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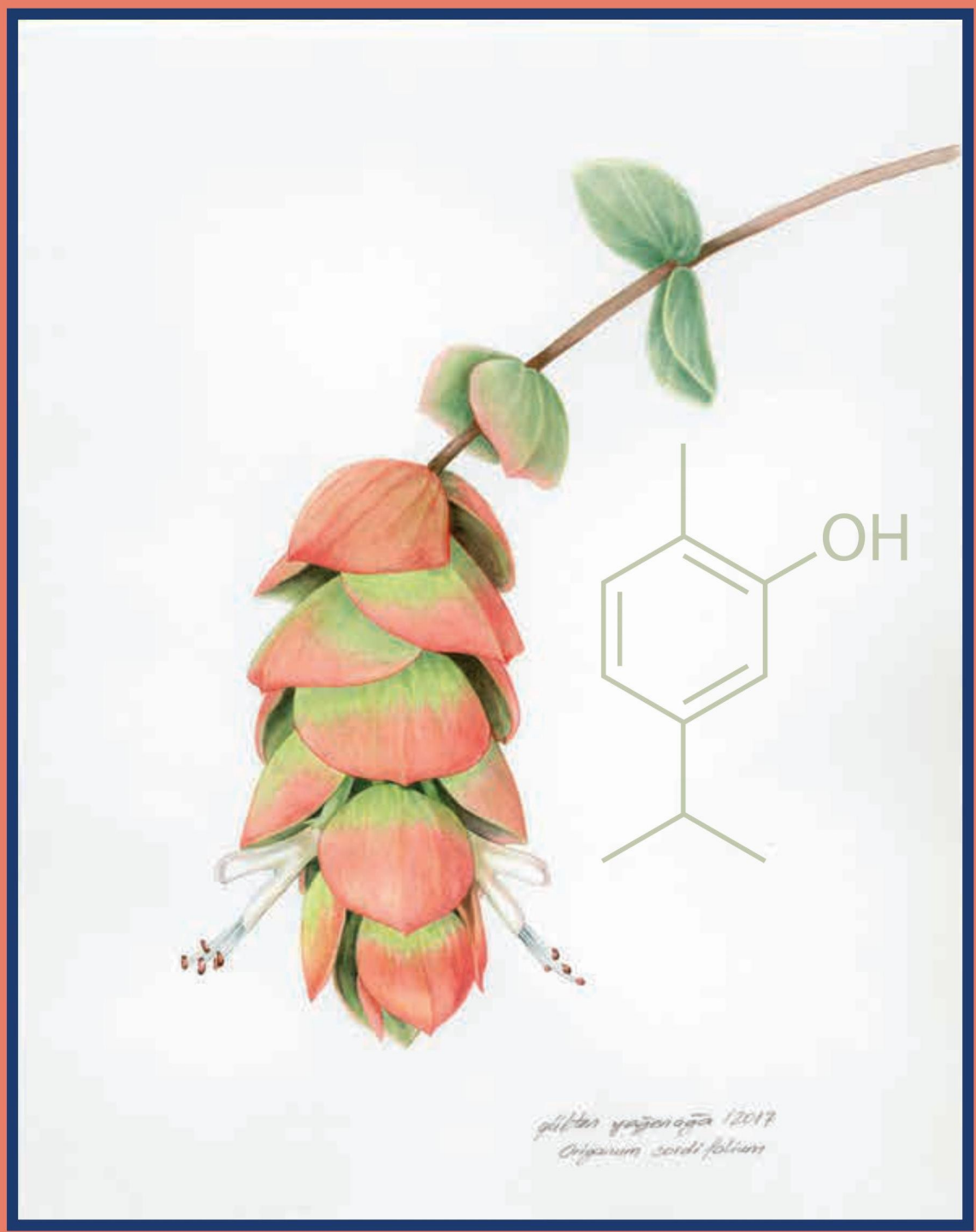

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Development and validation of new RP-HPLC method for estimation of pramipexole dihydrochloride in bulk and pharmaceutical formulation

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Abstract

A novel high-performance liquid chromatographic assay method was developed and validated for the quantitative determination of the anti-Parkinson agent pramipexole dihydrochloride monohydrate in bulk and its tablet dosage form. In this perspective, the chromatographic separation was accomplished on Eclipse XDB-12 C18 (150 mm x 4.6 mm, 5 µm particle size) column using UV detection at 263 nm. The mobile phase consisted of distilled water: acetonitrile (10: 90 v/v), run at a flow rate of 1.0 mL/min with isocratic elution. The method was validated in accordance with ICH guidelines by evaluating the system suitability, linearity, limits of detection (LOD) and quantitation (LOQ), precision, accuracy, specificity, selectivity and short-term stability. Our findings revealed that retention time for pramipexole dihydrochloride was found to be 5.2 minutes. The linearity range was established between 6.25-225.0 µg/mL with a mean recovery of 101.26 % ± 0.56. The limits of detection and quantification were determined to be 4.18 µg/mL and 12.66 µg/mL, respectively, indicating that the method is very sensitive. Intra and inter-day precision were within acceptable limits (RSD<2, n=6) and the typical excipients included in the pharmaceutical product did not interfere with the selectivity of the method. The proposed method was found to be simple, specific, accurate, precise and could be applied to the quantitative analysis of pramipexole dihydrochloride monohydrate in a bulk and in a its tablet dosage form.

Keywords

HPLC method development, pramipexole dihydrochloride, recovery, ICH.

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INTRODUCTION

Pramipexole (PRA) is a non-ergot dopamine agonist with high relative in vitro selectivity and full intrinsic activity at the D2 subfamily of dopamine (Dooley and Markham, 1998). The molecular weight of PRA is 302.3 g/mol, and its chemical name is (S)-2-amino-4,5,6,7-tetrahydro-6-(propyl amino) benzothiazole dihydrochloride monohydrate (Rambhade *et al.*, 2010*)* . The melting point of PRA is 296-305°C, while its solubility in water is 61 mg/mL, in DMSO is 41 mg/mL, and in ethanol is 1 mg/mL.

Its elimination half-life is around 8-12 hours (Benbir and Guilleminault, 2006). PRA is a drug used to treat the symptoms of Parkinson's disease (PD), a neurological disorder that causes difficulties with movement, muscle control, and balance, including body shaking, stiffness, slower motions, and balance deficits (Goldenberg, 2008).

Recently new therapeutic potential of PRA has been associated with restless legs syndrome (RLS; Willis–Ekbom illness) a sensory motor disorder characterized by strong need to move the leg, which is generally accompanied by unpleasant sensations. RLS symptoms are present during rest, subside with movement, and are usually at their worst in the evening or night. RLS is a prevalent disorder that affects about 5% and 15% of the population, and its frequency has been shown to increase with age (Deleu *et al.*, 2002; Lipford and Silber, 2012). RLS responds well to treatment, particularly to drugs that boost dopamine (DA) neurotransmission. PRA works by replacing dopamine, a natural substance found in the brain that governs movement, confirming that it belongs to the dopamine agonist drug class, despite this, the US Food and Drug Administration has only licensed one agonist, ropinirole, for use in the treatment of RLS (MacKie and Winkelman, 2015; Silber *et al.*, 2004).

The development and validation of methods for quantifying and identifying pharmaceutical active ingredients are key components of drug quality control (QC). Because of its relevance, the development of novel testing procedures for drug determination has gained substantial attention in recent years, particularly in assessing the potency of active ingredients.

Today, the literature reports a wide number of analytical procedures for assessing of PRA, ranging from spectrophotometric approaches (Gurupadayya *et al.*, 2009; Dey *et al.*, 2012; Muthu *et al.*, 2013; Thangabalan *et al.*, 2011), to HPLC methods (Pawar *et al.*, 2013; Sevim and Erk, 2015; Panditrao *et al.*, 2011; Pathare *et al.*, 2006), and GC/MC (Panchal *et al.*, 2011).

For routine QC testing of drugs, utilizing analytical methods that are not difficult, time consuming, and can be done with a lower cost make the analytical method more favorable and useful. The primary goal of this work was to validate and extend a new simple, effective, accurate, adaptable, and repeatable method for obtaining consistent results with similar input data for regular QC testing of PRA and its tablet dosage form. HPLC was utilized because of its precision, sensitivity, repeatability, and accuracy.

MATERIALS AND METHODS

PRA was obtained from Deva (Turkey). F-Melt® (Fuji Chem, Japan), Pearlitol® Flash (Roquette, Lestrem, France), Pharmaburst® 500 (SPI Pharma, New Castle, USA), Prosolv® Easytab SP (JRS Pharma, Rosenberg, Germany), Ludiflash® (BASF, Ludwigshafen, Germany), and Parteck® ODT (Merck, Darmstadt, Germany) ready-to-use ODT (Orally Disintegrating Tablet) excipients were used as received. Acetonitrile (ACN) was HPLC grade and purchased from Merck (Darmstadt, Germany). Double distilled water has been used for all experiments.

Instrumentation and chromatographic conditions

The Agilent 1260 Infinity HPLC system (Wilmington, DE, USA) was used for this study, which was outfitted with a solvent degasser, quaternary pump, auto sampler, column oven, and diode array detector. Agilent Chem Station software was used to process the data. The chromatographic separation in this item was achieved using an Eclipse XDB-12 C18 (150 mm x 4.6 m particle size) column with UV-detection at 263 nm wavelengths (λmax). The mobile phase consisted of distilled water: ACN (10: 90 v/v), run at a flow rate of 1.0 mL/min with 10 µL injection volume and isocratic elution.

Standard solutions and preparation of the samples

A standard stock solution was prepared by dissolving 10 mg of PRA in 10 mL of distilled water: ACN (10:90 v/v) mobile phase mixture. The solution was immersed in an ultrasonic bath (Selecta Ultrasound HD, Spain) for 30 minutes to achieve total dissolution.

Analytical method validation

The method has been validated in terms of linearity, limits of detection-LOD and quantitation-LOQ, precision, accuracy, specificity, and selectivity in accordance with ICH guidelines (The International Conference on Harmonization of Technical Requirements for Registration of

Pharmaceuticals for Human Use) (ICH, 2005). Linear calibration curve of the proposed method was evaluated by fitting least-squares regression analysis obtained by diluting stock solution with (10:90 v/v) mobile phase mixture and concentrations were 0.00 µg/mL, 6.25 µg/mL, 12.5 µg/mL, 25 µg/mL, 50 µg/mL, 75 µg/mL, 125 µg/mL and 225 µg/mL.

The specificity of the method was determined by analyzing chromatograms of excipient(s) interfering with PRA determination. To achieve this, drug-free excipients solution, PRA bulk solution, and mobile phase chromatograms were injected into the chromatographic process.

By comparing theoretical and experimental data of three PRA concentration levels with concentrations of 10 µg/mL, 100 µg/mL, and 200 µg/mL, the accuracy of the analytical technique was determined.

Intermediate precision was tested by two consecutive days and by two different analysts preparing six solutions of the 10 µg/mL same concentration and injected to HPLC system. All results were evaluated in terms of standard deviation (SD) and relative standard deviation (RSD).

The limits of detection and quantification value was determined based on the standard deviation (SD) of the responses and the slope (S). Equations (1) and (2) were used to calculate LOD and LOQ values.

$$LOD = 3.3\ SD/S \quad (1)$$

$$LOQ = 10\ SD/S \quad (2)$$

Assay procedure for analysis in tablet dosage form

Drug contents of the PRA in tablet dosage form was determined by weighing of twenty tablets and then finely powdered them in the mortar. A powder containing 10 mg of PRA was precisely weighed and placed into a 10 mL volumetric flask. Appropriate dilutions were made with the mobile phase. To obtain full dissolving of PRA at yield concentrations of 50 µg/mL, the solution was sonicated for 20 minutes. The resultant solution was then passed through 0.45 µm membrane filters before being injected to HPLC analysis.

Short-term stability of PRA

A solution of 50 µg/mL concentration of PRA was prepared from the stock solution. The prepared solution was kept at 37 °C for 48 hours. Samples were taken at 0, 24, and 48 hours, and HPLC analyzes were performed (n=3).

RESULTS AND DISCUSSION

Preliminary experiments were undertaken to determine suitable and optimal conditions to design an effective and easy RP-HPLC method for the analysis of the drug in bulk and tablet dosage forms. HPLC variables such as detection wavelength, optimum mobile phase & proportions, and flow rate were thoroughly investigated. For the trials, a variety of solvent combinations were utilized, including: Methanol: Distilled water; 10:90 v/v (Thangabalan *et al.*, 2011), Methanol: ACN; 10:90 v/v, and Ammonium Acetate Buffer (pH 4.4): ACN; 35:65 v/v (Sevim and Erk 2015) showing unsatisfactory results. The combination of ACN and distilled water (50:50 v/v, 60:40 v/v, 70:30 v/v, 80:20 v/v, and 90:10 v/v) yielded the best results, notably when ACN: distilled water (90:10 v/v) was utilized, which generated a well-defined peak and retention duration (5.2 minutes) for PRA. Table 1 summarizes the HPLC conditions, retention time, and symmetry factor used for this study.

Table 1: Data for optimized RP-HPLC method.

Parameters	
Mobile phase	Acetonitrile : Distilled water (90:10, v/v)
Flow rate	1.0 mL/min
Injection volume	10 µL
Wavelength	263 nm
Dilution solvent	Mobile phase
Retention time for PRA	5.2 min
Symmetry factor for PRA	0.23

By graphing the Area Under Curve (AUC) of PRA, a calibration curve was produced using the least squares approach. In the concentration range of 6.25-225.00 µg/mL, the calibration curves for PRA developed high linearity with an excellent regression coefficient (R^2=0.99). Figure 1 depicts the linearity findings.

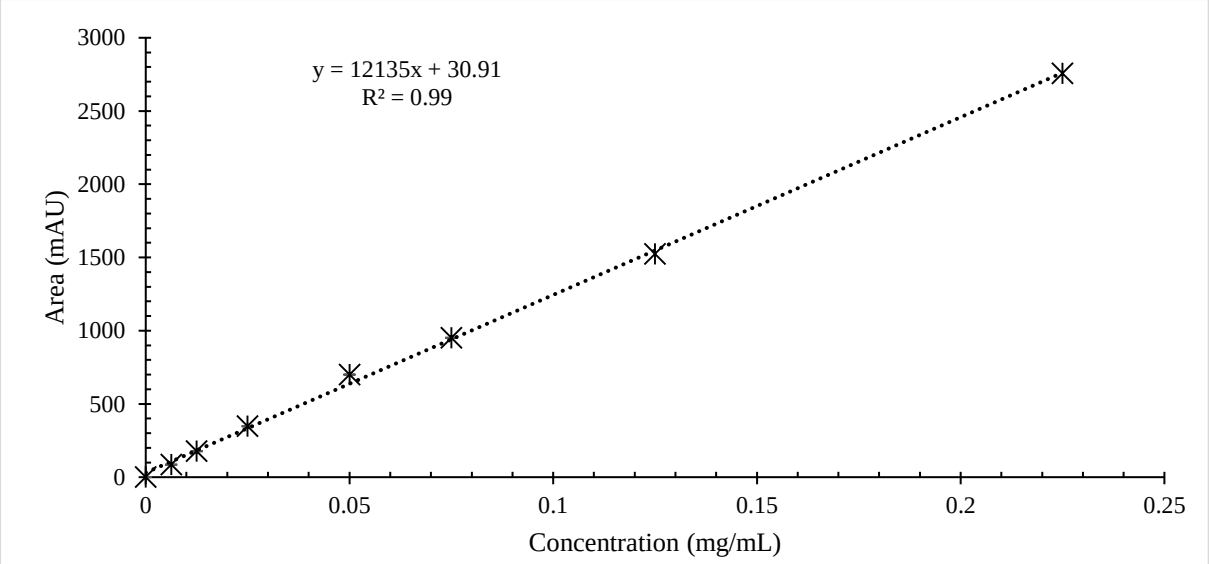

Figure 1: Calibration curve for PRA.

Specificity

Based on the comparation of the chromatograms of placebo (drug-free mixture of excipients), PRA solution and constituents of mobile phase, the methodology for specificity was determined to be unique. Figure 2 illustrates that no interference from excipients was found in the resulting derivative spectra and no other peak was observed other than the standard solution.

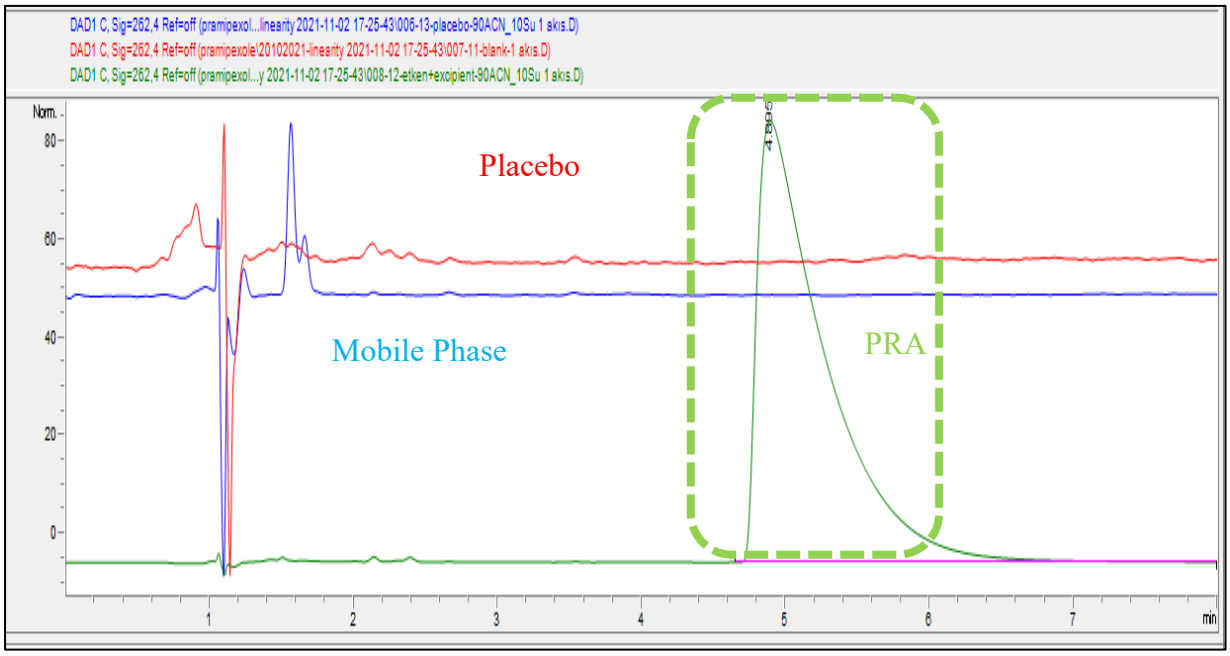

Figure 2: Specificity of the developed HPLC for PRA.

Accuracy and recovery

Using a stock solution containing PRA, three concentration sets (high, medium, and low) were prepared to test the accuracy of the analytical process. Using HPLC and first derivative spectroscopy techniques, the accuracy of the HPLC technique was determined and expressed as percent recovery. According to Table 2, percentage of total recovery values measured for PRA is below 2%, showing the accuracy of the process. The mean recovery and RSD data for the HPLC method were 100.50% and 1.10%, respectively.

Table 2: Recovery results for PRA convert

Drug	n	Theoretical concentration of the PRA (µg/ml)	Practical concentration of the PRA (µg/ml)	Recovery (%)	RSD (%)
	6	10.00	9.00	92.86	0.31
PRA	6	100.00	109.00	109.36	0.57
	6	200.00	203.00	101.55	0.79

Intermediate precision

There was no difference in peak area higher than 2% between the two successive days, showing that the procedure was very reproducible. The results (RSD values less than 2%) for intermediate precision reviewed by two analysts over two consecutive days met the precision criterion (Venkata Rajesh *et al.*, 2013). The intermediate precision results are presented in Table 3.

Table 3: Intermediate precision checked by two analysts and two different days.

Drug		1. Analyst	2. Analyst	1. Day	2. Day
PRA	Theoretical concentration: 100 µg/mL (n=6)	90.00	90.00	100.00	98.00
	RSD (%)	0.91	0.41	0.52	0.28

RSD: Relative standard deviation.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ values were determined using the above-mentioned equation to evaluate the method's sensitivity. Table 4 shows that the approach was found to be sensitive enough to evaluate PRA in low concentrations level.

Table 4: Limits of detection (LOD) and quantitation (LOQ) for PRA

	PRA (µg/mL)
Limits of detection - LOD	4.18
Limits of quantitation - LOQ	12.66

Assay procedure for analysis in tablet dosage form

A significant level of agreement with the labeled quantity was demonstrated. Table 5 presents the PRA analysis findings for Pexola® Tablet 1.0 mg using the established HPLC method.

Table 5: Assay of PRA for its tablet form

Tablet form of PRA (Pexola®)	n	Recovery for PRA (%) ± RSD (%)
	6	94.00 ±2.10

RSD: Relative standard deviation

Short-term stability of PRA

The short-term stability test results revealed no change in retention time or deterioration in the peak characteristics of the observed HPLC peaks. Table 6 reveals that the drug remained stable at 37 °C for 48 hours with an RSD value less than 2%.

Table 6: Short-term stability results for PRA

Time	0. Hour	48. Hour	Average	RSD (%)
PRA (µg/mL)	51.20	50.01	50.60	1.23

CONCLUSION

Validation is widely acknowledged as a vital step in the development of an analytical method. Following the development of the method, it was tested in accordance with the ICH guidelines. Validations of the suggested method demonstrated to be simple, specific, accurate, and precise and as a result, it might be a reliable HPLC approach for regular PRA analysis in bulk and tablet dose form.

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Pollen morphology of some taxa in the family Lamiaceae (Labiatae) from Turkey

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Abstract

The family Lamiaceae is often uttered as the mint family, and the plant family of flowering plants. In Turkey, 609 species of 46 genera in the Lamiaceae family are naturally distributed, and almost half of these are endemics. The aim of this study is to examine the pollen characteristics of some species in the Lamiaceae family. The family is a source of pollen and nectar, which is important for honey bees, and the medicinal and aromatic use of inflorescence reveal the importance of identifying the species.

In this study, pollen of 14 different species belonging to 12 genera in the Lamiaceae family were examined. The equatorial axis of the examined pollens is in the range of 50.6-22.4 μm and the polar axis of the examined pollens is in the range of 55.6-18.3 μm. It is stated that the pollen morphology of the Lamiaceae family can be used as an important character in the differentiation of taxa at the species level. It is also stated to be an important feature in the classification of the Lamiaceae family. As a result, these data obtained by light microscopy are fundamental data for taxonomic, morphological and melisopalynological studies.

Keywords

Endemic, Lamiaceae, LM, pollen, Turkey, wodehouse.

