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Morphological and Phytochemical Features in Fruits of *Pimpinella Anisoides* V. Brig. (Apiaceae)

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Abstract

In this study, the morphological characteristics, phytochemical composition of *Pimpinella anisoides* fruits (monocarps), an endemic plant of Italy was investigated.

The morphological analysis of the monocarps contributed to distinguish *P. anisoides* from other related *Pimpinella* species used for commercial purposes, in particular *P. anisetum* and *P. anisum*. Our study showed that these fruits are different for their surface and shape: in particular, *P. anisoides* monocarps are glabrous, whereas the other two species are strigose.

Two main compounds were identified in the essential oil of the five studied accessions: Limonene (46.78-50.74%) and Anethole (38.50-42.77%) whose sum ranged between 85.28 and 93.51%. Estragole and β-Phellandrene were other two representative compounds. All other volatiles accounted for less than 1% of the total volatile composition. One-way ANOVA analysis showed significant differences in almost all volatiles, whereas non-significant differences were found in the compounds detected in very low quantity. Hierarchical cluster analysis allowed to group the five accessions in three main clusters. Principal Components Analysis showed that the first two principal components explained 71.80 % of total variance.

Bartolucci et al. [19] reported 7 native species of *Pimpinella* and Galasso et al. [20] 1 alien. Following subsequent updates, it is now possible to recognize 10 taxa in total, including 3 subspecies, of which 9 are native (N) and 1 alien (A) (Portal to the Flora of Italy, 2021). However, the interpretation of the Italian taxa belonging to the genus *Pimpinella* L. is controversial. Indeed, Stinca and Ricciardi [21,22] reports 12 italian taxa of *Pimpinella*, including 9 species and 3 subspecies.

Pimpinella anisoides V. Brig. [Synonims: *Apium anisoides* (Brign.) Caruel, *Apium gussonei* (C. Presl) Calest., *Pimpinella bubonoides* DC., *Tobion bubonoides* Raf.; Italian name "Tragoselino

Introduction

The genus *Pimpinella* L. (Apiaceae Lindl., subfamily Apioideae, tribe Pimpinelleae) includes about 150-180 annual or perennial herbs distributed in the temperate and subtropical regions of Eurasia and Africa, but also in South America and in the western part of North America [1,2].

There have been numerous studies on this genus, but its taxonomy has not yet been fully clarified, representing one of the most complex genera of the Apiaceae family [3-7]. Fernández Prieto et al. [8] points out that the *Pimpinella* is a non-monophyletic group and that the *Pimpinella* taxa growing in Western Europe are part of phylogenetically independent groups that correspond to three different tribes of the Apioideae subfamily: Pimpinelleae (main group), Pyramidoptereae and Smyrnieae. The taxonomy of the genus *Pimpinella* but, in general, for different genera of Apiaceae family, is the fruit: shape, pubescence and anatomy [9-14] In particular, the anatomy of many species of this genus was studied [15,16,7,2] Another important character regarding this genus is its phytochemical composition. Tabanca et al. [17] studied the content of essential oils of 19 Turkish species of *Pimpinella* and established the phylogeny of *Pimpinella* species using gene sequence data and of essential oil analysis as a framework to ascertain whether there is a connection between the chemical and genetic profiles. Magee et al. [7] studied the phylogeny of 16 African and Malagasy species, including also 26 species from Eurasia. Fereidounfar et al. [18] analyzed 52 Southwest Asian species of *Pimpinella*. In the genus *Pimpinella*, the basic chromosome number is x = 8 to 11 [18].

The study of monocarps morphological and phytochemical features showed how characterize and recognize the ripe fruits of *P. anisoides* for distinguish this species from others to avoid commercial sophistications and for a better conservation *in situ* and *ex situ*.

Keywords: Black Anise; Calabria; essential oils; *ex situ* conservation; *in situ* conservation; Italy; sophistication.

meridionale"; vernacular Calabrian name "aranzu")] is an endemic species growing in some southern Italy regions, such as Lazio, Basilicata [23-25], Calabria [26,27], Campania [28], Sicily [29] , and Puglia [30,19,31,21,22], especially in the mountainous belt [27,25] occupied by mesophilic oak and chestnut woods. In Calabria, *P. anisoides* is reported for several locations. However, the practice of harvesting its fruits to flavor various traditional baked goods and liqueurs and as seasoning is known only for the Reventino Massif and Sila Catanzarese area [27].

Although the species is currently considered LC (Least Concern) [32], it is collected for commercial purposes in several regions: this is compromising the reproductive potential of the species with negative effects on the size of populations [27-25].

Some studies on the chemical composition of *P. anisoides* were carried out in the past. [33] Menichini et al. [33] studied acetylcholinesterase and butyrylcholinesterase inhibition of ethanolic extract and monoterpenes from *P. anisoides*, whereas Conforti et al. [34] described its chemical profile and in vitro properties, finding 23 major components in the ethanolic extract of its fruits.

Distinguish *Pimpinella* species among them is not so easy [35]. For this purpose, morphometric studies are very important in discriminating botanical taxa [36,37,38]. In particular, in the genus *Pimpinella*, as well as in most of the Apiaceae, the fruits take on considerable diagnostic significance and are considered essential in taxonomic analysis [35]. According to Spjut [39], Apiaceae fruits are classified as polachenarium, i.e. a schizocarpic fruit, originating from an inferior ovary separating in

Fruits were analyzed and measured using a stereomicroscope equipped with an HD camera Invenio

on *P. anisoides* fruits, to evaluate their characteristics for a proper valorization and protection of this endemic species.

Materials and Methods

The fruits (monocarps) were collected in five locations in the Sila Catanzarese, province of Catanzaro (Central Calabria, S-Italy) (Fig. 1), at altitudes between 700 and 900 m above sea level, in mountain forests of chestnut or deciduous oaks which represent their habitats (Tab. 1). The fruits were harvested at the time of ripening between July 2017 and August 2018 by local farmers who also deal with their marketing.

The fresh fruits were cleaned in the Geobotany laboratory of the Mediterranean University of Reggio Calabria (Italy), counted and analyzed for quality. To this purpose, 300 fruits were randomly sorted into 6 groups of 50. In each group, the fruits were observed and distinguished by an optical stereomicroscope Optech® into: "normal" (intact fruits, ripe, without evident macroscopic damages); "damaged" (for mechanical damages, malformations or with holes or injuries caused by insects); "aborted" (fruits that have not completed the ripening process correctly). Only "normal" fruits were used in the morphological and phytochemical analyses. Specimens of *P. anisoides* collected in the study area, are stored in the Herbarium of the Mediterranean University of Reggio Calabria (REGGIO).

2.1 Morphological analysis

single fruits (monocarps) from a longitudinal central axis (columella, or carpophore) often remaining attached to the axis at maturity.

In order to provide scientific support to preserve the small Calabrian natural populations of *P. anisoides* occurring mainly in the Reventino Massif and Sila Catanzarese area (Central Calabria, S-Italy), a series of botanical investigations have been launched on this important species. This work presents the first results obtained from phytochemical and morphological investigations carried out

5SII connected to a computer where its DeltaPix inSight© software is installed. This software allowed to reconstruct the image using a multifocal system, to determine the exact measurements of the fruits at different magnifications and to apply these measurements directly on the image of the examined objects (Fig. 1).

The observations were carried out on a sample of 20 fruits for each of the 5 locations examined. In detail, the following dimensions were measured: monocarp width and length, length from base to maximum width of monocarp, stylopodium width and length (Fig. 2 and Tab. 1). Fruit terminology is in agreement with Yeşil et al. [35].

2.2 Essential oil extraction

Fruits were harvested and air dried at room temperature until constant weight. For each sample, a 30 g aliquot was randomly taken out of 100 g. Fruits were powdered by a home grinder and blended with 300 mL water. At this point, fruits were hydro-distilled in an all glass Clevenger-type apparatus for 3 hours. The essential oil was separated from the water, placed in a glass vial and stored in a fridge in the dark at 4 °C before GC-MS analysis that was conducted within 48 h after extraction.

