T.R.
NİĞDE ÖMER HALİSDEMİR UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF PLANT PRODUCTIONS AND TECHNOLOGIES

GENETIC DIVERSITY AND BIOCHEMICAL CHARACTERIZATION OF COMMON PURSLANE (*PORTULACA OLERACEA* L.) POPULATIONS

MUHAMMAD YASIR NAEEM

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Doctor of Philosophy Thesis

Supervisor

Assoc. Prof. Dr. Khawar JABRAN

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The study titled "**Genetic Diversity and Biochemical Characterizatıon of Common Purslane (*Portulaca oleracea* L.) Populations**" is presented by Muhammad Yasir NAEEM under the supervision of Assoc. Prof. Dr. Khawar JABRAN and accepted as a Doctoral thesis by the jury at the Department of Plant Production and Technologies of the Niğde Ömer Halisdemir University, Graduate School of Natural and Applied Sciences.

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This thesis has been found appropriate at the date of ..../..../2023 by the jury mentioned above who have been designated by Board of Directors of Graduate School of Natural and Applied Sciences and has been confirmed with the resolution of Board of Directors dated …./…./2023 and numbered ……

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THESIS CERTIFICATION

To the best of my understanding and knowledge, I declare that the dissertation was written by me. The research given in this dissertation is all accurate and compliant with academic standards. In the dissertation, I've credited all the sources I utilized as well as any assistance I had while writing it.

Muhammad Yasir NAEEM

SUMMARY

GENETIC DIVERSITY AND BIOCHEMICAL CHARACTERIZATION OF COMMON PURSLANE (*PORTULACA OLERACEA* L.) POPULATIONS

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In addition to being a weedy and wild plant, common purslane (*Portulaca oleracea* L.) is also valued for its cooking and dietary benefits. The morphological, biochemical, and genetic variations of 25 accessions of common purslane were analyzed in the current investigation. The accessions' seeds were obtained from diverse areas around Turkiye. The study was repeated four times; twice for morphological data collection and twice for biochemical evaluation. The accessions BT-Altinkulak, Assem, and Yerli were found to have the maximum plant height, root fresh weight, and plant fresh weight. Accession Yagmur has a good total phenolic content (685.4 mg AEE 100^{-1}). Agr Tohum has the most anthocyanin concentration (1.09 g mL^{-1}). Cookies were made using a mixture of common purslane accessions and three distinct leaf powder proportions 10, 20, and 30 g were used. ISSR markers were employed to assess genetic variation. The number of recorded alleles, Polymorphic information content (PIC), and cluster analysis revealed no specific genetic variation in the collected common purslane accessions. The results demonstrated that common purslane accessions are an abundant supplier of numerous bioactive chemicals and have a multitude of morphological and biochemical features.

Keywords: Phytochemicals, weed, wild plants, phenolic compounds, product development.

ÖZET

SEMIZOTU (*PORTULACA OLERACEA* L.) POPÜLASYONLARININ GENETIK ÇEŞITLILIĞI VE BIYOKIMYASAL KARAKTERIZASYONU

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Semizotu hem yabani bir bitki hem de bir yabani ottur ve ayrıca yenilebilir ve besin değeri ile tanınır. Bu çalışma, 25 semizotu çeşidinin morfolojik ve biyokimyasal özelliklerini ve genetik çeşitlilik analizini belirlemiştir. Çeşit tohumları Türkiye'nin çeşitli yerlerinden toplanmıştır. Deney dört kez yapıldı; iki tekrarı morfolojik verilerin kaydı için, diğer ikisi ise biyokimyasal analiz için kullanıldı. Sonuçlar, BT-Altınkulak, Assem ve Yerli çeşitlerinin sırasıyla en yüksek bitki boyu, kök taze ağırlığı ve bitki taze ağırlığı için kaydedildiğini göstermiştir. Biyokimyasal karakterizasyon için en yüksek toplam fenolik madde içeriği Yağmur (685.4 mg AEE 100^{-1}) çeşidinde bulunmuştur. Antosiyanin en yüksek olarak Agr Tohum'da bulundu (1.09 µg mL^{-1}). Kurabiyeler, üç farklı yaprak tozu miktarı, yani 10 g, 20 g ve 30 g ile ortak semizotu girişlerinin bir karışımından hazırlandı. Kayıtlı alellerin sayısı, Polimorfik bilgi içeriği (PIC) ve küme analizi, toplanan ortak semizotu girişlerinde spesifik bir genetik varyasyon ortaya koymadı. Sonuçlar, yaygın semizotu girişlerinin çeşitli biyoaktif bileşikler açısından zengin bir kaynak olduğunu ve oldukça çeşitli morfolojik ve biyokimyasal özelliklere sahip olduğunu göstermiştir.

Anahtar Kelimeler: Fitokimyasallar, yabancı ot, yabani bitkiler, fenolik bileşikler, ürün geliştirme.

PREFACE

Common purslane (*Portulaca oleracea* L.) is a mid-summer plant with a broad geographical and ecological range and is a significant member of the Portulacaceae family. Common purslane is commonly presumed an unwanted plant, yet it is eaten as a vegetable in many regions. Common purslane has several traits that make it a challenging weed to manage, including the capacity for vegetative reproduction. Since antiquity, common purslane has been consumed and utilized as traditional medicine around the globe. It is recognized as highly nutritive due to its maximum amount of omega-3 fatty acids and an appropriate amount of vit. A and C, and antioxidants as well. Individuals who are prone to developing kidney stones shouldn't take it excessively because it also contains significant concentrations of oxalates (just like spinach does). It was also traditionally used as an ointment for burns and is occasionally administered to birds to lower egg cholesterol. Other common names for this plant are garden purslane, wild portulaca, small hogweed, and pusley.

Common purslane has the ability to thrive in a wide variety of soil types, ranging from dense clay to nutrient-rich organic soil when exposed to ample amounts of bright sunlight. It is simple to dig or hoe out undesired plants. For several years, seeds can survive in the soil. Common purslane takes 4 to 8 weeks to reach harvesting conditions. The plant can be taken whole, or the stems can be cut down to within two inches of the top, and the plant will regrow.

A slight bitter or tangy and salty flavour can be found in the stems, leaves, and flower buds. The physiology of the plant affects the flavour’s potency. Common purslane uses Crassulacean acid metabolism (C4) to conserve moisture in hot, dry circumstances. In this arrangement, the leaves gather carbon dioxide at night and transform it into malic acid instead of doing it during the day, when open stomata would enable water reserves to evaporate. The sour-tasting malic acid is then transformed into glucose and stored for use throughout the day. Therefore, the tartest-tasting leaves are those that are harvested earlier in the morning when malic acid levels are at their peak.

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SYMBOLS AND ABBREVIATIONS

Symbols	Descriptions
%	Percentage
℃	Celsius degree
µg	Microgram
µL	Microliter
µmol	Micromole
mmol	Milimole
cm	Centimeter
g	Gram
L	Liter
M	Molar
mg	Milligram
min	Minute
mL	Milliliter
mM	Millimolar
nm	Nanometer
rpm	Revolutions per minute
sec	Second
h	Hour
mm	millimeter

Abbreviations	Descriptions
PCR	Polymerase Chain Reaction
DNA	Deoxyribonucleic Acid
ddH2O	Double-distilled Water
DW	Dry Weight
FW	Fresh Weight
DW	Dry Weight
NaCl	Sodium Chloride
TE	Tris Ethylene Diamine Tetra Acetic Acid

bp	Base Pair
FAOSTAT	Food and Agriculture Organization Statistical
LSD	Least Significant Difference
H_2O_2	Hydrogen Peroxide
EDTA	Ethylenediaminetetraacetic Acid
TBE	Tris-borate-EDTA
MDA	Malondialdehyde
CAT	Catalase
SOD	Superoxide Dismutase
APX	Ascorbate Peroxidase
pH	Potential for Hydrogen
ROS	Reactive Oxygen Species
PCA	Principal Component Analysis

CHAPTER I

INTRODUCTION

The food industry's recent rapid development has limited our ability to consume a wide variety of edible plants, limiting our ability to eat a diverse and balanced diet. Plants have a wide range and quantities of vital nutrients that the human body is unable to provide on its own. Many plants include many factors that have been shown to protect the human body from ailments like malignancy and coronary heart disease and often assist in the development of different products. These defensive factors are termed nutraceutical compounds (Roy et al., 2019). Many findings show that several wild plants, typically not in the current diet, contain nutraceutical elements, helping the human body to prevent, defend and regulate several diseases currently commonly occurring (Åhlberg, 2021).

