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Medicinal and aromatic plants of Cape Verde: characterization of volatile metabolites of endemic and native species

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RESUMO

As plantas têm sido usadas como remédios pelo homem desde há milhares de anos e várias culturas incluem um amplo conhecimento sobre suas virtudes medicinais. Práticas tradicionais, apoiadas por conhecimentos empíricos, crenças e procedimentos, desempenham um papel importante, primeiramente nos cuidados de saúde das populações e, secundariamente, como evidências etnofarmacológicas disponíveis para o desenvolvimento de fármacos.

As plantas aromáticas são associadas a muitas aplicações biológicas e médicas. Elas biossintetizam uma grande variedade de metabolitos secundários voláteis que desempenham importantes papéis eco-fisiológicos, tais como, proteção contra organismos prejudiciais, predadores, patogénicos microbianos, stresse abiótico, calor, desidratação, etc ..., desempenhando também um papel importante na interação com outras plantas e organismos. Os metabolitos voláteis, distintivos das plantas aromáticas, são agentes naturalmente seleccionados para interagir com alvos biológicos.

Este trabalho está focado em plantas medicinais e aromáticas de Cabo Verde. Teve por base informação etnofarmacológica recolhida por inquéritos diretos à população, e que conduziu ao estudo dos metabolitos voláteis das espécies aromáticas endémicas e nativas utilizadas na medicina tradicional de Cabo Verde.

Reportamos a composição dos isolados voláteis das quatro plantas medicinais aromáticas, *Artemisia gorgonum*, *Satureja forbesii*, *Hytis pectinata* e *Cymbopogon citratus*, colhidas na ilha de Santiago. Os óleos essenciais foram obtidos por hidrodestilação foram analisados por combinação de técnicas de cromatografia de fase gasosa e espectrometria de massa.

A informação sobre a composição química dos recursos naturais de Cabo Verde é especialmente relevante, considerando a validação das práticas da medicina tradicional, bem como, a valorização da biomassa vegetal como fonte de compostos úteis para pesquisas, incluindo desenvolvimento de fármacos e outros fins.

Este trabalho também é uma homenagem aos terapeutas tradicionais de Cabo Verde e uma contribuição para o conhecimento da cultura e do património vegetal deste arquipélago Verde.

ABSTRACT

Plants have been used as remedies by man for thousands of years and various cultures have an extensive knowledge about their medicinal properties. Traditional healing practices, supported by empirical knowledge, beliefs and procedures, play important roles, immediately, in health care of populations, secondarily, as ethnopharmacological starting evidence for Drug Discovery.

Aromatic plants are very often associated with many biological and medical applications. They produce a wide range of volatile secondary metabolites with important ecophysiological roles, such as, protection against harmful organisms, predators, microbial pathogens, abiotic stress, heat, dehydration, etc..., or playing important interaction with other plants and organisms. Thus, volatile metabolites, distinctive of aromatic plants, are naturally skilled to interact with biological targets.

This work is focused on medicinal and aromatic plants from Cape Verde. Ethnopharmacological information, collected by direct inquires to locals, was in the base of a prospective chemical study on volatile metabolites of endemic and native aromatic species from Cape Verde used in traditional medicine.

We report on the composition of the volatile isolates of four aromatic medicinal plants, *Artemisia gorgonum*, *Satureja forbesii*, *Hytis pectinata* and *Cymbopogon citratus*, collected at Santiago Island. The essential oils were prepared at laboratory by hydrodistillation and analyzed by combination of gas chromatography and mass spectroscopy techniques.

Chemical information on these natural resources of Cape Verde is useful data in regard to the validation of traditional medicinal practices, as well as, in regard to the valorization of plant biomass as sources of compounds useful for research, including the development of drugs, and other industrial purposes.

This work is also homage to the traditional therapists of Cape Verde and contribution for the knowledge of the culture and the vegetal patrimony of this archipelago Verde.

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EQUATIONS

Equation I. Linear interpolation, according Van den Dool and Kratz: $IR_{(a)}$ - peak “a” retention indice, n – number of carbons of the n -alkane eluting before peak “a”; $Tr_{(a)}$ – Retention time of peak “a”; $Tr_{(n)}$ - Retention time of peak the n -alkane eluting before peak “a”; $Tr_{(n+1)}$ - Retention time of peak the n -alkane eluting after peak “a”25

I. Introduction

It is a fact, that there is a symbiotic relationship between human and plants. Over the ages, human have relied on nature for their basic needs, such as, production of food-stuffs, shelters, clothing, means of transportation, fertilizers, flavours and fragrances, and not the least, medicines. Plants have proven to be the basis of sophisticated traditional medicine systems, which exists for thousands of years (Fakim, 2011).

Human beings have been using plants as drugs for thousands of years. Nowadays, all the world's cultures have an extensively knowledge on medicinal herbs. Traditional medicine systems are based on empirical findings, beliefs and practices that existed long before the development of the so called "modern medicine" or "scientific drug therapy" (Abad *et al.*, 2012).

As previously said, plants are essential sources of important compounds in which human beings rely for their basic needs. This work will be focused on their medical use. These important compounds lead to drug discovery and, consequently, to health improvement. Hence, during the last decades, chemists and biologists have been intensively investigating tropical and subtropical plants species with potential medicinal properties in order to assess the feasibility of developing natural, sustainable, and affordable drugs and cosmetics (Koba *et al.*, 2009). The importance of ethnobotanical inquiries as cost-effective means of locating new and useful plant compound cannot be underestimated (Fakim, 2011). Plant biomass is considered an important reservoir of potential drugs. Nowadays, at least 80 per cent of the world's population still relies on traditional medicine for their health care.

Medicinal plants usually contains mixture of various and different chemical compounds that might act individually, additively or in synergy to improve health. For example, a single plant can contain compounds that may stimulate digestion together with anti-inflammatory compounds, reducing swelling and pain, phenolic compounds with antioxidant or venotonics proprieties and others with anti-bacterial and anti-fungal, etc. activities.

Natural products and their derivatives have traditionally been the most common source of drugs and represents more than 30% of the active ingredients of the registered pharmaceutical products. Roughly, 40% of the 1355 of the New Chemical Entities (NCEs) registered between 1981 and 2010, are natural occurring compounds, semi-synthetic derivatives or synthetic compounds inspired on natural product pharmacophores, (Newman

et al., 2012). Natural products are the major sources of innovative therapeutic agents for infectious diseases (bacterial, parasitic and fungal), cancer, lipid disorder and immunomodulation. Many of them have a certain complexity that may affect the scope of making chemical modifications to enhance their therapeutic properties, and this can cause the increase of the cost (Basso *et al.*, 2005).

The development of a new drug is a complex, expensive and time-consuming process, requiring about 12 years to produce practical results. The earliest phase of the discovery of a new drug involves the identification of active compounds holding the required characteristic (of drugability) to lead New Chemical Entities (NCEs) for drug development (Katiyar *et al.*, 2012).

The search of such valuable compounds in plants (screening of plant extracts for activities), is one of the tools considered in that earliest phase (Early Drug Discovery). Rational methodologies, alternatively to the casuistic evaluation of a certain plant, can be adopted to maximize the success of the screening programs for active compounds (hits, leads and NCEs). In fact the selection of plants (plant extracts) to be included in the screening, considering their phylogeny, biochemical features and relationships, geographic distribution and ethnopharmacological data, etc., impacts positively in the success of the research efforts.

Aromatic plants are frequently used in traditional medicine, as well as their characteristic isolates, the essential oils, mixtures of volatile compounds prepared by steam distillation. They are known since antiquity and associated to many biological properties and medicinal applications (Ortet *et al.*, 2011).

Aromatic plants produce a high diversity of secondary volatile metabolites with prominent functions: protecting them against harmful organisms, predators, microbial pathogens, abiotic stress, heat, dehydration, etc..., and accomplishing important functions in the interaction with other plants and organisms (Oraby *et al.*, 2013), thus metabolites able to interact with biological targets.

In this context we undertook a study on native and endemic species from Cape Verde used in the traditional medicine, with a special focus on the aromatic species and on the chemical characterization of their volatile metabolites. It was our intention to provide useful information on the natural resources of Cape Verde in what concerns to their use for medicinal purposes, as well as, on the value of the aromatic species as sources of compounds valuable for research, including drug development, and industrial purposes.

2. Cape Verde's Traditional Medicine systems - Use of medicinal plants

The United Nation Conference on Environment and Development (Earth Summit), held in Rio de Janeiro in June, 1992 can be considered a milestone in the whole world, impacting on consciousness about the tendency of environment degradation, leading to a big change in man attitude towards the environment.

Cape Verde was one of the many countries committed to preserve the biological diversity and joined the Biological Diversity Convention in 1995, thus agreed to make use of their sustainable components and share the benefits of the use of genetic resources and their access.

Like other countries, Cape Verde joined the environment concerns to their development plans in order to make some substantial changes. Since independence in 1975, several efforts have been made towards greater understanding, awareness and protection of the environment. For this purpose, Cape Verde has relied on several international cooperation programs for assisting the design and execution of the important instruments for environmental management (IV Relatório sobre o estado da biodiversidade em Cabo Verde, 2009).

Cape Verde climate is characterized by great disparities concerning the precipitation, with long periods of scarcity, which affects the type, the exuberance, and the diversity of the local flora. Despite of the subtropical dry climate of this archipelago, which does not allow the development of abundant vegetation, Cape Verde flora comprises about 740 species, consisting in more than one hundred families. 42% of those species are allochthonous, 13% are endemic, and 30% are from unknown origin. Most of the archipelago's flora is presently composed of exotic naturalized species. The use of local flora in folk medicine is common in Cape Verde, and 101 exotic plants and twenty percent of this exotic species are reported as medicinal in this archipelago. As a consequence of the hot and dry climatic conditions most of plant species are to annual or biannual (Romeiras *et al.*, 2011).

In what concern to the health systems, Cape Verde joins two different realities. Urban population, benefits from the availability and access of conventional modern health assistance and medicines. However, these conveniences are not easily accessible to rural population that preserves and depends of traditional therapeutic practices, as well as, of the knowledge of the preparation of natural remedies (*ramedi terra*) from local resources.

The few number plant species that grows in the archipelago play, thus, an important role for the health care of locals, attending their medicinal allegations.

Some of these species are aromatic, thus bearing volatile metabolites and allowing to isolate essential oils. Due to their intense scent they are easily recognized and remembered and, probably for those reasons, are the preferred and the most known species.

Artemisia gorgonum (Asteraceae), *Satureja forbesii* (Lamiaceae), and *Tornabenea annua* and *Tornabenea insularis* (Apiaceae), are endemic aromatic plants. Together with the allochthonous species, *Cymbopogon citrates* (Poaceae), *Hyptis pectinata* (Lamiaceae), *Tornabenea bischoffi* and *Tornabenea tenuissima* (Apiaceae) they represent the aromatic biodiversity of Cape Verde archipelago.

Table I- Most important medicinal plants used in the traditional medicine of Cape Verde. A = Santo Antão; B = Boa Vista; F = Fogo; L = Santa Luzia; M = Maio; N = São Nicolau; R = Brava; S = Sal; T = Santiago; V = São Vicente.

