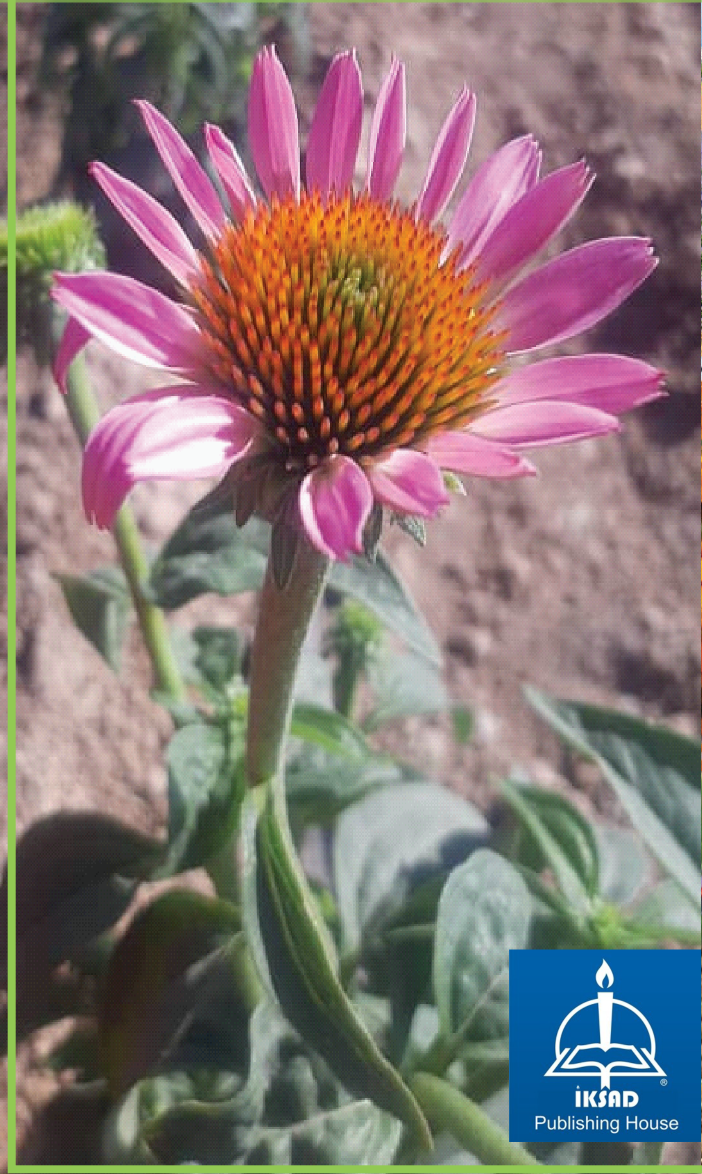
CURRENT STUDIES on MEDICINAL PLANTS-I

EDITOR: Assoc. Prof. Dr. Gülen ÖZYAZICI



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PREFACE

It is known that medicinal and aromatic plants have been used for health, food and dye purposes since the early ages of humanity. Despite the drugs and treatment methods developed by modern medicine, treatment with medicinal plants still maintains its importance. Medicinal plants, which were used by people living in rural areas in the past, are seen to be used by people living in all walks of life today. Medicinal and aromatic plants have a crucial place in helping people live a healthier life. A significant portion of the medicinal and aromatic plants traded in Türkiye are collected from nature, and very few medicinal plants are cultivated. For this reason, it is important to share new information by conducting studies on these plants.

This book, which deals with medicinal and aromatic plants from different aspects and was prepared with the participation of valuable researchers, consists of 11 chapters. I would like to thank all our authors who contributed to the preparation of this book titled 'CURRENT STUDIES ON MEDICINAL PLANTS-I' and the employees of IKSAD publishing house who contributed to the publication phase, and I hope that this book will be useful to the scientific community.

Best regards

Assoc. Prof. Dr. Gülen ÖZYAZICI

CHAPTER 1

BREEDING CRITERIA FOR MEDICAL AND AROMATIC PLANTS OF ECONOMIC IMPORTANCE

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INTRODUCTION

The use of medicinal-aromatic plants (food, cosmetics, agriculture, pharmacy, etc.), which has an obvious role in the protection of human health and the improvement of life quality for thousands of years, is quite wide.

Medicinal-aromatic plants are used as whole, fresh and dry, as well as plant parts such as leaves, roots, flowers, seeds, bark, tubers and aerial parts, their fragmented and ground forms, and extracts prepared in different ways. Today, there are two ways to obtain medicinal-aromatic plants: "collecting from nature" and "cultivation". The exact amount collected from nature is not known in Turkey's medicinal-aromatic plant statistics. The plants such as poppy, thyme, cumin, anise, fennel, black cumin, coriander, fenugreek, lemon balm, hops are cultivated in Turkey. The number of plant species cultivated continues to increase over the years. The cultivation area and production amounts of some medicinal-aromatic plants grown according to 2022 TUIK data are presented in Figure 1 (TUIK, 2023).

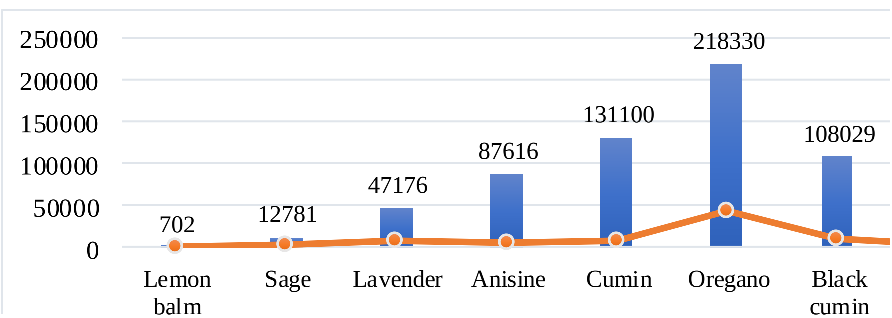

Figure 1: Cultivation area (da) and production (ton) amounts of some medicinal-aromatic plants grown in 2022

1. BREEDING CRITERIA FOR MEDICAL AND AROMATIC PLANTS OF ECONOMIC IMPORTANCE

1.1. Sage (*Salvia officinalis* L.)

Although *Salvia officinalis* L. does not grow naturally in our country, progress has been made in its cultivation in recent years. The plant, which has a semi-bush woody stem and can grow up to 60-100 cm, has violet blue, pink or white flowers and hairy leaves. The flowering period of perennial medicinal sage is from May to July. Originating from the Mediterranean Region, the species can grow in areas up to 800 m above sea level (Kokkini et al., 2003; Coşge Şenkal et al., 2012). Today, it is cultivated in many countries (Mirjalili et al., 2006; Raal et al., 2007). Due to its medicinal properties, *S. officinalis* has been used in folk medicine for therapeutic purposes for centuries (Kintzios 2000; El-Feky et al., 2016). It is generally used in folk medicine as a carminative, sedative, carminative, diuretic, stomachic, antiperspirant, externally wound healing and antiseptic. It has a wide range of biological activities (antibacterial, anti-inflammatory, antiseptic, antioxidant, antispasmodic, etc.) (Raal et al., 2007; Coşge Şenkal et al. 2012; Abu-Darwish et al., 2013).

The economically valued parts of medicinal sage are its leaves and essential oil. *S. officinalis* leaves contain 0.5-2.5% (ASTA, min. 1.5%) essential oil and essential oil contains α- and β-thujones, camphor and 1,8-cineol as basic components (Raal et al., 2007; Baydar, 2009). The amounts of these compounds determine the market value of the essential oil and the plant (Kuštrak et al., 1984).

Sage essential oils with more than 30% thujone and less than 20% camphor have a higher market value (Kokkini et al., 2003). In *S. officinalis* essential oil, α-thujone should be between 18-43%, β-thujone 3-8.5%, camphor 4.5-24.5%, 1,8-cineol 5.5-13% (ISO 9909:1997).

Breeding criteria

High drug leaf yield

High essential oil content (at least 0.5%)

Essential oil components in accordance with national and international standards, camphor-free or less than 0.5% camphor content due to its toxic effect

Disease and pest resistance

Good regeneration ability after cutting

Cold resistance

1.2. Anise (*Pimpinella anisum* L.)

Anise (*Pimpinella anisum* L.) is an annual, herbaceous, essential oil plant from the Apiaceae (Umbelliferae) family. Plant height is around 30-70 cm, flowers are umbrella-shaped and white. The leaves, which are simple at the base, are fragmented on the stems (Chevalier, 1996). Anise fruit is laterally flattened, 3-5 mm long and 2-3 mm wide, oval shaped (Ross, 2001). The gray-green or greenish-yellow fruits and shoots of the plant contain essential oil. Anise fruits contain 2-6% essential oil in the schizogenic oil channels (Lee et al., 1997). According to the European Pharmacopoeia, anise fruits to be considered as medicine should have an essential oil concentration higher than 2% (European Pharmacopoeia, 2000). The typical odor and taste component of fruit essential oil is trans-anethole, which makes up about 80-90% of the essential oil. The characteristic odor of Turkish raki stems from trans-anethole, the main component of anise essential oil (Tabanca et al., 2005; Orav et al., 2008). Anise fruits and essential oil are an important natural raw material used in the cosmetics, pharmaceutical, perfumery and food industries (Ross, 2001).

Breeding criteria

High fruit/seed yield

High biological efficiency

High 1000 fruit weight

Generally, genotypes with higher plant height, number of branches, number of main umbrellas, and number of fruits in the main umbrella have a low 1000 fruit weight. The number of branches and the excess number of umbrellas cause the fruits in the umbrella to become smaller. Low fruit weight negatively affects seed yield. Because there are positive and significant correlations between fruit yield and fruit number and 1000 fruit weight. Due to the negative correlation between phenological characteristics and fruit yield, early genotype selection should be made in anise in order to have genotypes with high fruit yield (Akan, 2016; Mehravi et al., 2020).

Drought stress tolerance

Drought stress is one of the most critical factors limiting anise production worldwide. A significant relationship was observed between fruit yield and relative water content under stress conditions. Therefore, this feature can be used as a physiological index to evaluate drought tolerance in anise. (Mehravi et al., 2020)

High essential oil content in fruits

The high rate of trans-anethole in the essential oil

Disease and pest resistance

1.3. Rosemary (*Rosmarinus officinalis* L.)

Rosemary (*Rosmarinus officinalis* L.), which is called by various names such as bird tongue, hasalbal and acuurene in our country, is an important medicinal and aromatic plant of the Lamiaceae family (Begum et al., 2013). Rosemary is a perennial herb that can be 50-100 cm tall, in the form of a bush, with evergreen flowers and pale blue flowers (Baytop, 1984). Rosemary (Ceylan, 1987), which can be grown mostly in places where the Mediterranean climate is dominant, is considered as a medicinal, aromatic and ornamental plant (Başkaya et al., 2016). Rosemary, which has a wide range of uses, is used in many areas such as food, cosmetics and pharmaceutical industry. Due to the essential oils, it contains, it is also used in traditional medicine, modern medicine and aromatherapy. (Sasikumar, 2004). Rosemary has anticancer (Bai et al., 2010; Valdes et al., 2012; Sanchez-Camargo et al., 2014), insecticide, antimicrobial (Hussain et al., 2010; Jordan et al., 2013; Angioni et al., 2004; Yosr et al., 2010; Jiang et al., 2011) and antioxidant (Hussain et al. 2010; Yosr et al., 2010) effects were also determined by studies. It has also been proven to have analgesic, anti-inflammatory and antitumor activities

(Genena et al., 2008; Nieto, 2017). Thanks to the bioactive substances (secondary metabolites) it contains, the rosemary plant, which is used in different areas, is produced and exported in our country, especially by being collected from the natural areas where it spreads. The breeding purposes of the rosemary plant, which has an important place both for our country and for other countries in the world, also have an important place.

Breeding criteria

Plant height

Plant stem yield

Leaf yield

Wet herb yield

Dry herb yield

Dry matter yield

Essential oil yield

Essential oil components

Borneol, camphor, 1-8 cineol amount in the essential oil

1.4. Fenugreek (*Trigonelle foenum-graecum* L.)

Fenugreek is an annual spice plant from the legumes (Fabaceae) family. The genus *Trigonella* generally spreads in places where the Mediterranean climate is dominant. 32 species and 2 subspecies of which 8 species and 1 subspecies are endemic grow naturally in the flora of Turkey (Güner et al., 2012). Among them, *Trigonelle foenum-graecum* L. species is cultivated. Fenugreek, popularly known as buy grass, is an annual and has a height of 30-60 cm. It usually has yellowish-white flowers. About 10 days after the flowers open, fruit setting takes place. The fruits, called pods, have an average of 10-20 seeds with hard corners, brown red or yellowish color (Gökçe and Efe, 2016). Seeds contain 4.3% moisture, 27.3% crude protein, 6.7% crude oil, 6.7% crude fiber, 51.2% nitrogen, 3.8% ash and mineral substances such as Na, K, Fe, Ca, P, Mn, Zn and Cu (Nour and Magboul, 1986). Due to the fact that fenugreek is a legume plant, it is included in the crop rotation systems and is grown for the purpose of green manuring and makes positive contributions to the improvement of the soil structure. The plant is evaluated in many different fields such as medicine, pharmacy and cosmetics. The ground form of its seeds is used in spice mixtures, especially in meat products. It is the main raw material of the mixture coated on bacon. Fresh leaves and sprouts of the plant are consumed as vegetables and spices in India (Leela and Shafeekh, 2008). Among the people, fenugreek is used for irritating, carminative, digestive, expectorant, etc. ailments. However, fenugreek is used in the treatment of diabetes and cancer due to its bronchitis, antipyretic, sore throat relief, wound healing and blood sugar lowering properties (Gökçe and Efe, 2016).

Breeding criteria

It varies according to the purpose of use (Malhotra, 2021)

High seed yield

High green grass yield

Adaptation to different climates

Resistance to abiotic stress conditions

Disease and pest resistance

Maturation time

High biological nitrogen fixation

For special use areas (medicine etc.):

High/low bitterness

High steroidal sapogenins (diosgenin and tigogenin)

High amino acids, polysaccharides (galactamannan)

High trigonelline content

High protein ratio

High essential oil content

1.5. Basil (*Ocimum basilicum* L.)

The genus *Ocimum*, which is a member of the Lamiaaceae family, contains about 150 species. The genus *Ocimum* has been used in folk medicine since ancient times, and herbal drugs are used in the treatment of various diseases. These species are extremely rich in carbohydrates, fiber, iron, b-carotene, vitamins, phosphorus, calcium, protein and essential oils (Gurav et al., 2022; Lal et al., 2018). Especially the *Ocimum basilicum* (sweet basil) species is widely cultivated. The plant is an annual or perennial herbaceous structure and the chromosome number is 2n=6x=48 (Purushothaman et al., 2018; Bilal et al., 2012).

Breeding criteria

Resistance to abiotic stress factors (Purushothaman et al., 2018)

The development of the disease factor should be prevented by preventing the development of resistant varieties against Fusarium wilt disease or by preventing mono-culture agriculture (in all-season production areas such as greenhouses) (Dudai et al., 2002).

Freshly cut leaves of basil are used in a wide variety of areas, such as being dried and used as a spice, used as a source of essential oil, used as a flavoring in various beverage and food industries, and grown in gardens as cleaning agents or ornamental plants. Therefore, it is necessary to improve the yield and quality characteristics for the purpose of use or to breed the varieties suitable for the purpose. The use of a single variety for all purposes negatively affects yield and quality in terms of the desired feature (Dudai et al., 2020).

New protocols should be developed for the production of active substances for medical use by producing in bioreactors (Lal et al., 2018).

New chemotypes with high essential oil and efficiency should be developed (Lal, 2014).

There is wide genetic diversity in the basil germplasm. This diversity should be defined and included in purposeful breeding programs and the development of new varieties should be paved (Varga et al., 2017).

1.6. Oregano (*Origanum onites* L.)

The flora of Turkey is quite rich in terms of Thymus (40 species), Origanum (27 species), Satureja (15 species) and Thymbra (3 species), known as thyme (Güner et al., 2012; Anonymous, 2023).

These species, which have an important place in the flora of Turkey, have been used in traditional and preventive medicine for years, and the biological activity they exhibit thanks to the secondary metabolites they contain has allowed these species to be used in large areas (Tepe et al., 2016; Erenler et al., 2018). In addition, the commercial value of oregano species increases the importance of these species. *Origanum onites* L., known as Turkey thyme or ball thyme, is the most prominent thyme species in terms of both production amount and trade volume (Baser, 2022). Oregano/Thyme is used in the preparation of essential oil, spice, herbal tea, folk medicine and various beverages (Yaldiz et al., 2005).

Oregano (*Origanum onites* L.) is a perennial herb and the plant height can reach up to 100 cm (Gönüz and Özörgücü, 1999). Essential oil can be obtained from the above-ground part of the plant by steam or water distillation method. The essential oil rate varies between 2-5% (Bozdemir, 2019). Its essential oil is rich in carvacrol, thymol and α-terpinene components. In chemotypes whose essential oil is rich in the carvacrol component, the proportion of the component rises above 80% (Bozdemir, 2019). The plant is fringe rooted and roots can reach 35 cm in depth. The plant is multi-stemmed and has between 15-50 green grass stems. Its leaves are heart-ovate in shape and the leaf surfaces are covered with a cuticle layer and contain abundant glandular hairs (Batıray, 2009).

Breeding criteria

High in essential oil

Drog herb yield is high

Increasing the ratio of carvacrol in chemotypes whose essential oil is carvacrol (>85)

The essential oil ratios change according to the vegetation period. While less essential oil synthesis takes place in cold and rainy vegetation periods, more essential oil is synthesized in hot and dry weather conditions. Selection of genotypes suitable for growing ecology will minimize the effect of adverse environmental conditions.

1.7. Coriander (*Coriandrum sativum* L.)

Coriander is a member of the Umbelliferae family and is cultivated for its seeds (Mandal and Mandal, 2015). It is an annual plant and its chromosome number is 2n=22. It is thought to originate from the Mediterranean basin and the Middle East (Bhat et al., 2014). Apart from the seeds of the plant, other parts are used as a spice to flavor foods and in traditional medicine. The largest producer is South Asian countries and the highest production is made in India (Coşkuner and Karababa, 2007; Ravi et al., 2007; Nadeem et al., 2013).

Plant height varies between 20 cm and 1.40 m. The seeds of the plant germinate epigeal. The one-year herbaceous plant is pile rooted. The plant usually branches from the top and there are flowers of the plant in the form of umbrellas at the ends of the branches, the leaf edges are fragmented and they are usually in a three-lobed structure (Diederichsen, 1996). The flowers of the plant are small, short-stemmed umbrella type, can be in various colors from light pink to white. Its fruits are spherical, with 2 pericarps and 6 mm in diameter (Sahib et al., 2013). The volatile oil rate in the ripe fruit varies between 0.03% and 2.6%. The main component of its essential oil is linalool. It is also found in fixed oil in its seeds and varies between 9.9% and 27.7%. In the fatty acid composition of its oil, petroselinic acid (60-68%) is the main component (Nadeem et al., 2013). The time between planting and harvesting maturity is 100 days, and in varieties with smaller seeds, the maturation period can be up to 120 days (Chahal et al., 2017).

Breeding criteria

Coriander fruit quality directly affects the product sales price, improving fruit quality criteria in line with market demands (Yilmaz et al., 2022)

Increasing seed yield

Increasing the essential oil ratio

Increasing the ratio of linolol compound, which is the main component of essential oil, in essential oil (>80%)

Improvement of yield and agricultural characteristics

1.8. Lavender (*Lavandula angustifolia* Mill.)

Lavender (Lavandula sp.) is a valuable essential oil plant belonging to the Lavandula genus from the Lamiaceae family (Kara and Baydar, 2012). Due to its high content and quality essential oil, lavender is used in different sectors such as perfume, cosmetics and medicine. Although there are about 39 species belonging to the genus Lavandula, the most important species are *Lavandula angustifolia* Mill. (lavender), *Lavandula indermedia* Emeric. (lavandin) and *Lavandula spica* L. (spike lavender) species (Kara and Baydar, 2013).

In general, high-quality lavender essential oil contains high levels of linalool and linalyl acetate and camphor, with various compounds present, but the odor of the essential oil deteriorates as the camphor content increases (Adam, 2006). This causes the oil quality to decrease. In addition, phenolic acids, flavonoids and tannins from the lavender plant are present in different amounts (Robu et al. 2011). Lavender oil is known for its antibacterial, carminative, antifungal, calming, nervous system stimulant and curative effects on various skin diseases (Sabara and Kunicka-Styczyńska, 2009). In addition to these, it is also used in phytotherapy for the relief of cough, neuralgia and insomnia (Ghelardini et al 1999, Cavanagh and Wilkinson 2002, Hritcu et al. 2012). Lavender, which is cultivated in almost all parts of the world, spreads most in the countries of Southern Europe and North Africa neighboring the Mediterranean, especially in the Mediterranean and Balkan countries (Akçay et al., 2021). In Turkey, it was first produced in the province of Isparta and spread rapidly in other provinces (Özyılmaz et al., 2020). In 2022, 7,722 tons of lavender was produced in an area of 47 176 decares in our country (TUIK 2022). Lavender gardens, which have been the focus of attention of both domestic and foreign tourists in recent years, contribute to the country's economy. For these reasons, the improvement studies in lavender are increasing from year to year.

Breeding criteria

Plant height

Number of plant branches

Number of leaves

Hairiness

Flower stem length

Flower spike length

Flower color / brightness

Seed size

Fresh branched flower yield

Dry branch flower yield

Essential oil content

Linelool and amount of linalyl acetate and camphor components

Cold resistance

1.9. Lemon Balm (*Melissa officinalis* L.)

A member of Lamiaceae family, lemon balm is a fragrant, perennial, shrubby plant. Known for its lemony scent, this plant has 3 subspecies (spp. *officinalis*, spp. *altissima*, spp. *inodora*) and is among medicinal and aromatic plants (Katar and Gürbüz, 2008; Kaçar et al., 2010). Balm, also known as lemon balm, can be grown in the Mediterranean climate, including our country, and throughout the Alpine Regions (Baytop, 1984; Sarı and Ceylan, 2002; Kızıl, 2009). It contains lower levels of essential oil compared to other medicinal-aromatic plants (Kızıl, 2009). Thanks to the valuable compounds it contains, it is used in different industries such as food, cosmetics and medicine. In our country, *M. officinalis* leaves, also known as lemon mint, lemongrass herb, are used because of their soothing, stomachic, gas-digesting, diaphoretic and antiseptic effects (Başkal et al. 2017). It also improves mood; It has been determined by various studies that it is beneficial when used in conditions such as anxiety and related insomnia, Alzheimer's, dementia, oxidative stress due to low-dose radiation exposure (Kennedy et al. 2003; Safdari-Dehcheshmehi et al. 2016). Contrary to the low amount of essential oil, the organic compounds it contains increase the importance of the plant and bring it to the fore in agricultural studies.

Breeding criteria

Plant height

Number of plant branches

Leaf yield (fresh and drog)

Leaf and seed size

Flowering time

Flower color

Germination purity

Increasing the number of annual formats

Weed resistance

Resistance to fungal diseases

Essential oil content

1.10. Mint (*Mentha* sp.)

Mint is a group of plants from the Lamiaceae family with a pleasant perennial, fragrant, stolon body structure (Baytop, 1992). The genus Mentha has 31 species spread all over the world and many subspecies and varieties of these species (Tucker and Nazcı 2007). It has been determined that there are 7 species of this genus and 14 taxa belonging to this genus in Turkey (Davis, 1982). The presence of various essential oil components in its content allows it to be used in different fields such as medicine, food, cosmetics and chemistry (Telci, 2010). It is consumed both fresh and dry. The most cultivated and economically important mint species in the world are Japanese mint (*Mentha arvensis*), English mint (*Mentha piperita*) and spearmint (*Mentha spicata*) (Forbidden, 2019). Peppermint has medicinal, carminative, refreshing, cooling, stomach relaxing and stimulating effects (Kumar and Chattopadhyay, 2007). While approximately 14 500 tons of production was made according to 2017 data, approximately 27 thousand tons of production was made according to 2022 data (TUIK, 2022). Peppermint, which spreads naturally in almost every region of our country, is cultivated in Marmara, Aegean, and Mediterranean regions and is also cultivated (Yılmaz and Taşkaya 2022). Peppermint, a member of the group of medicinal and aromatic plants, can be easily propagated by seeds, cuttings and underground shoots.