Natural herbs were utilized for a very long time to prevent and cure many ailments. Scientists have been looking for new pure natural elements for a long time. Synthetic medicines are only linked to a slew of negative adverse effects (Liao et al., 2022). Therefore, throughout the last several decades, researchers and scientists have been experimenting with the use of non-synthetic components from herbs, as well as green vegetables and fruits. Recent scientific evidence suggests that vegetables are significant producers. of different phytochemicals that can be exploited as a substantial source of new nutraceuticals in this respect. Plants that have a nutritional status like common purslane are well known for their high nutrient content, in particular, the presence in their leaves and stems of different nutrient components (Srivastava et al., 2021).

Lately, researchers have identified numerous nutritionally significant and underutilized wild crops for dietary diversification programs, including Indian spinach (*Basella alba, Basella rubra*), pearl millet (*Pennisetum glaucum*), amaranth (*Amaranthus viridis*), ground-cherry (*Physalis angulta*), winged bean (*Psophocarpus stetragono*) (Singh et al., 2018a; Singh and Abhilash, 2018; Singh and Abhilashm, 2019). Vitamins and other nutrients are abundant in these species. It's fascinating to notice that such untamed plants may survive brilliantly in scorching, humid tropical climates and are well adaptable to a variety of soil conditions (www.fao.org).

In this context, *Portulaca oleracea* L., often known as common purslane, can be employed since it has exceptional nutritional, therapeutic, pharmacological, and phytoremediation qualities.

This plant can be used commercially, mainly in developing nations where nutrient security is a concern, as it can replace other commonly used edible plant species and fulfill the majority of human nutritional needs while using very few resources and adaptable to changing soil conditions (even growing in harsh climatic conditions) (Srivastava et al., 2021). It is efficiently cultivable in a variety of climatic environments, where nutritious plants often do not flourish (El-Ramady et al., 2022).

An unhealthy or malnourished individual requires such nutrients promptly. Due to the obvious element of doubt between the old and young generations, this plant is not favored by the locals despite its numerous positive qualities. As a result, there is a need to reinvestigate this species for multiple advantages (Srivastava et al., 2021).

Common purslane (*Portulaca oleraceae* L.) is a mid-summer plant with, a slightly acidic and spinach-like taste, is a member of the Portulacaceae family and Portulaca genus which spread abundantly in temperate and tropical locations across the world. It is the 8th most widespread weed across the planet (Srivastava et al., 2021). The height of this plant can rise to 60 cm and has a fleshy, reddish-brown, and prostate-type branching stem. While the leaves are light green, fleshy, and oval-shaped, with many fibrous secondary roots (Srivastava et al., 2021). Common purslane flowers are mostly self-pollinated because no nectar is generated and just a few tiny insects visit the flowers. Common purslane may develop adventitious roots even though it lacks a specific structure for vegetative reproduction (Kumar et al., 2021). Common purslane is usually known by various names across the globe like rigla (Egypt), purslane (the USA and Australia), pigweed (England), pourpier (France), Semizotu (Turkiye), and kulfa saag (Pakistan). It yields up to 20 tons of fresh matter ha^{-1} (Ozturk et al., 2021).

According to the history of the common purslane, it was consumed in the 1st century A.D. and was also used as an antiseptic and vermifuges around the world (Srivastava et al., 2021). Common purslane is a major cultivated edible vegetable across the globe including Turkiye and other Mediterranean nations, in Southern Europe and Asia (Alu'datt et al.,

2019). Although the production of wild and cultivated common purslanes throughout Turkiye has reached around 5000 tons per year, East Marmara is the most commercial production area, with 3171 tons per year of manufactures (TUIK, 2010).

The global trend of producing medicinal plants and the desire for natural goods are rising. Therefore, common purslane has piqued the interest of the food and medicinal industries, as well as agriculturalists. It's used in salads, as cooked greens, and in soups, both fresh and dried. The stems, leaves, and flower buds are edible. This plant grows best in gardens, fertile fields, and row crops, although it may also be found in waste areas and along roads (Ozturk et al., 2021). Common purslane has been used for nourishment, animal chow, and for health care needs for a long time (Kamal-Uddin et al., 2020). According to numerous research, common purslane is highly valuable due to its nutritive, therapeutic, phytoremediation, and aesthetic qualities (Chugh et al., 2019; Petropoulos et al., 2019). It has a wide variety of anti-inflammatory, antioxidant, and injury-cure characteristics (Mattosinhos et al., 2022). The World Health Organization (WHO) lists it as a frequently utilized herbal plant, also given the title “Global Panacea” (Srivastava et al., 2021). Agriculturists and nutritionists must place a greater emphasis on purslane since it is rich in nutrients, particularly in nations where nutrition is a serious problem. Common purslane has long been used for nutraceutical applications by people across the world. Many ailments, including diarrhea and respiratory disorder, toothaches, obesity, snake bites, asthma, jaundice, ulcers, diabetes, and dysentery are treated using different aspects of the plant (Faruque et al., 2019; Mishra et al., 2020).

A dose of 9 to 15 μg of dry stem and leaves of common purslane is recommended for the treatment of dysentery, fever, diarrhea, eczema, and hematochezia (Mattosinhos et al., 2022). Common purslane has potent recommendations in Chinese Ethnomedicine for several illnesses (Chen et al., 2019). Consequently, several scholars from various nations have thoroughly established its usage as conventional healers (Kamal-Uddin et al., 2020; Mattosinhos et al., 2022; Mishra et al., 2020). Table 1.1 lists several purslane ethnomedical applications from throughout the world, including the components utilized. The ethnopharmacological common purslane consumption reports from 44 different nations are shown in Figure 1.1.

Table 1.1. Use of common purslane as an Ethno-medicinal in different countries.

S/No	Locations	Ethnopharmacological application	References
Europe			
1	Italy	Used as a treatment for kidney, stomach, intestinal, and headache discomfort.	Bosi et al., 2009
2	Greece	For inflammation treatment.	Brussell, 2004
3	Romania	Exterior baths for illnesses and weakened bodies.	Bosi et al., 2009
4	Spain	Upper area consumption for blood pressure control.	Carrió and Vallès, 2012
5	Turkiye	Diarrhea, diabetes, headaches, ulcers, urinary issues, wounds, and constipation are all treated with the leaves.	Sargin, 2021
Asia			
1	Pakistan	The leaves are utilized to treat scurvy, endothelial dysfunction, and nephrolithiasis. Drinking juice helps to purify the blood.	Kumar et al., 2021
2	China	It is used to treat diarrhea, inflammations, irregular uterine bleeding, hemorrhoids, skin disease, and dermatitis. It's also used to treat snake and bug bites.	Zhu et al., 2010
3	Iran	Common purslane roots, leaves, and seeds are used to treat diabetes.	Ghahramani et al., 2016
4	Bangladesh	To treat asthma and toothaches, use roasted seeds.	Faruque et al., 2019
5	India	Burning feelings, cough, and neurasthenia are treated with this Ayurvedic medication for disorders of the lungs, liver, kidney, bladder, and colon.	Prabhu et al., 2021
6	Afghanistan	Disorders of the mouth and diarrhea are treated with seeds.	Younos et al., 1987
7	Nepal	Scurvy, renal, and cardiovascular diseases can all be treated with leaves. Drinking juice helps to purify the bloodstream.	Singh et al., 2015
8	Sri Lanka	Ulcers, scrapes, wounds, and skin issues can all be treated using leaves.	Ranil et al., 2020
Gulf Countries			
1	UAE	Floral components are employed to lower temperature.	Iranshahy et al., 2017

2	Yemen	Leaf tea is utilized to treat stomach ache.	Hussein and Dhabe, 2018
3	Jordan	Seeds are utilized as aphrodisiacs and blood cleansers.	Lev and Amar, 2002
4	Egypt	used as a flavoring, a vegetable, and a source of herbals.	Sultana and Rahman, 2013