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***Corresponding author:** Mine Kocyigit **email:** minekocyigit@hotmail.com

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INTRODUCTION

Turkey is a country rich in plant species and diversity, as it is located at the intersection of different climatic zones. It is one of the leading countries in the world market in the export of tea plants and spices, and it is the first among the plant species traded in the Lamiaceae (Labiatae) family (Akalin *et al.*, 2020). In addition, our country is an important gene center in terms of Lamiaceae plants, which have an important place in alternative medicine (Kocabas and Karaman, 2001; Akalin *et al.*, 2020). The promising biological and pharmacological activities of these species within the family have been known for years (Bozin *et al.*, 2006; Akman *et al.*, 2007).

The Lamiaceae family is often uttered as the mint family, and the plant family of flowering plants. They consist of shrubs or herbs that produce and release the aromatic smell, which consists of more than 3,000 species in the Lamiaceae family (Secmen *et al.*, 1998). The family is a source of pollen and nectar for honey bees due to its aromatic properties (Ozhatay *et al.* 2012). The largest genera of Lamiaceae plant family are *Salvia* L., *Scutellaria* L., and *Stachys* L. (Michel *et al.*, 2020). In Turkey, 609 species of 46 genera in the Lamiaceae family are naturally distributed, almost half of these are also endemics, and the endemism rate is 44.5% (Guner *et al.*, 2012).

Most of the plants belonging to the Lamiaceae family are used as folk remedies in the treatment of various diseases, as well as in medicine, food industry, perfumery and cosmetics. In addition, the plants of this family are included in many preparations used in rational phytotherapy today (Saleem, 2000).

Some genera such as *Thymus* L., *Satureja* L., *Teucrium* L., *Sideritis* L., *Lamium* L., *Stachys* L. and *Ajuga* L. are known to be used therapeutically (Baytop, 1984; Baytop, 1991). Inflorescence and leaves of some species belonging to the genus *Sideritis* L., *Stachys* L. and *Phlomis* L. are widely used as an appetizer (Sezik and Ezer, 1983; Sezik 1984). Although it is known by many different names in Anatolia, the herba or inflorescence of *Sideritis* and *Salvia* species, which are generally called "Mountain tea, Yayla tea, Sage", have been used as tea and folk remedy for a long time (Duman, 2000; Duman *et al.*, 2005).

Pollen, the male reproductive unit of seed plants, was first described by Grew as spermatic globules. The term pollen was first used by Carl von Linné in his work titled "Philosophia Botanica" which was published in 1751 (Bryant *et al.*, 1990). Pollen shapes differ between taxa. This

difference varies according to the pollination patterns of the taxa, the environment in which they are located, the structure of the sporoderm layers, the aperture type and the ornamentation of the pollen (Karamanoglu *et al.*, 1975). The layer on the outer surface of the pollen is the exine layer. Exine stratification is quite distinct in the pollen of vascular plants, and various researchers have given different names to these layers. Terminologies used today in naming exine layers were developed by Erdtman (Fagrei and Iversen, 1975). Classification of the Lamiaceae family based on pollen morphology data was first made by Erdtman (1966). Erdtman formed two subfamilies in the Lamiaceae family, according to their pollen morphology (colpus numbers). He determined that there were two types of pollen with 3 colpus and 6 colpus, and he divided the family into two subfamilies, Lamioideae and Nepetoideae (Cantino and Senders, 1986).

The aim of this study is to examine the pollen characteristics of some species in the Lamiaceae family. The family is a source of pollen and nectar, which is important for honey bees, and the medicinal and aromatic use of inflorescence reveal the importance of identifying the species. Pollen analysis of the species examined in this study is intended to contribute to the identification of the species.

MATERIALS AND METHODS

The samples in the herbarium of Istanbul University, Faculty of Pharmacy were used in the study (Table 1). 14 taxa in the family Lamiaceae were investigated by light microscope. The pollen slides were prepared according to the Wodehouse (1935) technique. All measurements were determined on at least 20 pollen grains. The investigations and measurements of pollen grains were conducted with Olympus BX53 light microscope at magnifications ranging from ×200 to ×1000 with KAMERAM program.

Table 1: Voucher specimens of examined taxa in the family Lamiaceae with Turkish names and Voucher numbers.

	Scientific names	Turkish Names	Voucher number (ISTE)
1.	***Clinopodium graveolens* (M.Bieb.) Kuntze (Sin. *Satureja graveolens* (M.Bieb.) Caruel)**	Filiskin	100623
2.	***Lamium galeobdolon* (L.) L. (Sin. *Galeobdolon luteum* Huds.)**	Sarıbalıcak	98342
3.	***Lamium purpureum* L.**	Ballıbaba	50370
4.	***Nepeta obtusicrena* Boiss. & Kotschy ex Hedge (endemic)**	Kumpisiği	81761
5.	***Ocimum basilicum* L.**	Fesleğen	54441

6.	***Origanum acutidens* (Hand.-Mazz.) Ietsw. (endemic)**	Zemul	96917
7.	***Phlomis grandiflora* H.S.Thomps.**	Bahargülü	51272
8.	***Prunella vulgaris* L.**	Gelinciklemeotu	109843
9.	***Salvia rosmarinus* Spenn., (Sin. *Rosmarinus officinalis* L.)**	Biberiye	23054
10.	***Salvia virgata* Jacq.**	Fatmanaotu	54916
11.	***Scutellaria albida* L.**	Akkaside	92249
12.	***Sideritis libanotica* Labill.**	Gevreğen	83710
13.	***Stachys cretica* L.**	Deliçay	78026
14.	***Teucrium chamaedrys* subsp. *syspirense* (K.Koch) Rech.f.**	Sıcakotu	93761

RESULTS

In this study, pollen of 14 different species belonging to 12 genera in the family Lamiaceae were examined (Figures 1, 2, 3). Pollen characteristics of these species, such as pollen shape, pollen symmetry, polar axis length (P), equatorial axis length (E), P/E ratio, pollen shape, colpus number, colpus length, colpus width and exine layer thickness have been examined with a light microscope (Table 2). The studied pollen grains have isopolar polarity. Additionaly, their colpus number was either tricolpate or hexacolpate and their shape was either oblage-spheroidal or prolate-spheroidal.

The smallest polar diameter was observed in the pollen grains of *Scutellaria albida* (18.3 ± 0.5 μm), while the largest polar diameter was observed in the pollen grains of *Salvia virgata* (81 ± 1 μm). Additionally, the pollen grains of *Scutellaria albida* have the smallest equatorial axis length (22.4 ± 0.5 μm) and the pollen grains of *Salvia virgata* are the largest equatorial axis length (83.5 ± 0.3 μm) (Figure 2). The P/E ratio of the examined taxa have been ranged between 0.81-1.1. According to this measurement, *Origanum acutidens, Ocimum basilicum* and *Phlomis grandiflora* species have prolate-spheroidal pollen shapes, while in other species the pollen shapes are oblate-spheroidal.

The shortest colpus length was measured as 18.5 ± 0.4 μm in the pollen grains of *Scutellaria albida*, and the longest colpus length was measured as 72.2 ± 0.5 μm in the pollen grains of *Salvia virgata*. The narrowest colpus width was measured as 2 ±0.2 μm in the pollen grains of *Scutellaria albida*, and the widest was measured as 19.5 ± 0.7 μm in the pollen grains of *Stachys cretica*.

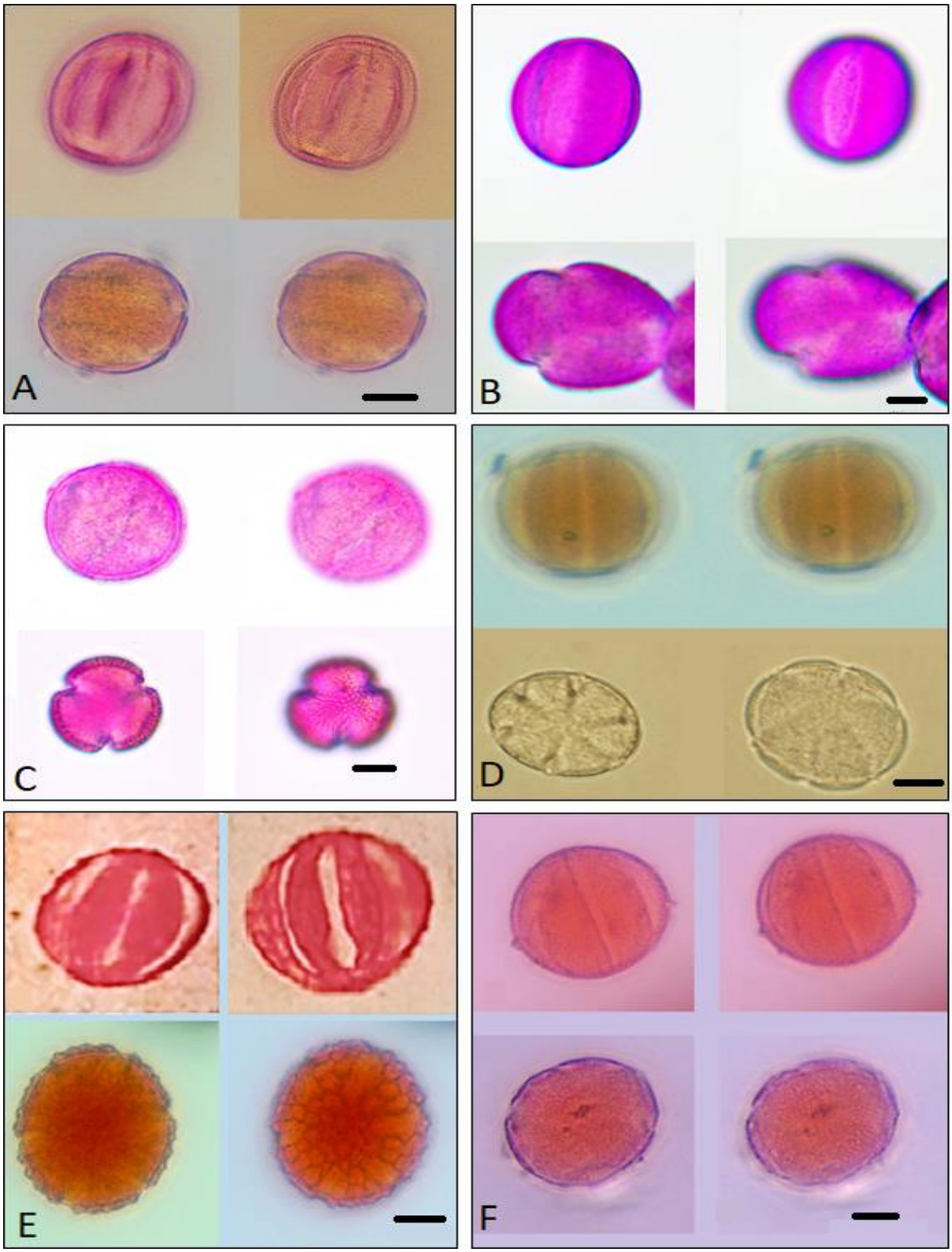

Figure 1: Light micrographs of pollen morphology in the examined species.
A) *Clinopodium graveolens* (Sin. *Satureja graveolens)*, B) *Lamium galeobdolon* (Sin. *Galeobdolon luteum*), C) *Lamium purpureum*, D) *Nepeta obtusicrena*, E) *Ocimum basilicum*, F) *Origanum acutidens* (Scale bars=0.01 mm).

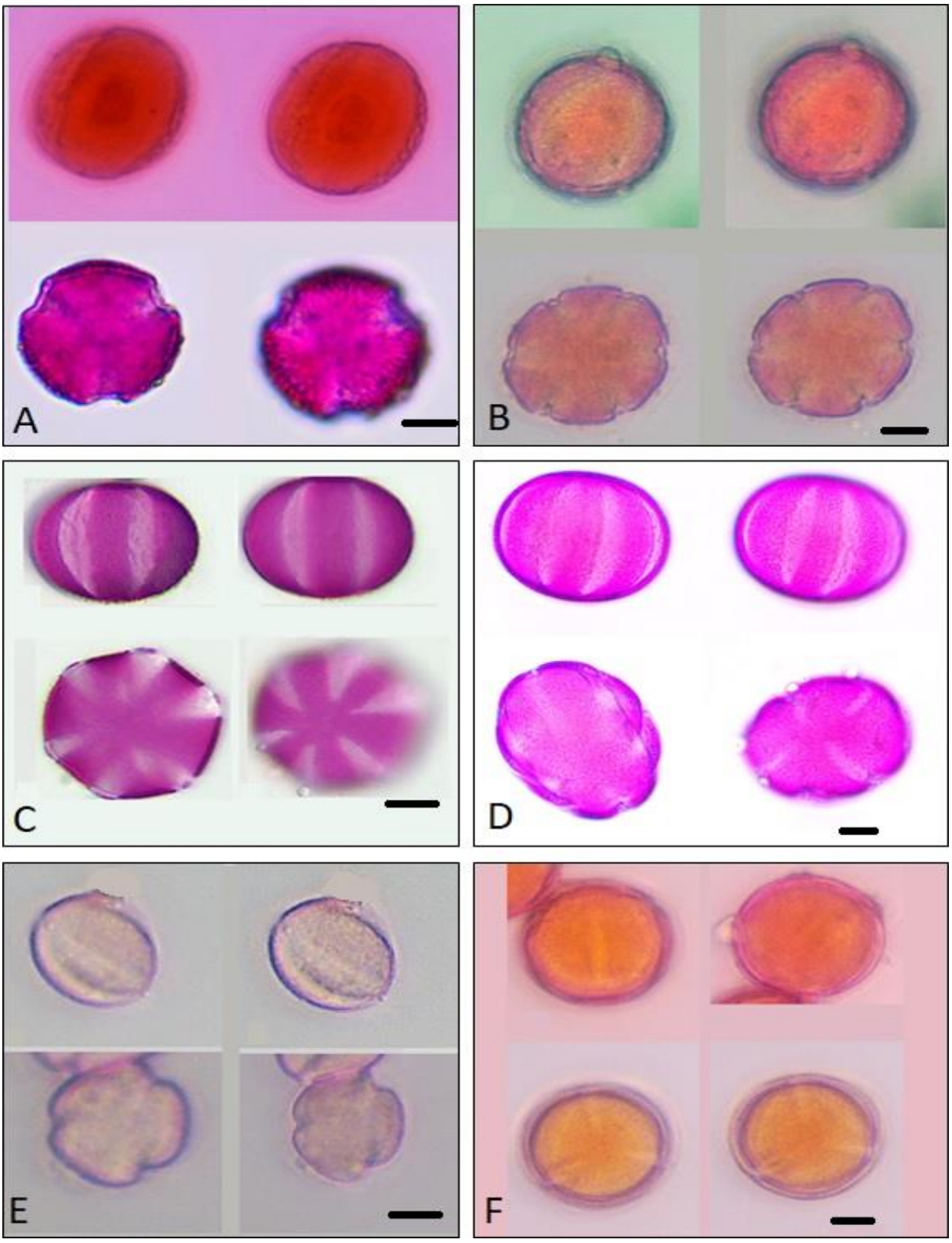

Figure 2: Light micrographs of pollen morphology in the examined species.
A) *Phlomis grandiflora*, B) *Prunella vulgaris*, C) *Salvia rosmarinus* (Sin. *Rosmarinus officinalis*), D) *Salvia virgata*, E) *Scutellaria albida*, F) *Sideritis libanotica* (Scale bars=0.01 mm).

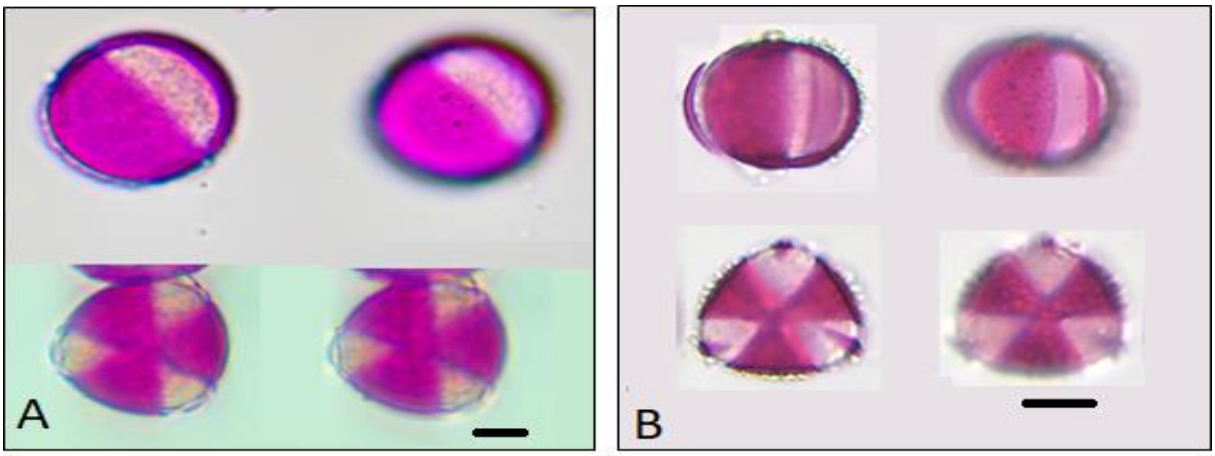

Figure 3: Light micrographs of pollen morphology in the examined species.
A) *Stachys cretica*, B) *Teucrium chamaedrys* subsp. *syspirense* (Scale bars=0.01 mm).

Table 2: Palynological features of the examined species in the family Lamiacaeae (Wodhouse).

Codes of examined species (Table 1)	Equatorial axis (E) (µm)	Polar axis (P) (µm)	Colpi	Colpus length (µm)	Colpus width (µm)	Exine thickness (µm)	P/E	Pollen shapes
1	37.3 ± 0.2	36.4 ± 0.2	6	32.5 ± 1.6	3.9 ± 0.4	2 ± 0.2	0.98	oblat-sferoidal
2	29.3 ± 0.5	28.4 ± 5.8	3	26 ± 0.6	11 ± 0.2	2.1± 0.4	0.97	oblat-sferoidal
3	30.2 ± 1.1	28.3 ± 1.2	3	26.7 ± 0.3	6.1 ± 0.2	3.2 ± 1.2	0.93	oblat-sferoidal
4	28.9 ± 0.5	24.9 ± 0.2	6	22.7 ± 0.7	5 ± 0.8	1.9 ± 0.3	0.86	oblat-sferoidal
5	50.6 ± 0.6	55.6 ± 0.6	6	48.2 ± 0.6	18.5 ± 0.7	2.14 ± 0.5	1.1	prolat-sferoidal
6	39.7 ± 0.5	40.6 ± 0.8	6	38.5 ± 1.2	3.1 ± 0.4	2.3 ± 0.1	1.02	prolat-sferoidal
7	48.5 ± 0.3	49.1 ± 1.1	3	40.5 ± 1.1	2.7 ± 0.3	2.6 ± 0.2	1.01	prolat-sferoidal
8	35.9 ± 1.1	33.8 ± 0.2	6	29.7 ± 1.4	5.6 ± 0.1	2.5 ± 0.4	0.94	oblat-sferoidal
9	33.5 ± 0.4	27.1 ± 0.1	6	30.9 ± 0.2	6.2 ± 0.2	1.2 ± 0.2	0.81	oblat-sferoidal
10	40.5 ± 0.2	39.2 ± 1.2	6	38.2 ± 0.5	13.7 ± 1.5	4.2 ± 0.7	0.97	oblat-sferoidal
11	22.4 ± 0.5	18.3 ± 0.5	3	18.5 ± 0.4	2 ± 0.2	1.4 ± 0.1	0.82	oblat-sferoidal
12	35.8 ± 0.6	35.1 ± 0.6	3	27.5 ± 1.6	2.4 ± 0.2	2.9 ± 0.5	0.98	oblat-sferoidal
13	30.1 ± 1.1	28.6 ± 1.7	3	24.2 ± 0.6	19.5 ± 0.7	2.6 ± 0.4	0.95	oblat-sferoidal
14	32.1 ± 0.7	27.9 ± 0.1	3	25.6 ± 1.2	15.1 ± 0.9	1.6 ± 0.9	0.87	oblat-sferoidal

DISSCUSSION

Erdtman, who examined the pollen morphology of the Lamiaceae family in detail, combined the results of his own studies with the results of other studies on this family and proposed a system in which each of the pollen type characterizes a subfamily (Erdtman, 1966). According to this system, the family is divided into two subfamilies: Lamioideae and Nepetoideae. Lamioideae contains pollen with 3 colpi (rarely 4 colpi), while Nepetoideae contains pollen with 6 colpi. Seven species included in this study, *Galeobdolon luteum, Stachys cretica, Lamium purpureum, Sideritis libanotica, Scutellaria albida, Phlomis grandiflora, Teucrium chamaedrys*, have tricolporate pollen which are in Lamioideae subfamily; and the other 7 species, *Salvia libanotica, Origanum acutidens, Prunella vulgaris, Ocimum basilicum, Salvia rosmarinus* (*Rosmarinus officinalis*), *Satureja graveolens* and *Nepeta obtusicrena* which are in the Nepetoideae subfamily with their hexacolpate pollen type.

According to the study of Pozhidaev, pollens with three colpi are considered more primitive than those with six colpi (Pozhidaev, 1991).

Abu - Asab and Cantino (1994), after examining the pollen morphology of the family in detail, determined that there are two basic pollen types with characteristic three colpi and six colpi. Brozova (1962) showed that the hexacolpate pollen is derived from the tricolpate pollen. Huynh (1972) supported this while working on the genus *Sideritis* and stated that the basic pollen type of the family Lamiaceae is tricolpate.

The genus *Galeobdolon* has been placed under the genus Lamium according to the systematic studies carried out in the recent years. In addition, the pollen characteristics support this similarity as well (Atalay *et al.*, 2016).

In a study involving pollens of *Ocimum basilicum*, the existence of different pollen types is mentioned (Khosla, 1993). Akolpate, monocolpate, bicolpate and hexacolpate pollen types can be observed in *O. basilicum* (Arogundade and Adedeji, 2009), but only hexacolpate pollen type was observed in this study.

Jamzad et al. (2003) and Jamzad (2013) examined the pollen morphologies of three new *Nepeta* L. species. They identified the species from Iran and stated that the palynological characteristics differed among the species supporting other morphological and molecular characters.

Perveen and Qaiser (2003) investigated the pollen morphology of family Lamiaceae in Pakistan and they stated that pollen

morphology can be used as an important character in the differentiation of various taxa at the species level in the Lamiaceae family. Also, Abu-Asab and Cantino (1994) stated that the pollen morphology is an important feature in the classification of Lamiaceae family. As a result, these data obtained with the light microscope are systematically important. This study is a basic data for taxonomical, morphological and melisopalynological researches.

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Richness of wild flowering plants and ferns in Northern Cyprus

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Abstract

Cyprus, the third largest island in the Mediterranean Sea, is situated just outside of the Gulf of Iskenderun, south of Turkey. Its topography can be summarized as 'The Coastal Belt, Northern Range, Southern Range and Central plain'. The island has been divided into 8 phytogeographical divisions by Meikle in 1977. The whole checklist of the vascular plants taxa, occurring in Northern Cyprus, has been prepared. The checklist comprises of 1564 wild taxa (species and subspecies) belonging to various families. Taxonomic status of the taxa has been updated according to two databases as follows 'The Plants Names' and 'International Plant Name Index (IPNI)'. Cyprus is a hotspot in Mediterranean basin and its number of the endemic taxa are 143; 73 of them occurring as endemic in Northern Cyprus and 15 of them are distributed only in the area of Northern Cyprus. The list of the vascular plants is given as tables and the endemic taxa is listed as a separate table in the article.