Breeding Criteria

Plant height

Number of branches

Number of plant leaves

Leaf thickness, width and length

Leaf color

Weed resistance

Resistance to root diseases

Drought tolerance

Fresh/drog leaf yield

Essential oil content

Ratios of menthol, menthone, carvone and menthofuran

1.11. Fennel (*Foeniculum vulgare* Mill.)

Fennel (*Foeniculum vulgare* Mill.) is one of the 300 genera in the Apiaceae family (Davis, 1978). Fennel grown in various parts of the world is mostly grown in India, Egypt, Turkey, China, Argentina, Pakistan and Indonesia (FAO, 2021). There are two different cultivars of fennel, bitter fennel (F. vulgare var. vulgare) and sweet fennel (F. vulgare var. dulce) (Baydar, 2016). There are single and perennial sub-varieties of bitter fennel species (Karataylı, 2020). *F. vulgare* var. *dulce*, which is sweet fennel. Although it is generally grown for one year, it is also rarely grown for two years (Muckensturm et al., 1997; Özyılmaz, 2015). Roots and fruits of fennel can be used as herbal medicine alone or in combination with drugs and different products, as a carminative, milk enhancer in nursing mothers, diuretic roots and leaves as wound healing, the remaining vegetative parts can be used as vegetables. (Davis, 1978; Baytop, 1999; Tamkoç, 1984; Tabata et al., 1993). In our country, fennel plant and its essential oil; It is used in the food industry, making alcoholic and non-alcoholic beverages, confectionery, meat products, pickles and salads. Although it is used in different fields, the production of fennel in our country is not sufficient. Increasing the production of the fennel plant, of which almost all parts are used, will also contribute to the country's economy. Various studies can be done in this context.

Breeding criteria

Plant height

Number of plant branches

Number of umbels

Fruit yield

Fruit size

A thousand fruit weight

Drought tolerance

Resistance to root diseases

Essential oil yield

Fixed oil yield

1.12. Black Cumin (*Nigella sativa* L.)

Black cumin is a medicine and spice plant grown in arid and semi-arid climatic conditions in the Ranunculaceae family. It is stated that the origin of the plant is Eastern Mediterranean, Southern Europe and Asia Minor (Gharibzahedi et al., 2010; Baydar, 2013; Özer et al.,2020). *Nigella sativa* is an annual, upright growing plant with a plant height of 20-70 cm. The leaves are three-part. An average of 4-6 branches are formed in a plant. There is a flower at the end of each branch, and the flowers have 5 light blue petals. Its fruit is in the form of a capsule with 5 chambers separated from each other by placentas. Seeds are arranged on both sides of each placenta. Its seeds are dark black with 3-4 corners. The 1000 seed weight of black cumin is 2-3 g

(Baydar, 2013; Salehi, 2016). The economically evaluated part of black cumin is its seeds. Its seeds contain on average 20% protein, 30% fixed oil, 35% carbohydrate and 0.3-0.5% essential oil. Thymoquinone, which constitutes 25-60% of the essential oil obtained from its seeds, has antioxidant, anti-histamine, analgesic and anti-inflammatory effects (Baydar, 2013). Black cumin seeds have appetizing, diuretic, germicidal, digestive, cholesterol-lowering, relief from headaches and toothaches, and preventive effects on eczema and back pain (Khan et al., 2011; Baydar, 2013). A significant number of studies have shown that black cumin has antioxidant, antihypertensive, antimicrobial, antibacterial, antifungal, antiviral, anti-inflammatory, anticancer and antidiabetic activities and neuroprotective and analgesic effects (Abdallah, 2017; Özdemir et al., 2018; Yimer et al., 2019).

Breeding criteria

High seed yield

The number of branches in the plant, grain filling rate and flowering time are the best indirect selection criteria to increase the yield of black cumin (Salehi et al., 2016).

High 1000 seed weight

High fixed oil content in seeds

High amount of essential oil in seeds

High amount of thymoquinone in essential oil

Resistance to diseases and pests

1.13. Caraway (*Carum carvi* L.)

Caraway (*Carum carvi* L.), a member of the Apiaceae family, has an herbaceous structure, has 2n=20 chromosomes, and is in the form of a two-year culture. It is cultivated in Western Asia, Europe and North Africa (Nemeth, 1998; Miraj and Kiani, 2016). It can be cultivated from Northern Europe to the Mediterranean region. It is among the countries producing in Russia, Iran, Indonesia, North America, Siberia, Egypt, Australia, China and Turkey (Agrahari and Singh, 2014). Caraway is often used as a spice or essential oil for the cosmetic industry. Caraway fruits contain many primary (sugar, oil, amino acids, free organic acids) and secondary metabolites (terpene, flavonoid, steroid, tannin and phenolic substances, etc.). Thanks to the primary and secondary metabolites it contains, it is used both in nutrition and in its medicinal properties (Mirmazloum et al., 2020). The fruits of the plant contain 1% to 6% essential oil. Carvone and limonene constitute 95% of its essential oil (Sedláková et al., 2003).

Breeding criteria

Caraway collection has significant differences in terms of morphological and yield parameters, these genotypes should be purified by studying the adaptation and selecting productive genotypes (Seidler-Łożykowska and Bocianowski, 2012)

Genotypes with high yield stability should be selected.

Genotypes with high essential oil content should be developed.

Studies should be carried out on genotypes that do not shed grain at harvest maturity (Šmirous et al., 2006)

There are studies on obtaining double haploid plants by an anther culture (Smykalova et al., 2005), these protocols should be developed and genotypes with high essential oil and yield potential should be purified.

The content of essential oil consists of carvone and limonene, the quality should be increased by choosing varieties with high carvone content and low content of limonene (Pank et al., 2005; Baydar, 2020)

1.14. Cumin (*Cuminum cyminum* L.)

Cumin is a diploid species with 14 chromosomes (2n=14) from the Apiaceae family. It is an annual, herbaceous structure that can grow up to about 60 cm. It has small, white or pink compound umbel flowers. It blooms in May-June. Its leaves are 5-10 cm long and threadlike (Roustakhiz and Raissi, 2017). Its fruits are schizocarp, 4-5 cm long and 2-3 mm wide. The economically used part of cumin is its fruits. Its fruits contain 2.5-5.0% essential oil. Cuminaldehyde (35-63%) and dihydrocuminaldehyde in essential oil are the most important fragrance components (Baydar, 2009). In addition, fruits contain fixed oil (20-24%), protein (9-11%), fiber (10-12%) and amino acids (Sowbhagya, 2013). It is widely used as a spice due to its unique aroma. Fruits have antimicrobial and antioxidant effects due to their chemical composition (phenolic compounds, essential oils, anthocyanins, etc.). In addition, it has been determined that it exhibits diuretic, cytotoxic, antitumor, anti-inflammatory, antifungal and antispasmodic activities (Agarwal et al., 2017).

Breeding criteria

High seed germination rate

High fruit yield

Increasing the fruit essential oil ratio

Essential oil composition

Disease resistance (especially fungal diseases)

Plant biomass has a positive and direct effect on essential oil yield. Since the number of umbrellas, canopy diameter and 1000 fruit weight in the umbrella constituting the plant biomass have the highest direct effect on essential oil yield, they are suitable characters for the development of the best cultivars in cumin breeding. In other words, selection based on plant biomass is more effective in improving essential oil and seed yield (Faravani et al., 2018; Kaya, 2022).

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CHAPTER 2

SWEET FENNEL (*Foeniculum vulgare* MILL. var. *dulce*): CULTIVATION AND INDUSTRIAL USE

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INTRODUCTION

Fennel (*Foeniculum vulgare* Mill.) is a valuable essential oil and spice plant from the Apiaceae family. Fennel, which is thought to be endemic in the Mediterranean region, originated from these regions and spread to a wide geography extending to Asia, Africa, Europe and Oceania (Chatzopoulou et al., 2006; Grover et al., 2013; Diao et al., 2014; Abdellaoui et al., 2017; Jadid et al., 2023). World fennel production data is not given alone, but cumulatively presented with some medicinal and aromatic plants such as anise, fennel and coriander. For this reason, official data about its production cannot be reached around the world, and information about its production is obtained with the own reports of local governments. It is known that the country with the highest production in the world is India with 90,000 ha. It is produced in significant quantities in India and Turkey, Syria, Egypt, Germany, Spain and Pakistan (Rahman et al., 2020) (Figure 1).

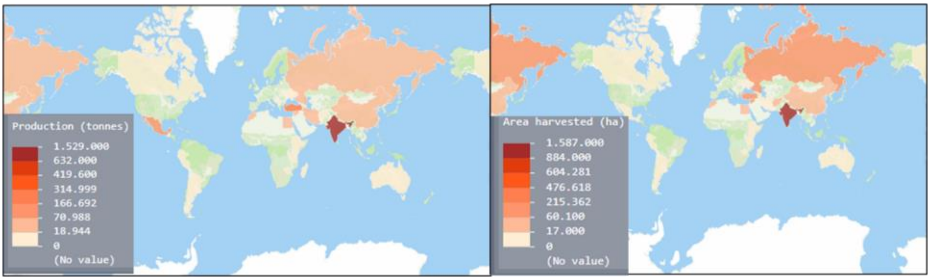

Figure 1: World fennel production (https://data.apps.fao.org/ accessed date: 29.05.2023)

According to 2022 production data in Turkey, a total of 2323 thousand tons of production was made with an average yield of 196 kg da^{-1} on an area of 11.875 decares (TUIK, 2023). Production data of the last 10 years in Türkiye are presented in Figure 2 and Figure 3. Burdur is the province with the highest production in Türkiye, followed by Antalya and Konya. Apart from these 3 provinces, a small amount of fennel is produced in Ankara, Afyonkarahisar and Manisa (TUIK, 2023).

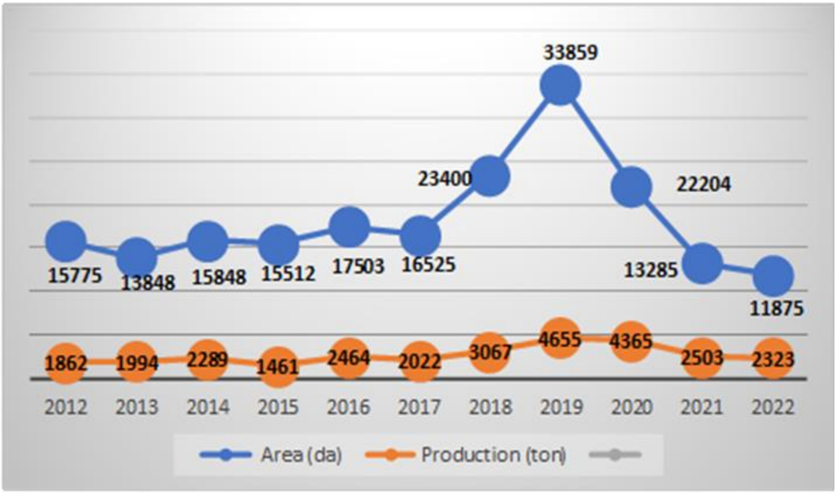

Figure 2: Fennel cultivation area and production amount for the last 10 years in Türkiye (TUIK, 2023).

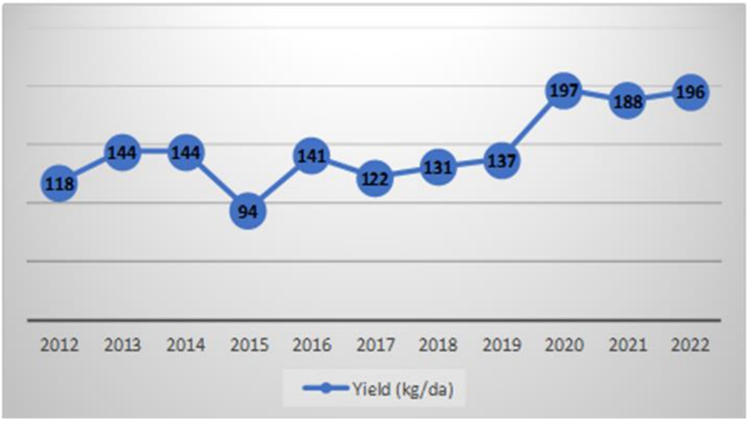

Figure 3: Fennel yield for the last 10 years in Türkiye (TUIK, 2023).

1. TAXONOMY AND PLANT CHARACTERISTICS

The taxonomy of the fennel plant is given in Table 1.

Table 1: Taxonomy of Fennel Plant (Menemen, 2012)

Regnum	**:** Plantae
Divisio	**:** Magnoliophyta
Subclassis	**:** Magnoliidae
Familia	**:** Apiaceae
Genus	**:** *Foeniculum*
Species	**:** *Foeniculum vulgare* Mill.

Fennel is an aromatic herb that can be grown as an annual, biennial or perennial (Mishra et al., 2016; Singh, 2017). There are varieties between wild and cultivated fennel that differ in size, odor and taste of the fruit. There are two subspecies of this species, "piperitum" (growing wild in the Mediterranean region) and "capillaceum" (widely cultivated), which are widespread throughout the world. There are three varieties of the Capillaceum subspecies. These:

1-*Foeniculum vulgare* var. *azoricum*

It is one-year. It has the ability to form bumps. It is consumed as a vegetable (Figure 4).

Figure 4: *Foeniculum vulgare* var. *azoricum*

(https://www.floragard.de/de-de/pflanzeninfothek/pflanze/gemuese/foeniculum-vulgare-var-azoricum) (Accessed date:17.06.2023)

2-*Foeniculum vulgare* var. *vulgare*

Bitter fennel. It is perennial. It is rich in fenchone. It is used in industry because of its aromatic bitterness and sharp aroma (Figure 5).

Figure 5: *Foeniculum vulgare* var. *vulgare*

(https://www.richters.com/Web_store/web_store.cgi?product=X2423&show=&prodclass=Herb_Seeds&cart_id=111.100) (Access date:17.06.2023)

3-*Foeniculum vulgare* var. *dulce*

Sweet fennel. It is usually an annual, sometimes biennial. It is grown for its aromatic fruits and essential oil (Figure 6).

Sweet fennel is foreign fertilized and the number of chromosomes in its somatic cells is 2n=22 (Malhotra, 2012). The stem of the plant is hollow and green in color. It grows upright and can grow up to 2.5 m in length (Dheebisha and Vishwanath, 2020). It has bisexual yellow oval and umbrella-shaped flowers (Rafieian et al., 2023) (Figure 6).

Figure 6: Sweet fennel flowers (left) and fruits (right) (Yozgat Bozok University Trial Area)

The fruits of the plant contain 9.5% protein, 10% fat, 42.3% carbohydrates, 18.5% fiber and various minerals and vitamins (C, E, B6, thiamine, riboflavin and niacin) (Abdellaoui et al., 2017). The stem, fruit and leaves of the plant are widely used in many culinary traditions (Nourimand et al., 2012; Abdellaoui et al., 2017).

The pale green, pale yellowish-brown fruits of sweet fennel contain volatile oils ranging from 1.0% to 6.0% (Cosge et al., 2008; Saharkhiz et al., 2011; Malhotra, 2012; Avci, 2013; Salami et al., 2016). Sweet fennel essential oil contains an average of 70-90% trans-anethole (Cosge et al., 2008; Saharkhiz et al., 2011; Salami et al., 2016; Borotová et al., 2021). Other important components in the essential oil were recorded as estragole (2.5-6.4%), limonene (2.18-7.12%), and α-fenchone (0.97-13.0%) (Cosge et al., 2008; Saharkhiz et al., 2011; Malhotra, 2012; Avci, 2013; Salami et al., 2016, Borotová et al., 2021). A maximum of 7.5% fenchone and 10% estragole is allowed in the essential oil obtained from sweet fennel fruits (Salami et al., 2016). However, there are types that do not contain any fenchone.

It is emphasized that the amount and chemical composition of essential oil obtained from fennel fruits are affected by many factors such as geographical origin, genotype, phenology, cultural practices, ecological conditions, extraction method (Cosge et al., 2008; Sahahat et al., 2012).

***Trans*-Anethole:** Anethole is an aromatic compound commonly found in essential oils in nature (Figure 7). It is a clear, colorless or amber liquid, sparingly soluble in water but highly soluble in ethanol. It has a sweet aroma. It is 13 times sweeter than sugar (Marinov and Valcheva-Kuzmanova, 2015). Trans-anethole is the main component of essential oil obtained from fennel fruits and is used in different industrial areas (Table 2).

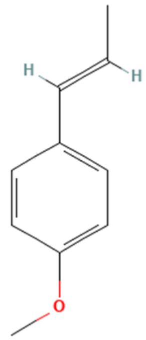

Figure 7: Chemical structure of *trans*-anethole (https://pubchem.ncbi.nlm.nih.gov/compound/637563#section=Structures) (Accessed date:12.06.2023)

Table 2. Some Chemical Properties and Uses of Trans-Anethole[1]

Molecular Formula	$C_{10}H_{12}O$
Molecular weight	148.20 g/mol
Chemical Classes	Other aromatic compounds (A natural product)
Pharmacological Classification	Flavoring agent (substances added to foods and drugs to improve taste)
USES	
1-Cleaning and home care products	Household air fresheners, including scented candles
2- Flavoring	
3- Fragrance	
4- Fragrance component	
5- Personal care	
a- Dental care	Toothpastes and teeth cleaning products
b-Fragrance	Fragrances, colognes and perfumes
6-Vehicle-Car interior	
a-Automatic air freshener	Products to mask odors or add fragrance to cabin air

[1] https://pubchem.ncbi.nlm.nih.gov/compound/637563 (Accessed date: 11.06.2023).

Anethole;

-It is added to alcoholic beverages (like Turkish raki).

It has the feature of covering unpleasant odors. Due to this feature, it is considered as a masking agent in many products (toothpastes, mouthwashes, toilet soaps, etc.) (=MAIN USAGE AREA).

-It is used as flavoring and fragrance in some food industry products (bakery products, candies, etc.).

- It is included in chewing gum and cigarettes for both aroma and fragrance (Marinov and Valcheva-Kuzmanova, 2015).

2.USES OF THE FENNEL PLANT

Food: Fennel is grown mainly for its fruits, which have a pleasant smell and aromatic taste. It is widely used in various Indian dishes to season soups, sauces, pastries, confectionery, breads, liqueurs, meat dishes and pickles. Its essential oil is used as a flavoring agent in various foodstuffs, pickles, perfumes, soaps, liqueurs and liqueur ice candies. It is used in the production of herbal tea (Saber and Eshra, 2019).

Medical: Fennel contains anethole, which, along with other pharmacologically active substances, is antispasmodic. It is reported to be effective in the treatment of diseases of bacterial, viral and fungal origin (Noreena et al., 2023). It has a wide range of biological activity (Kwon et al., 2002; de Marino et al., 2007; Badgujar et al., 2014) (Figure 8). It has carminative, expectorant and spasmolytic activity, especially at the respiratory and digestive level. It also shows estrogenic activity. It is also used externally; in which case it acts as an anti-inflammatory. Fennel is also beneficial in amenorrhea and dysmenorrhea (Mahboub, 2019). It is traditionally used in medicines to treat tremors and stomach problems (Raghavan, 2007).

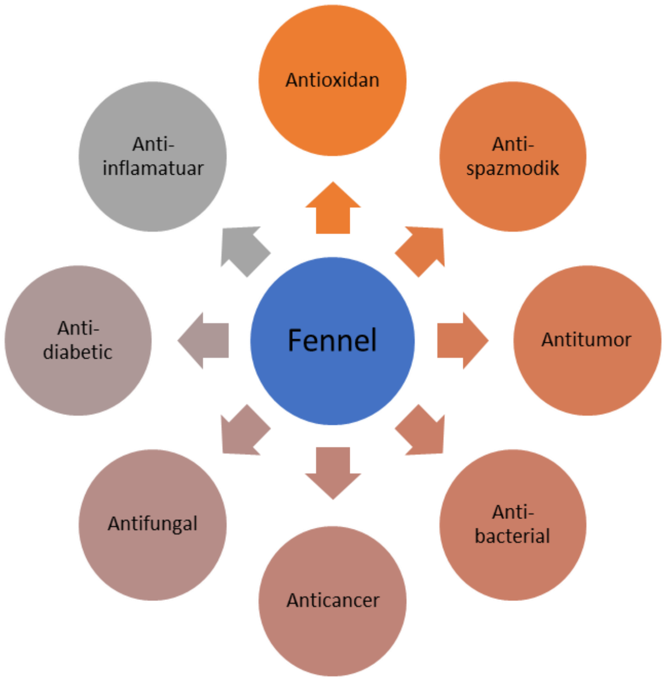

Figure 8: Biological activity of fennel plant

Its fruits are aromatic, act as a stimulant and are carminative. It is used in diseases such as cholera, bile, nervous disorders, cough and cold, constipation, dysentery and diarrhea, as well as diseases affecting the chest, lungs, spleen and kidneys (Saber and Eshra, 2019). In folk medicine, a hot infusion of the fruit is used to increase milk secretion. Its essential oil is used in cough lozenges. Fennel is used as a sweetener in natural toothpastes (Badgujar et al., 2014; Noreena et al., 2023, Kim et al., 2023).

Perfume-Cosmetics: Its essential oil is used in perfumes and soaps. Due to its antimicrobial properties, it is used in cosmetic creams, body lotions and moisturizers. The bulb, leaves, and seeds of the fennel plant are used in different ways in cuisines around the world (Stefanini et al., 2006).Fennel pollen has very strong antioxidant properties.

Fennel fruits contain 12-15% fatty oil and 80-85% of the oil is petroselinic acid ($C_{18}H_{34}O_2$, unsaturated fatty acid). It can be used in the edible oil and biodiesel industry with its high melting points. It can also be evaluated in the food and perfumery industry due to its antimicrobial effect (Baydar, 2016).

To Feed Animal: The residue remaining after distillation of the essential oil from the fruit is used as a rich cattle feed.

3. CULTIVAION

Fennel is grown in warm and temperate climatic regions where the Mediterranean climate is dominant (Jaitawat, 2007). The soil selectivity of the fennel plant is low. It can be grown in all kinds of soils, only the cultivated areas should not be affected by excessive salinity and flooding (Grover et al., 2013). It generally grows well in lime-rich, humus, sandy, sandy-loam soils with a pH between 4.8-8.3. It is also reported that it can grow in sodic soils. It has been reported that fennel can take part in the improvement of sodic soils because it accumulates a large amount of sodium in its own structure and removes a large amount of sodium from the soil (Garg, et al. 2004).

Fennel seeds can be planted directly in the field, or it can be produced by planting the seedlings formed in the nursery. The yield is higher when planted as a seedling (Jaitawat, 2007). The optimum temperature should be 15-20°C during the development phase of the plant. During the seed setting stage, dry and cool air increases seed yield and product quality (Dheebisha and Vishwanath, 2020).

In fennel cultivation, fertilization can be made with 4 kg N and 6 kg P2O5 per decare. The planting norm of fennel should be 60 x 2-3 cm, with 1.5 kg of seeds per decare. Sowing time is autumn or early spring (Baydar, 2020). It can be irrigated 1-3 times according to the sprinkler or drip method, especially at the beginning of flowering and during the seed setting period. Irrigation increases yield. With the increase of weed density with irrigation, it is struggled by hand hoeing or mechanized.

Fennel fruits are harvested by mowing or removing at a time when they are just starting to turn brown. Harvest time usually coincides with August. Harvested plants are blended after drying for a few weeks (Baydar, 2020) (Figure 9).

A

B

C

Figure 9: The beginning of flowering (A), full flowering (B), and seed maturity (C) stages in sweet fennel (Yozgat Bozok University-2022)

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CHAPTER 3

LEGUME FORAGE CROPS WITH MEDICINAL VALUE AND THEIR SECONDARY METABOLITE CONTENTS: *Vicia* sp., *Trifolium* sp., *Trigonella foenum-graecum* L., *Lathyrus* sp.

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INTRODUCTION

Secondary metabolites are effective in developing defense mechanisms against disease-causing bacteria, fungi, viruses and parasites in plants. In addition to this, they play a role in resistance to biotic and abiotic stresses, attracting insects for pollination, and interacting with rhizobium bacteria for nodule formation in legumes (Ku et al., 2020; Kaushik et al., 2021; Elshafie et al., 2023). Additionally, there are also a wide variety of secondary metabolites with pharmaceutical, nutraceutical and toxicological effects in humans (Wink, 2013; Kazlauskaite et al., 2023). Plant-specific metabolites have received great attention in recent years; they are used as a source of pharmaceuticals, nutrients, agricultural chemicals and chemical additives (Tiwari and Rana, 2015).

The structure of secondary metabolites and their components vary according to plant species. In this sense, legume forage crops are the main sources of secondary metabolites such as polyphenols, saponins and alkaloids, as well as being rich in proteins, lipids, vitamins and carbohydrates. Many studies have reported that legumes show secondary metabolite content and antioxidant activity (Pastor-Cavada et al., 2009; Xu and Chang, 2010; Zhao et al., 2014; Llorent-Martínez et al., 2017a; Yazici Ozbek et al., 2020). In this section, secondary metabolites of some legume forage crops genera and species and their medicinal properties are pointed out.

1. VETCHES (*Vicia* sp.)

The genus *Vicia* belongs to the Fabaceae family; it contains important species considered as forage, silage, cover crop and green manure crops in different countries of the world (Figure 1).

Figure 1. *Vicia* species

Vetch seeds are a good food source for animals with their high nutritional value. The common vetch (*Vicia sativa*) (Figure 2) is the most cultivated vetch species around the world in this genus and stands out with its wide adaptability (Piergiovanni and Taranto, 2005; Huang et al., 2017). Common vetch is also the most important source of quality roughage for livestock enterprises worldwide with its rich protein, mineral matter and vitamin content. At the same time, they also contain a large number of chemical compounds, antinutritional and/or bioactive compounds with various pharmacological activities that have significant potential benefits for human health (Megías et al., 2009; Saleem et al., 2014). Although *Vicia* species are a quality forage source, it has been reported that many species are toxic as a result of chemical analyzes (Ressler et al., 1997; Sadeghi et al., 2004). In this sense, in a study with 12 *Vicia* species, 184 metabolites were identified in *Vicia* seeds (Abozeid et al., 2018).