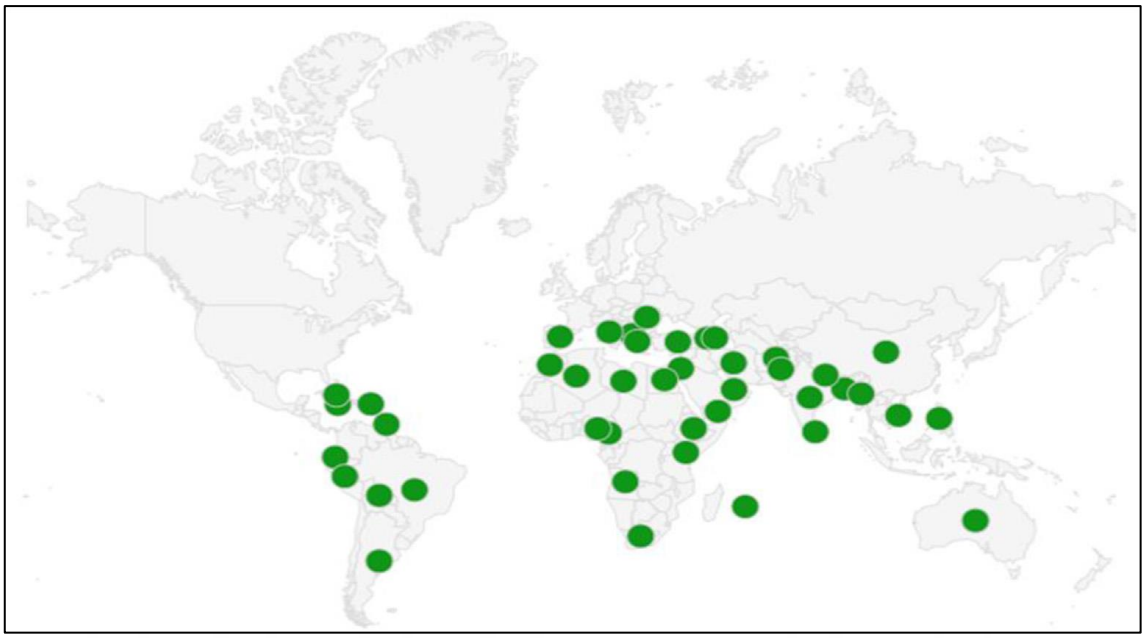

Figure 1.1. Map displaying common purslane as a conventional method of medicine remedy in 44 nations for various ailments around the globe.

Although this plant is not widely consumed, interest in its output has risen in the past several years due to research demonstrating its maximum level of fatty acids, in particular, α-linolenic acid and linoleic acid (Melilli et al., 2020; Gu et al., 2022). Common purslane, which grows wild, has the highest amount of fatty acid in its fresh leaves when compared to other common plants. The tremendous nutritional value of common purslane is noticeable when you compare the nutritional content of common purslane with other prominent vegetables, fruit, or seeds such as sunflower, avocado, olive, or flax (Table 1.2). The concentration of protein, carbohydrates, and fiber is particularly high. In addition, the fat content of common purslane is lower than that of other plants except for lettuce, particularly in comparison to sunflower, flax, and avocado (Srivastava et al., 2021).

Table 1.2. Nutrient contents estimated for 100g (FW) of plant tissue.

S/No	Crop	Fat	Protein	Fibre	Ash	Carbohydrate
1	Spinach	5.5	28	8	13	40
2	Lettuce	0	2.1	0.5	1.2	3
3	Sunflower	47	24	3.8	4	19
4	Avocado	12.7	2	6.7	1.6	8.5
5	Olive	22	1.6	0.1	0.1	19
6	Flax	35.5	19	6.8	3.5	35.4
7	Common purslane	4	26	11.5	20	50

It's been mentioned that common purslane is a plant with significant nutraceutical content that provides people with an excellent supply of nutrients and prevents numerous ailments (Nemzer et al., 2020). Although known as weed, in many countries' human ingestion of the common purslane was observed (Melilli et al., 2020; Gu et al., 2022). Common purslane is also high in antioxidants including vit. E and C, and other minerals are mentioned in Table 1.3.

Table 1.3. Content of vitamins and fatty acids of common purslane, sunflower, spinach, avocado, lettuce, olive, flax (mg $100g^{-1}$ FW).

S/No	Plants	Vit. A	Vit. B1	Vit. B2	Vit. C	α-Linolenic	Linoleic
1	Common purslane	1500	0.35	2.4	350	457	200
2	Sunflower	30	1.96	0.23	0	74	1200
3	Spinach	50	0.7	2	600	138	26
4	Avocado	3	0	0	17	110	1440
5	Lettuce	2200	0	0	15	0	0
6	Olive	0	0	0	0	64	810
7	Flax	0.03	0.17	0.16	0	280	55

The intake of fatty acids and antioxidants found in common purslanes, such as flavonoids, betalains, carotenoids, vitamin C, and polyphenols, has a significant influence on health.

These are said to prevent and treat many maladies, such as cardiovascular disease, endothelial dysfunction, hypertension (Nemzer et al., 2020), kidney problems (Chugh et al., 2019), rheumatoid arthritis disease, pneumonia, as well as other immune-related diseases. The phytochemicals are crucial to the growth, development, prevention, and maintenance of a healthy immune system for many cardiovascular illnesses (Iranshahy et al., 2017).

Genetic variation is crucial element of diversification and is correlated with the geographic dispersion of the population. The use of DNA-based molecular markers has various benefits for evaluating genetic variation. Inter-simple sequence repeats (ISSR) are a few of the easiest and most extensively utilized indicators among PCR-based markers techniques. According to the Mendelian hypothesis, two morphologies may be discriminated at each locus using ISSR markers, which are regarded as dominant markers (Tsumura et al., 1995). Ratnaparkhe et al. (1998) stated that ISSR markers might offer critical data for plant species' genome analyses. For this marker, genomic DNA is amplified by PCR using a primer that is commonly connected at the 5' or 3' end and has one to four random, frequently degenerate bases extending into the bordering regions (Joshi et al., 2000). When used on duplicates sample, ISSR markers have a high degree of reproducibility and can distinguish between closely related cultivars (Joshi et al., 2000). ISSR indicators were utilized for cultivar characterization, genetic variability evaluation and phylogeny investigations of distinct species, the discovery of DNA markers associated with agronomic features, and plant breeding (Singh et al., 2007).

Polymorphic bands are useful for studying genetic diversity and relatedness among individuals, populations, and species. Polymorphism refers to the presence of two or more alleles at a given locus within a population. The detection of polymorphic bands provides information on the variation in DNA sequences among individuals, which can be used to infer evolutionary relationships, estimate genetic diversity, and investigate gene flow (Gupta et al., 2003).

Dominant and codominant markers are two types of molecular markers that are widely used in various fields of biology, including genetics, genomics, and breeding. The main difference between the two is the way they express genetic variation (Tsumura et al., 1995).

Dominant markers are molecular markers that reveal the presence or absence of a particular allele or DNA fragment, without distinguishing between homozygous and heterozygous genotypes. In other words, dominant markers do not provide information about the genotype of the individual, only whether they possess a particular allele or not. For example, in a population of diploid organisms, a dominant marker may show the presence of a band on a gel indicating the presence of a particular allele, regardless of whether the individual is homozygous or heterozygous for that allele (Singh et al., 2007).

In contrast, codominant markers are molecular markers that reveal both the presence and the genotype of two different alleles. Codominant markers can distinguish between homozygous and heterozygous genotypes. For example, in a population of diploid organisms, a codominant marker may produce two different bands on a gel, indicating the presence of two different alleles and whether the individual is homozygous or heterozygous for each allele (Tsumura et al., 1995).

Therefore, this research aims to understand and emphasize the relevance of nutraceutical components in this wildly cultivated plant, known as the common purslane. To learn how to grow marketable common purslane, a novel edible weed with significant health advantages for humans, to assess the viability of producing high-quality common purslane for the fresh market, and to supply a green vegetable rich in nutraceutical components and antioxidants. This ubiquitous weed offers unique marketing options to enterprising producers.

1.1 Aims and Objectives

Several authors have measured the concentrations of numerous key compounds in common purslane. As per Ezekwe et al. (1999), Liu et al. (2000), Omara-Alwala et al. (1991), and Simopoulos et al. (1992) differed greatly. Differences in the levels of various nutraceutical components and antioxidants can be due to variations in experimental circumstances, sample materials, and analytical techniques (Palaniswamy et al., 2001). The goal of this study is to look at the genetic variation in common purslane accessions by comparing the number of nutraceutical chemicals found in the accessions that were collected.

The accessions with the utmost antioxidant content will be chosen for breeding and selection of common purslane as a newly cultivated vegetable. Common purslane, its appetizing stems, and foliage are constantly consumed, might be a viable candidate species for this purpose, as the market needs alternative sources for various natural antioxidants instead of synthetic ones. The evaluation of this study will give entrepreneurial farmers marketing options as well as the maximum nutritious components necessary for the human body.

Therefore, the objective of the project, in particular, will be:

1. To study the genetic variation of common purslane accessions collected from different geographical regions using ISSR markers.
2. Growth comparison of collected common purslane populations in greenhouse conditions to find out the fastest-growing population.
3. To evaluate the nutraceutical quality of common purslane by carrying out biochemical analysis (determination of total chlorophyll content, anthocyanin content, β-carotene content, total phenols analysis, total flavonoids, and isolation of alkaloids to find out populations with the best nutritional values.
4. Product development by using common purslane-dried powdered forms.