Keywords

Endemic taxa, Northern Cyprus, wild vascular plants.

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INTRODUCTION

Cyprus ranks as the third largest Mediterranean island with an area of 9251 km^2 after Sicily and Sardinia as shown in Figure 1 (Pariona, 2018). The island is located in the south of Turkey, west of Syria and Lebanon, north of Israel and Egypt, and southeast of Greece. Due to the political reasons, the island is subdivided into four main segments. The Republic of Cyprus occupies the southern two-third of the island (59.56%), the Turkish Republic of Northern Cyprus occupies the northern one-third (35.04%) of the island, and the United Nations-controlled Green Line provides a buffer zone that separates the two and covers 2.64% of the island. Lastly, two bases under British sovereignty are located on the island called Akrotiri and Dhekelia, covering the remaining 2.76% (Ilseven *et al.*, 2006).

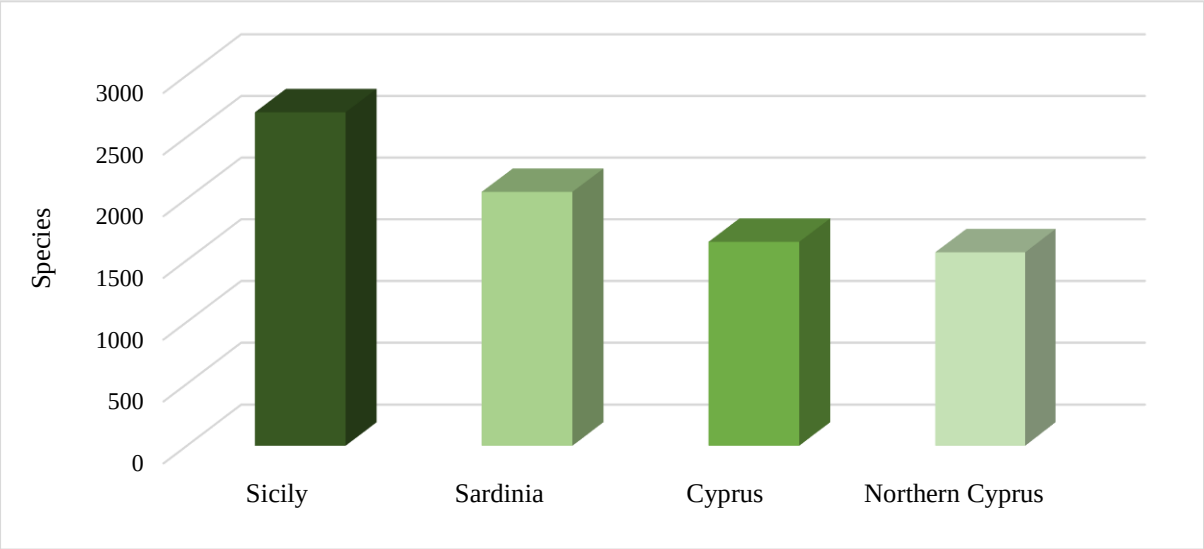

Figure 1: Species richness versus land surface within 3 islands in the Mediterranean (Sicily 25,708 km², Sardinia 24,090 km², Cyprus 9,251 km², and Northern Cyprus 3,355 km²).

Cyprus has remarkable flora for the following reasons: Its geographically isolated, located within the Mediterranean climate and contains unique habitat and altitude. On the island of Cyprus, 85-92 million years ago, geologically and biogeographically isolated regions were formed by the rise of sea beds after the formation of the Troodos Mountains. This isolation has caused many animal and plant species to be colonized on the island and to gradually form endemic species (Hadjikyriakou and Hadjisterkotis, 2002). The movement of floral elements from other climatic regions to the Mediterranean region during the ice age and the presence of volcanic rock types and the agro-sylvo-pastoral systems in the last century were effective in increasing the rate of endemism within Cyprus (Vural *et al.*, 2010).

The most comprehensive Flora for Cyprus is written by Robert Desmond Meikle

(1923-2021). Mr. Meikle spent approximately 30 years on the Flora of Cyprus. He visited the island multiple times, collected various plant specimens and kept them in his herbarium. The first volume of the Flora of Cyprus was published in 1977 and the second volume was published in 1985 (Meikle, 1977; Meikle, 1985). They include more than 1700 taxa and 157 species illustration.

He introduced a system of subdividing the island into eight different phytogeographical regions according to their geographical features and differences in vegetation, which is still in use today as shown in Figure 2 (Hand *et al.*, 2011). The boundaries of these regions have been drawn by the roads and streams as much as possible.

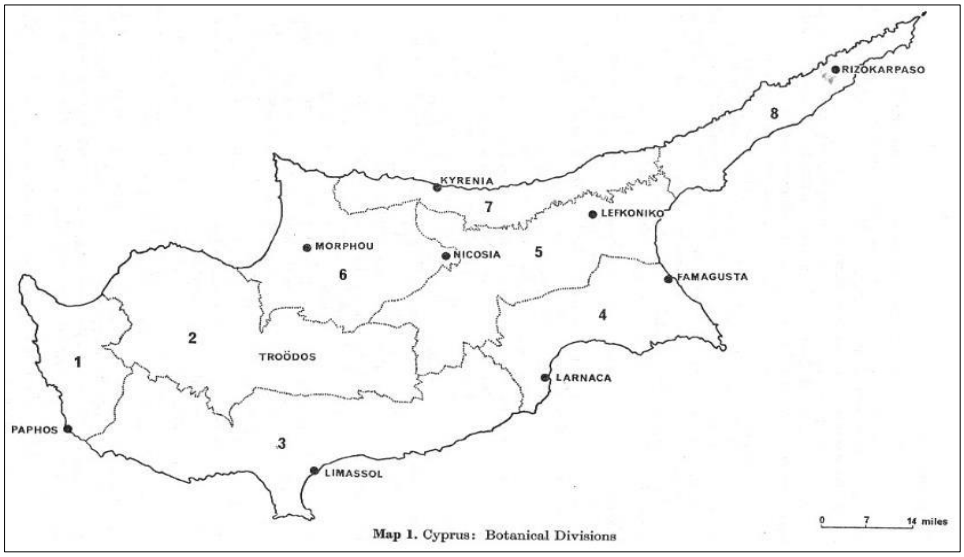

Figure 2: Phytogeographical 8 divisions of Cyprus.

Northern Cyprus includes partials of regions 4, 5, 6, and whole of regions 7 and 8. The following is a summary of the flora of these regions (Hand *et al.*, 2011; Hadjichambis *et al.*, 2004):

Division 4 is mostly cultivated or heavily grazed with typical Mesaoria cornfields in the north and with numerous barrens, eroded chalk or limestone hills in the south. Larnaca Salt Lake provides a habitat for interesting *Limonium* species and other halophytes.

Division 5 is mostly occupied by cereal fields of Mesaoria with interesting weed communities, but now through the general use of herbicides, it is almost weed free and the region is uninvitingly monotonous for the botanists. However, the Kyrenia range in the north of the division has rich and characteristic flora.

Division 6 is heavily cultivated, with cornfields in the centre and east, and it has extensive *Citrus* groves about Morphou. Botanically, the most important regions in

this division are Kormakiti and Ayia Irini. *Tulipa cypria* is locally abundant in fields about Diorios and Mrytou.

Division 7 has the richest flora among all of the island. The number of endemics and the rarities are too many to list. This division is mainly uncultivated with extensive areas of *Pinus brutia* and *Cupressus sempervirens* forests on the upper slopes.

Division 8 is an area with low hills and sand or rocky shores. This division includes many rare plant species. To date, 352 taxa containing 65 families and 217 genera have been identified on the coastal dunes of Cyprus. This corresponds to about 19% of the flora of the island.

The flora of Northern Cyprus, 'An illustrated flora of Northern Cyprus', is edited by Deryck E. Viney (1921-2016). Dr. Viney moved to Northern Cyprus and lived in Karaman after his retirment in 1981. He spent years travelling throughout the Northern Cyprus collecting plant specimens. He drew remarkable detailed illustrations of each specimen. The first volume of Viney's flora was published in 1994 and second volume was published in 1996 (Viney, 1994, 1996). Besides the illustration, the flora includes keys for identification as well. All of these properties of his flora makes it easy to use for everyone. Dr. Viney created field guide on the vascular plants of Northern Cyprus based on the scientific information but it was still meant to be suitable for amateurs as well.

The herbarium, which was prepared in cooperation with the TRNC Ministry of Agriculture and Forestry Department of Forestry and Deryck Viney, was opened on 9 November 1989. Northern Cyprus Herbarium is located within Alevkayası in the Turkish Republic of Northern Cyprus. There are approximately 1250 plant specimens in the herbarium. Dr. Viney kept all of his extensive collection he had accumulated over the years within Northern Cyprus in this herbarium. Unfortunatelly, it is closed since 2016 due to restoration.

Aim of this study

The purpose of this study is to prepare an up-to-date list of natural vascular plants growing in the Northern Cyprus within the illumination of recent studies. The list provided here is based on all of the published papers, books, monographs and revisions in regards to the Northern Cyprus vascular flora. The list prepared in this study is an updated list of natural plants growing in Northern Cyprus.

MATERIALS AND METHODS

The pathway that was followed in the preparation of the the indigenous species list of flowering plants and ferns (vascular plants) in Northern Cyprus:

1. The taxa in volumes 1 and 2 of the illustrated flora of Northern Cyprus were surveyed and listed (Viney, 2011). The list was arranged in different groups as Ferns (Table 1), Gymnosperms (Table 2), Monocotyledones (Table 3) and Dicotyledones (Table 4). Then, in each group was rearranged in terms of the families, genera and species in alphabetical order.

2. Additionally, published papers about the species in Northern Cyprus were examined. Several books and other articles were surveyed and additional species were detected and listed (Anonim, 2003; Brandes, 2020; Hadjikyriakou and Hand, 2008 a, b; Hand *et al.*, 2021; Hand, 2018; Hand, 2019; Hand, 2001; Ilseven, 2004; Sekerciler and Merakli, 2013; Tamson, 2014).

3. Moreover, this draft list was compared with the Dynamic Checklist of flora of Cyprus and the list was updated accordingly (Hand *et al*., 2011).

4. Lastly, the prepared list was updated through 'IPNI' and 'The Plant List' data bases.

RESULTS

The checklist is documented as four different tables according to the taxanomic groups: Ferns (Table 1), Gymnosperms (Table 2), Monocotyledones (Table 3) and Dicotyledones (Table 4). Endemics are marked with **(E)** in each table. Species, whose existence needs to be investigated within Northern Cyprus, are marked with **(?)** in each table.

Table 1: Checklist of Ferns.

FERNS	
Aspleniaceae	**Marsileaceae**
Asplenium ceterach L.	*Marsilea aegyptiaca* Willd.
A. onopteris L.	**Ophioglossaceae**
A. adiantum-nigrum L.	*Ophioglossum lusitanicum* L.
Dryopteridaceae	**Polypodiaceae**
Dryopteris pallida (Bory) Fomin subsp. *libanotica* (Rosent.) E. Nardi	*Polypodium cambricum* L.
Dennstaedtiaceae	**Pteridaceae**
Pteridium aquilinum (L.) Kuhn.	*Adiantum capillus-veneris* L.
Equisetaceae	*Anogramma leptophylla* (L.) Link
Equisetum ramosissimum Desf.	*Cheilanthes acrostica* (Balb.) Tod.
E. telmateia Ehrh.	*C. pteridoides* C. Chr.
	C. vellea (Aiton) Domin
	Selaginaceae
	Selaginella denticulata (L.) Link

Table 2: Checklist of Gymnosperms.

GYMNOSPERMS	
Cupressaceae	**Ephedraceae**
Cupressus sempervirens L. var. *horizontalis* (Mill.) Aiton)	*Ephedra foeminea* Forrsk.
Juniperus oxycedrus L. subsp. *oxycedrus*	*E. nebrodensis* Tineo subsp. *procera* (Fisch. & C. A. Mey.) K. Richt.
J. phoenicea L.	**Pinaceae**
Tetraclinis articulata (Vahl) Mast.	*Pinus brutia* Tenore
	P. holepensis Mill.
	P. pinea L.

Table 3: Checklist of Monocotyledones.

ANGIOSPERMS MONOCOTYLEDONES	
Alismataceae	*B. trifoliata* Kunth.
Alisma lanceolatum With.	*Drimia aphylla* (Forssk.) J. C. Manning&Goldblatt
Baldellia ranunculoides (L.) Parl.	*D. maritima* (L.) Baker
Damasonium alisma Mill.	*Hyacinthella millingenii* L. **(E)**
D. bourgaei Coss.	*Muscari comosum* Mill.
Amaryllidaceae	*M. inconstrictum* Rechinger f.
Allium amethystinum Tausch	*M. neglectum* Ten.
A. ampeloprasum L.	*M. parviflorum* Desf.
A. autumnalis P. H. Davis. **(E)**	*Ornithogalum divergens* Boreau
A. cupani Rafin. subsp. c*yprium* Meikle **(E)**	*O. narbonense* L.
A. curtum Boiss.	*O. neurostegium* Boiss. & C.I. Blanche ex Boiss.
A. cyprium Brullo, Pavone & Salmeri	*O. pedicellare* Boiss et. Kotschy **(E)**
A. cyprium subsp. *lefkarense* (Brullo, Pavone & Salmeri) Christodoulou & Hand. **(E)**	*O. umbellatum* L.
	O. trichophyllum Boiss.
A. junceum Sm.	*Ruscus aculeatus* L.
A. dentiferum Webb & Berthel.	*Scilla autumnalis* L.
A. guttatum subsp. *sardoum* (Moris) Stearn	*S. cilicica* Meikle
A. neapolitanum Cirillo.	*S. morrisii* Meikle **(E)**
A. nigrum L.	**Colchicaceae**
A. oreintale Boiss.	*Colchicum pusillum* Sieber
A. pallens L.	*C. stevenii* Kuntz.
A. paniculatum L.	*C. troodi* Kotschy **(E)**
A. rubrovittatum Boiss.	**Cymodoceaceae**
A. stamineum Boiss.	*Cymodocea nodosa* Ascherson
A. trifoliatum Cyr.	**Cyperaceae**
A. willeanum Holmboe **(E)**	*Bolboschoenus glaucus* (Lam.) S. G. Sm.
Narcissus obsaletus (Haw.) Steud.	*B. maritimus* (L.) Palla
N. serotinus L.	*Carex cyprica* A.M.Molina, Acedo & Llamas **(E)**
N. tazetta L.	*C. divisa* Huds.
Pancratium maritimum L.	*C. divulsa* Stokes
Sternbergia lutea (L.) Spreng.	*C. egorovae* A.M.Molina, Acedo & Llamas
Araceae	*C. extensa* Gooden.
Arisarum vulgare Targ. Tozz.	*C. flacca* Schreb.
Arum dioscoridis Sm.	*C. halleriana* Asso
A. hygrophilum Boiss.	*C. hispidis* Schkuhr
A. italicum Mill. subsp. *italicum*	*C. illegitima* Cesati
Arum sintenisii (Engl.) P. C. Boyce	*C. otrubae* Podp.
Arecaceae	*Cladium mariscus* (L.) Pohl
Phoenix dactylifera L.	*Cyperus capitatus* Vand.
Asparagaceae	*C. flavidus* (Retz.) Koyama
Agave americana L.	*C. fuscus* L.
A. sisalana Perrine	*C. glober* L.
Asparagus acutifolius L.	*C. involucratus* Rottb.
A. horridus L.	*C. laegivatus* L.
A. officinalis	*C. longus* L.
Bellevalia nivalis Boiss. et Kotschy	*C. rotundus* L.

Eleocharis palustris (L.) Roem et Schultz
E. vulgaris (Walters) Á. Löve & E. Löve
Fimbristylis ferruginea (L.) Vahl.
Isolepis cernua (Vahl) Roem. Et Schult
Pycreus flavidus (Retz) Koyama
Schoenoplectus litoralis (Schrader) Palla
Schoenus nigricans L.
Scirpoides holoschoenus (L.) Sojak
Dioscoreaceae
Tamus communis L.
Elatinaceae
Elatine macropoda Guss.
Hydrocharitaceae
Halophila stipulacea Ascherson
Najas marina L. subsp. *armata* Horn
Iridaceae
Crocus hartmannianus Holmboe **(E)**
C. veneris Teppeiner **(E)**
Gladiolus italicus Mill.
G. triphyllos (Sm.) Ker-Gawler
Iris germanica L.
Moraea sisyrinichium (L.) Ker Gawl.
Rumulea columnae Seb. subp. *columnae*
R. ramiflora Ten. subsp. *ramiflora*
R. tempskyana Freyn
Juncaceae
Juncus acutus L.
J. articulatus L.
J. articulatus x fontanesii subsp. *pyramidatus*
J. bufonius L.
J. capitatus Weigel
J. fontanesii Leharpe subsp. *pyramidatus* (Leharpe) Snogerup
J. heldreichianus Parl. subsp. *heldreichianus*
J. hybridus Brot.
J. inflexus L.
J. littoralis C. A. Mey.
J. maritimus Lam.
J. rigidus Desf.
J. subulatus Forssk.
Luzula forsteri (Sm) DC. subsp. *rhizomata* (Ebinger) Z. Kaplan
Juncaginaceae
Triglochin barrelieri Loisel
Liliaceae
Fritillaria acmopetala Boiss.
F. persica L.
Gagea chlorantha (M. Bieb.) J. A. et J. H. Schulets
G. fibrosa (Desf.) J. A. et J. H. Schultes
G. graeca (L.) A. Terracc.
G. juliae Pascher
G. peduncularis (J. et C. Presl.) Pascher
Smilax aspera L.
Tulipa cypria Stapf. **(E)**
Orchidaceae
Anacamptis collina (Banks. S. & Sol. ex Russell) R. M. Batemen. Pridgeon. M. W. Chase
A. coriophora (L.) R. M. Batemen. Pridgeon. M. W. Chase
A. morio L. subsp. *syriaca* (E.G.Camus) H. Kreutz
A. pyramidalis (L.) Rich
A. sancta (L.) R. M. Batemen. Pridgeon. M. W. Chase
Himantoglossum robertiana Greuter
Dactlorhiza romana (Seb.) Soo.
Limodorum abortivum (L.) Swartz
Neotinea maculata (Desf.) Stearn
Ophrys apifera Huds.
O. argolica H. Fleischm. subsp. *elegans* (Renz) F. Nelson **(E)**
O. fuciflora (F. W. Schmidt) Moench subsp. *bornmullerii* (M. Schulze) B. Willing & E. Willing
O. fuciflora (F. W. Schmidt) Moench subsp. *grandiflorum* (Fleischm. et Soo.) Faurh.
O. fusca Link. subsp. *fleischmannii* (Hayek) Soo.
O. fusca Link. subsp. *iricolor* (Desf.) K. Richt.
O. kotschyi H. Fleischm. & Soo
O. omegaifera H. Fleischm subsp. *fleischmannii* (Hayek) Del Prete
O. scolopax Cav. subsp. *rhodia* (H. Baumann & Kunkele) H. A. Pedersen & Faurh.
O. sphegpodes Mill. subsp. *mammosa* (Desf.) Soo. ex E. Nelson
O. umbilicata Desf. subsp. *umbilicata*
O. umbilicata Desf. subsp. *laptehica* (Gölz & H. R. Reich.) Faurh. & H. A. Pedersen
Orchis anatolica Boiss.
O. intacta Link.
O. italica Poir.
O. morio L. subsp. *syriaca* E. G. Camus
O. punctulata Lindl.
O. pyramidalis L.
O. quadriloba E. G. Camus
O. sezikiana B.Baumann & H.Baumann
O. simia Lam.
O. tridentata Scop.
Serapias bergonii E. G. Camus
S. levantina H. Baumann & Künkele
Spiranthes spiralis (L.) Chevall.
Poaceae
Achnatherum bromoides (L.) P. Beauv.
Aegilops bicornis (Forssk.) Jaub. & Spach.
Ae. biuncialis Vis. subsp. *biuncialis*
Ae. comosa Sm. subsp. *comosa*
Ae. geniculata Roth
Ae. × insulae-cypri H. Scholz
Ae. kotschyi Boiss.
Ae. paniculata Roth
Ae. peregrina (Hackel) Maire var. *peregrina*
Ae. peregrina (Hackel) Maire var. *brachyathera* (Boiss.) Maire
Ae. triuncialis L. var. *persica* (Boiss.) Eig
Ae. triuncialis L. var. *truincialis*
Ae. ventricosa Tausch
Aeluropus lagopoides L.
Ae. lagopoides x littoralis
Ae. littoralis (Gouan) Parl.
Agrostis stolonifera L.
Aira elegans Willd. ex Roem. & Schult.
Alopecurus myosuroides Huds.