Family	Scientific name	Common name	Habit	Used Parts	Therapeutic	Distribution
Asteraceae	<i>Artemisia gorgonum</i>	Losna, lasna	Shrub	All plant	Deworming and digestive	A;T;F
	<i>Campylanthus glaber</i>	Alecrim bravo,	Shrub	All plant	Muscle aches	
Boraginaceae	<i>Echium hipernopicum</i>	Lingua de vaca	Shrub	Seed oil	Dietetic	A;T
Boraginaceae	<i>Echium stenosphon</i>	Lingua de vaca	Shrub		Cough syrup	A;V;L;N;B
Boraginaceae	<i>Echium vulcanorum</i>	Lingua de vaca	Shrub	Seed oil	Dietetic	F
Asteraceae	<i>Conyza feae</i>	Losna-bravo	Shrub	Leaf	Menstruation Treatment. Bath done with infusion	A;V;N;T;F;R
	<i>Forsaeolia pocridifolia</i>					
Fabaceae	<i>Lotus purpureus</i>	Piorno	Shrub	Leaf	Fever, back and chest pain. tea leaves	A;V; N; B; T; F
Lamiaceae	<i>Satureja forbesii</i>	Erva cidreira	Shrub, herbaceous	Plant	Constipation and labor stimulation. tonic for stomach and intestinal disorders	T;F;R;A;
	<i>Paranychia illecebroides</i>					
Asclepiadaceae	<i>Sarcostema daltonii</i>	alvatão, alcatrão, ervatão, gestiva sistiba	candent herb	Plant	Decayed teeth by subtracting the pain and fragmenting the tooth	A;V;N;T;F;R
	<i>Periploca chevalieria</i>					
	<i>Verboscum cystolithicum</i>					
Euphorbiaceae	<i>Euphobia tuckeyana</i>	Tortolho, tortilho tortoinho, lentisco	Shrub	Plant	The milky caustic and sicative sap is dangerous for the eyes. Used against gonorrhoea and syphilis.	V; L; N; S.; B; T; F;
Apiaceae	<i>Tornabenea insularis</i>	Aipo, funcho	perennial herb	Leaf and fruits	cough, boiled with sugar	A;V;N;T;F;R
Asparagaceae	<i>Asparagus squarrosus</i>	Espargo	Shrub	Shoo	Diuretic	A;V;L;N;S;B
Asteraceae	<i>Nidorella varia</i>	Tabua, tabuinh		Leaf	External inflammations	A;V;N;T;F;R
Asteraceae	<i>Sonchus daltonii</i>	Coroa-de-rei	Shrub			A;V;N;T;F
Brassicaceae	<i>Erysimum caboverdeanum</i>	Cravo-bravo	Subshrub	Plant	Infusion for emmenagogue	F
Campanulaceae	<i>Campanula jacobaea</i>	Contra bruxas-azul, dedal	Subshrub	Plant		A;V;N;T
Caryophyllaceae ,	<i>Paronychia illecebroides</i>	Agrião-de-rocha	Herb	Plant	Syrup mixed with agrião de água, for bones lesions	A;V
Dracaenaceae	<i>Dracaena draco L.</i>	Dragoeiro	Arboreal plant	Sap	Sap (blood Draco) and the resin has healing applications. Topic of scams and trauma. Analgesic and anti-inflammatory and anti-viral	A;V;N;T;F;R
Gentianaceae	<i>Centaurium tenuifloru</i>	Fel-da-terra	Herb	Aerial part	Increases the secretion of gastric juice, used in dyspepsia and to increase appetite	F
Globulariaceae	<i>Lytanthus amygdalifolius</i>	Mato-botão, argueiro	Shrub	Leaf	Tooth aches	A; N; T; F; R

Papaveraceae	<i>Papaver gorgoneum</i>	Papoila	Perennial herb	Fruits and seeds	Antitussive	N;F;A
Scrophulariaceae	<i>Celsia cystolithica</i>	Erva-de-são-joão	Herb	Plant	For colds, with tea egg yolk and a tablespoon of "grogue"	T;F
Scrophulariaceae	<i>Celsia insularis</i>	sabão-de-lagartixa sabugo,sabão-de-feiticeira	Herb, and some shrub variation	Roots, fruits and leaves	Leaves and green fruit are used in liver diseases	A; V; N; B; T
Solanaceae	<i>Withania chevalieri</i> A	Malagueta-de-galinha,	Shrub	Roots and leaves	Sedative, hypnotic, analgesic, laxative and diuretic	A; V; N; B; F; R
Urticaceae	<i>Forssakaolea procrifolia</i>	Urtiga, língua-de-vaca-branca	Shrub	Leafs	Tooth ache and Infusion against asthma.	A; V; L; N; S; M;T; F; R

3. Aromatic plants, essential oils and health care

Therapeutic properties of medicinal plants are related to their secondary metabolites diversity that includes a huge number of compounds classified in numerous groups of natural organic compounds: phenolic acids, stilbenes flavonoids, tannins, coumarins, alkaloids, terpenoids, among others.

Aromatic plants characteristically biosynthesize and accumulate volatile compounds in specialized cells, in secretion ducts or cavities or in glandular trichomes. They are lipophilic and low molecular weight (<300 Da) compounds, the most of them monoterpenoids, sesquiterpenoids, phenylpropanoids or aliphatic compounds. Few volatile diterpenoids, as well as, some nitro and sulphur- containing compounds (Wink, 1999) can also be found in certain species.

Monoterpenoids and sesquiterpenoids (also diterpenoids) share the metabolic origin, resulting from the condensation of a 5 carbon precursors, the isopentenyl-pyrophosphate and the dimethylallyl-pyrophosphate, synthesized in the cytosol and in the plastids (Figure 2 and 3).

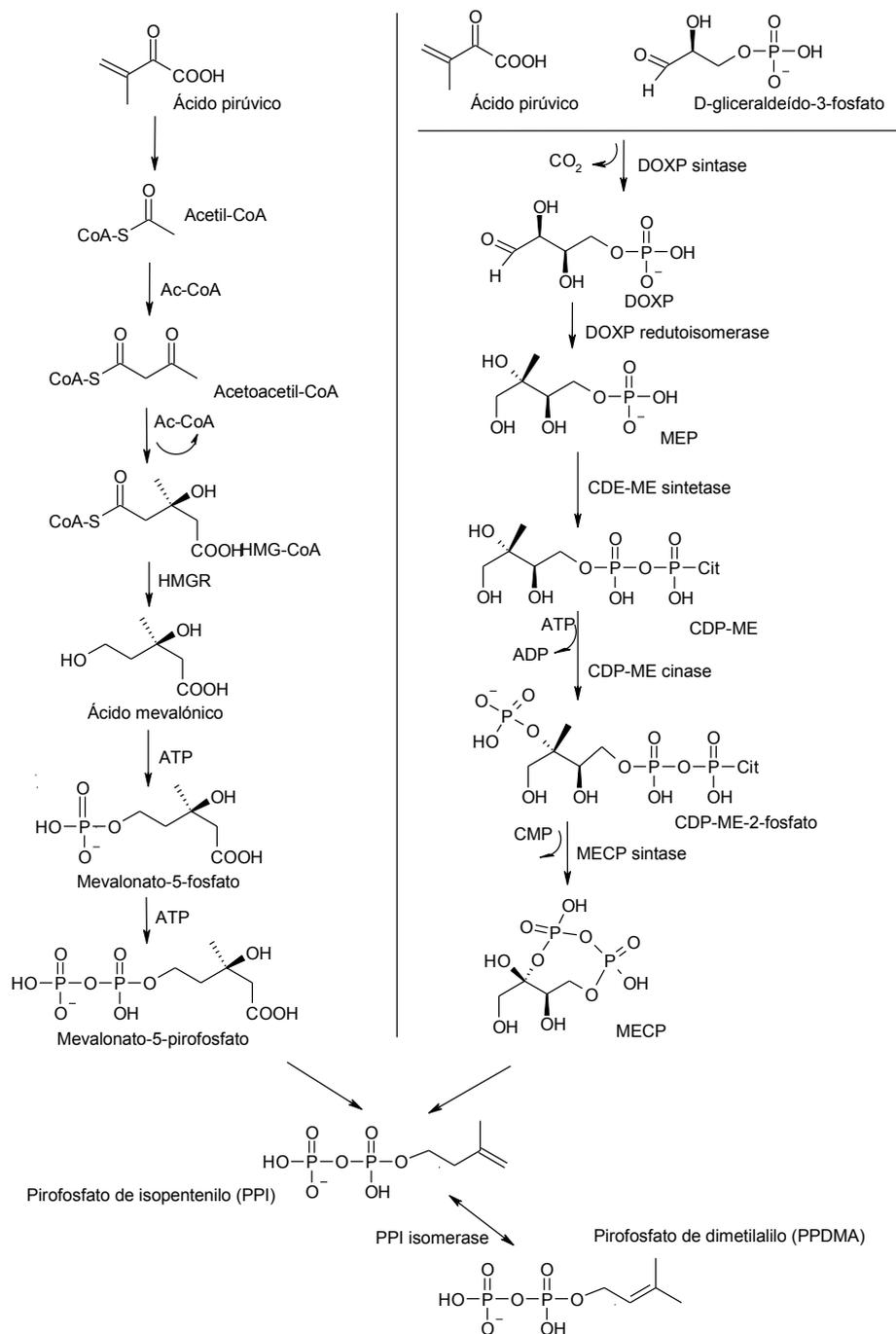


Fig.1- Synthesis of the isopentenyl pyrophosphate: acetate-mevalonate (in cytosol), and 2-methylerythritol-4-phosphate (MEP) (in plastids) pathways. (Adapted from Cavaleiro C, 2012)

Ac-CoA = Acetyl-CoA; HMG-CoA = 3-hidroximetilglutariil-coenzime-A; HMGR = 3-hidroximetilglutariil-coenzime-A-redutase; DOXP = Desoxixilose-5-fosfato; MEP = 2C-Metil-D-eritritol-4-fosfato; CDP-ME = 4-difosfocitidil-2C-metil-D-eritritol; MECP = Metil-D-eritritol-2,4-difosfato.

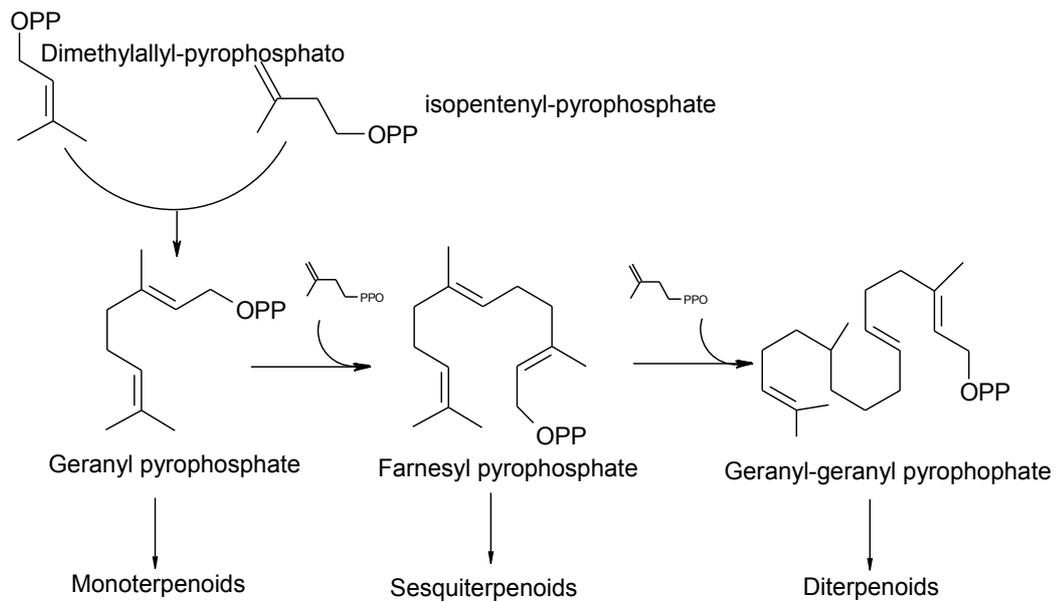


Fig.2- Biosynthesis of terpenoids from isopentenyl pyrophosphate. (Adapted from Cavaleiro C, 2012)

The diversity of terpenoids results from the carbon skeleton arrangements (linear, cyclic, polycyclic, etc.) and also from functionalization, (alcohols, aldehydes, ketones, acids, esters, oxides...).