CHAPTER II

LITERATURE REVIEW

2.1 Common Purslane Characteristics and Description

Common purslane is a member of the Portulacaceae family of plants. It's a prostrate annual herb with a dense taproot and several fibrous secondary roots. Its prostate growth habitat produces a 60 cm-diameter mat per plant. The leaves are often succulent, and the stems are dark red, hairy, succulent, and fleshy. The yellow-petaled flowers are tiny. The circular lid on the spherical, many-seeded pods (globular pointed capsules) opens (Miyanishi and Cavers, 1980).

2.1.1 Habitat

The common purslane grows from the earth's surface to 12582 feet and is found all across the world. It flourishes in vineyards, agriculture fields, groomed regions, lawns, wayside canals, and other outdoor spaces (Mitich, 1997).

2.1.2 Temperature

The growing season for common purslane is restricted to the hottest months of the year (June to September). The seed needs moderate germination temperatures optimally higher than 30ºC and exceedingly low lower than 24ºC. Chilling temperatures destroy plants because they are vulnerable to frost. On the other hand, common purslane seeds can thrive in temperatures as low as -30ºC in the winter and as high as 50ºC in the summer (Miyanishi and Cavers, 1980). Common purslane flower growth is influenced by light intensity and temperature.

2.1.3 Pollination

The flowers of the common purslane are normally self-pollinated, so no nectar is produced and only a few small insects visit the flowers. Common purslane has the

potential to grow adventitious roots even though it lacks a particular sequence for asexual reproduction (Miyanishi and Cavers, 1980).

2.1.4 Vegetative Reproduction

Proctor et al., 2011 stated that this plant is a common summer herb. It can renew vegetative propagation by forming unintentional roots from cutting of stem at the cut end of the stem. After 21 days, a greenhouse study evaluated the survival and growth of three cuts from two stem sites, as well as a leaf from one stem position. Cuttings contained a stem node with or without leaves, a stem internode, all from the intermediate and distant location of a stem with relation to the root core, and a leaflet from a node near the basal stem. The cutting's position on the main stem had no bearing on life. The newest leaves were formed via cuttings with connected leaves and only nodded cuttings produced new leaves. Common purslane may generate new plants from either root sections or vegetative parts following disturbances such as hoeing or cultivation. The capacity of the plant to generate new plants after cultivation and hoeing makes it difficult to manage the plant using non-chemical, mechanical methods.

2.1.5 Common Purslane Global Variation and Dispersion

Rashed et al., 2003 mentioned that common purslane was reportedly a common Roman vegetable. Common purslane's origin is unknown; however, it has been believed to have existed for over 4,000 years. Common purslane's succulent stems and squishy leaves suggest that it originated in India and the Middle East, where it adapted to arid conditions.

The origin of common purslane is a point of contention among scientists. Several sources claim that it is indigenous to South America (Rydberg, 1932), North Africa (Holm et al., 1977), western Asia, and Europe (Mitich, 1997).

Caballero-Salazar et al., 2002 stated that it was originally discovered in the United States in Massachusetts in 1672. Because the plant has a milky liquid, The Latin words "oleracea" and "Porto," which both mean "pertaining to kitchen gardens," are also said to have contributed to the name "portulaca," which means "to carry" and "lac," which means

"milk" alluding to its usage as a green vegetable. Since the time of the ancient Egyptians, this herb has been consumed as a vegetable, spice, and medicine. During the Medieval Era, it was also widely utilized in Britain.

According to Simopoulos, 2004 common purslane is a weed all over the world, although it's primarily grown as a leafy vegetable in Europe, Asia, and Mexico. It's used in salads, as cooked greens, and in soups, both fresh and dried. Along with all plant components, the stems, leaves, and flower buds are edible. Common purslane is grown and used throughout the world, mostly in Mediterranean nations. Most individuals in modern civilizations, on the other hand, eat only a few cultivated vegetables. Nonetheless, manufacturing and usage are restricted.

Common purslane is a plant that may be found all over the world. This weedy plant infests vegetable gardens, barren spaces, lawns, decorative plants, and agricultural many areas of the globe. It grows in the warm, humid conditions that irrigated agricultural and ornamental areas. Early spring is the optimum time to sow the seed, which may then be put out during late spring (Simopoulos, 2004).

Holm et al., 1991 mentioned that common purslane can be found in both temperate and tropical climates. This plant grows well in gardens, green fields, and row crops, but it can also be found in waste areas and all along the roads. While common purslane is measured as a weed across the world, it is primarily used as a leafy vegetable in Asia, Mexico, and Europe. It is consumed as both fresh and dried in salads, as fried greens, and in soups as well. Its flower, leaves, and roots are comestible.

2.2 Common Purslane Nutritional Content

Simopoulos, 2004 stated that although this plant is not widely consumed, interest in its cultivation has grown in recent years. It has a moderately high protein, starch, and fiber content in total. Common purslane's nutritional value is apparent when compared to the nutritional quality of other popular vegetables, fruits, or seeds such as sunflower, avocado, olives, or flax. Common purslane also has lower fat content than all of the plants except lettuce, particularly when compared to sunflower, flax, and avocado.

According to studies by Simopoulos, 1995, it is rich in fatty acids. Common purslane which grows wild has the highest amount of fatty acid in its fresh leaves as opposed to other typical edible plants including borage (*Borago officinalis* L.), sorrel (*Rumex acetosa* L.), beet greens (*Beta vulgaris* L.), spinach (*Spinacia oleracea* L.), buttercrunch lettuce (*Lactuca sativa* L.) and mustard greens (*Brassica juncea* L.).

Kamal et al., 2014 studied common purslane as a weedy plant that grows naturally in grasslands and farm crops. It contains gelatinous texture substances that have medicinal value. It is high in potassium, magnesium, and calcium. It contains a high amount of alpha-linolenic acid and gamma-linolenic acid (4 mg g^{-1} fresh weight (FW)) of omega-3 fatty acids in any leafy green vegetable. It had the most alpha-tocopherol (22 mg and 130 mg 100 g^{-1} of FW and dry weight (DW)) as well as ascorbic acid (27 mg and 506 mg 100 g^{-1}FW and DW). Common purslane leaves have a high oxalate content. Common purslane's antioxidant content and nutritional value have been confirmed to be 671–870 mg 100 g^{-1} FW. It emerged that this herb has a lot of nutritional potentials and that it may be used in the future.

Okafor et al., 2014 mentioned that the common purslane is a nutritious herb that can be eaten. Common purslane's chemical structure, fatty acids research, phenolic compound, and radical scavenging behaviour were all investigated in their study. Additionally, the impact of common purslane cooking at various temp (60, 80, and 100ºC), for different times (5, 10, 15, and 20 min) was investigated. After reducing the oxalate content of biscuits fortified with common purslane powder, a sensory assessment of consistency characteristics and fatty acid analysis were performed. Common purslane contained 5.64, 5.30, 23.42, and 16.03% protein, fat, ash, and fiber, on a dry matter basis. The mineral with the most abundant was K (4694 mg $100g^{-1}$), and the mineral with the minimal quantity was Zn (0.94 mg). Common purslane had 193 mg $100g^{-1}$ and 87 % total phenolic. The majority of the fatty acids in common purslane (40.4%) are unsaturated fatty acids, particularly linolenic acid. On the other hand, common purslane boiled for 20 min at 60, 80, and 100ºC exhibited solubility fractions of total oxalate contents that were, respectively, 34.65, 19.84, and 15.84%. During therapies, biscuits were fortified with common purslane powder, which improved the product's consistency characteristics. Common purslane may be used in nutritional foods and nutraceuticals as a beneficial source of vitamins, minerals, and omega-3 fatty acids.

Petropoulos et al., 2015 stated that the common purslane is an annual herb that is eaten for its nutritious leaves and stems and is high in omega-3 fatty acids. The nutritional value and chemical composition of the food is determined by genotype. Genotype D has the lowest oxalic acid content, while genotypes E and F, which have medium oxalic acid content, are more promising for commercial cultivation. Omega-3 and omega-6 fatty acid ratios were well-balanced in genotype E, and it also had strong antioxidant potential. When compared to the other genotypes, genotype A had the largest yield.