A. utriculatus Banks & Sol.
Amophilla arenaria (L.) Link
Andropogon distachyos L.
Aristida adscensionis L. subsp. *coerulescens* (Desf.) Auquier & Duvign.
Arundo donax L.
A. micrantha Lam.
Avelliana festucoides (Link) Valdes & Scholz
Avena barbata Link subsp. *barbata*
A. barbata Link subsp. *wiestii* (Steud.) Mansf.
A. byzantina Koch
A. eriantha Durieu
A. ludoviciana Durieu
A. sativa L.
A. sterilis L. subsp. *ludoviciana* (Durieu) Gillet & Magne
A. sterilis L. subsp. *sterilis*
A. ventricosa Coss.
A. wiestii Steud.
Brachiaria eruciformis (Sm.) Griseb.
Brachypodium distachyon (L.) P. Beauv.
B. pinnatum (L.) P. Beauv.
B. sylvaticum (Huds.) P. Beauv. subsp. *sylvaticum*
Briza maxima L.
B. minor L.
Bromus alopecuros Poir. subsp. *caroli-henrici* (Greuter) P. M. Sm.
B. arvensis L.
B. bidentatus Holmstr. & H. Scholz **(?)**
B. chrysopogon Viv.
B. diandrus Roth
B. fasciculatus Presl. subsp. *delilei* (Boiss). H. Scholz
B. fasciculatus Presl. subsp. *fasciculatus*
B. hordeaceus L. subsp. *molliformis* (J. Lloyd. ex Billot) Mairea & Weiller
B. intermedius Guss. subsp. *intermedius*
B. intermedius Guss. subsp. *optimae* H. Scholz
B. japonicus Thunb.
B. lanceolatus Roth
B. madritensis L. subsp. *madritensis*
B. madritensis x rubens
B. molliformis Lloyd.
B. rigidus Roth
B. rubens L. subsp. *rubens*
B. scorparius L.
B. squarrosus L. subsp. *squarrosus*
B. sterilis L.
Carynephorus divaricatus (Pourr.) Breistr.
Catapodium marinum (L.) C. C. Hubb.
C. rigidum (L.) C.E Hubb
Corynephorus articulatus (Desf.) P. Beauv.
Crithopsis delileana (Schult.) Roschev.
Cutandia dichotoma (Forssk.) Trabut
C. maritima (L.) Richter
Cynodon dactylon (L.) Pers.
Crypsis aculeata (L.) Aiton
C. factorovski Eig
C. schoenoides (L.) Lam.
Cynosurus coloratus Nees
C. effusus Link
C. elegans Desf.
Dactylis glomerata L. subsp. *hispanica* (Roth) Nyman
Dactyloctenium aegyptium (L.) P. Beauv.
Digitaria sanguinalis (L.) Scop. subsp. *sanguinalis*
Echinochloa colona (L.) Link
E. crus-galli (L.) P. Beauv.
Elymus elongatus (Host) Runemark subsp. *haifensis* (Rech. f.) Haneen & Runemark
E. farctus (Viv.) Meld.
Eragrostis cilianensis (All.) Vign-Lut
E. minor Host
Festuca arundinaceae Schreb.
Gastridium phleoides (Nees et. Mey.) C.E Hubb.
Hainardia cylindrica (Willd.) Greuter
Hordeum bulbosum L.
H. distichon L.
H. geniculatum All.
H. glaucum Steudel.
H. leporinum Link.
H. marinum Huds.
H. spontaneum Koch
H. vulgare L. subsp. *agriocrithon* (Aberg) D. Love & A. Löve
Hyparrhenia hirta (L.) Stapf
Imperata cylindrica (L.) Raeus.
Lagurus ovatus L.
Lamarckia aurea (L.) Moench
Lolium multiflorum Lam.
L. perenne L.
L. rigidum Gaud. subsp. *rigidum*
L. rigidum x temulentum
L. subulatum Vis.
L. temulentum L.
L. x hubbardii
Maillea crypsoides (d'Urv.) Hack.
Melica minuta L.
Milium pedicellare (Bornm.) Meld.
Moorochloa eruciformis (Sm.) Veldkamp
Panicum miliaceum L.
P. repens L.
Parapholis cylindrica (Willd.) Romero Zarco
P. incurva (L.) C.E Hubb.
P. marginata Runemark
Paspalum dilatatum Poir.
P. distichum L.
Phalaris aquatica L.
P. brachystachys Link
P. minor Retz.
P. paradoxa L.
Phleum crypsoides (d'Urv.) Hack.
P. subulatum (Savi) Aschers.
Phragmites australis (cav.) Trin.
P. frutescens H. Scholz
Piptatherum coerulescens (Desf.) P. Beauv.
P. miliaceum (L.) Coss.
Poa angustifolia L.
P. annua L.
P. bulbosa L.

P. compressa L.
P. infirma Kunth
P. trivialis L.
Polypogon maritimus Willd.
P. monspeliensis (L.) Desf
P. viridis (Gouan) Breistr
Psilurus incurvus (Gouan) Schinz et. Thell
Rostraria amblyantha (Boiss.) Holub
R. cristata (L.) Tselev
R. hadjikyriakou Christodoulou & Hand **(E)**
R. hispida (Savi) M. Doğan
R. obtusiflora (Boiss.) Holub.
R. smyrnacea (Trin.) Scholz.
Saccharum spontaneum L.
Schismus arabicus Nees
Sclerochloa dura P. Beauv.
Secale cereale L.
Setaria italica (L.) P. Beauv.
S. pumila (Poir.) Roem.
S. verticillata (L.) P. Beauv.
S. viridis (L.) P. Beauv.
Sorghum halepense (L.) Pers.
Sphenopus divaricatus (Gouan) Reichb.
Sporobolus virginicus (L.) Kunth
Stenotaphrum secundatum (Walt.) Kuntze
Stipa arabica Trin. & Rupr.
S. barbata Desf.
S. bromoides (L.) Doerfl.
S. capensis Thunb.
S. holosericea Trin.
Taeniatherum caput-medusae (L.) Nevski subsp. *crinitum* (Scherb.) Melderis
Trachynia distachya (L.) Link
Tripidium ravennae (L.) H. Scholz.
Triplachne nitens (Guss.) Link
Trisetaria linearis Forssk.
Triticum aestivum L.
T. durum Desf.
T. spelta L.
T. turgidum L.
Urocloa panicoides P. Beauv.
Vulpia brevis Link
V. ciliata Boiss.
V. fasciculata (Forssk.) Samp.
V. muralis (Kunth) Nees
V. myurus (L.) C. C. Omel.
Posidoniaceae
Posidonia oceanica (L). Del.
Potamogetonaceae
Potamogeton nodosus Poir.
P. pectinatus L.
P. perfoliatus L.
Ruppiaceae
Ruppia drepanensis Tineo
R. maritima L.
R. spiralis L. ex Dumort.
Typhaceae
Typha domingensis Pers.
Xanthorrhoeaceae
Aloe vera (L.) Burm. f.
Asphodelus fistulosus L.
A. ramosus L.
A. tenuifolius Cav.
Asphodeline brevicaulis (Bertol.) Gay
A. lutea (L.) Reichb.
Zannichelliaceae
Althenia filiformis E. Petit
Zannichellia palustris L.

Table 4: Checklist of Dicotyledones.

ANGIOSPERMS DICOTYLEDONES	
Acanthaceae	*Arthrocnemum macrostachyum* (Moric.) K. Koch
Acanthus mollis L.	*Bosea cypria* Schintz **(E)**
Adoxaceae	**Anacardiaceae**
Sambucus ebulus L.	*Pistacia atlantica* Desf.
S. nigra L.	*P. lentiscus* L.
Viburnum tinus L.	*P. terebinthus* L.
Aizoaceae	*P. vera* L.
Aizon hispanicum L.	*Schinus molle* L.
Aptenia cordifolia (L. f.) N. E. Br.	**Apiaceae**
Carpobrotus edulis (L.) N. E. Br.	*Ainsworthia trachycarpa* Boiss.
Mesembryanthemum nodusum L.	*Ammi majus* L.
M. crystalinum L.	*A. visnaga* (L.) Lam.
M. nodiflorum L.	*Apium graveolens* L.
Altingiaceae	*A. nodiflorum* (L.) Lag
Liquidambar styraciflus L.	*Bifora testiculatus* (L.) DC.
Amaranthaceae	*Bupleurum lancifolium* Hornem.
Amaranthus albus L.	*B. orientale* Snogerup
A. blitoides S. Watson	*B. semicompositum* L.
A. graecizans L.	*B. sintenisii* Huter **(E)**
A. hybridus L.	*B. subovatum* Spring
A. retroflexus L.	*B. trichopodum* Boiss.
A. viridis L.	*Bunium ferulaceum* Sihth et. Sm.

Cachrys scabra (Fenzl) Meikle
Coriandrum sativum L.
Crithmum maritimum L.
Cyclospermum leptophyllum (Pers.) Britton
Daucus aurea Desf.
D. broteri Ten.
D. carota L.
D. durieua Lange
D. glaber (Forssk.) Thell.
D. guttatus Sm.
D. involucratus Sibth. et Sm.
D. pumilus (L.) Hoffm. et Link
Dichoropetalum kyriakae (Hadjik. & Alziar) Hand & Hadjik.
Echinophora tenuifolia L.
Eryngium campestre L.
E. creticum Lam.
E. glomeratum Lam.
E. maritimum L.
Ferula communis L.
F. cypria Post.
F. glauca L.
Ferulago cypria H.Wolff. **(E)**
F. syriaca Boiss.
Foeniculum vulgare Mill.
Glaucosciadium cordifolium (Boiss.) B. L. Burtt & P. H. Davis
Helosciadium nodiflorum (L.) W. D. J. Koch
Krubera peregrina (L.) Hoffm.
Lagoecia cuminoides L.
Lecokia cretica (Lam.) DC.
Opopanax hispidus Griseb.
Orlaya daucoides Greuter
Petroselinum crispum (Mill.) A. W. Hill
Pimpinella cretica Poir.
P. cypria Boiss. **(E)**
P. peregrina L.
Pseudorlaya pumila L.
Ridolfia segetum (Guss.) Moris
Scaligeria alziari Hand & al. **(E)**
S. napiformis (Spreng.) Grande
Saligeria cretica (Mill.) Boiss.
Scandix australis L.
S. grandiflora L.
S. pecten-veneris L.
Smyrnium connatum Boiss.
S. olusatum L.
Tordylium aegyptiacum (L.) Poir.
T. apulum L.
T. syriacum L.
T. trachycarpum (Boiss.) Jury & Al-Eisawi
Torilis africana Spreng.
T. heterophylla (L.) Reichb.
T. leptophylla (L.) Rchb.
T. nodosa (L.) Gaerth.
T. pseudonodosa Bianca
T. purpurea (Ten.) Guss.
T. tenella (Del.) Rchb
T. veneris (Huds.) Link
Turgenia latifolia (L.) Hoffm.
Zosima absinthiifolia (Vent.) Link

Apocynaceae

Cynanchum acutum L.
Cyprinia gracilis (Boiss.) Browicz
Nerium oleander L.
Trachomitum venetum (L.) Woodson
Vinca major L.

Araliaceae

Hedera helix L.

Aristolochiaceae

Aristolochia parvifolia Sm.
A. sempervirens L.

Asteraceae (Compositae)

Achiella arabica Kotschy
A. biebersteinii Afan.
A. cretica L.
A. maritima L. subsp. *maritima*
A. millefolium L.
A. santolina L.
Aethiorhiza lubosa (L.) Cass.
Ambrosia maritima L.
Anthemis amblyolepis Eig.
A. chia L.
A. cotula L.
A. palaesting (Kotschy) Boiss.
A. parvifolia Eig.
A. pseudocotula Boiss.
A. rigida Heldr.
A. tomentosa L.
A. tricolor Boiss. **(E)**
Artemisia arborescens L.
Aster squamatus (Spreng.) Hieron.
Asteriscus aquaticus (L.) Less.
Atractylis cancellata L.
Bellis annua L.
B. perennis L.
B. sylvestris Cyr.
Bidens frondosa L.
Bombycilaena discolor (Pers.) Lainz
Calendula arvensis L.
C. officinalis L.
Cardopatium corymbosum (L.) Pers.
Carduus argentatus L.
C. pycnocephalus L.
Carlina involucrata Poir.
C. lanata L.
C. libonotica Boiss.
C. pygmaea (Post.) Holmboe **(E)**
Carthamus boissieri Hal.
C. caeruleus L.
C. dentatus Vahl
C. lanatus L.
C. tenuis (Boiss. et. Blanche) Bornm.
Catanche lutea L.
Centaurea aegiolophila Wagenitz
C. benedicta (L.) L.
C. calcitrapa L. subsp. *angusticeps* (Lindberg f.) Meikle **(E)**
C. calcitrapa L. subsp. *calcitrapa*
C. cyanoides Wahlenb.

C. iberica L.
C. hyalolepis Boiss.
Chlamydophora pycnocephalus L
Chondrilla juncea L.
C. tridentata (Del.) Ehrenb.
Chrysanthemum coronarium L. var. *discolor* d'Urv.
C. segetum L.
Cnicus benedictus L.
Conyza bonariensis (L.) Cronq.
Cichorium endivia L.
C. intybus L.
C. pumilum Jacq.
C. spinosum L.
Cirsium arvense (L.) Scop.
Crepis aspera L.
C. foetida L. subsp. *commutata* (Spreng.) Babc.
C. foetida L. subsp. *foetida*
C. fraasii Sch. Bip.
C. micrantha Czerep.
C. palaestina (Boiss.) Bornm.
C. pulchra L.
C. pusilla (Sommier) Merxm.
C. reuteriana Boiss.
C. sancta (L.) Bornm.
C. zacintha (L.) Loisel
Crupina crupinastrum (Moris.)Vis.
Cynara cardunculus L.
C. cornigera Lindley
C. scolymus L.
Dittrichia graveolens (L.) Greuter
D. viscosa (L.) Greuter subsp. *viscosa*
D. viscosa (L.) Greuter subsp. *angustifolia* (Beg.) Greuter
Echinops spinosissimus Turra
Erigeron bonariensis L.
E. canadensis L.
Eupatorium cannabinum L.
Evax contracta Boiss.
E. eriosphaera Boiss.
E. pygmaea (L.) Brot.
Filago aegaea Wagenitz subsp. *aristata* Wagenitz
F. contracta (Boiss.) Chrtek & Holub
F. eriocephala Guss.
F. eriosphaera (Boiss. & Heldr.) Chrtek & Holub
F. gallica L.
F. mareotica Delile
F. pygmaea L.
F. pyramidata L.
Garhadiolus hedynois Jaub. & Spach
Geropogon hybridus (L.) Sch.
Glebionis coronaria (L.) Spach
G. segetum (L.) Fourr.
Gundelia tournefortii L.
Hedypnois rhagadioloides (L.) F. W. Schmidts
Helichrysum conglobatum (Viv.) Steudel.
H. luteoalbum (L.) Rchb.
Helminthotheca echioides (L.) Holob.
Hirtellina lobelii (DC.) Dittrich
Hyoseris scabra L.
Hypochaeris achyrophorus L.
H. glabra L.
Inula crithmoides L.
Klasea cerinthifolia (Sm.) Greuter & Wagenitz
Koelpinia linearis Pallas.
Lactuca saligna L.
L. serriola L.
L. tuberosa Jacq.
L. undulata Ledeb.
L. viminea (L.) J. Presl & C. Presl
Launea resedifolia (L.) O. Kuntze
L. fragilis (Asso) Pau subsp. *fragilis*
Leontodon tuberosus L.
Limbarda crithmoides (L.) Dumort. subsp. *longifolia* (Arcang.) Greuter
Mantisalca salmantica (L.) Briq. & Cavill.
Matricaria aurea (Loefl.) Sch.
M. recutita L.
Notobasis syriacus (L.) Cass.
Onopordum cyprium Eig. **(E)**
Osteospermum ecklonis (DC.) Norl.
Otanthus maritimus (L.) Hoffsgg.
Pallenis spinusa (L.) Cass.
Phagnalon rupestre (L.) DC.
Picnomon acarna (L.) Cass.
Picris cyprica Lack
P. altissima Del.
P. rhagadioloides (L.) Desf.
Ptilostemon chamaepeuce (L.) Less. subsp. *chamaepeuce*
Ptilostemon chamaepeuce (L.) Less. subsp. *cyprius* (Greuter) Chrtek & B. Slavik **(E)**
Pulicaria arabica (L.) Cass.
P. dysenterica (L.) Bernh.
Reichardia intermedia (Sch. Bip.) Coutinho
R. picroides (L.) Roth.
R. tingitana (L.) Roth.
Rhagadiolus edulis Gartner
R. stellatus (L.) Gartner
Scariola viminea (L.) F. W. Schmidt
Scolymus hispanicus L.
S. maculatus L.
Scorzonera alpigena (K. Koch) Grossh.
S. jacquiniana (Koch) Celak
S. laciniata L.
S. troodea Boiss. **(?)**
Senecio aegyptius L.
S. angulatus L. f.
S. cineria DC.
S. glaucus L. subsp. *cyprius* Meikle **(E)**
S. leucanthemifolius Poir.
S. vulgaris L.
Serratula cerinthifolia (Sm.) Boiss.
Silybum marianum (L.) Gartner
Sonchus asper (L.) Hill.
S. bulbosus (L.) N. Kilian & Greuter
S. oleraceus L.
S. tenerrimus L.
Staehelina lobelii DC.
Steptorhamphus tuberosus (Jacq.) Grossh.

Symphyotrichum squamatum (Spreng.) G. L. Nesom
Tagetes minuta L.
Taraxacum aphrogenes Meikle **(?)**
T. cyprium Lindberg
T. hellenicum Dahlst.
Tolpis virgata (Desf.) Bertol.
Tragopogon sinnatus Ave-Lall.
T. porrifolius L. subsp. *longirostris* (Sch. Bip.) Greuter
Urospermum picroides (L.) F. W. Schmidt
Xanthium spinosa L.
Xanthium strumarium L.
Xeranthemum inspertum (L.)Mill.

Basellaceae

Anredera cordifolia (Ten.) Steenis

Berberidaceae

Bongardia chrysogonum (L.) Endl.
Leontice leontopetalum L.

Boraginaceae

Alkanna lehmanii (Tineo) A. DC.
A. orientalis Boiss.
A. tinctoria (L.) Tausch
Anchusa aegyptiaca DC.
A. arzurea Mill.
A. humilis (Desf.) I. M. Johnston
A. strigosa Labill.
A. undulata L.
Asperugo procumbens L.
Borago officinalis L.
Buglossoides arvensis (L.) I. M. Johnston
B. incrassata (Guss.) I. M. Johnst. subsp. *splitgerberi* (Guss.) E. Zippel & Selvi
B. tenuiflora (L. f.) I. M. Jonhston
Cordia myxa L.
Cynoglossum creticum Mill.
Echium angustifolium Mill.
E. arenarium Guss.
E. glomeratum Poir.
E. plantagineum L.
Heliotropium dolasum De Not.
H. europaeum L.
H. hirsutissimum Grauter
H. supinum L.
Lithodora hispidula (Sm.) Griseb
Myosotis ramosissima Rochel.
Neatostema apulus (L.) I. M. Johnston
Nonea echioides Roem. & Schult.
N. philistea Boiss.
N. ventricosa (Sm.) Griseb
Onosma caespitosa Kotschy **(E)**
O. *fruticosa* Sm. **(E)**
O. giganteum Lam.
O. orientalis (L.) L.

Brassicaceae

Aethionema arabicum (L.) Andrz. ex Lipsky
A. carneum (Banks & Sol.) Fedtsch
Alyssum strigosum Banks et Sol.
Arabidopsis thaliana (L.) Heynh.
Arabis cypria Holmboe **(E)**
A. kennedyae Meikle **(?)**
A. verna (L.) R. Br. In W. T. Aiton
Biscutella didyma L.
Brassica hilarionis Post **(E)**
B. nigra (L.) W. D. J. Koch
B. tournefortii Govan
Cakile maritima Scop.
Capsella bursa-pastoris (L.) Medike
Cardamine hirsuta L.
Cardaria draba Desv.
Carrichtera annua (L.) DC.
Clypeola jonthlaspi L.
Conringia orientalis (L.) Dumort.
Coronupus squamatus Aschers.
Crampe hispanica L.
Didesmus degyptius (L.) Desv.
Diplotaxis viminea DC.
Draba minima (C. A. Mey.) Steud.
D. praecox Steven
D. verna L.
Enarthrocarpus arcnatus Labill
E. lyratus (Forssk.) DC.
Erophila verna (L.) Chevall.
Eruca vesicaria (L.) Cav.
Erucaria hispanica (L.) Druce
E. minima C. A. Mey.
Hirschfeldia incana (L.) Lagreze-Fossat
Hornungia procumbens (L.) Hayek
Hymenolobus procumbens (L.) schinz et. Thell.
Iberis odorata L.
Lepidium coronopus (L.) Al-Shehbaz
L. draba L. subsp. *draba*
L. latifolium L.
Lobularia maritima (L.) Desv.
L. libyca (Viv.) Meisn.
Malcolmia africana (L.) W. T. Aiton
M. chia (L.) DC.
M. flexuosa Sibth. et Sm
M. nana (DC.) Batt.
Maresia nana (DC.) Batt.
Matthiola fruticulosa (L.) Maire
M. incana (L.) R. Br.
M. longipetala (Vent.) DC.
M. tricuspidata (L.) R.Br.
Microthlaspi natolicum (Boiss.) F. K. Mey. subsp. *sporadium* F. K. Mey.
M. perfoliatum (L.) F. K. Mey.
Nasturtium officinale R.Br.
Neotorularia torulosa (Desf.) Hedge & J. Léonard
Neslia apiculata C.A.Mey.
N. paniculata (L.) Desv.
Raphanus raphanistrum L.
R. sativus L.
Rapistrum rugosum All.
Sinapis alba L.
S. arvensis L.
Sisymbrium irio L.
S. officinale (L.) Scop.
S. orientale L.
S. polyceratium L.

Thlaspi perfoliatom L.
Torularia torulosa (Desf.) O.E. Schulz
Cactaceae
Opuntia ficus-indica (L.) Mill.
O. humifusa (Raf.) Raf.
Callitrichaceae
Callitriche brutia Petagne
Campanulaceae
Campanula delicatula Boiss.
C. erinus L.
C. fastigiata Dufour ex A. DC.
Legousia falcata (Ten.) Jauchen
L. hybrida (L.) Delarbre
L. speculum-veneris (L.) Chais
Solenopsis antiphonitis Hadjik. & Hand. **(E)**
S. bivonae (Tineo) M. B. Crespo, Serra & A. Juan
Cannabaceae
Cannabis sativa L.
Celtis australis L.
Capparaceae
Capparis spinosa L.
Caprifoliaceae
Lonicera etrusca Santi
Caryophyllaceae
Arenaria leptoclados (Reichb.) Guss.
A. pamphylica Boiss. et Heldr. subsp. *pamphylica*
A. pamphylica Boiss. et Heldr. subsp. *kyrenica* McNeill
Cerastium brachypetalum Pers.
C. comatum Desv.
C. dichotomum L.
C. glomeratum L.
C. illyricum Ard.
Dianthus cyprius A.K. Jackson et Turill **(E)**
D. strictus Banks et Sol. subsp. *troodi* (Post.) Burdet & Greuter **(E)**
D. tripunctatus Sibth. et Sm.
Gypsophila linearifolia (Fisch. & C. A. Mey.) Boiss.
G. pilosa Huds.
Herniaria cinerea DC.
H. hemistemon J. Gay
H. hirsuta L.
Kohlrauschia velutina (Guss.) Reichb.
Minuartia geniculata (Poir.) Thell.
M. globulosa (Labill.) Schinz & Thell.
M. hybrida (Vill.) Schischk.
M. intermedia (Boiss.) Hand.-Mazz.
M. mediterranea (Ledeb.) K.Mel
M. picta (Sibth. et. Sm.)Bornm.
M. thymifolia (Sibth. et. Sm.) Bornm.
Paronychia argentea Lam.
P. macrosepala Boiss.
Petrorhagia cretica (L.) P.W.Ball
P. dubia (Raf.) G. López & Romo
P. kennedyae (A. K. Jacks. & Turrill) P. W. Ball & Heywood
Polycarpon tetraphyllum L.
Pteranthus dichotomus Forssk.
Rhodalsine geniculata (Poir.) F. N. Williams
Sagina apetala Ard.
S. bocconii (Scheefe) Ascher.
S. marina (L.) Griseb.
S. maritima G. Don
Saponaria mesogitana Boiss.
Spergularia bocconii (Scheele) Asch. & Graebn.
S. diandra (Guss.) Sart. & Heldr.
S. marina (L.) Besser
Silene aegyptiaca (L.) L.f.
S. alexandrina (Asch.) Danin
S. behen L.
S. colorata Poir.
S. conoidea L.
S. cretica L.
S. discolon Sibth. et Sm
S. fraudatrix Meikle **(E)**
S. fruticosa L.
S. fuscato Link
S. galataea Boiss.
S. gallica L.
S. gigantea L.
S. kotschyi Boiss.
S. laevigata Sm.
S. longipetala Vent.
S. macrodonta Boiss.
S. nocturna L.
S. rubella L.
S. sedoides Poir.
S. tridentata Desf.
S. vulgaris (Moench) Garcke
Stellaria apetala Ucria
S. cilicica Boiss. & Balansa
S. cupaniana Jord & Fourr
Vaccaria pyramidata Medik.
V. hispanica (Mill.) Rauschert subsp. *hispanica*
Velezia rigida L.
Casuarinaceae
Casuarina equisetifolia L.
Chenopodiaceae
Arthrocnemum macrostachyum (Moric.) K. Koch
Atriplex davisii Aellen
A. halimus L.
A. patula L.
A. portulacoides L.
A. prostrata DC.
A. rosea L.
A. tatarica L.
Bassia indica (Wight) A. J. Scott
Beta adanensis Pamukç.
B. macrocarpa Guss.
Chenopodium album L.
C. botrys L.
C. murale L.
C. opulifolium Koch
C. vulvaria L.
Dysphania botrys (L.) Clemants & Mosyakin
Halimione portulacoides (L.) Aellen
Halocnemum strobilaceum (Pall.) M. Bieb.
Halopeplis amplexicaulis (Vahl.) Cesati
Noaea mucronata (Forssk.) Aschers.