GC-MS Analysis

It was applied for the qualitative analysis. The samples were analysed by gas chromatography with mass spectrometry (GC-MS) using a GCMS-QP2010 Ultra (Shimadzu, MI, Italy) operated by Lab solution software. The chromatographic separation was conducted using a fused capillary column (Mega SE52, 0.25 μm, 0.25mm, 30m). MS source temperature: 280 °C; interface temperature: 280 °C; ionization energy: 70 eV; ionization current: 60A. The oil components were identified by computer matching against Nist library. The samples were analysed on three replicates

GC-FID Analysis

The fruits are always glabrous in all their parts with 5 well evident dorsal ribs ± prominent (Fig.3a).

solution (Shimadzu, MI, Italy) equipped with flame ionization detectors (FID), and fused capillary column (Mega SE52, 0.25 µm, 0.25 mm, 30 m). The oven temperature was programmed: isothermal 40 °C for 3 min, from 40 to 220 °C (6 °C/min), from 220 °C to 250 °C (15 °C/min), and isothermal for 5min; injector temperature: 270 °C (injection mode: split 1/100); detector temperature: 270 °C; carrier gas: helium.

One µl of essential oil was directly injected. The relative proportions of the essential oil constituents were expressed as percentages obtained by peak-area normalization.

Statistical analysis

Means and standard deviations were calculated by Excel© 2010 software. Statistical differences were calculated by one-way ANOVA and Tukey test for post hoc analysis at $p < 0.05$ using the SPSS® 23.0 software (SPSS Inc., Chicago, IL, USA). Principal Component Analysis (PCA) were applied using SPSS® software for Windows©, version 17.0 (Chicago, IL, USA). Then, a cluster analysis was performed and a classification obtained through the mean link on the Euclidean distance squared similarity function.

Results and Discussion

Morphology

A preliminary morphological analysis shows that 50% of the fruits were 'normal', 30% 'aborted' and 20% 'damaged'. The "normal" fruits are oblong-ovoid (length/width ratio 1.09) to oblong-cylindrical (length/width ratio 2.05), on average 2.77 (± 0.30) mm long and 1.33 (± 0.14) mm wide; the monocarp length from base to maximum width is 1.12 (± 0.14) mm. The stylopodium is on average 0.45 (± 0.06) mm long and 0.33 (± 0.09) mm wide (Tab.2).

GC analysis was performed to quantify compounds using a GC2010 Plus Tracera operated with GC

The monocarp is compressed dorsally. The ventral surface is flat, longitudinally is well visible the columella that joins the two monocarps (Fig.3 b).

Examination of the fruit cross-section showed that *P. anisoides* monocarps are characterised by a smooth, thin cuticle (Fig. 4). Five small vascular bundles (vb) are present in the lower half of the dorsal and lateral ribs.

The vittae present in the mesocarp containing the essential oils in which the species is rich, are divided into:

- vallecular vittae (vv): three between the central groove and the median groove and four in the median grooves closest to the commissural side. They are larger than the vascular bundles (vb);
- commissural vittae (cv): located on the commissural side are four, two larger and more obvious as typical feature of fruits of the genus *Pimpinella*, the other two smaller located near the lateral vascular bundles (vb).

The endosperm (e) occupies the largest part of the section (Fig. 4).The analysis of variance of results (Anova test) (Table 2) shows a high significance value of the data (*** = $p < 0.001$).

The morphological analysis of the monocarps, allows to distinguish the fruits of *P. anisoides* from related *Pimpinella* species used for commercial purposes. In particular, the monocarps of *P. anisetum* Boiss. & Balansa are strigose with sharp, stiff, slanting hairs, hair-like scales or bristles which are appressed to the surface and ovoid in shape, whereas *P. anisum* L. has strigose, ovoid-subglobose fruits [35]. In *P. anisoides* the monocarps are completely glabrous and vary in form from oblong-ovoid to oblong-cylindrical (Table 3).

These distinctive characters are very important and necessary to know in order to prevent the sold material from being sophisticated with other types of fruits of inferior quality, which are often mixed with those of *P. anisoides*.

5.67% and 5.54% in the essential oil extracted by a Clevenger-type apparatus with steam distillation

Essential oils are an important discriminating factor between different taxa [40]. They can have several applications in preventing some diseases [41], but they are also a constituent of fragrances and flavours which are fundamental in a food or a beverage with regard to the approach of a consumer which: see, smell and taste a product before to eat or drink.

Little is known about the essential oil of the *Pimpinella* genus with very scarce findings for the *P. anisoides* and *P. anisetum* species and some information more for the *P. anisum* species.

Thirty-two components were identified in the *P. anisoides* essential oil studied in this work (Table 4). The four most preponderant compounds of the five studied accessions were: Limonene, Anethole, Estragole and β-Phellandrene, here listed in decreasing order for the highest quantity detected.

The most representative was limonene ranging between 50-74% (Pa_3) and 46.78% (Pa_5) ($p < 0.001$ (Table 4). Limonene is a hydrocarbon monoterpene used to prepare flavourings, perfumes and in local medicine for its anaesthetic, antiseptic, expectorant, antiviral, antilithic and antimicrobial properties [42-45] even if Boughendjioua and Boughendjioua have found a lower antimicrobial activity of limonene than alcohols and phenols. Studies in aromatherapy have shown an anti-migraine activity [46,47]. Menichini et al [33] found a 13.5% limonene content in *P. anisoides* essential oil from Calabria region after fruits maceration in ethanol, this allows us to speculate that water used as a solvent facilitate the limonene extraction from fruits if compared with ethanol. *P. anisoides* was found to have a higher limonene content than *P. anisum*, in fact in the latter, the limonene content was: 2.06% in Iranian fruits [48]; 0.63 g/kg, 0.51 g/kg and 2.11 g/kg in Iranian fruits respectively obtained from plants grown in a soil without Zn addition and with 0.1% and 0.2% Zn addition (w/v) [49]; 0.06% in Serbian seeds [50]; 0.2% in Turkish seeds from Fadliya region, Mosul Province [51]. Limonene is characteristic of the essential oil of *Citrus* genus and it was quantified for: 84.73% in lemon peel [52]; 39.74% in lemon flower [*Citrus* ×*limon* (L.) Osbeck] [53]; 60.8% in *Citrus medica* cv. 'Diamante' peel [54], 31.10% to 44.48% in bergamot (*Citrus bergamia* Risso) peel [55,56]. Limonene is also contained in the essential oil of other genus and species but in lower quantity: 6.05%

Phytochemical

respectively from flowers, fruits and leaves of wild fennel [*Foeniculum vulgare* Mill. subsp. *piperitum* (Ucria) Bég.] [57], 3.80% to 10.11% in essential oil of needles of Calabrian pines (*Pinus nigra* J.F. Arnold subsp. *laricio* Palib. ex Maire) [58].