Figure 2. *Vicia sativa* L.

In general, legumes are rich in polyphenols and other phytochemicals; in this respect, they are known as products with great potential (Megías et al., 2009). In this meaning, *Vicia* species are also rich in polyphenols; the highest polyphenol content was found in *V. sativa*, which is widely grown in Mediterranean countries such as Türkiye, Syria, Morocco, Algeria and Spain (Robertson et al., 1996). In a study, it was emphasized that the polyphenol extracts obtained from *V. sativa* showed promising antioxidant and antiproliferative activities, and that this ancient product is an interesting plant in terms of re-evaluation in this respect (Megías et al., 2009). In different studies, various secondary metabolites obtained from *V. sativa*; it has been reported that it exhibits cytotoxic activities against human tumor cells, especially HeLa cells (Liu et al., 2020).

The seeds of *V. cracca* and *V. palaestina* species contain polyphenols, lectins and the non-protein amino acid canavanin (Megías et al., 2018). Lectins, which are carbohydrate-binding proteins that may have antinutritional activity by interfering with food absorption in the small intestine (Rüdiger and Gabius, 2001), are potential anticancer agents (Mazalovska and Kouokam, 2020); it is found in *V. unijuga*, *V. cracca, V. faba* and *V. ervilia* species (Fornstedt and Porath, 1975; Hemperly et al., 1979; Baumann et al., 1982; Yanagi et al., 1990). *Vicia palaestina* Boiss., an annual herbaceous vetch that grows in arid regions of Eastern Mediterranean countries, is a source of lectins with health-promoting and pharmacological potential due to its seeds antiproliferative activities, in addition to its high nutritional value (Elamine et al., 2021). A total of sixteen essential oil components, representing 82.2% of the total composition, were detected in the above-ground parts of another *Vicia* species, *V. ochroleuca* Ten., and the results suggested that *V. ochroleuca* essential oil could be considered as a natural bioactive source with anticancer activity (Boussaha et al., 2023).

Vicia sativa extract has antioxidant, anti-inflammatory and antinociceptive activities due to some flavonoid components such as apigenin, kaempferol, luteolin, quercetin, and these activities may help in the prevention and/or treatment of inflammation and pathological disorders (Gamal-Eldeen et al., 2004).

Vicia sativa is a medicinal plant traditionally used in skin infections, asthma, bronchitis, urinary tract diseases; it is also used as an antiseptic, anti-poison, aphrodisiac, anti-rheumatic and antipyretic. *Vicia sativa* also has significant antibacterial activity (Saleem et al., 2014).

In a study comparing *V. sativa* and *V. faba* seed extracts; while total antioxidant activity was higher in *V. sativa* extracts, radical scavenging activity, cholinesterase and tyrosinase inhibitory activities were higher in *V. faba* extracts. In the same study, the total polyphenol content was higher in *V. sativa*, and both species were characterized by the presence of apigenin and quercetin glycosides. These results showed that *V. sativa* seeds have health-promoting properties with their higher antioxidant activity (Megías et al., 2016).

A total of 27 varieties belonging to ten different *Vicia* species were examined in terms of flavonoid content, total polyphenol content and 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2' azinobis (3-ethylbenzothiazoline 6-sulfonic acid) (ABTS). The results reported that *V. monantha* has the highest values in terms of total flavonoid and *V. hyrcania* has the highest total polyphenol content. Among the flavonoids, naringenin has been reported to be in high concentrations in *Vicia* species. In the same study, the highest antioxidant activity was found in *V. faba*. Researchers reported that the results of this research with *Vicia* species are important in terms of expanding the flavonoid database and providing valuable information about *Vicia* species for the development of functional foods or feed additive sources (Lee et al., 2017).

Figure 3. *Vicia ervilia* (L.) Willd

Vicia ervilia (Figure 3), one of the oldest plants of the Mediterranean Region, is a useful protein (24.1%) source for food and animal feed. The plant also contains bioactive components. In the analysis of seed samples from multiple *V. ervilia* populations, polyphenol contents ranged from 0.09% to 0.19%. Luteolin, kaempferol, apigenin and quercetin were identified as major aglycones. Seed extracts showed antioxidative effects by simultaneously inhibiting the proliferation of Caco-2 colon tumor cells. Therefore, *V. ervilia* seeds may represent a source of high-value food and feed ingredients as well as functional components (Vioque et al., 2020). *Vicia ervilia* seeds have anti-inflammatory, analgesic, antiulcerogenic, antihyperglycemic and antiviral activities. It is an excellent choice for the treatment of many diseases in developing countries due to its easy accessibility, affordability and newly proven important broad biological activities (Okba et al., 2017).

Among the six common vetch (*V. sativa* L.) cultivars tested, some cultivars (Istros and M-6900) exhibited an interesting phytochemical profile characterized by high phenolic content, significant antioxidant potency and remarkably high fatty acid indices. These findings are indicative of the great potential of these cultivars to function as suitable candidates for inclusion in livestock diets as dry biomass (straw) or cereal feed additives (Myrtsi et al., 2023).

Vicia species contain various bioactive compounds such as flavonoids, phenolic acids and phytic acid, which have antioxidant properties. This makes *Vicia* strains valuable sources of natural antioxidants that can help prevent a variety of health problems, including cancer, heart disease, and aging. In addition to their antioxidant properties, some *Vicia* strains also have cytotoxic activity, which is, the ability to kill cancer cells or inhibit their growth (Hajibayli et al., 2023).

Plants of the genus *Vicia* are of great interest as many bioactive compounds. Besides the traditional medicinal uses of *Vicia* plants, the wide range of major biological activities attributed to *Vicia* species have potential health beneficial properties, particularly anti-Parkinson, anticholinesterase, antidepressant, anticonvulsant, antimicrobial, cytotoxic, antioxidant, anti-inflammatory, antinociceptive, antidiabetic, antihemolytic, anticoagulant, estrogenic, diuretic, antihypoxic activities are discussed (Salehi et al., 2021).

2. CLOVERS (*Trifolium* sp.)

Phenolic compounds are one of the most prominent secondary metabolites of plants. Therefore, the biological activity of plants in general is primarily attributed to these compounds (Kazlauskaite et al., 2023). Clovers are also rich in phenolic compounds.

The genus *Trifolium* which is an important member of the Fabaceae family, includes annual and perennial species. The most common and well-known species among clovers is red clover (*Trifolium pratense* L.) (Figure 4). Red clover which is originating in Asia Minor and Southeast Europe (Yan et al., 2022) is common in temperate and subtropical regions of the world (Özyazıcı et al., 2021). There are also secondary metabolites in different parts of the red clover plant, which is rich in crude protein, minerals and vitamins (Acar et al., 1996; Özyazıcı and Manga, 1995, 1996). Terpenes and (iso)flavonoids constitute the most important secondary metabolite group in red clover (Medina, 2022). The main polyphenolic compounds in red clover are biochanin A, formononetin, daidzein and genistein (Lee et al., 2020; Kanadys et al., 2021; de Rus Jacquet et al., 2021).

Figure 4. *Trifolium pratense* L.

Red clover flower extracts are rich in isoflavones which have estrogenic activity. In addition to this, it also has antioxidant and antibacterial activities, which may be due to the presence of other flavonoids and phenolic compounds such as phenolic acids, clovamides and saponins (Vlaisavljević et al., 2014; Esmaeili et al., 2015). In a study conducted with *Glycyrrhiza glabra* L. and *T. pratense* L. extracts and *Myristica fragrans* Houtt. essential oil, *T. pratense* extract has been the highest antioxidant (26.27 ± 0.31 and 638.55 ± 9.14 µg TE/g dw by the DPPH and ABTS methods, respectively) and antiviral activity (56 times decreased titer of virus). In the same study, total phenol

(74.00 ± 0.15 mg GA/g dw) and flavonoid (19.50±0.04 mg RU/g dw) contents were found to be quite high in *T. pratense* compared to other plant samples. Researchers stated that it is possible to obtain a ready-to-use drug with strong antibacterial and antioxidant properties by using *T. pratense* extract together with other plants (Kazlauskaite et al., 2023).

The most well-known use of substances of natural origin in menopause is the use of isoflavones (Gosciniak et al., 2023). Soybean is one of the plants from which isoflavones are obtained. However, in recent years, one of the raw material sources rich in these secondary metabolites (Depypere and Comhaire, 2014) and from which isoflavones can be effectively isolated is red clover (Naseer et al., 2022; Gosciniak et al., 2023). Gosciniak et al. (2023) reported that while high total polyphenol content was determined in red clover flowers, higher isoflavone content was determined in leaves, but the isoflavone content also varied according to red clover cultivars. Although studies have focused on soybean as the most popular product for menopause, there is also scientific evidence for the health-promoting effects of red clover for this purpose (Gosciniak et al., 2023). Because of these secondary metabolites, red clover is used to treat and alleviate symptoms (flush, cardiovascular health effects, breast cancer and osteoporosis) that occur in postmenopausal women (Vlaisavljević et al., 2014; Won et al., 2023). Red clover extract has been reported to have therapeutic potential in diseases associated with ferroptotic cell death, particularly ferroptosis induced by cellular iron metabolism dysfunction. This report is also the first report on red clover in this manner (Won et al., 2023).

Phytoestrogen-isoflavones which is found in *T. pratense* show potential neuroprotective effects, especially in neurodegenerative disorders. It has been reported that experimental key findings have been obtained for the clinical use of prescriptions containing *T. pratense*-derived isoflavones for these effects (Al-Shami et al., 2023).

Red clover has also been used in traditional medicine to treat whooping cough, asthma, eczema, and eye diseases (Booth et al., 2006). In another study presented evidence that red clover is effective in lowering blood sugar and has beneficial effects in preventing cardiovascular diseases (Luís et al., 2018; Błaszczuk et al., 2022). An in vitro experiment also documented the anti-inflammatory and antioxidant effects of red clover (Lee et al., 2020; Masuda et al., 2021). Also, *T. pratense* has been used to treat edema, psoriasis, and prostate cancer (Burdette et al., 2002). In addition, it has been proven that *T. pratense* extract can be used as an anticariogenic agent (Kavishri and Rajasekar, 2023).

White clover (*T. repens*) (Figure 5), another plant of the *Trifolium* genus that is most commonly grown worldwide, has high quality with its high crude protein content (Petrović et al., 2016) and is known for its large amount of secondary metabolite groups (Ponce et al., 2004). In other words, white clover is a plant with high feeding and medicinal value (Qi et al., 2021). Petrović et al. (2016) determined that the total phenolic content of *T. repens* was approximately 37.2 (mg of GA/g of extract), the flavonoid concentration was 43.3 (mg of Ru/g of extract), and the antioxidant activity was 702 IC50 (µg/ml). In another research, total phenolic content, total flavonoid content and total tannin content of white clover (*T. repens* L.) 1.81 (mg GAE/g FW), 0.10 (mg QE/g FW) and 1.17 (mg CE/g FW), respectively (Iqbal et al., 2022). Phenolic acid and flavonoids are indicative of strong antioxidant activity. The plant is used in Turkish traditional medicine with these features (Sabudak and Guler, 2009). Products such as flavonoids obtained from white clover extract have multiple effects such as reducing blood fat, preventing cancer, strengthening immunity and antiaging (Marshall et al., 2004; Li et al., 2018).

Figure 5. *Trifolium repens* L.

Dried flowers of Berseem clover (*T. alexandrinum* L.) (Figure 6) are used in the treatment of asthma, cough and ulcer (Khan et al., 2014); strawberry clover (*T. fragiferum* L.) as an ointment for wounds and burns (Günbatan et al., 2016); white clover (*T. repens* L.) cough and cold (Amjad et al., 2015), skin diseases (Ghasemi et al., 2013), stomach disorders (Kilic and Bagci, 2013), neonatal jaundice (Tahvilian et al., 2014) and also as expectorant (Dolatkhahi et al., 2014), antipyretic (Ijaz et al., 2015) and antirheumatic (Ummara et al., 2013); Persian clover (*T. resupinatum* L.) is used to heal skin wounds (Sharma and Rana, 2014) and liver ailments (Gulshan et al., 2012) in traditional medicine.

Figure 6. *Trifolium alexandrinum* L.

From a pharmacological point of view, *T. pratense* is the most recognizable of the *Trifolium* species. On the other hand, preliminary in vitro and in vivo evaluations of antioxidant properties of many clover species were investigated. Despite these developments, chemical composition researches of plant extracts of clover species mostly focused on *T. pratense* and *T. repens*. More detailed characterization of the chemical composition of extracts of different clover species, including these clover species, should be made and their biological activities should be evaluated in more detail with various in-vitro and ex-vivo experimental models (Kolodziejczyk-Czepas, 2016).

3. FENUGREEK (*Trigonella foenum-graecum* L.)

Fenugreek (*Trigonella foenum-graecum* L.) (Figure 7) is one of the oldest known medicinal plants belonging to the Fabaceae family (Acharya et al., 2008; Mehrafarin et al., 2010; Naika et al., 2022), is used both as a spice plant and as a forage plant by making use of its grass and grain in many parts of the world (Naika et al., 2022). Fenugreek, which can also be used for silage (Özyazıcı et al., 2022); thanks to the high protein, vitamins and minerals contained in its hay and grain, it is an important source of roughage and an important component of the feed rations of ruminants in terms of protein supplement.

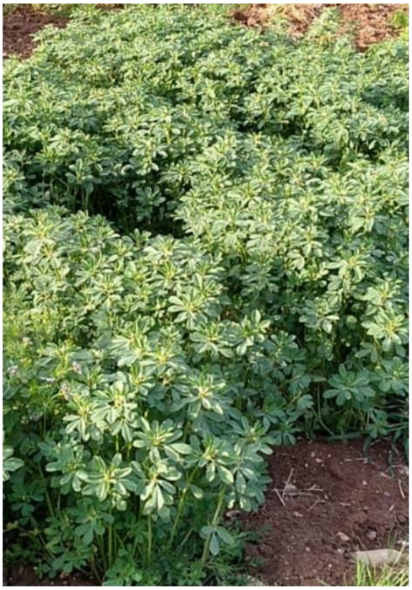

Figure 7. *Trigonella foenum-graecum* L.

Fenugreek plant contains secondary metabolites such as volatiles (eugenol, linalool), phenolics, steroids, terpenoids, saponins (diosgenin, fenugrin, foenugracin, yamogenin, tigonenin), flavonoids (quercetin, rutin, luteolin, naringenin, vitexin, kaempferol, isovitexin), alkaloids (trigonelline, glycine-betaine, choline, gentianine, trimethylamine, neurin), modified amino acids (4-hydroxyisoleucine) (Gupta et al., 2001; Kaviarasan et al., 2007; Ahmad et al., 2016; Özyazıcı, 2020; Güzel and Özyazıcı, 2021; Joshi et al., 2022; Naika et al., 2022; Srivastava et al., 2022). Having a wide repertoire in terms of secondary metabolites reveals the nutraceutical and pharmaceutical properties of fenugreek (Naika et al., 2022). As a matter of fact, Petropoulos (2002) reports that the pharmacological effects of fenugreek depend on the diversity of components such as steroids, nitrogen compounds, polyphenolic substances, volatile compounds, amino acids. Due to these secondary metabolites that fenugreek contains, it has many health benefits such as antioxidant, antimicrobial, antiseptic, anti-inflammatory, analgesic, anticancer, anti-infectious, antidiabetic, antimigraine, antibacterial, antiviral, anti-tumor, antidiabetic effects, antipathogenic properties and has some other medicinal properties (Srinivasan, 2006; Yadav and Baquer, 2014; Allaoui et al., 2019: Syed et al., 2020; Joshi et al., 2022; Naika et al., 2022; Falana et al., 2023). Secondary metabolites such as steroidal sapogenins (diosgenin, tigogenin, neotigogenin and yamogenin) are also part of the seed composition. These ingredients of fenugreek may reduce serum cholesterol levels by increasing bile excretion (Zameer et al., 2018). Diosgenin has also been attributed to treatments such as anticarcinogenic and hepatoprotective (Syed et al., 2020).

Nevertheless, the medicinal value of fenugreek in general is due to two important secondary metabolites called diosgenin and trigonelline (Petropoulos, 2002). In this sense, fenugreek is the original source of trigonelline which is the smallest alkaloid and multifunctional plant hormone (Lambein et al., 2008; Mandal and DebMandal, 2016). Trigonelline is considered as a physiological active compound that induces leaf movements in plants (Auerbach, 1961), accumulates as a result of stress (Rosser, 1985) and acts like an osmoprotectant (Shimizu and Mazzafera, 2000). It has been reported that trigonelline plays a role in various stress situations (Lambein et al., 2008) and may support the regeneration of neuronal networks in humans and prevent memory loss (Tohda et al., 2005). Among the other pharmacological activities of trigonellin include hypoglycaemic, hypolipidemic, antimigraine, sedative, antibacterial, antiviral, anti-inflammatory, antinociceptive, and anti-tumour activities (Mandegary et al., 2012; Zhou et al., 2012; Naika et al., 2022).

Diosgenin, a saponin group secondary compound, is an important raw material for the preparation of many steroidal drugs in the pharmaceutical industry (Jesus et al., 2016; Chaudhary et al., 2018). Plant sources rich in diosgenin include fenugreek (*T. foenum-graecum* L.) (Chaudhary et al., 2018). This secondary metabolite that is found in the fenugreek component has been reported to show high potential in the treatment of different types of disorders such as cancer (Raju et al., 2004; Jesus et al., 2016), hypercholesterolemia, inflammation and various types of infections (Jesus et al., 2016).

In addition, it has been reported that the saponin compound contained in fenugreek also reduces cholesterol in human blood (Sowmya and Rajyalakshmi, 1999), and fenugreek extracts have a chemopreventive effect against breast cancer (Amin et al., 2005) and oxidative damage (Kaviarasan et al., 2004). It has also been stated that fenugreek has properties against obesity prevention and this feature of fenugreek is making it a good herb candidate that is likely to be used to treat obesity (Liew et al., 2023).

Another bioactive component, 4-hydroxyisoleucine, which is abundant in fenugreek seeds (Naika et al., 2022), has more antidiabetic properties (Broca et al., 1999, 2004; Avalos-Soriano et al., 2016).

Secondary metabolites that are found in fenugreek leaves and seeds may vary depending on plant age and genotypes. As a matter of fact, Singh et al. (2013) reported that the polyphenol content ranged between 95.50-148.50 mg/100 g, the phytic acid content between 102.2 and 213.2 mg/100 g and the saponin content between 1245.0-1780.7 mg/100 g in fenugreek genotypes, and this variation was found to be significant between genotypes. Gupta and Singh (2002) reported that in some genotypes of fenugreek, total phenols and flavonols increased with the growth of leaves (leaf age), while in some genotypes they increased until the second form and then decreased. At the same time, the amounts of diosgenin determined in fenugreek seeds originating from Poland and Africa were found to be similar and varied between 0.12-0.18% (Król-Kogus et al., 2018).

Since ancient times, fenugreek has been used as a dietary condiment alongside its various therapeutic properties (Liew et al., 2023). Numerous studies have shown that the secondary metabolites in the fenugreek plant are responsible for its therapeutic properties.

4. *LATHYRUS* L. (*Lathyrus* sp.)

The most important species of the genus *Lathyrus* L. is the common grass pea (*Lathyrus sativus* L.) plant. Grass pea (*L. sativus* L.) (Figure 8) is a forage legume plant that is not soil-selective, resistant to drought, and is grown both for grass and grain (Lambein et al., 2008;

Özyazıcı and Açıkbaş, 2019). The plant is also more resistant to biotic and abiotic stress than other legumes and needs very little input (Lambein et al., 2008; Chandrashekharaiah et al., 2023). It is an ideal plant for sustainable agriculture due to its superior agricultural properties (Chandrashekharaiah et al., 2023), such as rich mineral content like calcium, magnesium, phosphorus, iron, zinc, manganese, copper (Parida and Ghosh, 2016; Al-Snafi, 2019; Özyazıcı and Açıkbaş, 2023), vitamin amount (Parida and Ghosh, 2016) and its rich protein content (Chandrashekharaiah et al., 2023; Edwards et al., 2023). Many legume forage plants contain biologically active compounds with antinutritional properties. The main compounds found in grass pea are phytic acid, tannins and β-*N*-oxalyl-1-α,β-diaminopropionic acid (β-ODAP) (Lambein et al., 1993; Ramachandran and Ray, 2008; Arslan et al., 2022). Bhattacharjee et al. (2018) reported the presence of alkaloids, flavonoids, terpenes, phenols and tannins in grass pea plant extract. In a study that is conducted with grass pea genotypes (Bandana et al., 2022), the tannin, phenol, flavonoid, phytic acid and BOAA/ODAP contents of the genotypes in dry weight were varied between 5.58-8.39 mg/g, 0.27-2.15 mg GAE/g, 0.02-0.34 mg QE/g, 5.33-9.57 mg/g, and 0.47-2.23 mg/g, respectively. Pastor-Cavada et al. (2009) reported that the total phenolic compound values in 15 *Lathyrus* species ranged from 3.8 to 29.2 mg/g. The same researchers reported that there was a negative relationship between phenolic content and seed size, such that smaller seeds contained more phenolic substances. These secondary compounds in grass pea indicate that it has potentially diverse pharmacological activities.

Figure 8. *Lathyrus sativus* L.

Members of the genus *Lathyrus* are used as food and traditional medicine (Llorent-Martínez et al., 2017b). Lathyrus species is used as a pain reliever and anti-rheumatic (Altundag and Ozturk, 2011), the oil which is obtained from *L. sativus* seeds is used locally in Bangladesh as homeopathic drug (Duke, 2012), traditional medicine to treat scabies, eczema and allergies (Ahsan et al., 2010) and used in traditional medical treatments. Additionally; it has been reported by various studies that *L. sativus* seeds have antioxidant (Sarmento et al., 2015) and hypoglycemic (Sultana and Rahmatullah, 2016) activities. Laboratory experiment results support the traditional use of this herb as a Central Nervous System (CNS) depressant, analgesic, and antipyretic agent (Bhattacharjee et al., 2018). It has been emphasized that grass pea plant can be considered as therapeutic functional foods to manage and prevent various chronic diseases in humans because of its significant amount of functional protein and carbohydrate content, as well as secondary metabolite reserves such as polyphenols and tannins (Fratianni et al., 2014).

In studies conducted with *L. pratensis*, and *L. aureus* species, it has been stated that flavonoids and saponins are the main compound groups of extracts from both plants, they exhibit remarkable antioxidant and enzyme inhibitory effects in methanol and water extracts, these plants may be used more in phytopharmaceutical or food industry applications (Llorent-Martínez et al., 2016).

In another study carried out with *Lathyrus* species (*L. czeczottianus* and *L. nissolia*), it was reported that flavonoids were the main components in the extracts, *Lathyrus* extracts were rich in biologically active components and therefore these species could be used to design new phytopharmaceutical and nutraceutical formulations (Llorent-Martínez et al., 2017b).

In a research performed with five *Lathyrus* species growing in Türkiye, *L. armenus*, *L. aureus*, *L. cilicicus*, *L. laxiflorus* subsp. *laxiflorus* and *L. pratensis*, it has been reported that the species has anti-inflammatory properties, and the membrane stabilizing activity of *Lathyrus* species is due to the flavonoids that they contain (Heydari et al., 2019).

In a study to evaluate the phenolic content and antibacterial and antioxidant activities of *Lathyrus* L. (*L. hierosolymitanus* and *L. annuus*) species, phenolic compounds were extracted from a whole seed, seed coat and cotyledons. In this study, the total phenolic compound of extracts ranged from 0.12 mg to 6.53 mg GAE/gdw, it has been observed that the seed coat extracts generally provide higher total phenolic compound and antioxidant activities. High Performance Liquid Chromatography (HPLC) analysis results of the extracts has been suggested that the phenolic content in the extracts of *Lathyrus* species contributes to their antioxidant and antibacterial activities (Genc et al., 2022).

In addition to its good agronomic properties, unfortunately, grass pea seeds contain the neurotoxin β-ODAP (Murti et al., 1964; Rybiński et al., 2018). β-ODAP is lathyrogens that is a toxic compound found in *Lathyrus* plant species (Hailu et al., 2015). Depends on the β-ODAP content, high consumption of grass pea causes neurodegenerative disorder called neurolathyrism in humans and domestic animals (Getahun et al., 2002; Lambein and Kuo, 2009). Thus, ODAP is a neurotoxic secondary metabolite that can cause neurolathyrism (Yan et al., 2006; Enneking, 2011). Neurolathyrism is one of the oldest diseases known by human being and is caused by overconsumption of grass pea food over an uninterrupted long period of time (Hailu et al., 2015). The β-ODAP content of traditional grass pea cultivars varies between 0.5-2.5%, and this ratio has decreased to 0.10% and below with breeding studies (Kumar et al., 2011). However, soaking and boiling significantly reduces the β-ODAP content in the seeds (Khandare et al., 2018).