Salicornia europaea L.
S. fruticosa L.
S. macrostachya Mori.
S. vera Forssk.
Salsola inermis Forssk.
S. kali L. subsp. *kali*
S. kali L. subsp. *ruthenica* Soo.
S. soda L.
S. tragus L. subsp. *pontica* (Pall.) Rilke
S. tragus L. subsp. *tragus*
Sarcocornia fruticosa (L.) A. J. Scott
S. perennis (Mill.) A. J. Scott
Suaeda aegyptiaca (Hasselq.) Zohary
S. maritima (L.) Dumort. subsp. *maritima*
S. vera Forssk. ex J. F. Gmel.
Cistaceae
C. creticus L. subsp. *creticus*
C. creticus L. subsp. *eriocephalus* (Viv.) Greuter & Burdet
C. monspeliensis L.
C. parviflorus Lam.
C. ×pauranthus
C. salviifolins L.
C. monspeliensis × *parviflorus*
Fumana arabica (L.) Spach
F. laevis (Cav.) Pau
F. thymifolia (L.) Verlot.
Helianthemum aegyptiacum (L.) Mill.
H. ledifolium (L.) Mill. subsp. *lasiocarpum* (Jacq. & Hérincq) Nyman
H. ledifolium (L.) Mill. subsp. *ledifolium*
H. obtisifolium Dunal **(E)**
H. salicifolium (L.) Mill.
H. stipulatum (Forssk.) C. Christens
H. syriacum (Jacq.) Dum.-Cours.
Tuberaria guttata (L.) Fourr.
T. inconspicua (Thib. ex Pers.) Willk.
Cleomaceae
Cleome iberica DC.
C. ornithopodioides L.
Convolvulaceae
Calystegia sepium (L.) R. Br.
Convolvulus althaeoides L.
C. arvensis L.
C. althaeoides L.
C. betonicifolius Mill.
C. coelesyriacus Boiss.
C. dorycnium L.
C. humilis Jacq.
C. oleifolium Desr.
C. pentapetaloides L.
C. siculus L.
Cressa cretica L.
Cuscuta campestris Yuncker
C. monogyna Vahl
C. palaestina Boiss.
C. planiflora Ten.
Ipomoea imperati (Vahl) Griseb.
I. indica (Burm.) Merill
I. purpurea (L.) Roth
I. sagitata Poir.
I. stolinifera (Cyr.) J. F. Gmel.
Crassulaceae
Crassula alata (Viv.) Berger
C. vaillantil (Willd.) Roth
Rosularia cypria (Holmboe) Meikle
R. globulariifolia (Fenzl) A. Berger
R. pallidiflora (Holmboe) Meikle **(E)**
Sedum aetnense Tineo
S. caespitosum (Cav.) DC.
S. eriocarpum Sm. subsp. *porphyreum* (Kotschy) 't Hart **(E)**
S. lampusae (Kotschy) Boiss. **(E)**
S. litoreum Guss.
S. microcarpum (Sm.) Schönland
S. sediforme (Jacq.) Pau.
Telmissa microcarpa (Sm.) Boiss.
Umbilicus horizontalis (Guss.) DC
U. rupestris (Salisb.) Dandy
Cucurbitaceae
Bryonia cretica L.
Citrullus colocynthis (L.) Schrad.
Ecballium elaterium (L.) A.Rich.
Cytinaceae
Cytinus hypocistis L.
Datiscaceae
Datisca cannabina L.
Dipsacaceae
Cephalaria syriaca (L.) Schrader
Lomelosia brachiata (Sm.) Greuter & Burdet
L. divaricata (Jacq.) Greuter & Burdet
L. prolifera (L.) Greuter & Burdet
Pterocephalus brevis Coult.
P. multiflorum Poech subsp. *Multiflorum* **(E)**
P. multiflorum subsp. *obtusifolium* Holmboe **(E)**
Scabiosa brachiata Sm.
S. sicula L.
S. prolifera L.
Elaeagnaceae
Elaeagnus angustifolia L.
Ericaceae
Arbutus andrachne L.
Erica manipuliflora Salisb.
E. sicula Guss.
Euphorbiaceae
Andrachne telephioides L.
Chrozophora obliqua (Vahl) Spreng.
C. tinctoria (L.) Rof.
Euphorbia aleppica L.
E. arguta Banks et Sol.
E. berythea Boiss. & C. I. Blanche
E. cassia Boiss.
E. chamaepeplus Boiss.
E. chamaesyce L.
E. dimorphocaulon P. H. Davis
E. exigua L.
E. falcata L. subsp. *falcata*
E. falcata L. subsp. *macrostegia* (Bornm.) O. Schwarz
E. helioscopia L.

E. hirsuta L.
E. hirta L.
E. nutans Lag.
E. paralias L.
E. peplis L.
E. peplus L.
E. petiolata Banks & Sol.
E. pubescens Vahl.
E. sintenisii Freyn
E. terracina L.
E. valerianifolia Lam.
Mercurialis annua L.
Ricinus communis L.
Fabaceae (Leguminosae)
Acacia farnesiana Willd.
A. karroo Hayne
A. saligna (Labill.) H. L. Wendl.
Alhagi maurorum Medik. subsp. *graecorum* (Boiss.) Awmack & Lock
A. maurorum Medik. subsp. *maurorum*
Anagyris foetida L.
Argyrolobium uniflorum (Becne.) Jaub.
Astragalus asterias Ledeb.
A. boeticus L.
A. caprinus L. subsp. *caprinus*
A. cyprius Boiss. **(E)**
A. epiglottis L.
A. hamosus L.
A. palecinus L.
A. sinaicus Boiss.
A. suberosus Banks. & Sol.
Bauhinia variegata L.
Bituminaria bituminosa (L.) C. H. Stirt.
Calycotome villosa (Poir.) Link
Ceratonia siliqua L.
Cersis siliquastrum L.
Cicer arietinum L.
C. repanda (Poir.) Guss. subsp. *repanda*
C. scorpioides (L.) Koch
Dorycnium graecum (L.) Ser.
D. rectum (L.) Ser.
Erophaca baetica (L.) Boiss.
E. baetica (L.) Boiss. subsp. *orientalis* (Chater & Meikle) Podlech
Genista fasselata Decne.
Glycyrrhiza glabra L.
Hedysarum cyprium Boiss. **(E)**
H. spinosissimum L.
Hippocrepis ciliata Willd.
H. emerus (L.) Lassen
H. multisiliquosa L.
H. unisiliquosa L. subsp. *bisiliqua* (Forskl.) Bornm.
H. unisiliquosa L. subsp. *unisiluqosa*
Hymenocarpos circinnatus (L.) Savi
Lathyrus annuus L.
L. aphaca L.
L. blepharicarpos Boiss.
L. cicera L.
L. gorgonei Parl.
L. ochrus (L.) DC.
L. sativus L.
L. saxatilis (Vent.) Vis.
L. setifolius L.
L. sphaericus Retz.
Lens culinaris Medik.
L. ervides (Brign.) Grande
L. orientalis (Boiss.) Hand.-Maz
Lotus edulis L.
L. corniculatus L.
L. cytisoides L.
L. halophilus Boiss. & Spruner
L. longisiliquosus R. Roem.
L. ornithopodioides L.
L. palustris Willd.
L. peregrinus L.
L. tenuis Willd.
L. tetragonolobus L.
Lupinus micranthus Guss.
Medicago acicularis (L.) Mill.
M. arabica (L.) Huds.
M. bianchaena Boiss.
M. bonarotiana Arcang.
M. ciliaris (L.) All.
M. constricta Dur.
M. cornuta (L.) Bartal
M. disciformis DC.
M. hypogaea E. Small.
M. intertexta (L.) Mill.
M. littoralis Lois.
M. lupulina L.
M. marina L.
M. minima (L.) Bartal
M. monspeliaca (L.) Trautv.
M. orbicularis (L.) Bartal
M. polymorpha L.
M. praecox DC.
M. rigidula (L.) All.
M. rotata Boiss.
M. rugosa Desr.
M. sativa L.
M. scutellata (L.) Mill.
M. truncatula Gaertn.
M. turbinata (L.) All.
Melilotus indicus (L.) All.
M. italicus L.
M. messanensis (L.) All.
M. siculus (Turra) B. D. Jacks.
M. sulcatus Desf.
Onobrychis aequidentata (Sibth. et. Sm.) d'Urv.
O. caput-galli (L.) Lam
O. cristata-galli (Murr.) Lam.
O. venosa (Desf.) Desv. **(E)**
Ononis biflora Desf.
O. diffusa Ten.
O. mitissima L.
O. ornithopodioidea L.
O. pubescens L.
O. pusilla L.
O. reclinata L.
O. serrata Forssk.

O. sicula Guss.
O. spinosa L. subsp. *leiosperma* (Boiss.) Sirj.
O. variegeta L.
O. viscosa L. subsp. *breviflora* (DC.) Nyman
Ornithopus compresus L.
Parkinsonia aculeata L.
Pisum sativa L. subsp. *biflorus* (Raf.) Soldano
Prosopis farcta (Banks et Sol.) Macbride
Robinia pseudoacacia L.
Scorpirus muricatus L.
Securigera parviflora (Desv.) Lassen
S. securidaca L.
Sulla spinosissima (L.) B. H. Chol & H. Ohashi
Trifolium angustifolium L.
T. boissieri Soyer-Willamet.
T. campestre Schreb. subsp. *campestre*
T. cherieri L.
T. clypeatum L.
T. dasyurum C. Presl.
T. diffusum Ehrh.
T. echinatum M. Bieb.
T. fragiferum L. subsp. *bonannii* (C. Presl.) Sojak
T. globosum L.
T. lappaceum L.
T. leucanthum M. Bieb.
T. nigrescens Viv. subsp. *petrisavii* (Clem.) Holmboe
T. pamphylicum Boiss. et. Heldr.
T. pilulare Boiss.
T. repens L.
T. resupinatum L.
T. scabrum L.
T. sculatum Boiss.
T. spumosum L.
T. stellatum L.
T. striatum L.
T. suffocatum L.
T. tomentosum L.
Trigonella berythea Boiss.
T. cariensis Boiss.
T. foenum-graecum L.
T. monspeliaca L.
T. spicata Sibth.
T. spinosa L.
T. spruneriana Boiss.
T. strangulata Boiss.
Tripodion tetraphyllum (L.) Fourr. subsp. *tetraphyllum*
Vicia amphicarpa L.
V. angustifolia L.
V. assyriaca Boiss.
V. bithynica L.
V. cretica Boiss.
V. cypria Kotschy.
V. ervilla (L.) Willd.
V. faba L.
V. hybrida L.
V. johannis Tamamsh.
V. lathyroides L.
V. laxiflora Brot.
V. lunata (Boiss. et Bal.)
V. monantha Retz. subsp. *monantha*
V. narbonensis L.
V. palestina Boiss.
V. pannonica Crantz
V. parviflora Cav.
V. peregrina L.
V. pubescens (DC.) Link
V. sativa L. subsp. *sativa*
V. villosa Roth. subsp. *eriocarpa* (Hauskkn.) P. W. Ball

Fagaceae
Quercus coccifera L.
Q. infectoria Oliv.

Frankeniaceae
Frankenia hirsuta L.
F. pulverulenta L.

Gentianaceae
Blackstonia acuminata (Koch et Ziz) Domin subsp. *acuminata*
B. perfoliata (L.) Hudson subsp. *intermedia* (Ten.) Zeltner
Centaurium erythraea Rafn subsp. *rhodense* (Boiss & Reut.) Melderis
C. mairei Zeltner
C. maritimum (L.) Fritsch
C. pulchellum (Swartz) Druce subsp. *pulchellum*
C. tenuiflorum (Hoff et Link) Fritsch

Geraniaceae
Erodium botrys (Cav.) Bertol.
E. ciconium (L.) L. 'Herit
E. cicutarium (L.) L'Herit subsp. *cicutarium*
E. crassifolium subsp. *crassifolium* L. 'Herit
E. gruinum (L.) L. 'Herit
E. laciniatum (Cav.) Willd.
E. malacoides (L.) Willd.
E. moschatum (L.) L. 'Herit
E. touchyanum Godr.
Geranium columbinum L.
G. dissectum L.
G. lucidum L.
G. molle L.
G. purpureum Vill.
G. pusillum Burm.
G. rotundifolium L.
G. tuberosum L.

Haloragaceae
Myriophyllum spicatum L.

Hypericaceae
Hypericum empetrifolium Willd.
H. hircinum L.
H. lanuginosum Lam.
H. perforatum L. subsp. *veronense* (Schrank) H. Lindb.
H. repens L. **(E)**
H. triquetrifolium Turra.

Juglandaceae
Juglans regia L.

Lamiaceae (Labiatae)
Acanthoprasium integrifolium (Benth.) Ryding **(E)**

Acinos exiguus (Sm.) Meikle **(E)**
Ajuga chamaepitys Schreber subsp. *palastina* (Boiss.) Bornm.
A. chamaepitys Schreber subsp. *cypria* P.H. Davis
A. iva Schreber
Ballota nigra L. subsp. *rudelaris* (Sw.) Briq.
Calamintha incana (Sm.) Benth
Dracocephalum triflorum L.
Lamium amplexicaule L.
L. garganicum L. subsp. *garganicum*
L. moschatum Mill. subsp. *moschatum*
L. moschatum Mill. subsp. *micranthum* (Boiss.) Mennema
Lavandula stoechas L.
Marrubium vulgare L.
Melissa officinalis L.
Mentha aquatica L.
M. longifolia L. subsp. *cypria* (Heinr. Braun) Harley **(E)**
M. pulegium L.
M. spicata L. subsp. *condensata* (Briq.) Harley
Micromeria microphylla (Urv.) Benth
M. myrtifolia Boiss. & Hohen.
M. nervosa (Desf.) Benth
Moluccella laevis L.
M. spinosa L.
Origanum laevigatum Boiss.
O. majorana L. var. *tenuifolium* Weston **(E)**
O. onites L.
O. syriacum L.
Phlomis brevibracteata Turrill
P. cypria L. subsp. *cypria*
P. fruticosa L.
Prasium majus L.
Rosmarinus officinalis L.
Salvia fruticosa Mill.
S. hierosolymitana Boiss.
S. lanigera Poir.
S. pinnata L.
S. veneris Hedge **(E)**
S. verbenaca L.
S. viridis L.
Satureja thymbra L.
Scutellaria sibithorpii (Benth) Hal. **(E)**
Sideritis curvidens Stapf.
S. cypria Post. **(E)**
Stachys cretica L.
Teucrium creticum L.
T. cyprium Boiss.
T. divaricatum Heldreich subsp. *canescens* (Celak.) Holmboe **(E)**
T. karpasiticum Hadjik & Hand **(E)**
T. kyreniae (P. H. Davis) Hadjik & Hand **(E)**
T. micropodioides Rouy **(E)**
T. salaminium Hadjik & Hand **(E)**
T. scordium L. subsp. *scordioium*
T. scordium L. subsp. *scordioides* (Schreb.) Arcang.
T. scordium L. subsp. *scordium*
Thymbra capitata (L.) Cav.
Thymus capitatus (L.) Hoffsgg.
T. integer Griseb. **(E)**
Vitex angus-castus L.
Wiedemannia orientalis Fisch. & C. A. Mey.
Ziziphora capitata L.

Lauraceae
Laurus nobilis L.

Linaceae
Linum bienne Mill.
L. corymbulosum Reichb.
L. grandiflorum Desf.
L. nodiflorum L.
L. pubescens Banks & Sol.
L. strictum L. subsp. *spicatum* (Pers.) H. Lindb.
L. trigynum L.
L. usitatissimum L.

Lythraceae
Lytrum hyssopifolia L.
L. junceum Banks et. Sol.
L. tribracteatum Sprengel
Punica granatum L.

Malvaceae
Alcea acaulis (Cav.) Alef.
Althaea hirsuta L.
A. setosa Boiss.
Corchorus olitarius L.
Hibiscus trionum L.
Malva aegyptia L.
M. cretica Cav.
M. multiflora (Cav.) Soldano et al.
M. nicaeensis All.
M. parviflora L.
M. punctata (All.) Alef.
M. sylvestris L.
M. verticillata L.
Malvella sherardiana (L.) Jaub. & Spach

Meliaceae
Melia azedarach L.

Molluginaceae
Glinus lotoides L.

Moraceae
Ficus carica L.
F. cycomorus L.
Morus alba L.

Myrtaceae
Eucalyptus camaldulensis Dehnhardt
E. gomphocephala DC.
E. tereticornis Sm.
E. torquata Luehm.
Myrtus communis L.

Neuradaceae
Neurada procumbens L.

Nitrariaceae
Peganum harmala L.

Oleaceae
Olea europaea L.
Phillyrea latifolia L.

Onagraceae
Epilobium hirsutum L.

Orobanchaceae
Bellardia trixago (L.) All.
Odontites cyprius Boiss. **(E)**
Orobanche aegyptiaca Pers.
O. alba Willd.
O. crenata Forssk.
O. minor Sm.
O. mutelii F. W. Schulz.
O. orientalis Beck-Managetta
O. pubescens d'Urv.
O. ramosa L.
Parentucellia latifolia (L.) Cuatrec.
Oxalidaceae
Oxalis corniculatis L.
O. pes-caprae L.
Papaveraceae
Ceratocapnus palaestinus Boiss.
Fumaria bracteosa Pomel.
F. capreolata L.
F. densiflora DC.
F. gaillardotii Boiss.
F. judaica Boiss.
F. macrocarpa Parl. subsp. *macrocarpa*
F. parviflora Lam.
Glaucium corniculatum (L.) J.H. Rudolph
G. flavum Crantz. subsp. *leiocarpum* (Boiss.) Author
Hypecoum imberbe Sm.
H. pendulum L.
H. procumbens L. subsp. *procumbens*
Papaver cyprium (Chrtek & B. Slavik) M. V. Agab., Christodoulou & Hand. **(E)**
P. gracile Boiss.
P. hybridum L.
P. rhoeas L. subsp. *rhoeas*
P. setigerum DC.
Roemeria hybrida (L.) DC.
Passifloraceae
Passiflora caerulea L.
Pedaliaceae
Sesamum indicum L.
Phytolaccaceae
Phytolacca americana L.
P. dioica L.
Plantaginaceae
Antirrhinum majus L.
Callitriche brutia Petagna
C. pulchra Schotsm.
Chaenorhinum gerense (Stapf) Speta
C. rubrifolium (DC.) Fourr.
Cymbalaria longipes (Boiss. & Heldr) Chev.
Kickxia commutata (Reichb.) Fritsch subsp. *graeca* (Bory & Chaub) R. Fernandes
K. elatine (L.) Dumort. subsp. *sieberi* (Rchb.) Hayek
K. lanigera (Desf.) Hand-Mazz
Linaria albifrons (Sm.) Spreng.
L. chalepensis (L.) Mill.
L. haelava (Forrsk.) Del.
L. micrantha (Cav.) Hoffsgg.
L. pelisseriana (L.) Mill.
L. simplex Desf.
Misopates orontium (L.) Raf.
Plantago afra L.
P. albicans L.
P. amplexicaulis Cav.
P. bellardii All.
P. coronopus L. subsp. *coronopus*
P. cretica L.
P. lagopus L.
P. lanceolata L.
P. major L.
P. maritimus L.
P. notata Lag.
P. ovata Forssk.
P. sarcophylla Zohary
P. squarrosa Murr.
Veronica anagallis-aquatica L.
V. arvensis L.
V. cymbalaria Bodard
V. polita Fries
Platanaceae
Platanus orientalis L.
Plumbaginaceae
Limonium albidum (Guss.) Pignatti
L. ammochostianum Erben, Christodoulou, Hand & Kefalas **(E)**
L. aucheri (Girard) Greuter & Burdet
L. aucheri x virgatum
L. avei (De Not.) Brullo & Erber
L. cyprium (Meikle) Hand & Buttler **(E)**
L. echioides (L.) Mill.
L. karpasiticum Kefalas, Erben, Christodoulou & Hand **(E)**
L. meyeri (Boiss.) O. Kuntze
L. mucronulatum (H. Lindb.) Greuter&Burdet **(E)**
L. sinuatum (L.) Mill.
L. virgatum (Willd.) Fourr.
Plumbago europaea L.
Polygalaceae
Polygala monspeliana L.
P. venulose Sm.
Polygonaceae
Emex spinosa (L.) Campdera
Persicaria lapathifolium (L.) Delarbre subsp. *lapathifolium*
Polygonum aviculare L.
P. equisetiforme Sm.
P. maritinum L.
P. salicifolium Willd.
Rumex bucephalophorus L. subsp. *bucephalophorus*
R. bucephalophorus L. subsp. *gallicus* (Steinh.) Rech. f.
R. conglomeratus Murr.
R. crispus L. subsp. *crispus*
R. cyprius Murb.
R. dentatus L. subsp. *mesopotamicus* Rech. f.
R. pulcher L.