Anethole was the second major volatile in *P. anisoides* fruit essential oil and ranged between 42.77% (Pa_5) and 38.50% (Pa_3) ($p < 0.001$ (Table 2). Anethole is a phenylpropanoid. It is sweet (13 times sweeter than sugar) and it is used in alcoholic beverages and in aromatised foods [59]. It is recognised to have the following properties: antioxidant , anti-fungal and anti-microbial [60,25,61], spasmolytic [62], gastro-protective [63,64], sedative [65] and in reducing the risk of Parkinson's disease [66]. Studies in aromatherapy have shown that anethole might produce anti-migraine effects via the inhibition of neurogenic inflammation through the MAPK and NF- κB pathways [67]. In the literature it is reported as characteristic for *Pimpinella* genus. In a previous study conducted on *P. anisoides* fruits collected in the region of Calabria (South Italy), anethole was found to be 54.5% of the total volatiles, from essential oil extracted by maceration with ethanol [33]. Anethole was found to be the predominant volatile in *P. anisetum* oil from Turkey obtained by a Clevenger apparatus (77.1% in fruit oil and 55.2% in herb oil respectively) [68]. Anethole also predominated in *P. anisum*: 93.5% in seed essential oil from Turkey obtained by hydrodistillation [51]; 74.40% in seed essential oil obtained by a Sohlet extractor and distilled water as a solvent [48]; 88.49% in seed essential oil from Serbia obtained by hydrodistillation and *n*-hexane as collecting solvent [50]. Anethole is also contained (even if in lower quantity) in seed essential oil of fennel (*Foeniculum vulgare* Mill.) (49.76-81.21%) 69 and guarana [*Paullinia cupana* H.B.K. var. *sorbilis* (Mart.) Ducke] [70].

Estragole (methyl chavicol) ranged between 5.76% (Pa_5) and 6.67% (Pa_2) ($p < 0.001$) (Table 4). Estragole is phenylpropanoid, more in detail an alkenylbenzene for which, if consumed in high quantity, a carcinogenic and genotoxic activity was found (Scientific Committee on Food, 2001a, 2001b, 2001c) even if estragole orally administered to mice for 10 mg/kg/day for 14 days showed no toxicity [71].

morphometric data have distinguished two main clades. Pa_1 and Pa_2 are at a distance of 1 and are *anisoides* of the present work: 22.4% in fruit oil and 41.9% in herb oil. In studies conducted on the essential oil of *P. anisum*, estragole was found to be: 2.4% in seeds submitted to hydrodistillation with double-distilled water [72]; 1.39% and 0.63% from Algerian seeds respectively submitted to hydrodistillation and to microwave assisted hydrodistillation [73]; 1.73% from seeds produced in Turkey and submitted to hydrodistillation [51]; 1.87% from Iranian seeds and distilled water as a solvent [48].

β-Phellandrene was detected in a range between 1.99% (Pa_5) and 1.55% (Pa_1) ($p < 0.001$). It is a cyclic monotepene that was not listed as the major constituents of the *P. anisoides* essential oil in the only existing study conducted from fruits collected in the region of Calabria [33]. β-Phellandrene is one of the minor volatiles found in the peel essential oil some *Citrus* species: 0.40% in *Citrus reticulata* Blanco grown in Algeria [74]; 0.19% and 0.07% respectively in *Citrus x aurantium* L. (sub *Citrus sinensis*) and *Citrus x limon* (L.) Osbeck (sub *Citrus lemon*) taxa grown in Spain.Higher quantity of β-phellandrene were found in the essential oil of wild populations of *Echinophora cinerea* (Boiss.) Hedge & Lamond from Iran (13.11% - 10.29%) [75] and in *Artemisia dracunculus* L. growing in Tajikistan (3.2%) [76].

Each one of all other volatiles of the five studied accessions of *P. anisoides* was detected for less than 0.50% (Table 4). No significant differences ($p > 0.05$) were found for cis-Limonene-oxide (0.05%-0.07%), Cyclohexene, trans-Carveol (0.03%-0.05%), 4-Terpenyl acetate (0.01%-0.03%), *p*-Acetonyl anisole (0.03%-0.06%), Methyl eugenol (0.03%-0.05%), Farneseol (0.02%-0.03%), Limonene 1,2-epoxide (0.01%-0.03%), Nerolidol (0.01%-0.03%), α-Bisabolene (0.01%-0.02%), Cariophyllene oxide (0.07%-0.10%) and Spatulenol (0.03%-0.05%) (Table 4).

Cluster Analysis

The cluster analysis was conducted both on the basis of morphometric data and of the volatile composition. Two different dendrograms were appreciated. The results obtained on the basis of the

Baser et al. [68] in a study on *P. anisetum* found estragole in a higher quantity if compared with *P.* closely related to Pa_3 at a distance of 3, whereas Pa_4 and Pa_5 are grouped in the second clade at a distance of 16. This was due to the fruits shape which is oblong-ovoid in the Pa_1, Pa_2 and Pa_3 accessions, whereas is oblong-cylindrical in the Pa_4 and Pa-5 accessions. More in detail, Pa_1, Pa_2 and Pa_3 show a fruit having a minor length/width ratio than Pa_4 and Pa_5 (Fig.5).

The results obtained on the basis of the volatile composition permit to appreciate three main clusters (Fig.6). The first cluster grouped Pa_1, Pa_2 and Pa_4 at a distance of 6. Pa_1 and Pa_2 were at a distance of 1, this was due to the lowest *β*-Phellandrene content and a high limonene content. The second cluster distinguished Pa_4 in relation with a significantly high limonene percentage and a significantly low Anethole percentage. Finally, the third cluster distinguished the Pa_3 essential oil which showed the lowest limonene and Estragole contents and the highest Anethole content (Fig.6). In brief, for three out of five accessions (Pa_3, Pa_4 and Pa_5) there was no relationship between morphometric data and volatile composition in contrast to Pa_1 and Pa_2 which were in both cases grouped together at a distance of 1.

Principal Components Analysis (PCA)

The analysis of chemical data by the technique of PCA permitted to group chemically the samples in three groups, in a way to express and evidence their similarities and differences. The significant factor loadings from the principal component analysis (PCA) of chemical constituent variables were obtained as suggested by D'Agostino et al. [77], after Varimax rotation. To explore the relationship between the samples from various areas and their relation to specific volatile compounds, the GC-MS data was subjected to PCA (Fig. 7). PCA results revealed that the first two principal components explained 71.80 % of total variance. The loadings of first and second principal components (PC1 and PC2) accounted for 44.08 and 27.72 % of the variance, respectively. The first component (PC1) is highly positively correlated with *α*-Pinene, *β*-Phellandrene, *β*-Myrcene, Farnesol, *β*-Cariophyllene, Limonene 1,2 epoxide, Nerolidol. The second component (PC2) is characterized by the positive

8. Fernández Prieto JA, Sanna M, Bueno Sánchez Á, Molero-Mesa J, Llorens García L Cires E.

Carveol, trans Carveol, Carvone D and Spatulenol. In the Figure 7, the biplot graph PC1 x PC2 of the loadings and scores is presented, relating the data of chemical constituents of the essential oils of the five collection areas with their essential oils, in order to correlate them, grouping them in 3 groups. Thus, Anethole, α-Bisabolene, Nerolidol, α-Bisabolol, β-Myrcene, β-Santalol and Farnesol were the principal components that contributed to the clustering of the samples obtained of Pa_4 and Pa_5 accessions. PCA statistical analyses indicate the existence of three groups. The first group of clustered accessions is formed by Pa_4 and Pa_5, the second by only Pa_3 and the third by Pa_1 and Pa_2.

REFERENCES

1. Pimenov MG, LeonovMV, 1993. In: The Genera of the Umbelliferae. Royal Botanic Gardens, Kew.
2. Akalın Emine, Yeter Yeşil, Aşkın Akpulat (2016) Fruit anatomy of the Turkish *Pimpinella* species, Flora, 223: 62-73.
3. Wolff H. 1927. Umbelliferae-Apioideae-Ammineae-Carinae, AmmineaeNovemjugatae et Genuinae. In: Engler A (ed.) Das Pflanzenreich, Heft 90 (IV.228). W. Engelmann, Berlin.
4. Matthews VA, 1972. Pimpinella L. In: Davis, P.H. (Ed.), Flora of Turkey and the EastAegean Islands, vol. 4. University Press, Edinburgh, pp. 352–364.
5. Engstrand L (1987). *Pimpinella* L. In: Flora des Iranischen Hochlandes und der Umrahmenden Gebirge, no: 162 (Rechinger KH, eds.). Graz: Akademische Druck-u, Verlagsanstalt. 311-333.
6. Pu FT, Watson M, 2005. *Pimpinella* Linnaeus. In: Wu, Z.-Y., Raven, P.H. (Eds.), Flora of China 14. Science Press & St. Louis, Beijing, Missouri Botanical Garden,pp. 93–104.
7. Magee AR, Van Wyk BE, Tilney PM, Downie SR. A taxonomic revision of *Capnophyllum* (Apiaceae: apioideae). *S. Afr. J. Bot.* 2009, 75, 283–291.

correlation with cis-p-Mentha-2,8 dienol, *trans-p*-Mentha-2,8 dienol, Limonene-oxide, Geraniol, cis

Polyphyletic origin in Pimpinella (Apiaceae): evidence in Western Europe. *J. Plant Res.* 2018, 31: 747-758.