In 2009 Aberoumand explained that the mineral components of common purslane stem and leaves have been studied. They comprised 23% ashes, 23% protein, 5% fat, 8.0% fiber, and 41% carbs. Mineral concentrations (100 mg g^{-1} DW) were as follows: K (15), Na (7), Ca (19), Fe (0.5), and Zn (0.5). The results showed that common purslane provided more minerals than the recommended dietary requirements when the mineral compositions of the leaves and stems were compared.

2.2.1 Common Purslane Impact on Health

Dkhil et al. in 2011 mentioned that the intake of fatty acids and antioxidants contained in common purslanes, such as flavonoids, betalains, carotenoids, vitamin C, and polyphenols, has a significant effect on human health. Several disorders, including coronary heart disease, kidney disease, stroke, renal disease, chronic obstructive pulmonary disease, and other inflammatory and autoimmune diseases, are said to be prevented and treated by common purslane.

Palaniswamy et al., 2004 stated that this edible plant (common purslane) is high in nutrients; it also contains a significant amount of oxalic acid when consumed in high concentration. The presence of this acid has several harmful effects on humans, including kidney stones, hyposidermia, and reduced iron and calcium levels in the blood. The degree to which oxalic acid-rich foods pose a health risk ranges from person to person. Individuals that are more sensitive to oxalates, such as those with kidney disease, gout, rheumatoid arthritis, or certain types of chronic pain, should limit their oxalic acid consumption.

Rahimi et al., 2019 stated that the common purslane is a grassy annual plant. Several of the therapeutic actions of common purslane include an anesthetic, antimicrobial, muscle tissue relaxation, wound healer, anti-inflammatory, and radical's protector. The immunomodulatory, anti-oxidant, and anti-tumor functions of the common purslane are the subject of this review paper. common purslane's immunomodulatory, antioxidant, and anti-tumor properties were researched until the end of August 2018 using different databases. Common purslane works to reduce inflammation and, under some circumstances, to balance the immune system's innate and adaptive systems. Common purslane functions as an immune modulator and antioxidant in inflammatory states characterized by a dominant Th2 reaction, including atopic dermatitis, cancer, and triggered Th1 illnesses like hepatitis and sclerosis.

Jingrong and Ramesh, 2009 mentioned that the common purslane is a Chinese medicinal herb that has been used as a home cure for some illnesses. Common purslane extracts compounds were tested using thin-layer chromatography, and counteracting kinetic stress was assessed using a forced swimming exercise. Common purslane extracts significantly reduced kinetic fatigue in mice, increasing overall swimming potential by boosting the use of fat while delaying the ammonia and lactate deposits in the plasma.

2.3 Phytochemical and Pharmacological Profile of Common Purslane

Agha-Hosseini et al. (2010) stated that common purslane is also high in antioxidants including vitamin E, vitamin C, β-carotene, and glutathione. A 100 g fresh common purslane leaves have fatty acids between 200-460 mg, and a high amount of flavonoid, β-carotene, betaxanthin, glutamine, methionine, folic acid, vitamins A and C along with high levels of alpha-tocopherol.

Dkhil et al. in (2011) explained the antioxidant properties of common purslane. He claims that mature male Waster albino rats were used to investigate the antioxidant capacity of common purslane aqueous juice. Rat liver, kidney, and testis were examined for reduced glutathione, and catalase, as well as for the inhibition of lipid oxidation and nitric oxide. The role of the liver and kidneys was also assessed. Administering purslane aqueous juice to rats resulted in significant improvements in all examined parameters. The results suggest that common purslane may be a natural compound that is advantageous in the

protection of cardiac, neurologic, and certain other chronic disorders brought on by oxidative stress.

Chugh et al. (2019) mentioned that common purslane is regarded as the initial vegetable ever eaten by people and has traditionally been used in western cuisine and herbal remedies. It has been labelled a "future superfood" because of its abundance in vitamins, flavonoids, alkaloids, polysaccharides, omega-3 fatty acids (particularly alpha- and gamma-linolenic acids), terpenoids, sterols, proteins, and minerals. Common purslane has been confirmed to have potent pharmaceutical actions such as hepatoprotective, neuroprotective, anti-inflammatory, antimicrobial, antidiabetic, antioxidant, anticancer, and antihypertensive actions due to its diverse phytoconstituents.

Okafor and Ezejindu, 2014 stated that common purslane is utilized for medical and wellness purposes across the globe, demanding an investigation into its phytochemicals. For this analysis, upper portions of common purslane were harvested, air-dried, and powdered. To examine the phytoconstituents, standard procedures were employed to execute chemical assays on the aqueous leaf extract and powdered material. The presence of alkaloids, saponins, tannins, flavonoids, cardiac-glycosides, terpenoids, glucocorticoids, proteins, and starch were determined qualitatively, and the presence of flavonoid, tannin, alkaloid, and saponin was measured quantitatively.

Almashad et al., 2019 described the common purslane as a vegetable with a variety of uses in both the healthcare and food industries. Fatty acids and volatile compounds in fresh leaves were also measured. Saponin and alkaloid concentrations were the largest phytochemicals contained in both common purslane leaf extracts. In comparison to the aqueous extract, the ethanol extract of the leaves had the largest percentage of tannins (3.15%). (2.65%). In comparison to the aqueous extract, the ethanol extract has the largest saponin content 4.98%. While the ethanol extract was more effective in phytochemicals such as tannins and saponins, both extracts were virtually identical in phenolic and flavonoid extracts. Pyrogallol was the dominant phenolic compound in ethanol and aqueous extracts, with concentrations of 24.85 and 23.65%, respectively, according to HPLC research. Common purslane water extract, on the other hand, had elevated levels of pyrogallol, chlorogenic, and salicylic flowed by, as well as catechin, rosmarinic, vanillic, and rutin. The antioxidant activity of the ethanolic extract of common purslane

leaves was high. -linolenic acid (51.72%), palmitic acid (24.03%), and linoleic acid were the most concentrated fatty acids in common purslane leaves (9.46%).

Chen et al. in (2019) stated that common purslane, a plant that is both edible and therapeutic is widespread across China. Numerous chemical constituents in common purslane have anti-inflammatory, anti-cancer, hypoglycaemic, antioxidant, and anticancer effects. The evaluation of common purslane included consideration of its botanical resources, ethnopharmacological activity, chemical makeup, pharmacological and pharmacokinetic, safety assessment and toxicity, and clinical applications.

2.3.1 Chemical and Remedial Effects of Common Purslane

Hassan et al., 2014 described that the common purslane is considered one of Egypt's most significant unknown plants. Common purslane dry powder and leaf extract of the young plant were examined for their constituents. When rats with toxic hepatitis were fed aqueous extract instead of silymarin medication, the serum concentration of hepatic enzyme, nitric oxide, uric acid, lipid, and liver levels did not rise. The findings of anti-cancer activity revealed that plant dry powder (0.547 HEPG2 liver cell) had the largest HEPG2 dead cell proportion (0.6) at a level of 12 g. The number of dead cells rose to 0.7 at a level of 100 g, and the cytotoxic impact was assessed using Ic50 values of 17 g in the cell line.

Almasoud and Eman, 2014 revealed that common purslane was referred to be "a potent food" in the future because of its nutritional and antioxidant content. The concentration of Common purslane is high (179.89 mg $100gm^{-1}$) as well. Scavenging activity is intense (RSA) (89.23%). The fattiest acid in common purslane was linolenic acid (44.29%). Therefore, crackers were strengthened by three amounts of common purslane powder that have been previously prepared in the current report (5, 10, and 15%). The samples analyzed for crackers were examined for physical and sensory characteristics. Through the inclusion of a bucket, the quality of protein, ash, and fiber from crackers has improved considerably with the number of buckets. Adding the common purslane also allowed the content of zinc and calcium to be increased to nearly 2 plants and the content of iron to three plates. Through an increase in common purslane, the overall phenol content of the

reinforced crackers has risen. A 15% rise in the number of antioxidants was achieved in comparison to 20.6% in the regulation of 51.25%.

Dabbou et al., 2020 explained about the common purslane, is an herb with a wide range of therapeutic applications owing to its elevated amounts of phytochemical compounds. However, few experiments on the biochemical characterization of Common purslane have been performed to date.

Dabbou et al., 2020 stated that Tunisia is a potential origin country, as are other North African nations. The proximal composition, pigmentation, and volatiles of the samples were analysed, and the phenolic component content and in vitro tests of the ethanolic and aqueous extracts were studied. While leaves had higher chlorophyll concentrations, the stem exhibited more moisture (89.9%) and anthocyanins (4.61 mg g^{-1} dry matter) (7.42 mg g^{-1}). Ethanolic extracts exhibited considerably larger quantities of phenolic compounds and antioxidant capabilities than water extracts, regardless of the plant component under study (p 0.05).