Portulucaceae
Portulaca cypria Danin
P. granulata – stellulata (Poellin.) Ricceri & Arrigoni
P. nitida (H. Baker & Danin) Arrigoni & Ricceri
P. oleracea L.
P. rausii Danin
P. sativa Haw.
P. trituberculata Danin et al.
Primulaceae
A. arvensis L. var. *arvensis*
A. arvensis L. var. *caerulea* Gouan
A. arvensis L. var. *foemina* (Mill.) Schinz et Thell
Androsace maxima L.
Astrolinon linum-stellatum (L.) Duby
Cyclamen cyprium Kotsch **(E)**
C. graecum Link subsp. *anatolicum* Ietsw.
C. persicum Mill.
Lysimachia dubia Sol.
Samolus valerandi L.
Punicaceae
Punica granatum L.
Ranunculaceae
Adonis annua L.
A. dentata Del.
A. microcarpa DC.
Anemone blanda Schott & Kotschy
A. coronaria L.
Ceratocephala falcata (L.) Pers.
Clematis cirrhosa L.
Delphinium caseyi B.L. Burtt **(E)**
D. peregrinum L.
Ficaria chrysocephala (P. D. Sell) Galasso & al.
Nigella ciliaris DC.
N. domascena L.
N. fumariifolia Kotschy
Nigella nigellastrum (L.) Willk.
N. sativa L.
Ranunculus arvensis L.
R. asiaticus L.
R. bullatus L.
R. chius DC.
R. constatinopolitanus (DC.) Urv.
R. cornutus DC.
R. cytheraeus (Halacsy) Baldini
R. isthmicus Boiss.
R. marginatus Urv.
R. millefoilatus Vahl. subsp. *millefoilatus*
R. millefoilatus Vahl. subsp. *leptaleus* (DC.) Meikle **(E)**
R. millefolius Banks
R. muricatus L.
R. neopolitanus Ten.
R. paludosus Poir.
R. peltatus Schrank subsp. *fucoides* (Freyn) Munoz Garm.
P. sphaerospermus Boiss. & C. I. Blanche
Staphisagria macrosperma Spach
Resedaceae
Reseda alba L.
R. lutea L.
R. luteola L.
R. minoica Martin-Bravo & Jim.
R. orientalis (Muell. Arg.) Kotschy
Rhamnaceae
Rhamnus alaterius L.
R. oleoides L.
R. lycioides L. subsp. *graeca* (Boiss. & Reut.) Tutin
Ziziphus lotus (L.) Lam.
Z. spina-christi (L.) Willd.
Z. zizyphus (L.) Meikle
Rosaceae
Aphanes arvensis L.
Crateagus azarolus L.
C. monogyna Jacq.
Eribotrya japonica Lindl.
Potentilla reptans L.
Poterium verrucosum G. Don
Pyrus syriaca Boiss.
Rubus sanctus Schreb.
Sarcopoterium spinusum (L.) Spach
Rubiaceae
Asperula arvensis L.
A. cypria Ehrend. **(E)**
A. stricta Boiss.
Crucianella aegyptiaca Boiss.
C. latifolia L.
C. macrostachya Boiss.
Cruciata articulata (L.) Ehrend.
Galium aparine L.
G. canum Req.
G. divaricatum Lam.
G. humifusum M. Bieb.
G. murale (L.) All
G. pisiferum Boiss.
G. setaceum Lam.
G. tricornutum Dandy
G. verrucosum Huds.
Rubia laurae (Holmboe) Airy-Shaw **(E)**
R. tenuifolia d'Urv.
R. tinctorium
Sherardia arvensis L.
Theligonum cynocrambe L.
Valantia hispida L.
V. muralis L.
Rutaceae
Haplophyllum buxbaumii (Poir.) G. Don
Ruta chalepensis L.
Salicaceae
Populus alba L.
P. nigra L.
Salix alba L.
Santalaceae
Osyris alba L.
Thesium humile Vahl.
Sapindaceae
Acer obtisifolium Sibth. et. Sm
Dodonaea viscosa (L.) Jacq.

Saxifragaceae
Saxifraga hederacea L.
S. tridactylites L.
Scrophulariaceae
Limosella aquatica L.
Scrophularia peregrina L.
S. peyronii Post
Verbascum levanticum I. K. Ferguson
V. orientale (L.) All.
V. sinuatum L.
Simaraubiaceae
Ailanthus altissima (Mill.) Swingle
Solanaceae
Cestrum nocturnum L.
Datura innoxia Mill.
D. stramonium L.
Hyoscyamus albus L.
H. aureus L.
Lycium ferocissimum Miers
L. schweinfurthii U. Dammar
Mandragora officinarum L.
Nicotiana glauca Graham
Physalis angulata L.
Solanum angustifolium Mill.
S. cornutum Mill.
S. elaeagnifolium Cav.
S. nigrum L.
S. villosum Mill.
Withania somnifera Dunal
Styracaceae
Styrax officinalis L.
Tamaricaceae
Tamarix aphylla (L.) H. Karst.
T. hampeana Boiss. ex Heldr.
T. smyrnensis Bunge.
T. tetragyna Ehrenb.
T. tetrandra M. Bieb.
Thymelaeaceae
Thymelaea hirsuta (L.) Endl.
T. passerina (L.) Coss. & Germ. subsp. *pubescens* (Guss.) Meikle
T. tartonraira All. subsp. *argentea* (Sm.) Holmboe
Ulmaceae
Celtic australis L.
Ulmus canescens Melville
Zelkova abelicea (Lam.) Boiss.
Urticaceae
Parietaria cretica L.
P. judaica L.
P. lusitanica L.
Urtica cypria (H. Lindb.) Hand
U. membranacea Poir.
U. pilulifera L.
U. urens L.
Valerianaceae
Centranthus calcitrapa (L.) Dufr. subsp. *orbiculatus* (Sm.) Meikle **(E)**
C. ruber (L.) DC
Valeriana italica Lam.
Valerianella coronata (L.) DC.
V. discoides (L.) Lois.
V. echinata (L.) DC
V. lasiocarpa (Stev.) Betcke
*V. muralis (*Stev.) Baxt.
V. muricata (Stev.) Baxt.
V. orientalis (Schlech.) Boiss.
V. vesicaria (L.) Moench
Verbanaceae
Lantana camara L.
Phyla canescens (Kunth) Greene
P. nodiflora (L.) Greene
Verbena officinalis L.
V. supina L.
Vitex agnus-castus L.
Zygophyllaceae
Peganum harmala L.
Tetraena alba (L. f.) Beler & Thulin
Tribulus terrestris L.

Cyprus is a hotspot in the Mediterranean region and number of the endemic taxa are about 143 within the whole island (Hand *et al.*, 2011). The table given below is the list of taxa that naturally grow in the northern part of the island of Cyprus, in Northern Cyprus, according to our current knowledge. In the list, those with distribution in both the northern and southern parts are indicated in black and those that grow only in Northern Cyprus are indicated in **red**.

Table 5: Checklist of endemic taxa in Northern Cyprus.

Amaranthaceae
Bosea cypria

Amaryllidaceae
Allium autumnale
A. cupani subsp. *cyprium*
A. cyprium subsp. *lefkarense*
A. willeanum

Apiaceae
Bupleurum sintenisii
Ferulago cypria
Pimpinella cypria
Scaligeria alziarii

Asparagaceae
Hyacinthella millingenii
Ornithogalum pedicellare
Scilla morrisii

Asteraceae
Anthemis tricolor
Carlina pygmaea
Centaurea calcitrapa subsp. *angusticeps*
Onopordum cyprium
Ptilostemon chamaepeuce subsp. *cyprius*
Scorzonera troodea
Senecio glaucus subsp. *cyprius*
Taraxacum aphrogenes

Boraginaceae
Onosma caespitosa
O. fruticosa

Brassicaceae
Arabis cypria
A. kennedyae
Brasica hilarionis

Campanulaceae
Solenopsis antiphonitis

Caryophyllaceae
Dianthus cyprius
D. strictus subsp. *troodi*
Petrorhagia kennedyae
Silene fraudatrix

Cistaceae
Helianthemum obtusifolium

Colchicaceae
Colchicum troodi

Crassulaceae
Rosularia pallidiflora
Sedum eriocarpum subsp. *porphyreum*
S. lampusae

Cyperaceae
Carex cyprica

Dipsacaceae
Pterocephalus multiflorus subsp. *multiflorus*
P. multiflorus subsp. *obtusifolius*

Fabaceae
Astragalus cyprius
Hedysarum cyprium
Onobrychis venosa

Hypericaceae
Hypericum repens

Iridaceae
Crocus hartmannianus
C. veneris
Gladiolus triphyllus

Lamiaceae
Acanthoprasium integrifolium
Acinos exiguus
Mentha longifolia subsp. *cyprica*
Origanum majorana var. *tenuifolium*
Phlomis brevibracteata
Salvia veneris
Scutellaria sibthorpii
Sideritis cypria
Teucrium divaricatum subsp. *canescens*
T. karpasiticum
T. kyreniae
T. micropodioides
T. salaminium
Thyms integer

Liliaceae
Tulipa cypria

Orchidaceae
Ophrys argolica subsp. *elegans*

Orobanchaceae
Odontites cyprius

Papaveraceae
Papaver cyprium

Plumbaginaceae
L. ammochostianum
L. cyprium
L. karpasiticum
L. mucronulatum

Poaceae
Bromus bidentatus
Rostraria hadjikyriakou

Primulaceae
Cyclamen cyprium

Ranunculaceae
Delphinium caseyi
Ranunculus millefoliatus subsp. *leptaleus*

Rubiaceae
Asperula cypria
Rubia laurae

Valerianaceae
Centranthus calcitrapa subsp. *orbiculatus*

*The endemic taxa with distribution in both the northern and southern parts are indicated in black; and those that distributed only in Northern Cyprus are indicated in red.

CONCLUSION

In this paper, the checklist of wild vascular taxa occurring in the Northern Cyprus has been presented (Tables 1, 2, 3, and 4). The list comprises of 1564 wild vascular taxa that was obtained through the data from all of the published documents. The list was updated according to 'The Plant List' and 'IPNI'. The statistical values for the richest families consisting of endemic taxa and total endemism percentage was updated accordingly as presented in Figure 3 and 4, respectively.

Table 6: The five richest families of endemic taxa in the Northern Cyprus flora.

Family	Total taxa	Endemic taxa	Endemism (%)
Lamiaceae	61	14	22.95
Asteraceae	175	8	4.57
Amaryllidaceae	24	4	16.67
Caryophyllaceae	67	4	5.97
Apiacaeae	72	4	5.55

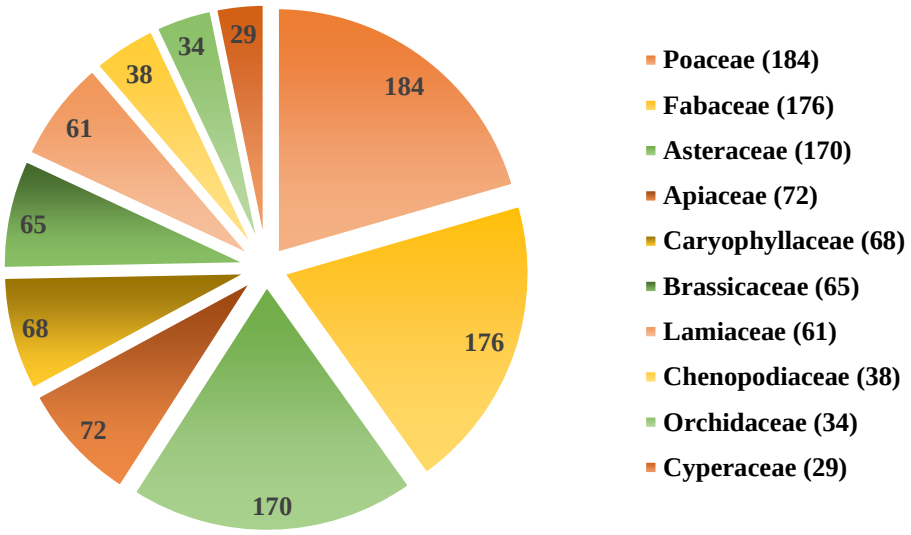

Figure 3: The ten richest families within Northern Cyprus in terms of species diversity.

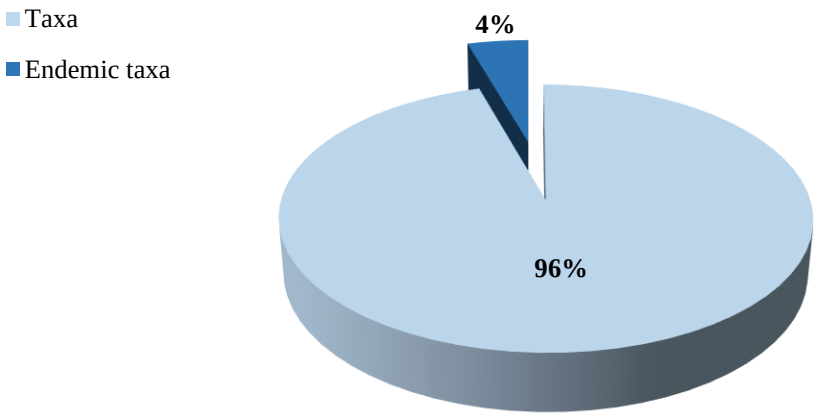

Figure 4: The percentage of endemic taxa compared to the total number of taxa within Northern Cyprus (73 endemic taxa in comparison to 1564 total taxa).

In this updated list of the vascular plants of the Northern Cyprus, there are total of 75 endemic taxa (Table 5) of which 21 of them have been named after Cyprus as carrying *cypria*, *cyprium* or *cyprius* epithets (Viney, 1992; Yildiz and Guzel, 2008). The five richest genera for endemic taxa in the Northern Cyprus area was shown to represent an overall endemism richness (Table 6). To sum up, according to our current knowledge, 15 taxa of 75 endemic taxa are distributed only in northern part of the island, within Northern Cyprus, and the rest are distributed both in north and south regions.

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The website Dynamic checklist the flora of Cyprus has made a great contribution to the preparation of this list. We would also like to thank those who prepared this web page. Hand R., Hadjikyriakou G. N. & Christodoulou C. S. (ed.) 2011– (continuously updated): Flora of Cyprus – a dynamic checklist. Published at http://www.flora-of-cyprus.eu.

Also, on the 100th anniversary of his birth, we would also like to commemorate Dr.Viney. His two volumes ‘’An Illustraded Flora of North Cyprus ‘’ introducing the plants growing in Northern Cyprus is extremely useful.

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Influence of carnauba wax on the release profile of ibuprofen implants

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Abstract

Pharmaceutical implants are small sterile solid masses usually cylindrical consisting of a highly potent and purified drug intended to be subcutaneously implanted beneath the skin by suitable special injector or by surgical incision for the purpose of providing the continuous release of the active medicament over a prolonged period of time. The purpose of this study was to evaluate the influence of carnauba wax on the release profile of ibuprofen implants. The implants were prepared with gelatin, hydroxypropyl methylcellulose admixture (80:20) and varying amount of carnauba wax (2.5%, 5%, 7.5%) using the solvent casting technique. Another batch of the implant was formulated without the incorporation of carnauba wax. Glycerin was used as the plasticizing agent. The physicochemical properties and the release kinetics of the implants were evaluated. The implant pellets had a similar appearance with minimal batch to batch variation. The mean diameter/thickness of the implants ranged from 2.46±0.10-2.86±0.03 mm, the percentage drug content was ≤96.92±0.12% and the swelling index values were between 2.68±0.01 – 4.87±0.01%. The rate of drug release from the ibuprofen implants was significantly affected by the incorporation of carnauba wax. The higher the amount of carnauba wax incorporated in the formulation, the more retarded the rate of drug release. This can be exploited in the formulation of sustained release ibuprofen implants for the management of chronic diseases such as arthritis.

Keywords

Carnauba wax, ibuprofen, subcutaneous, implant.

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INTRODUCTION

Pharmaceutical implants are small sterile solid masses containing a highly potent and purified active pharmaceutical ingredient that are intended to be subcutaneously implanted beneath the skin using a suitable special injector or surgical incision to provide continuous release of the active medicament over a long period of time (Wang *et al.,* 2010; Rajgor *et al.,* 2011). Implants have several advantages such as convenience, improved drug delivery, improved adherence to therapy, reduction in the frequency of dosing, potential for zero order controlled release, flexibility in therapy termination, potential for bio-responsive release and flexibility in the choice of polymers as well as the method of manufacture (Alissa *et al.,* 2009; Isesele *et al.,* 2021).

Implants have been used therapeutically in cancer chemotherapy, dental applications, immunization, as ocular drug delivery systems in the treatment of ocular diseases such as glaucoma (e.g., an ocular insert containing pilocarpine) and in the formulation of some long-acting contraceptives such as levonorgestrel, which is used to prevent pregnancy (Tian *et al.,* 2012, Mohammed *et al.,* 2012).

Carnauba wax is a vegetable wax obtained from the fronds of the carnauba tree (*Copernicia cerifera*). It is valued among the natural waxes for its hardness and high melting temperature. It consists primarily of esters of long-chain alcohols and acids. It has a melting point of 85°C and it is normally used pharmaceutically in melt granulation for sustained release of highly soluble tablets (Garcia *et al.,* 2002).

Ibuprofen is an analgesic, antipyretic, and anti-inflammatory drug that belongs to the non-steroidal anti-inflammatory drug (NSAID) class of medications. Its pharmacological activity is elicited by blocking the enzyme cyclooxygenase (COX), which converts arachidonic acid to prostaglandin H_2 (PGH_2), reducing the synthesis of other prostaglandins in the body, which are mediators of pain, swelling and inflammation (Grosser *et al.,* 2010).

It is available as cream, tablet, gel, suppositories and oral suspension. It is used in the treatment of pain associated with sprains, bone fracture, arthritis, dysmenorrhoea and also for the treatment of fever. Isesele *et al.,* (2021) formulated ibuprofen biodegradable subcutaneous implants and investigated the *in vivo* analgesic activities using mice. They found out that the subcutaneous ibuprofen implants significantly inhibited acetic acid-induced writhing in mice as compared to the control.

The aim of this study was to evaluate the influence of carnauba wax on the release profile of subcutaneous implants of ibuprofen (Gisella *et al.*, 2010).

MATERIALS AND METHODS

Ibuprofen sample was obtained as a gift from Edo Pharmaceuticals Limited (Nigeria). Gelatin, carnauba wax and hydroxypropyl methylcellulose (HPMC) were purchased from Pyrex Chemical Industries (London). Glycerin, acetone and formaldehyde were obtained from Aarti Industries Ltd, (India). Other chemicals used were of analytical grade.

Preparation of implants

Gelatin (24 g) was sprinkled on top of 100 mL of water in a beaker and left to hydrate for 30 min. Hydroxypropyl methylcellulose (HPMC) (6 g) and varying amount of carnauba wax were then added to the hydrated gelatin (Table 1). An extra batch was prepared without the addition of carnauba wax. Glycerin (20 mL) was added as a plasticizing agent while stirring continuously and the solution was heated over a hot water bath at 60°C until the gelatin was completely dissolved. Separately, 4 g of ibuprofen was dissolved in 5 mL acetone before being added to the heated gelatin, HPMC and carnauba wax mixture in the beaker. The resulting liquid was poured into a glass petri-dish and allowed to gel for 30 min while the petri-dish was placed on an ice pack. The congealed mass was allowed to air dry for 72 h at room temperature in an aseptic cabinet. After drying, the implants were removed from the petri dish and cut into 4 mm wide and 2 mm long rods with a stainless-steel cutter (Rajgor *et al.,* 2011).

Hardening/cross-linking of implants

A petri dish containing formaldehyde solution (37%v/v) was placed in an empty glass desiccator which was quickly closed after the sliced implants were kept on top of the petri dish in a wire mesh. The implants were exposed to formaldehyde vapour for 12 h. They were then removed from the desiccator and air-dried for 72 h to ensure that the formaldehyde and gelatin had fully reacted. The implants were then stored for one week in an open atmosphere under aseptic conditions to ensure that any leftover formaldehyde was totally evaporated (Rao *et al.,* 2010).

Table 1: Composition formula of ibuprofen implants.

Formulation	Drug (g)	Gelatin (g)	HPMC (g)	Carnauba wax (%)	Glycerin (mL)
IC0	04.0	24.0	6.0	-	20.0
IC1	06.0	24.0	6.0	0.25	20.0
IC2	08.0	24.0	6.0	0.50	20.0
IC3	10.0	24.0	6.0	0.75	20.0

Evaluation of subdermal implants

Thickness of implants

The thickness of a sample of three implants from each batch was measured with a micrometer screw gauge (Begemann GMBH, Germany) and the mean value was recorded.

Weight uniformity of implants

Implant samples were chosen randomly from each batch (n=3) and weighed separately on an analytical scale (Mettler Tolledo, Switzerland). The average weight and the percentage deviation from the mean were calculated.

Drug content uniformity

The drug content of the implants was determined by micronizing three (3) randomly picked implants and transferred to a 50 mL volumetric flask. Then, 45 mL of 0.1 M sodium hydroxide (NaOH) was added and vigorously shaken for 30 min at 500 rpm with a flask shaker, the volume was then made up to 50 mL. To estimate the amount of ibuprofen present, serial dilutions were done and the absorbance was measured using a UV spectrophotometer (UNICO ^{Tm,} 2011, UK). The technique was performed in triplicate. The mean and standard deviations were calculated (Purushotham *et al.*, 2010).

Swelling Index

Three (3) sliced implant samples were immersed in a phosphate buffer pH 7.4 swelling solution and the weight of each implant was calculated 1 h later after the excess fluid was gently wiped away with a dry piece of tissue paper (Kanzaria *et al.*, 2012). The degree of swelling of each implant formulation at a particular time was calculated using equation 1.

$$H = \frac{W_t - W_o}{W_o} \times 100 - - - - - - eqn\ 1$$

where W_t and W_o are the weight of the implant at any given time and in the dry state respectively and H is the swelling index.

Percentage moisture content

For each batch, five (5) cut implant samples were weighed on a weighing balance and placed in a dessicator with activated silica gel as the dessicant. The implants were removed and weighed on a regular basis until they attained a constant dry weight (Onishi *et al.,* 2005). The percentage mass loss on drying (moisture content) was calculated using equation 2:

$$mass\ loss(\%) = \frac{initial\ weight - dry\ weight}{initial\ weight} X\ 100 - - - eqn\ 2.$$

Moisture sorption studies

Under various simulated relative humidity (RH) conditions, the cut implant formulations were tested for stability. Saturated sodium chloride (75% RH), magnesium chloride (45% RH), water (100% RH) and activated silica gel (0% RH) were used in the experiment. Individually wrapped in aluminum foil paper, the implant formulations were stored

in relative humidity tanks at 30°C ambient room temperature. For a maximum of three months, the physical parameters of the implants and their weight were documented at predetermined intervals. The average values were calculated and plotted against time in days.

Preparation of standard calibration curve

Pure ibuprofen sample (100 mg) was dissolved in sufficient quantity of the dissolution medium (0.1 M NaOH) to yield a 100 mL solution and a stock solution of 1 mg/mL was obtained. Using the dissolution medium, serial dilutions of the stock solution were made to obtain the following concentrations: 0.5, 1, 2, 4, 6, 8, 10 µg/mL. The absorbance of the diluted samples was measured using a UV spectrophotometer at a maximum wavelength of 227 nm. The measurements were carried out in triplicate and a graph of the mean absorbance versus concentration was plotted (Beer-Lambert plot).