Species belonging to the genus *Lathyrus* L. are known as forage plants and medicinal plants widely used in traditional medicine and homeopathy (Solovyeva et al., 2019). With the consumption of these species as food by animals and humans, there is insufficient biological activity research on *Lathyrus* (Heydari et al., 2019). For all that, especially *L. sativus*, *L. pratensis* and *L. tuberosus* were the most extensively studied species in terms of their chemical composition and pharmacological properties. Datas on the medicinal properties of *Lathyrus* species, which is a quality legume forage plant, confirms that it has a promising potential in terms of medicinal product. Therefore, considering that the concentration of flavonoids, which are phenolic compounds with antioxidant activity, varies according to cultivation practices (Dykes et al., 2005), it is important to reveal the secondary metabolite contents and pharmacological properties of *Lathyrus* species grown under different cultural practices in different ecological conditions.

5. CONCLUSIONS

Legume forage plant species, some of which are also used as human food and are among the indispensable sources of roughage in animal nutrition, have created a worldwide awareness with their many medicinal properties, especially antioxidant activity, due to the presence of a wide variety of secondary metabolites, especially phenolic compounds. A significant portion of exogenous natural antioxidants are derived from traditional medicinal plants, including legume forage plants. Plants belonging to legume forage plant species that contain many secondary bioactive compounds (polyphenols, anthocyanins, isoflavones) have also been plants with strong antioxidant activity.

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CHAPTER 4

MORPHOLOGY AND ESSENTIAL OILS OF COMMERCIAL *LAVANDULA* SPECIES

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INTRODUCTION

Lamiaceae (Labiatiae) is a well-known family of medicinal plants containing a wide variety of taxa with traditional medicinal uses (Mamadalieva et al., 2017; Uritu et al., 2018). It is one of the largest angiosperm plant families with approximately 240 genera and ~7,000 species worldwide (Napoli et al., 2020). The *Lavandula* genus plants, which belongs to this family, is a plant that has been evaluated and used in many branches of industry, especially in pharmacology.

Lavandula genus which contains about 39 species classified into 3 subgenus and 8 divisions and numerous hybrids (Passalacqua et al., 2017) and also about 400 registered cultivars (Salehi et al., 2018). Within the scope of this study, lavender species with commercial value and their herbal characteristics, geographical distribution, essential oil component and quality values are mentioned.

1. COMMERCIAL *LAVANDULA* SPECIES

Within the *Lavandula* genus, ***Lavender*** (English lavender) (*Lavandula angustifolia* Mill. = *L. officinalis* = *L. vera*) and ***Lavandin*** (*Lavandula x intermedia* Emeric ex Loisel.= *L. hybrida* L.) are the most important species, most commonly cultivated for commercially essential oil production and horticultural values.

L. angustifolia produces the best quality essential oil among the *Lavandula* species and is a beautiful ornamental plant. There are many lavender varieties for the agricultural sector. Among them, some of the best known are: for example, Hidcote, Loddon Blue, Munstead, Rosea, Nana Alba (Lis-Balchin, 2002), Alba, Ashdown, Ashdown Forest, Baby Blue, Betty's Blue, Blue Cushion, Bosisto, Buena Vista, Cedar

Blue, Compacta, Dwarf Blue, Dwarf White, Folgate, Forest, Hidcote Superior, Jean Davis, Lavance Purple, Little Lottie, Maillette, Martha Roderick, Melissa, Mitchum Grey, Norfolk, Royal Purple, Royal Velvet, Sachet, Sharon Roberts, Twickle Purple, Thumbelina Leigh, Tucker's Early Purple, and Violet Intrigue (Anonymous, 2020). Crişan et al. (2023) reported in detail the genotypes of *L. angustifolia* cultivated in various regions.

L. x intermedia is a sterile hybrid of *L. angustifolia* and *L. latifolia*, most widely used in the world for essential oil production and horticulture in agriculture and trade.The essential oil quality of *L. x intermedia* is lower than *L. angustifolia.* There are many cultivars grown for the essential oil trade of *L. x intermedia.* Alba, Dutch Group, Grappenhall, Grosso (most popular for oil production), Hidcote Giant, Lullingstone Castle, Old English, Seal (Lis-Balchin, 2002), Super, Abrialii, Provence (Karık et al., 2017), Fred Boutin, Gros blue, Margaret, Silver Gray , Sussex, Vera, White Spike (Anonymous, 2020) and Tesseract, Bridget Chloe (Pokajewicz et al., 2023) are the most well-known lavandin varieties.

Among the other *Lavandula* species, the most grown are **Spike lavender** (*Lavandula latifolia* Medik.) and **Spanish lavender** (*Lavandula stoechas* L.) species (Crișan et al., 2023). *L.latifolia* is grown both for its oil and as an ornamental plant. Although *L. stoechas* has essential oil, it is no demanded in the world's cosmetic and perfumery markets, and is used for home gardens or dried flowers in floristry (Giray, 2018). There are its cultivated varieties: 'Snowman' 45 cm tall and white flowers, 'Kew Red' - 45 cm tall and pink flowers (Upson et al., 2002).

2. GEOGRAPHICAL DISTRIBUTION OF COMMERCIAL *LAVANDULA* SPECIES

The genus *Lavandula* belongs to the Lamiaceae family and is generally distributed from Cape Green Island in the Azores to India, the Canary Islands, North Africa, the Mediterranean region, and southern Arabia (Chaytor, 1937). However, six species were reported in Europe (Guinea, 1972), and four different *Lavandula* species (*L. angustifolia* and *L. latifolia*, *L. x intermedia*, *L. dentata*) were mentioned from the Mediterranean region (Ibrahim et al., 2017).

Within the genus *Lavandula*, the *Lavandula* section is the most well-known agriculturally and includes three species from Central and Southwest Europe. Among them, ***L. angustifolia*** grows naturally in Italy, southern France, Spain and northeast Africa. As indicated in Figure 1, the places indicated with green color are the regions where the *L. angustifolia* species natively spread, but it was later introduced to the places marked with purple color (KEW, 2023).

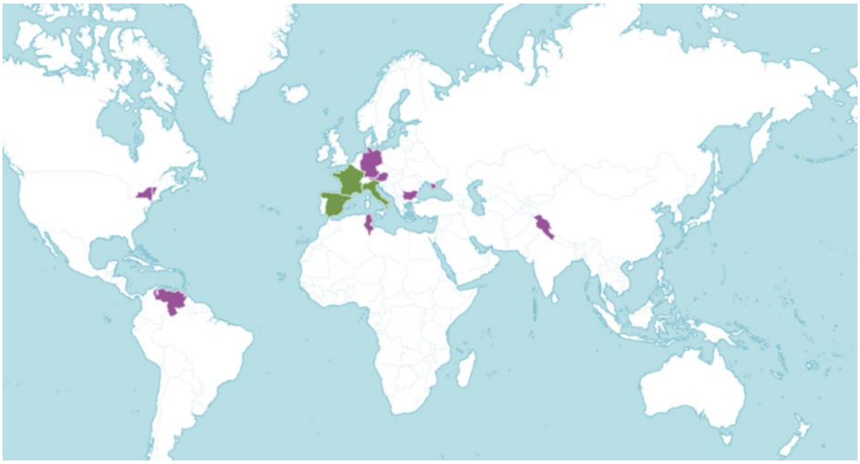

Figure 1: Geographic Distribution of *Lavandula angustifolia* Mill.in the World (Green:Native, Purple:Introduced) (KEW, 2023)

L. latifolia, grows wild mainly in France, Spain, Italy, Portugal and the former Yugoslavia (Morales, 2010). It grows in forest clearings, especially on dry grasslands or limestone rocky on sunny slopes and alluvial sands (Morales, 2010), in areas between 600-1000 m altitude (Burillo Alquézar, 2003). KEW (2023) has recorded that the native distribution of *L. latifolia* is France, Spain and Italy (Figure 2).

Figure 2: Geographic Distribution of *Lavandula latifolia* Medik. in the World (Green:Native, Purple:Introduced) (KEW, 2023)

L. x intermedia 's natural geographical distribution is thought to be france and spain (Figure 3) (KEW, 2023).

Figure 3: Geographic Distribution of *Lavandula × intermedia* Emeric ex Loisel. in the World (Green:Native, Purple:Introduced) (KEW, 2023)

L. stoechas are spread over three continents (Africa, Asia and Europe). This species grows in the Mediterranean basin, including Algeria, France, Greece, Italy, Morocco, Spain, Tunisia, and Türkiye (Figure 4). It is also found in Iran and Saudi Arabia (Upson and Jury, 2002). It is distributed in northern, northeastern and Cap Bon regions at altitudes between 400 and 1000 m in Tunisia (Chograni et al., 2008). Also, *L. stoechas* is found in Bihar and Bengal in India (Siddiqui et al., 2016). It has subsequently been introduced throughout Europe and to temperate/sub-temperate regions in the Americas, Asia and Australia (Lim, 2012).

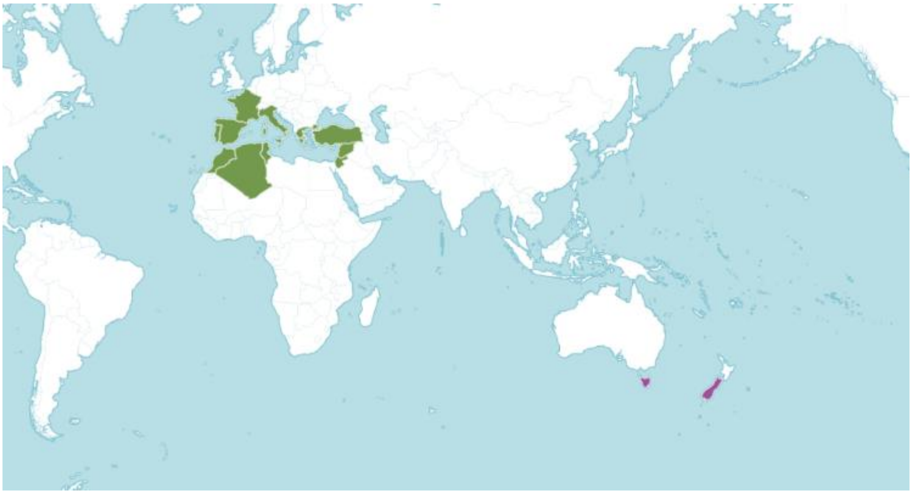

Figure 4: Geographic Distribution of *Lavandula stoechas* L. in the World (Green:Native, Purple:Introduced) (KEW, 2023)

3. MORPHOLOGY OF COMMERCIAL *LAVANDULA* SPECIES

Taxa in *Lavandula* genus are perennial shrub-shaped plants. *Lavandul*a sp., which belongs to the group of dicotyledonous plants, has a strong taproot. Their roots can go down to 80-100 cm deep in the soil, depending on the soil and climatic conditions. They are in full bloom between mid-June and mid-July and prefer acidic soils. Stems are 4-angled, bare or hairy. Stems are 4-angled, bare or hairy, gray-green color. The plants of this genus give a large number of side branches. Aging branches become woody over time. The leaves are attached with or without a very short stalk in the nodes of the stems standing upright. Inflorescence is common in the *Lavandula* genus. Flowers, in spirals, and cylindrical or quadrangular cylindrical cyma. They are usually purple, blue, violet, pink or mauve. Although the product is obtained economically for 10-15 years from *Lavandula* sp., the plants can live up to 30 years if it is regularly care. As the age of the Lavandula plant progresses, their lower branches become woody, also, the number of branches increases and the plant projection area expands (Anonymous, 2020).

L. angustifolia can grow up to 80 cm (Figure 5). The leaves are gray hairy when young, becoming greener with age. Leaves are clustered on leafy shoots but widely spaced on flowering shoots. The leaves are opposite each other at the nodes. The leaves are 17 mm long and 2 mm wide, respectively, on the flowering shoots, and 2–6 cm long and 3–6 mm wide at the base. Petiole is very short and linear-lanceolate to linear blade. Therefore, the leaves are pointed, straight edges, inwardly curved, strip-shaped. The flower size with stalk is 10-25 cm and a flower spike size is 5-8 cm. There are 4-6 flower clusters on the spike axis. Each flower cluster has between 6 and 14 florets depending on some factors. Lavender flowers with very short stems are in shades of blue/lilac, white or rarely violet-pink. The flowers are protected by 4 longitudinally striped sepals, up to 5 mm long, smooth inside and hairy outside. Calyx 4–7 mm long, densely gray star-shaped, hairy outside, with 13 longitudinal ribs. Bracts broadly ovate-rhombic to obovate, bracteoles present but very small. They bloom from mid-June to July. The fruit (seed) of lavender has a width of 1 mm. The color of the fruit usually ranges from dark brown to black. The 1000-grain weight of the plant's seed is less than 1g (Koç, 1997; WHO, 2017).

Figure 5: The appearance of Lavender (*Lavandula angustifolia*) and lavandin (*Lavandula x intermedia*) cultivars under field conditions (Anonymous, 2020)

L. x intermedia is a vigorous bush plant that can grow up to 1.5 m (Figure 5). Leaves linear-lanceolate to spathulate, usually gray hairy. The peduncle is branched, the spike is usually loose and occasionally interrupted. Fertile bracts are oval-rhombic, but variable in precise shape and size, bracteoles 1-4 mm long. Calyx with thirteen ribs, rounded to elliptical extension. The crown is bilaterally symmetrical, variable in color, usually lilac-purple to white (Lis-Balchin, 2002). They bloom from mid-June to July. The 1000-grain weight of the seed is less than 1 gram (Anonymous, 2020).

L. latifolia, plant height 50–70 (100) cm (Figure 6). Leaves gray, linear-lanceolate to spatulate. The peduncle of the flower cyme is distinctly branched, forming a tridentate flower spike, usually up to 25 cm high. The spike is often interrupted, 5-8 cm long. The bracts extending below to wrap flower cyma are linearly lanceolate, the bracteoles are prominent, 4 mm long. Calyx, with thirteen ribs, rounded extension. Crown strongly symmetrical bilaterally, blue to mauve (Lis-Balchin, 2002). Flowering lasts from July to October (Herrera et al., 2016).

Figure 6: General view of *Lavandula latifolia* Medik. (Herrera et al., 2016)

L. stoechas is a shrub-shaped plant that can grow 40–70 cm tall. The leaves are usually gray hairy. The peduncle is sessile or no longer than the length of the spike. At the apex, 1–2 (4) cm long, enlarged, sterile, flowering bracts obovate or spathulate. Fertile bracts broadly oval to obovate, briefly acuminata. The calyx is sessile, an elongated inside, the middle lobe of the modified calyx is thirteen ribs. Corolla black-purple to mauve, variants with white and rose-red or pink flowers (Figure 7) (Upson, 2002).

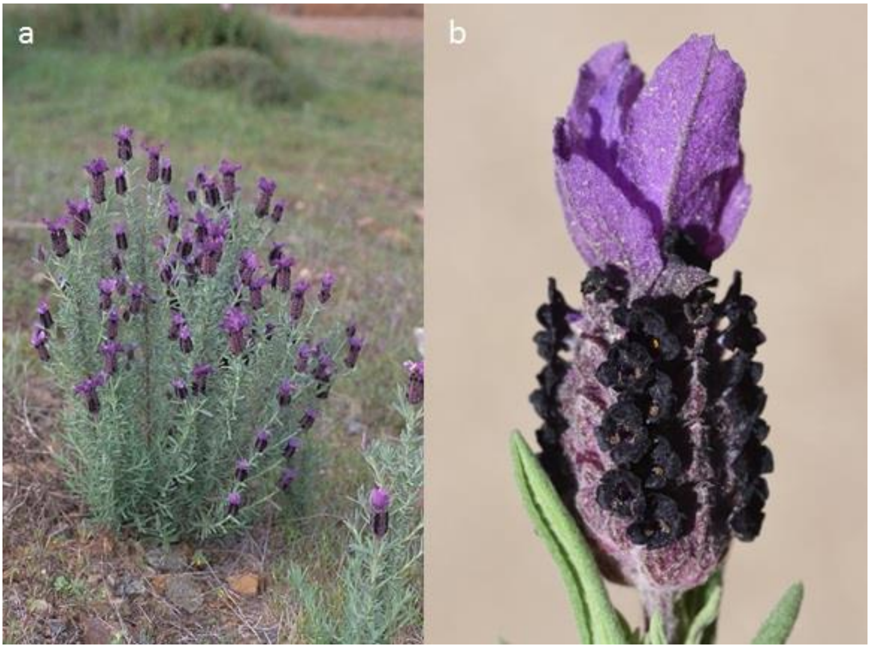

Figure 7: The appearance of *Lavandula stoechas* in its natural flora: aerial plant parts (a) and flower spike (b) (Kew, 2023)

4. ESSENTIAL OILS OF COMMERCIAL *LAVANDULA* SPECIES

4.1. CHEMICAL CONSTITUENTS

The most important ingredient of *Lavandula* flowers is essential oil. According to the codex, it is required that the genuine lavender flower contains at least 1% essential oil (Başer, 2014). *Lavandula* essential oils is a colorless to pale yellow liquid with a characteristic sweet, fresh, floral-herbaceous and slightly balsamic-woody undertone (Upson and Andrews, 2004).

The qualitative and quantitative composition of *Lavandula* essential oils are affected by many parameters such as genotype and location, extraction process (Hassiotis et al. 2010; Détár et al., 2020; Sharifzadeh et al., 2022). The general essential oil components of commercially grown *Lavandula* species (*L. angustifolia, L.×intermedia* and *L. latifolia*) are given in Table 1.

Table 1: Essential Oil Compositions of Commercially Grown *Lavandula* Species (100% flowering period): *L. angustifolia, L.×intermedia* and *L. latifolia* (Erland and Mahmoud, 2016)

	L. angustifolia	*L.×intermedia*	*L. latifolia*
Linalyl acetate	12–54%	19–38%	0–1.5%
Linalool	10–50%	20–35%	26–44%
1,8-cineole	–	4–10%	25–36%
Camphor	–	6–12%	5.3–15.3%
Borneol	–	1.5–3.7%	0.8–4.9%
Lavandulyl acetate	0.1–14%	0.5–3%	0.2–1.5%

Composition is given as ranges to account for variation due to various reasons (genotype, location and others).

There are differences in the main components and their ratios of essential oils of these three species. This difference also affects the essential oil quality. Among the *Lavandula* species with high commercial value, *L. angustifolia* has the highest quality essential oil. The essential oil of *L. angustifolia* is rich in linalool and linalyl acetate, the main aroma components, and has a low camphor content, which gives lavender oil its off-flavor (Adams and Yanke, 2008). The main components of the essential oils of *L. angustifolia* and *L.×intermedia* are the same, but differ in their concentrations, and also the camphor content of *L.×intermedia* is higher. Similarly, camphor content is higher inessential oil of *L. latifolia*. Therefore, the essential oil quality of *L.×intermedia* and *L. latifolia* is lower. Even, *L. latifoli* contains 1,8-

cineol, linalool and camphor, which make up about 80% of the oil (Boelens, 1986; Lis-Balchin, 2002).

Table 2 is shown the chromatographic profile of essential oil components of commercial *L. angustifolia*, *L.×intermedia* and *L. latifolia* according to ISO, European pharmacopoeia and World Health Organization.

Table 2: ISO (11024), European pharmacopoeia (Ph. Eur.) and World Health Organization (WHO) standards for the chromatographic profile of essential oils of Commercially Grown *Lavandula* Species (100% flowering period): *L. angustifolia, L.×intermedia* and *L. latifolia*

Constituents	**ISO LA**	**Ph. Eur. LA**	**Ph. Eur. LL**	**WHO LA ve Li**
Linalool	25−38	20–45	34–50	20–45
Linalyl acetate	25−45	25–47	<1.6	25–46
1,8-cineole	-	<2.5	16–39	<2.5
Camphor	-	<1.2	8–16	<1.2
Cis-β-osimene	3−9	-	-	-
Limonene	-	<1	0.5–3	<1
Terpinen-4-ol	-	0.1–8	-	1.2–6.0
α-terpineol	-	<2	0.2–2	<2.0
Lavandulyl acetate	>1,0	>0.2	-	>0.1
Lavandulol	-	>0.1	-	-
1-Octen-3-yl-acetate	<1.8	0.1–5	-	<2.5
trans-α-Bisabolene	-	-	0.4-0.25	-
Reference	ISO 11024-1:1998., 2013; ISO 11024-2:1998., 2013	Ph. Eur., 2020	Ph. Eur., 2020	WHO, 2007

L. angustifolia, LA. *L.x intermedia*, Li. *L. latifolia* (LL).

In addition to this, the standard values for the chromatographic profile of essential oils of Lavender (*Lavandula angustifolia* Mill.) essential oil vary in different countries origins according to ISO 3515:2002 (ISO, 2002).

4.2. USE

The use of the essential oils of the lavender plant dates back to Greek and Roman times due to its pleasant smell. In the Middle Ages, it was stated in the records of traditional medicine that the lavender plant was used for the treatment of many different diseases such as paralysis, head lice, migraines, epilepsy, fainting, panting, heart diseases (likely panic attacks, heart palpitations, or other heart problems), colds, apoplexy, bites, cramps, and congestion (Erland and Mahmoud, 2016). Lavender essential oil was used to disinfect and heal wounds during the Second World War in England (Lis-Balchin, 2002). The ancient Egyptians used lavender flowers in the mummification process. In Traditional Chinese Medicine, lavender has been used to treat a variety of conditions including infertility, infection, anxiety, and fever (Chu and Kemper, 2001).

In addition to the extensive history of lavender use, its use has recently been promising for health, due to its powerful antimicrobial (antifungal, antibacterial), antidepressant, anti-inflammatory, carminative, analgesic, and sedative properties and neurological effects when inhaled or absorbed through the skin. Because of its diverse activities, lavender has become a popular natural health product used directly or incorporated into oils, creams, and formulations (Crișan et al., 2023).

The widespread use of *Lavandula* essential oil in traditional medicine and the pharmaceutical industry has led to increased interest in various other fields. It has started to be of great curiosity in the food industry due to its strong antimicrobial activity as well as a pleasant smell and taste (Engels, 2007; Da Porto et al., 2009; Gómez-Estaca et al., 2010; Djenane et al., 2012). In all its applications in food science, its most promising activity is its potent biological control in both agricultural fields and stored products (Rozman et al., 2007; Cosimi et al., 2009; Radev, 2020).

In recent years, preparations and essential oils of *Lavandula* species and varieties have begun to gain value in different branches of industry due to many reasons such as the rapid development of technology, the ability to analyze essential oil components, and the detailed examination of chemical differences between plant species.

Lavender (*Lavandula angustifolia*) essential oil is the most widely known and used essential oil from ancient history to the present. *L. angustifolia* essential oil contains up to 60% of the two main aroma components (linalool and linalyl acetate) (Tomi et al., 2018) and the low camphor content that gives lavender oil its malodor (Adams and Yanke, 2008). Therefore, its oil is among the best and most desirable *Lavandula* essential oils, mostly in the perfume, cosmetic and pharmaceutical industries (Kara and Baydar, 2013). While supporting varieties of *L. angustifolia* with a desirable profile for the pharmaceutical and fine perfumery trade (eg Fine, Mailette, Munstead, Raya, etc.), other varieties are traditionally used by aromatherapists, including those that yield higher concentrations of camphor (Bejar, 2020).

The camphor content in essential oils of *L.×intermedia* and *L. latifolia* species is higher than that of *L. angustifolia*. These species, which have camphor-rich essential oils, are often more appreciated in aromatherapy and phytotherapy for their therapeutic properties (Herraiz-Peñalver et al., 2013).

4.3. COMMERCIAL VALUE

Commercially, the most valuable/quality *Lavandula* oil is obtained from *L. angustifolia*. Although *L. x intermedia* species has approximately 5-6 times higher amounts of essential oil than *L. angustifolia*, its essential oil quality is lower (Beetham and Entwistle, 1982; Kara and Baydar, 2013; Katar et al., 2020). *L. angustifolia* oil is considered to be of higher quality than *L.x intermedia* oil and is marketed at a price approximately 3-7 times higher (Stanev et al., 2016; Bejar, 2020). *L. latifolia* oil is also much cheaper (Pokajewicz et al., 2022).

In 2018, the price of *L. angustifolia* oil ranged from 66-188 $/kg and the price of *L. x intermedia* oil ranged between 32-51 $/kg (Giray, 2018).

The contents of essential oils from *L. angustifolia* are variable and depends primarily on genotype, climatic conditions, morphological characteristics and others (Chrysargyris et al., 2016; Dušková et al., 2016; Najar et al., 20219; Gorgini Shabankareh et al., 2021). Therefore, its commercial value also changes. For example, in 2018, *L. angustifolia* oil from France is $188/kg, while Hungarian *L. angustifolia* oil (66 $/kg) is almost a third of the value of French oil (Giray, 2018).

L. x intermedia provides the majority of the essential oil in the *Lavandula* oil trade, but the quality of the oil is lower, as it has a distinctive camphor odor (Stahl-Biskup et al., 2016). *L. x intermedia* oil varies according to its variety and growing location. For example, in 2018, while French Abrial variety oil was 48 $/kg and French Sumian variety oil was 51 $/kg, Grosso variety prices were reported as $32-34/kg depending on the country of origin and $46-49/kg for Super variety (Giray, 2018). Başer reported that there is a price range of 35-75 $/kg for *L. x intermedia* oils in 2019, depending on the quality (Başer, 2019).

L. latifolia yields about three times more essential oil than *L. angustifolia*. Although it has higher yields and can be grown at lower altitudes, the price of essential oil is cheaper due to the dominant scent of camphor (Stahl-Biskup et al., 2016).