2.4 Genetic Description

Alam et al. (2015) clarified that genetic variation and links between 45 common accessions obtained using SSR markers have been assessed. There were 167 bands on the 28 primers, 163 of which were polymorphic (97.6%). Shannon's data index measured the genetic variation to be 0.513, which reveals a very large amount of genetic diversity within the germplasm. The media of alleles, efficient allele, heterosity, PICs, and NEI's indexes were 6, 1.6, 0.4, 0.4, and 0.4. The whole set of alleles was 0.25. Dependent on Nei's genetic gap the UPGMA dendrogram divided the entire germplasm into 7 separate clusters. A molecular variance analysis (AMOVA) showed that 89% of the total difference was found in the population and 11% between populations.

Ren et al. (2011) explained that drought stress is a natural occurrence that causes significant production loss in agriculture. Finding additional genetic resources is critical for enhancing crop drought resistance. Common purslane is a xerophyte that thrives in extremely dry environments all over the world. They provide genetic variance in drought tolerance traits as well as AFLP-based genetic diversity in purslane accessions obtained

from geographically different locations. To evaluate the genetic variability of common purslane accessions, AFLP fingerprinting was employed. UPGMA-cluster analysis identified four different groups using average genetic similarity as a cut-off value, and Tokombiya was determined to be unique from all other purslane accessions. These findings show that there are considerable genetic differences across purslane accessions and that Tokombiya is a unique accession with high drought resistance. Further research into this accession might aid attempts to increase agricultural drought resistance.

Mohebodini and Fathi, 2020 stated that using PCR-based markers, diversity analysis at the molecular level is an inexpensive and fast way to classify genotype relationships and variations. In the investigation, ISSR markers were used to assess the genetic relationships and diversity among 20 common purslane accessions. The genotyping data were used to figure out how the collected accessions were related and to find genetically diverse common purslane accessions. There were 92 bands overall from the 25 primers, 62 of which were polymorphic (67.4%). Shannon's knowledge index measured genetic variation to be 0.55, demonstrating that the germplasm has a high level of genetic variation. On average, the polymorphic information content (PIC) and index of the Nei are 2, 1.65, 0.37, and 0.37, respectively,

Jia and his colleagues 2017 stated that ornamental common purslanes are annual plants. So yet, the genetic characterization of common purslane species has received little public attention. They examine the genetic variability and relationships of a group of ornamental common purslanes from *Portulaca umbraticola* and *P. grandiflora* growing in China using SRAP markers. According to the genotyping, an average of 16.31 informative fragments were produced by each of the 16 SRAP primer combinations, for a total of 261 informative fragments.

According to Tatikonda et al. (2009), amplified fragment length polymorphism was used to evaluate the top germplasm pool of Jatropha curcas, which has recently gained tremendous relevance as a bioenergy plant both in India and numerous other regions (AFLP). Using seven distinct AFLP primer combinations, 48 accessions obtained from six different Indian states resulted in a total of 770 fragments. The RP was acknowledged as the genuine attribute for AFLP to assess the discriminating potential of the primer

despite the PIC values ranging from 0.20 to 0.3 with an average of 0.27 per primer combination and the MI values ranging from 17 to 32.3 with an average of 25 per primer combination. The RP value varies from 23.1 to 46.9, with an average of 35. To choose the best accessions for plant improvement using both traditional and molecular breeding approaches, the estimated genetic diversity in the current work together with databases on other morphological/agronomic features would be very helpful.

D'Andrea et al., in 2015 stated that common purslane-rich sources of fatty acids as well as other substances of potential nutritional value. It makes a suitable candidate for studies on abiotic stress resistance mechanisms since it is well-grown in dry, arid regions, and well-watered environments. It can endure extreme drought and then recover after being rehydrated. Using differential display and qRT-PCR, the transcriptome level response to drought and subsequent recovery were evaluated. Several abiotic stressors were used to test a subset of common purslane genes. Several proteins, lipid metabolism, and stress-related genes have altered expression in water stress and recovered plants. Numerous unidentified genes parameters and genes that react to different abiotic stimuli were also found. It is investigated whether candidate genes may be used to improve drought resistance and recovery.

Egea-Gilabert et al. in 2014 stated about molecular, morphological, agronomic, and biochemical studies were used to assess the appropriateness of twelve common purslane accessions. A scanning microscope was used to classify the accessions according to their seed coat features. The ISSR method was used to do the molecular study, this produced 141 DNA bands, of which 85 varied across accessions. With a Jaccard's genetic similarity value of 0.32, a dendrogram constructed from a comparison vector using the UPGMA method indicated that the 60 common purslane plant samples could be divided into two main clusters. Due to the substantial inter-population variability identified for the researched variables employed, a study of the phenotypic, crop yield, and biological aspects proved to be a less successful approach to identifying the accessions. CM 13-00809 had the best yield, highest dry matter content, highest potassium level, lowest oxalate level, and lovely green stem and leaf color.

2.5 Notion of Molecular Markers

The variation in DNA nucleotide sequence between distinct animals or species that is close to or intricately related to a target gene that exhibits a characteristic is known as a molecular or DNA marker. Usually, the tightly coupled molecular marker and the target gene-expressed phenotype, or biological function are transmitted simultaneously (Collard et al., 2005). Although the genes involved may be known or unknown, and regardless of whether the gene(s) can be detected or not, the close affiliation of molecular markers to a trait or gene of a specific biological feature ends up making the markers represent practical signs or flags that signal a particular gene locus and aid in the detection or identification of the associated traits (Reddy et al., 2021). If changes known as variants occur in the indicator nucleotide sequences across or among individuals or species, DNA markers may be useful for identifying individual genotypic variances in the same or distinct species. Molecular marker polymorphisms result from a variety of DNA mutations that alter the nucleotide sequence of one or more species (Mandal et al., 2018).

Based on the detection approach, DNA markers are divided into three classes: hybridization, polymerase chain reaction (PCR), and DNA sequence-dependent markers. Cary Mullis created the PCR method for the mass amplification of a small DNA segment in 1986. The automation of PCR that followed (Mullis et al., 1986) represented a significant technical advance in the field of the genome and molecular biology research. Since then, plant molecular breeders have found PCR to be a highly helpful technology for the generation and study of DNA markers. Taq DNA polymerase quality and type are crucial factors to take into account for effective product amplification in any PCR-based marker system (Moazen et al., 2012).

Agarwal et al., 2008 stated that biomarkers may or may not correspond with the phenotypic manifestation of a genetic characteristic. They have several benefits over traditional, phenotypic expression approaches since they are persistent and observable in all sections independent of the cell's growth, diversification, development, or defensive state. Moreover, they are not muddled by environmental, pleiotropic, or epistatic effects. Any ideal biomarker ought to have the specific traits: (1) be variable and distributed evenly all across the genome; (2) facilitate sufficient settlement of genetic variations; (3) create various, impartial, and dependable markers; (4) be simple, quick, and reasonably priced; (5) require minimal quantities of tissue and DNA samples; (6) connect to distinguishable phenotypes; and, (7) necessitate no prior knowledge of an organism's

genome. All of the aforementioned benefits are not, however, offered by every molecular marker.

According to Luz et al., 2020 ISSR (Inter-Simple Sequence Repeat) markers are a type of molecular marker that amplify the DNA segments between microsatellite repeats, using a single universal primer that anneals to the microsatellite repeats. ISSR markers are highly polymorphic and can detect a large number of alleles within a population, making them useful for studying genetic diversity and relatedness. ISSR markers are dominant, meaning that they provide information about the presence or absence of a particular allele, but not about the heterozygosity or homozygosity at a specific locus. ISSR markers are cost-effective compared to other molecular markers, such as SSR and SNP markers. These markers can be analysed using gel-based or capillary electrophoresis methods and can be scored visually, making them useful for researchers who do not have access to advanced genetic analysis software.

Soliman et al., 2014 mentioned that ISSR markers have high reproducibility, which allows for consistent results across different laboratories. ISSR markers can be used in high-throughput genotyping assays, which allows for the analysis of large numbers of samples in a short amount of time. Furthermore, ISSR markers are versatile, being applicable in a wide range of genetic studies, including population genetics, phylogenetics, and molecular breeding.

ISSR markers can be used to study genetic structure, identify specific cultivars or varieties of crops or trees, and are stable, allowing for the study of genetic variation over time. These markers have been used in a range of applications, including the study of the genetic basis of disease resistance in plants and animals, the genetic basis of behaviour and other complex traits, and forensic science.