***In vitro* drug release studies**

The dissolution test was carried out using the reciprocating disc method (Apparatus 7; ST7, G.B. Caleva Ltd, England). Individual implants were placed in a dissolution basket and immersed into an 800 mL 0.1 M NaOH solution heated to 37±0.5°C and agitated at 50 rpm dissolution medium. Using a pipette, 5 ml aliquots of the dissolving fluid were withdrawn at various time intervals of 1, 4, 8, 16, 32 h, etc. and placed in suitable sample test tubes for testing. Sink condition was maintained by replacing the withdrawn dissolution medium with fresh 5 mL of 0.1 M NaOH. The drug concentration in the obtained samples of dissolution fluid was determined spectrophotometrically at a wavelength of maximum absorption (max) of 227 nm after suitable dilution with the dissolution medium.

***In vitro* drug release kinetics**

The results of the dissolution rate tests of the ibuprofen implants were subjected to several drug release models to analyze the release kinetics and the models used were zero order, first order, Higuchi square root of time and Korsmeyer-Peppas. The linear regression coefficient (r^2) was calculated for each rate order. The dissolution release profile was regarded to have followed a specific release order if the r^2 value was greater than 0.95 (Higuchi, 1963; Korsemeyer *et al.,* 1983).

Drug excipients interaction

The potassium bromide pellet method was used to generate the spectra for ibuprofen and the various formulations on a Fourier transform infra-red (FTIR) spectrophotometer (Perkin Elmer, Series model 1615, England), and the spectra were evaluated for any interactions or incompatibilities.

RESULTS AND DISCUSSION

Evaluation of physical parameters of implants

The physical appearances of the formulated implants are shown in Figure 1. They conform to the physical properties of implants designed for long-term ibuprofen administration. The implants were yellowish in colour. The cut implants appeared firm and smooth after 12 h of hardening in formaldehyde solution. The contact of the implants with formaldehyde vapour improved the degree of cross linking of the polymer matrix, resulting in an increase in the tensile strength of the implants (Oalta *et al.,* 2015).

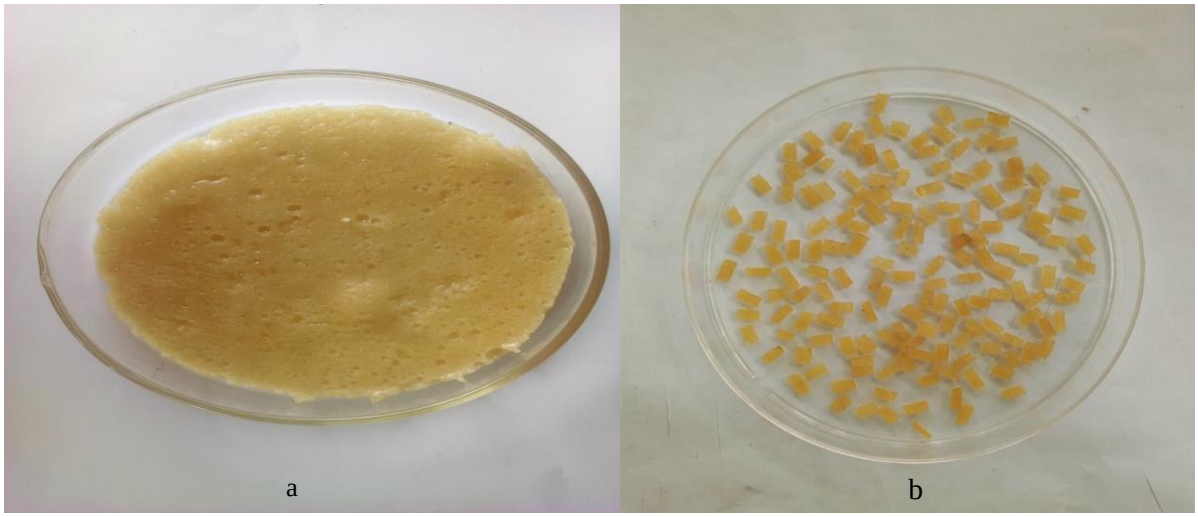

Figure 1: (a) Formulated ibuprofen implants (b) Cut ibuprofen implant.

Evaluation of the physicochemical parameters of implant formulations

The physical parameters of the formulated implants are shown in Table 2. In all batches of implant formulations, the mean diameter/thickness of the implants was between 2.46±0.10 and 2.86±0.03 mm. The computed percent weight variation for all implant formulations was within official limits, indicating that the formulated implants passed the weight variation test (BP, 2012). The implant formulations weighed between 120±0.2 and 126±0.1 mg. This is an important feature since it shows the amount of particulate matter embedded within the implant polymer matrix.

In the formulated implants, the percentage drug content of ibuprofen was ≤96.92±0.12%. The results, however, demonstrate a high level of entrapment efficiency and drug loading and they are within officially permissible limits (BP, 2012).

The swelling index of the various implant formulations ranged from 2.68±0.01 - 4.87±0.01% after 1 h of immersion in a

phosphate buffer swelling solution (pH 7.4). When exposed to an aqueous solution, the polymer expands owing to the uptake of water. The polymer hydrophobicity determines how rapidly the implant absorbs water. The encapsulated drug diffuses out through the pores generated by the swelling of the implant (Michael *et al.*, 2015).

The percentage mass loss on drying (moisture content) data reveal moisture content values ranging from 24.47±0.01% - 28.89±0.02%, which are within the official moisture content limits for biodegradable gelatinous polymers. Gels are formed when biodegradable gelatinous polymers come into contact with a suitable solvent. As a result, matrix implants made of biodegradable gelatinous polymers that form a random network infiltrated by liquid-filled pores are known to have a high moisture content (Satish, 2017).

Table 2: Results of the physical parameters of ibuprofen implant formulations.

Formulation	Thickness (mm) $\pm S.D$	Weight (mg) ± S.D	Drug content (%)	Swelling index (%)	Moisture content (%)
IC0	2.46 ± 0.10	120± 0.2	95.69± 0.11	2.68± 0.01	24.47± 0.01
IC1	2.68 ± 0.01	121± 0.1	96.38± 0.10	3.64±0.02	26.72± 0.02
IC2	2.79 ± 0.02	123± 0.1	96.54 ± 0.12	4.28±0.01	28.64± 0.01
IC3	2.86 ± 0.03	126± 0.1	96.92 ± 0.12	4.87±0.01	28.89± 0.02

Influence of formulation variables on the *in vitro* dissolution profiles of ibuprofen loaded implants

Figure 2 shows *in vitro* drug release studies of ibuprofen implant formulations (IC0 - IC3) in 0.1 M NaOH. In general, factors such as the swelling and dissolution of polymeric drug carriers, as well as diffusion of the active drug over a long period of time, have been shown to influence the rate of drug release from hydrophilic matrices (Isesele *et al.*, 2021).

Implantable drug delivery systems have been shown to successfully sustain the release of drugs held within their matrices over a long period of time when compared to conventional drug formulations, which are expected to release over 85% of their drug content during the first hour (BP, 2012). As shown in Figure 2, all implant formulations showed a sustained release of the drug over a 6-day period. Ibuprofen has a short biologic half-life of 3 h, hence it must be taken 2-3 times a day. However, based on *in vitro* dissolution studies, the implant formulations revealed a sustained modified release of ibuprofen that was similar to the zero-order release profile.

The rate of drug release was faster for batch IC0 formulated without the incorporation of carnauba wax as compared to formulations IC1-IC3 which showed a sustained release of drug over a long period of time. For example, the maximum drug release for

batch IC0 was 96% and the time to attain maximum drug was 80 h as compared to batches IC1, IC2 and IC3 which had drug released rate of 72%, 64% and 52% respectively in 80 h. Batches IC1-IC3 formulated with the incorporation of carnauba wax had their maximum drug release and were able to sustain the rate of drug release for up to 140 h. The higher the amount of carnauba wax incorporated in the formulation, the more retarded the rate of drug release. Previous studies have reported that the mechanism of drug release from carnauba wax involves the leaching of drug by the dissolution medium and the diffusion of drug from the polymeric matrix (Onyechi and Okafo, 2016). The findings from this research indicate that carnauba wax, a hydrophobic wax was slowly permeated by the dissolution media as a function of time and this delayed the rate of drug release form the implant formulations compared to the formulations that were prepared without the incorporation of carnauba wax which were highly permeated by the dissolution medium leading to the faster release of drug from the formulation. Drug release from the carnauba wax was also diffusion–controlled and simulated the Higuchi's Square root model. There was a significant difference between the drug release rate of the formulations without carnauba wax and those with carnauba wax ($P>0.05$).

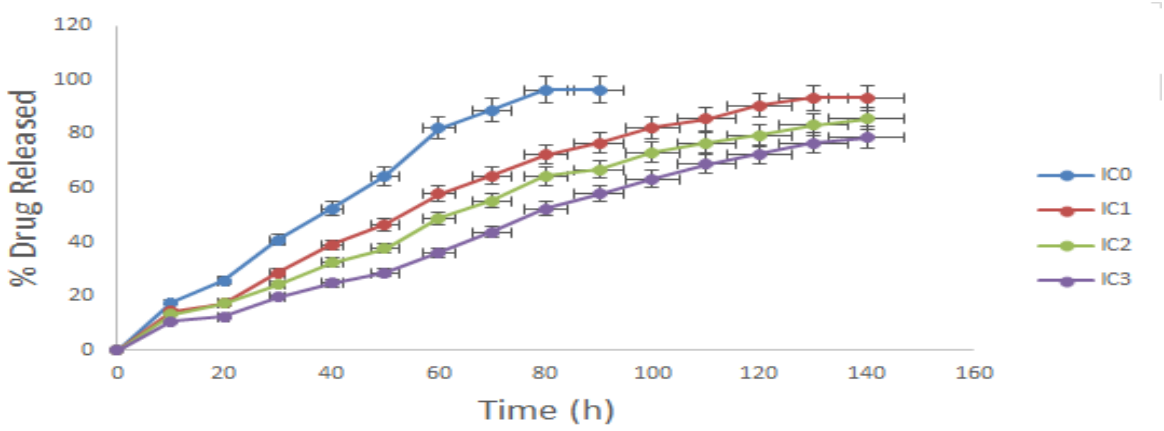

Figure 2: Drug release profiles of ibuprofen implants formulated with gelatin and HPMC.

Release kinetics of ibuprofen implants

The release kinetics analysis shows that the drug initially was released from the formulation rapidly, followed by a sustained release over time. Previous researches have shown that the mechanism of drug release from biodegradable implants is frequently controlled by diffusion, and degradation (Gisele *et al.*, 2010). Table 3 shows that the release mechanism of the various batches of ibuprofen implant formulations simulated the Higuchi model (r^2=0.998), indicating that the drug was homogeneously diffused

throughout the polymer matrices and that drug release kinetics were diffusion controlled (Higuchi, 1963). The results of the Korsmeyer-Peppas diffusion model (n > 0.5) show that the diffusion was non-Fickian (Korsmeyer *et al.*, 1983, Oalta *et al.*, 2015).

Table 3: Correlation coefficient and release kinetics of ibuprofen implants.

Models	**Zero**		**First**		**Higuchi**		**Korsmeyer and Peppas**	
Formulations	r^2	K_0	r^2	K_1	r^2	K_H	r^2	n
IC0	0.926	4.14	0.958	-0.052	0.992	19.28	0.569	0.56
IC1	0.954	3.87	0.959	-0.026	0.993	17.61	0.634	0.62
IC2	0.959	2.68	0.962	-0.037	0.996	16.73	0.652	0.63
IC3	0.964	3.75	0.968	-0.048	0.998	18.92	0.673	0.68

FTIR Analysis

The drug/excipient compatibility was determined using FTIR analysis. The peaks of the pure ibuprofen sample and the various formulations of ibuprofen implants did not differ significantly. The internal structure of the pure ibuprofen sample and the ibuprofen implant formulations were identical at the molecular level, as shown in the FTIR spectra below (Figure 3). As a result, there were no significant interactions between the drug and the excipients used in the formulation of the ibuprofen implants.

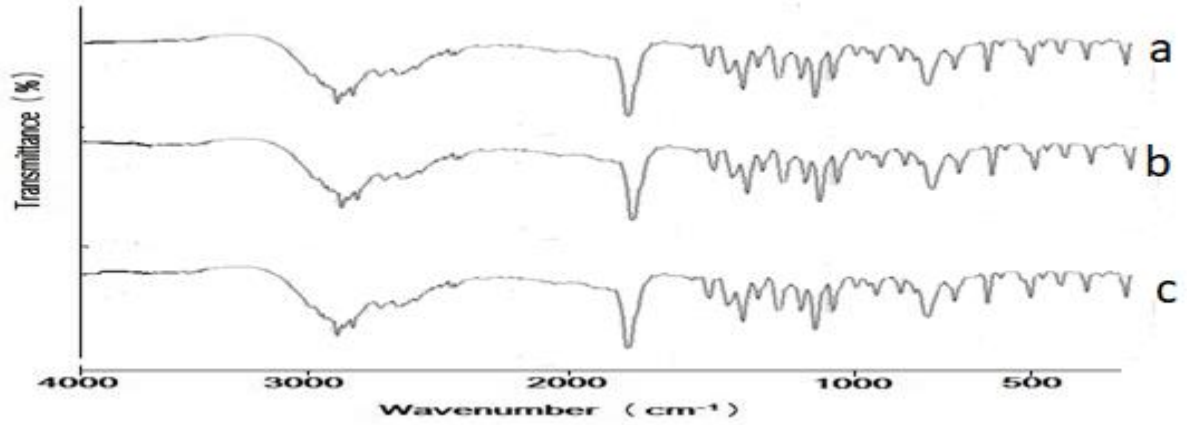

Figure 3: FTIR spectra (a) pure sample of ibuprofen (b) physical mixture of ibuprofen, gelatin and HPMC (c) implant of ibuprofen, carnauba wax, gelatin and HPMC.

Influence of relative humidity on the stability profile of the implants

The data for the change in implant weights over time under various relative humidity conditions at 30^{o}C is shown in Figure 4. In saturated sodium chloride (75% RH) and magnesium chloride (45% RH) solutions, the implants showed a relative stable weight but a rapid weight gain was observed in water (100% RH) and a

significant weight loss in activated silica gel (0% RH). Stability testing enables the determination of recommended storage conditions, shelf-lives and retest periods by revealing how the quality of a drug product changes over time as a result of a variety of environmental factors such as temperature, humidity and light (Isesele *et al.*, 2021).

There was no significant weight increase or change in the organoleptic properties of the implants stored at relative humidity of 45% and 75% at a temperature of 30^{o}C over the 3 months test period, according to the results of the moisture sorption isotherm of the ibuprofen implant formulations. The implants can be safely stored under similar environmental conditions.

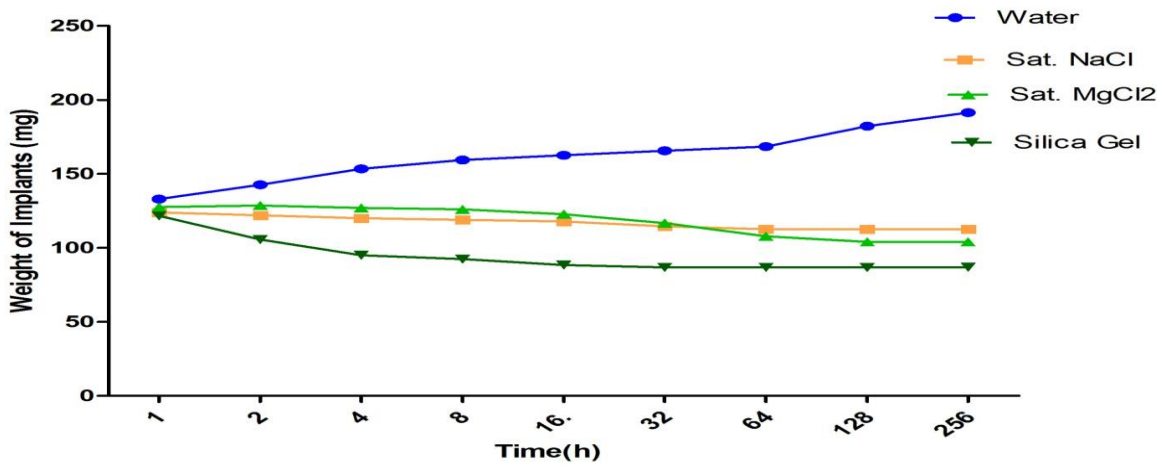

Figure 4: Moisture sorption isotherm of implant formulations under different conditions of relative humidity.

CONCLUSION

The rate of drug release from the ibuprofen implants was significantly affected by the incorporation of carnauba wax. The higher the amount of carnauba wax incorporated in the formulation, the more retarded the rate of drug release. There was a significant difference between the drug release rate of the formulations without carnauba wax and those with carnauba wax ($P>0.05$). This could be exploited in the formulation of sustained release ibuprofen implants for the management of chronic diseases like rheumatoid arthritis.

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Tea Tree (*Melaleuca alternifolia* (Maiden & Betche) Cheel) Oil: An important medicinal essential oil

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Abstract

Melaleuca alternifolia (Maiden & Betche) Cheel oil (Tea Tree Oil, TTO) is an essential oil appropriate for medicinal and cosmetic usage. Tea tree oil is composed of complex formulation with more than 100 components; however, the most pharmaceutically active one is terpinen-4-ol. TTO can be implemented for decolonization of multi-resistant *Staphylococcus aureus*, anti-tumor therapy and antifungal activity based on different doses and exposure-duration proportionate with the targeted species. Antioxidant activity is related to α-terpinene, α-terpinolene and γ-terpinene. Hypersensitivity may occur as mild dermatitis or being aggravated to hepatitis and central nervous system reactions due to chronic or acute poisoning. Acne treatment prognosis shows significant improvement after TTO application proceeding by *Propionibacterium acnes* colony destruction. Plus, TTO usage psoriasis is also possible. Further investigations have premised TTO's insecticidal effects performed by anticholinesterase activity. Destructive ability of the oil on *Pityrosporum ovale* is also indisputable and including TTO as the active ingredient has been highly beneficial for curing scalp dandruff. Expeditious antiviral activity is also considered as the promising characteristic suggested for this oil. Still, little information is available about feasibility of *in vivo* utilization.

Keywords

Dermo-cosmetic, *Melaleuca alternifolia*, pharmacological properties, tea tree oil, terpinen-4-ol.

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INTRODUCTION

Dominant member of Australian domestic forest, *Melaleuca alternifolia* is an ever-green woody shrub from Mytraceae family. In 1770, the name of tea tree mentioned by Captain James Cook due to its aromatic scent (Saller *et al.*, 1998). Tea tree oil, obtained by steam distillation from leaves, have been consumed by native Australian people, Aboriginals, for its germicidal property (Carson and Riley, 1998). First scientific examination about TTO's antiseptic activity had been performed by Penfold *et al.* (Penfold and Grant, 1925). First officially approved tilt of the oil's chemical composition was discovered and published by Brophy et al. (Brophy *et al.*, 1989). Six distinct chemotypes based on genetic differences may exhibit variable antimicrobial ranges; terpinen-4-ol chemotype is the one used in commercial oil production (Keszei *et al.*, 2010). While terpinen-4-ol is the core of biological activities, 1,8-cineole is assumed to be the beginner of dermatologic hypersensitivity; a prevalence side effect in topical applications (Carson *et al.*, 2019). TTO's mode of action may show sharp switch from bacteriostatic to bactericidal according to the applied concentration (Oliva *et al.*, 2018). High selectivity among different strains emphasizes oil's destructive effect on pathogenic microorganisms (Cueva *et al.*, 2010). Synergism is, also, another crucial term used while analyzing TTO performance (D'Arrigo *et al.*, 2010, Mickiene *et al.*, 2011). Bacterial resistance linked with frequent antibiotic prescription creates a demand for alternative agents. TTO, with remarkable antibacterial spectrum, not only does it exhibit acceptable range of *in vivo* activity against MRSA and resistant *Escherichia coli* strains, but also preparation of TTO containing sanitizing products for ICU personals is feasible (Blackwood *et al.*, 2013). TTO, with an unknown mechanism, declines the count of lesions originated by *Tobacco mosaic* (Bishop, 1995) plus application of this oil speeds up re-epithelization in recurrent *Herpes labialis* (RHL) involution (Carson *et al.*, 2001); and, also, it can be preferred for curing fungal infections in a dose-dependent manner (K. A. Hammer *et al.*, 2003). As an excellent antioxidant, it accelerates the rate of tissue renovation (Kim *et al.*, 2004). Improvement observed in subcutaneous mesothelioma prognosis after topical TTO administration revealed anti-cancer potential of the oil as well. TTO is accepted as an ideal active ingredient for cosmetology in order that it can cover both infectious and

inflammatory related skin disconfirms. The usage of TTO in pharmaceutical industry is not limited to the 'active ingredient' section; it also is preferable as a natural preservative option (Zhang *et al.,* 2018). In this review, tea tree oil was evaluated according to its botanical and chemical properties as well as biological activity.