Phenylpropanoids derived from the shikimic acid pathway (Figure 3). The diversity of volatile phenylpropanoids is lesser than those of terpenoids. However aromatic species from certain families, such as Apiaceae, are rich in volatile phenyl propanoids.

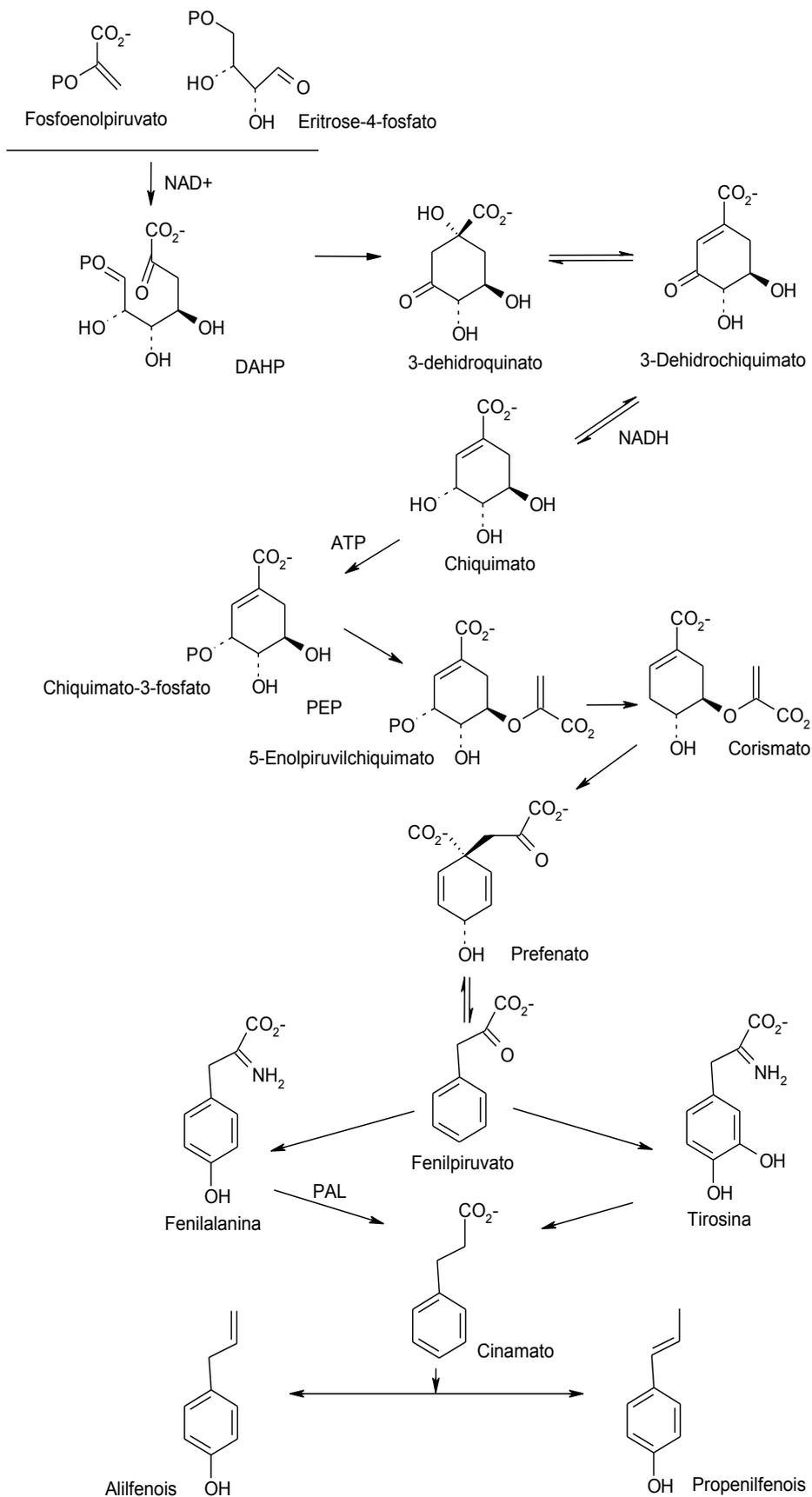


Fig.3- Biosynthesis of allyl- and propenylphenols. Shikimate pathway. (PAL) = phenylalanine ammonia-lyase. (Adapted from Cavaleiro C, 2012)

Essential oils are the typical isolates from aromatic plants, products obtained by steam or water distillation of plant parts (leaves, stems, bark, seeds, fruits, roots and plant exudates). Thus, an essential oil may contain up to several hundred chemical compounds, the mixture or each one of its constituents with potential usefulness for industrial purposes (Junior *et al.*, 2012).

Because of their multipurpose applications, the production and consumption of essential oils, is continuously increasing. They are used in perfumery, the food industry, households, condiments, making sweets, beverages as well as pharmaceutical and aroma therapeutic products. Nowadays, according to market data, essential oils are produced on a large scale and commercialized from 400 species from 67 families. The most important families from this point of view are Asteraceae, Lamiaceae and Apiaceae (Bernáth, 2000).

In other perspective an essential oil, as a complex mixture, can be considered as collection of organic compounds, available to investigate for their potential biological activities. Essential oils are long-term used, safely, by humans (for different purposes including ethnomedicine) allowing to infer that their constituents are likely to be safer than those derived from plant species with no history of human use. So essential oils can also be perceived for their value in the context of drug discovery, offering a huge diversity of organic compounds that can be screened for potential biological activities.

4. Objectives

It was our purpose to study the native and endemic aromatic plant species from Cape Verde used in the traditional medicine, with a special focus on the characterization of their volatile metabolites. It was our intention to provide useful information on the natural resources of Cape Verde, in particular in what concerns to their use in the traditional medicine.

To define the object of study we have considered the two above criteria:

A- The geographic and ethnopharmacological criterion restrained the object of our study to plant species used in the traditional medicine of Cape Verde;

The Cape Verde islands are located in the mid-Atlantic ocean, about 454 km from the West African coast. It consists on a set of 10 islands and 5 islets, which occupy an area of 4033 km². They are divided in two major groups, Barlavento islands formed by Santo Antão, São Vicente, Santa Luzia, São Nicolau, Sal and Boa Vista, and the Sotavento islands consisting in Santiago, Maio, Fogo and Brava.

The islands are volcanic with mountainous areas and valleys covered with vegetation. Climate is tropical dry, mild, characterized by a mean annual temperature of 25°C, low temperature ranges and relative humidity between 20 and 60%. The climate is strongly influenced by the following atmospheric currents: *tradewinds from NE*, which are responsible for the fresh and dry season between November and July; winds from the SW, *monsoon* from the South Atlantic, which depends on the Inter Tropical Convergence Zone, that affects the region between August and October, therefore the season is considered "wet"; *harmatão*, the hot, dry wind that blows in January and February. The landscape varies from dry plains to highest active volcanoes with cliffs rising steeply from the ocean (Gomes *et al.*, 2008).

The vegetation and reliefs are directly affected by the climate factors, thus the trade winds, by causing orographic precipitation in the higher areas are responsible for the characteristic vegetation of Santo Antao, São Vicente, Santa Luzia, São Nicolau, Santiago, Fogo and Brava. Sal, Boavista and Maio islands, are affected by the Harmatão, giving them their dry characteristics. The combination of climatic factors and relief leads to climate zones, responsible for different frames landscaped and distribution of plant species and plant community types.

This confined territory, isolated from the African mainland, is the habitat of several native or endemic plant species used by locals for healing.

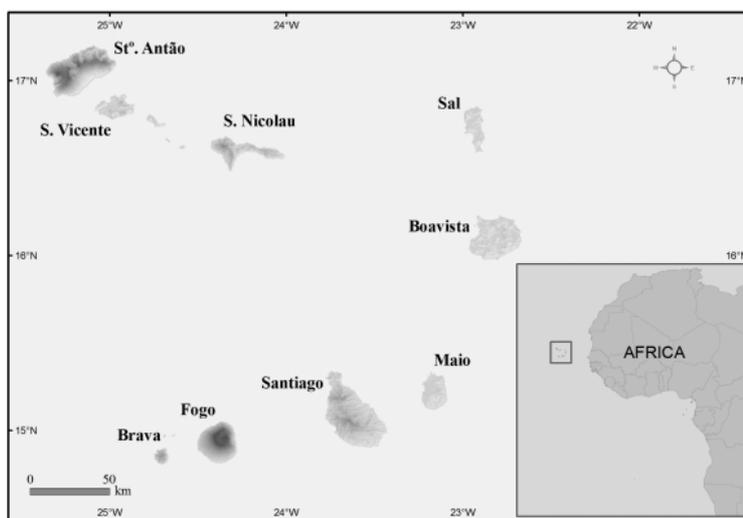


Fig.4- Geographic location of Cape Verde archipelago in the North Atlantic Ocean (Romeiras *et al.*, 2011).

B- A more limited set of plant species was defined, considering a biochemical criteria to select those that are able to biosynthesize and accumulate low molecular weight and lipophilic compounds that meet physical and chemical characteristics compatible with good pharmacokinetic features (drug-ability parameters). Coincidentally, that compounds are the volatile metabolites responsible for the characteristic aroma of that plants (aromatic plants) (Bernáth, 2000).

5. Methods

5.1. Aromatic plants from Cape Verde

The knowledge on the aromatic plants from Cape Verde is scarce. Only four papers are indexed at ISI Web of Knowledge (accessed 18/6) dealing with aromatic plants from Cape Verde and their essential oils.

These papers report on the essential oils of *Tornabenea bischoffii*, *Tornabenea annua*, *T. insularis*, *T. tenuissima*, *Artemisia gorgonum* and *Satureja forbesii*.

We abridge below the available compositional data of the essential oils of *Tornabenea* species. Data on the essential oils of *Artemisia gorgonum* and *Satureja forbesii* will be discussed later, together with our results.

Grosso *et al.* (2009), studied the composition of the oils from *Tornabenea annua*, *T. insularis* and *T. tenuissima*. Myristicin was found to be the major component of *T. annua* oil, representing 95-98% of the whole composition of this oil; similarly, the oil of *T. tenuissima* is mainly composed of elemicin attaining 90.0%; the oil of *T. insularis* is mainly composed of myristicine (47.3-87.1%); elemicine is an important constituent of the oils from plants growing at S. Antão island (5.1%), as well as, from plants propagated in the Botanic Garden of Lisbon (11.8%) from seeds from plants of Santiago island. Surprisingly, in the oils from plants growing in Santiago island elemicine was not detected. This variability can be due to the climatic and edaphic conditions, influencing the metabolic pathways of the plant and its volatile metabolites.

Ortet *et al.* (2011) studied the composition of *Tornabenea bischoffii* through gas-chromatography and gas-chromatography / mass spectroscopy, and identified sixty volatile compounds of which myristicin was the major one (33,6 %). The aim of that study was the evaluation of the antioxidant activity, which was confirmed by *in vitro* essays.

None of these papers mentions any biological activity, nor relationships among compositions and the use of the plants in the traditional medicine of Cape Verde.

5.2. Plant material

5.2.1. *Artemisia gorgonum* Webb

Family: Asteraceae

Genus: *Artemisia*

Species: *Artemisia gorgonum*

Common name: Losna, lasna or lorna



Fig.5- *Artemisia gorgonum* webb.

Artemisia sp. is a large and widespread genus, including some 390 species distributed in the Old and New world, predominantly in Northern Hemisphere. The small capitula are usually arranged in many capitula, and achenes lack pappus. The genus is represented by a single, endemic species in Cape Verde Islands (Brochman et al., 1997).

Description

Strongly branched, erect, aromatic shrub up to 2m high. Branches robust, whitish tomentose when young, glabrescent and brownish with age. Leaves bi- to tri-

pinnatisected, up to 8 cm long and 6 cm wide, whitish tomentose, with narrowly elliptical to almost linear lobes. Involucre bracts more or less imbricate, broadly ovate; inner bracts brownish, scarious, herbaceous only along the midrib. Flowers yellowish, tubular, outer flowers female; central flowers hermaphrodite. Achens obovoid, without pappus.

Variation

No essential variation from each island.

Chromosome number

$2n=18$ – Sto Antão, Lombos da Pedras, 1300 m (Borgen, 1975).

Related taxa

Closely related to Canarias's species *Artemisia thuscula* cav. Differs in flowers and involucre characters.

Distribution and ecology

A. *gorgonum* is a western mesophyte recorded from mountain areas in Sto. Antão, Santiago and Fogo. The localities are evenly distributed among the semiarid, and humid zone, mainly between 800 m and 2000 m. The lowermost record is about 400m (Sto. Antão), and the uppermost ones at 2200-2400 m on Pico Novo and the old crater rim on Fogo. The most species grows mainly in gravelly mountain slopes and plains. It is a characteristic component of the scrub vegetation in these areas, which today are largely destroyed except from some rather poor fragments (Brochman *et al.*, 1997).

Abundance in Cape Verde

Although many of the records of the species are dated after 1970, many populations are clearly diminishing, and some populations are probably already extinct

because of the habitat destruction and cutting. The species is vulnerable (VU) on Sto Antão and Fogo. It has only been recorded twice in Santiago, first by Chevalier in 1934 in the Serra do Pico da Antonia Mountains, and it was not rediscovered on this well-explored island. The rediscovered population consists only of 10-15 plants, some of them very old, and occurs on cultivated slopes near Rui Vaz in the Serra do Pico da Antonia mountains. *A. gorgonum* is considered Critically Endangered (CR) on Santiago, and it is generally considered to be Vulnerable (VU) (Brochman *et al.*, 1997).

5.2.2. *Satureja forbesii* Benth

Family: *Lamiaceae*

Genus: *Satureja*

Species: *Satureja forbesii*

Common name: Erva-cidreira



Fig.6- *Satureja forbesii* Benth.

This genus comprise about 100, mostly subshrubby species distributed from the mid-Atlantic archipelagos and the Mediterranean, which constitutes one center of diversity, to the Himalayas and SW China and North America. *Satureja* is represented by a single, endemic species in the Cape Verde islands.

Description

Strongly branched, ascending dwarf shrub up to 0,3 m high; usually strongly aromatic, occasionally odourless. Leaves ovate to elliptical, up to 1,2 cm long and 0,8 wide, more or less pubescent, sessile to shortly petiolate, apex acute, margin sometimes slightly revolute. Inflorescent axillary, with 3-6 small flowers. Calyx purplish, tubular, slightly zygomorphic. Corolla pinkish to white, hairy. Mericarps dark brown, 0,8 mm long.

Variation

It is very polymorphic; Perez de Paz (1978) distinguished three varieties (var. *forbesii*, *inodora* and *altitudinum*) based on odour, leaf petiolation and leaf density. The variation appears to be ecoclinal and too complex for delimitation of any infraspecific taxa.

Chromosome number

Unknown.

Related taxa

Closely related to the Canarian *Satureja tenerifae*. Differs in several leaf and floral characters (Brochman *et al.*, 1997).

Distribution and ecology

It is a mesophyte occurring on Sto Antão, S.Nicolau, Santiago, Fogo and Brava but absent from S.Vicente. Occurs in semiarid, sub humid zone mainly between 800 m and 1600 m. Being the lower most record about 500 m on Santiago, and the uppermost at 2830 m at the top of volcanic cone of Fogo (Gilli 1976). The plants grow in cliffs and mountain gravelly plains and slopes.

Abundance in Cape Verde

It is still common but probably declining on Sto Antão and Fogo, where it comprises some large population. It is vulnerable (VU) on Santiago and endangered

(EN) on S. Nicolau, where it is confined to the Monte Gordo area, and it is Indeterminate (I) on Brava. But it is generally considered indeterminate (I) in Cape Verde.

5.2.3. *Hyptis pectinata* L. Poit

Family: Lamiaceae

Genus: *Hyptis*

Species: *Hyptis pectinata*

Common name: Rosmaninho



Fig.7- *Hyptis pectinata* L. Poit.

Description

Slender erect herbaceous subshrub with 4-angled puberulent stems; foliage aromatic if rubbed and crushed; the leaves are ovate or ovate-elliptic, cuneate to rounded (even subcordate) at base, acute or blunt at tip, puberulent or glabrescent dorsally, crenate-serrate, 2-9 cm long, 1-6 cm wide; flowers subsessile, white to pale violet, in cymules axillary to reduced leaves, subtended by linear pubescent bracts 1-3 mm long; calyx about 2 mm, enlarging in fruit to 4 mm; corolla 2.5 mm, lower lip

darker; filaments somewhat pubescent; nutlets oblong, 1 mm long, black. Robust plants may reach up to 4 m tall.

Distribution and ecology

Hyptis pectinata is a weedy plant native to tropical America, commonly distributed from Mexico, South Florida to Venezuela and also naturalized to Hawaii.

Abundance in Cape Verde

In Cape Verde it's a rather vulgar species, occurring in Santo Antão, São Nicolau, Santiago, Fogo and Brava (Monteiro and Benton, 2012).

5.2.4. *Cymbopogon citratus* Stapf

Family: Poaceae

Genus: *Cymbopogon*

Species: *Cymbopogon citratus*

Common name: Xa-li



Fig.8- *Cymbopogon citratus* Stapf.

Description

It is a tall, aromatic, perennial grass with culms (stems) up to 2 m tall. The leaves are linear, up to 1 m long and 2 cm wide, tapering towards the sheath. They are smooth and hairless, white on the upper surface and green beneath. The ligules (appendage between the leaf sheaf and blade) are less than 2 mm long, and are rounded or truncate (ending abruptly as if cut off).

As for the flowers, the inflorescence is a loose, nodding panicle, about 60 cm long and reddish to russet in colour. The pedicels are tinged with purple.

Distribution and Ecology

Cymbopogon citratus is native to Indonesia, and introduced and cultivated in most of the tropics, including Africa, South America and Indo-China.

Africa: north, Macaronesia, west tropical, west-central tropical, east tropical, southern tropical, middle Atlantic ocean, and western Indian ocean. Asia-temperate: China and eastern Asia. Asia-tropical: India, Indo-China, Malaysia and Papuasia. Australasia: Australia. Pacific: southwestern, south-central, northwestern, and north-central. North America: Mexico. South America: Caribbean, northern South America, western South America, Brazil, and southern South America.

5.3. Analysis of the chemical composition of volatile oils

After inquiries to the population regarding the use of aromatic plant species for medicinal purposes we undertook a botanic prospection of *Artemisia gorgonum*, *Satureja forbesii*, *Hyptis pectinata* and *Cymbopogon citratus* viewing the collection of samples of plant material.

Plant material collection

Table II- Data about the sampling of the native and endemic species from Cape Verde.

Species	Harvested Part	Local	Data
<i>Artemisia gorgonum</i>	Aerial	Longueira (Santiago)	February 5 th 2013
<i>Satureja forbesii</i>	Aerial	Rui Vaz ((Santiago)	February 5 th 2013
<i>Hyptis pectinata</i>	Aerial	Rui Vaz((Santiago)	February 5 th 2013
<i>Cymbopogon citratus</i>	Aerial	Orgãos (Santiago)	February 5 th 2013

The vouchers samples were pressed, dried and assembled with on paper and then deposited at the herbarium of the Faculdade Farmácia de Universidade de Coimbra (FFUC).

5.3.1. Isolation of the volatile components

In order to isolate selectively the volatile metabolites we proceed to the preparation of essential oils by water distillation, during three hours, using a Clevenger-type apparatus, following the procedure described in the European Pharmacopoeia (1999) (Figure 9).

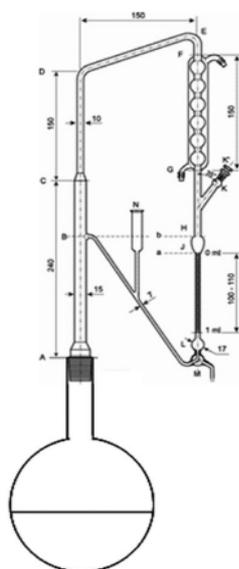


Fig.9- Clevenger circulatory distillation apparatus, as reported in the European Pharmacopoeia.

Essential oils were kept in the dark at 4°C until prior use. For analysis, essential oils solutions were prepared by dilution (1:8) in *n*-pentane.

5.4. Analysis of the composition of the volatile oils

The analysis of an essential oil involves identification and quantitative determination of its components. Volatility of the essential oils's components makes the gas chromatography an appropriate technical base for such purpose (Rubiolo *et al.*, 2010).

The analysis was achieved by combination of gas-chromatography retention data for columns with different stationary phases, and mass spectra acquired by gas-chromatography tandem mass spectroscopy (GC/MS).

Analytical GC was carried out in a Hewlett-Packard 6890 (Agilent Technologies, Palo Alto, CA, USA) chromatograph with a HP GC ChemStation Rev. A.05.04 data handling system, equipped with a single injector and two flame ionization detectors (FID). A graphpak divider (Agilent Technologies, part no. 5021-7148) was used for simultaneous sampling to two Supelco (Supelco, Bellefonte, PA, USA) fused silica capillary columns with different stationary phases: SPB-1 (polydimethylsiloxane 30m×0.20mm i.d., film thickness 0.20m), and SupelcoWax-10 (polyethyleneglycol 30m×0.20mm i.d., film thickness 0.20m). Oven temperature program: 70–220 °C (3°Cmin⁻¹), 220 °C (15 min); injector temperature: 250 °C; carrier gas: helium, adjusted to a linear velocity of 30cms⁻¹; splitting ratio 1:40; detectors temperature: 250 °C.

GC–MS was carried out in a Hewlett-Packard 6890 gas chromatograph fitted with a HPI fused silica column (polydimethylsiloxane) 30m×0.25mm i.d., film thickness 0.25m), interfaced with an Hewlett-Packard mass selective detector 5973 (Agilent Technologies) operated by HP Enhanced ChemStation software, version A.03.00. GC parameters as described above; interface temperature: 250 °C; MS source temperature: 230 °C; MS quadrupole temperature: 150 °C; ionization energy: 70 e V; ionization current: 60 A; scan range: 35–350 units; scans s⁻¹: 4.51 (Fig.6).

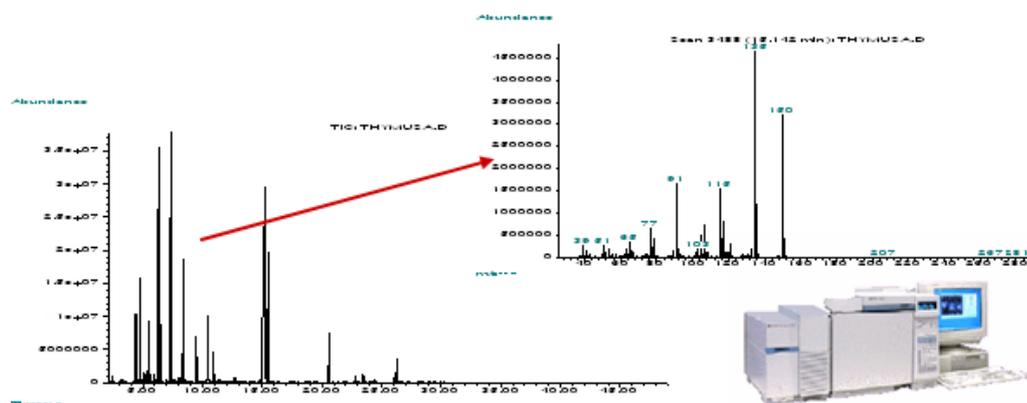


Fig.10- MS detectors coupled to CG, enables not only obtaining the elution profile of the compounds in the sample (total ion current chromatogram), but also the acquisition of mass spectra of each one of the resolved compounds.

Components of the volatile oils were identified by: (i) their retention indices on both SPB-1 and SupelcoWax-10 columns, calculated by linear interpolation, according Van den Dool and Kratz (1963) relative to retention times of C8–C24 of n-alkanes (equation 1) and compared with those of reference compounds included in CEF laboratory database or literature data (Adams RP, 2007; WebBook; Pherobase; Flavournet) (ii) their mass spectra by matching with reference spectra from the CEF laboratory own spectral database, Wiley/NIST database or literature data (McLafferty, 2009).

$$IR(a) = (n * 100) \frac{Tr(a) - Tr(n)}{Tr(n+1) - Tr(n)} \times 100$$

Equation 1. Linear interpolation according Van den Dool and Kratz: IR_(a)- peak “a” retention indice, n – number of carbons of the n-alkane eluting before peak “a”; Tr_(a) – Retention time of peak “a”; Tr_(n) - Retention time of peak the n-alkane eluting before peak “a”; Tr_(n+1) - Retention time of peak the n-alkane eluting after peak “a”.

For quantification, relative amounts of individual components were calculated based on GC raw data without further correction.

6. Results and Discussion

6.1. Ethnopharmacological data on *Artemisia gorgonum*

This endemic species, known among folks as “losna” or “lorna” is used by locals for treatment of many ailments. Prepared as infusion, is useful for fever, headache, dewormer, flu and digestive disorders. For constipation and digestive colic leaves are prepared as an infusion and added with salt. *A. gorgonum* is also used, prepared as a decoction to promote abortion. There is no data in literature to support these assumptions.

6.2. *Artemisia gorgonum* essential oil

Distillation of *A. gorgonum* produced a yellowish essential oil, yield 0.4% (v/m), calculated considering plant material fresh weight. During the distillation color of the distillate changed from light yellow to yellow-brown and reddish yellow. Thirty-nine components were identified, accounting for 94,6% of the whole composition of the essential oil (table III). The oil is mainly composed of oxygen containing monoterpenes (76.4%) and monoterpenes hydrocarbons (14.4%). Sesquiterpenes hydrocarbons (3.1%) and oxygen containing sesquiterpenes (0.7%) were also detected. The major components were chrysanthenone (44.2%), camphor (13.7%), α -phellandrene (6.3%), iso-chrysanthenone (3.1%), bornyl acetate (3.0%), camphene (2.9%), geranyl propanoate (2.0%) and neryl isovalerate (2.0%) (Fig. I I).

Table III- Composition of the essential oil of *Artemisia gorgonum*.

RI ^a	RI ^a _{Lit}	RI ^P	Compounds	%
921	921	1020	Tricyclene	t
923	923	1029	α -Thujene	0.4
930	930	1029	α -Pinene	0.8
943	942	1075	Camphene	2.9
959	965	1338	6-methyl-5-hepten-2-one	t
965	964	1125	Sabinene	0.7
970	970	1118	β -Pinene	0.2
981	980	1161	Myrcene	0.6
987	988	n.d.	Isobuthylisovalerate	t
998	997	1168	α-Phellandrene	6.3
1012	1012	1273	<i>p</i> -Cymene	1.5
1012	1009	1185	α -Terpinene	0.2
1020	1020	1204	Limonene	0.6
1020	1020	1213	β -Phellandrene	0.2
1037	1035	1250	<i>E</i> - β -Ocimene	t
1046	1047	1249	γ -Terpinene	t
1080	1082	1437	Filifolone	3.5
1083	1088	1483	iso-Chrysanthenone	3.1
1100	1100	1506	Chrysanthenone	44.2
1117	1118	1514	Camphor	13.7
1144	1146	1695	Borneol	t
1147	1146	1673	Lavandulol	0.1
1160	1159	1594	Terpinene-4-ol	0.1
1242	1244	1563	<i>cis</i> -Chrysantenyl acetate	1.4
1265	1266	1573	Bornyl acetate	3.0
1273	1274	1599	Lavandulyl acetate	0.8
1362	1359	1751	Geranyl acetate	0.2
1369	1025	2000	3,7-Dimethyl-keto-1,3,6-octatriene	1.9
1381	1382	1585	β -Elemene	0.2
1406	1208	1590	<i>E</i> -Caryophyllene	0.4
1445	1444	1658	<i>E</i> - β -Farnesene	0.2
1466	1464	1763	<i>ar</i> -Curcumene	0.4
1466		1681	γ -Curcumene	1.4
1470	1470	1709	β -Selinene	0.4
1493	1485	1763	Geranyl propanoate	2.0
1563	1565	n.d.	Neryl isovalerate	2.0
1586	1590	n.d.	Geranyl isovalerate	0.4
1661		2206	α -Bisabolol	0.7
1694		n.d.	Chamazulene	0.1
Monoterpene hydrocarbons				14.4
Oxygen containing monoterpenes				76.4
Sesquiterpene hydrocarbons				3.1
Oxygen containing sesquiterpenes				0.7
Total identified				94.6

RI ^a - Retention indices from the SPB-I columnRI ^a_{Lit} - Reference retention indices from literatureRI ^P - Retention indices from the SupelcoWax 10 column

t – traces (<0.05%)

n.d. – not determined

Major compounds ($\geq 2\%$)

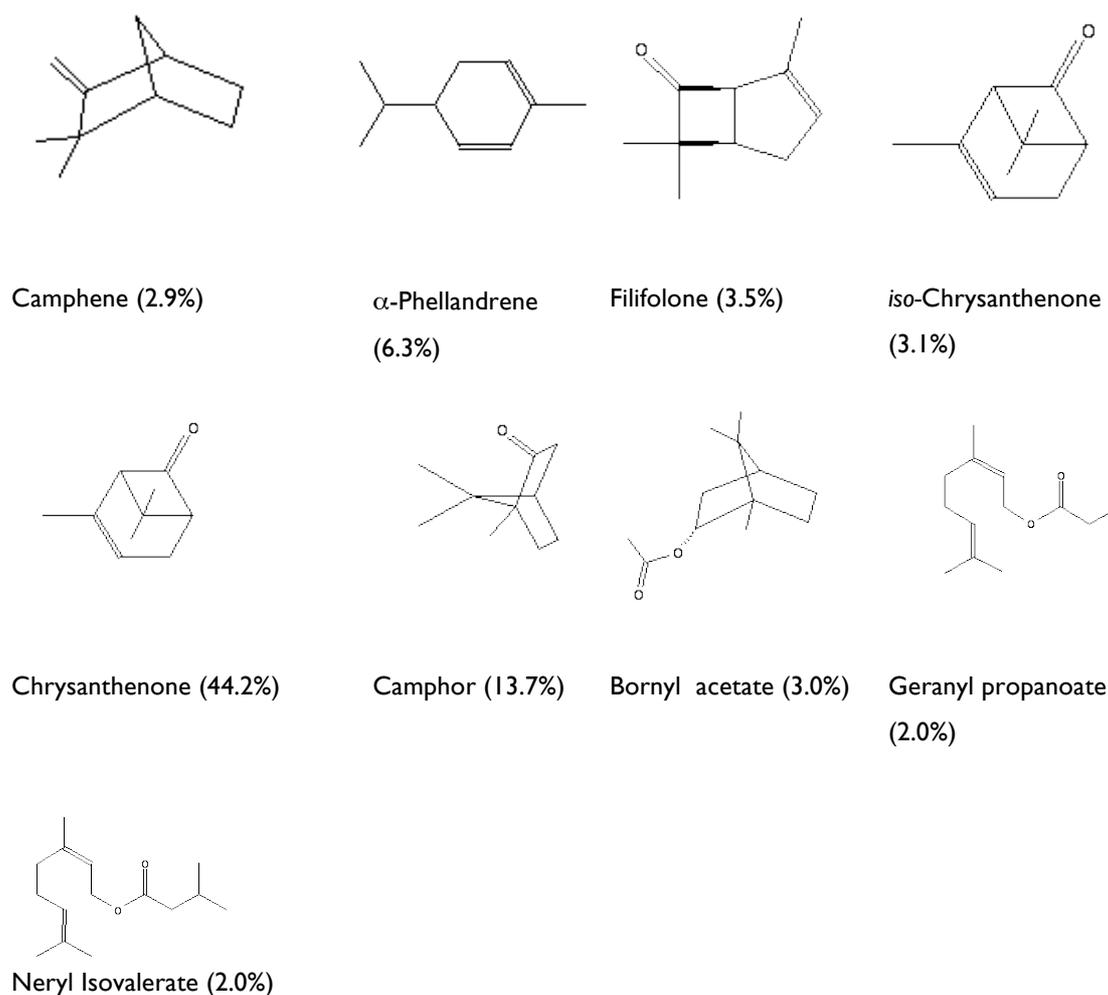


Fig. II - Chemical structures of the major compounds ($\geq 2\%$) of *Artemisia gorgonum* essential oil.

Only four papers indexed at ISI Web of Knowledge (accessed 01/08) deal with *Artemisia gorgonum* and only one of them (Ortet *et al.*, 2010) describes the volatile compounds of the species.

Although some differences in the preparation of the plant samples for distillation - Ortet *et al.* (2010) considered steam distillation of air-dried aerial parts, we adopted the hydrodistillation of fresh material – the essential oil yields were similar.

However, Ortet *et al.* (2010) reported a composition noteworthy different from that we achieved. These authors mention camphor (28.7%), chrysanthenone (10.8%), lavandulyl 2-methylbutanoate (9.5%), α -phellandrene (5.5%), lavandulyl propanoate (4.2%), camphene

(4.0%), and p-cymene (3.4%) as major constituents. In our sample, the relative amount of chrysanthenone is 44.2% and camphor only attains 13.7%. Additionally, iso-chrysanthenone was not detected by Ortet *et al.* while it is one of the major components in our sample (3.1%),

Artemisia genus has been a subject of various studies. Many species of *Artemisia* have shown to have medicinal properties, including antimalarial, antiviral, antitumor, spasmolytic, and others.

One of the four papers reports *A. gorgonum* as a subject of a phytochemical study, which revealed the presence of 13 sesquiterpene lactones that exhibited antiplasmodial activities. Many species of the genus *Artemisia* have proved to be rich in substances with several medicinal uses, including antimalarial, antiviral, antitumor, spasmolytic, and others. Among them, artemisinin is undoubtedly a lead compound as a potent antimalarial agent (Ortet *et al.*, 2010). As said, the composition is very similar to our samples, even the percentage.

6.2.1. Identification of 3,7-dimethyl-keto-1,3,6-octadiene

Spectrum 1371 (and others immediately next) acquired over the a peak at 19.66 minutes of the total ion chromatogram (TIC), suggests an oxygenated monoterpene (MW=150 Da), probably with a carbonyl function. The query of the Wiley275 MS database pointing to safranal. The relative amount calculated from the TIC peak area was quantified with 21%. However, in the chromatogram produced in the SPB-I column with a FID, the peak registered at the retention index 1369 has an area that corresponds to a relative amount of 1.95%. In addition in the chromatogram produced in the SupelcoWax column no peak was compatible with the retention of safranal. Considering this facts we hypothesize on the existence of thermal artifacts. According to Asfaw *et al.* (2001) chrysanthenone can suffer thermal degradation producing filifolone, isochrysanthenone and geranic acid. The process of involves rearrangements through an intermediary compound, a 150 Da ketone, 3,7-dimethyl-keto-1,3,6-octadiene. Its structure is consistent with that can be inferred from the mass spectrum acquired on the peak at retention index 1371.

Although the absence of reference retention indices to confirm the we are convicted of the robustness of the identification considering that is a known product from the thermal degradation of chrysanthenone. The polar retention index (SupelcoWax 10 column) was found at value 2000. Mass spectra was used to recognize the peak.

The difference among the areas of the peaks in the GC/MS and GC-FID produced chromatograms can be explained by the difference in experimental conditions, particularly the temperature of detection devices.

Proved the occurrence of this thermal artifact from degradation of chrysanthenone it was expected that other products could appear in the analysis . According Asfaw *et al.* (2001), *iso*-chrysanthenone and filifolone can result also from chrysanthenone thermal rearrangement.

So, we looked for the presence of *iso*-chrysanthenone. According literature (El-Sayed, 2012) retention indices (polydimethylsiloxane and PEG) of *iso*-chrysanthenone are, respectively 1088 and 1434. In fact, mass spectra acquired on a peak revealed at retention 1083 lead to different identification proposals, depending on their position on time scans: filifolone is proposed by the later MS scans on the peak; the earliest MS scans on the peak proposed chrysanthenone, however with a poor matching quality. Facing this we hypothesized on the co-elution of filifolone and *iso*-chrysanthenone at retention index 1083. The hypothesis was confirmed by the apolar and polar reference retention indices (El-Sayed, 2012; Cozzani *et al.*, 2005).

6.3. Ethnopharmacological data on *Satureja forbesii*

To date, informations on *Satureja forbesii* properties are purely empirical. From our inquiries this endemic medicinal plant, known as “erva-cidreira” or “cidreirinha”, is used in folk medicine to prepare an infusion for the treatment of insomnia. A blended drink is also used to treat cough, indigestion, diarrhea using the infusion of *Satureja forbesii* mixed with wine, sulfur and rhubarb.

6.4. *Satureja forbesii* essential oil

Distillation produced a golden yellowish essential oil, yield 0.03% (v/m) calculated considering plant material fresh weight.

Twenty-two volatile compounds were identified, accounting for 98.7% of essential oil. The oil was largely composed of sesquiterpenes hydrocarbons (65.2%), followed by oxygen containing monoterpenes (25.0%), oxygen containing sesquiterpenes (4.8%) and compounds from other classes (3.7%). Monoterpene hydrocarbons were not identified (table

IV). The major compounds were *E*-caryophyllene (32.2%), germacrene D (18.8%), geranial (14.1%), neral (10.6%), α -humulene (6.8%), caryophyllene oxide (3.2%), β -bourbonene (2.3%), geranyl acetate (2.1%) and *E*- α -bergamotene (2.0%) (Fig.12).

Table IV- Composition of *Satureja forbesii* essential oil.

RI ^a	RI ^a _{Lit}	RI ^P	Compound	%
968	965	1338	6-Methyl-5-hepten-2-one	0.3
968	962	n.d	3-Octanone	0.2
1081	1082	1539	Linalool	0.3
1215	1214	1676	Neral	10.6
1244	1249	1727	Geranial	14.1
1362	1359	n.d	Geranyl-acetate	2.1
1375	1376	1513	β-Bourbonene	2.3
1382	1382	1583	β -Elemene	0.8
1407	1208	1590	<i>E</i>-caryophyllene	32.2
1417	1418	n.d	β -Gurjunene (Calarene)	0.6
1426	1426	1577	<i>E</i>-α-Bergamotene	2.0
1430	1428	n.d	Aromadendrene	0.4
1439	1442	1661	α-Humulene	6.8
1465	1466	1700	Germacrene D	18.5
1495	1493	1749	<i>E,E</i> - α -Farnesene	1.1
1507	1498	1749	δ -Cadinene	0.4
1558	1558	1974	Caryophyllene oxide	3.2
1584	1582	2031	Humulene epoxide	0.7
1632	1628	2222	α -Cadinol	0.9
1645	1161	2365	Caryophylla-2(12),6-5-beta-ol	0.1
1833	1828	n.d	Hexahydrofarnesyl acetone	0.1
2082		n.d	8- β -Hidroxsandaracopimaradiene	1.0
			Monoterpene hydrocarbons	-
			Oxygen containing monoterpenes	25.0
			Sesquiterpene hydrocarbons	65.2
			Oxygen containing sesquiterpenes	4.8
			Others	3.7
			Total identified	98.7

RI^a - Retention indices from the SPB-I column

RI^a_{Lit} - Reference retention indices from literature

RI^P - Retention indices from the SupelcoWax 10 column

t – traces (<0.05%)

n.d. – not determined

Major compounds ($\geq 2\%$)

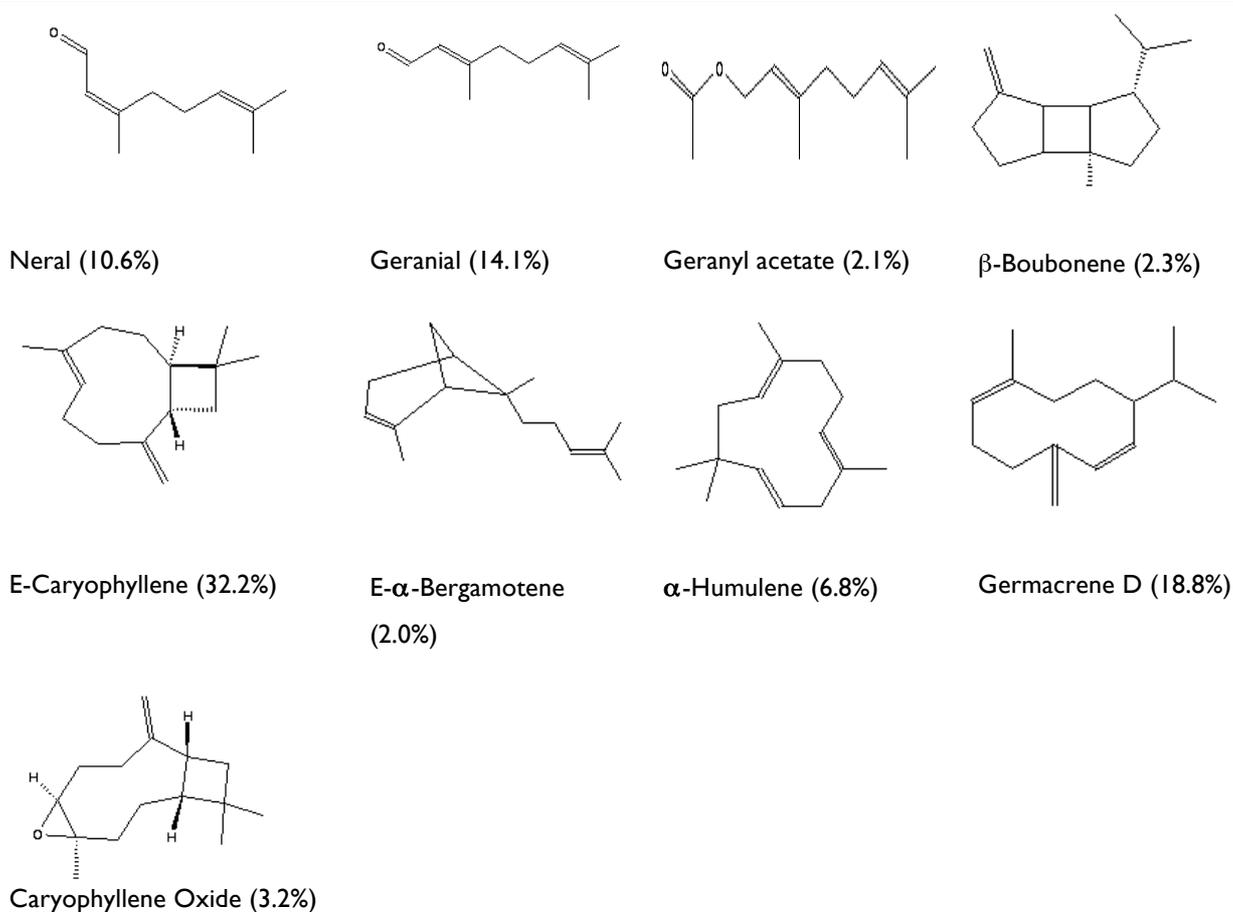


Fig.12- Major constituents of *S.forbesii* essential oil and their structure.

There is only a single paper (Ortet *et al.*, 2010) indexed at ISI Web of Knowledge (accessed 01/08) dealing *Satureja forbesii*.

Ortet *et al.*, 2010 identified a total of 39 volatile compounds, representing 90% of the total composition. Geranial (42.0%) and neral (31.2%) are the major constituents. This composition is rather different from that we determined, since geranial and neral are in lower concentrations in our sample (14.1% and 10.6% respectively). It is worthy to note that in the composition of our sample, sesquiterpenic compounds are dominant. This can be due to differences in sample preparation, not excluding the possibility of resulting from differences in the metabolism of plants, regarding the phase of the vegetative cycle or external influencing factors.

6.5. Ethnopharmacological data on *Hyptis pectinata*

Hyptis pectinata leaves, prepared as infusion, are useful to mitigate symptoms associated to strokes (cerebrovascular accidents - CVA). Additionally is used to relieve nasal congestion rhinopharyngitis, gastric disorders and fever.

6.6. *Hyptis pectinata* essential oil

The distillation of *Hyptis pectinata* produced an opaque yellow essential oil, yield of 0.1% (v/m), calculated considering plant material fresh weight. There were a total of fifty-two volatile compounds, accounting for 95.2% of essential oil identified (table V).

The essential oil was mainly composed by sesquiterpene hydrocarbons (50.7%), followed by monoterpene hydrocarbons (39.2%), oxygen-containing sesquiterpenes (3.8%), oxygen containing monoterpenes (1.1%) and other compounds (0.3%). The major compounds were β -pinene (17.1%), *E*-caryophyllene (14.1%), β -elemene (11.5%), sabinene (10.7%), germacrene D (7.7%), bicyclogermacrene (6.3%), γ -terpinene (2.8%) and α -pinene (2.3%) (Fig.13).

Table V- Composition of the essential oil of *Hyptis pectinata* .