Overall, ISSR markers are valuable tools for studying genetic diversity, relatedness, and structure within and among populations, as well as for studying the genetic basis of various traits in different species.

2.6 Inter Simple Sequence Repeat (ISSR) Markers

ISSRs are genomic sites that are surrounded by microsatellites or simple sequence repeat sequences. Microsatellite sequences are genome areas that are made up of small, repetitive DNA sequences that are often di-, tri-, tetra-, or pentanucleotide in size. ISSRs are widely present and dispersed at random across the genome. The ISSR marker method is based on the creation of multi-locus markers by the PCR amplification of genomic areas bordered at each end by similar parts of the microsatellites repeat that are orientated in the opposite direction (Sarwat et al., 2016). The ISSR-PCR reaction is a single-primer PCR amplification, in contrast with numerous additional marker systems (Sharafi et al., 2017).

Targeting genomic microsatellite regions, ISSR primers are typically lengthy, between 15 and 30 bases in length, and made up of repeating simple DNA sequences. Based on the ISSR primer melting point, ISSR-PCR is often conducted at a high annealing temperature of 45 to 60°C. Using ISSR primers that are challenging to tune using a conventional PCR approach, it is helpful to have a touch-down PCR reaction profile that covers this range of annealing temperatures (45-60°C) (Yadav et al., 2019). The ISSR multi-locus markers are made up of 10 to 60 fragments from several loci during PCR, on average. Moreover, the ISSR PCR products had a range of 100 to 3000 bps, illustrating the variance in distance between adjacent and oppositely oriented microsatellite areas (Sujata et al., 2012).

The ISSR-PCR DNA products are electrophoresed in agarose or polyacrylamide gel and profiled before being seen under UV light. Depending on certain sizes, the bands are classified as present or missing. ISSR markers are referred to as dominant markers and are highly polymorphic (Yadav et al., 2019). The fundamental benefit of ISSRs is that primer sequences may be determined without any prior knowledge of DNA data. Moreover, using a little DNA in ISSR analytical processes is made possible by the use of PCR. Typically, 10–50 ng of DNA of high quality are used for each assay. Most of the practical restrictions of SSR, AFLP, and RAPD analysis are avoided by ISSR marker applications (Padmakar et al., 2015; Ebrahimi et al., 2015). In investigations of genetic variation and phylogenetic, genomic identification, and evolutionary biology, ISSR has

been used to generate helpful data in strongly linked or remote species (Thakur et al., 2016).

2.7 ISSR vs SSR Markers

SSR (Simple Sequence Repeats) and ISSR (Inter-Simple Sequence Repeats) are two types of molecular markers commonly used in genetic studies. Here's a description of each marker type:

SSR markers are based on short tandem repeat sequences that occur in the genome. This tandem repeats typically consist of 1-6 base pairs, which are repeated multiple times within a particular locus. SSR markers are useful in identifying genetic variations in a particular individual or population (Yadav et al., 2019). The repeat sequences are highly variable and can be used to construct genetic maps, conduct population genetics studies, and identify different genetic variations. These markers are widely used in a variety of plant and animal species, including humans. SSR markers have high levels of polymorphism and are highly reproducible. They are useful for the study of different populations due to their high resolution and sensitivity (Thakur et al., 2016).

ISSR markers are based on the amplification of regions between microsatellites using primers anchored in these regions. ISSR markers are highly polymorphic and can be used to generate genetic fingerprints for the identification of different populations (Ebrahimi et al., 2015). ISSR markers are widely used in plant breeding and have been useful in detecting genetic variations in plants, including hybridization and mutation events. ISSR markers are highly reproducible, which makes them ideal for different applications (Sujata et al., 2012).

ISSR (Inter-Simple Sequence Repeat) markers have several advantages over SSR (Simple Sequence Repeat) markers, making them a preferred choice in certain applications. Here are some advantages of ISSR markers over SSR markers with references:

No need for prior genomic sequence information: ISSR markers do not require prior knowledge of the genome sequence, as they are designed to amplify regions between microsatellite sequences. This makes ISSR markers useful for studying organisms with

limited genomic information or for identifying new microsatellite regions in a genome (Shivani et al., 2014).

High level of polymorphism: ISSR markers have a high level of polymorphism due to the amplification of regions between microsatellites that are more variable than the microsatellite regions themselves. This makes ISSR markers useful for studying genetic diversity, genetic relationships, and population genetics (Abdulmajeed et al., 2018).

Low cost and high throughput: ISSR markers require fewer primers than SSR markers, reducing the cost of the experiment. Additionally, ISSR markers can be multiplexed to analyse multiple loci simultaneously, further reducing the cost and increasing the throughput (Hizem et al., 2013).

Less sensitivity to DNA quality: ISSR markers are less sensitive to DNA quality than SSR markers, as they can amplify regions with more variation in DNA quality. This makes ISSR markers useful for analysing degraded DNA samples or samples with low DNA quantity (Yavari et al., 2019).

Overall, ISSR markers are a useful tool in genetic studies, particularly in organisms with limited genomic information, where high levels of polymorphism are required, and in studies involving degraded or low-quality DNA samples.

2.8 Product Development

Mastud et al. (2018) described how to make common purslane powder from popular common purslane for use in a variety of products. In addition to sating hunger and supplying vital nutrients for people, food is also created to prevent diseases. Functional foods play a significant role in this respect. Common purslane is a vegetable and an herb that is used for medicinal and therapeutic uses all over the world. Common purslane powder is made by drying it in a tray at 60°C. Common purslane powder was prepared and analyzed chemically. Common purslane powder was applied to ice cream and cookies in quantities of 1, 2, 3 g, and 2, 4, 6, and 8 g, respectively. According to the findings, common purslane powder, which is often used in ice cream and cookies, provides a large amount of protein and antioxidants with possible health benefits and nutritional

properties. Sensory analyses of all existing ice cream and cookie formulations were also conducted, and it was discovered that 2 g of common purslane powder in ice cream and 6 g of common purslane powder in cookie formulations were appropriate, demonstrating improved nutritional and physicochemical properties.

Almasoud and Saleem, 2014 explained that common purslane has been termed a "future power food" because of its high nutritional and antioxidants. Common purslane has maximum phenol concentration and strong radical scavenging activity. Linolenic acid was the most abundant fatty acid in this plant. As a result, three different common purslane powder dosages (5, 10, and 15%) were used to fortify wafers with common purslane. The physicochemical parameters and sensory qualities of the cracker samples examined were assessed. As the proportion of common purslane increased, it significantly increased the protein, ash, and dietary fiber of biscuits. Antioxidant levels rose to 51% with the addition of 15% common purslane as opposed to 20.6% for the control.

Tarkergari et al., 2013 used common purslane leaves and stalks were used to create palatable, nutrient-dense products to investigate the possibilities of incorporating fresh and dried leaves and stalks into everyday recipes to boost greens consumption as a source of micro and macro nutrients. Ten food items were created and standardized, six of which used dried powder and four of which used dhal and vegetable mixtures. A control of spinach was utilized. The nutritional content of the prepared dishes was higher than the control, notably in terms of dietary fiber, protein, calcium, and iron. There were significant variations in sensory characteristics (appearance, flavour, taste, and overall acceptability) between common purslane and control dishes in a few of the recipes.

Renu and Waghray, 2016 explained that papads are protein-rich preserved grain or legume preparations. In India, they are served as a side dish after deep-fat frying. In this work, common purslane leaves were used as green leafy vegetables to give value to the traditional savory. Legume-based papads were made with varying quantities of black gram and purslane leaves (10, 15, 20, 25, and 30%).

Minh et al., 2019 increased that agriculturalists and dietitians should pay special attention to common purslane. It's a widespread weed both in turfgrass and field crops. Its protective and health benefits are caused by a mixture of phytonutrients that are present

in a variety of plants. The plant's edible stems and leaves have a flavour that is similar to spinach in that it is slightly acidic and sour. A combination of phytochemicals found in many of these plants contributes to their defensive and health advantages.

Mohamed and Hussein, in 1994 explained the chemical composition stating that common purslane was taken at three different development phases and its complete plants, leaves, stems, and roots were examined for total solids, total protein, ash, soluble carbohydrates, and fructose. During plant maturity, total solids and protein levels increased significantly. In the third development stage, leaves possessed the most protein (44.25g $100g^{-1}$ dry matter). As the plant matured, the protein content in the roots decreased. Soluble carbohydrate levels were considerably greater in growth stages 1 and 3. Compared to stems and roots, leaves have a much higher total phosphorus content. Throughout different stages of growth, the iron content differs widely, with roots and leaves containing the highest iron levels. Manganese build-up was shown to be significant at various stages of development. Mn concentration was considerably greater in the leaves and roots than in the stems.