DISCUSSION

Melaleuca alternifolia is an evergreen, little sclerophyllous with woody texture reaching up to 7 meters, which is covered with paper-like bark and decorated with flowers, each attached to a separate bract and collected in bottlebrush shaped clusters (Altman, 1988). Seeds can be observed with naked eye, being protected by a round-shaped woody capsule (Craven, 1999). Tea tree is not the only member of *Melaleuca* genus, it represents 330 other species. Important for determining ecological properties of wetlands, Myrtaceae family constitutes a dominant part of Australian natural flora (Franklin *et al.,* 2007; Edwards *et al.,* 2019) and most of them contain aromatic extracts stored in glandular sacs under their leaves (Serbesoff-King, 2003). After *M. alternifolia*, *M. cajuputi* and *M. leucadendron* are famous species due to their essential oil. Although it is possible to obtain equivalent extracts from different *Melaleuca* plants (Falci *et al.,* 2015), only tea tree oil (TTO) can provide a high degree of germicidal efficacy (Sharifi-Rad *et al.,* 2017). *M. alternifolia* grows in the coastal regions of the country from port Macquarie to New South Wales. Aborigines used to implement it as an antiseptic agent in their traditional medicines (Edmondson *et al.,* 2011); prepared pomades were utilized for accelerating wound healing prognosis plus persistent respiratory discomforts were suppressed with the aid of TTO (Cox *et al.,* 2001; Carson *et al.,* 2019). Moreover, there were healing lakes localized under tea trees where leaves naturally fell down and gave the water disinfecting ability (Craven, 1999). All knowledge and practical skills were conveyed from one generation to another, but the chain of transition was cleaved at some point (Carson *et al.,* 2019). At 1770's, Captain James Cook and his sailors named this tree as 'tea tree' due to its spicy smell (Ian Southwell, 1999). First medical research was published in 1920s to 1930s, performed by Penfold *et al.* (Penfold and Grant, 1925), a comparison-based study carried out between essential oils with antibacterial activity and phenol. Acquisition of optimistic outcomes from TTO, 11-13 times more effective than phenol, supervised bias toward natural germicidal

products. During Second World War TTO was added to navy's first aid kits due to its wide antibacterial spectrum (Lis-Balchin *et al.,* 2000). *Obstetrics and Gynecology* published a journal authored by Pefia (Pefia, 1962) named "*Melaleuca alternifolia* oil – its use for Trichomonal Vaginitis and other Vaginal Infections". 0.5% diluted preparations of TTO as washing solution cured all cases after six sessions. In 1985, three discrete studies were performed by Belaiche (Belaiche, 1985) on vaginal *Candida albicans* cystitis and onychomycosis in which TTO speeded up treatment prognosis associated with a safe regimen. In addition, topical treatment of toenail onychomycosis with 100% *Melaleuca* oil can be as effective as 1% clotrimazole solution (Buck *et al.,* 1994). After successful feedbacks from first synthetic antibiotics (*e.g.* penicillin) popular notion was inclined to artificial choices, but occurrence of resistance, disturbance of natural flora and subsequent superinfections caused by arbitrary prescriptions was discouraging (Carson and Riley, 1998; Larson and Jacob, 2012). Cross-contamination caused by aerosol microbes may be a questionable situation after dental surgeries. Although, there is no doubt that chlorhexidine digluconate performs expeditiously among famous disinfectant agents, however; utilization of TTO as a mouthwash before manual or ultrasonic scaling suppresses the microbial colonies dramatically (Shetty *et al.,* 2013). Success of a TTO containing washing gel, due to its anti-inflammatory activity, in chronic gingivitis management in comparison with chlorhexidine was also remarkable (Hart *et al.,* 2000; Soukoulis and Hirsch, 2004). TTO has been mentioned among first-line agents of veterinary medical history (Mozelsio *et al.,* 2003). Studies suggested TTO usage as air sanitizer against airborne microbes (influenza virus, *E. coli* and *Pseudomonas fluorescens*) for animal houses, stables (May, 2000; Mickiene *et al.,* 2011) and air tunnel system of industrial environments (Pyankov *et al.,* 2008, 2012). TTO disinfectant products can be implemented by caregivers for preventing nosocomial infections prevalence (Blackwood *et al.,* 2013). In 1970, plantation of tea tree has been established (Carson *et al.,* 2019). Conditions of natural environment, soil type, climate, humidity and water content, must be imitated while cultivation (Rodney *et al.,* 2015). Sandy loam is proposed as the native soil texture with high amount of moisture and slightly acidic pH circulating around 5.0. In addition, tea tree desires a mild subtropical weather that may has annual raining height between 1200 and 1600 mm. It is crucial to maintain the soil temperature above 17 ^{o}C, because the temperature reduction may trigger

'dormancy' of the tree (Colton and Murtagh, 1999). Surprisingly, *M. alternifolia* prefers dense tree orientation. However, the spacing pattern does not affect the oil components, but it shows boosting effect on oil yield (Small, 1981). Pest damaging in natural lands is uncommon due to low leaf/wood ratio. Increased leaf ratio in cultivation area is accompanied by greater demand for suitable pesticide. Attention must have be given to *Purana tigrina*, mites and psyllids; the most dangerous ones for oil yield (Campbell and Maddox, 1999). Using any type of chemical materials in wrong dose, rate or technique may lead to impurity creation (Rowe, 1999; Larkman, 2016). Visual examination can be applied for determining oil purity. While TTO is colorless in its pure form, any discoloration is considered as the presence of impurity. Examination of odor abnormality, caused by inappropriate distillation methods, is a crucial step in QC (Rowe, 1999). Appropriate time for harvesting may vary, from 1 to 3 years, depending on the growth conditions. Quality peak time for *M. alternifolia* can be determined as nine months after planting, preferring dry seasons to minimize the risk of fungal infection development. Tree should be cut 15-30 cm above the soil level to assure tree re-growth. TTO is extracted from leaves and not woody terminal branches mainly by steam distillation. Solvent extraction is another method in which ethanol is used as the solvent; however, a sharp drop in terpene concentration turns it into an undesirable option. New methods such as microwave heating are also available. Additionally, more precise techniques may be applied to *in vitro* media; such as microwave technology (Carson *et al.,* 2019) and Static Headspace Gas Chromatography (HS-GC) (Homer *et al.,* 2000). TTO's chemical composition needs to be controlled before marketing to assure the concentration of pharmacologically active terpenes. Naturally growing trees may expose to different environmental factors triggering genetic mutation and subsequent intra-specific variation (Sharififar *et al.,* 2007). Even though morphological characteristics are similar among different foliar chemotypes, ecological and chemical properties show significant differences (Bustos-Segura *et al.,* 2017). Six foliar chemotypes, based on dissimilarity of terpinen-4-ol, terpinolene and 1,8-cineole concentrations (Keszei *et al.,* 2010), are currently accepted by **The International Standard, ISO 4730**. Similar *in vitro* and *in vivo* bioactivity is expected from oils with identical chemotype (Homer *et al.,* 2000). Differences between biosynthetic pathways for terpene production may be the reason for chemotype variation (Keszei *et al.,*

2010), which is significantly complex (Padovan *et al.,* 2017). The only commercially valuable chemotype is the one with highest terpinen-4-ol as well as lowest 1,8-cineole and *d*-limonene level. Geographical separation is observable according to dominant TTO chemotype (Homer *et al.,* 2000); however different sources can be used for this purpose even if chemotype variability is detected (Keszei *et al.,* 2010).

Functional groups are important in determining pharmaceutical value of an essential oil (Kumari *et al.,* 2018). TTO is composed of cyclic monoterpenes; half of them are oxygenated and the other half remains as simple hydrocarbons (Noumi *et al.,* 2011). Terpenes are single structural units of terpene (C_5H_8, isoprene) polymers (Dorman and Deans, 2000). First official list of 'chemical composition of tea tree oil' was published by Brophy et al. It has a complex formulation with more than 100 components; main constituents are as follows: terpinen-4-ol, 1,8-cineole, α-terpineol, terpinolene, α- and γ-terpinene involving 90% of the whole composition (Brophy *et al.,* 1989). Catechins and polyphones are critical components managing the antibacterial action in cooperation with terpinen-4-ol and 1,8-cineole. Bacterial cell membrane is damaged by these compounds, leading to vital defects in respiration, permeability, and osmoregulation (Kumari *et al.,* 2018). Presence of trace components, sabinene, globulol and viridiflorol, creates a favorable synergism effect (Mickiene *et al.,* 2011). TTO, with density between 0.885 and 0.906, exhibits low aqueous solubility. Surfactant, Tween 20 and Tween 80 from 0.001% to 0.5% (v/v), addition to agar medium would be beneficial (Kumari *et al.,* 2018); however, involvement of suspending agents may cause turbidity and decrease the accuracy of 'inhibition zone' measurements. Presence of triphenyl tetrazolium chloride (TTC) in the bacterial culture puts out a tricky point; TTC 0.005% (w/v) changes from transparent to red color simultaneously with bacterial colonization; a 'growth detector' (Hammer *et al.,* 1998). Terpinen-4-ol is considered as the active part responsible for antimicrobial activity, and 1,8-cineole acts as a skin and mucous irritant (Mondello *et al.,* 2006); however, recent researches have proved that calculating the best ratio between these two dominant terpenes is the most appropriate perspective for achieving maximum potency associated with minimum hypersensitivity (Mickiene *et al.,* 2011). A premise about the interaction between different oil constituents was suggested after discriminative examinations of TTO's components. While terpineol-4-ol was found to be effective

against *Pseudomonas aeruginosa*, this predestinate result could not be achieved in the complete-oil testing (Papadopoulos *et al.,* 2006; Rodney *et al.,* 2015). It may, also, explain the empowered bactericidal activity resulted from cooperation of the two essential oils. *Melaleuca alternifolia* and *Cymbopogon citrarus* are two plants with remarkable antimicrobial feedbacks; an underestimate MIC (0.05%) expressed by the combined agent indicated increased antimicrobial activity against *S. aureus, P. mirabilis, C. albicans*, and *E. coli*. On the other hand, *P. aeruginosa* and *E. faecium* colonies were more stable in the media inoculated by mixed product with MIC increased from 5.0% to 8.0% (Mickiene *et al.,* 2011).

TTO can be consumed as a bactericidal agent against both gram-negative and gram-positive pathogens and shows sufficient destructive activity by obstructing cellular respiration interfered with enzymatic reactions in cell membrane together with increasing permeability of cytoplasmic membrane established by measuring the amount of propidium iodide uptake (Hammer *et al.,* 1998) as well as morphological examination of treated organism (Carson *et al.,* 2002). It may also cause potassium leakage and destroy chemiosmotic control of microorganism. This premise is empowered by presence of nucleic acid residue in extracellular fluid. Target sensitivity can vary depending on penetration rate of monoterpenes (Cox *et al.,* 2001). Moreover, diabetic gangrene, leg ulcer, and catarrh are cases that have been treated by this oil. Presence of blood or any other organic material augments antibacterial ability of the oil (Edmondson *et al.,* 2011). TTO shows an acceptable decolonization degree at 1% concentration, in specific cases higher concentrations up to 5% in term of MIC may be needed. (Mickiene *et al.,* 2011). It is classified as a bactericidal agent, but bacteriostatic effect is also observable at higher concentrations (Oliva *et al.,* 2018). Although mupirocin is the first-line drug for MRSA decolonization, frequent application in prophylaxis manner elevates resistance risk (Caelli *et al.,* 2000). Colonization of multi drug resistance (MDR) *Staphylococcus aureus* in Intensive Care Units (ICU) is among most life-threatening situations for hospitalized patients. Laboratory trials revealed that TTO can be preferred for MRSA treatment; however clinical trials did not support it. A randomized controlled study was designed to determine the effect of *M. alternifolia* oil on MRSA decolonization in patients without systemic infection. Including 1080 patients hospitalized in ICU, a comparison protocol has been followed between 5% TTO body wash and Johnson's baby soft wash for 21 months. While results were not adequate,

clinical improvement may be reached by preferring a leave-on medication with higher TTO concentration (Blackwood *et al.,* 2013*).* A limitation mentioned in MRSA infectious lesions treatment is healing rate. In this theme, TTO might be helpful by decreasing the size of the lesions as well as the healing period (Edmondson *et al.,* 2011). TTO as a volatile agent with remarkable colony clearance potency can be utilized in its vapor form for pneumonia treatment caused by *Klebsiella pneumoniae* (Oliva *et al.*, 2018). This oil can speed up recovery speed by deactivating pro-inflammatory mediators and preventing or curing present fungal infections. A study was performed around killing capacity of thirteen phenolic acid structures premising that sensitivity of pathogenic *E. coli* O157:H7 (CECT 5947) is twice more than non-pathogenic *E. coli* (ATCC) 25922 (Cueva *et al.,* 2010). This conclusion can be expanded to the plants containing phenolic structures such as *Melaleuca alternifolia.* Moreover, combination of TTO with tobramycin can express high bactericidal capacity and subsequent post antibiotic effect (PAE), even at doses lower than MIC. In addition to empowered bioactivity, diminished drug dosage increases drug tolerability and patient compliance (D'Arrigo *et al.,* 2010). TTO in phosphate-buffered saline (PBS) solution was tested on salt adapted *Enterococcus faecalis* sample with 6.5% NaCl, triggering cross protection; a reduction in TTO's colony eradication ability. It can be explained by TTO's mode of action; empowered cell membrane will suppress antibacterial activity of all agents with mutual side of action (Lim and Hammer, 2015). Another study suggested equivalent efficacy of TTO and 3% Sodium hypochlorite. Sodium hypochlorite is the number one root canal irrigant agent utilized during dental operations. TTO with significant *in vitro* activity gives hope for replacing old-fashion medications with undesirable effects (Sheth *et al.,* 2013). While occurrence of single-step mutation leading to bacterial resistance was uncommon, gradual increase after several sub-culturing with underestimated TTO concentration was observable (McMahon *et al.,* 2007; Hammer *et al.,* 2008,). Antimicrobial spectrum can be a challenging characteristic while applying on a particular area with sensitive microflora like vagina. While TTO might be helpful for treatment of discomforts caused by *Bacteroides, Prevotella, Fusobacterium* and *Peptostreptococcus* with MIC_{90} less than 0.5% (v/v), the natural microflora of vagina stays untouched due to high MIC_{90} reaching up to 2% in lactobacilli (Carson and Riley, 1998). Furthermore, antibacterial property of this oil can protect the irritated skin

patches from pathogenic microorganisms like *Staphylococcus aureus* and accelerate healing process (Edmondson *et al.,* 2011). TTO can also be used as a sanitizing agent for nurses and devices (Blackwood *et al.,* 2013). Being in direct contact with the infected patients, there is a huge necessity for effective hand washers to break the transmission chain of the intended pathogen. TTO can be defined as a preferable agent for this purpose; expanded antimicrobial spectrum distinguishing between host and transient microorganisms plus lipophilic nature enabling oil penetration to the skin's outer layers. (Carson and Riley, 1998). Fungal infections caused by 'filamentous fungi' after traumatic events (Fanfair *et al.,* 2012) are treated by surgical discharge of infected tissue and subsequent support with systemic antifungal agents, especially when the rotten tissue is out of access or too tiny (Austin *et al.,* 2014). Usage of topical antifungal agents, such as Dakin's solution, seems to be rational for accelerating healing process with minimizing systemic toxicity (Barsoumian *et al.,* 2013). Antifungal achievements of TTO are mainly related to terpinen-4-ol (Brophy *et al.,* 1989). Several fungal species can be target for TTO with dose-variation (Hammer *et al.,* 2003); highlighted performance of 2% butenafine hydrochloride TTO solution in curing toenail onychomycosis (Syed *et al.,* 1999) and *in vitro* activity against *Madueralla mycetomatis* are vital examples (van de Sande *et al.,* 2007). *Exophialia* spp., *Actinomucor* spp. and *Fusarium* spp. were strains with highest susceptibility, while Aspergillus *terreus* and *Absidia* spp were resistant even in 100% oil concentration. Increasing exposure time, mutually, increased efficacy. (Homeyer *et al.,* 2015). There has been a growing concern about prevalence of resistant to common antifungal therapies, especially among immune deficient and cancerous patients, (Hammer *et al.,* 2003); infections generated by *C. albicans* strains have been highly insistent to treatment with azoles (Casalinuovo *et al.,* 2004). TTO has been reported to display potent antifungal performance against azole-resistant yeast types (Mondello *et al.,* 2003) and specific species of oral Candidiasis (Bagg *et al.,* 2006). The planktonic *C. albicans* are susceptible to TTO components, terpinen-4-ol and α-terpinol, with MIC_{50} 0.5% and 0.25% (Ramage *et al.,* 2012). Local treatment is a preferable option for strengthening *in situ* pharmacological action as well as minimizing the systemic toxicity. Curing superficial cancerous tissues with topical chemotherapy drugs, imiquimod and 5-fluorouracil, is also possible, but limitations leading to little patient satisfaction are present: low-rate

elimination, local unwanted effects, and long duration of treatment. Moreover, treatment prognosis may show variation depending on the nature of cancerous tissue (Greay *et al.,* 2010). Nowadays natural therapeutic agents such as TTO and ingenol mebutate are at the center of attention for pre-clinical trials. While topical application of 10% diluted TTO together with dimethyl sulphoxide (DMSO) to subcutaneous AE17 mesothelioma suppressed the tumor's size and growth rate, skin irritation can be considered as a disadvantage. The complexity of the mechanism became clear after analyzing the involved cells by flow cytometry, immunohistochemistry, and transmission electron microscopy. TTO starts a local immunization. Although, first expression about the mechanism is T cell mediated anti-tumor cytotoxicity, subsequent examinations eliminate this option. Skin irritation is the weak point in topically applied TTO. After *i.p.* administration of Gr –1 mAb, a reduction in neutrophil concentration together with skin irritation was observed; however, cytotoxicity degree of the medication did not diminish. High specificity in mode of action can be another breakpoint of novel cancer therapy methods (Ireland *et al.*, 2012). Unsatisfied amount of the oil (3-5%) was combined with prolonged therapy interval to compensate the shortage of the agent. Although skin irritation was suppressed, it also underachieved the pharmaceutical effect as well (Greay *et al.,* 2010). *In situ* observations of the target tissue emphasized the importance of the penetration degree for a successful treatment. Layers that were close to the exposure area showed higher level of destruction. The effect of DMSO on penetrability was also notable (Ireland *et al.,* 2012). Moreover, five pharmaceutically valuable parts of the oil were isolated and examined. None of them could reach the desirable concentration in epidermal and dermal layers by themselves; while using these five terpenes as a unit showed enough penetration. (Greay *et al.,* 2010). *M. alternifolia* essential oil is one of the momentous extracts with remarkable antioxidant property. An evaluation by three methods gave hopeful background information. TTO was examined by DPPH$^{\bullet}$ (2,2-diphenylpicrylhydrazyl) and TBARS (thiobarbituric acid reactive substances) assays together with Hydroxyl Radical Scavenging Activity. Intended substance was compared to well-known antioxidant agents such as quercetin, α-lipoic acid, vitamin C and E. Earlier premise was completely supported by the final outcomes. District investigation with the aim of recognizing the responsible compounds for this task made a clear point

to phenol functional groups (Zhang *et al.*, 2018). Studies clarified TTO compositions with the highest antioxidant property: α-terpinene, α-terpinolene and γ-terpinene (Kim *et al.*, 2004). Little information is available about essential oil's antiviral activity. Investigations concerning healing capacity of TTO on lesions caused by *Tobacco mosaic* suggested its efficacy on decreasing lesion numbers within 10 days after inoculation with an unknown mechanism (Bishop, 1995). Moreover, while average time required for re-epithelialization after recurrent *Herpes labialis* (RHL) contamination for TTO treated group was 9 days, control group with placebo needed 12.5 days; plus, a modest reduction was highlighted in median duration of culture positivity. viral titer appeared lower in the TTO group (Carson *et al.*, 2001).

Jacobs *et al.* (Jacobs and Hornfeldt, 1994) reported a case of systemic toxicity after TTO ingestion; 23-month-old white patient suffering from disorientation showed complete recovery after 5 hours of hospitalization. Reversible systemic toxicity may be related to suppressed central nervous system activity. Systemic contact dermatitis, semi-consciousness or comatose are serious conditions associated with TTO ingestion. Apart from the nature of the oil constituents, appearance of toxic reactions may be linked to inappropriate storing. Inauguration of specified storage standards about air and light availability in storage milieu must have been established for preventing the formation of impurities such as peroxides and p-cymene (Southwell, 2006). Decreased amount of terpinene coincidently with increased cymene concentration indicates variation in oil composition (Pazyar *et al.*, 2013). Dose adjustment is also essential (Hammer *et al.*, 2006); for example, fungal nail infection, as an acute-type of infection, needs high TTO concentrations, treatment prognosis is considerably long and the risk of toxicity is assumed to be high (Syed *et al.*, 1999). Although TTO usage in antimicrobial products is widespread, there is no clarity about the exact toxic dose. Two main detoxifying procedures in TTO metabolism are glycine and glucuronide conjugative pathways in which metabolites can cause acute hepatotoxicity (Meesters *et al.*, 2009). Infraclass analysis revealed that authentic TTO triggered hypersensitivity; therefore, hypersensitivity associated with intended TTO ascended concurrent with aging. Furthermore, terpinolene, α-terpinene and terpinen-4-ol were the least stable terpenes against aging (Avonto *et al.*, 2016). Although toxicity after topical application may be under control, direct contact with inner layers shows unpredictable interactions. Analyzing fibroblasts, keratinocytes, osteoblasts and

HUVECs suggested a perception about the relationship between toxicity and concentration; cell viability was suppressed by increasing the dose. Different LD_{50}s were obtained from different cell types; HUVECs showed highest resistance with 13.4% $LD_{50.}$ In addition, single cell destruction was more obvious compared to the whole tissue. Long-term application together with concentration under 25% may give us both efficacy and safety (Homeyer *et al.,* 2015). Analysis of TTO influence on treatment prognosis ascended bias toward being optimistic about Dermocosmetics studies (Kulkarni, 2012). *M. alternifolia* extract destroys *Propionibacterium acnes* colonies; a commercial microorganism causing acne (Kabir Mumu and Mahboob Hossain, 2018). TTO can compete with benzoyl peroxide and topical erythromycin with low toxicity (Hammer, 2015). The main reason of dandruff is overgrowth of a yeast type named *Pityrosporum ovale* (Piérard-Franchimont *et al.,* 2006; Turner *et al.,* 2012). In a 4-week research, satisfactory results were obtained in a group with 126 members using 5% TTO product. A high degree of curing with well-tolerated toxicity profile made the oil successful in this competition with placebo-controlled group. Dilution of the oil with daily shampoo or direct application of a few drops to the hair scalp can be helpful in long term (Satchell *et al.,* 2002). Moreover, *M. alternifolia* leaf extract exhibits antiseptic property, an advantage while curing dermatitis (Davis, 1999). In a study searching for replacements for corticosteroids in dermatitis treatment, suppressed allergic contact dermatitis caused by nickel up to 40.5% was linked with anti-eczematic properties of TTO. Initial TTO concentration was 50%, but erythema development leaded to dose reduction to 20%. Comparison of anti-eczematic effectiveness among TTO, zinc oxide and clobetasol butylate determined high potential of the oil. Although, skin hypersensitivity associated with nickel was well-treated with TTO, augmenting effect of the oil on histamine-induced weal (52.5%) emphasized the necessity of etiological analysis for this pathologic condition. (Wallengren, 2011). Head mice or scabies, named *Pediculosis capitis*, is a persistent parasitic infection with severe itching. Skin lesions appearing as holes and secondary infections are the consequences of untreated *Pediculosis capitis*. (Leung *et al.,* 2005; Nutanson *et al.,* 2008). *Melaleuca alternifolia* extract as shampoo is a preferable option. Not only for head scalp but also all affected body parts can be treated (Walton' *et al.*, 2000). This insecticidal effect is the result of anticholinesterase activity of TTO (Mills *et*

al., 2004). A chronic skin disease with high genetic tendency showing itself before 20's called psoriasis. Existence of red or brown patches with different sizes is a strong sign of psoriasis, but morphometric details like vasodilation of the problematic area must be analyzed. Pathophysiological examinations premise TNF-α as the reason for underlying inflammatory reaction. *Melaleuca alternifolia* oil, with antioxidant efficacy, can control over-expression of TNF-α, PGE 2, IL-1 and IL-8 (Pazyar and Yaghoobi, 2012). Anti-psoriatic 5% TTO transdermal patches based on micro-emulsion technology were design for direct and continuous drug delivery (Sonia and Anupama, 2011).

CONCLUSION

Naturally obtained medical agents are always one step ahead. *M. alternifolia* oil has been proceeding successfully in considerable numbers of assessments. Even though the promising abilities as an antimicrobial, anti-inflammatory and anti-tumor agent are well-known, *in vivo* evaluations must be done to assure the safety and reproductivity.

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