4.4. TRADE

Lavandula essential oil is the most sought-after product obtained from the lavender crop due to its wide range of uses (Gallotte et al., 2020). Essential oils obtained from species and hybrids of *Lavandula* genus constitute approximately 1500 tons per year (Wells et al., 2018). Worldwide, the production of *L. angustifolia* essential oil is estimated to be around 300-500 tons per year (Botha et al., 2018; Crișan et al., 2023). In addition, the amount of *L. angustifolia* essential oil in trade each year is difficult to assess because official figures do not take into account the amount of adulteration or synthetic lavender essential oil on the market (Peter, 2004; Crișan et al., 2023).

Although *Lavandula* species and varieties are cultivated in many countries, only a few countries dominate the *Lavandula* essential oil supply and trade. The leading countries in *Lavandula* production are Bulgaria and France, followed by Russia, Ukraine, Moldova, Romania, Hungary, Poland, Italy, Spain, Türkiye, Morocco, United Kingdom, United States, Australia, South Africa and China (Giray, 2018). Commercially, France, Bulgaria, China, Russia and some other Eastern European countries are the most regular suppliers of *Lavandula* oil, and New Zealand can be considered a new entrant to the world market (Giray, 2018). However, the world's largest *Lavandula* essential oil producers are Bulgaria and France. Together, these two countries produce two-thirds of the world's *Lavandula* essential oil production (Giray, 2018).

5. CONCLUSION AND RECOMMENDATIONS

The interest for this commercial *Lavandula* species (*L. angustifolia*, *L.x intermedia* and *L. latifolia*) is increasing day by day.One of the general reasons for this is that it has many uses and offers a serious source of income in terms of tourism. Surprisingly, the demand for essential oils for pharmacological and aromatherapy applications, is on the rise. For this reason, *Lavandula* production areas have been increasing in recent years. The these *Lavandula* species has a wide range of adaptations due to its perennial and bushy form. Even, the cultivations of these *Lavandula* species can be grown in marginal and less fertile lands as well as being low input. Considering all these, it is important to select species and varieties suitable for growing conditions in standards that can have commercial value. And even, new varieties need to be developed to the standards that may be suitable for the region, especially, to take place in world trade.

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CHAPTER 5

DISTRIBUTION OF VOLATILE OILS IN DIFFERENT PLANT PARTS OF *Passiflora incarnata* L. AND USES IN FOLK MEDICINE

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INTRODUCTION

The name Passiflora is derived from the Latin word "Passio," which was originally found by Spanish explorers in 1529 and was identified as a symbol for "Christ's Passion." (Dhawan et al., 2004; Kinghorn, 2001). The largest genus in the Passifloraceae family, Passiflora, contains roughly 400 species (Montanher et al., 2007; Beninca et al., 2007). A sizable genus of herbaceous or woody tendril climbers that is primarily found in warm, temperate, and tropical climates worldwide (The Wealth of India, 2001), while they are far less common in Asia, Australia, and tropical Africa (Beninca et al., 2007). This genus of plants is known for its diverse active ingredients with medicinal efficacy and biological action against a number of ailments. Numerous pharmacological effects of these plants have been documented.

1. GENERAL CHARACTERISTICS OF *Passiflora incarnata* L.

Enlightened Passiflora other names include passion vines and passion flower. The passion flower is a perennial plant with a woody stem that can grow up to 10 meters. Only a flower is contained on the 8 cm long axillary pedicle. Flowers have a lined diameter ranging from 5 to 9 cm. The five sepals are white on the inside and gray on the outer. Contains an additional corolla inside the petals that is made up of a 4-fiber ring placed radially in the flower's axis and is both white on the inside and purple on the outside. Three carpels and three styles make up the ovary, which ends in a thick stigma.

The bases of the five stamens are bound. A number of reviews have also discussed the medicinal and traditional usage of these substances for the treatment of minor symptoms of mental stress and to promote sleep. Dosage information is only found in one reference (Lutomski et al., 1981), which also references data from Commission E and ESCOP. The length of use is not mentioned in any of the reviews. (Dhawan et al., 2003) presented a thorough analysis of the botany, chemistry, pharmacology, and clinical application of plants in the genus Passiflora, including *Passiflora incarnata*.

Figure 1: Flower morphology leaf and seed shape of the three *Passiflora*

1.1. CHEMICAL CONTENT of *Passiflora incarnata L.*

The genus is reported to contain alkaloids, phenols, glycosyl flavonoids, and cyanogenic chemicals.

All vascular plants contain flavonoids, which are chemical phenylbenzopyrones that are typically conjugated with sugars (Zanoli et al., 2000). According to reports, *P. incarnata*'s primary phyto-constituents are flavonoids. It primarily comprises C-glycosylflavones derived from luteolin and apigenin. Additionally, according to Poethke et al. (1970), *P. incarnata* contains simple indole alkaloids with the carboline ring system, such as harman, harmol, harmine, harmalol, and harmaline.

Various other constituents which have been reported from P. incarnata include γ-benzo-pyrone derivative maltol (Aoyagi et al., 1974), carbohydrates such as raffinose, sucrose, D-glucose and Dfructose (Gavasheli et al., 1975); essential oil containing hexanol (1.4%), benzyl alcohol (4.1%), linalool (3.2%), 2-phenylethyl alcohol (1.2%), 2-hydroxy benzoic acid methyl ester (1.3%), carvone (8.1%), trans-anethol (2.6%), eugenol (1.8%), isoeugenol (1.6%), βionone (2.6%), α-bergamotol (1.7%) and phytol (1.9%); various constituents responsible for typical odor of P. incarnata such as limonene, cumene, α-pinene, prezizaene, zizaene, and zizanene (Buchbauer & Jirovetz, 1992), twenty one amino acids (Gavasheli et al., 1974), and a cyanogenic glycoside gynocardin (Dhawanet al., 2004).

The distribution of volatile oil components in different plant parts of *Passiflora incarnata* L. and their uses in folk medicine will be discussed.

2. MATERIALS AND METHODS

2.1. Plant material

In the study; Different parts of *Passiflora incarnata* L. (flesh, seeds, leaves, flowers) were used as study material.

2.2. Sample preparation and volatile oil analysis

Analysis was performed using a GC-MS Device (Shimadzu, Japan) outfitted with SPME. Ground plant samples weighing 1.00 g were then put into a 10 mL vial and sealed with a silicone-rubber septum lid. In order to pre-condition the fiber, manufacturer guidelines were followed. The fiber was left in the headspace at equilibrium for one minute at room temperature. The fiber was placed into the needle after sampling and then moved to the injection port of the GC or GC-MS device. The column was a CP 5MS (30 m x 0.25 mm i.d., film thickness 0.25 m). The oven temperature was set to isothermal at 220 °C for 20 minutes after being scheduled to rise from 40 °C to 240 °C at 2 °C/min. As a carrier gas, helium was employed at a constant flow rate of 1 mL/min. The Wiley, NIST Mass Spectral, and aroma technique databases were used to determine the constituents of essential oils (Yurteri et al., 2021).

2.3. Data Analysis

The XLSTAT 2023 (Addinsoft, 2023) was used to perform Biplot Analysis. To visualize current variation in *Passiflora incarnata* L. plant portions examined for chemical variability, use a statistical program. Current data was used to produce scatter plot diagrams (Backhaus et al., 1989). Using the results of the GC-MS study, a separate biplot diagram was also produced..

3. RESULTS AND DISCUSSION

The aqueous extract solution of *Passiflora edulis* was analyzed using GC-MS. 44 compounds were found. Saturated fatty acids, volatile oils, sugar, carboxylic acids, and aldehydes were a few of the substances that were found (Table 1).

Table 1: Volatile oil composition of *Pasiflora incarnata* L. plant parts

Numeric	Compounds (%)	RI*	Flower	Fruit Peel	Seed	Leaf
1	Vinylbutanol	790	-	-	5.08	-
2	Pentanol <1-methyl->	792	-	-	7.99	-
3	Adipic ketone	793	-	-	9.38	-
4	Lactate <Ethyl>	798	8.81	-	18.34	-
5	Capronaldehyde	801	-	-	3.13	2.00
6	Piruvate <ethyl->	819	-	-	1.16	-
7	Furfural	831	-	-	-	-
8	Hex-3(E)-enol	832	-	-	9.11	-
9	Hexanol <n->	869	-	-	-	1.55
10	Formate < hexyl->	912	-	--	27.00	-
11	Vinyl amyl carbinol	980	-	-	-	1.91
12	Benzyl alcohol	1033	1.09	-	-	-
13	Hex-2(E)-enyl acetate	1036	-	12.82	-	-
14	Phenylacetaldehyde	1042	-	2.37	-	-
15	Butyrate <isoamly->	1058	-	-	0.75	-
16	Pelargonaldehyde	1105	-	2.70	-	2.10
17	Acetate <octyl->	1142	-	-	3.50	-
18	β-Artemisia acetate	1184	0.84	-	-	6.49
19	Dodecane	1201	-	-	3.58	-
20	Pulegone	1221	-	3.04	-	-
21	Carvone <(E)-, dihydro->	1232	-	-	-	2.00
22	Tridecane	1301	-	-	6.44	-
23	Geranyl acetone	1454	-	3.72	-	-
24	Theaspirane <6-hydroxydihydro->	1464	-	-	-	4.27
25	β-Lonone (E)	1489	-	21.35	-	2.19
26	Pentadecane	1497	0.80	2.30	-	-
27	Citronellyl butyrate	1532	0.61	12.67	1.92	12.91
28	Nonanoate <isoamyl->	1543	-	2.58	-	-
29	Hexadecane	1600	0.50	3.62	-	2.08
30	α-Muurolol	1649	1.03	-	-	-
31	Heptadecane	1700	0.83	-	-	2.81
32	Myristate <methyl->	1726	1.39	-	-	1.84
33	Octadecane	1801	1.00	-	-	1.63
34	Salicylate <2-ethylexyl->	1810	-	2.92	-	-
35	Pyhtone	1847	3.93	-	-	-
36	Caffeine	1847	-	4.06	2.62	-
37	Phytone	1847	-	-	-	30.36
38	Cetyl alcohol	1891	-	8.74	-	-
39	Nonadecane	1901	1.86	-	-	-
40	Palmitate <methyl->	1927	13.62	11.86	-	17.00
41	Eicosane	2000	7.84	-	-	-
42	Heneicosane	2101	51.95	-	-	-
43	Phytol	2103	-	5.25	-	8.85
44	Docosane	2201	3.91	-	-	-

Volatile oil components detected only in flower parts were Beznyl alcohol (1.09 %), Capronaldehyde (28.7 %), α-Muurolol (1.03 %), Phytone (3.93 %), Nonadecane (1.86 %), Eicosane (7.84 %), Heneicosane (51.95 %) and Docosane (3.91).

A number of 8 volatile oil components, namely Hex-2(E)-enyl acetate (12.82 %), Phenylacetaldehyde (2.37 %), Pulegone (3.04 %), Geranyl acetone (3.72 %), β-Lonone (E) (21.35 %), Pentadecane (2.30 %), Salicylate <2-ethylexyl-> (2.92 %) and Cetyl alcohol (8.74 %) were only detected in fruit peel. In seeds, 10 volatile oil components weer present: Vinylbutanol (5.08 %), Pentanol <1-methyl-> (7.99 %), Adipic ketone (9.38 %), Piruvate <ethyl-> (1.16 %), Formate < hexyl-> (27.00 %), Butyrate <isoamly-> (0.75 %), Acetate <octyl-> (3.50 %), Dodecane (3.58 %), Tridecane (6.44 %) and Caffeine (2.62 %).

The leaf parts contained 10 volatile oil components, respectively Hexanol <n-> (1.55 %), Vinyl amyl carbinol (1.91 %), β-Artemisia acetate (6.49 %), Carvone <(E)-, dihydro - > (2.00 %), Theaspirane <6-hydroxydihydro-> (4.27 %), β-Lonone (E) (2.19 %), Heptadecane (2.81 %), Myristate <methyl-> (1.84 %), Octadecane (1.63 %) and Phytone (30.36 %). Volatile oil components detected in highest amounts in different plant parts were Heneicosane (51.95 %) in flower parts, β-Lonone (E) (21.35 %) in fruit peel, Formate < hexyl-> (27.00 %) in seeds and Phytone in leaf parts.

Dülger et al. (2018) detected hexanal (1.4%), benzyl alcohol (4.1%), linalool (3.2%), 2-phenylethyl alcohol (1.2%), 2- hydroxy benzoic acid methyl ester (1.3%,) carvone (8.1%), trans-anethole (2.6%), eugenol (1.8%), isoeugenol (1.6%), β-ionone (2.6%), α-bergamotol (1.7%), palmitic acid (7.2%) and oleic acid (6.3%) in flowers of *Passiflora incarnata* L..

The chemical composition of the essential oils of *Passiflora sexocellata* and *Passiflora trifasciata* (Passifloraceae, subgenus Decaloba) were studied. For *P. sexocellata* leaves, 33 compounds (75% of the total oil composition) and 29 (74% of the total oil composition) in flowers were detected. Regarding *P. trifasciata*, 35 compounds (76% of the total oil composition) were detected in leaves and 32 (71% of the total oil composition) in flowers (della Cuna et al., 2021).

Calevo et al. (2016) investigated the chemical composition of the volatile oil from flowers and leaves of new Passiflora hybrids. Several compounds were identified, with a peculiar distribution in the hybrids: benzyl alcohol (7.6%), geraniol (13.7%), phytol (14,3%), eugenol (3.9%), 2-phenylethanol (4.7%), cis-3-hexenal (2.8%) and palmitic acid (2%) were the main compounds of the essential oil of fresh leaves of the hybrid P. ‘FSO-040711’; the highest percentages of benzyl alcohol (12.2%) and 2-phenylethanol (13.6%) were found in fresh flowers of P. ‘FSO-130913’ and the highest amount of phytol (38.5%) was present in the fresh leaves of P. ‘FSO-080415’.

In oils from *Passiflora sexocellata* the oil was dominated by pentadecanoic acid (9.6%), dodecanoic acid (8.7%), (Z)-2-hexenal (7.0%), hexanal (2.2%), 1-eicosene (7.0%) and 3-tetradecene (1.5%). In the flower oil the same species α-humulene (47.5%), β-caryophyllene (3.6%), pentadecanoic acid (14.4%), 9-tricosene (10.4%), 3-tetradecene (1.7%), 4-vinylguaiacol (3.0%) and 1-nonanol (1.7%) were detected.

In *Passiflora incarnata* L., hexanol (1.4%), benzyl alcohol (4.1%), linalool (3.2%), 2-phenylethyl alcohol (1.2%), 2-hydroxy benzoic acid methyl ester (1.3%), carvone (8.1%), trans-anethol (2.6%), eugenol (1.8%), isoeugenol (1.6%), β-ionone (2.6%), α-bergamotol (1.7%) and phytol (1.9 %) were detected by Buchbauer & Jirovetz (1992).

Some *Passiflora* species have been widely investigated for the presence of bioactive compounds in all the plant organs, including leaves, flowers, fruits and seeds, and a series of flavonoids, glycosides, alkaloids, and phenols have been reported (Chassagne et al., 1998; Bendini et al., 2006; Patil, 2010; Zeraik et al., 2010; Patel et al., 2011; . Zucolotto et al., 2012; Argentieri et al.; 2015; Dos Santos et al., 2016; Farag et al., 2016)

Volatile oil components with a content over 10 % Lactate <Ethyl>, Hex-2(E)-enyl acetate, β-Lonone (E), Citronellyl butyrate, Phytone, Palmitate <methyl-> and Heneicosane were used to create Biplot Analysis (Figure 2).

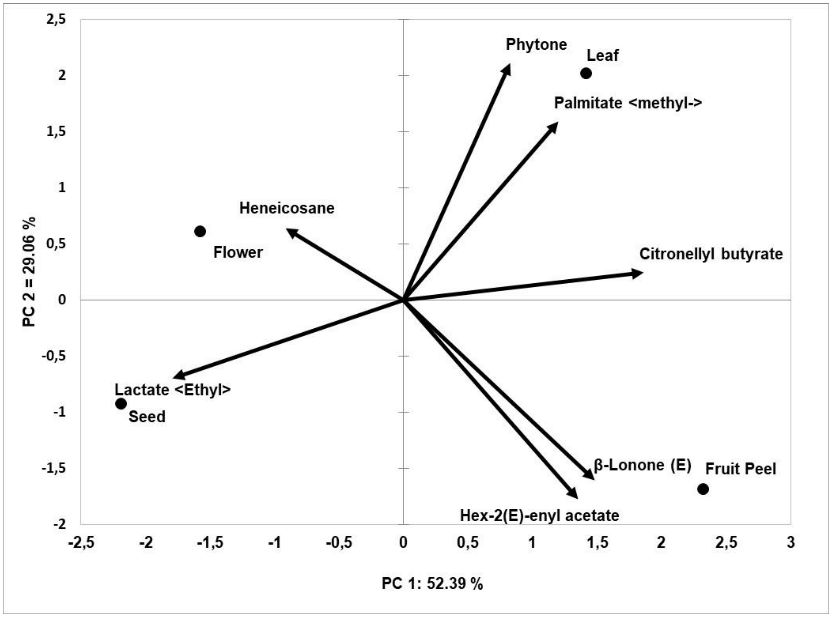

Figure 2: Biplot analysis of volatile oil composition of investigated *Passiflora incarnata* L. plant parts.

Totally 81.45 % of the present total variation could be explained using the first two principal components. Based on evaluated volatile oil it can be said that the plant parts of this investigated plant differed based on their chemical content and all parts could be differentiated from each other.

The investigations about the volatile oil composition of different plant parts of *Passiflora incarnata* L. is given in the scientific literature. Based on obtained results we can declare that the findings of the present study were different from given literature and different from volatile oil contents of different Passiflora species.

The use of therapeutic ethnomedical items has been proven in recent years, however there have been side effects. *Passiflora incarnata* is a nutraceutical that is rich in bioactive chemicals that have been used for many years as traditional medicine and may help in the treatment and prevention of a variety of diseases. To evaluate whether *Passiflora incarnata* plant parts might be used in the pharmaceutical industries, more research is necessary.

4. CONCLUSION

For thousands of years, nature has served as a source for therapeutic agents, and an astounding number of contemporary drugs have been separated from natural sources, many as a result of their traditional medical applications. Many civilizations have employed plants from the genus *Passiflora* in traditional medicine. The main phyto-components of the Passiflora species have been identified as flavonoids, glycosides, alkaloids, phenolic compounds, and volatile constituents.

Passiflora species and hybrids are particularly desirable for the horticulture business as well as for the herbal and medicinal industries due to their exotic allure, natural adaptability, and great inter and intraspecific genetic variability. Passion flowers display a variety of distinctive floral characteristics, such as numerous series of vividly colored corona filaments, varied operculum morphology, a prominent androgynophore, and ornate nectary structures. They are also surrounded by dyed sepals and elaborate bracts. These plants are highly sought-after in the global wellness industry due to their captivating blossoms, luxuriant foliage, tasty fruits, and presence of valuable bioactive ingredients.

Numerous opportunities for research still exist in the somewhat more recent domains of these plants' functionality and their medicinal applications. Therefore, these plants' phytochemicals and minerals will make it possible to utilise them for therapeutic purposes. Therefore, additional research may be done to demonstrate the potential of these plants. Due to the fact that this species is currently becoming an endangered species, more effort can be put into improving the agricultural and climatic circumstances for this plant.

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CHAPTER 6

QUALITY BREEDING IN *Camellia sinensis*

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INTRODUCTION

Nearly all communities consume tea, *Camellia sinensis* (L.) O. Kuntze, which is the source of this aromatic beverage. The tea plant's young shoots, known as the flush, are picked or plucked so that they can be used to make tea. The flush is made up of two to three of the top, immature leaves and buds (Modder and Amarakoon, 2002). Water is the most popular drink in the world, with tea coming in at number two, far behind carbonated soft drinks, coffee, beer, and wine (Costa et al., 2002). Additionally, many disorders are treated with tea as a therapeutic beverage. According to estimates from Mc Kay and Blumberg (2002), more than half a billion cups of tea are consumed daily around the world, with a daily average intake of 120 ml. Due to the chemicals in tea's components that have health benefits, tea has gotten a lot of attention in recent years. Tea leaves contain more than 700 chemical components, including flavonoids, amino acids, vitamins, alkaloids, and polysaccharides (Thomas et al., 2006), as well as more than 500 taste compounds (Rawat and Gulati, 2008). The majority of them are said to have anti-diabetic, anti-cancer, anti-obesity, anti-aging, and anti-histamine properties (He et al., 2006; Sharangi, 2009).

Plant production and breeding are crucial in the effort to fulfill the global issue of supplying enough nutritious food for a growing population. Plant breeding is a technique for modifying and enhancing plant species to satisfy human demands and preferences. It is a field that is crucial to both our continued existence and the long-term viability of our agricultural landscapes. Breeding is essential for improving traits that can improve the quality of life for people all around the world, including resilience to diseases and pests, drought, and temperature extremes. Additionally, plant breeding can be utilized to assist in crop adaptation to new environments around the globe, enhancing food security and assisting local and regional food systems.

Studies on *C. sinensis* have expanded significantly in recent years, covering everything from the examination of potential uses in various fields of research to the enhancement of extraction process conditions. Studies employing *C. sinensis* have been established in a variety of study areas, including food science, plant science, applied chemistry, molecular biology, and biochemistry, among others. The primary research areas concern the bioactive composition, antioxidant qualities, biological activities, and usage of extracts or isolated components from teas to create novel products that are appealing to the food and pharmaceutical industries (Bortolini et al., 2021).

In order to increase the yield and nutritional value of food crops and ensure that people may live healthy lives, plant breeding is required. The quality breeding of *C. sinensis* will be thoroughly examined in the foolowing review.

Camellia sinensis breeding

In order to improve tea plant breeding, it is crucial to identify and assess the genetic resources of the tea plant, *C. sinensis* (L.) O. Kuntze. Recent years have seen the evaluation of tea germplasm resources using a variety of techniques, including morphology, biochemistry, molecular markers, and sensory evaluation (Gai et al., 2019).

The germplasm of the tea plant, *C. sinensis* (L.) O. Kuntze, is a valuable source of genetic material for tea breeding and biotechnology studies. The extensive variety in the phenotypic and biochemical characteristics of the tea germplasms provided a wealth of resources for developing the breeding effort (Das and Bhagobaty, 2010). To choose attractive cultivars, it is also crucial to evaluate the germplasm using various biochemical factors that determine tea quality. To evaluate tea germplasm resources, a variety of germplasm appraisal techniques, including morphology, biochemistry, molecular markers, and sensory evaluation, have been applied recently (Feng et al., 2014; Li et al., 2016; Wambulwa et al., 2016). For the evaluation of tea germplasm, phenotype can be considered a good benchmark. According to Gunasekare (2007), physical features are the most straightforward way for evaluating genetic diversity. For instance, the production of tea depends on the size of the leaf, the density of the shoots, the weight of the buds, and the germination period, however the biochemical makeup of the tea shoots is what mostly determines the quality of the tea. According to Chen and Zhou (2005) and Kilel et al. (2013), studies on these biochemical elements can help us understand how they influence the qualitative traits of different tea kinds. As of late, the use of molecular markers has emerged as one of the most reliable ways to distinguish between various tea kinds (Chen et al., 2005; Chen and Yamaguchi, 2010; Yao et al., 2008). According to Gunasekare (2012) and Yao and Chen (2012), traditional tea breeding and selection programs are multistage and involve the generation of variation, individual selection of promising genotypes, evaluation and screening for yield, quality, biotic and abiotic stresses, local adaptability testing, and cultivar release. All of these processes can take 20-25 years.

Breeding aims in crop plants

The process of modifying and enhancing a plant's genetic makeup is known as plant breeding (Poehlman, 2013). The knowledge and use of genetic concepts are the foundation of contemporary plant breeding. It assumes knowledge of the species' botanical traits, plant diseases and their epidemiology, insect pests that prey on the various plant species, physiological aspects of plant adaptation, and biochemical traits influencing utilization and nutritional value (Poehlman, 2013).

All cultivated agricultural plants have breeding objectives. The key, however, is that each breeding objective serves the objectives of yield and quality in crop plants (Miedaner, 2016). In other words, if you create plants that are resistant to pests and diseases, you will help to increase the yield and quality of the crop plant you are interested in. This information needs to be taken into account when discussing breeding goals.

The entire varietal evolution of tea and other *Camellia* species has been accomplished through conventional breeding since its inception in 1939, notwithstanding the challenges of its applications (Shehasen, 2019). Since then, various genetic and breeding advancements have been made with the goals of creating high-quality tea varieties, increasing yield, enhancing cup tea quality, and strengthening tolerance to biotic and abiotic stress. This is accomplished using processes for improving the genetic makeup of tea, such as traditional selection, hybridization, marker-assisted selection, mutation breeding, polyploidy, genetic engineering, and micropropagation.

Quality definition in plant breeding

Quality breeding, which involves genetically modifying crop plants in relation to multiple quality traits, calls for understanding the right growth pattern of the crop plant and the metabolism of the desired quality trait. According to the usage of producing output, quality is the degree of excellence for a certain use.

Quality breeding refers to the genetic modification of crop plants in respect to certain quality traits. Every crop has a different set of quality features.

Breeding objectives in *Camellia sinensis*

The general breeding objectives of *C. sinensis* are listed in Table 1. The goals of tea (*C. sinensis*) breeding range from enhancing quality and productivity to increasing resistance to various illnesses and pests. Breeding objectives for this significant crop can include increased winter hardiness, compatibility to various manufacturing processes, and resilience to low soil pH.

Table 1: Breeding objectives of tea

Objectives	Importance	Regions
Improving quality	Directly linked to the profitability	Black tea producing countries such as India, East-Africa, Sri Lanka, Bangladesh and Indonesia
Increasing yield	Horizontal increase of production by extension planting is limited	Worldwide

Table 1: Breeding objectives of tea (continued)

Objectives	Importance	Regions
Improving quality	Directly linked to the profitability	Black tea producing countries such as India, East-Africa, Sri Lanka, Bangladesh and Indonesia
Increasing yield	Horizontal increase of production by extension planting is limited	Worldwide
Drought tolerance	Reduce productivity and occur all tea-growing regions of the world	Worldwide where tea grown as rain-fed crop
Reduce winter dormancy	No leaf production during winter months and occurs in North-East India, Japan and China, etc	Tea plantation near the equator
Hail/frost resistance	Causes economic loss as young leaves during rainy season are mostly affected	Hilly region of the tea-producing countries
Water log tolerance	Reduce productivity during rainy season. Generally occurs in northeastern India	North-East India

Table 1: Breeding objectives of tea (continued)

Objectives	Importance	Regions
Cold hardiness	Reduced productivity during winter due to snow. Generally occurs in China, Japan and Russia	Mainly India, Sri Lanka, Indonesia and Japan
Diseases resistance, such as blister blight, stem canker, etc.	Blister blight causes severe damage as only young leaves are infected. Generally occurs in Japan, Sri Lanka, South India and Darjeeling hills of North-East India	Mainly India, Sri Lanka, Indonesia and Japan
Pest resistance, such as red spider mite, tea mosquito bug, leaf-sucking pest, etc.	Most important biotic stress - causes severe damage to the leaves. Generally, in all tea regions in the world	Worldwide
Suitability to type of manufacturing	For matching the customer's demand as well as better recovery percentage in made tea	Black-tea-producing countries such as India, East-Africa, Sri Lanka, Bangladesh and Indonesia
Low input responsive Clone	Required for organic tea farming	Organic tea
Resistance to low soil pH		Worldwide

Mondal (2009, 2014)

According to Shehasen (2019), the breeding goals for *C. sinensis* breeding stock have evolved from high yield to high quality, then to a variety of goals like high quality, high efficiency, high functional component contents, and high stress tolerance.

Breeding Strategies in *Camellia sinensis*

Any tea breeding strategy must first decide whether to use clones or seed varieties to increase productivity. Although the current populations of tea were grown from seedlings, the demands for increased output and quality necessitated the crossing and back crossing of numerous characters (Banarjee, 1992).

Quality Breeding

Every effort made to create new clones and cultivars naturally results in certain plants exhibiting the necessary qualities, which in turn help to create the quality traits that will be accessed. The methods for enhancing *C. sinensis* quality will be discussed.

Hybridization breeding

Since both the parents and the hybridized offspring are heterozygous and heterogeneous, hybridization is one of the primary techniques for gaining genetic variety and is crucial in the breeding of novel tea cultivars (Chen et al., 2007). To induce genetic variety, two parents chosen for their desired traits are crossed. All subsequent propagation steps are carried out by vegetative propagation after the initial genetic recombination. In the production of tea, genotype selection is done in stages starting with seed progeny and continuing with plants that have been vegetatively propagated.

Clonal selection used the same genetic material even though it sometimes helped in significant increases in tea yields. Planting materials deteriorate due to selection within a population because it prevents genetic recombination (Bezbaruah and Saikia, 1977). Conversely, plants grown from seeds have a greater genetic diversity because they frequently result from unchecked hybridization between dissimilar tea kinds that are allowed to flourish freely (Wood and Barna, 1958). However, because they were not bred specifically for yield or quality, natural hybrids are not always excellent yielders. Therefore, it becomes vital to cross-breed choices that have desirable qualities for production and quality in order to synthesize extra variety.

Mutation breeding

The goal of mutant breeding is to create favorable mutations that will increase yield and quality (Banerjee, 1992). However, preliminary research was primarily limited to irradiating cuttings, pollen grains, and seeds in the hopes that some of the treated plants would perform better in tea than the untreated ones because it was unknown where the foci of the genes responsible for these traits were located. X-rays and y-rays as well as the chemical mutagen ethyl methane sulphonate are utilized as mutagens.

According to Ahloowalia et al. (2004), the primary goal of mutation-based breeding has been to improve well-adapted plant types by changing one or two major characteristics that inhibit their productivity or quality improvement.

Systematic analysis was done on the combined impacts of c-ray and chemical mutagens on the biological harm to the tea plant (Yang and Lin, 1992). Under Co60c-ray radiation, one outstanding novel strain with spring sprouting that is extremely early, high cup tea quality, disease resistance, and suitability for fine green tea has been chosen from the progeny of Longjing 43 cuttings (Yang et al., 2003).In 2004 (Oowalia et al.).

Molecular marker assisted selection (MAS)

In a study employing the association mapping method, Jin et al. (2016) looked at the genetic link between the tea caffeine synthase 1 (TCS1) gene and the amount of caffeine found in the tea plant and related species. They discovered CAPS markers that were created from SNP4318 sequence variants linked to the caffeine content of tea plants, and the markers may be used in MAS in the future to enhance the quality of tea. Additionally, Koech et al. (2018) reported using the DArTseq platform to identify 6 caffeine QTLs, 25 catechin QTLs, 3 theaflavin QTLs, and 9 QTLs for tea-taster score. For tea grown in temperate climates, the time of spring bud flush (TBF) is a crucial agronomic feature, and Tan et al. (2018) confirmed two QTLs that may be applied in MAS for TBF.

Micropropagation

Tea can be micropropagated to increase the number of premium cultivars of tea quickly and to create high-quality planting materials to meet the rising demand in tea estates. Aseptic in vitro culture establishment, explant type optimization, nutrient composition of culture media, and subsequent multiplication were the primary goals of the initial efforts (Mukhopadhyay et al. 2015). Different explant types were used for micropropagation such as nodal segments (Agarwal et al. 1992), shoot tips (Banerjee and Agarwal 1990), zygotic embryos (Iddagoda et al. 1988; Ranaweera et al. 2013; Seran et al. 2006), embryogenic axes (Seran et al. 2006), epidermal layers of stem segments (Kato 1985), axillary buds (Nakamura 1990) and leaves (Sarwar 1985). For multiple shoot proliferation, various base media formulations, including Murashige and Skoog's (1962), White's medium (White 1963), Woody Plant Medium (WPM) (Lloyd and McCown 1980), and Schenk and Hildebrandt (1972), were supplemented. These growth regulators included BAP, Kinetin, NAA, GA3, IBA, Zeatin, and TDZ in varying concentrations (Das et al. 2012). The results varied greatly depending on the tea cultivar employed, and no one uniform medium and hormone combination for micropropagation was discovered.

Polpyploidy

Although tea usually has two chromosomes per cell (2n = 30), triploids naturally occur in some Japanese types (Simura and Inabe, 1953), and one from south India is thought to have qualities that contribute to both high yield and quality (Sharma and Ranganathan, 1985). Additionally, strong tetraploids and superior diploids have been crossed artificially to produce triploids with excellent yield potential and quality traits (Sarmah and Bezbaruah, 1984). Due to their resilience, these triploids can act as progenitors in hybridization programs.

Breeding for seed quality

Tea is a crop that is both heavily heterozygous and cross-pollinated. When closely related people mate, there is a noticeable decrease in fertility and vigor. Cross-pollinated cultivars tend to be heterozygous to some extent, and groups of seedlings are frequently varied, which causes greater production issues with seed propagation (Patel et al., 2018). Therefore, it is not unexpected that this subject received little research.

However, the current seed oil quality in *C. sinensis* seed oil offers a viable alternative for breeding edible oil quality.

Tea is gathered for its fresh leaves in the majority of tea-producing nations, including Turkey, and seed is only utilized to establish new regions or raise seedlings. Consequently, the seed yield per area is low.

An oleaginous seed is produced by all *Camellia* species. Tea seed was used to make crude edible oil in native mills in West Bengal, Himachal Pradesh, Assam, and Northern Indochina (Owuor et al., 1985). Commercial-scale production of tea seed oil began in China, where 180,000 tons of the oil were produced in 1958 (Sengupta et al., 1976). The tea seed oil is yellow in color, freely flowing, and has a pleasant aroma. It may be kept at room temperature for three months without losing any quality, according to Roberts and De Silva (1972). According to Rajaei et al. (2008), the fatty acid content of *C. sinensis* seed oil included 21.5% palmitic acid, 2.9% stearic acid, 56% oleic acid, 22% linoleic acid, and 0.3% linolenic acid. According to Rajaei et al. (2005), oleic acid made up 50% of the total oil in the *C. sinensis* seed oil and was the main fatty acid. As a result, in terms of oleic acid, *C. sinensis* seed oil can be compared to olive oil and sunflower oil (Sahari et al., 2004).

The plant used to make tea is primarily grown for its robust vegetative development. Additionally, the tea plant produces considerable amounts of oil (30-32%), and its seeds serve as a useful commodity with a variety of uses. Due to its high level of unsaturated fatty acids, particularly vital linoleic acid, and low content of saturated fat, this oil is one of the most significant vegetable oils (Sahari and Amooi, 2013).

In reality, different types of tea are processed using fresh tea plant leaves. Specific regions might be taken care of for the development of *C. sinensis* seed oil and, of course, *C. sinensis* honey in nations where there is an excess of national tea production.

Leaf dependent quality

This subject requires more clarification. Naturally, each tea clone exhibits its genetic potential in future qualities that may be of interest. Therefore, it is essential to create outstanding clones with the right characteristics for producing various types of tea in various tea-producing regions. This is crucial for the collection of uniform leaf material, which will aid in the creation of high-quality tea.

Soil dependent quality

Tea (*C. sinensis*) really loves acid soils (pH 4.0-5.5) in contrast to most crops, whose production is severely limited by soil acidity (Ding et al., 2021). Tea, in particular, is particularly tolerant of aluminum (Al) toxicity increased by acidity, a significant factor that restricts the output of most other crops, and even requires Al for optimum growth.

Fertilizer use is crucial for the commercial production of tea (*C. sinensis* (L.) O. Kuntze), which is produced as a perennial monoculture (Woldegebriel, 2007). Fertilizers enhance the soil's and plants' nutritional status. The ideal soil conditions for growing tea are characterized as deep soils with good air circulation and an acidic pH range (4.5-5.6). Application of fertilizer is a crucial component of the typical intensive tea production (Drinnan, 2008), and it is one of the regular field management activities that has a big impact on tea output and quality (Anonymous, 2002).

Harvest technology dependent quality

The chemical makeup of tea (*C. sinensis*) changes depending on a wide range of variables, including geographical origin, cultivar, climate, plucking position, agricultural techniques, and of course harvest technology (Rubel et al., 2020).Unknown, 2002).

Tea leaves are plucked from the field either manually, with shears, or automatically. Depending on the methods used for harvest, different tea leaves have different polyphenol contents. In comparison to mechanically harvested leaves, which are often more mature, and shear plucked leaves, which are coarser and harmed, hand plucked fresh tea leaves typically have higher quantities of phenolic chemicals. In terms of organoleptic evaluation, tea brewed from hand-plucked leaves was discovered to be superior to that made from shear-plucked leaves (Ravichandran et al., 1998).

At pre-harvest, dryness and insect herbivory favor polyphenols and fragrance compounds, while cold and high light intensity stress mostly increase soluble sugar content and polyphenol/amino acid ratio, respectively. On the other hand, after harvest, low-temperature stress, drying, and leaf wounding can all significantly alter tea scent (Shao et al., 2021).

Harvesting the leaf is the most crucial task on a tea estate or tea farm. This must be done at the appropriate time, as determined by the factory, when the shoots are at the proper size and developmental stage. The shoots, which typically consist of two or three leaves and an unopened terminal bud, must be removed with the least amount of leaf damage possible to allow the sustainable production of high yields of high-quality tea. Small shoots, such as those with one leaf and an unopened bud, should be left on the shrub after selectively plucking (Carr, 2018).

Plucking criteria are crucial in determining the quality of black tea. The standard of plucking has a significant impact on tea quality and yield. The plucking standard can often be described as fine, medium, or coarse. Only the first two leaves and the bud are removed when using delicate plucking, while three or four leaves and the bud are often removed when using coarse plucking (Wright, 2005). The optimal balance between yield and quality is said to be achieved by plucking two leaves and a bud (Banerjee, 1992).

Factory Depending Quality

The biochemical makeup and sensory quality of black tea are influenced by withering, cutting and rolling, fermentation, drying, storage method, and length (Teshome, 2019). Every factory has a unique processing environment and capacity. Due to capacity, each factory will therefore have a unique manufacturing method, which will affect factory yield and quality.

Effect of Processing on Tea Quality

The unit procedures used to process tea have a direct impact on its chemical make-up, which affects its bioactive qualities, such as antioxidant and sensory activity (Sun et al., 2019). Up to five separate processes—withering, panning or steaming, sweating, rolling, and drying—are used to produce tea herbs. According to Braibante et al. (2014), the concentration of bioactive chemicals, primarily phenolic compounds, is reduced with the number of stages. Additionally, these processes change the bioactive qualities of methylxanthines, theaflavins, amino acids, and volatile components, resulting in various types of teas (Figure 1). Different processing steps and types of *C. sinensis* tea result in phenolic component concentrations that vary.

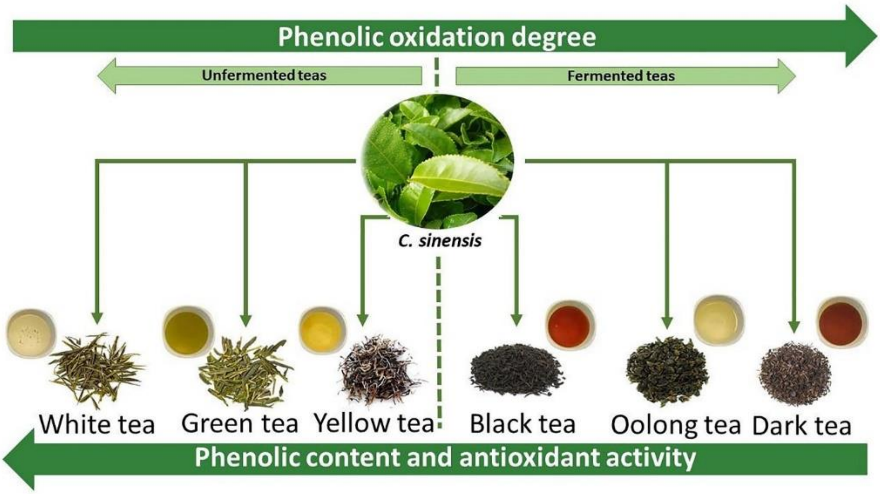

Figure 1: Effect of tea processing on phenolic content and antioxidant activity of infusions (Bortolini et al., 2021)

Different tea varieties reflect different palates around the globe. These could be a possibility for export tea production of several sorts.

Main research emphases of tea genetics and breeding in the future

Identifying new/superior clones

If selections are performed from seedlings developed from seed produced as a result of cross-pollination between two interplanted exceptional clones (biclonal seed) or from cross-pollination between two high-performance clones from an orchard, the likelihood of finding a superior clone is boosted.

Tea seedlings are heterogeneous, hence no seed, with the exception of biclonal stocks, could ensure the generation of better planting material (Njuguna, 1990). For improved production and quality of tea, emphasis should be placed on the selection and planting of vegetatively propagated material, specifically high yielding quality clones (Dutta and Alam, 2001). The most common method for creating superior cultivars in tea is clonal selection. Due to the allogamous nature of the tea plant, the current population of seedlings and their cross-pollinated offspring vary greatly from bush to bush. Through selection procedures, this variety is taken advantage of to create new tea cultivars with improved attributes (Ranatunga et al., 2009). Clonal selection is conducted in a largely uniform manner across all nations that produce tea (Tubbs, 1946; Visser and Kehl, 1958; Wight, 1961; Barua, 1964; Waheed et al., 2001).

The chemical composition, leaf shape, and physiological activity of plants in various genetic groups (wild or cultivated species) of tea exhibit a high degree of biological variety, according to previous studies (Feng et al., 2014; Tang et al., 2015).This current plant material can be used to create new clones.

Low pH

In fact, as was previously established, tea (Camellia sinensis) favours acid soils (pH 4.0–5.5) (Ding et al., 2021). According to the USEPA (2008), the ideal soil pH for tea plants is between 4.5 and 6.0, with 5.5 being the ideal pH. When the soil pH is higher than 6.5, tea plants gradually stop growing, and when it is higher than 7.0, they die (Su, 2012). Tea plant growth is impeded when the pH is below 4.0, endangering human health (Aloway, 1995) and decreasing both the amount and quality of tea production (Su, 2012). Soil acidification undermines the safety of tea intake in addition to causing soil nutrients to be lost.

Tea is a unique crop that demands acidic soil and contributes to soil acidification. The biogeochemical cycling of aluminum (Al) in tea leaves can produce soil acidification, and it can absorb and deposit significant amounts of Al in the leaves (Song and Liu, 1990; Ding and Huang, 1991).

Tea may prove to be a valuable new model for the investigation of both Al tolerance and Al growth-stimulatory processes given its exceptional performance on acidic soils and the recent publication of its entire genomic sequence. For the production of crops that are entirely adapted to acid soils in the future, a precise understanding of these pathways may be essential (Ding et al., 2021). The creation of novel clones that are pH-adapted will lead to the production of high-quality teas, as will the application of appropriate and right agronomic practices.

Enhancing the basic genetics research for breeding in tea

In general, tea production increases, quality, and tolerance of or resistance to biotic and abiotic challenges were achieved via processes including conventional selection, hybridization, marker-assisted selection, mutation breeding, polyploidy, genetic engineering, etc. The degree of intense use of such breeding procedures is correlated with the volume of tea produced (Shehasen, 2019).

Recently, chromosome-scale genome assemblies that enhance the original reference genome for this species—which is highly significant from an agronomic and economic standpoint, particularly in Asia—have been released. These research have also produced a high-resolution genomic variation map of wild and cultivated tea plants and an investigation of the ancestry of significant Chinese cultivars, providing fresh insights into the evolutionary dynamics of long terminal repeat (LTR) retrotransposons in the tea genome. Meanwhile, important genes influencing flavonoid and catechin contents have been discovered by genome-wide association analyses. The provided reference and resequenced/RNA-sequenced tea plant accessions' genomic sequences serve as useful resources for upcoming functional genomics research, tea plant genetic improvement, and the manufacture of healthy natural chemicals.

The current situation of collection, conservation, appraisal and evaluation of tea germplasm

The levels of output in various countries that produce tea are related to the stages of genetic resource collection, conservation, appraisal, and evaluation. Tea germplasm is currently one of the most valuable basic resources for tea breeding and biotechnology, and it has significant future promise for the entire tea industry.

Tea germplasms need to be collected, conserved, appraised, and evaluated with great care in order to effectively utilize their abundance, breed more novel kinds, and serve the tea business.

Every nation should build its genetic library in accordance with regional requirements. Maintaining the current genetic diversity in tea farms is another crucial concern.

The establishment and development of tea breeding system

Over the years, the breeding goals have evolved from high yield to high quality, then to a variety of goals like high quality, high efficiency, high functional component contents, and high stress tolerance. The breeding process, however, is constantly improving. A highly effective tea breeding system has now been constructed and is being steadily developed (Liang et al., 2007), in which controlled hybridization and individual selection are the primary breeding tactics along with molecular marker-assisted selection and micro propagation techniques.

Of course, reproduction is dynamic. Particularly Türkiye has a special position because of the interspecific character of its tea, based on Chinese-Assam hybridizations, and the possibility of existing diversity these countries have to build their own tea breeding programs.

Individual selection, mutation breeding, hybridization breeding, the formation of national tea germplasms, the use of molecular markers, poliploidy breeding, and, of course, their biochemical characterization should all be included in these systems. After the introduction of new, improved cultivars, morphological descriptors are the requirements that tea growers have the most. All planned actions should, of course, relate to national priorities, which can be shared with nations that produce tea.

Climate Change

One of the biggest issues facing tea growers today is the influence of global climate change in locations where tea is grown. Rising temperatures and drier weather have been predicted to result in a decrease in tea output. Intense soil deterioration and the occurrence of extreme weather conditions, such as protracted droughts and sudden downpours, can result in decreased soil fertility (Wijeratne 2018). Additionally, it is anticipated that the effects of global climate change may make these areas more susceptible to pests, illnesses, and weeds (Lal 2005). In addition, inadequate wintertime exposure to cold temperatures in places where tea is grown may interfere with the timing of the first flush's blooming (Tanaka 2012). The consistency of budding is crucial for preserving the caliber of first flush leaves.. Therefore, it is essential to create new tea cultivars that are compatible with various ecological settings and novel cropping patterns (Wijeratne 2018).

CONCLUSION

The current diversity must be protected and characterized for upcoming crop development projects, which form the essential framework for the tea business.

Climate change, new pest and disease pressures, and customer demand for tea grown with fewer chemical inputs are just a few of the hurdles that future tea cultivars will need to overcome. However, breeders and scientists are aware that the genetic diversity of cultivars now found in farmers' fields is insufficient to address these issues. The diversity present in genebanks, gardens, vacant fields, and the wild must be readily accessible for usage in the long term in order for tea farming to be sustainable and lucrative.

The primary goal of tea breeding, like that of any crop, is to increase the quantity and quality of the finished product. However, tea is distinct from other woody perennials in that the shoot tips, or the two leaves and a bud, are what must be harvested. About 10–18% of the biomass and dry matter produced by the plant is made up by the harvest.

Although traditional breeding programs—which included earlier creation of enhanced seed varieties, superior clones, and clonal seeds—increased tea yields, little progress has been made in raising the tea harvest index, which is currently quite low. High photo-respiratory losses, which lower net photosynthesis and hence tea productivity (Roberts and Keys, 1978), are a related genetic issue. Both of these elements place a cap on productivity that is challenging to exceed using traditional methods. Only with effective light energy interception, absorption, and photosynthetic use are quantum leaps in yield achievable (Banerjee, 1986). Therefore, the foliar configuration of the conceptual ideotypes of tea must allow for the best possible utilization of light energy, water, and nutrients, resulting in greater productivity. The optimal partitioning of photosynthates into the organs that provide economic output is possible with the right leaf arrangement. However, there hasn't been any attempt at tea selection for this global variety (Allard and Bradshaw, 1964), mainly because the physiological, morphological, and architectural elements for high yield, quality, and environmental adaptations are unknown. It is unknown if the ideal tea plant architecture proposed by Yao et al. (1987) would be appropriate for all settings. Increasing the genetic diversity of tea, which should not be restricted or limited to the generation of hybrids alone, is one strategy to boost output.

Chromosome alterations in somatic cells can be induced, detected, and recovered in order to increase genetic variety. It is possible to modify the genetic makeup of somatically created hybrid tissues before they regenerate into plants. However, superior plant models require a grasp of the genetic structure of the plant, whether by bioengineering or conventional breeding.

A priceless resource for crop development is the genetic variety of tea that has been conserved in germplasm repositories. The development of new tea cultivars has been facilitated by efforts to collect, preserve, and describe germplasm. The future research and development of tea breeding must concentrate on incorporating biochemical and metabolite profiling methodologies into the traditional tea breeding program in light of the enormous genetic variety of tea germplasm.

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CHAPTER 7

RECENT DEVELOPMENTS IN OMIC-BASED APPROACHES IN HEMP (*Cannabis sativa* L.)

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INTRODUCTION

Dioecious hemp (*Cannabis sativa*), family Cannabaceae, grows as annual (Moliterni et al., 2004), plant species with gene center in Central Asia (Zhang et al., 2018). The seeds are a rich protein and vegetable oil (Yang et al., 2017) and the plants are rich fiber used in the manufacture of thread, clothes, paper money etc. (van der Werf, 1994). Due to the therapeutic properties of the high resin content; it has found use in medical, religious, cultural and recreational applications (Andre et al., 2016).

1. CANNABIS (*Cannabis* sp.); CLASSIFICATION AND IMPORTANCE

Some variations of the plants have narcotic effects and were prohibited for cultivation during the first quarter 20th century and is heavily regulated in several countries under the United Nations convention on narcotic drugs. (Bridgeman, 2017).

Its cultivation as medicinal plant has increased rapidly in recent years, scientific research has also increased in parallel (Dolgan, 2018) at the heart of this increase is the relaxation of legal limitations for its cultivation in some developed countries. With the controlled legalization of *Cannabis* and hemp-based products, a production and marketing network has emerged above the expected, reaching a market volume of billions of dollars in the USA alone (Parker et al., 2019; Conneely et al., 2021).