RI ^a	RI ^a _{Lit}	RI ^p	Compounds	%
924	923	1031	α -Thujene	0.6
931	930	1031	α-Pinene	2.3
944	942	1076	Camphene	0.1
965	964	1127	Sabinene	10.7
971	970	1117	β-Pinene	17.7
982	980	1160	Myrcene	0.9
999	997	1168	α -Phellandrene	0.2
1012	1009	1185	α -Terpinene	t
1013	1012	1273	<i>p</i> -Cymene	0.5
1020	1020	1204	Limonene	1.9
1026	1025	n.d.	Z- β -Ocimene	0.1
1036	1035	1246	E- β -Ocimene	1.1
1047	1047	1246	γ-Terpinene	2.8
1051	1051	n.d.	Z-Sabinene hydrate	t
1078	1077	n.d.	Terpinolene	0.1
1084	1082	1436	Filifolone	0.4
1100	1100	n.d.	Chrysanthenone	0.4
1118	1118	n.d.	Camphor	0.1
1122	1119	1644	E-Pinocarveol	0.1
1160	1159	1598	Terpinene-4-ol	0.1
1168	1165	1621	Myrtenal	0.1
1170	1169	n.d.	α -Terpineol	t
1172	1176	1783	Myrtenol	t
1242	1244	1563	Farnesyl acetone	t
1266	1266	1571	Bornyl acetate	0.1
1330	1329	1464	δ -Elemene	1.6
1363	1364	1485	α -Ylangene	t
1368	1368	1485	α -Copaene	1.2
1374	1376	n.d.	β -Bourbunene	1.6
1382	1382	1585	β-Elemene	11.5
1401	1405	1565	α -Cedrene	0.1
1408	1208	1592	E-Caryophyllene	14.1
1420	1428	1848	Geranyl acetone	0.3
1439	1442	1659	α -Humulene	1.2
1466	1466	1699	Germacrene D	7.7
1470	1470	n.d.	β -Selinene	0.5
1481	1482	1724	BicycloGermacrene	6.3
1485	1480	1708	α -Selinene	0.2
1489	1490	1756	Germacrene A	1.4
1496	1498	1752	γ -Cadinene	0.3
1506	1508	1752	δ -Cadinene	0.7
1519		n.d.	α -Cadinene	1.0
1526	1527	n.d.	Selina-3,7(11)-diene	0.5
1538	1539	1818	Germacrene B	0.9
1552	1551	2111	Spathulenol	1.1
1556	1558	1972	Caryophyllene oxide	1.8
1615	1615	2167	T-Muurolol	0.4
1628	1628	n.d.	α -Cadinol	0.4
1672	1675	n.d.	Juniper camphor	0.1
1904	1900	1900	Nonadecane	t

Monoterpene hydrocarbons	39.2
Oxygen containing monoterpenes	1.1
Sesquiterpene hydrocarbons	50.7
Oxygen containing sesquiterpenes	3.8
Other Compounds	0.3
Total Identified	95.2

RI^a - Retention indices from the SPB-I column
 RI^a_{Lit} - Reference retention indices from literature
 RI^P - Retention indices from the SupelcoWax 10 column
 t – traces (<0.05%)
 n.d. – not determined

Major compounds (≥2%)

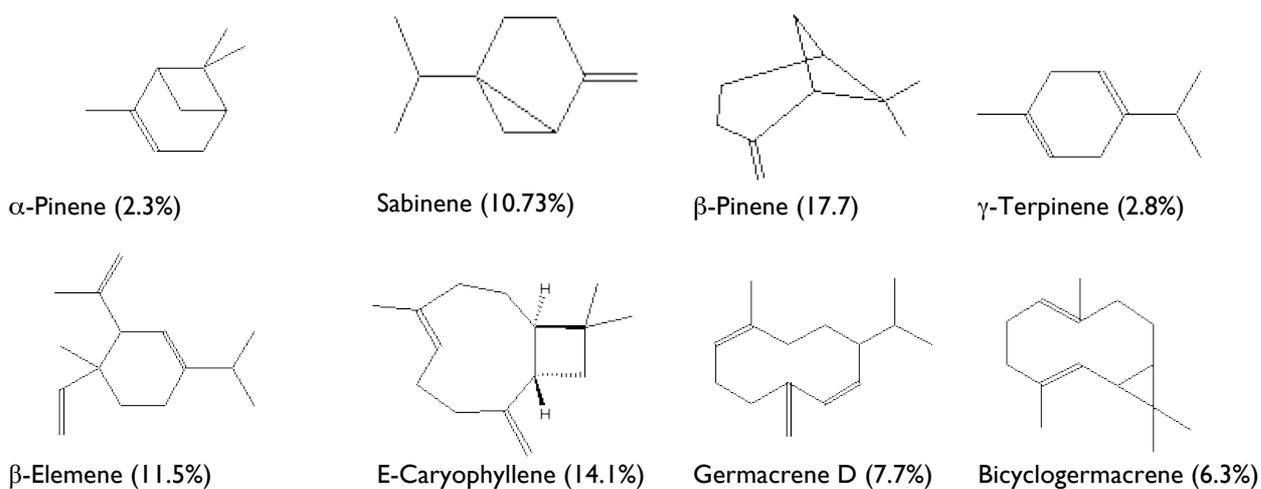


Fig.13- Chemical structures of the major compounds (≥ 2%) of *H. pectinata* essential oil.

Forty-three papers are indexed at ISI Science Direct (accessed 01/08) dealing with of *Hyptis pectinata*, however, not all of them under the subject *H. pectinata* essential oil.

Raymundo *et al.* (2011) obtained a yield of 0.5% of essential oil, five times more than what we attained.

Nascimento *et al.* (2008) report the composition of the essential oil from leaves of *H. pectinata* cultivated in Sergipe (Brazil). The main constituents were β-caryophyllene, caryophyllene oxide, and β-pinene. Differently the essential oil from *H. pectinata* cultivated in western Africa revealed cymene and thymol as the major compounds, accounting more than 60% of the whole composition. Caryophyllene, caryophyllene oxide and β-pinene are trace constituents (Malan *et al.*, 1988). Comparing these compositions with our results, the oil from Cape Verde is completely different from that reported by Malan *et al.* (1988). In fact

we did not detect cymene or thymol in our samples being β -pinene, *E*-caryophyllene, β -elemene, sabinene, germacrene D, bicyclogermacrene, γ -terpinene and α -pinene, the major constituents.

Differences are so sharp that it is difficult to believe that they are due to environmental conditions, climate or soil. It is acceptable to speculate on the existence of different chemotypes supporting the variability found in the oils of this species.

The essential oil from *H. pectinata* was found to be very effective against *Streptococcus mutans*. This bacterium rapidly metabolizes dietary carbohydrates, resulting in the formation of acid end products that can contribute to the demineralization of tooth enamel during caries development (Nascimento *et al.*, 2008).

According to Raymundo *et al.* (2011), *Hyptis pectinata* essential oil exhibits antinociceptive effects, mediated by opioid and cholinergic receptors, and anti-inflammatory activity through the inhibition of nitric oxide and PGE2 production.

6.7. Ethnopharmacological data on *Cymbopogon citratus*

Cymbopogon citratus, commonly known by capeverdean folk as “xá-li”, is used as soothing, as well as for digestive disorders. Together with *Rocella tinctoria*, locally known as urzela, is used to prepare a decoction useful for fertility treatments for dysmenorrhea and metrorrhagia.

6.8. *Cymbopogon citratus* essential oil

Hydrodistillation of *Cymbopogon citratus* produced a golden yellow essential oil, with 0.3% (v/m) yield. There were a total of thirty-nine volatile compounds accounting for 85.3% of the whole composition of the essential oil (table VI).

The essential oil extracted from *C. citratus* was essentially composed by oxygen-containing monoterpenes (68.8%) followed by monoterpenes hydrocarbons (9.9%), oxygen-containing sesquiterpenes (3.6%), sesquiterpenes hydrocarbons (1.8%) and at last, other compounds (1.2%). The major compounds were geranial (38.3%), neral (26.4%), myrcene (7.6%), α -cadinol (2.3%) and geraniol (2.0%) (Fig. 14).

Table VI- Composition of the essential oil of *Cymbopogon citratus*.

RI ^a	RI ^a _{Lit}	RI ^P	Compound	%
923	921	1020	Tricyclene	0.0
932	930	1031	α -Pinene	0.1
944	942	1078	Camphene	0.2
963	965	1337	Hept-5-ene-6-methyl-2-one	1.1
965	964	1125	Sabinene	t
971	970	1118	β -Pinene	0.2
982	980	1162	Myrcene	7.6
998	997	1168	α -Phellandrene	t
1012	1012	1275	<i>p</i> -Cymene	t
1021	1020	1214	1,8-Cineole	0.2
1021	1020	1205	Limonene	0.1
1026	1025	1233	Z- β -Ocimene	1.1
1036	1035	1250	E- β -Ocimene	0.6
1046	1047	1249	γ -Terpinene	t
1080	1082	1539	Linalool	0.1
1118	1117	n.d.	<i>allo</i> -Ocimene	0.1
1132	1132	1476	Citronellal	0.2
1140	1145	1661	<i>iso</i> -Borneol	1.4
1172	1169	1687	α -Terpineol	0.2
1218	1214	1679	Neral	26.4
1240	1233	1839	Geraniol	2.0
1248	1249	1731	Geranial	38.3
1361	1359	1757	Geranyl acetate	0.1
1381	1382	1583	β -Elemene	0.2
1407	1208	1590	E-Caryophyllene	0.5
1430	1434	1577	E- α -Bergamotene	0.1
1444	1444	1661	E- β -Farnesene	0.1
1465	1466	1700	Germacrene D	0.2
1468	1469	n.d.	γ -Selinene	0.1
1475	1475	1717	δ -Selinene	0.1
1485	1485	1721	α -Muurolene	0.1
1497	1498	1749	γ -Cadinene	0.2
1506	1508	1749	δ -Cadinene	0.3
1526	1526	2069	Elemol	0.2
1628	1628	2221	α-Cadinol	2.3
1666	1675	n.d.	Juniper camphor	0.8
1691	1696	2351	<i>E,E</i> -Farnesol	0.1
1718	1715	2261	<i>E,E</i> -Farnesal melange	0.2
1833	1828	n.d.	Hexahydroxyfarnesyl acetone	t
			Monoterpene hydrocarbons	9.9
			Oxygen containing monoterpenes	68.8
			Sesquiterpene hydrocarbons	1.8
			Oxygen containing sesquiterpenes	3.6
			Other Compounds	1.2
			Total identified	85.3

RI^a - Retention indices from the SPB-I columnRI^a_{Lit} - Reference retention indices from literatureRI^P - Retention indices from the SupelcoWax 10 column

t – traces (<0.05%)

n.d. – not determined

Major Compounds ($\geq 2\%$)

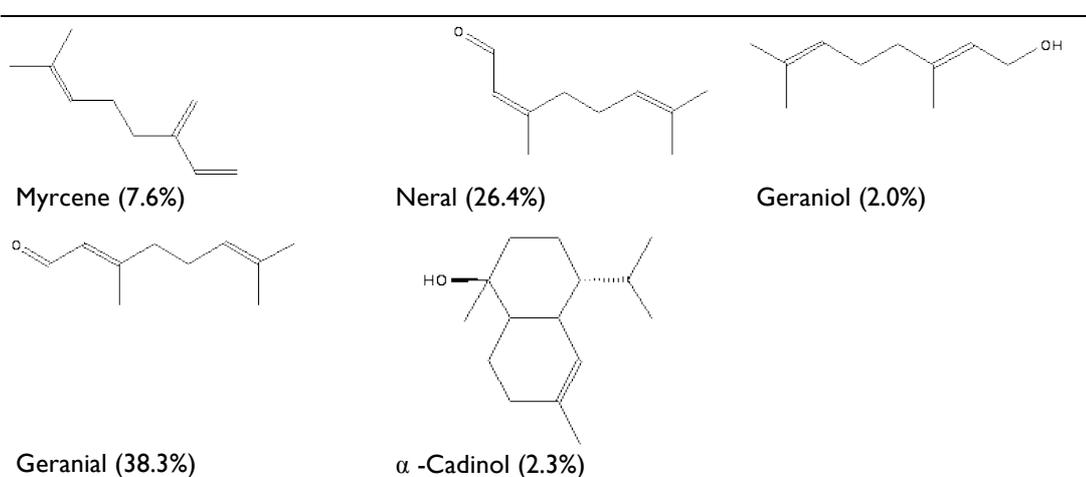


Fig.14- Chemical structures of the major compounds ($\geq 2\%$) of *Cymbopogon citratus* oil.

Schuck *et al.*, 2001, reported a yield of 0,6% and Gbenou *et al.*, 2013 0.9%. Other authors like Masamba *et al.*, 2003, Matasyoh *et al.* 2011 and Gbenou *et al.* 2013 etc, found myrcene, neral and geranial to be the major components. With the exception of α -cadinol, compositions do not seem different.