Delvarianzadeh et al., 2020 described that common purslane is a vegetable and herb that can be utilized in medicinal and culinary applications. The results revealed that as the content of Common purslane in samples grew, so did the water absorption capacity, mixer stability, and softening level. The addition of 15% common purslane powder dramatically reduced the harmonograph quality number. Common purslane powder dramatically reduced the extensibility, energy, and viscosity of the dough while improving resistance to extension. In terms of sensory characteristics, the sample containing 15% common purslane powder received the lowest score, whereas the other samples were found to be satisfactory in terms of taste, texture, colour, odour, and overall acceptability. In conclusion, it is feasible to include common purslane in dense bread, and 10% of powdered common purslane yields the greatest sensory results.

Shanshan and Bushuty, 2020 explained the consequences of supplementing wheat flour with purslane seeds powder (extraction of 72%) and the potential of utilizing it to produce various baked goods Three varieties of baked items (batun salih, croissants, and pizza) were made for this purpose, with three levels of purslane seeds powder (6%, 10%, and 14 % replacement) and sensory characteristics for these products studied. The chemical

composition and rheological characteristics of wheat flour were investigated. Several 21 male and female rats were categorized into 3 equal groups (7 rats each): control group C, group PS1 (6% purslane seeds powder), and PS2 (6% common purslane seeds powder) (10% common purslane seeds powder). Serum lipid profile (TC, TG, LDL-c), liver enzymes (AST, ALT, ALP), renal functions (urea and creatinine), and blood glucose were measured after the 6-week trial. The heart, liver, and kidney histopathology were also evaluated. The findings showed that adding common purslane seeds powder to wheat flour caused large increases in protein, fat, ash, and fiber but significant decreases in carbohydrates and calories (P 0.05). Sensory tests revealed that supplemented baked products (batun salih, croissants, and pizza) containing 14 % common purslane seeds powder, as well as croissants containing 10% common purslane seeds powder, were not accepted, whereas batun salih and pizza containing 6% and 10% common purslane seeds powder, respectively. The results of the farinograph and extensible graph tests showed a rise in water uptake, dough consistency, proportionate quantity, and height limit, as well as a reduction in arrival, bread including designing, susceptibility to stretch, flexibility, and energy. Histopathological study revealed that purslane seeds powder had no negative effects on the liver, kidney, or heart.

Hassan, 2014 mentioned that the common purslane is regarded as one of Egypt's most significant unknown plants. Aqueous extract and dried powder of common purslane were examined for their chemical makeup. Infested rats with toxic hepatitis were fed aqueous extract orally instead of silymarin medication. The results of the liver's histological examination also supported the use of common purslane as a hepatoprotection in treating various liver disorders since it reduced the frequency of liver lesions and signs of hepatic toxicity.

In 2016, Hussien and Saleem reported how common purslane is one of the most often used pharmacological herbs, according to the WHO. Because of its strong nutritional and anti-oxidant qualities, it has been dubbed a "future power food." Common purslane has a strong radical scavenging activity. Three quantities of common purslane powder were added to rice flour, and the snacks' physiochemical and sensory quality was assessed. As the proportion of common purslane rose, it significantly boosted the proteins, ash, and dietary fiber of baked products. Antioxidant levels rose to 87.28% after 10% common purslane was added, as opposed to 67.44% for the control. Common purslane, as a result,

may be successfully utilized to enhance snacks, providing an alternative usage and nutritious choice.

2.9 Common Purslane as a Weed

The plant known as common purslane spreads rapidly and poses a significant threat to several crops all over the globe. According to several sources, common purslane is the seventh most widespread plant on earth. Purslane is classified as a hazardous plant, and it is recognized as one of the ten most noxious weeds.

According to Smith and Figueiredo, 2010, common purslane is frequently classified as one of the most destructive weeds in the entire globe, some believe it to be harmless due to its shallow roots and the fact that it is a healthy green vegetable that can be readily harvested and utilized. It hardly ever establishes itself in highly mulched areas, and mulch poured over it strangles plants and prevents seeds from germinating.

Proctor et al., 2013 explained that common purslane can be an issue on thin turf, beside walkways and garages. Common purslane-controlling herbicides that are also intended for turfgrass have not received much attention in published studies. Nine PRE herbicides were applied on new perennial ryegrass in experiments carried out in 2011 and 2012 to determine their effectiveness at half-maximum and maximum label rates (*Lolium perenne* L.). On mature common purslane plants, 25 POST herbicides were evaluated in 2011 and 2012 at full-label rates. The POST herbicides fluroxypyr, triclopyr, and metsulfuron-methyl worked well to control common purslane.

Doohan and Felix in 2012 mentioned that weed control in green onions remains a concern for food producers in Ohio. Green onion yield was unaffected by the transitory damage. The yield of green onions fluctuated between 1.8 and 2 kg $plot^{-1}$ in 2006 and between 1.3 and 1.5 kg $plot^{-1}$ in 2009. The rate of oxyfluorfen enhanced the control of common purslane. Across the years, The most effective method for dealing with common purslane was provided by oxyfluorfen at 70 to 105 g ha^{-1}. These results show that oxyfluorfen rates of 70 to 105 g ha^{-1} are tolerated by green onion.

Haar and Fennimore, 2003 conducted research based on current cultural and chemical control approaches as well as a knowledge of common purslane biology. When bensulide or pro-namide was applied in a wide band rather than a small band, common purslane emergence was decreased. More quickly than the narrow-band pro-namide therapy, the wide-band pro-namide therapy accelerated thinning. Generally, pro-namide treatments had lower herbicide weed management costs than bensulide and the control. Weed management costs were lower with wide-band bensulide than with narrow-band bensulide, but not with small ranges. Plants removed one or two weeks after emergence (WAE) produced no mature seed. Uprooted common purslane produced fewer seeds after being treated with flame and 2% (v/v) glyphosate. Nomenclature: POROL; lettuce, *Lactuca sativa* L.; bensulide; pronamide; common purslane. Banded herbicide, and weed seed production are two additional index terms. Days after treatment, growing degree day, and weeks after emergence are all abbreviated as DAT, GDD, and WAE, respectively.

Grichar in 2008 mentioned that during the 2003–2005 growing seasonsField tests were conducted to evaluate the effectiveness of soil-applied weed killers either individually or in a mixture with postemergence (POST) herbicides for the control of Palmer amaranth, common purslane, and smell melon in peanuts. Preplant-integrated (PPI) pendimethalin alone did not successfully suppress any of the three weeds. Preemergence (PRE) administration of pendimethalin and diclosulam, followed by imazethapyr, or postemergence application of acifluorfen or imazapic (POST. The soil-applied herbicides flumioxazin, imazethapyr, S-metolachlor, and dimethenamid alone were ineffective at controlling horse purslane and smell melon (75%).

El-Rokiek et al., 2013 conducted pot tests in March 2009 and 2010 to assess the physiological reaction of common purslane and two recombinant innate lines (RILs) of normal bean (Phaseolus vulgaris) RIL-115 and RIL-147 to post-development utilization of the herbicide bentazone at the endorsed rate (1L Fed^{-1}. (2.4 L ha^{-1}) and at the diminished rate [½ L Fed^{-1} (1.2 L ha^{-1})]. Bentazone was applied in all pots after plant development with or without phosphorus (P) at a pace of 27.7 kg Fed^{-1} (66 kg ha^{-1}). Weed control of common purslane expanded with a higher portion of bentazone. The expansion of phosphorus worked on the control of common purslane. The combined application of P and bentazone at low and high rates reduced the dry weight of common purslane in

RIL-115 by 79.4 and 95.7% at harvest, respectively. In RIL-147, the equivalent figures were 66.8 and 84.4%. Control of common purslane weed by bentazone alone or in combination with phosphorus was accompanied by increases in growth and yield of the two common bean genotypes, RIL-115 and RIL 147. In general, the increase in growth and yield in the two genotypes was greater than 100% when compared to the matching controls. Increases in bean growth and production were accompanied by increases in phosphorus and nitrogen content in bean leaves, as well as metabolic activities reflected in increases in the percentage of carbohydrates and protein in the produced seeds.

Karkanis et al., 2017 stated that common purslane is a waterlogging weed grown for its fresh leaves and stems. An outdoor test was performed. to evaluate plant growth and photosynthesis of different well-known common purslane types in a high-density planting strategy. Four local populations of common purslane were investigated, as well as two commercial cultivars. Significant variations in plant height and biomass were detected among the genotypes tested, according to the findings of our study. The genotype 'