	Section *Anthemis*											**Section *Maruta* (Cass.) Griseb**							**Section *Chia* Yavin.**	
Component	***S*(l)**	***T*(a)**	***U*(a)**	***V*(a)**	***W*(a)**	***W*(r)**	***X*(a)**	***Y*(a)**	***Z*(f)**	***Z*(l)**	***AA*(a)**	***AB*(a)**	***AC*(f)**	***AC*(l)**	***AD*(a)**	***AE*(a)**	***AF*(a)**	***AG*(a)**	***AH*(a)**	***AI*(a)**
Hydrocarbons	**0.0**	**0.0**	**0.0**	**0.0**	**30.0**	**27.5**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**10.8**	**11.0**	**0.0**	**0.0**	**0.0**	**0.0**	**7.2**	**0.0**
1-Eicosane														11.0						
Heptacosane					8.1	6.8														
n-Nonadecane													10.8							
Nonacosane					21.9	20.7														
Tricosane																			7.2	
Carbonylic Compounds	**0.0**	**0.0**	**0.0**	**0.0**	**6.8**	**5.9**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**5.2**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Hexahydrofarnesylacetone					6.8	5.9														
Nonanal															5.2					
Monoterpene Hydrocarbons	**39.8**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**22.4**	**8.0**	**25.3**	**30.9**	**0.0**	**0.0**	**0.0**	**11.9**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
cis-β-Ocimene								7.5	2.1	4.3										
Myrcene									3.6	16.9					5.4					
p-Cymene								7.1												
Sabinene	6.1																			
Santolinatriene	27.3																			
trans-β-Ocimene								7.8							6.5					
α-Pinene									2.3	4.1	30.9									
β-Pinene	6.4																			
Sesquiterpene Hydrocarbons	**0.0**	**6.0**	**0.0**	**15.2**	**0.0**	**0.0**	**5.4**	**20.6**	**12.7**	**5.4**	**0.0**	**51.4**	**15.2**	**7.1**	**14.3**	**32.1**	**0.0**	**0.0**	**12.1**	**4.5**
(*E*)-β-Farnesene																11.1				
(*E*,*E*)-α-Farnesene													6.0							
Aromadendrene														7.1						
Bicyclogermacrene				15.2					3.5											
Calarene												30.5								
Cedrane													9.2							
Farnesene												20.9								
Germacrene D								9.5	5.1						8.9	7.2			4.6	1.0

β-Caryophyllene		6.0						11.1	4.1	5.4									7.5	1.9
β-Cedrene																13.8				
β-Selinene																				1.6
γ-Curcumene							5.4													
γ-Muurulene															5.4					
Oxygenated Monoterpenes	**5.0**	**19.7**	**39.5**	**6.9**	**5.2**	**0.0**	**0.0**	**4.9**	**19.9**	**0.0**	**38.4**	**2.2**	**0.0**	**0.0**	**0.0**	**0.0**	**21.5**	**64.3**	**4.0**	**3.8**
1,8-Cineole	5.0		12.1	1.5													7.5	39.4		
4-Thujen-2α-yl acetate																				2.9
Artemisiaketone																		5.7		
Borneol												2.2								
Camphor									11.6									9.4		
Carvacrol											38.4									
Chrysanthenone																	5.1			
Eugenol					5.2															
Filifolene																		5.1		
Limonen-10-ol				5.4																
Linalool			14.5					4.9									8.9		4.0	0.9
Linalyl acetate			12.9																	
Nopol		14.7																		
Terpinen-4-ol		5.0							8.3											
α-Terpineol																		4.7		
Oxygenated Sesquiterpenes	**0.0**	**0.0**	**4.8**	**0.0**	**0.0**	**0.0**	**9.4**	**14.6**	**14.9**	**22.1**	**0.0**	**15.3**	**0.0**	**0.0**	**6.4**	**10.1**	**0.0**	**0.0**	**10.2**	**52.3**
Caryophyllene oxide			4.8																2.8	1.6
cis-Crhysantenyl acetate									14.9	17.8									3.0	47.4
Elemol																4.0				
Farnesol												15.3								
Isospathulenol										4.3										
Spathulenol							9.4								6.4	6.1			4.4	1.3
α-Eudesmol								14.6												
β-Eudesmol																				2.0
Diterpenes																				
14-Labdadien-8-ol		12.1																		
Phytol																4.0				

Fatty Acid	**0.0**	**0.0**	**0.0**	**0.0**	**8.2**	**32.1**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**13.5**	**0.0**	**0.0**	**0.0**
Palmitic acid					8.2	32.1											13.5			
Other	**4.6**	**0.0**	**12.9**	**0.0**	**0.0**	**0.0**	**8.4**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**8.9**	**0.0**	**0.0**	**4.1**	**0.0**	**0.0**	**0.0**
Benzyl salicylate														8.9						
cis-3-Hexenyl benzoate		9.1																		
n-Hexyl-2-methylbutanoate	4.6																			
n-Octyl-2-methylbutanoate							8.4													
α-Phenylpropanoid																	4.1			
TOTAL	**49.4**	**46.9**	**44.3**	**22.1**	**50.2**	**65.5**	**23.2**	**62.5**	**55.5**	**52.8**	**69.3**	**68.9**	**26.0**	**27.0**	**37.8**	**46.3**	**39.1**	**64.3**	**33.5**	**60.6**

l = leaves, a = aerial parts, r = root, f = flowers, S: *A. melampodina* Del. Collected in Egypt, T: *A. werneri* L. subsp. *werneri* Stoj. and Acht. Collected in Greece, U: *A. tomentosa* L. collected in Greece Nomos Attikis, V: *A. tomentosa* L. collected in Greece Nomos Korinthias W: *A. tomentosa* L. collected in Greece Skianthos, X: *A. auriculata* Boiss. Collected in Greece Nomos Korinthias Y: *A. auriculata* Boiss.Greece Nomos Viotias, Z: *A. hyalina* DC. (syn. *A. crassipes* Boiss.) collected in Iran, AA: *A. hyalina* DC. (syn. *A. crassipes* Boiss.) collected in Iran, AB: *A. cotula* L. collected in Argentina, AC: *A. cotula* L. collected in Iran, AD: *A. cotula* L. collected in Greece, AE: *A. cotula* L. Serbia Montenegro, AF: *A. pseudocotula* Boiss. Collected in Turkey, AG: *A. pseudocotula* Boiss. Collected in Turkey, AH: *A. chia* L. collected in Greece Nomos Achaia, AI: *A. chia* L. collected in Greece,

***Table 3**: continuation*

Genus *Anthemis* L.																			
Subgenus *Cota* (J. Gay) Rouy																			
	Section *Cota*																**Section *Anthemaria* Dumort**		
Component	***AJ*(a)**	***AK*(f)**	***AK*(l)**	***AK*(s)**	***AL*(f)**	***AL*(l)**	***AM*(f)**	***AM*(l)**	***AN*(f)**	***AO*(a)**	***AP*(a)**	***AQ*(a)**	***AR*(a)**	***AS*(a)**	***AT*(a)**	***AU*(a)**	***AV*(a)**	***AW*(a)**	***AX*(a)**
Hydrocarbons	**0.0**	**0.0**	**0.0**	**0.0**	**13.2**	**0.0**	**0.0**	**0.0**	**4.4**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
1-Eicosane					7.0														
Tricosane									4.4										
Carbonylic Compounds	**27.1**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**13.8**	**0.3**	**0.0**	**0.0**	**8.3**	**0.0**	**0.0**	**8.3**
Benzaldehyde	27.1											13.8	0.3						
Hexahydrofarnesylacetone																8.3			8.3
Nonanal																			
Monoterpenes Hydrocarbons	**8.2**	**0.0**	**0.0**	**0.0**	**6.2**	**0.0**	**17.9**	**15.5**	**9.2**	**10.9**	**0.0**	**11.2**	**4.2**	**19.5**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
p-Cymene												11.2	4.2						
Sabinene					6.2									19.5					
Santolinatriene							9.4	9.5											

Δ^2-Carene	4.2																		
α-Pinene	4.0						8.5	6.0											
β-Pinene									9.2	10.9									
Sesquiterpenes Hydrocarbons	**7.6**	**37.4**	**17.2**	**2.6**	**0.0**	**7.4**	**8.0**	**12.7**	**0.0**	**0.0**	**15.8**	**0.0**	**0.0**	**16.6**	**0.0**	**0.0**	**0.0**	**4.0**	**0.0**
(*E*)-β-Farnesene				2.6															
Germacrene D		6.9									10.2			12.6					
Isocaryophyllene						7.4													
α-Humulene		5.2																	
β-Caryophyllene	7.6	25.3	17.2				8.0	6.0			5.6			4.2					
δ-Cadinene								6.7											
γ-Cadinene																		4.0	
Oxygenated Monoterpenes	**4.7**	**0.0**	**3.5**	**0.0**	**0.0**	**0.0**	**9.7**	**8.2**	**34.3**	**18.2**	**0.0**	**8.0**	**13.3**	**12.3**	**27.0**	**17.8**	**17.6**	**0.0**	**0.0**
Carvacrol			3.5													5.2			
Camphor																	2.4		
Chrysantenone													3.3		5.7				
Chrysanthenol												4.4				3.2			
Linalool	4.6									4.9					12.8	3.2			
Terpinen-4-ol								4.5	4.1					6.2					
trans-Verbenol												3.6	10.0						
α-Borneol																	1.8		
α-Terpineol									26.4	4.5									
β-Thujone							9.7	3.7											
1,8-Cineole									3.8	8.8				6.1	8.5	6.2	13.4		
Oxygenated Sesquiterpenes	**0.0**	**11.9**	**27.0**	**0.0**	**28.0**	**27.7**	**16.6**	**0.0**	**11.7**	**26.2**	**4.0**	**1.5**	**5.7**	**4.2**	**0.0**	**0.0**	**36.0**	**22.9**	**29.7**
(*E*)-Nerolidol							10.6												
Blobulol									5.4										
Caryophyllene oxide		6.5	9.6		9.3	9.5						1.5	5.7				3.4		5.9
cis-Chrysantenyl acetate									6.3	19.8									
Elemol																	4.5		
epi-α-Cadinol														4.2					11.5
epi-α-Muurolol										6.4							18.1	4.0	
Eudesmol																	4.1		

Spathulenol		5.4	17.4		18.7	18.2	6.0				4.0						5.9		12.3
α-Eudesmol																		10.2	
γ-Cadinol																		8.7	
Fatty Acid And Derivatives	**0.0**	**6.1**	**0.0**	**38.9**	**0.0**	**8.0**	**0.0**	**0.0**	**0.0**	**0.0**	**13.5**	**0.0**	**0.0**	**0.0**	**6.1**	**22.7**	**0.0**	**0.0**	**6.4**
9,12-Octadecadienoic acid																12.2			
Decanoic acid		6.1																	
Palmitic acid				39.6							13.5				6.1	10.5			
Linoleic acid				36.2															6.4
Methyl hexadecanoate						8.0													
Pentadecanoic acid				3.1															
Other	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**26.9**	**33.6**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
2-Phenyl-1-ethanol													33.6						
Benzyl alcohol												26.9							
Isobutyl-*o*-phtalate																			5.5
n-Octyl-2-methylbutanoate																			5.3
TOTAL	**47.6**	**55.4**	**47.7**	**41.5**	**47.4**	**43.1**	**52.2**	**36.4**	**59.6**	**55.3**	**33.3**	**61.4**	**57.1**	**42.6**	**33.1**	**48.8**	**53.6**	**26.9**	**55.2**

a = aerial parts, f = flowers, l = leaves, s = steem, AJ: *A. altissima* L. (syn. *Cota altissima* (L.) J. Gay) collected in Greece, AK: *A. altissima* L. (syn. *Cota altissima* (L.) J. Gay) collected in Iran, AL: *A. altissima* L. var. *altissima* colleceted in Iran, AM: *A. altissima* L. var. *altissima* colleceted in Iran, AN: *A. altissima* L. var. *altissima* colleceted in Iran, AO: *A. austriaca* Jacq. Collected in Serbia Montenegro, AP: *A. dipsacea* Bornm collected in Turkey, AQ: *A. melanolepis* Boiss. (syn. *Anthemis palestina* (Reut. Ex. Kotschy) Boiss; *Cota palestina* Kotschy) collected in Greece, AR: *A. melanolepis* Boiss. (syn. *Anthemis palestina* (Reut. Ex. Kotschy) Boiss; *Cota palestina* Kotschy) collected in Greece, AS: *A. segetalis* Ten. (syn. *Cota segetalis* (Ten.) Holub) collected in Montenegro, AT: *A. wiedemanniana* Fisch. & C. A. Mey collected in Turkey, AU: *A. wiedemanniana* Fisch. & C. A. Mey collected in Turkey, AV: *A. tinctoria* L. collected in Serbia Montenegro, AW: *A. tinctoria* L. collected in Estonia, AX: *A. tinctoria* L. var. *parnassica* collected in Greece

***Table 3**: continuation*

Genus *Anthemis* L.												**Genus *Chamaemelum* P. Mill**					
	Subgenus *Cota* (J. Gay) Rouy											.					
	Section *Anthemaria* Dumort								***Anthemis* species with unresolved status:**								
Component	***AY*(fh)**	***AZ*(fh)**	***BA*(a)**	***BB*(a)**	***BC*(a)**	***BD*(a)**	***BD*(f)**	***BE*(a)**	***BF*(f)**	***BF*(l)**	***BG*(a)**	***BH*(a)**	***BH*(r)**	***BI*(a)**	***BJ*(a)**	***BK*(a)**	***BL*(f)**
Carbonylic compounds	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**17.1**	**0.0**	**3.0**	**0.0**	**0.0**	**0.0**	**0.0**
6-Methyl-5-hepten-2-one											17.1						
Hexahydrofarnesylacetone													3.0				

Monoterpenes Hydrocarbons	**11.7**	**11.7**	**31.3**	**14.0**	**0.0**	**5.0**	**0.0**	**0.0**	**0.0**	**0.0**	**5.7**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Camphene											5.7						
α-Fenchene						5.0											
α-Pinene	4.4	4.4	14.4	7.6													
β-Pinene	7.3	7.3	16.9	6.4													
Sesquiterpenes Hydrocarbons	**0.0**	**0.0**	**0.0**	**13.7**	**9.3**	**0.0**	**8.6**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Germacrene D				5.5	2.1												
trans-Caryophyllene				8.2	7.2												
α-Copaene							8.6										
Oxygenated Monoterpenes	**7.9**	**7.9**	**20.8**	**25.0**	**45.3**	**0.0**	**0.0**	**13.3**	**47.2**	**67.5**	**29.0**	**15.5**	**0.0**	**0.0**	**4.5**	**0.0**	**0.0**
1,8-Cineole	7.9	7.9	5.8	5.2	8.4				5.5	16.7							
Artemisia alcohol												3.8					
Borneol								13.3	31.8	30.2	11.5						
Camphor			15.0	14.4	3.5						17.5						
cis-Crhysantenol				2.2	27.0												
Santolina alcohol												11.7					
Terpinen-4-ol				3.2	6.4				4.8								
Thujone										12.1							
trans-pinocarveol															4.5		
Yomogi alcohol									5.1	8.5							
Oxygenated Sesquiterpenes	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**23.0**	**23.8**	**34.4**	**0.0**	**5.5**	**11.0**	**20.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Caryophyllene oxide												3.8					
cis-Chrysantenyl acetate										5.5							
Elemol						4.0	15.8	7.6									
epi-α-Cadinol												6.7					
epi-α-Muurolol												3.0					
Humulene oxide II							8.0										
Ledol						4.2											
Sesquicineole											11.0						
Spathulenol												6.5					
α-Copaen-8-ol						7.6											
α-Eudesmol								18.2									
β-Eudesmol						7.2											

γ-Eudesmol								8.6									
Phenolyc Compounds	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**7.9**	**0.0**	**12.7**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**5.0**	**0.0**	**0.0**
Elemicin							7.9										
Estragol															5.0		
Phenol									12.7								
Fatty Acid	**5.4**	**5.4**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**9.5**	**0.0**	**0.0**	**0.0**	**15.2**	**61.0**	**0.0**	**0.0**	**0.0**	**0.0**
Decanoic acid	5.4	5.4															
Dodecanoic acid													4.2				
Palmitic acid								9.5				15.2	52.0				
Tetradecanoic acid													4.8				
Esters	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**12.3**	**66.0**	**65.5**	**58.8**	**66.7**
1,2-Benzendicarboxylic acid bis (2-Methylpropyl) ester													12.3				
2-Butenyl angelate															7.3		
2-Methylbutyl angelate															17.4	20.3	13.0
2-Methylbutyl isobutanoate															4.3		
3-Methylbutyl isobutyrate																	5.2
Isoamyl angelate														27.0	7.6		6.6
Isoamyl-2-methyl Butyrate																	4.1
Isobutyl angelate														39.0	24.5	38.5	25.8
Isobutyl isobutanoate															4.4		
Propyl tiglate																	12.0
TOTAL	**25**	**25**	**52.1**	**52.7**	**54.6**	**28.0**	**40.3**	**57.2**	**59.9**	**73.0**	**62.8**	**50.7**	**76.3**	**66.0**	**75.0**	**58.8**	**66.7**

fh = flowerheads, a = aerial parts, f = flowers, l = leaves, r = root, AY: *A. tinctoria* L. collected in Slovakia, AZ: *A. tinctoria* L. collected in Slovakia, BA: *A. triumfetti* (L.) DC. (syn. *Cota triumfetti* (L.) J. Gay) colleced in Montenegro, BB: *A. triumfetti* (L.) DC collected in Serbia Montenegro, BC: *A. triumfetti* (L.) DC collected in Serbia Montenegro, BD: *A. triumfetti* (L.) All. subsp. *Triumfetti* collected in Iran, BE: *A. talyshensis* A. Fedor. (syn. *Anthemis triumfetti* (L.) DC; *Cota triumfetti* (L.) J. Gay) collected in Iran, BF: *A. xylopoda* O. Schwarz collectd in Turkey, BG: *A. tenuisecta* Ball collected in Morocco, BH: *Chamaemelum mixtum* (L.) All.(= *Anthemis mixta* L.) collected in Sicily, BI: *Chamaemelum nobile* L. All. (syn. *Anthemis nobilis* L.), BJ: *Chamaemelum nobile* L. All. (syn. *Anthemis nobilis* L.) collected in France, BK: *Chamaemelum nobile* L. All. (syn. *Anthemis nobilis* L.) collected in Italy, BL: *Chamaemelum nobile* L. All. var. *flora plena* collected in Hungary.

***Table 4*:** MIC (µg/mL) and MBC* (µg/mL) of essential oils from *Anthemis* species growing wild in Sicily

Strain ***Gram positivi***	***A*1f**	***A*1l**	***A*2a**	***A*2f**	***A*3a**	***A*4f**	***A*4l**	***A*5f**	***A*6a**	***A*7a**	***A*8a**	***Chloramphenicol***
Bacillus cereus (ATCC 11778)	50 (100)	50 (100)	50	25 (50)	50 (100)	50	25 (50)	50	50 (100)	25 (50)	50	12.5
Bacillus subtilis (ATCC 6633)	50 (100)	50	50 (100)	50 (100)	100	50 (100)	50	50 (100)	50 (100)	50	25 (50)	12.5
Staphylococcus aureus (ATCC 25923)	>100	>100	>100	>100	>100	>100	100 (>100)	>100	>100	>100	>100	25
Staphylococcus pidermidis (ATCC 12228)	25 (50)	50 (100)	25 (50)	50 (100)	25	12.5 (25)	25 (50)	50	50 (100)	12.5 (25)	25	3.12
Streptococcus faecalis (ATCC 29212)	100	100	>100	100	>100	100	100 (>100)	>100	50 (100)	25 (50)	25 (50)	25
Strain ***Gram negativi***	***A1f***	***A1l***	***A2a***	***A2f***	***A3a***	***A4f***	***A4l***	***A5f***	***A6a***	***A7a***	***A8a***	***Chloramphenicol***
Escherichia coli (ATCC 25922)	50 (100)	50	50 (100)	50 (100)	50	50 (100)	25 (50)	50	50 (100)	25 (50)	50	12.5
Klebsiella pneumoniae (ATCC 10031)	100	>100	>100	100 (>100)	>100	100 (>100)	100 (>100)	>100	100	100	100	50
Proteus vulgaris (ATCC 13315)	100	>100	100	100	>100	100	100 (>100)	>100	>100	100	100	25
Pseudomonas aeuriginosa (ATCC 27853)	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	100
Salmonella typhi Ty2 (ATCC 19430)	>100	>100	100 (>100)	100 (>100)	>100	>100	>100	>100	100 (>100)	100	100	6.25

MBC are reported in brackets when different from MIC

a = aerial part, f= flowers, l = leaves, A1: *A. montanA*, A2: *A. cupaniana*, A3: *A. arvensis* subsp. s*phacelata*, A4: *A. affine cupaniana*, A5: *A. aetnensis*, A6: *A.* collected in cava grande, A7: *A. messanensis* on the rocks, A8: *A. messanensis* in the greenhouse.

7.2. TABLE OF THE COMPOSITION OF ESSENTIAL OILS FROM *SALVIA ARGENTEA* AND *SALVIA ARGENTEA* TAXA AND OF THE EXRACTS FROM *SALVIA ARGENTEA*

***Table 5*:** Chemical constituents of the essential oil from *Salvia argentea* L. and *Salvia argentea* taxa published in literature arranged by class.

KI[a]	KI[b]	COMPONENT[d]	*Si*[e]	*Ma*[f]	*S*[g]	*T1*[h]	*T2*[i]	*Mo*[l]	Id.[c]
		Hydrocarbons	**0.1**	**9.4**	**4.0**	**0.0**	**0.0**	**0.0**	
1358	1634	α-ionene	t						1,2
2300	2300	Tricosane	t	0.5					1,2,3
2500	2500	Pentacosane	0.1	0.9					1,2,3
		Heneicosane		0.5					
		Heptacosane		1.0	2.4				
		Nonacosane			1.6				
		Pentadecane		0.5					
		Tetradecane		4.0					
		2-methyldocosane		0.7					
		2-methyltetracosane		0.5					
		2-methyltetradecane		0.8					
		Carbonylic Compounds	**0.1**	**5.4**	**4.1**	**0.6**	**0.4**	**0.0**	
1480	1807	Tridecan-2-one	t						1,2
1619	1934	Tetradecanal	0.1						1,2
		Benzaldehyde			t	0.1	0.1		
		Benzene acetaldehyde			0.6	0.5	0.3		
		Hexahydrofarnesyl acetone		4.6	3.5				
		Nonanal		0.8					
		Monoterpene Hydrocarbons	**21.4**	**0.0**	**0.5**	**14.5**	**13.5**	**28.7**	
925	1035	α-thujene	t	t		t	t		1,2
928	1014	Tricyclene	0.1						1,2
938	1032	α-pinene	2.2		0.5	3.9	1.8	9.3	1,2,3
953	1076	Camphene	t		t	0.7	0.6	19.4	1,2
980	1118	β-pinene	2.6	t	t	0.8	0.5		1,2,3
1005	1150	Limonene	t		t	1.0	0.9		1,2,3
1025	1278	*p*-cymene	3.4		t	4.2	6.4		1,2,3
1057	1256	γ-terpinene	1.0		t	1.5	1.2		1,2,3
1108	1409	1,3,8-*p*-menthatriene	12.1						1,2
		α-phellandrene	t			0.3	0.1		
		(*E*)-β-ocimene				0.5	0.5		
		Myrcene				0.7	0.5		
		Terpinolene				0.1	0.3		
		α-terpinene				0.6	0.5		
		δ^3-carene				0.2	0.2		
		Sesquiterpene Hydrocarbons	**13.6**	**30.2**	**12.7**	**9.2**	**13.1**	**0.0**	
1337	1468	δ-elemene	0.1						1,2

1351	1467	α-cubenene	3.9						1,2
1364	1527	Silphiperfola-5,7(14)-diene	0.8						1,2
1396	1616	7-*epi*-sesquithujene	2.1						1,2
1432	1612	β-cubebene	0.1						1,2
1487	1679	α-amorphene	t						1,2
1490	1694	*ar*-curcumene	0.8		t				1,2
1522	1785	β-sesquiphellandrene	5.8						1,2
		γ-cadinene			t	0.1	0.2		
		(*E*,*E*)-α-farnesene		t					
		γ-elemene		t					
		γ-muurolene			t	0.4	0.6		
		6,9-guaiadiene		1.2					
		allo-aromadendrene			t	0.3	0.3		
		Bicyclogermacrene		t					
		Cadalene		3.0					
		cis-calamenene			t	0.3	0.6		
		Germacrene B		1.3					
		Germacrene D		3.9	t	0.1	0.8		
		Sesquithujene		t					
		Valencene			t	0.2	0.1		
		α-calacorene		1.3		0.1	0.2		
		α-colocalene		0.3					
		α-copaene		8.5	t	0.4	0.1		
		α-cubenene			t	0.1	0.1		
		α-humulene		0.6	10.7	4.1	5.3		
		β-bourbenene		0.6					
		β-calacorene		1.1					
		β-caryophyllene		6.1	2.0	2.5	3.3		
		β-elemene		0.4					
		δ-cadinene		1.9	t	0.6	1.5		
		Oxygenated Monoterpenes	**0.0**	**0.6**	**13.1**	**22.1**	**20.6**	**64.1**	
		1,8-cineole			3.1	4.2	4.0	2.5	
		Borneol		t	1.0	0.1	0.1	9.0	
		Bornyl acetate		t		0.6	0.2		
		Camphor			t	3.2	2.1	45.1	
		Carvacrol			t	0.4	0.1		
		Isoborneol				0.1			
		Linalool		0.6	t	2.2	1.3		
		Terpinen-4-ol			t	0.8	0.8		
		Thymol			t	0.2	0.2		
		α-terpineol			t	0.8	1.0		
		α-thujone			1.7	7.3	8.1	7.5	
		β-thujone			7.3	2.2	2.7		
		Oxygenated Sesquiterpenes	**58.6**	**51.0**	**39.4**	**36.6**	**29.6**	**0.0**	

1582	2095	(*E*)-sesquisabinene hydrate	4.1						1,2
1585	2135	β-copaen-4-α-ol	1.4	0.5					1,2
1587	2098	Globulol	7.4						1,2
1593	2103	Viridiflorol	0.3		32.4	26.9	18.7		1,2
1602	2037	Salvial-4(14)-en-1-one; (Mintketone)	t	2.4					1,2
1634	2089	1-*epi*-cubenol	3.1		t				1,2
1640	2187	τ-cadinol	1.1						1,2
1642	2209	τ-muurolol	0.2						1,2
1645	2145	Torreyol (α-muurolol)	0.9						1,2
1716	2478	14-Hydroxy-α-humulene	40.1						1,2
		10-*nor*-calamenen-10-one		1.2					
		10-peroxy-murolan-3,9(11)-diene		1.2					
		Caryophyllene oxide		37.6		3.3	4.1		
		Germacra-4(15),5,10(14)-trien-1-α-ol		1.3					
		Humulene epoxyde I		0.5					
		Humulene epoxyde II		6.3	2.3				
		Spathulenol		t	4.0	1.3	2.7		
		α-cadinol				3.4	2.2		
		β-eudesmol			0.7	1.7	1.9		
		Diterpenes	**t**	**0.4**	**14.8**	**6.1**	**13.6**	**0.0**	
1950	2622	(*Z*)-phytol	t						1,2
		(*E*)-phytol			1.2				
		Isocembrene		0.4					
		Manool			14.6	6.1	13.6		
		Phenolic Compounds	**0.0**	**0.0**	**t**	**t**	**0.1**	**0.0**	
		Eugenol			t	t	0.1		
		Fatty Acid	**0.0**	**0.0**	**3.6**	**0.0**	**0.0**	**0.0**	
		Tetradecanoic acid			3.6				
		Others	**0.0**	**2.7**	**1.0**	**0.8**	**1.3**	**0.0**	
1285	1485	Dihydroedulan II	t						1,2
		Sclareol oxide		2.7					
		β-ionone			1.0	0.8	1.3		
		TOTAL	**93.8**	**99.7**	**94.2**	**89.9**	**92.2**	**92.8**	

[a]: retention index on a HP-5MS column; [b]: retention index on a HP-Innowax column; [c]: Identification, 1 = comparison of retention index; 2 = comparison of mass spectra with MS libraries identification; 3 = co-injection with authentic compounds; [d]: t = trace, less than 0.05 %, [e]: Si = collected in Sicily, [f]: Ma = collected in Macedonia,[32] [g]: S = collected in Serbia,[31] [h]: T1 = collected at Sers (Tunisia), [I]: T2 = collected at Makther [19] (Ben Farhat et al. 2013a); [l]: Mo = collected in Morocco.[30]

Table 6: Chemical constituents of the extracts from *Salvia argentea* L.

KI[a]	KI[b]	COMPONENT[d]	ETP1	ETP2	DCM1	DCM2	Id.[c]
		Hydrocarbons	**60.1**	**17.0**	**63.1**	**26.8**	
2300	2300	Tricosane	1.5				1,2,3
2478		Methyltetracosane[#]	3.2		1.4	0.7	1,2
2500	2500	Pentacosane	4.4	0.6	2.3	1.3	1,2,3
2700	2700	Heptacosane	8.4	2.9	10.5	4.6	1,2,3
2863		Methyloctacosane[#]	2.8	0.6	1.9	1.0	1,2
2900	2900	Nonacosane	6.3	1.8	8.3	3.0	1,2,3
3052		Methyltriacontane[#]	3.2	0.5	1.0	2.2	1,2
3100	3100	Hentriacontane	8.3	3.2	10.9	4.4	1,2,3
3264		Methyldotriacontane[#]	7.9	2.4	7.6	3.4	1,2
3300	3300	Tritriacontane	9.9	4.1	14.1	5.0	1,2,3
3500	3500	Pentatriacontane	4.2	0.9	5.1	1.2	1,2
		Carbonylic Compounds	**18.3**	**8.9**	**17.5**	**11.4**	
1480	1807	Tridecan-2-one	2.5	1.1		1.0	1,2
1517	1829	Tridecanal	3.9	1.8	3.8	2.5	1,2
1619	1934	Tetradecanal	8.4	4.7	10.2	5.7	1,2
1703	2036	Pentadecan-2-one	2.7	1.3	3.5	1.8	1,2
1908	2219	Heptadecan-2-one	0.8			0.4	1,2
		Monoterpene Hydrocarbons	**4.0**	**0.0**	**5.5**	**0.6**	
938	1032	α-pinene	1.2		5.5		1,2,3
980	1118	β-pinene	0.3				1,2,3
1057	1256	γ-terpinene	2.5			0.6	1,2,3
		Sesquiterpene Hydrocarbons	**3.9**	**0.9**	**7.1**	**0.9**	
1432	1612	β-cubebene	2.6	0.9	7.1	0.9	1,2
1494	1735	α-zingiberene	1.3				1,2
		Diterpenes					
2132	2625	(*E*)-phytol	0.5				1,2
		Ester	**0.1**	**63.7**	**0.0**	**50.4**	
1703		9-tetradecenoic acid methyl ester; Methyl myristoleate		10.5		18.5	1,2
1712	2021	Tetradecanoic acid methyl ester; methyl myristate				5.6	1,2,3
1891	2237	(*Z*)-9-hexadecenoic acid methyl ester; methyl palmitoleate		2.4			1,2,3
1928	2208	Hexadecanoic acid methyl ester; methyl palmitate	0.1	8.0		1.9	1,2,3
2085	2505	(*Z,Z*)-9,12-octadecadienoic acid methyl ester methyl linoleate		3.0		1.6	1,2,3
2135	2487	(*Z,Z,Z*)-9,12,15-octadecatrienoic acid methyl ester; Methyl linolenate		36.6		13.5	1,2,3

2139		Octadecanoic acid methyl ester; Methyl stearate		1.6		1.5	1,2,3
2298		Methyl eicosenoate; Methyl gadoleate		1.6		3.6	1,2
3132		Octacosanoic acid methyl ester				2.2	1,2
3317		Triacontanoic acid methyl ester; Methyl melissate				2.0	1,2
		Others	**3.3**	**0.0**	**0.0**	**0.0**	
1472	1973	Dodecanol	0.4				1,2
1677	2193	Tetradecanol	2.0				1,2
1893	2384	Hexadecanol	0.9				1,2
		TOTAL	**90.2**	**90.5**	**93.2**	**90.1**	

[a]: retention index on a HP-5MS column; [b]: retention index on a HP-Innowax column; [c]: Identification, 1 = comparison of retention index; 2 = comparison of mass spectra with MS libraries identification; 3 = co-injection with authentic compounds; [d]: t = trace, less than 0.05 %,

7.3. TABLES OF THE COMPOSITION OF ESSENTIAL OILS FROM *PULICARIA SICULA*, *PULICARIA VULGARIS* AND *PULICARIA* TAXA

Table 7: Percentage composition of the essential oils from aerial parts of *Pulicaria sicula* (L.) Moris and *Pulicaria vulgaris* Gaertn. var. *graeca* (Sch.-Bip.) Fiori arranged by class.

KI[a]	KI[b]	COMPONENT[d]	*P.s.*	*P.v.g.*	Id.[c]
		Hydrocarbons	**1.4**	**7.2**	
2500	2500	Pentacosane	0.7	3.3	1,2 3
2600	2600	Hexacosane		0.1	1,2,3
2700	2700	Heptacosane	0.4	1.4	1,2,3
2800	2800	Octacosane		0.2	1,2,3
2900	2900	Nonacosane	0.3	1.2	1,2,3
3100	3100	Hentriacontane		1.0	1,2,3
		Carbonylic Compounds	**3.4**	**2.8**	
854	1231	(*E*)-2-hexenal	0.1		1,2
963	1543	Benzaldehyde	0.3		1,2,3
1044	1663	Phenylacetaldehyde	t	0.2	1,2
1102	1616	Nonanal	0.3	0.3	1,2
1206	1510	Decanal	0.2		1,2
1315	1827	(*E,E*)-2,4-decadienal	t		1,2
1359	1787	(*E*)-β-damascenone	0.4		1,2
1434	1869	Neryl acetone	t		1,2
1452	1867	(*E*)-geranyl acetone	0.4		1,2
1715	2038	Pentadecanal	0.3		1,2
1845	2131	Hexahydrofarnesylacetone	1.4	2.3	1,2
		Monoterpene Hydrocarbons	**1.9**	**0.0**	
938	1076	α-pinene	0.1		1,2,3
953	1076	Camphene	1.4		1,2

980	1118	β-pinene	0.2		1,2,3
1025	1278	*p*-cymene	0.2		1,2,3
1030	1203	Limonene	t		1,2,3
		Sesquiterpene Hydrocarbons	**4.7**	**31.9**	
1356	1579	Longipinene	0.1	0.2	1,2
1383	1732	β-maaliene	0.1		1,2
1387	1600	β-Elemene		t	1,2
1394	1543	Modhephene		0.3	1,2
1397	1503	Italicene		0.2	1,2
1414	1612	β-caryophyllene	2.9	14.3	1,2,3
1415	1593	1,7-Di-epi-β-cedrene (β-Funebrene)		3.5	1,2
1432	1612	β-Cubebene		t	1,2
1438	1573	trans-α-Bergamotene		0.3	1,2
1454	1668	(Z)-β-Farnesene		t	1,2
1455	1689	α-humulene	0.2		1,2
1475	1715	β-selinene	0.3	t	1,2
1478	1704	γ-Muurolene		0.3	1,2
1482	1692	γ-Curcumene		4.6	1,2
1490	1694	ar-Curcumene		3.8	1,2
1510	1743	β-Bisabolene		2.5	1,2
1515	1776	γ-cadinene	0.4		1,2
1524	1776	β-Sesquiphellandrene		1.9	1,2
1526	1773	δ-cadinene	0.2		1,2
1541	1918	α-calacorene	0.1		1,2
1677	2256	Calarene	0.4		1,2
		Oxygenated Monoterpenes	**43.2**	**9.2**	
989	1197	1,8-dehydrocineole	1.7		1,2
1034	1213	1,8-Cineole		0.4	1,2,3
1098	1553	Linalool	0.5		1,2,3
1105	1430	α-Thujone		0.1	1,2
1117	1571	*trans*-*p*-menth-2-en-1-ol	0.1		1,2
1145	1532	Camphor	0.3		1,2,3
1152	1523	Camphene hydrate	0.1		1,2
1152	1683	trans-Verbenol		t	1,2
1153	1482	Nerol oxide	0.2	t	1,2
1167	1719	Borneol	23.7		1,2,3
1176	1611	Terpineol-4		t	1,2,3
1185	1856	*p*-cymen-8-ol	0.2		1,2
1189	1706	α-terpineol	0.6	0.1	1,2,3
1223	1628	β-cyclocitral	0.3		1,2
1226	1809	Nerol		0.4	1,2,3
1227	1589	Bornyl formate	0.2		1,2
1235	1857	Geraniol	0.1		1,2,3
1286	1567	Bornyl acetate	6.5		1,2,3
1425	1882	Thymohidroquinone dimethyl ether	1.0		1,2

1479	1835	Geranyl propionate		8.2	1,2
1548	1948	Isothymol isobutyrate	6.2		1,2
1602	1893	Geranyl isovalerate	1.5		1,2
		Oxygenated Sesquiterpenes	**35.7**	**7.2**	
1553	2076	*cis*-α-copaen-8-ol	2.1	2.8	1,2
1565	1903	Geranyl butyrate	1.3		1,2
1571	2035	α-Caryophyllene alcohol	0.4		1,2
1579	2208	Caryophyllene oxide	10.2		1,2,3
1592	2035	Caryophylla-2(12),6-dien-5-one; Caryophyllenone	t		1,2
1599	2178	Widdrol		0.3	1,2
1612	2018	Humulene oxide II	0.6	t	1,2
1621	2324	Caryophylla-4(12),8(13)-dien-5-α-ol; Caryophylladienol II	1.2	0.2	1,2
1632	2371	Caryophylla-3,8(13)-dien-5α-ol		t	1,2
1638	2316	Caryophylla-4(12),8(13)-dien-5-β-ol; Caryophylladienol I	4.3	1.3	1,2
1640	2185	τ-cadinol	0.2		1,2
1645	2145	Torreyol	3.2	0.1	1,2
1649	2371	Caryophylla-3,8(13)-dien-5-β-ol	4.3		1,2
1652	2255	α-cadinol	2.8	t	1,2
1654	2267	Eudesm-11(13)en-4α-ol; Kongol; Selin-11-en-4-α-ol	0.3		1,2
1668	2357	14-hydroxy-β-caryophyllene; α-Betulenol	1.0	t	1,2
1672	2393	14-hydroxy-*iso*-caryophyllene; β-Betulenol	2.0	0.2	1,2
1675	2272	cis-(Z)-α-Bisabolene epoxide		0.5	1,2
1687	2137	1-naphthalenol, decahydro-1,4-α-dimethyl-7-(1-methylethenyl); *Neo*-intermedeol	1.6	0.1	1,2
1709	2247	(Z)-nerolidyl isobutyrate	0.2		1,2
1727	2242	Valerenal		1.1	1,2
1734	2483	Ledene oxide		0.6	1,2
		Diterpenes	**0.8**	**7.6**	
2134	2625	(*E*)-phytol	0.8	1.9	1,2
1989	2393	Manoyl oxide		5.7	1,2
		Fatty Acids	**0.0**	**27.2**	
1958	2931	Hexadecanoic acid		21.7	1,2,3
2122	3157	(Z,Z)-9,12-Octadecadienoic acid		5.5	1,2,3
		Others	**0.7**	**0.5**	
977	1452	1-octen-3-ol	t		1,2
992	1394	3-octanol	t		1,2
995	1243	2-pentylfuran	0.5	t	1,2
1307	1815	Benzyl isobutyrate	0.2		1,2
2821	3047	Squalene		0.5	1,2
		TOTAL	**91.8**	**93.6**	

[a]: HP-5 MS column; [b]: HP Innowax column [c]: 1, retention index, 2: mass spectrum, 3: co-injection with authentic compound; [d]: t: trace, <0.05%.

Table 8: Composition of the essential oils from *Pulicaria* taxa published in literature.

COMPONENT	***A*(a)**	***B*(s)**	***B*(f)**	***B*(l)**	***C*(a)**	***D*(a)**	***E*(a)**	***F*(a)**	***G*(a)**	***H*(a)**	***I*(a)**	***J*(l)**	***K*(a)**	***L*(a)**	***M*(l)**	***N*(a)**	***O*(a)**	***P*(l)**	***Q*(a)**	***R*(a)**
Hydrocarbons	**0.0**	**1.5**	**0.3**	**0.1**	**0.0**	**0.6**	**0.0**	**0.0**	**0.0**	**0.5**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Various		1.5	0.3	0.1		0.6				0.5										
Carbonylic Compounds	**2.0**	**0.0**	**0.6**	**0.2**	**0.0**	**0.0**	**0.0**	**0.1**	**4.5**	**0.5**	**0.0**	**0.0**	**0.0**	**0.4**	**0.2**	**0.0**	**0.0**	**0.0**	**0.0**	**3.0**
(*Z*)-3-methylnon-2-en-4-one								0.1												
(*Z*)-jasmone	2.0		0.1												0.2					
2-(*E*)-hexenal										0.2										
Decanal														0.2						0.1
Ethyl cinnamate																				2.8
Geranyl acetone														0.1						
Hexahydrofarnesylacetone			0.5							0.1										
Isobutyl isobutyrate														0.1						
Nonanal										0.2										0.1
Pentadecan-2-one				0.2																
p-hydroxy-cyclohexanone									4.5											
Monoterpene Hydrocarbons	**0.0**	**0.0**	**0.5**	**0.5**	**0.0**	**0.0**	**0.6**	**36.3**	**6.4**	**5.4**	**0.0**	**0.0**	**0.0**	**0.3**	**1.6**	**0.6**	**51.9**	**0.1**	**14.4**	**2.4**
(*Z*)-β-ocimene															0.1					
1,3,8-*p*-menthatriene																			0.2	
4-carene																				0.3
Camphene								0.1									0.2			
Limonene			0.4	0.4				0.2		0.1					0.1					0.2
Myrcene							0.6												0.1	
p-cymene								0.7		0.2				0.1		0.6	0.9			1.2
Sabinene								0.1							0.9		0.2		0.2	
Verbenene																	0.1			
α-fenchene																			0.1	
α-phellandrene																	0.2		0.1	0.3
α-pinene			0.1	0.1				34.1		5.0				0.1	0.2		45.7	0.1	0.5	0.4
α-terpinene								0.2									1.0		4.0	
α-terpinolene																	0.2		2.1	
α-thujene								0.3									0.6		0.1	
β-pinene								0.6	6.4					0.1	0.1		1.2			
δ^3-carene															0.1					
γ- terpinene										0.1					0.1		1.6		7.0	

Sesquiterpene Hydrocarbons	**8.3**	**15.5**	**38.9**	**28.7**	**39.6**	**18.1**	**25.6**	**5.1**	**12.5**	**1.6**	**6.4**	**0.0**	**41.8**	**0.0**	**27.4**	**10.5**	**3.1**	**0.1**	**29.3**	**3.9**
(*Z*)-β-farnesene						1.2	2.2													
Alloaromadendrene															0.2					
Aromadendrene				0.5				0.4		0.3										
Bicyclogermacrene															1.2					
Cadalene								1.1											1.9	
Cadina-1,4-diene		0.2	0.7																	
Calacorene								0.1												
Calarene; β-gurjunene		1.7	0.5	6.9	1.3								0.1		0.1		0.5		0.1	
cis-cadina-1(6)-4-diene															0.2					
cis-calamene											1.5									
cis-calamenene								0.1		0.2									13.4	
cis-α-bergamotene	0.7												0.1							
Clovene	1.9																			
Daucene								0.3					0.6							
Dehydroaromadendrene		1.4	3.0						12.5				1.7							
epi-bicyclosesquiphellandrene			3.2	2.9																
Germacrene A			0.3										1.5		0.1					
Germacrene B											2.0									
Germacrene D													5.9		2.5					
Isocaryophyllene; (*Z*)-caryophyllene		0.4																		
Isoledene		0.2	0.8	1.3																
Italicene					1.1															
Junipene; α-longifolene																			8.7	
Patchoulane																2.4				
trans-cadina-1(6)-4-diene															0.2					
trans-calamenene								0.1											2.7	
trans-muurola-4(14),5-diene															0.2					
trans-β-bergamotene													0.3							
trans-γ-bisabolene																			0.6	
Unidentified compd $C_{15}H_{24}$																6.4				
Unidentified compds $C_{15}H_{24}$	0.5																			
Valencene			3.5	1.3																
α-amorphene		0.3		2.5							1.1								0.1	
α-cadinene					4.9								0.5		0.6					
α-caryophyllene; α-humulene			0.1	0.1									0.5		1.5	0.1				

α-copaene										0.1			0.3		0.6		0.2		0.1	
α-cubebene		0.3	0.6	0.1									0.8		0.1					
α-curcumene; *ar*-curcumene					28.3	4.9	3.0	0.4			0.4									0.1
α-elemene																0.1				
α-guaiene																				0.4
α-gurjunene			0.2	0.3		1.2	0.9	0.1		0.1							0.3			
α-humulene																				
α-muurolene		1.0	2.7	1.0	0.7			0.3		0.1			1.5		0.2		0.2		0.3	
β-cadinene			1.3	1.3																
β-caryophyllene			0.3	0.2	2.5	4.4	12.8	0.1					1.0		13.4			0.1	0.1	3.0
β-cedrene	2.5																			
β-copaene															0.1					
β-cubebene			0.2	0.3																
β-elemene													15.4		0.2					
β-guaiene	0.2																			
β-longipinene							1.2													
β-selinene			0.3										1.2		0.2		0.4			
δ-cadinene	1.2	9.1	9.5	7.9		5.3	2.0	1.4		0.6	1.4		5.4		3.9		1.0		1.0	
δ-guaiene; α-bulnesene	0.9																			
δ-selinene																				
γ-cadinene		0.9	11.7	2.1	0.8			0.7		0.2			3.6		1.5		0.5		0.3	0.4
γ-curcumene						1.1	3.5													
γ-muurolene	0.4												1.4		0.4	1.5				
Oxygenated Monoterpenes	**7.1**	**15.3**	**0.1**	**0.1**	**5.5**	**11.7**	**11.0**	**26.4**	**50.1**	**48.9**	**15.4**	**98.6**	**0.2**	**92.5**	**2.8**	**39.1**	**36.6**	**97.8**	**52.7**	**69.6**
(*E*)-citral										1.8									1.3	
(*Z*)-chrysanthenyl acetate									22.4	1.2										
(*Z*)-citral										1.4									1.0	
(*Z*)-isogeranic acid								0.4		0.6										
(*Z*)-tagetone										0.2										
1,8-cineole; Eucalyptol							0.8	11.9	5.6	1.0				0.2	1.0		27.1	0.1	1.0	0.1
1-methyl-carvacrol			0.1																	0.8
3,7-DiMe-(*E*)-2,6-octadien-1-ol; Geraniol	0.7							0.1		5.3										
3-methyl-4-iso-propyl phenol; *p*-thymol																				0.6
4-caranol																1.1				

Artemisia alcohol										0.1										
Borneol	0.3									0.6				0.2				0.7		0.6
Bornyl acetate																				0.1
Bornyl formate								0.1												
Camphor									1.2	0.4				0.5	0.3			0.3		0.2
Carvacrol														0.9				0.6		
Carvenone														0.2				0.3		
Carvotanacetone												98.6		87.3				91.4		55.9
Chrysanthenone								2.0												
cis-carvotanacetone																		0.2		
cis-chrysanthenol								2.3												
cis-myrtanol										0.5										
cis-piperitone oxide							2.0													
cis-*p*-menth-2-en-1-ol		0.1																		
cis-*p*-menthan-2-one																		0.2		
cis-sabinene hydrate								0.8							0.3		0.2		8.3	
cis-sabinol																0.6				
Citronellol								0.2		9.0	4.4									
Citronellyl acetate										1.0	2.6									
Citronellyl formate										0.2	0.9									
Citronellyl valerate																			0.3	
Dehydro-1,8-cineole								0.1												
Dill ether																		0.2		
Filifolone										0.1										
Geranyl acetate										0.4	0.6								0.1	
Geranyl formate										0.1										
Isobornyl formate																				2.7
Isolimonenol;																			2.4	
Isopulegol										0.2										
Isothujol																2.8				
Lavandulol						0.9														
Linalool	3.3									0.6			0.2	1.2	0.1	6.4		0.1	5.6	4.5
Linalool oxide	1.9															6.0				
Myrtenal																				
Myrtenol								0.4		13.2	0.6						0.4		5.8	
Myrtenyl acetate										4.1									0.9	

Neral											0.5									
Neric acid										0.2										
Nerol						6.2	4.0	0.2									0.6			
Nerol oxide							1.1			0.3										
Neryl 2-methylbutyrate								0.1												
Neryl acetate										0.9									0.3	
Neryl isobutyrate						4.6	2.2			1.6								0.4		
Neryl isovalerate					5.5					0.3	2.6							0.2		
p-cymen-8-ol									2.1					0.6						
p-cymene-7-ol acetate										0.2										
Piperitenone oxide; *p*-menth-4(8)-en-3-one							0.9													
p-menth-6-en-2-one																6.3				
p-mentha-1,5-dien-8-ol										0.3										
Pulegone oxide																0.3				
Santolina alcohol										0.1										
Terpineol-4								3.1	2.2	0.4	0.4			0.2	0.4		4.4		20.1	
Thujol																1.4				
Thymohydroquinone dimethyl ether														0.7				2.6		
Thymol		15.2		0.1				0.3		1.1				0.3	0.6	6.6	0.5	0.2	0.5	3.0
Thymol methyl ether										0.2										0.3
trans-myrtanol											1.9									
trans-carveol								0.2		0.3							0.2			
trans-pinocarveol										0.2										
trans-*p*-menth-2-en-1-ol								0.2												
trans-*p*-menthan-2-one																		0.3		
trans-sabinene hydrate								0.8							0.1		0.6		2.7	
trans-sabinyl acetate																				0.4
Unidentified																7.3				
Verbenol									16.6					0.2						
Verbenone																0.1				
Yomogi alcohol										0.2										
α-campholenal								0.1									0.6			
α-campholenyl formate								0.1												
α-terpineol	0.9							2.9		0.2	0.9						2.0		2.4	0.4
β-terpineol																0.2				
γ-campholenyl formate										0.4										

Oxygenated Sesquiterpenes	**40.7**	**9.7**	**9.6**	**19.7**	**38.0**	**44.2**	**34.8**	**25.1**	**0.0**	**29.1**	**67.2**	**0.0**	**39.2**	**1.6**	**58.1**	**0.8**	**0.0**	**0.5**	**0.8**	**0.8**
(*E*)-nerolidol						6.6	6.9								8.5					
(*E*)-nuciferal										3.4										
(*E*)-nuciferol								0.1		5.2										
(*E*)-nuciferyl formate								0.2		0.2										
(*E*)-nuciferyl isobutyrate								0.1		0.1										
(*E*)-nuciferyl isovalerate								0.1		0.9										
(*E*)-nuciferyl-2-methyl butyrate								0.1		1.9										
(*E*)-γ-curcumen-12-ol								0.2		0.9										
(*Z*)-nerolidol						11.2														
(*Z*)-nuciferal								0.6		0.6										
(*Z*)-nuciferol								0.8												
(*Z*)-nuciferyl acetate								0.2		0.2										
(*Z*)-nuciferyl isobutyrate								0.5												
(*Z*)-nuciferyl isovalerate								1.6		0.5										
(*Z*)-nuciferyl-2-methyl butyrate								1.6		0.4										
(*Z*)-α-santalol							2.5													
(*Z*)-γ-curcumen-12-ol								0.6		1.9										
(*Z*)-γ-curcumen-12-yl 2-methylbutyrate								1.6												
(*Z*)-γ-curcumen-12-yl acetate								0.1		1.4										
(*Z*)-γ-curcumen-12-yl formate										0.2										
(*Z*)-γ-curcumen-12-yl isobutyrate								0.5		0.6										
(*Z*)-γ-curcumen-12-yl isovalerate								1.6												
1,10-di-*epi*-cubenol															0.4					
1,8-epoxycadin-4-ene								0.5												
10,11-epoxycalamenene											4.3									
10-*epi*-1,8-epoxycadin-4-ene								0.2												
10-*epi*-italicen-12-ol										0.4										
10-*epi*-italicen-12-yl 2-methylbutyrate								0.2												
10-*epi*-italicen-12-yl isobutyrate								0.1		0.4										
10-*epi*-italicen-12-yl isovalerate								0.2		0.1										
10-*epi*-italicene ether					4.1															
10-*epi*-italicene-12-yl acetate										0.9										
10-*epi*-α-muurolol						4.8	2.4	1.6		0.5										
14-hydroxy-9-*epi*-β-caryophyllene						2.6	1.0						2.6		2.0					
14-hydroxy-δ-cadinene											5.5									

1-*epi*-cubenol													1.1		0.8					
1-*epi*-cubenol										0.2										
4-*epi*-cubebol								0.3					2.0		1.2					
6-oxo-cyclonerolidol														0.5						
6*R*,7*R*-bisabolone											2.5									
6*S*,7*R*-bisabolone											1.6									
Amorpha-4,9-dien-2-ol															0.5					
ar-curcumen-15-al											5.6									
Aristolone		0.2	0.5	0.8																
Aromadendrenepoxyde			0.1																	
Bergamotol acetate													3.0							
Cadina-4,10(14)-dien-8α-ol								11.4												
Cadina-4,10(14)-dien-8β-ol										0.1										
Calamenene-10-one											12.2									
Caryophylla-4(12),8(13)-dien-5α-ol;															0.6					
Caryophylla-4(12),8(13)-dien-5β-ol; Caryophylladienol I															1.3					
Caryophylla-4(14),8(15)-dien-5α-ol							1.4													
Caryophylla-4(14),8(15)-dien-5β-ol							4.4													
Caryophyllene oxide					2.6	9.1	12.8	0.2			4.2		3.4	0.6	8.5			0.4	0.6	
cis-cadinene ether											0.6									
cis-calamene-10-ol											1.2									
cis-muurol-5-en-4-β-ol															0.2					
cis-sesquisabinene hydrate										1.2										
cis-α-copaen-8-ol								0.5		0.3										
Cubebol											2.4				1.5					
Curcumenol											4.9									
Elemol	1.5																			
Farnesol	0.3															0.3				
Germacra-4(15),5,10(14)-triene-1α-ol															1.3					
Germacrene D-4-ol		2.9			4.5								2.6		3.4					
Globulol																				
Helifolen-12-al A					1.8			0.3												
Helifolen-12-al B								0.3												
Italicene-12-ol										0.7										
Italicene-12-yl 2-methylbutyrate								0.2												

Italicene-12-yl isobutyrate								0.2												
Italicene-12-yl isovalerate								0.1		0.2										
Ledol					0.4			0.4							0.5					
Liguloxide													1.1							
Longifoliol											6.0									
Muurola-4,10(14)-dien-8α-ol								3.0												
Oplopanone															0.2					
Salvial-4(14)-en-1-one; Mintketone															0.7					
Shiromool; Germacr-1(10)-en-6-ol, 4,5-epoxy-										5.1										
Spathulenol															6.8					0.8
trans-calamene-10-ol											5.1									
trans-isolongifolanone					1.2															
Unidentified compds $C_{15}H_{22}O$	5.0																			
Unidentified compds $C_{15}H_{24}O$	25.3																			
Unidentified compds $C_{15}H_{24}O_2$	1.8																			
Unidentified compds $C_{15}H_{26}O$	1.6																			
Valerenol			0.4	0.6															0.2	
Viridiflorol						2.3														
τ –muurolol															2.4					
α-bisabolol					2.5															
α-cadinol			8.6	10.3	4.5	6.2	2.1				1.2		8.4	0.2	8.2					
α-costol															0.3					
α-*epi*-cadinol (τ-Cadinol)		6.6		8.0	16.4						4.0		14.2	0.1	4.7			0.1		
α-humulene epoxide II						1.4	1.3			0.2										
α-muurolol;															1.3					
β-bisabolol											2.5									
β-costol															0.3					
β-eudesmol														0.2		0.5				
β-maaliene alcohol	1.8																			
β-oplopenone													0.8		1.5					
β-selinen-2α-ol; Jatamol A	3.4							0.7												
β-sinensal															0.3					
γ-curcumen-15-al											3.4									
γ-costol															0.7					

Diterpenes	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**3.4**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.2**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
(*E*)-phytol														0.2						
Manool						3.4														
Phenolic Compounds	**0.0**	**0.0**	**0.3**	**0.2**	**1.1**	**0.0**	**0.0**	**0.1**	**0.0**	**0.7**	**0.0**	**0.0**	**0.0**	**0.0**	**0.1**	**26.6**	**0.0**	**0.5**	**0.0**	**0.0**
2-(4-Isopropylphenoxy)ethanol																5.0				
3-methoxy-2,4,6-trimethyl-phenol																2.5				
4-isopropylveratrole																		0.2		
Buthyl-hydroxy-anisole; (BHA)																6.6				
Coniferyl alcohol					1.1															
Eugenol			0.3	0.2																
Methyl eugenol								0.1		0.7					0.1	6.1		0.3		
tert-bythyl-4-hydroxy-anisole																6.4				
Fatty Acid	**0.0**	**5.3**	**0.0**	**0.3**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Oleic acid		1.3																		
Palmitic acid		3.5																		
Linolenic acid				0.1																
Pentadecanoic acid				0.2																
Tetradecanoic acid		0.5																		
Others	**0.6**	**3.4**	**3.7**	**7.6**	**0.0**	**0.0**	**0.0**	**0.5**	**18.5**	**0.1**	**2.8**	**0.0**	**0.0**	**0.2**	**0.1**	**4.8**	**0.0**	**0.0**	**0.0**	**4.6**
(*E*)-hex-3-enol								0.2												
(*Z*)-hex-3-enol								0.1												
1,4-ethanonaphthalene																				0.2
1-octen-3-ol															0.1					
2,6-octadien-1-ol																				0.2
2L-4L-dihydroxy-eicosane									18.5											
2-pentyl furan										0.1										
2-*tert*-butyl-1,4-dimethoxy-benzene																				2.2
Benzene derivatives			2.1	3.9																
Bicyclohexane	0.6															1.0				
Cyclododecyl-1-ethanone																				2.0
Dihydroedulan														0.2						
HexaH-1,6-2-OMe-3-Me-6,6diipr-naphtalene				1.9																
HexaH-1,6-diMe-4-(MeEt)-naphtalene		1.2	1.6	1.8																
Linolenyl alcohol		2.2																		
Naphtalene 2, acetyl											2.8									
n-hexanol								0.2												

Tetramethyl benzene																3.8				

a = aerial parts, s = stems, f = flowers, l = leaves. A: *P. arabica* collected in Saudi Arabia, Najd,[229] B: *P. arabica* collected in Tunisia,[159] C: *P. dysenterica* collected in Iran, Malayer,[230] D: *P. dysenterica* collected in Greece, Katara,[168] E: *P. dysenterica* collected in Greece, Arahova,[168] F: *P. gnaphalodes* collected in Iran, Elbrus mountains,[231] G: *P. gnaphalodes* collected in Iran, Birjand,[232] H: *P. gnaphalodes* collected in Iran, Qom,[233] I: *P. gnaphalodes* collected in Iran, Mashhad,[234] J: *P. jaubertii* collected in S. Arabia,[160] K: *P. glutinosa* collected in UAE, 1997,[235] L: *P. mauritanica* collected in Morocco,[162] M: *P. stephanocarpa* collected in Soqotra,[169] N: *P. undulata* collected in Saudi Arabia, Medinah,[229] O: *P. undulata* collected in Iran, Ramsar,[236] P: *P. undulata* collected in Yemen, Zingibar,[163] Q*: P. undulata* collected in Iran, Saravan,[237] R*: P. undulata* collected in Sudan, El-Fiteehab.[164]

Table 9: MIC (µg/mL) and MMC* (µg/mL) of essential oil from aerial parts of *Pulicaria vulgaris* Gaertn. var. graeca (Sch.-Bip.) Fiori

Strain	Oil	Chloramphenicol
Bacillus cereus (ATCC 11778)	25(50)	12.5
Bacillus subtilis (ATCC 6633)	25(50)	12.5
Staphylococcus aureus (ATCC 25923)	50(100)	25
Staphylococcus epidermidis (ATCC 12228)	50(100)	3.12
Streptococcus faecalis (ATCC 29212)	100	25
Escherichia coli (ATCC 25922	100	12.5
Klebsiella pneumonia (ATCC 10031)	100	50
Proteus vulgaris (ATCC 13315)	100	25
Pseudomonas aeruginosa (ATCC 27853)	100	100
Salmonella typhi Ty2 (ATCC 19430)	100	6.25

*MBC are reported in brackets when different from MIC

[229] Mossa J. S., Hifnawy M. S., Al-Yahya M. A., Al-Meshal I. A., Mekkawi A. G., *International Journal of Crude Drug Research*, **1987**, *25*, 113.
[230] Mumivand H., Rustaii A. R., Jahanbin K., Dastan D., *Journal of Essential Oil Bearing Plants,* **2010**, *13*, 717.
[231] Weyerstahl R., Marschall H., Christiansen C., Rustaiyan A., Mirdjalili F., *Flavour and Fragrance* Journal, **1999**, *14*, 121.
[232] Khani A., Asghari J., *Journal of Insect Science* (Madison, WI, United States), **2012**, *12*, 73.
[233] Kazemi M., Nagafi G. R., Azad A., *Asian Journal of Chemistry*, **2013**, *25*, 3215.
[234] Bashi D. S., Ghani A., J. Asili, *Journal of Essential Oil Bearing Plants,* **2013**, *16*, 252.
[235] Yousuf M. A., Bashir A., Veres K., Dobos A., Nagy G., Mathe I., Blunden G., *Journal of Essential Oil Research*, **2001**, *13*, 454.
[236] Nematollahi F., Rustaiyan A., Larijani K., Nadimi M., Masoudi S., *Journal of Essential Oil Research*, **2006**, *18*, 339.
[237] Ravandeh M., Valizadeh J., Noroozifar M., Khorasani-Motlagh M., *Journal of Medicinal Plants Research*, **2011**, *5*, 2035.

7.4. TABLE OF THE COMPOSITION AND OF THE BIOLOGICAL ACTIVITY OF ESSENTIAL OILS FROM *BALLOTA HISPANICA*

***Table 10*:** Percent composition of the essential oils of aerial parts of *Ballota hispanica* (L.) Benth. arranged by class.

KI[a]	KI[b]	COMPONENT[d]	*B.h*	Id.[c]
		Hydrocarbons	**13.8**	
1293	1295	1-Tridecene	3.0	1,2
1493	1495	1-Pentadecene	3.7	1,2
2100	2100	Heneicosane	0.3	1,2,3
2300	2300	Tricosane	1.3	1,2,3
2500	2500	Pentacosane	1.4	1,2,3
2600	2600	Hexacosane	0.2	1,2,3
2700	2700	Heptacosane	2.8	1,2,3
2800	2800	Octacosane	0.2	1,2,3
2900	2900	Nonacosane	0.9	1,2,3
		Carbonylic Compounds	**3.8**	
1102	1401	Nonanal	0.4	1,2
1204	1508	Decanal	0.5	1,2
1380	1835	β-damascenone	t	1,2
1845	2131	Hexahydrofarnesyl acetone	2.9	1,2
		Sesquiterpene Hydrocarbons	**23.9**	
1348	1466	α-cubebene	0.5	1,2
1363	1492	Cyclosativene	0.8	1,2
1373	1493	α-ylangene	8.5	1,2
1377	1497	α-copaene	1.2	1,2
1385	1535	β-bourbonene	1.3	1,2
1387	1600	β-elemene	0.9	1,2
1407	1538	α-gurjunene	0.1	1,2
1418	1612	(*E*)-caryophyllene	0.9	1,2,3
1432	1583	β-copaene	0.5	1,2
1432	1612	β-cubebene	0.4	1,2
1437	1628	Aromadendrene	0.3	1,2
1438	1573	α-bergamotene	0.6	1,2
1438	1652	γ-elemene	t	1,2
1475	1679	α-amorphene	0.3	1,2
1475	1715	β-selinene	0.5	1,2
1476	1669	γ-gurjunene	t	1,2
1477	1726	Germacrene D	3.5	1,2
1485	1675	Epizonarene	0.4	1,2
1494	1687	Viridiflorene	0.4	1,2
1495	1740	Valencene	t	1,2
1504	1740	α-muurolene	0.3	1,2
1515	1776	γ-cadinene	0.9	1,2

1526	1173	δ-cadinene	0.6	1,2
1554	1856	Germacrene B	1.0	1,2
		Oxygenated Monoterpenes	**0.5**	
1098	1553	Linalool	0.5	1,2,3
1187	1706	α-terpineol	t[e]	1,2,3
		Oxygenated Sesquiterpenes	**32.8**	
1542	2096	α-elemol	10.9	1,2
1553	2076	*cis*-α-copaen-8-ol	0.5	1,2
1565	2057	Ledol	0.3	1,2
1577	2148	Spathulenol	0.9	1,2
1581	2008	Caryophyllene oxide	t	1,2,3
1593	2104	Viridiflorol	0.5	1,2
1598	2108	Guaiol	0.8	1,2
1625	2127	10-*epi*-γ-eudesmol	0.4	1,2
1636	2185	γ-eudesmol	4.2	1,2
1640	2185	τ-cadinol	0.5	1,2
1642	2209	τ-muurolol	0.8	1,2
1650	2258	β-eudesmol	3.7	1,2
1652	2253	α-cadinol	0.8	1,2
1654	2206	α-eudesmol	2.9	1,2
1679	2334	Khusinol	2.5	1,2
1753	2535	γ-costol	0.8	1,2
1774	2068	14-hydroxy-α-muurolene	1.6	1,2
1780	2607	β-costol	0.7	1,2
		Diterpenes	**9.9**	
1949	2622	(*Z*)-phytol	0.2	1,2
1989	2393	Manoyl oxide	4.8	1,2
1994	2406	13-*epi*-manoyl oxide	1.9	1,2
2054	2524	Abietatriene	0.2	1,2
2132	2625	(*E*)-phytol	2.8	1,2
		Others	**5.4**	
1680	2396	γ-dodecalactone	5.1	1,2
2828	3048	Squalene	0.3	1,2
		TOTAL	**90.1**	

[a]: K_i: Retention index on a HP-5MS column; [b]: K_i: Retention index on a HP-Innowax column; [c]: Identification: 1 = comparison of retention index; 2 = comparison of mass spectra with MS libraries; 3 = comparison with authentic compounds; [d]: t = trace, less than 0.05 %

***Table 11*:** MIC (µg/mL) and MBC* (µg/mL) of essential oil from *Ballota hispanica* (L.) Benth. growing wild in Sicily

Strain	Oil	Chloramphenicol
Bacillus cereus (ATCC 11778)	50(100)	12.5
Bacillus subtilis (ATCC 6633)	100	12.5
Staphylococcus aureus (ATCC 25923)	100	25
Staphylococcus epidermidis (ATCC 12228)	50(100)	3.12
Streptococcus faecalis (ATCC 29212)	100	25
Escherichia coli (ATCC 25922)	100	12.5
Klebsiella pneumoniae (ATCC 10031)	>100	50
Proteus vulgaris (ATCC 13315)	>100	25
Pseudomonas aeuriginosa (ATCC 27853)	>100	100
Salmonella typhi Ty2 (ATCC 19430)	>100	6.25

*MBC are reported in brackets when different from MIC

***Table 12*:** Scavenging ability of *B. hispanica* oil on DPPH radical

Concentration of oil (mg/mL)	1.25	2.5	5	10
Inhibition percentage (%)	11	14	19	26

***Table 13*:** ABTS assay of *B. hispanica* oil

Concentration of oil (mg/mL)	1.25	2.5	5	10
Inhibition percentage (%)	24.51	23.19	26.11	26.61

7.5. TABLE OF THE COMPOSITION AND BIOLOGICAL ACTIVITY OF ESSENTIAL OILS FROM *MOLUCCELLA SPINOSA*

***Table 14*:** Percent composition of the essential oils of aerial parts of *M. spinosa* arranged by class.

KI[a]	KI[b]	COMPONENT[d]	*Ms*	Id.[c]
		Hydrocarbons	**16.3**	
2300	2300	Tricosane	0.4	1,2,3
2400	2400	Tetracosane	2.3	1,2,3
2500	2500	Pentacosane	2.5	1,2,3
2600	2600	Hexacosane	0.3	1,2,3
2700	2700	Heptacosane	5.3	1,2,3
2900	2900	Nonacosane	5.5	1,2
		Carbonylic Compounds	**8.6**	
1101	1401	Nonanal	0.7	1,2
1153	2027	Ethylbenzaldehyde	3.4	1,2
1385	1836	(*Z*)-β-damascenone	0.7	1,2
1453	1867	(*E*)-geranyl acetone	0.5	1,2
1484	1958	(*E*)-β-ionone	1.1	1,2,3
1845	2131	Hexahydrofarnesylacetone	1.9	1,2
1915	2387	(*E*,*E*)-farnesyl acetone	0.3	1,2
		Monoterpene Hydrocarbons	**39.6**	
929	1035	α-thujene	5.9	1,2
938	1076	α-pinene	26.6	1,2,3
973	1132	Sabinene	2.2	1,2
980	1118	β-pinene	1.4	1,2,3
1025	1280	*p*-cymene	1.5	1,2,3
1030	1203	Limonene	0.8	1,2,3
1129	1383	*allo*-ocimene	1.2	1,2
		Sesquiterpenes Hydrocarbons	**9.3**	
1415	1612	β-caryophyllene	8.6	1,2,3
1455	1689	α-humulene	0.4	1,2
1477	1726	Germacrene D	0.3	1,2
		Oxygenated Monoterpenes	**2.0**	
1034	1213	1,8-cineole	0.7	1,2,3
1117	1571	*trans*-*p*-menth-2-en-1-ol	0.3	1,2
1128	1487	α-campholenal	0.4	1,2
1176	1611	Terpineol-4	0.3	1,2,3
1208	1723	*cis*-verbenone	0.3	1,2
		Oxygenated Sesquiterpenes	**18.1**	
1571	2035	α-caryophyllene alcohol	0.2	1,2
1578	2150	Spathulenol	0.3	1,2
1580	2008	Caryophyllene oxide	16.8	1,2,3
1599	2178	Widdrol	0.8	1,2

		Diterpenes	**0.5**	
2132	2625	(*E*)-phytol	0.5	1,2
		Others	**1.4**	
995	1243	2-Pentylfuran	1.1	1,2
1573	2077	Tridecanol	0.3	1,2
		TOTAL	**95.8**	

[a]: HP-5 MS column; [b]: HP Innowax column [c]: 1, retention index, 2: mass spectrum, 3: co-injection with authentic compound; [d]: t: trace, <0.05%,

Table 15: MIC (mg/mL) and MMC* (mg/mL) of aerial parts of *M. spinosa*.

Strain	Oil	Chloramphenicol	Amphotericin B	Ketoconazole
B. cereus (ATCC 11778)	50 (100)	12.5	NT	NT
B. subtilis (ATCC 6633)	50 (100)	12.5	NT	NT
S. aureus (ATCC 25923	100 (>100)	25	NT	NT
S. epidermidis (ATCC 12228)	25 (50)	3.12	NT	NT
S. faecalis (ATCC 29212)	50 (100)	25	NT	NT
E. coli (ATCC 25922)	50 (100)	12.5	NT	NT
Klebsiella pneumonia (ATCC 10031)	100	50	NT	NT
P. vulgaris (ATCC 13315)	100(100)	25	NT	NT
P. aeuriginosa (ATCC 27853)	>100	100	NT	NT
Candida albicans (ATCC 10231)	100	NT	1.56	NT
F. oxysporum (ATCC 695)	100 (>100)	NT	NT	3.12
A. niger (ATCC 16401)	50 (>100)	NT	NT	1.56

*MMCs are reported in brackets when different from MIC; NT: not tested.

7.6. TABLE OF THE COMPOSITION AND BIOLOGICAL ACTIVITY OF ESSENTIAL OILS FROM *THAPSIA GARGANICA*

Table 16: Percent composition of the essential oils of *Thapsia garganica* L. (Apiaceae) flowers (**T.f.**) and leaves (**T.l.**).

Ki[a]	Ki[b]	Component	*T.f.*	*T.l.*	Id.[c]
		Hydrocarbons	**23.9**	**6.0**	
2300	2300	Tricosane	8.2	1.1	1, 2, 3
2492	2497	1-Pentacosene	7.5		1,2
2500	2500	Pentacosane	8.2	4.9	1, 2, 3
		Monoterpene hydrocarbons		**0.1**	
938	1032	α-Pinene	t		1, 2, 3
1029	1218	β-Phellandrene		0.1	1, 2, 3
1049	1262	(*E*)-β-Ocimene		t	1, 2
		Sesquiterpene hydrocarbons	**63.0**	**67.7**	
1735	2434	Chamazulene	58.3	49.2	1, 2
1553	2289	1,4-Dimethylazulene	4.7	18.5	1, 2
		Oxygenated sesquiterpenes	**11.3**	**5.3**	
1498	2078	Curzerene; 5-Isopropenyl-3,6-dimethyl-6-vinyl-4,5,6,7-tetrahydro-1-benzofuran	2.3	1.4	1, 2
1612	2018	Humulene oxide II	9.0		1,2
1876	2707	Furanoeremophil-1-one		3.9	1,2
		Diterpenes		**11.4**	
1838	1992	Neophytadiene		5.1	1, 2
2135	2625	(*E*)-Phytol		6.3	1, 2
		TOTAL	**98.2**	**90.5**	

[a]: HP-5 MS column; [b]: HP Innowax column [c]: 1: retention index, 2: mass spectrum, 3: co-injection with authentic compound; [d]: t: trace, <0.05%; **: irregular terpenes.

Table 17: Main volatile constituents of the essential oils from *Thapsia* ssp. reported in literature.

COMPONENT	***A*(r)[a]**	***A*(r)[b]**	***A*(fr)[b]**	***A*(f)[b]**	***B*(r)**	***C*(f)**	***C*(l)**	***C*(s)**	***D*(f)**	***D*(fr)**	***E*(a)**	***F*(r)**	***F*(f)**	***F*(l)**	***F*(s)**	***G*(f)**	***H*(f)**	***I*(f)**	***L*(fr)**	***L*(r)**
Hydrocarbons	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**47.8**	**0.0**	**0.0**	**0.0**	**8.2**	**22.9**	**0.0**	**7.4**	**0.0**	**0.0**
n-heneicosane											17.4									
Pentadecane											12.5									
Tricosane											17.9				8.2	22.9		7.4		
Carbonylic compounds	**2.2**	**4.1**	**25.8**	**7.7**		**0.0**	**0.0**	**0.0**	**4.8**	**0.0**	**0.0**	**3.7**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
2-hexenal				3.5																
2-nonenal	2.2																			
Acetophenone			22.1																	
Heptanal		4.1	3.7																	
Latifolone					19.6-32.7															
Nonal				4.2																
Pentadecanal												3.7								
Phenylacetaldehyde									4.8											
Sulcatone			3.7																	
Monoterpenes Hydrocarbons	**2.9**	**2.8**	**0.0**	**14.3**	**0.0**	**5.3**	**5.1**	**4.9**	**0.0**	**0.0**	**34.5**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**34.1**	**0.0**
Limonene						5.3	5.1	4.9											34.1	
Sabinene											14.2									
α-pinene											14.5									
α-terpinene				3.5																
β-myrcene	2.9	2.8		10.8																
δ^3-carene											5.8									
Sesquiterpenes Hydrocarbons	**21.1**	**37.9**	**0.0**	**0.0**	**0.0**	**6.6**	**6.3**	***6.2***	**7.0**	***10.3***	**0.0**	**21.6**	**27.1**	**30.8**	**35.1**	**27.1**	**22.0**	**24.1**	**0.0**	**7.5**
1,4-dimethylazulene						6.6	6.3	6.2	7.0	6.3										
7-acetyl-1,4-dimethyazulene										4.0										

bicyclogermacrene												21.6	27.1	30.8	35.1	27.1		24.1		
Cadina-1,4-Diene																	6.6			
Longifolene													3.5							
α-copaene																				4.4
α-elemene																				3.1
β-elemene	2.1																3.3			
β-sesquiphellandrene																	12.1			
δ-cadinene	16.7	34.8																		
δ-guaiene	2.3	3.1																		
Oxygenated Monoterpenes	**2.2**	**0.0**	**7.9**	**16.2**	**0.0**	**7.2**	**6.5**	**6.1**	**17.8**	**12.8**	**0.0**	**6.9**	**19.1**	**14.0**	**31.4**	**9.3**	**0.0**	**6.2**	**62.8**	**6.3**
Borneol												3.1								
Bornyl Acetate																				6.3
Geranial												3.8	6.7	3.1	14.8	4.1		6.2		
Geraniol									3.1	4.2										
Geranyl Acetate			7.9	10.1																
Isoeugenol															16.6					
Linalool						7.2	6.5	6.1	11.6	8.6			9.1	10.9		5.2				
Methyl Eugenol																			62.8	
trans-sabinene hydrate													3.3							
α-terpineol									3.1											
β-citral	2.2			6.1																
Oxygenated Sesquiterpene	**0.0**	**25.2**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**26.8**	**0.0**	**0.0**	**58.7**
Caryophyllene Oxide																				20.7
Elemol		25.2																		
Epicedrol																	3.6			
Epicubenol																	15.7			

Humulene Oxide																				17.7
Sphatulenol																				20.3
α-cadinol																	3.9			
α-cedrol																	3.6			
Diterpenes	**0.0**	**0.0**	**0.0**	**0.0**		**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**4.5**	**4.2**	**5.2**	**3.5**	**0.0**	**0.0**	**0.0**	**0.0**	**24.0**
(*E*)-phytol												4.5	4.2	5.2	3.5					
Elemicin					53.7-73.0															24.0
Fatty Acids and Derivatives	**0.0**	**0.0**	**0.0**	**0.0**		**0.0**	**0.0**	**0.0**	**4.8**	**0.0**	**0.0**	**4.0**	**3.8**	**5.8**	**0.0**	**3.4**	**0.0**	**6.4**	**0.0**	**0.0**
Fereulic Acid Derivatives					2.6-6.6															
Hexadecanoic Acid												4.0	3.8	5.8		3.4		6.4		
Other	**0.0**	**4.9**	**3.1**	**23.1**		**63.2**	**61.6**	**50.9**	**60.7**	**61.3**	**0.0**	**0.0**	**0.0**	**3.3**	**0.0**	**13.2**	**0.0**	**0.0**	**0.0**	**0.0**
1-octanol				4.8																
2-ethylhexenol		4.9		18.3																
Coumarin									12.7											
Hexenol			3.1																	
Myristicin					1.3-3.2															
p-vinylguaiacol						63.2	61.6	50.9	48.0	61.3										
Tetradecanol														3.3						
α-kaurane																13.2				
TOTAL																				

r = root, fr = fruit, f = flowers, l = leaves, s = stems, a = aerial parts, a = a Steam distillation; b = SPME;, A: *T. garganica* L. collected in France[120], B: *T. garganica* L.collected in Italy[118], C: *T. garganica* collected in Algery[119], D: *T. garganica* collected in Italy[117], E: *T. garganica* collected in Greece[121], F: *T. garganica* collected in Tunisia (Morgane)[122], G: *T. garganica* collected in Tunisia (Oued Rmal), H: *T. garganica* collected in Tunisia (Utique), I: *T. garganica* collected in Tunisia (Ghar El Mehl), L: *T. maxima* Mill. Type I (*T. maxima*)[c] collected in Portugal[238,185]; c = According classification by Weitzel et al. 2014.

[238] Avato P., Jacobsen N., Smitt U. W., *Journal of Essential Oil Research*, **1992**, *4*, 467-473.

Table 17: *continuation*

COMPONENT	***M*(fr)**	***M*(r)**	***N*(a)**	***O*(r)[a]**	***O*(f)[a]**	***O*(fr)[a]**	***O*(r)[b]**	***O*(f)[b]**	***O*(fr)[b]**	***O*(s)[b]**	***P*(fr)**	***Q*(fr)**	***R*(fr)**	***S*(fr)**	***T*(r)**	***U*(r)**	***V*(r)**	***Z*(ap)**
Carbonylic compounds	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**4.4**	**0.0**	**0.0**	**4.0**	**0.0**	**0.0**		**0.0**				**0.0**
Hexanal							4.4											
Octanal										4.0								
Monoterpenes Hydrocarbons	**27.2**	**0.0**		**0.0**	**2.1**	**2.7**	**0.0**	**4.4**	**6.6**	**57.5**						**0.0**	**0.0**	**57.6**
2,5-dimethoxy p-cymene															0-11.9			
Limonene	27.2								2.5	8.1	0.2-3.1	0.1-8.3	14.0-26.4	30.9-55.0				57.6
p-cymene			2.3-3.1								0.8-2.6	0.4-3.1						
Sabinene										7.3								
α-pinene										9.4								
α-terpinene										3.2								
α-thujene										7.0								
α-cymene										10.6								
β-myrcene					2.1	2.7		4.4	4.1	4.3								
δ^3-carene																		
γ-terpinene			3.0-3.2							7.6		0.5-1.0						
Sesquiterpenes Hydrocarbons	**0.0**	**6.4**	**0.0**	**2.6**	**0.0**	**0.0**	**23.3**	**4.2**	**13.1**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**				**0.0**
Bicyclogermacrene																2.0-4.8		
Germacrene D																2.3-3.2		
α-copaene		3.0																
α-elemene		3.4																
α-guaiene							6.7											
α-humulene							3.5		2.6						0-4.2			
β-caryophyllene								4.2	10.5							138-21.2	t-1.0	

δ-cadinene				2.6			4.0									1.4-1.8	1.0-3.3	
δ-guaiene							9.1											
γ-cadinene																	0.6-2.2	
γ-muurolene																	1.2-2.7	
Oxygenated Monoterpenes	**59.4**	**13.6**		**0.0**	**41.3**	**51.7**	**6.4**	**52.8**	**39.2**	**3.4**								**35.9**
2,5-dimethoxy--p-cymene																0.0-7.3	0.9-4.7	
Bornyl Acetate		6.8													2.0-5.0			
Carvone													1.6-3.1	0.1-4.1	0-3.9			
cis-linalool oxide											0.05-4.8							
Geranial						2.2												
Geraniol					3.2	2.2		9.7			1.0-7.6	1.1-1.5						
Geranyl Acetate			82.3-83.0		36.5	45.1	6.4	43.1	39.2	3.4	78.5-92.2	81.7-88.5		0-7.3				
Isoeugenol																		
Linalool					1.6	2.2					0.4-4.2	1.2-5.1			t-5.9			
Methyl Eugenol	59.4	6.8											45.7-62.4	33.3-66.1	0-12.4		0.0-5.4	35.9
trans-carveol													1.8-3.3					
Oxygenated Sesquiterpene	**0.0**	**27.1**	**0.0**	**25.2**	**0.0**	**0.0**	**21.6**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**				**0.0**
8-hydroxy isobornyl isobutyrate																	6.3-6.4	
Caryophyllene Alcohol															0-3.7			
Caryophyllene Oxide		13.2													0-8.5	9.3-18.6	0.0-1.2	
Elemol				9.9														
Farnesol																3.0-4.3		
Farnesyl Acetate																0.0-13.0		
Guaiol		10.8		15.3			21.6									0-24	35.0	

Humulene Oxide		3.1													0-7.3			
Isobornyl-2-Methyl-Butyrate															0-5.4			
Sphatulenol		11.8													11.5-15.3	7.1-7.5	4.0-6.6	
β-copaen-4-α-ol															0-7.5			
β-oplopenone															0-50.8			
Diterpenes	**0.0**	**29.7**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**		**0.0**				**0.0**
Elemicin		29.7											t-30.1		2.3-22.4	t-8.4	9.7-25.3	
Other	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**		**0.0**				**0.0**
α-Irone																1.7-12.1	0.0-3.2	
Myristicin															0-3.9		1.6-2.1	
Calamenene-1,11-Oxide															3.3			
TOTAL																		

r = root, fr = fruit, f = flowers, l = leaves, s = stems, a = aerial parts, a = a Steam distillation; b SPME; M: *T. maxima* Mill. Type II (*T. smittii* Simonsen, Rønsted,Weitzel & Spalik)[c] collected in Portuga,l[238,185] N: *T. minor* Hoffgg. and Link collected in Portugal,[239] O: *T. villosa L.* type II (*T. laciniata* Rouy)[c] collected in France,[120] P: *T. villosa L.* type I+III (*T. minor* Hoffgg. and Link)[c] collected in Portugal, Spain,[240] Q: *T. villosa L.* type II (*T. laciniata* Rouy)[c] collected in Portugal, Spain,[240] R: *T. villosa L.* (tetraploid) collectet in Portugal, Spain,[241] S: *T. villosa L.*(hexaploid) collectet in Portugal, Spain, T: *T. villosa L.* type IV+V (polyploid) 2n=44 e 2n=66 collectet in Portugal, Spain,[185] U: *T. villosa L.* type I+III (diploid and tetraploid) (*T. minor* Hoffgg. and Link)[c] collectet in Portugal, Spain, V: *T. villosa L.* type II (diploid) (*T. laciniata* Rouy)[c] collectet in Portugal, Spain, Z: *T. villosa* collectet in Portugal,[242] c According classification by Weitzel et al. **2014**.

[239] Goncalves M. J., Cruz M. T., Tavares A. C., Cavaleiro C., Lopes M. C., Canhoto J., Salgueiro L., *Industrial Crops and Products*, **2012**, *35*, 166-171.
[240] Avato P., Trabace G., Smitt U. W., *Phytochemistry*, **1996a**, *43*, 609-612.
[241] Avato P., Trabace G., Smitt U. W., *Journal of Essential Oil Research*, **1996b**, *8*, 123-128.
[242] Rufino A. T., Ferreira I., Judas F., Salgueiro L., Lopes M. C., Cavaleiro C., Mendes A. F., *Pharmaceutical Biology*, **2015**, *53*, 1220-1230.

Table 18: MIC (µg/mL) and MMC* (µg/mL) of essential oils from *Thapsia garganica*

Strain	*T.f.*	*T.l.*	Ch	Am	Ke
B.subtilis ATCC 6633	12.5 (25)	50 (100)	12.5	NT	NT
S.aureus ATCC 25923	50 (100)	25 (50)	25	NT	NT
S. epidermidis ATCC 12228	50 (50)	12.5 (25)	3.12	NT	NT
S.faecalis ATCC 29212	50 (100)	50	25	NT	NT
E.coli ATCC 25922	50 (100)	25 (50)	12.5	NT	NT
Klebsiella pneumoniae ATCC 10031	100	50 (100)	50	NT	NT
P. vulgaris ATCC 13315	100	100	25	NT	NT
P. aeuriginosa ATCC 27853	100	100 (>100)	100	NT	NT
Candida albicans ATCC 10231	6.25 (12.5)	12.5	NT	1.56	NT
F. oxysporum ATCC 695	12.5	12.5	NT	NT	3.12
A. niger ATCC 16401	50	50	NT	NT	3.12

*MMC are reported in brackets when different from MIC; NT: not tested; **Ch**: Chloramphenicol; **Am**: Amphotericin B; **Ke**: Ketoconazole

7.7. TABLE OF THE BIOLOGICAL ACTIVITY OF THE EXTRACTS OF *TETRACLINIS ARTICULATA*

Table 19: In vitro antiproliferative activity of *Tetraclinis articulata* (TA) extracts against three tumor lines: J774.A1 macrophages, A-375 human melanoma cells and MCF-7 breast cancer cells, at 72 h

IC_{50} 72h			
	J774.A1	**A-375**	**MCF-7**
TA/HEX	0.82±0.09	142.23±3.22	6.82±0.94
TA/DCM	0.94±0.08	180.42±2.25	8.94±0.82
TA/MeOH	75.22±2.42	N.D.	140.22±3.42
6-mercaptopurine	$0.456\ 10^{-6}$	$7.33\ 10^{-3}$	$21.3\ 10^{-3}$

IC_{50} values for different cancer cell lines are expressed in mg /mL for extracts and for 6-MP, used as reference drug. The IC50 value is the concentration of compound that affords 50% reduction in cell growth after 3 days incubation. Values are expressed as mean ± SD, n = 3, N.D.: not detected

8. NMR DATA

8.1. COMPOUNDS ISOLATED FROM EXTRACTS OF *TETRACLINIS ARTICULATA*

Table 24: NMR data for componud ***3, 4*** and ***5*** in $CDCl_3$ at 600MHz for ^1H and 150.9MHz for ^{13}C (δ in ppm, J in Hz)

position	δ ^1H of ***3***	δ ^{13}C of ***3***	position	δ ^1H of ***4***	δ ^{13}C of ***4***	position	δ ^1H of ***5***	δ ^{13}C of ***5***
1 CH_2	0.91(m) 1.89 (m)	40.8	1 CH_2	α 0.97 (m) β 1.69 (m)	42.0	1 CH_2	1.16 (m) 1.64 (m)	38.1
2 CH_2	1.53 (m) 1.62 (m)	18.0	2 CH_2	1.51 (m)	18.0	2 CH_2	1.45 (m) 1.70 (m)	18.1
3 CH_2	1.29 (m) 1.41 (td, J=3.9, J=13.2)	35.1	3 CH_2	α 1.50 (m) β 1.22 (m)	36.1	3 CH_2	1.65 (m) 1.79 (m)	36.9
4 C	-	37.5	4 C	-	38.5	4 C	-	47.0
5 CH	1.27 (m)	48.3	5 CH	1.62 (d, J=6.5)	54.8	5 CH	1.93 (d, J=12.28)	48.3
6 CH_2	1.72 (m) 1.81 (m)	17.4	6 CH	β 3.95 (m)	67.4	6 CH_2	1.29 (m) 1.48 (m)	24.5
7 CH_2	1.57 (m) 1.78 (m)	42.4	7 CH_2	α 1.71(m) β 1.90 (dd, J=5.2, J=15.2)	42.2	7 CH_2	2.10 (m) 2.25 (dd, J=2.9, J=14.4)	34.4

8 C	-	73.0	8 C	-	75.0	8 C	-	136.5
9 CH	1.28 (m)	54.4	9 CH	1.41 (dd, J=4.7, J=12.3)	60.6	9 CH	2.12 (m)	51.1.
10 C	-	38.5	10 C	-	35.6	10 C	-	37.4
11 CH_2	1.22-1.55 (m) 1.22-1.55 (m)	19.3	11 CH_2	1.42 (m) 1.62 (d, J=4.7)	19.8	11 CH_2	1.56 (t, J=12.2) 1.80 (m)	23.9
12 CH_2	1.54 (m) 1.75 (m)	30.7	12 CH_2	α 1.80 (m) β 1.58 (m)	32.6	12 CH	4.82 (dd, J=3.7, J=12.2)	76.0
13 C	-	35.5	13 C	-	34.3	13 C	-	41.4
14 CH_2	1.51 (m) 1.82 (d, J=14.4)	47.6	14 CH_2	α 1.65 (d, J=14.6) β 1.32 (d, J=14.6)	48.7	14 CH	5.14 (s)	127.9
15 CH	6.01 (dd, J_{cis}=10.7, J_{trans}=17.6)	148.9	15 CH	6.07 (dd, J_{cis}=10.8, J_{trans}=17.7)	151.0	15 CH	5.73 (dd, J_{cis}=10.6, J_{trans}=17.3)	145.6
16 CH_2	*cis* 5.06(d, J=11.0) *trans* 5.12 (d, J=17.8)	111.6	16 CH_2	*trans* 5.07 (d, J=17.7) *cis* 4.98 (d, J=10.8)	110.6	16 CH_2	4.98 (d, J=10.5) 4.99 (d, J=17.6)	112.5

17 CH_3 (s)	0.95 (s)	32.6	17 CH_3	1.01 (s)	30.6	17 CH_3	1.13 (s)	19.37
18 CH_2	α 3.11 (d, J=10.7) β 3.40 (d, J=10.7)	72.1	18 CH_2	α 3.45 (d, J=11.7) β 3.05 (d, J=11.7)	72.6	18 COOH	-	183.9
19 CH_3 (s)	0.77 (s)	17.9	19 CH_3	0.87 (s)	18.2	19 CH_3	1.22 (s)	16.8
20 CH_3 (s)	1.06 (s)	19.0	20 CH_3	0.82 (s)	17.0	20 CH_3	0.86 (s)	14.8
						21 O***C***OCH_3	-	170.7
						22 OCO***CH_3***	2.62 (s)	21.2

Figure 20: Roesy spectra of ***3***

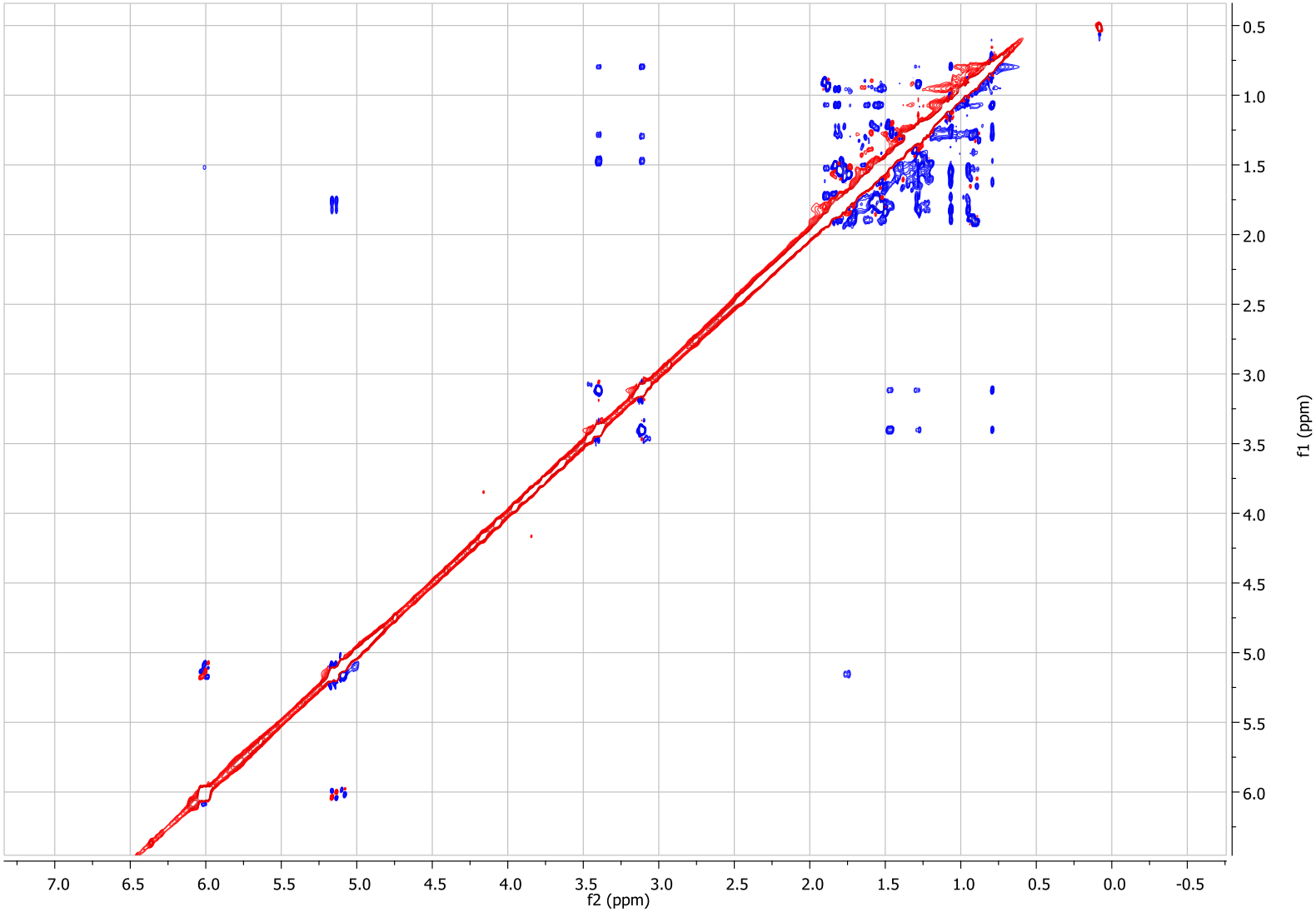
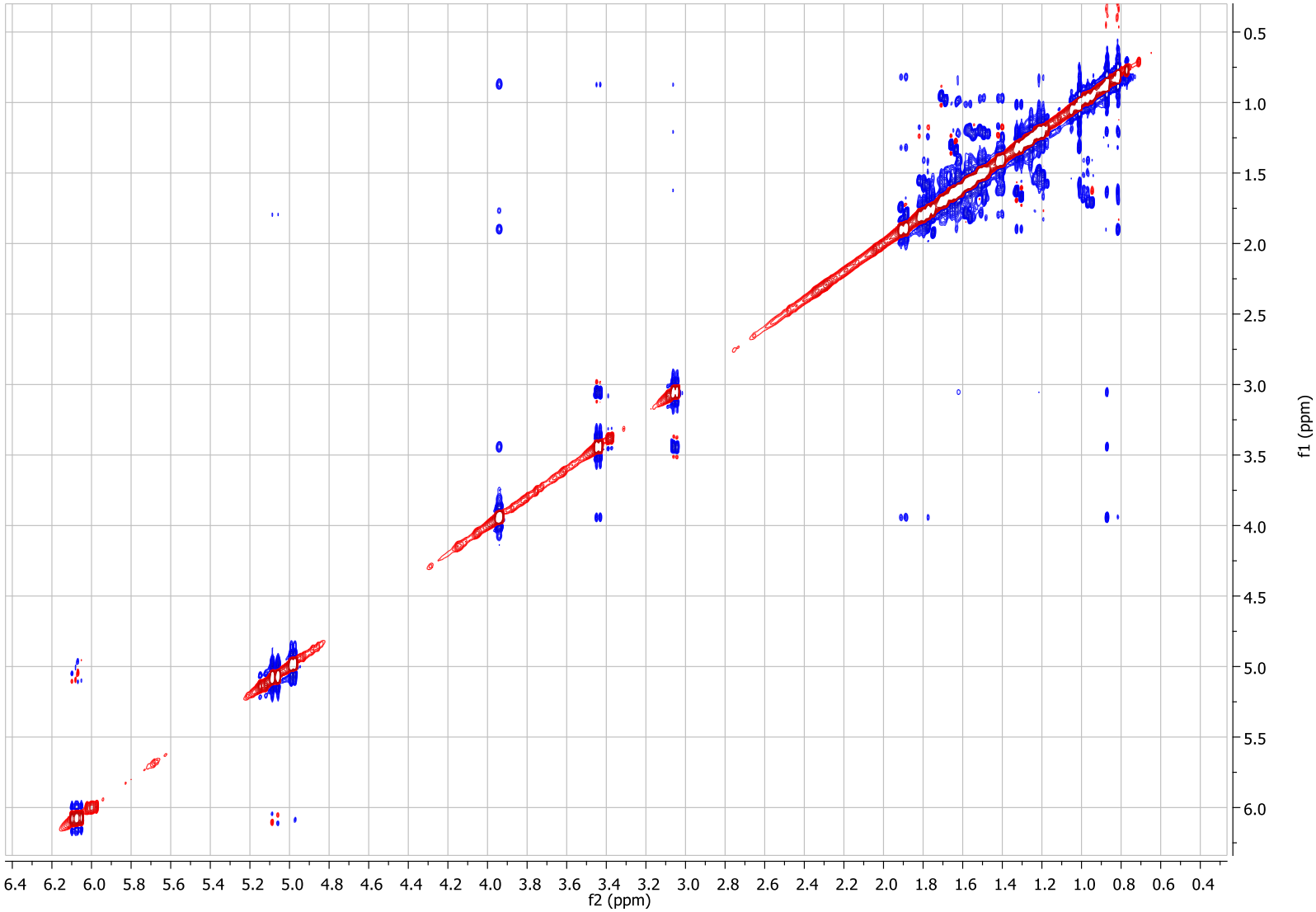


Figure 21: Roesy spectra of ***4***



9. SCIENTIFIC ACTIVITY

9.1. PUBLICATIONS

1. A. Maggio, L. Riccobono, V. Spadaro, A. Scialabba, M. Bruno*, F. Senatore, “Chemical composition of the essential oils of three endemic species of *Anthemis* [sect. Hiorthia (DC.) R. Fern.] Growing Wild in Sicily. Chemotaxonomic volatile of the genus *Anthemis* L.: an update”, *Chemistry & Biodiversity* **2014**, *11* (4), 652-672.

2. A. Maggio, L. Riccobono, S. Bancheva, M. Bruno*, F. Senatore, “Chemical composition of the Essential Oil of the Local Endemics *Centaurea davidovii* and *C. parilica* (Asteraceae, sect. Lepteranthus) from Bulgaria”, *NPC Natural product Communications*, **2014**, *9* (9), 1373-1376.

3. A. Maggio, L. Riccobono, V. Spadaro, P. Campisi, M. Bruno*, F. Senatore, “ Volatile constituents of aerial parts of *Pulicaria sicula* (L.) Moris Growing Wild in Sicily. Chemotaxonomic Volatile Markers of the Genus *Pulicaria* Gaertner”, *Chemistry & Biodiversity*, **2015**, *12* (5), 781-799.

4. S. Casiglia, M. Ben Jemia, L. Riccobono, M. Bruno*, E. Scandolera, F. Senatore, "Chemical composition of the essential oil of *Moluccella spinosa* L. (Lamiaceae) collected wild in Sicily and its activity on microorganisms affecting historical textiles”, *Natural Product Research*, **2015**, *29* (13), 1201-1206.

5. L. Riccobono, M. Ben Jemia, M. Bruno*, F. Senatore, “Chemical composition and free radical scavenging activity of the essential oil of *Ballota hispanica* (L). Benth. Growing in Sicily”, *Plant Biosystem* (in press).

6. L. Riccobono, A. Maggio*, S. Rossellia, V. Ilardi, F. Senatore, M. Bruno, “Chemical composition of essential oil and fixed oils from *Salvia argentea* L. (Lamiaceae) growing wild in Sicily”, *Natural Product Research,* (in press).

7. S. Casiglia, L. Riccobono, M. Bruno*, F. Senatore, F. Senatore "Chemical composition of the essential oil from *Pulicaria vulgaris* var. graeca (Sch.-Bip.) Fiori (Asteraceae) grown wild in Sicily and its antimicrobial activity", *Natural Product Research* (in press).

8. S. Casiglia, L. Riccobono, M. Bruno*, S. Rosselli, F. Senatore, F. Senatore, "Chemical composition of the essential oil from *Thapsia garganica* L. (Apiaceae) grown wild in Sicily and its antimicrobial activity", *Natural Product Research*, (in press).

9. L. Riccobono, A. Maggio, M. Bruno*, S. Bancheva, F. Senatore, F. Senatore, "Chemical Composition of the Essential Oil of *Centaurea grinensis* Reuter and *Centaurea apiculata* Ledeb. Growing Wild in Croatia and Bulgaria, Respectively and PCA Analysis of Subgeus Lopholama (Cass.) Dobrocz", (submitted).

9.2. COMMUNICATIONS TO CONGRESS

9.2.1. Poster communication

1. S. Casiglia, L. Riccobono, M. Bruno, F. Senatore, F. Senatore, "Chemical Composition of the Essential Oil from *Pulicaria Vulgaris* Var. *graeca* (Sch.-Bip.) Fiori (Asteraceae) Grown Wild in Sicily and Its Antimicrobial Activity", Plovdiv, Bulgaria 14-17 October 2015, 2nd International Conference on Natural Products: Utilization From Plants to Pharmacy Shelf (ICNPU 2015).

2. L. Riccobono, M. Ben Jemia, S. Rosselli, A. Maggio, M. Bruno, "Chemical composition and cytotoxic activity of *Tetraclinis articulata* (vahl) mast. Growing in Tunisia" Padova 10-12 June 2015, XIV Congresso Società Chimica di Fitochimica.

3. A. Maggio, L. Riccobono, M. Bruno, S. Bancheva, F. Senatore, “Chemical composition of the essential oil of *Centaurea grinensis* reuter and *Centaurea apiculata* ledeb. Growing wild in Croatia and Bulgaria, respectively”, Padova 10-12 June 2015, XIV Congresso Società Chimica di Fitochimica.

4. L. Riccobono, M. Ben Jemia, S. Rosselli, A. Maggio, M. Bruno, “ Chemical composition and cytotoxic activity of *Tetraclinis articulata* (Cupressaceae)”, Rende (CS) 7-12 September 2014, XXV Congresso Nazionale della Società Chimica Italiana.

5. O. Fici, M. Ben Jemia, F. Senatore, L. Riccobono, M. Bruno, “Chemical composition of the essential oli *Teucrium fruticant* L. and its free radical scavenging activity”, Catania 2-3 December 2013, Congresso Società Chimica Italiana, Convegno Congiunto delle Sezioni Sicilia e Calabria.

6. L. Riccobono, M. Ben Jemia, F. Senatore, M. Bruno “Chemical composition and free radical scavenging activity of the essential oil of *Ballota hispanica* (L.) Benth”, Catania 2-3 December 2013, Congresso società chimica italiana, Convegno Congiunto delle Sezioni Sicilia e Calabria.

7. A. Maggio, L. Riccobono, V. Spadaro, F. M. Raimondo, M. Bruno, F. Senatore, “Chemical Composition of the essential oils of three endemic species of *Anthemis* (Asteraceae) growing wild in Sicily. Chemotaxonomic volatile markers of *Anthemis* L. (Sect. HIORTHIA): an update.”, Baselga del Pinè (TN) 18-20 September 2013, 108° Congresso Società Italiana di Botanica.

9.2.2. Oral communication

1. L. Riccobono, S. Rosselli, A. Maggio, M. Bruno, "Diterpenes from *Teraclinis articulata*", Messina, Italy 17-18 september 2015, RMN e Salute: Diagnostica e Alimentazione (Second Ediction).

2. Luana Riccobono, Mariem Ben Jemia, Gianluigi Lauro, Sergio Rosselli, Antonella Maggio, Giuseppe Bifulco, Maurizio Bruno, "Chemical Composition and Potential Biological Activity of Tetraclinis Articulata (Vahl) Mast. Growing in Tunisia", Naples Stazione Zoological "Anton Dohrn" 6-10 July 2015, International Summer School on Natural Products (ISSNP).

3. L. Riccobono, G. Catinella, A. Maggio, G. Fontana, S. Rosselli, M. Bruno, "Chemical composition of *Salvia argentea* L. (Lamiaceae)", Palermo 1-2 December 2014, Congresso Società Chimica Italiana, Convegno Congiunto delle Sezioni Sicilia e Calabria.

9.2.3. Schools

1. International Summer School on Natural Products (ISSNP), 6-10 July 2015, Stazione Zoological "Anton Dohrn", Naples, Italy.

2. National School of Photochemistry, 13-17 September 2010, University of Bologna, Italy

9.3. EXTERNAL COLLABORATION

1. From 16 June 2014 to 30 July 2014 at University of the Study of Salerno in the research group of prof. Giuseppe Bifulco.
2. From November 2013 to February 2014 at CSIC of Barcelona in the research group of prof. Angel Guerrero Perez.