The volatile oil contains mostly geranial and neral (the mixture called citral), besides myrcene. The antimicrobial and antifungal activity of *C. citratus* is cited by Shuck *et al.*, 2001 and suggests that citral is the main responsible for this activity. The volatile oil has antioxidant properties and deodorant. Due to its strong odor, is used as a fragrance in soaps and detergents. Also, is employed as the insect repellent due mainly to the presence of citral.

Matasyoh *et al.*, (2011) evaluated the antifungal activity of essential oil of *Cymbopogon citratus* against five mycotoxigenic species of the genus *Aspergillus* (*Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus ochraceus*, *Aspergillus niger* and *Aspergillus fumigatus*). The antifungal activity tests showed that the oil was active against all the five *Aspergillus* species.

Gbenou *et al.*, 2013 carried out a study with the purpose of characterizing the inflammatory and analgesic activity of *C. citratus* on Wistar rats under laboratory conditions. The anti-inflammatory effect of the essential oils was investigated on formol-induced edema in the animals. Treatments with *C. citratus* essential oil reduced the edema over time in a dose dependent manner. *C. citratus* essential oil was found to have a preventive effect. The

analgesic activity of the essential oils was tested by tail immersion test. The essential oil treated animal tails were immersed in hot water kept at 50 °C. Animals treated with essential oils were able to keep their tails longer in a hot water bath (50 °C) than the untreated animals demonstrating the analgesic activity of the essential oils and significantly reduced the hyperthermia. This paper can at least explain its use for dysmenorrhea treatment.

6.9. Biological relevance of the major compounds of the studied plant species

Some of the compounds identified in the essential oils have biological activities, and are responsible or contribute for the healing features of some medicinal plants. This knowledge is also useful considering these compounds as leads for drug discovery and development.

***E*-Caryophyllene**

Ghelardini *et al.*, (2001) evaluated the anesthetic activity of β -caryophyllene using *in vivo* and *in vitro* models, rabbit conjunctival reflex test in a rat phrenic nerve-hemidiaphragm preparation. *E*-Caryophyllene was able to drastically reduce, in a dose dependent manner, the electrically evoked contractions of the rat phrenic nerve-hemidiaphragm. In the rabbits treated with a solution of *E*-caryophyllene, conjunctival reflex test treatment increased the number of stimuli necessary to provoke the reflex. The same essays were made with caryophyllene oxide, proving to be ineffective, either *in vivo* as *in vitro*. In conclusion, data evidence the local anaesthetic activity of *E*-caryophyllene, which appears to be strictly dependent on its chemical structure.

Soares *et al.*, (2003) investigated leishmanicidal activity against *Leishmania amazonensis* in some essential oils, and pointed out that *E*-caryophyllene as an effective antileishmanial compound.

Cavaleiro *et al.* (2011) shown the inhibition of dermatophyte fungi by *E*-caryophyllene as well as by its oxygenated derivatives, β -betulenal (isocaryophyllen-14-al), 14-hydroxy- β -caryophyllene and caryophyllene oxide.

The anti-inflammatory activity of *E*-caryophyllene was also reported by Fernandes et al. (2007). In fact, *E*-caryophyllene, was proved to be an agonist of CB2 (peripheral cannabinoid receptors) involved in the anti-inflammatory response (Gertsch et al.,2008). The same mechanism explains the antinociceptive effects of the compound (Katsuyama et al, 2013).

Camphene and Geranyl Acetate

Junior et al., (2012), made *in vivo* and *in vitro* experiments to evaluate antinociceptive and redox properties of monoterpenes. Camphene and geranyl acetate presented high antinociceptive activity, although both had antioxidant effect.

Yamaguchi et al., (2009) reported that the antifungal properties of thiosemicarbazide are enhanced when it is associated with camphene.

Vallianou et al. (2011) reported camphene alternative lipid lowering agent, reduces Plasma Cholesterol and Triglycerides.

α -Phellandrene

Several biological properties, such as analgesic and anti-inflammatory, are assigned to α -phellandrene. Besides, Jen-Jyh Lin (2012), showed that α -phellandrene can induce apoptosis in leukemia cells which is very useful in cancer treatment.

Lima et al. (2012), showed that this component has antinociceptive effects and it possibly involves the glutamatergic, opioid, nitrenergic, cholinergic and adrenergic systems.

According to Iscan et al. 2012, microbial biotransformation of α -phellandrene is a important resource for natural pharmaceutical. Its metabolites proved to be very effective against *Candida* species.

Neral and Geranial

According to Albuquerque et al. (2007), neral and geranial present nematicidal and larvicidal potencial against the nematode *M. incognita* and the *A. acgypti* larvae and anti-fungal activity.

Silva *et al.* (2008) displayed that lemongrass oil and citral have a potent in vitro activity against *Candida* spp. (*Candida albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis* and *C. tropicalis*).

Citral, the mixture of neral and geranial, was proved to be useful for giardiasis, inhibiting the grow of *Giardia lamblia* trophozoites (Machado *et al.* (2010). Similarly it is active against other flagellate protozoa, such as, *Leishmania* species (Machado *et al.*, 2012).

Citral is also known and used as insect repellent.

Camphor

Camphor's alleged medicinal benefits currently include local anesthetic, antipruritic, antiseptic, skin permeability enhancer and mild expectorant activity. It has a characteristic odor and is used commercially as a moth repellent and as a preservative in pharmaceuticals and cosmetics.

The cytotoxicity of camphor, was evaluated by Cherneva *et al.*, (2012), on rat thymocytes. The cells were incubated at different concentrations of the component and the results showed that camphor increases, significantly, at the highest concentration the thymocyte viability, enhancing the immune system.

Germacrene D

The sesquiterpene germacrene D seems to play a significant role in host plant location by females of the American tobacco budworm moth *Heliothis virescens*. Electrophysiological recordings from single olfactory receptor neurones have shown that a major type of the antennal receptor neurones responds with high sensitivity and selectivity to germacrene D. The behavioural significance of this sesquiterpene has been studied in a two-choice wind-tunnel where mated females could choose between a plant containing a low release rate of (–)-germacrene D dispensers and a plant with control dispensers. There were an increased attraction to and oviposition on plants with the germacrene D dispensers, which suggested that it has a positive effect on mated *H. virescens* females, by acting either as an attractant or as an attractant as well as a stimulant for oviposition (Stranden *et al.*, 2003).

α -Pinene and β -Pinene

The antimicrobial activities of pinene isomers and enantiomers were evaluated against bacterial and fungal cells. The work of Silva *et al.* (2012) intended to evaluate the antimicrobial effects of the different isomers and enantiomers of these monoterpenes against *Candida albicans*, *Cryptococcus neoformans*, *Rhizopus oryzae* and methicillin-resistant *Staphylococcus aureus*.

The minimal inhibitory concentration values of α -pinene and β -pinene enantiomers were determined. Only the positive enantiomers exhibited a microbicidal effect against all of the microorganisms tested. No antimicrobial activity was detected with the negative enantiomers. Fungi, especially *C. neoformans*, were more sensitive to (+)- α -pinene and (+)- β -pinene than MRSA.

Him *et al.*, (2008) investigated α -pinene effects in mice, and it proved to have antinociceptive properties.

Neves *et al.* (2010) evidenced the dual inhibition of NO synthase and Nf-kB expression on chondrocytes stimulated by IL-1 β , proving the anti-inflammatory and anti-arthritic potential of α -pinene.

γ -Terpinene

The mechanism of antimicrobial activity of essential oils components α -terpineol, γ -terpinene and eugenol was studied to evaluate their effect on the bacterial membrane against four strains of bacteria: *Listeria monocytogenes*, *Streptococcus pyogenes*, *Proteus vulgaris* and *Escherichia coli* (Oyedemi *et al.*, 2008). The study was conducted observing changes in membrane composition, assaying for the leakage of protein and lipid using Bradford and van Handel's method. The oils components were capable of inducing cell lyses by the leakage of protein and lipid contents. Eugenol was highly effective toward protein content leakage after 120 min of exposure. α -Terpineol and γ -terpinene showed similar effect under the same condition. γ -Terpinene displayed the highest activity toward lipid content leakage, while α -terpineol and eugenol showed similar effect after 120 min of exposure. The result revealed that both cell wall and membrane of the treated gram-negative and gram-positive bacteria were significantly damaged.

Thus, according to Rudbäck *et al.* (2012) α -terpinene can as it easily autoxidizes to form allergens, and maybe used in products for topical applications like cosmetics and skin care products.

α -Humulene

Hadri *et al.*, (2010) studied the essential oil of *Salvia officinalis*, with the purpose of evaluating its effect on “*in vitro*” tumor cells lines. α -Humulene, the major constituent of the oil, exhibited high cytotoxic activity in murine macrophage cells, colorectal adenocarcinoma cells and breast melanoma cells, which means, it has potential to inhibit cancer cell growth.

Acheflan® is a pharmaceutical made with essential oil, standardized with 2.3 to 2.9% of α -humulene. It is indicated for tendinitis, musculoskeletal disorders associated with pain and inflammation, such as myofascial pain, back pain, low back pain in painful inflammatory conditions associated with limb trauma, sprains and bruises.

Bornyl acetate

Wang *et al.*, (2010) investigated the anti-abortive effects of quercetin and bornyl acetate and their immunological modulation at maternal-fetal interface. It is recorded in the Chinese Veterinary Pharmacopoeia (the 2005 edition) that some of the species containing bornyl acetate as major constituent, has anti-abortive properties. The study provided evidence that alterations in both the CD4+/CD8+ and IFN- γ /IL-4 ratios participate in LPS-induced fetal resorption, and that quercetin and bornyl acetate have an antiabortive effect via maintenance of the CD4+/CD8+ T lymphocytes and IFN- γ /IL-4 balance in uterus.

β -Elemene

Li *et al.* (2009) evaluated the therapeutic application of β -elemene in sensitizing lung cancer cells to cisplatin and it considerably enhanced the inhibitory effect of cisplatin on cell proliferation in a time- and dose-dependent manner in the human non-small cell lung cancer (NSCLC) cell lines.

Li *et al.* (2013) displayed that some synthetic analogs of β -elemene may inhibit brain cancer cell growth and proliferation, showing that synthetic analogs of β -elemene hold promise for patients with brain tumors

Yang *et al.* (1997) reported mechanisms of antitumor activity of β -elemene.

Geraniol

Lorenzi *et al.*, (2009), evidenced the efflux pump inhibition of essential oils containing geraniol. They were able to demonstrate that the essential oil from *Helichrysum italicum* modulate drug resistance in several gram-negative bacterial species by targeting efflux mechanisms. Geraniol appeared to be a potent inhibitor of efflux mechanisms

Kim *et al.* (1995) showed that citral and geraniol has a potent antibacterial activity against *Salmonella typhimurium*.

7. Conclusion

Natural products demand is increasing every day, from manufacture of food, cosmetics and pharmaceuticals. Hence, the magnitude of conducting studies on essential oils lies not only in the identification and biochemical characterization of the species but also find out their functions and maybe connect the structure of the chemical contents with their biological activities. As previously mentioned, very few papers concerning folk plant traditions have been published for Cape Verde Islands. It was made a series of interview, but only in Santiago Island. Further field studies must be organized for a better characterization of the medicinal Cape Verde flora. Although various studies have been done with *C. citratus* and *H. pectinata* more investigation is necessary to confirm the use of these species in Cape Verde. Though is known and used worldwide, it is possible to say that their application, as any other species, varies with the location.

We were able to establish the composition of the essential oils from aromatic medicinal plants from Cape Verde. With the exception of *Satureja forbesii*, it was possible to compare the results with other author's results.

Clinical trials are required to confirm the effects of the endemic species. Whether it is the primary function, or whether it is a consequence. More analysis of the pharmacokinetic and pharmacodynamics parameters of active compounds are necessary before starting the clinical trials in order to determine the dosage, side effects and toxicity.

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