There are two basic compounds of Cannabis named THC (Tetrahydrocannabinol) and CBD (Cannabidiol). While cannabidiols are mostly used for medical (sedative, anxiety prevention and sleep disorders) and cosmetic (shampoo, soap, skin care products) purposes, varieties containing high levels of THC are abused as drugs, although they contain more psychoactive components. Since 2001 (EC 2860/2000), the European Union has permitted the growth of fiber hemp types with a tetrahydrocannabinol (THC) level of 0.2 percent or lower in dry material. Varieties/species containing up to 5-20% THC mostly in dry material are called drug-type and are restricted due to their narcotic effects (Bruci et al., 2012). Cannabinoids are produced by phenols preserved in vacuoles and terpenes emitted by plastids in disc cells, according to the current hypothesis. Terpenes were discovered to be secreted from spherical plastids using electron microscopes.

2. OMICS-BASED APPROACHES

In its simplest definition, the suffix -omics functions as a derivational suffix (inspiration, neologism) in studies in the field of biology in English. Examples of omics are genomics, proteomics, and metabolics. Apart from these, transcriptomics and ionomics constitute a separate field of study in the field they specialize in. The field of omics research focuses on the collective characterization of an organism's molecules that translate into multiple biological structures, functions, and dynamics.

Omic-based methods; These uses high-throughput technologies such as proteomics, transcriptomics, ionomics, and metabolomics for large-scale analysis of various types of molecules. These high-throughput techniques are used on a large scale for purifying, identification, and characterization of DNA proteins, RNA, and other compounds (Coughlin, 2014; Kumar et al., 2019).

Development of these methodologies have helped in rapid analysis of the compounds present in medicinal plants through integrated omic approaches (next-generation sequencing (NGS) technologies) methods (precursor feeding, overexpression, mutant selection). Analyses of huge and several samples could be performed rapidly, accurately and clearly in a short time (Porter and Hajibabaei, 2018). In addition, with the identification and characterization developed within the scope of these methods, the modification of the genes responsible for secondary metabolites can be included in homogeneous and heterogeneous systems. With the advancement of transcriptomics and genomics methods, it is now possible to describe several genes at the same time.

2.1. Genomics

The single gene level has been the main focus of molecular investigation of plants up until recently. This paradigm has been altered by recent technical developments, which now make it possible to evaluate organisms in terms of how their genomes are organized, expressed and interact.

Tremendous studies and data have been collected using genomic data since the beginning of the twenty-first century; more and more plants are being sequenced through for RNA and whole genome sequencing for characterization of genes to elucidate and regulate several metabolic and cellular pathways (Michael and Cristobal, 2013).

Genomic science focuses on genetics, material which are organized inside genome, using techniques used to gather and examine this data, and how this arrangement affects how the genes work biologically. As they rely on information recorded in DNA, genomic techniques permeate every element of plant biology and with expanded molecular analyses using a single level to multiple species. The previous paradigm to identify the genes underlying biological functions has been reversed by plant genomics, which now focuses on figuring out the biological functions underlying the genes. Additionally, it narrows the genetic gap between genotypes and phenotypes aiding in understanding of how a gene's genetic context and the genetic networks it interacts to modulate their activities in addition to the gene's isolated effects. (Campos-De Quiroz, 2002).

2.2. Transcriptomics

Transcriptome have important function in identifying potential genes involved in important metabolite biosynthesis for plants. Transcriptomics technology has gained momentum with next-generation sequencing (NGS) methods. Large-scale transcriptomes help define the mechanisms, cellular processes and responses of specialized metabolites in which candidate genes participate (Strickler et al., 2012).

2.3. Metabolomics

According to Hall et al. (2002), the term "metabolomics" refers to extensive analyses of complex metabolite mixtures in plant samples.

These simultaneous analyses facilitate complex metabolites in organisms and cells. It effectively analyzes the various chemical compounds induced in plant cells in this way (Pichersky and Gang, 2000).

2.4. Proteomics

Proteomic approaches include large-scale proteome analysis of tissues, organs and cells. It is an essential component of omics technologies and aids in the discovery of modifications in the proteome profiles of plants' responses to exterior factors. Proteome analytical tools for proteomic analysis are electrophoresis combined with the use of tandem mass spectrometry and electrophoresis have facilitated profiling in systematics one-dimensional polyacrylamide gel electrophoresis and two-dimensional electrophoresis combined with tandem mass spectrometry have enabled profiling of entire proteomes. (Hussain and Huygens, 2012). Proteomics approaches are useful in the identification of large-scale specific protein changes in organs, tissues and cells in response to the formation of plant-based formulations or purified plant-derived compounds. Their understanding the localization of proteins in various tissues may reveal information related to biosynthesis of specific metabolites (Martinez-Esteso et al., 2015).

3. RESULTS

Recently, omic-based methods have come to the fore in studies on plants with medicinal properties, including *Cannabis* (Aliferis and Bernard-Perron, 2020; Backer et al., 2020; Romero et al., 2020;

Vujanovic et al., 2020). When the omic-based studies on *Cannabis* are examined, it is seen that they mostly focus on trichomes (Aliferis and Bernard-Perron, 2020; Romero et al. 2020; Conneely et al., 2021). In a study by Andre and Guerriero (2016), *Cannabis* trichomes were named as small phytochemical factories. Trichomes, which are epidermal outgrowths, are primarily found on the Cannabis plant's leaf, bracket, and stem surfaces. The bracket and floral leaves of female plants are where glandular trichomes are most frequently found. Cannabinoids are therefore more abundant, in the tips of female plants. (Huchelmann et al., 2017; Monthony et al., 2021). As a defense mechanism, glandular trichomes have a function of secreting and storing secondary metabolites. Single or non-glandular trichomes are mentioned as non-glandular trichomes without any function as secretory organs. According to theory, non-glandular trichomes have genes that stop the plant from losing water or enable the synthesis of a few secondary metabolites. (McDowell et al., 2010; Monthony et al., 2021).

Because of their rich biochemical potential, some studies have characterized on the characterization of specialized trichome structures using omic methods. (Schilmiller et al., 2010; Jin et al., 2014; Aliferis and Bernard-Perron, 2020).

TrichOME (http://www.planttrichome.org/trichomedb/), an omics database on this subject, enables comparative analysis in plant trichomes and provides researchers with data mining about metabolites, genes and gene expressions (Dai et al., 2009). TrichOME is a functional omic database that includes mass spectrometry (MS)-based trichome metabolite profiles of both trichome and control tissues. A study conducted by Conneely et al. (2021) can be used as an example for trichome characterization in this context. Researchers isolated the protein from the head and stem parts of the glandular trichomes (100 mg for each sample) as well as in flower samples (1 g) taken at the end of the flowering period. Protein samples were analyzed by nanoHPLC mass spectrometry method and protein identifications were compared with nanoHPLC MS/MS results with proteins from peptides from *C. sativa* cs10 Project (https://www.ncbi.nlm.nih.gov/genome/gdv/browser/genome/?id=GCF_900626175.1) with the reference proteome. Among a total of 1820 proteins, 370 (20%) were seen in all three samples; 569 proteins were detected only in flowers (31.3%), 122 proteins (6.8%) in glandular trichome heads and 1 protein (0.1%) in glandular trichome stems. In line with the results obtained; A total of 1682 proteins were found in the flowers of *C. sativa* taken at the end of the flowering period, 1240 proteins in the head of the glandular trichomes and 396 proteins in the stem of the glandular trichomes. The mitotic DNA binding, molecular functions and cell cytoskeleton, nucleozone, and microtubules were found to be overrepresented in the mitotoic cell cycle, molecular function, DNA binding, cytoskeleton, nucleozone, and microtubules as a result of Gene Ontology (GO) enrichment analysis. It has been determined that 569 proteins in flower samples are mainly effective in the biosynthesis of chlorophyll and in the photosynthesis process (Biological process and gene ontology: BP, GO terms). has been done. Therefore, many protein mapping studies have determined that glandular trichome head structures play a role in terpene, phenylpropanoid, and phenolic biosynthesis tasks and are the center of secondary metabolites.

In addition to secondary metabolite synthesis, glandular trichome head structures were discovered. Glandular trichome stems, on the other hand, do not participate in biosynthesis and instead serve a variety of specialized functions on the trichomes. As a result, the main cannabinoids stored in the trichome heads contain at least 15% of the amount contained in the dried inflorescence of *C. sativa*. At the same time, trichome heads are important in the pollination period as they are a rich carbon source (Conneely et al., 2021).

Another omic-based study on *Cannabis* is carried out by Marti et al. (2014). Researchers subjected *C. sativa* leaves exposed to UV-C radiation, which has a metabolic inducing effect on secondary metabolite production, to LC-MS-based metabolomics and antioxidant analysis. There was no significant change in cannabinoid content as a result of the study, but dehydrostilbenes and cinnamic acid amide excessive derivatives. It has been reported that induced cinnamic acid amide derivatives have a functioning tissue damage cell wall strengthening. (Clarke, 1982). In the antioxidant activity results, however, no significant changes were observed in *C. sativa* leaves after UV-C radiation exposure.

Another metabolomic study on *Cannabis* is Pec et al. (2010) conducted by Researchers used jasmonic acid and pectin as elicitors. Jasmonic acid and pectin were used to treat cannabis cell cultures, and nuclear magnetic resonance was treated to the dual cell line. Chloroform extracts of pectin-treated cells differed from control and jasmonic acid treatments, as shown by principal component analysis. In the methanol/water extracts, the cells treated with jasmonic acid showed a clear difference compared to the pectin and control treatments. Antioxidant metabolite tyrosol was found in *Cannabis* cell cultures, but it was more abundant when jasmonate acid elicitor was present.

4. CONCLUSION

A collection of newer and high-throughput methods, omics (genomics, metabolomics, ionomics, and metabolic engineering) are useful tools for large-scale production, diagnosis, and characterization of plant-derived substances in both in vivo and in vitro settings. Thanks to these new methods used, gene-protein-metabolite networks offer better opportunities in terms of increasing the production of specialized metabolites and molecular improvement. It is thought that understanding genetic, protein and metabolic activities and determining their mechanisms of action will be of great importance in plant breeding and secondary metabolite production in the future.

Omic methods used in hemp (*C. sativa*) plant were applied in drug types rather than fiber types. When omic-based studies in other medicinal plants are examined, it helps to better understand the protein-metabolism mechanisms of proteomic and metabolomic methods within secondary metabolites.

The controlled legalization of *Cannabis* plants and Cannabis-derived products in certain regions of countries such as the United States and Canada, particularly in the last 20 years, has created a very large market volume and increased interest in these products. When examined on a product basis, secondary metabolites stand out mostly compared to fiber production, and technological developments (cannabinomics, proteomics, metabolomics) in this direction are increasing year by year. Since omics-based studies on *Cannabis* plants are a new field of study, the databases used are not plant-specific and bio-informatic analyzes of the data obtained include many databases. It is expected that in the coming years, with the concurrent formation metabolites and cannabis specific softwares, it will significantly contribute to Cannabis-related metabolomics applications.

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CHAPTER 8

EFFECT OF DROUGHT STRESS ON SECONDARY METABOLITES IN MEDICINAL PLANTS

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INTRODUCTION

Phytodiversity, has an important place in provide for nutrition of people, their medical needs and economic development. For this reason, agricultural, phytochemical, biological and genetic researches that support the development of plants are increasing day by day (Sharma et al., 2020; Husen, 2022; Asfaw et al., 2022) However, ever-increasing industrialization, climate change, environmental pollution and population growth not only reduce plant diversity, but also increase the need for medicinal plants due to deteriorating health problems (Boit et al., 2016; Husen, 2023). Climate change can cause changes on the natural habitat of plants and they are very sensitive to these environmental changes (Hoffman et al., 2019; Pant et al., 2021). Plants activate stress mechanisms against the negative situations created by their changing environment. These negative situations are; light stress, salinity, drought, low or high temperature, ultaviolet radiation, heavy metal exposure, air pollution. Plants can create positive or negative responses in growth, flowering, fruit and seed yield as a result of the mechanisms to have developed to cope with these biotic or abiotic stress conditions (Wani et al., 2016; Jan et al., 2021).

Medicinal plants are rich in aromatic compounds such as terpenoids, steroids, phenols, tannins and flavonoids, and these compounds are widely used as raw materials in the pharmaceutical, cosmetic and food industries (Efferth and Greten, 2012) These chemical compounds are known as "secondary metabolites", which do not actually play a main role for plant growth and development, but are necessary defense components for adaptation to environmental conditions (Ramakrishna et al., 2011; Isah, 2019). There are more than two million known secondary metabolites, and many unidentified secondary metabolites are still thought to exist (Kumar et al., 2019) Due to this chemical diversity, the demand for medicinal plants has increased in recent years, especially in the pharmaceutical industry in terms of low cost, less adverse effects and reliability (Verpoorte and Memelink, 2002; Erb and Kliebenstein, 2020) Many of the studies have revealed that these secondary metabolites can be used as potential agents in the prevention and treatment process of many diseases such as cancer, diabetes, ulcer, Alzheimer's disease and cardiovascular diseases (Jansen et al., 2008; Crozier et al., 2009; Li et al., 2016; Ergül et al., 2021)

Medicinal and aromatic plants activate the mechanisms necessary for their physiological development to keep pace with changes in environmental conditions. Various abiotic factors can affect the growth and development of plants. This enables the formation of secondary metabolic pathways to develop stress tolerance and establish defense mechanisms (Mahajan et al., 2023). Various environmental factors trigger different signalling pathways that increase the production of secondary metabolites. These metabolites are used in the production of food additives, sweeteners and pharmaceuticals of industrial importance. Secondary metabolites can increase or decrease depending on the genotype, growth factors and developmental stages of the plant (Vardhan and Shukla, 2017).

Abiotic stress factors such as drought, salinity, temperature, deficient minerals, heavy metal toxicity, radiation, gaseous pollutants may cause inhibition or activation of these metabolites by affecting the secondary metabolite mechanisms in plants (Figure 1). These mechanisms provide adaptation and defense of plants to climatic conditions. However, the role of these regulatory factors is still not fully understood (Qaderi et al., 2023).

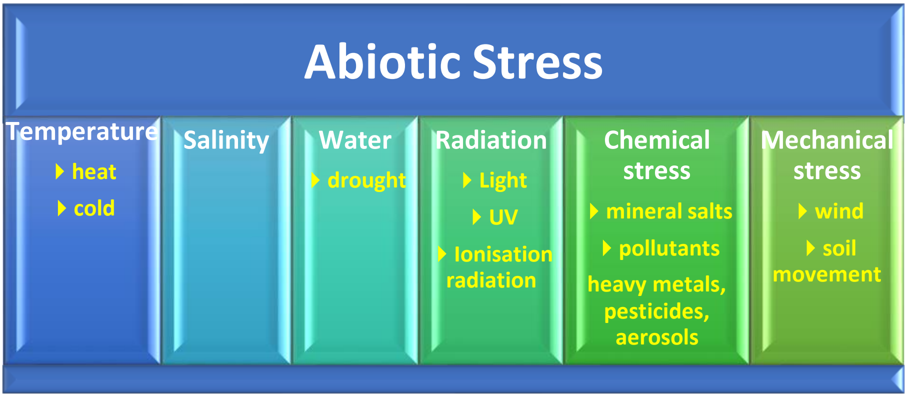

Figure 1: Different abiotic stress signals that cause stress in plants (modified from Akula and Ravishankar, 2011).

To increase the production of secondary metabolites induced by environmental stress signals, researchers use stress factors in in vitro studies on plant cell cultures (Goyal et al., 2012). Some researchers have applied various elicitors to increase the production of secondary metabolites in plant cell, tissue and organ cultures (Karuppusamy, 2009). It can be said that nutrient stress has a significant effect on phenolic compounds in plant tissues. Changes in growth conditions affect the concentrations of plant secondary products and the responses of metabolic pathways are influenced by the accumulation of natural products (Gautam et al., 2023). Salt and drought stress are common responses in plants. In both stress conditions, osmotic stress occurs and with dehydration, water is withdrawn from the cytoplasm to the vacuoles (Rahman et al., 2023).

Drought Stress Effects on Plant

Drought stress (DS) is one of the most important abiotic stress factors affecting plant growth and development (Xu et al., 2010) (Table 1). DS occurs when long-term water loss occurs under atmospheric conditions and the water in the soil drops to a level that is not sufficient for the plant. All plants show DS tolerance. This is often the case when solar radiation and temperature are high and water is reduced. This situation is considered a global problem, especially when it comes to ensuring the viability of agricultural products and sustainable food production (Massacci et al., 2008). Drought usually causes oxidative stress and it has been reported that the amount of flavonoids and phenolic acids in willow leaves increases (Al Ghamdi, 2009). Hovewer, plants are not always seriously damaged by DS. Instead, acclimatisation may occur slowly over a few weeks, resulting in metabolic adjustments and reduced overall growth (Bowne et al., 2012).

If one evaluates many studies, one can be said that flavonoids generally increase as a result of the response of plants to drought stress (Qaderi et al., 2023) The increase in non-flavonoid phenolic compounds varies according to the drought resistance of the plants (Hura et al., 2008; Rebey et al., 2012; Aninbon et al., 2016).

Table 1: Effect of drought stress on the production of secondary metabolites in plants

Plant Species	Secondary Metabolite	References
Dysoxylum binectariferum	Rohitukine	Kumara et al. (2016)
Artemesia annua	Artemisinin	Vashisth et al. (2018)
Mentha piperata	Terpenoids and alkaloids	Alhaithloul et al. (2019)
Andrographis paniculata	Andrographolides	Chen et al. (2020)
Codonopsis pilosula	Obetyolin and Syringin	Liang et al. (2021)
Achillea pachycephalla.	Flavonoids, apigenin, luteolin	Gharibi et al. (2019)
Scutellaria baicalensis Georgi	Baicalin	Cheng et al. (2018)
Larrea divaricata	Polyphenols	Varela et al. (2016)
Salvia dolomitica Codd	Sesquiterpene	Caser et al. (2019)
Oryza sativa L	Vanillic acid, p-hydroxybenzoic acid	Quan et al. (2016)
Adonis amurensis	Flavonoids	Gao et al. (2020)
Dendrobium moniliforme	Flavonoids, and alkaloids	Wu et al. (2016)

Effect of Drought Stress on the Production of Phenolic Compounds in Medicinal Plants

Phenolic compounds are a large family that contains phenol groups in their structure and has about 10000 known members. They have important functions in several physiological and biochemical processes in plants (Ma et al., 2014). In plants, drought often results in oxidative stress. This increases the activity of antioxidant enzymes and therefore the amounts of flavonoids and phenolic acids (Anjum et al., 2008). *Achillea pachycephala* Rech.f. is a medicinal herb grows endemically in Iran. The plants exposed to drought during t 0, 7, 14, 21 and 28 days and HPLC analysis revealed that most of the phenolic compounds increased during drought stress (Gharibi et al., 2019). In a study ten sesame genotypes were used to find out the influence of drought stress and relationships between seed coat colors. It was found that drought increased total phenolic content, especially, polyphenolic components and sesamolin. They investigated that the dark-seeded genotypes consisted higher total phenolic content whereas the light-seeded consisted higher sesamin and sesamolin (Kermani et al., 2019). It was observed that the total amount and concentration of phenolic compounds increased significantly in Hypericum brasiliense plants exposed to drought stress compared to control plants (de Abreu and Mazzafera , 2005).

On the other hand, after exposing the *Mentha piperita* L. and *Catharanthus roseus* plants to drought for seven and fourteen days Alhaithloul et al. (2019) indicated that total phenolic compound contents decreased when compared to control. However, the amount of phenolic compound is higher after seven days than after 14 days. Hence the contents vary according to the time sets (Alhaithloul et al., 2019). Likewise, in *Rehmannia glutinosa* (Gaertn.) DC, irrigation deficit decreased the number and amount of phenolic compounds. From this point of view, it cannot be said that under drought conditions phenolic compounds content increases.

In a study in which melatonin was applied to the leaves of two lemon species (*Citrus latifolia Tanaka* and *Citrus aurantifolia*) reported that overall flavonoid and phenolic concentrations increased in response to DS (Jafari and Shahsavar, 2021). In another study conducted in 2019, melatonin was applied to the plant *Dracocephalum moldavica* and as a result, it was stated that it regulated the activity of enzymes such as ammonia lyase and polyphenol oxidase and secondary metabolites in response to DS (Naghizadeh et al., 2019)

Since metabolic genes are responsible for various metabolic pathways in drought stress tolerance, they play an important role (Kumar et al., 2021). It has been reported that tolerance of transgenic Arabidopsis thaliana overexpressing CeWR14 gene from *Cyperus esculentus* increases when drought stress increases and cuticular wax accumulation occurs (Cheng et al., 2020).

It was determined that the levels of phenolic compounds increased in *Cuminum cyminum* L. leaves in severe drought, while the levels of phenolic compounds increased in seeds with over irrigation (Alinian et al., 2016).

This change tells us that secondary metabolite production in plants may differ in different tissues and organs of plants. However, it shows that metabolite production can change during the developmental stages of the same plant species or during exposure to stress.

Effect of Drought Stress on the Production of Alkaloids in Medicinal Plants

Alkaloids are a class of nitrogenous heterocyclic compounds derived from amino acids. They have important roles in the response of several stress conditions.

Most of the data on the amount of nitrogen-containing natural products in plants exposed to drought stress are related to the concentrations of these compounds in specific organs (Kleinwächter and Selmar, 2015) In most plants, alkaloid contents have been shown to rise upon drought stress. It has been observed that drought increases alkaloid levels, especially morphine, in *Papaver somniferum*, which is an important medicinal plant in the world (Szabó et al., 2003). There was a significant increase in alkaloid accumulation by the expression of genes in the isoquinoline alkaloid biosynthesis pathway in the leaves of *Catharanthus roseus* L. (Liu et al., 2017). Alkaloids and terpenoids contents of *Mentha piperita* L. and *Catharanthus roseus* plants showed increased alkaloid levels after exposure of drought stress. In these plants, in contrast to the phenolic compounds, the amount of alkaloids increased with the increase of the stress time (Alhaithloul et al., 2019) Nevertheless, in contrast to these results, Liu et al. revealed that indole alkaloid amounts were increased after drought stress in *C. roseus* (Liu et al., 2017).

Effect of Drought Stress on the Production of Terpenoids in Medicinal Plants

Terpenes are derived from the five-carbon isoprene unit. They are the largest group of secondary metabolites with at least 25,000 members (Krauss and Nies, 2015) Terpenes are involved in the response or communication against abiotic stress in plants (Singsaas, 2000) Terpene compounds have functions as antioxidants because they scavenge ROS species to prevent oxidative damage (Loreto et al., 2001).

It was determined that the monoterpene concentration increased greatly as a result of drought stress inn *Salvia officinalis* plants that of well-watered (Nowak et al., 2010). Similar research revealed a concentration increase in monoterpene amounts in leaves of *Petroselinum crispum* (Petropoulos et al., 2008) It was observed that the increases in monoterpene levels were temporary in thyme plants exposed to drought stress. It has been measured that drought stress reaches its highest level about 2 weeks after its onset and eventually stabilizes. Of course, it is stated that the values may decrease as time progresses Kleinwächter et al., 2015) *Artemisia annua* L. accumulates sesquiternoid artemisinin and related phytomolecules in glandular trichomes. Under prolonged drought stress artemisinin, arteannuin-B, artemisinic acid content were negatively regulated by water deficit stress. On the other hand, some of minor monoterpenes and all sesquiterpenes were increased by stress treatment. The researchers thought that this might because of the decrease in glandular trichome density (Yadav et al., 2014) Terpenoid amounts in plants do not always increase with drought stress. For example, the overall terpenoid content of *Melissa officinalis* plant, grown under drought stress, was found to be lower than well-watered controls (Manukyan, 2011).

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CHAPTER 9

INVESTIGATION OF ANTIBACTERIAL ACTIVITIES OF *LAVANDER*, *SAGE* AND *TILIA*

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INTRODUCTION

Medicinal and aromatic plants (MAPs) have been an important part of daily life and culture all over the world since the existence of humanity. These plants are used in the fields of health, pharmacy, cosmetics and food. The cultivation and collection of medicinal and aromatic plants has been going on since ancient times.

The oldest and most common use of medicinal and aromatic plants is seen as medicine. Today, it has become a basic material that is still used in hygiene, spices, dyes for coloring, preservatives and many other fields.

The use of plants for therapeutic purposes varies according to the level of development of countries. In developing countries, treatment with plants varies between 80-95%, while the use of herbal treatment methods is lower in developed countries (Acıbuca and Bostan Budak, 2018).

Medicinal and aromatic plants contain steroids, flavonoids, saponins, terpenes, alkaloids and phenolic compounds. Due to this structure, they have painkillers, cardiovascular system protection, anticancer, antihistamine, antifungal and antibacterial properties (Zehra et al., 2019). Plants can be used in fresh and dried form. All parts of plants such as roots, stems, leaves, flowers, seeds and peel are used for different purposes and methods.

In this chapter, antibacterial properties of medicinal and aromatic plants and essential oils obtained from them were evaluated and sage, linden and lavender plants, which are used in many fields, were discussed.

1. LAVENDER (*Lavandula* sp.)

It is a purple flowering plant of the genus *Lavandula* of the *Lamiaceae* family. Lavenders are divided into four main categories: *Lavandula latifolia*, *Lavandula angustifolia*, *Lavandula stoechas, L. angustifolia* (Cavanagh and Wilkinson, 2002). The majority of the chemical components and ethnobotanical characteristics of different species of lavender are identical, yet distinct species have diverse purported medicinal applications. Both the dry bud and the essential oil of the lavender plant are used. Especially the high quality essential oil in its content enables its use in perfume, cosmetics and medicine (Cavanagh and Wilkinson, 2005; Jugreet et al., 2020).

Lavender oil has recently become popular in the textile industry. It is preferred in sleep textiles due to its sleep-inducing and calming properties by stimulating the central nervous system (Boh and Knez, 2006).

In addition, the antiseptic property of lavender oil makes it the most preferred oil in aromatherapy applications (Bagheri-Nesami et al., 2017).

It has been discovered that lavender oil is effective against a wide variety of pathogens and fungi. Lavender essential oil is additionally related to the potential recovery of bacterial illnesses that are resistant to treatment. For instance, Lavender oil has been demonstrated to exhibit in vitro activity against methicillin-resistant *S. aureus* and vancomycin-resistant *E. faecalis* at a concentration of less than 1%.

Adaszynska et al. (2013) analysed the essential oils obtained from five lavender cultivars (Munstead, Munstead Strain, Lavender Lady, Ellegance Purple and Blue River). The chemical composition of essential oils and their biological activities against *S. a*ureus and *P. Aeruginosa* pathogenic bacteria were compared. It was reported that the chemical composition of lavender varieties had the same main compounds, but the percentage ratios of the compounds were different. They determined that the main components of essential oils were linalool, linalyl anthranilate, 1-terpinen-4-ol, p-menth-1 en-8-ol and linalool oxide. The antibacterial activities of lavender varieties 'Munstead' and 'Blue River' were found to be more effective against *S. aureus* and *P. aeruginosa*.

Hossain et al. (2017) investigated the antibacterial activity of lavender essential oil against pathogenic bacteria (*A. hydrophila, A. caviae, A. dhakensis, C. freundii, P. mirabilis, S. enterica and P. aeruginosa*) from pet turtles. Antibacterial activity was determined by disc diffusion, minimum inhibitory concentration and minimum bactericidal concentration assays. The results showed that lavender essential oil was effective against all turtle-borne pathogenic bacteria tested except *P. aeruginosa.*

3. SAGE (*Salvia* sp.)

Sage (*Salvia officinalis*) is a plant of the family *Labiatae/Lamiaceae*. It is native to the Middle East and Mediterranean regions, but is now cultivated all over the world. In ancient times, it was used by people to treat various ailments such as ulcers, gout, rheumatism, dizziness and diarrhoea (Ghorbani and Esmaeilizadeh, 2017). With the development of science and technology, the biological effects and content of this plant, which has been used with traditional methods for many years, has become a subject of curiosity and has become a focal point for studies on it. Studies have revealed the microbiological and pharmacological effects of *S. officinalis.*

The antibacterial activities of *S. officinalis* are supported by a number of lines of evidence. Gram positive and gram negative bacteria are both very susceptible to the bactericidal and bacteriostatic actions of *S. officinalis* essential oil and ethanolic extract. Gram positive bacteria such as *S. epidermidis, B. cereus, B. megaterium, B. subtilis, E. faecalis,* and *L. monocytogenes* have great sensitivity to *S. officinalis* (Veličković et al., 2003; Mitic-Culafic et al., 2005; Bozin et al., 2007). The type of extract utilized determines how *S. officinalis* affects gram negative bacteria. The growth of *Aeromonashydrophila, Aeromonassobria, E. coli, K. oxytoca, K. pneumonia, Pseudomonas morgani, S.anatum, S. enteritidis, S. typhi*, and *S. sonnei* are all significantly inhibited by the essential oil of *S. officinalis* Veličković et al., 2003; Mitic-Culafic et al., 2005) .

Salvia officinalis has also been linked to antiviral and antifungal activities in addition to its antibacterial effects (Tada et al., 1994; Badiee et al., 2012).

Terpenes and terpenoid chemicals present in *S. officinalis* are thought to be responsible for this plant's antibacterial properties.

Medicinal and aromatic plants are also used to ensure food safety. Moghimi et al. (2016) investigated the antibacterial activity of sage (*Salvia officinalis*) essential oil and nanoemulsion obtained using this oil against *E. coli, S. dysentery* and *S. typhi* pathogens. For this purpose, the essential oil was sonicated with nonionic surfactants (Tween 80, Span 80) and nanoemulsion was formed. The antibacterial activities of nanoemulsion and pure essential oil were compared. The antibacterial activity of nanoemulsion was found to be four times more effective than pure oil against *E. coli* and *S. typhi*. In the kinetic studies, it was determined that the nanoemulsion destroyed *E.coli* within 10 minutes. These results showed that the antibacterial activity of sage in nanomeulsion form increased.

The antibacterial activity of sage was investigated against *Streptococcus mutants* (SM) bacteria causing dental plaque (Beheshti-Rouy et al., 2015). In a clinical study, SM was determined by SM colony counting method in intraoral samples. At the end of 21 days, a significant decrease in the number of SM colonies was determined after mouthwash with sage.

3. LİNDEN (*Tilia* sp.)

The linden plant (Tilia) has applications in both traditional medicine and nutrition worldwide. Tea made from linden is used for colds, flu, inflammation, migraine, hypertension and as a sedative. Due to its moisturising properties, linden tea is also found in cosmetic products (Karioti et al., 2014).

Several types of phenolic chemicals, primarily quercetin glycosides (rutin, quercitrin, and isoquercitrin), kaempferol glycosides (tiliroside), procyanidins, and phenolic acids (caffeic acid, p-coumaric acid, and chlorogenic acids) make up the complex chemical make up of linden tea (Toker et al. 2001; Karioti et al., 2014).

Fitsiou et al. (2007) investigated the content and antibacterial activity of linden species found in Greece and Romania. Investigations were done on the essential oils of *Tilia tomentosa* and *T. cordata*. Hydrodistillation was used to produce essential oils, which were then analyzed using GC and GC/MS. Tricosan made up the majority of the oils from *T. tomentosa* and *T. cordata* inflorescences, which had significant hydrocarbon content (32.3 and 60.4%, respectively), with tricosan. Using the disc diffusion technique, the oils' antibacterial activity was evaluated against gram positive and gram negative bacteria as well as a pathogenic fungus. Gram positive bacteria were significantly resistant to the leaf oil of *T. cordata*.

4. CONCLUSION

There are many researches showing that medicinal and aromatic plants and essential oils obtained from plants have been used since ancient times due to their effective antibacterial activity. In recent years, there has been an increase in the use of medicinal aromatic plants as people move away from synthetic products containing chemicals and turn to natural products. Medicinal and aromatic plants have a wide range of uses. They are used in medicine, pharmacy, food industry, perfumery, cosmetics, textile industry and biofuels. Nowadays, the emergence of bacteria that are extremely resistant to antibiotics and the side effects of synthetic drugs have led scientists to search for drugs of natural origin. Determining the antimicrobial effects of the active components of medicinal and aromatic plants is thought to be a solution to prevent rapidly increasing antibiotic resistance. In this section, the antibacterial activities of linden, sage and lavender plants were discussed and it was determined that they were effective against the bacteria examined in the studies.

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CHAPTER 10

PURIFICATION, CHARACTERIZATION AND APPLICATION AREAS OF MEDICINAL AND AROMATIC PLANTS

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INTRODUCTION

From past to present, plants have been used in many areas such as nutrition, defense, shelter and heating, especially in the treatment of diseases. The interest in medicinal aromatic plants, which are the raw materials of many sectors such as food, pharmacy, agriculture and cosmetics, has increased after the negative effects of synthetic and chemical drugs on human health have been observed. Medicinal and aromatic plants have been used for centuries as flavor, flavor, color, aroma and preservative in foods, as well as for cosmetic purposes, coloring matter and traditional treatment. While traditional prescriptions are used by 80% of the population in undeveloped countries, this rate is around 40% in developed countries; In addition, 25% of pharmaceutical drugs are produced from medicinal plants today (Karadag, 2019). It has been reported by the World Health Organization (WHO) that the number of medicinal aromatic plants used for spice, food and therapeutic purposes is around 20,000. It is known that approximately 1/3 of nearly 300 plant families in nature contain volatile fatty acids. Medicines have been prepared from plant extracts since 2700 BC, and many plants that were found by trial and error method, which are known as medicinal plants among the people, are used in the treatment of diseases (Faydaoğlu and Sürücüoğlu, 2013). Medicinal and aromatic plants are gaining increasing importance in terms of supplying raw materials to the food industry and using them in alternative or complementary medicine, which has become widespread in recent years. It is known that approximately 50% of the medicinal and aromatic plants traded in the world are used in the food industry, 25%

in the cosmetics industry and 25% in the pharmaceutical industry. From this point of view, it can be said that the demand for medicinal aromatic plants will increase gradually.

Applications

Medicinal aromatic plants, as the name suggests, are increasing in many sectors, especially in the fields of medicine and veterinary medicine. Innovative and value-added applications also enable the use of medicinal aromatic plants in food, livestock and agriculture fields. Essential oils contained in medicinal and aromatic plants, especially in the food industry, soft drinks, confectionery; In the cosmetics industry, it is used in perfumes, skin and hair care products, and aromatherapy (Christaki et al., 2012; Ribeiro-Santos et al., 2017). Essential oils can be considered as antiseptic and antimicrobial agents for personal use.

Although the pharmaceutical industry has focused on new artificial drugs, it is known that more than 50% of existing medicinal drugs using natural products are produced from plants. In addition, these plants have areas of use as insect repellent in food preservation (Bakkali et al., 2008).

The use of medicinal aromatic plants in agricultural control also attracts attention. The use of substances obtained from harmless natural plant extracts in order to increase the productivity and resistance of the plant and the soil is also very important for human and environmental health.

In animal nutrition, aromatic plants and essential oils obtained from them on animals; It is also known that they have many positive effects such as resistance to environmental conditions, herbal insecticide, use against pests and pathogens, increase in taste in feed, increase in feed efficiency, digestion-stimulating and antiseptic properties (Şengezer and Güngör 2008).

Plant species, which are also used as natural dyes, are known and used with their non-toxic and non-carcinogenic properties that do not cause environmental pollution.

In the food industry, the use of plant extracts is increasing day by day in order to extend the food preservation period. It is estimated that plants will find value as an important antimicrobial especially in organic food production because they are natural and do not cause residue problems (Cerit, 2008).

Due to the antioxidant, antibacterial and antimicrobial properties of plant species, their use in many different industrial areas will continue to increase.

Purification

Components that play an active role in applications in medicinal aromatic plants are obtained by different methods. Distillation, extraction, anfluorage, maceration and pressing are among the most basic techniques (Ou et al., 2015).

In recent years, modern techniques such as supercritical fluid, microwave, pressure solvent, enzyme assisted extractions and solid-phase microextraction have also been preferred (Handa, 2008; Dahmoune et al., 2014; Gligor et al., 2019). Each of these methods has advantages and disadvantages depending on the purpose of use.

After the plant samples are collected, they should be dried quickly in the first stage so that they do not deteriorate, and in this way, their moisture content should be reduced. Drying can be done in the shade, in the sun, in an oven, in a lyophilizer, and in the microwave.

After the drying process, the moisture content of the plants decreases, the formation of some chemical reactions is prevented, the growth of microorganisms is slowed down, the organic component composition is preserved and the shelf life is increased (Arslan et al., 2010; Hamrouni-Sellami et al., 2013; Jin et al., 2018). If the plants are not to be used as fresh and if they are not to be dried immediately after collection, they should be stored in a deep freezer. The plants to be used must be ground after drying. This process is important in terms of the interaction of the plant with the solvent and increasing the amount of substance transferred to the solvent during the purification and separation process. Grinders and mills are mostly used in the grinding process (Handa, 2008). After these pre-treatments, the separation and purification methods mentioned above can be applied to the plant samples.

Differences in compound composition, yield and activity obtained from plant samples may also result from postharvest laboratory practices such as sample preparation, solvent type and extraction method (Yakoub et al., 2018).

For example, it has been reported that the solvent used especially in the extraction of plants has a significant effect on the amount and structure of secondary metabolites. For this reason, it is important to choose the appropriate solvent to ensure active substance activity (Truong et al., 2019).

On the other hand, temperature has an important effect during the separation and purification processes of plants. It has been reported that increasing the temperature, especially in the extraction process, leads to higher permeability of cell walls, higher solubility of phenolic compounds, and higher heat and mass transfer throughout the plant matrix (Efthymiopoulos et al., 2018). It can be said that the temperature increases the productivity of the product to be obtained, as long as it does not cause the compounds in the structure of the plants to decompose.

Factors such as processing time and particle size also play an important role in the separation and purification of plants (Arslan et al., 2021).

The supercritical fluid extraction method, which is considered among the modern separation and purification methods, is attracting great attention. Because the treatment of natural products with organic solvents has become an undesirable phenomenon in recent years in terms of both environmental and health. In this respect, the supercritical extraction method gains importance with its feature of separating compounds that consume less solvent, have a shorter extraction time and dissolve at high temperatures (Yamani et al., 2008).

Another method is the microwave extraction method. This method, which is based on heating the plant sample with a solvent by applying microwave, is a closed system extraction with temperature and pressure control. In this method, while product losses decrease, the extraction time and the amount of solvent used are also significantly reduced (Kaufmann et al., 2007).

After the purification method is selected and applied, the last step is the characterization step.

In the characterization of plant extracts, techniques such as high performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), electrospray mass spectrometry (ESI-MS), nuclear magnetic resonance spectrometry (NMR), fourier-transform infrared (FT-IR) spectroscopy are used.

Characterization

Plant extracts exist in the form of mixtures of various functional compounds, and the process of characterization of compounds by their separation poses great challenges. It is common practice to use a number of different separation techniques, such as thin-layer chromatography (TLC), column chromatography, flash chromatography, and HPLC to obtain pure compounds from plant extracts. A simple, fast and inexpensive technique, TLC is a quick-response procedure for how many ingredients are in a mix. It is also used to support the identity of the compound in a mixture by comparing the Rf of the compound to be separated with the Rf of a known compound (Sasidharan et al., 2011). HPLC, GC-MS, ESI-MS, NMR and FT-IR techniques are mostly used in the characterization of the components in this mixture.

Widely used for the characterization of plant extracts, the HPLC technique is ideal for rapid processing of multicomponent samples at both analytical and preparatory scales (Iordache et al., 2009; Sasidharan et al., 2011; Augusti and Athayde, 2014**)**. In the HPLC technique, a detector is first selected to identify any compound, and a separation test is performed after optimum detection settings are made. The parameters of this method should be such that a clear peak of the sample known from the chromatograph is observed and well separated from foreign peaks. In HPLC, UV detectors are popular among all detectors because they offer high sensitivity. Phenolics are often identified using UV-VIS and Photodiode Array (PDA) detectors. Besides UV, other detection methods are also used to detect phytochemicals, such as the Diode Array Detector (DAD) coupled with Mass Spectrometry (MS) (Iordache et al., 2009; Sasidharan et al., 2011; Augusti and Athayde, 2014**).**

GC-MS is an advantageous method with high reproducibility used in the analysis of plant extracts, with extended data libraries for the identification of unknown compounds (for example, degradation products), and the possibility of simultaneous detection of molecules in complex matrices in a single step. Especially for the analysis of flavones, flavonols and isoflavones in plant extracts, the application of the GC method gives satisfactory results (Iordache et al., 2009; Degani et al., 2014). The GC-MS method is also a useful analytical technique for approaching archaeometric problems and its applicability is high in historical objects.

The ESI-MS approach by direct infusion for the detection of bioactive compounds in complex matrices of plant extracts is specific, sensitive, rapid and provides structure elucidation without requiring pre-purification steps. This method is a useful technique as it allows the simultaneous detection of different components in plant extracts. It is also important for the qualitative control of plant extracts (Mauri and Pietta, 2000; Amaral et al., 2009).

NMR technique, which provides information about the chemical structures of molecules, can also be used in the characterization of components in plant extracts (Sasidharan et al., 2011). NMR is an advantageous method as any organic compound can be easily detected without any detection limitations. In addition, it is an important and special technique in terms of providing information about the chemical interactions of the components in the mixture.

The FT-IR technique is also used for the characterization of functional groups of compounds in a plant extract mixture. However, it is not an adequate technique for identification alone. It is a complementary technique that can be used in conjunction with other methods (Sasidharan et al., 2011).

CONCLUCSION

When all the methods used in the purification of medicinal aromatic plants are evaluated, it is seen that the properties of the components and the purpose of producing the product are important in the selection of the method. In recent years, it can be said that modern methods have attracted more attention due to their advantages such as shorter time, less toxic waste and higher product purity. After obtaining the plant extracts with appropriate separation and purification methods, it is important to characterize them with appropriate techniques and to elucidate their structures. Different analysis techniques are used in the characterization of plant extracts. When the literature is examined in detail, it is seen that methods such as HPLC, GC-MS, ESI-MS, NMR and FT-IR are widely used in the characterization of plant extracts. Comparisons can be made by using one or a combination of these analysis techniques to determine the content of plant extracts. The purer the plant extracts can be obtained in terms of functional components, the easier it will be to identify the components.

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CHAPTER 11

EVALUATION OF ANTIOXIDANT ACTIVITY OF *Ferula communis L.* AND *Malva sylvestris*

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INTRODUCTION

The genus *Ferula* are from the Umbelliferae family (Yaqoob and Nawchoo, 2016). It is known that the genus *Ferula* has been used as a medicinal plant for many years. Its pharmacological properties in humans and animals have been demonstrated in numerous studies (Rahali et al., 2018) Previeous phytochemical investigations revealed that the *Ferula* genus contain active compenents like coumarin derivatives, sesquiterpene coumarins, aromatic lactones and phenylpropanoid compounds (Appendino et al., 1988; Miski and Jakupovic, 1990; Kojima et al., 2000; Isaka et al., 2001; Lahouel et al., 2007; Kasaian et al., 2014; Zhou et al., 2017). This genus have biological effects like antitumor, anti-inflammatory, antibacterial, antidiabetic, anticoagulant, mitochondrial dysfunction and antiviral activities (Appendino et al., 2004; Appendino et al., 2006; Monti et al., 2007; Gliszczyńska and Brodelius, 2011). Some species of the genus *Ferula* are also used in traditional medicine, but *F. communis* has been noted to be harmful to both people and animals (Marchi et al., 2003).

Malva sylvestris from the Malvaceae family is an annual herb with lobed, purple petals. This plant is also known as mallow. It can be said that this plant, which is widespread in Europe, Southwest Asia and North Africa, is found in moist environments such as salt marshes, meadows and riverbanks. The plant is very commonly used in herbal medicine. In Southwest Asia, it is also used as a vegetable. This herb is reportedly used to treat stomach problems, cystitis, bronchitis and other respiratory disorders and skin diseases (Billeter et al., 1991; Gasparetto et al., 2012). Studies have shown that plant anthocyanins cause a decrease in plasma total triglyceride and cholesterol levels. (Liu et al., 2016). The leaves of this plant are rich in mucilages and flavonoids, terpenoids, vitamins E and C, coumarins (Billeter et al., 1991; Farina et al., 1995; Cutillo et al., 2006; Barros et al., 2010; Najafi et al., 2017).

Hydrogen peroxide, superoxide radical and hydroxyl radical are oxygen-centric free radicals. They have the ability to oxidize proteins, lipids, and DNA, which causes cell death (Ames, 1983; Özsoy et al., 2008; Bhattacharya, 2011; Sathuvan et al., 2012; Halliwell and Gutteridge, 2015; Boran and Uğur, 2017; Caro et al., 2019). Phenols, which are abundant in medicinal and aromatic plants, defend the body from the negative effects of free radicals, thanks to their antioxidant properties (Do et al., 2004). From this perspective, it is crucial to find novel plants that have antioxidant properties.

In this work, 80% ethanol extracts of *Ferula communis* L. and *Malva sylvestris* were investigated for their antioxidant activities, total phenolic concentration and flavonoid content.

1. MATERIAL AND METHODS

The aerial parts of *Malva sylvestris* were collected from the Cumhuriyet University campus, while *Ferula communis L.* was collected from the natural area in Sivas. After the fresh plants were dried and the experiments were done in triplicate by the Pharmacognosy lab of pharmacy faculty at Sivas Cumhuriyet University in Sivas in 2020.

1.1. Preparation of extracts

A blender was used to grind dried plants (Bluehouse). A 10 g sample from the ground plant was placed in 50 ml of 80% ethanol (Sigma) and shaken periodically for 48 hours. The filter paper was used to filter the mixture. The final product was concentrated in triplicate under reduced pressure on a rotary evaporator at 35°C until completely dry.

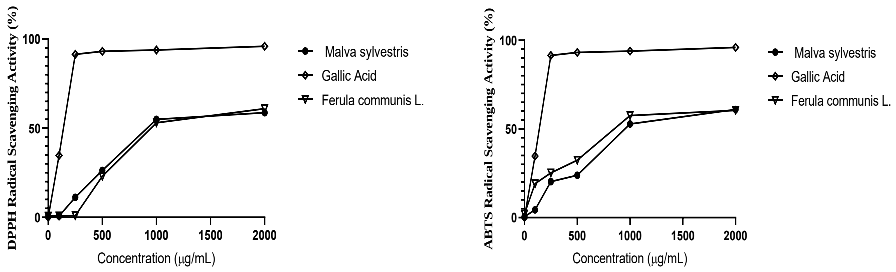
1.2. *In vitro* antioxidant activity

The DPPH radical scavenging activity (RSA) of the extracts was assessed using a slightly modified version of Blois' method from 1958, and the ABTS radical scavenging activity (RSA) was assessed using the method of Re et al. from 1999. Using the Folin-Ciocalteu spectrophotometric technique, the total phenolic substance in the extracts was calculated and stated as gallic acid equivalents (Clarke et al., 2013). The amount of total flavonoids was calculated using Molan and Mahdy's (2014) aluminum chloride colorimetric method,and the results were reported as milligrammes of catechin equivalent per gram of dry weight of the extracts.

2. DISCUSSION and CONCLUSIONS

In the present study, the antioxidant potential of %80 ethanol extracts of *Ferula communis L.* and *Malva sylvestris* were measured by two different biochemical assay on DPPH and ABTS radicals. As can be seen from Figure 1a and 1b, the DPPH and ABTS radical scavenging activity of *Ferula communis L.* and *Malva sylvestris* demonstrated concentration dependent manner in terms of RSA. However, the RSA was lower than the reference compound gallic acid at every concentration tested.

Phenolic compounds are known as the important secondary metabolites with strong antioxidant activity. When compared to each other, the total phenolic and flavonoid content of *Ferula communis L.* extract (251.08±0.5 mg GAE/g and 78.77±0.05mg QE/g, respetively) were higher than the extract of *Malva sylvestris* (140.82±0.04mg GAE/g and 49.23±0.06mg QE/g, respectively).



(a) (b)

Figure 1: DPPH (a) and ABTS (b) RSA of *Malva sylvestris* and *Ferula communis L.*

In a study conducted with the methanol extract of *F.communis* in 2018, the antioxidant activities of various organs of the plant were investigated using the ORAC method. As a result, it was reported that the flower organ of the plant has higher antioxidant capacity than other organs (Rahali et al., 2019). In another study in which different extracts (hexane, chloroform, ethanol, ethanol-water (50%)) of *F. communis* were prepared, it was reported that the antioxidant activity of the plant was not stronger than that of ascorbic acid determined by default (Aydın et al., 2021). Studies suggest that there is not yet sufficient and reliable data on the antioxidant capacity of this plant.

In the other study investigating the antioxidant activity of *Malva sylvestris* using the enzyme activities CAT, POD, AxPOD and SOD, it was reported that the enzyme activity CAT was high. The total phenolic content was determined as 5.34 (Kordalı et al., 2021). In the same year, a more comprehensive study was carried out, four different (hexane, methanol, water and dichloromethane) extracts of *M. sylvestris* were prepared and their antioxidant activities were investigated (DPPH assay), with the highest antioxidant activity in the water extract (IC_{50}: 60.61) compared to ascorbic acid used as standard. ± 0.07) was determined. The highest phenolic and falvonoid content was determined in the dichloromethane extract (73.31 ± 0.16 and 69.22 ± 0.25, respectively).

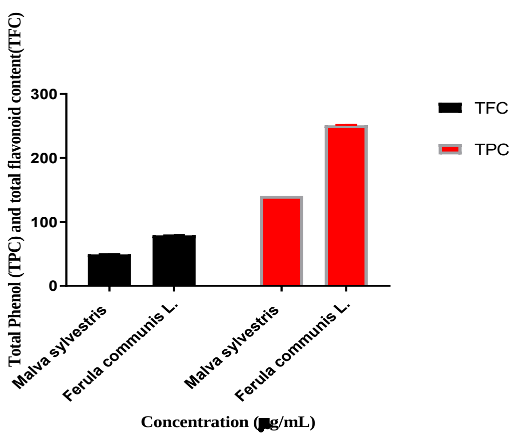


Figure 2: Total phenolic and flavonoid content of *Malva sylvestris* and *Ferula communis L.*

In conclusion, the plant *Malva sylvestris* and *Ferula communis L.* evaluated showed some level of antioxidant potential. The findings for *Ferula* here in shown indicate further phytochemical investigation need to identify the active compounds which are attributed for antioxidant. In general, it can be said that ethanol or aqueous etanol extracts containing strongly polar compounds are more effective.

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