9. Özcan T. Analysis of the fruit surfaces in *Bupleurum* L. (Umbelliferae) with SEM. *Plant Syst Evol.* 2004, 247: 61–67.
10. Akalın UrusE, Kızılarslan, C. Fruit anatomy of some *Ferulago* (Apiaceae)species in Turkey. *Turk J Botany*. 2013, 37: 434–445.
11. Lyskov D, Degtjareva G, Samigullin T, Pimenov M. 2015. Systematic placement of the Turkish endemic genus *Ekimia (Apiaceae)* based on morphological and molecular data. *Turk J Botany* 2015*;* 39: 673– 680.
12. Bani B, Ulusoy F, Karakaya M.A, Koch M.A. 2016a. Taxonomic implications from morphological and anatomical studies in the section *Stenodipterafrom* the genus *Grammosciadium (Apiaceae). PhytoKeys* 2016a; 68: 73–89.
13. Bani B, Karakaya MA, Çeter T. Fruit micromorphological characters of the genus *Grammosciadium* DC. *(Apiaceae)* in Turkey. *Phytotaxa* 2016b, 246: 184–91.
14. Liu M, Downie SR. The Phylogenetic Significance of Fruit Anatomical and Micromorphological Structures in Chinese *Heracleum* Species and Related Taxa *(Apiaceae). Syst Botany* 2017, 42 (2): 313–325.
15. Khajepiri M, Ghahremaninejad F, Mozaffarian V., Fruit anatomy of thegenus *Pimpinella* L. (Apiaceae) in Iran. Flora 2010, 205: 344–356.
16. Aksenov ES, Tikhomirov VN. Klyuch dlya opredeleniya po plodam vidovroda *Pimpinella* L. flory SSSR. Glavn. Bot. Sada (Moscow) 1972, 85, 35–45, Illustrations; key Geog: 1–2 Systematics: ANGIOSPERMAE (Umbelliferae:Pimpinella).
17. Tabanca N, Douglas AW, Bedir E, Dayan FE, Kirimer N, Baser KHC, Aytaç Z, Khan IA Scheffler BE. 2005. Patterns of essential oil relationships in *Pimpinella (Umbelliferae)* based

28. Rosati L, Salerno G, Del Vico E, Lapenna MR, Villani MC, Filesi L, Fascetti S, Lattanzi E. 2005, 3 (2): 149–169.

18. Fereidounfar S, Ghahremaninejad F. Khajehpiri M. 2016. Phylogeny of the Southwest Asian *Pimpinella* and related genera based on nuclear and plastid sequences. *Genet Molecul Res.* 2016, 15 (4): 1–17.

19. Bartolucci F, Peruzzi L, Galasso G, et al. An updated checklist of the vascular flora native to Italy. *Plant Biosyst.* 2018, 152(2): 179–303.

20. Galasso G, Conti F, Peruzzi L, Ardenghi NMG, Banfi E, Celesti-Grapow et al. An updated checklist of the vascular flora alien to Italy. *Plant Biosyst.* 2018, 152(3): 556–592.

21. Stinca A, Ricciardi M. 2018a - *Pimpinella* L.: 549-553. In: Pignatti S., Guarino R., La Rosa M. (Eds.), Flora d'Italia 3. Seconda edizione. Edagricole, Bologna.

22. Stinca A, Ricciardi M. 2019b - *Pimpinella* L.: 799-800. In: Pignatti S., Guarino R., La Rosa M. (Eds.), Flora d'Italia 4. Seconda edizione. Edagricole, Bologna.

23. Gavioli O. Synopsis florae lucanae. N. Giorn. *Bot. Ital.* 1948, 54: 1–278.

24. Laghetti G, Hammer K, Olita G, Perrino P. 1993. Collecting crops in Basilicata/Italy. *Plant Genet. Res. Newslett.* 1993, 96: 35.

25. Hammer K, Laghetti G, Cifarelli S, Spahillari M, Perrino P. *Pimpinella anisoides* Briganti. *Genet Resour Crop Evol.* 2000, 47(2): 223–225.

26. Laghetti G, Ghiglione G, De Lisi A, Maruca G, Losavio F, Pignone D, Hammer K. Survey for The Conservation of Agrobiodiversity in Three Italian Linguistic (Occitan, Croatian and Franco-Provençal) Islands. *J BiolLife Sci.* 2013, 4(1).

27. Maruca G, Spampinato G, Turiano D, Laghetti G, Musarella CM. (2019) Ethnobotanical notes about medicinal and useful plants of the Reventino Massif tradition (Calabria region, Southern Italy). *Genet ResourCrop Evol.*2019, 66**,** 1027–1040.

on phylogenetic relationships using nuclear and chloroplast sequences. *Plant Genet Resour* Un aggiornamento alla flora del Cilento e della Campania *Informatore Botanico Italiano* 2012, 44: 111-119.

29. De Leonardis W, De Santis C, Ferrauto G, Fichera G, Zizza A. Palinologia di alcune specie presenti in Sicilia. *Informatore Botanico Italiano* 2007, 41(1): 47-52.
30. Conti F, Abbate G, Alessandrini A, Blasi C, editors. 2005. An annotated checklist of the Italian vascular flora. Rome: Palombi Editori.
31. Pignatti, S. (1982) Flora d'Italia. Vol. 2. Edagricole, Bologna.
32. Orsenigo S, Montagnani C, Fenu G, Gargano D, Peruzzi L, Abeli T. Red Listing plants under full national responsibility: Extinction risk and threats in the vascular flora endemic to Italy. *Biol Conserv*. 2018, 224: 213-222.
33. Menichini F, Tundis R, Loizzo MR, Bonesi M, Marrelli M, Statti GA, Menichini F, Conforti F. Acetylcholinesterase and butyrylcholinesterase inhibition of ethanolic extract and monoterpenes from *Pimpinella anisoides* V Brig. (Apiaceae). *Fitoterapia* 2009, 297–300.
34. Conforti F, Tundis R, Marrelli M, Menichini, F, Statti GA, De Cindio B, Menichini F, Houghton PJ. Protective effect of *Pimpinella anisoides* ethanolic extract and its constituents on oxidative damage and its inhibition of nitric oxide in lipopolysaccharide-stimulated RAW 264.7 macrophages. *J Med Food*. 2010, 13: 137–141.
35. Yeşil Y, Akalın E, Akpulat A, Vural C. (2018) Fruit morphology of the genus *Pimpinella* (Apiaceae) in Turkey. Anales del Jardín Botánico de Madrid 2018, 75 (2): e072.
36. Cano E, Musarella CM, Cano-Ortiz A, Piñar Fuentes JC, Spampinato G, Pinto Gomes CJ. Morphometric analysis and bioclimatic distribution of *Glebionis coronaria* s.l. (Asteraceae) in the Mediterranean area. *PhytoKeys* 2017, 81: 103-126.
37. Yu X, Shi P, Hui C, Miao L, Liu C, Zhang Q, Feng C. Effects of Salt Stress on the Leaf Shape and Scaling of *Pyrus betulifolia* Bunge. *Symmetry* 2019a, *11*: 991.

47. Rafieian-Kopaei M, Hasanpour-Dehkordi A, Lorigooini Z, Deris F, Solati K, Mahdiyeh F. of 12 Rosaceae Species. *Symmetry* 2019b, *11*: 1255.

39. Spjut RW. 1994. A systematic treatment of fruit types. Memoirs of the New York Botanical Garden 70: 1–182.

40. Perrino EV, Wagensommer RP. Crop Wild Relatives (CWR) Priority in Italy: Distribution, Ecology, In Situ and Ex Situ Conservation and Expected Actions. *Sustainability* 2021, *13*, 1682.

41. Benelli G, Pavela R, Iannarelli R, Petrelli R, Cappellacci L, Cianfaglione K, Heshmati Afshar F, Nicoletti M, Canale A, Maggi F. Synergized mixtures of Apiaceae essential oils and related plant-borne compounds: Larvicidal effectiveness on the filariasis vector *Culex quinquefasciatus* Say, *Ind. Crops Prod.* 2017, 96: 186-195.

42. Boulos L. In medicinal plants of North Africa. 218 St. Clair River Drive, Box 344, Algonac, Michigan 48001: Reference publications, Inc. 1993, p. 286

43. Burt S. Essential oils: their antibacterial properties and potential applications in foods - a review. *Int. JoFood Microbiol.*, 2004, 94, 3, 223-253.

44. Duke JA, Beckstrom-Sternberg SM. Hand book of medicinal mints (*aromathematics*) phytochemicals and biological activites. Washington. CRC Press. 2001; 402.

45. Reichling J, Schnitzler P, Suschke U, Saller R. Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties—an overview. *Forsch Kompl.* 2009, 2 (2), 79–90.

46. de Groot A, Schmidt E. 2016. Essential oils, Part V: peppermint oil, lavender oil, and lemongrass oil. *Dermatitis* 2016, 27: 325–332.

38. Yu X, Hui C, Sandhu HS, Lin Z, Shi P. Scaling Relationships between Leaf Shape and Area Comparing the effect of intranasal lidocaine 4% with peppermint essential oil drop 1.5% on migraine attacks: a double-blind clinical trial. *Int. J. Prev. Med*. 2019, 10: 121.

48. Mahdavi V, Hosseini SE, Sharifan A. Effect of edible chitosan film enriched with anise (*Pimpinella anisum* L.) essential oil on shelf life and quality of the chicken burger. *Food Sci Nutr*. 2018, 6: 269–279.

49. Tavallali V, Ramati S, Bahmanzadegan A. Antioxidant activity, polyphenolic contents and essential oil composition of *Pimpinella anisum* L. as affected by zinc fertilizer. *J Sci Food Agric*. 2017; 97: 4883–4889.

50. Samojlik I, Mijatović V, Petković S, Škrbić B, Božin B. The influence of essential oil of aniseed (*Pimpinella anisum*, L.) on drug effects on the central nervous system. *Fitoterapia* 2012, 83: 1466–1473.

51. Kiralan M, Bayrak A, Abdulaziz OF, Özbucak T. Essential oil composition and antiradical activity of the oil of Iraq plants, *Nat Prod Res.* 2012, 26(2): 132-139.

52. Al-Jabri NN, Hossain MA. Chemical composition and antimicrobial potency of locally grown lemon essential oil against selected bacterial strains. *Journal of King Saud University – Science* 2018, 30: 14–20.

53. Ben Hsouna A, Ben Halima N, Smaoui S, Hamdi N. Citrus lemon essential oil: chemical composition, antioxidant and antimicrobial activities with its preservative effect against *Listeria monocytogenes* inoculated in minced beef meat. *Lipids in Health and Disease* 2017, 16: 46.

54. Poiana M, Sicari V, Mincione B. A Comparison between the Chemical Composition of the Oil, Solvent Extract and Supercritical Carbon Dioxide Extract of *Citrus medica* cv. Diamante. *J Essential Oil Res. 1998,* 10 (2):, 145-152.

64. Ibrahim AM, Mohamed AS, Saleh A, Mansour AS, Mohammed AD, Mohamed AY et al. bergamot fruit of Reggio Calabria (South Italy). *Emir J Food Agric* 2020, 32(7): 522-532.

56. Gioffrè G, Ursino D, Labate MLC, Giuffrè AM. The peel essential oil composition of bergamot fruit (*Citrus bergamia*, Risso) of Reggio Calabria (Italy): a review. *Emir J Food Agric.* 2020, 32(11): 835-845.

57. Marrelli M, Amodeo V, Viscardi F, De Luca M, Statti G, Conforti F. Essential Oils of *Foeniculum vulgare* subsp. *piperitum* and Their *in Vitro* Anti-Arthritic Potential. *Chem. Biodiversity* 2020, *17*, e2000388.

58. Foti V, Araniti F, Manti F, Alicandri E, Giuffrè AM, Bonsignore CP, Castiglione E, Sorgonà A, Covino S, Paolacci AR, Ciaffi M, Badiani M. Profiling volatile terpenoids from Calabrian Pine Stands Infested by the Pine Processionary Moth. *Plants* 2020, 9, 1362.

59. Marinov V, Valcheva-Kuzmanova S. Review on the pharmacological activities of anethole. *Scripta Scientifica Pharmaceutica,* 2015, 2(2): 14-19.

60. Astani A, Reichling J, Schnitzler P. Screening for antiviral activities of isolated compounds from essential oils. *Evid Based Complement Alternat Med.* 2011, 253643: 1-8.

61. Maruzzella J, Freundlich M. Antimicrobial substances from seeds. *J Am Pharm Assoc.*1959, 48: 356-358.

62. Cambar PJ, Aviado DM. Bronchopulmonary effects of paraquat and expectorants. *Arch Env Health.* 1970, 20: 488-494.

63. Coelho-de-Souza AN, Lahlou S, Barreto JEF, Yum MEM, Oliveira AC, Oliveira HD, Celedonio NR, Feitosa RGF, Duarte GP, Santos CF, de Albuquerque AAC, Leal-Cardoso JH. Essential oil of *Croton zehntneri* and its major constituent anethole display gastroprotective effect by increasing the surface mucous layer. *Fundam Clin Pharmacol.* 2013, 27: 288-298.

55. Giuffré AM, Nobile R. *Citrus bergamia*, Risso: the peel, the juice and the seed oil of the Fennel "*Foeniculum vulgare*" treatment protects the gastric mucosa of rats against chemically-induced histological lesions. *Int J Pharmacol.* 2013, 9:1 82-189.

65. Mileni Versuti Ritter A, Queiroz Ames F, Otani F, Weffort de Oliveira R.M, Nakamura Cuman R.K, Bersani-Amado C.A. Effects of Anethole in Nociception Experimental Models. *Evid. Based Complement Alternat Med.* 2014, ID 345829, 7.

66. Drukarch B, Flier J, Jongenelen CAM, Andringa G, Schoffelmer ANM. The antioxidant anethole dithiolethione inhibits monoamine oxidase-B but not monoamine oxidase A activity in extracts of cultured astrocytes. *J Neural Transm.* 2006, 113: 593–598.

67. Chainy GB, Manna SK, Chaturvedi MM, Aggarwal BB. Anethole blocks both early and late cellular responses transduced by tumor necrosis factor: effect on NF-kappaB, AP-1, JNK, MAPKK and apoptosis. *Oncogene* 2000, 19: 2943–2950.

68. Baser KHC, Özek T, Tabanca N, Duman H. Essential Oil of *Pimpinella anisetum* Boiss. et al. *J Ess Oil Res.* 1999, 11: 4, 445-446.

69. Ben Abdesslem S, Boulares M, Elbaz M, et al. Chemical composition and biological activities of fennel (*Foeniculum vulgare* Mill.) essential oils and ethanolic extracts of conventional and organic seeds. *J Food Process Preserv*. 2021, 45:e15034.

70. Benoni H, Dallakian P, Taraz K. Studies on the essential oil from guarana. *Z LebensmUnters Forsch*. 1996, 203: 95-98.

71. Ponte EL, Sousa PL, Rocha MVAP, Soares PMG, Coelho-de-Souza AN, Leal-Cardoso JH, Assreuy AMS. Comparative study of the anti-edematogenic effects of anethole and estragole. *Pharmacol Rep.* 2012, 64: 984-990.

72. Bluma RV, Etcheverry MG. Application of essential oils in maize grain: Impacton *Aspergillus* section *Flavi* growth parameters and aflatoxin accumulation. *Food Microbiol.* 2008, 25: 324–334.

hydrodistillation of the essential oil from Algerian *Pimpinella anisum* seeds. *Flavour Fragr J*. 2021, 36: 34–46.

74. Boughendjioua H., Boughendjioua Z. Chemical Composition and Biological Activity of Essential Oil of Mandarin (*Citrus reticulata)* Cultivated in Algeria. *Int J Pharm Sci Rev Res.* 2017, 44(1): 179-184

75. Ghasemi Pirbalouti A, Gholipour Z. Chemical composition, antimicrobial and antioxidant activities of essential oil from *Echinophora cinerea* harvested at two phenological stages. *Jo Ess Oil Res*. 2016, 28 (6) 501–511.

76. Sharopov FS, Salimov A, Numonov S, Bakri M, Sangov Z, Habasi M, Aisa H.A. Setter WN. Phytochemical Study on the Essential Oils of Tarragon (*Artemisia dracunculus* L.) Growing in Tajikistan and Its Comparison With the Essential Oil of the Species in the Rest of the World. *Nat Prod Communicat.* 2020, 15(12): 1–7.

77. D'Agostino MF, Sanz J, Martínez-Castro I, Giuffrè AM, Sicari V, Soria AC. Statistical analysis for improving data precision in the SPME GC–MS analysis of blackberry (*Rubusulmifolius* Schott) volatiles. *Talanta* 2014, 125: 248-256.

Tab. 1. Sources of *Pimpinella anisoides* V. Brig. fruits and their collection data in the province of Catanzaro (Calabria, S-Italy).

Accession Id	Location	Municipality	Month and year of collection	Collector Farm	Altitude (m a.s.l.m.)
Pa_1	Monticelli	Cicala	August 2018	Azienda Muraca	900 m
Pa_2	Cutura	Sorbo San Basile	August 2018	Azienda Muraca	780 m
Pa_3	Pietra di Fota	San Bernardo di Decollatura	July 2018	Azienda Talarico	840 m
Pa_4	Cutura	Cicala	July2017	Azienda Talarico	700 m

Pa_5	Marignano	Decollatura	August 2017	Azienda Talarico	900 m

73. Boumahdi Y, Moghrani H, Nasrallah N, Ouarek S, Maachi R. Microwave-assisted

Table 2. Main characteristics of *Pimpinella anisoides* V.Brig. monocarps in the 5 locations reported with their Id accession. *** significance at $p < 0.001$; ** significance at $p < 0.01$;

	Pa_1	Pa_2	Pa_3	Pa_4	Pa_5	
	Mean±DS	Mean±DS	Mean±DS	Mean±DS	Mean±DS	Sig.
Monocarp width	1.58±0.12ab	1.64±0.17a	1.51±0.11b	0.84±0.14d	1.10±0.15c	***
Monocarp length	3.10±0.35a	3.10±0.34a	2.90±0.32a	2.25±0.30b	2.51±0.21b	***
Monocarp length from base to maximum width	1.23±0.14a	1.23±0.16a	1.22±0.16a	0.88±0.13c	1.05±0.12b	***
Stylopodium width	0.46±0.07ab	0.48±0.07a	0.46±0.06ab	0.41±0.05b	0.43±0.05b	**
Stylopodium length	0.39±0.09a	0.38±0.15ab	0.30±0.05c	0.31±0.08bc	0.28±0.06c	***
Length/width ratio	1.96±0.20c	1.90±0.22c	1.93±0.22c	2.72±0.35a	2.32±0.30b	***

Table 3 - Monocarps measurements and features of three commercial *Pimpinella* L. species. The features of *P. anisoides* come from this study, those of *P. anisetum* and *P. anisum* from Yeşil et al. (2018).

Species	Fruit length and width (mm)	Length/width ratio	Indumentum	Shape of fruit
P. anisoides V. Brig.	2.53-3.84 x 1.44-2.07	(1.90) 2.2 (2.72)	glabrous	oblong-ovoid/ oblong-cylindrical
P. anisetum Boiss. & Balansa	1.5–1.57 × 1–1.08	1.5	strigose	ovoid
P. anisum L.	3.9–4 × 1.5–1.53	2.6	strigose	ovoid-subglobose

Table 4. Fruit essential oil composition of the five accessions of *Pimpinella anisoides* collected in the Region of Calabria (South Italy). Values are expressed as percentage content ± Standard Deviation. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; n.s. = $p > 0.05$.

	Pa_1	Pa_2	Pa_3	Pa_4	Pa_5	
COMPOUND	Mean±DS	Mean±DS	Mean±DS	Mean±DS	Mean±DS	**Sig.**
α-Pinene	0.03±0.01ab	0.02±0.01b	0.03±0.01ab	0.05±0.01a	0.03±0.01ab	*
β-Phellandrene	1.55±0.03c	1.59±0.03c	1.77±0.07b	1.83±0.03b	1.99±0.01a	***
β-Myrcene	0.23±0.02c	0.22±0.02c	0.25±0.03c	0.34±0.02b	0.44±0.04a	***
Limonene	49.10±0.11b	49.11±0.07b	50.74±0.10a	48.00±0.05c	46.78±0.08d	***
p-Cresol	0.05±0.01ab	0.04±0.01ab	0.05±0.02ab	0.03±0.01b	0.06±0.01a	*
trans-p-Mentha-2,8 dienol	0.04±0.01c	0.05±0.01c	0.10±0.02b	0.14±0.01a	0.09±0.02b	***
cis-Limonene-oxide	0.07±0.01a	0.07±0.02a	0.07±0.02a	0.05±0.01a	0.07±0.01a	n.s.

cis-p-Mentha-2,8 dienol	0.03±0.01b	0.04±0.02b	0.09±0.03a	0.05±0.01ab	0.06±0.01ab	*
trans-Limonene-oxide	0.03±0.01b	0.04±0.01b	0.09±0.02a	0.05±0.01b	0.05±0.01b	**
Cyclohexene	0.10±0.02a	0.10±0.02a	0.09±0.03a	0.09±0.01a	0.08±0.01a	n.s.
Geraniol	0.01±0.01b	0.02±0.01b	0.05±0.01a	0.02±0.01b	0.02±0.01b	***
Estragole	6.49±0.05b	6.67±0.04a	6.40±0.06b	6.19±0.03c	5.76±0.04d	***
cis-Carveol	0.04±0.01b	0.06±0.01b	0.13±0.03a	0.08±0.01b	0.07±0.01b	***
trans-Carveol	0.04±0.01a	0.03±0.01a	0.05±0.02a	0.05±0.01a	0.04±0.01a	n.s.
Carvone D	0.03±0.01c	0.05±0.02bc	0.13±0.03a	0.07±0.00bc	0.09±0.01ab	***
p-Anisaldehyde	0.39±0.02cd	0.45±0.02bc	0.49±0.05b	0.37±0.02d	0.66±0.02a	***
Anethole	40.98±0.05c	40.57±0.09d	38.50±0.07e	41.36±0.03b	42.77±0.09a	***
4-Terpinyl acetate	0.02±0.01ab	0.02±0.01ab	0.03±0.01a	0.01±0.01b	0.02±0.01ab	n.s.
p-Acetonyl anisole	0.03±0.01a	0.06±0.02a	0.04±0.01a	0.04±0.01a	0.03±0.01a	n.s.
Methyl eugenol	0.04±0.01a	0.04±0.01a	0.05±0.01a	0.04±0.01a	0.03±0.01a	n.s.
β-Caryophyllene	0.38±0.03bc	0.38±0.03c	0.47±0.05b	0.64±0.03a	0.45±0.02bc	***
Humulene	0.03±0.01ab	0.03±0.01a	0.03±0.01a	0.03±0.01ab	0.01±0.01b	**
Farnesol	0.02±0.01a	0.02±0.01a	0.02±0.01a	0.03±0.01a	0.02±0.01a	n.s.
Limonene 1,2-epoxide	0.01±0.01a	0.01±0.01a	0.02±0.02a	0.03±0.01a	0.02±0.01a	n.s.
β-Copaene	0.06±0.01ab	0.05±0.02b	0.10±0.03a	0.09±0.01a	0.07±0.01ab	**
β-Santalol	0.02±0.01ab	0.02±0.01b	0.01±0.01b	0.04±0.01a	0.03±0.01a	**
Isohomogenol	0.04±0.01a	0.03±0.01ab	0.03±0.01ab	0.02±0.01b	0.03±0.01ab	*
Nerolidol	0.01±0.01a	0.01±0.01a	0.01±0.01a	0.03±0.01a	0.02±0.01a	n.s.
α-Bisabolene	0.02±0.01a	0.01±0.01a	0.01±0.01a	0.02±0.01a	0.01±0.01a	n.s.
Cariophyllene oxide	0.08±0.02a	0.10±0.02a	0.08±0.02a	0.10±0.01a	0.07±0.01a	n.s.
Spatulenol	0.04±0.01a	0.03±0.01a	0.05±0.01a	0.03±0.01a	0.03±0.01a	n.s.
α-Bisabolol	0.05±0.01bc	0.05±0.01bc	0.04±0.01c	0.07±0.01ab	0.09±0.01a	***

Fig. 1. Map of *Pimpinella anisoides* V. Brig. fruits collection locations in the province of Catanzaro (CZ), Calabria region (S-Italy): 1. Monticelli, 2. Cutura, 3. Pietra di Fota, 4. Cutura, 5. Marignano.

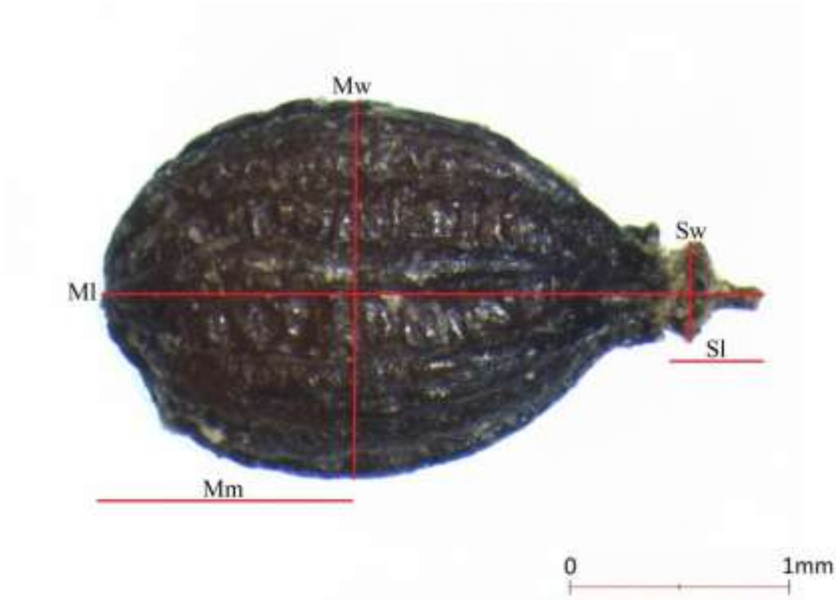

Figure 2 - Monocarp of *Pimpinella anisoides* V. Brig. photographed and measured under a stereomicroscope using the DeltaPix inSight© software. Mw: Monocarp width; Ml: Monocarp length; Mm: Monocarp length from base to maximum width; Sw: Stylopodium width; Sl: Stylopodium length.

Figure 3 - Monocarp of *Pimpinella anisoides* V.Brig. obvserved in dorsal (a) and ventral (b) view, with a stereomicroscope using the DeltaPix inSight© software.

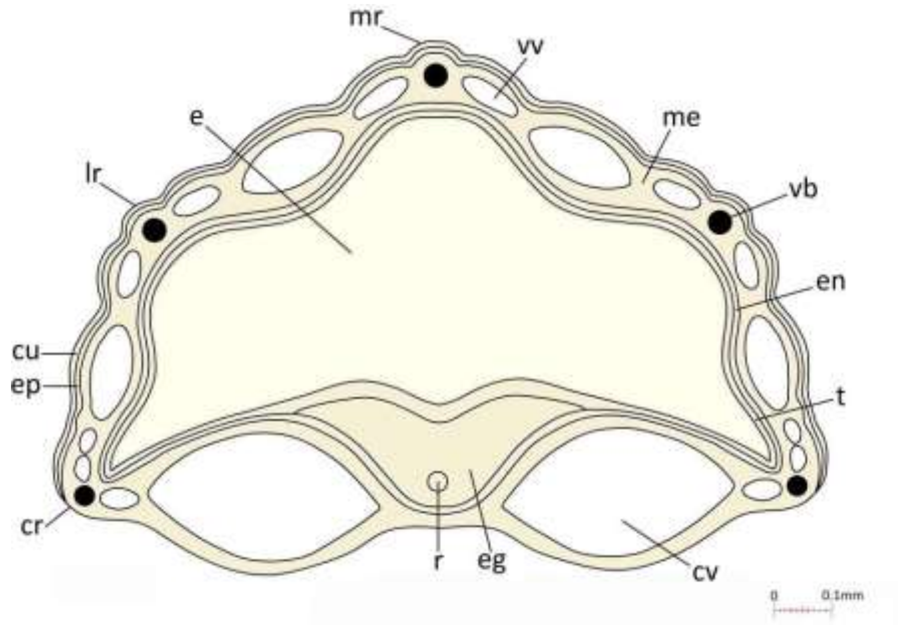

Fig. 4- Monocarp transverse section of *Pimpinella anisoides* V.Brig. cr: commissural rib; cu: cuticula; cv: commissural vittae; eg: endosperm groove; en: endocarp; e: endosperm; ep: epiderm; lr: lateral rib; me: mesocarp; mr: median rib; r: raphe; vb: vascular bundle; vv: vallecular vittae; t: testa.

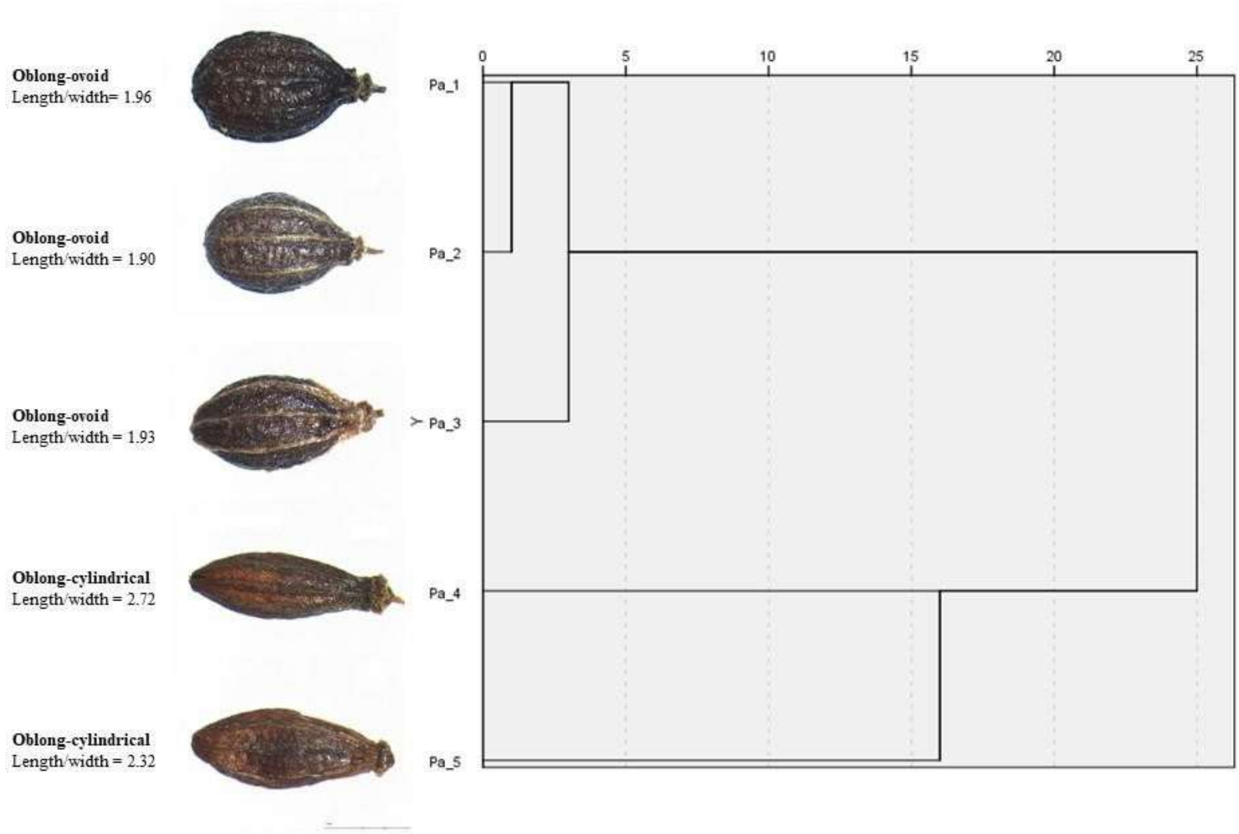

Fig.5. Cluster analysis and corresponding monocarps shape of the different accessions of *Pimpinella anisoides* V.Brig.

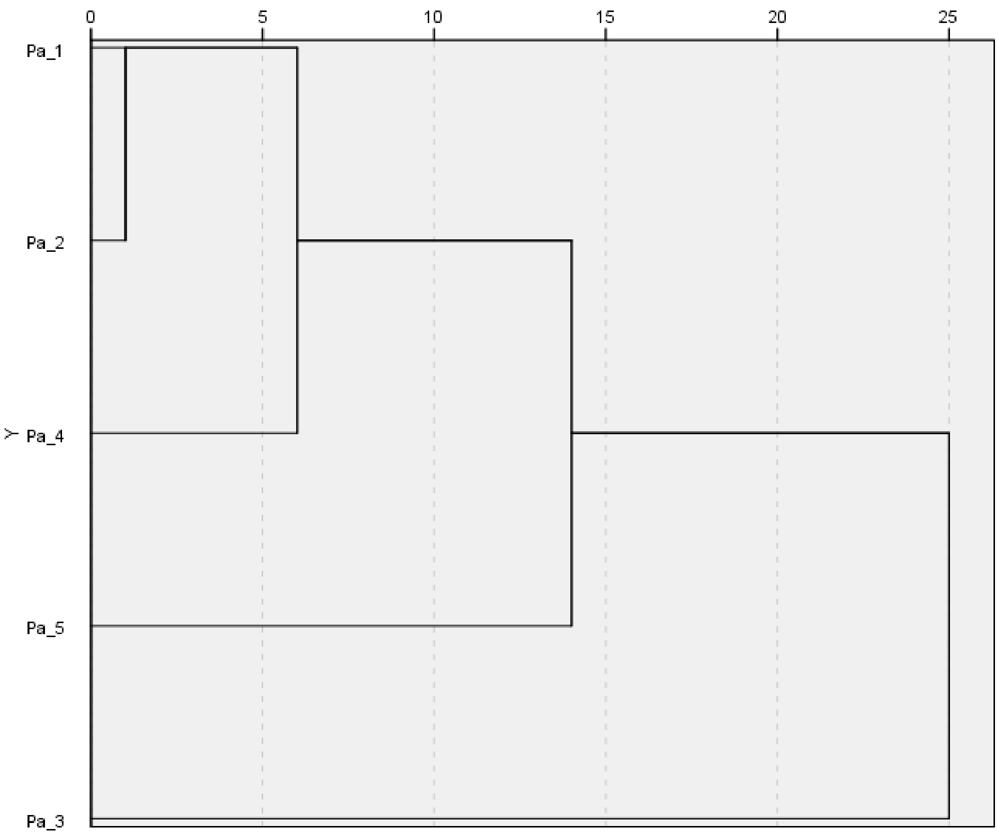

Fig.6. Cluster analysis and corresponding essential oil of the different accessions of *Pimpinella anisoides* V.Brig.

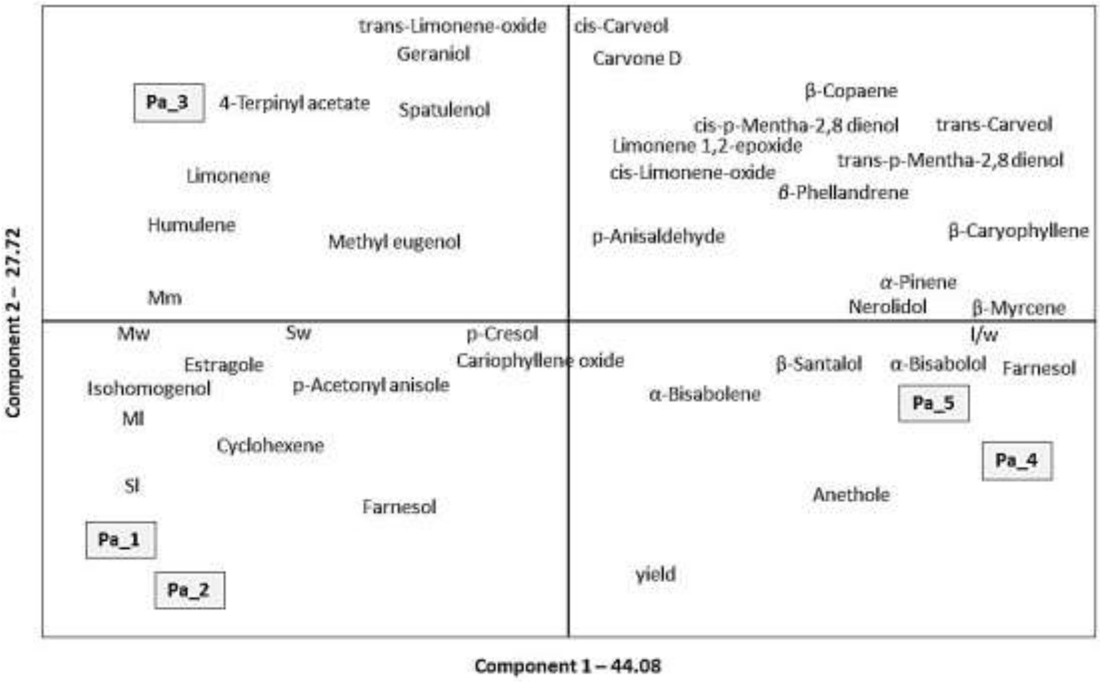

Figure 7 - Biplot graph, PC1 versus PC2, using loadings and scores for different essential oils of *Pimpinella anisoides* V. Brig. growing in different locations. Mm: Mericarp length from base to maximum width; Mw: Mericarp width; Ml: Mericarp legth; Sw: Stylopodium width; Sl: Stylopodium length; l/w: Length/width ratio.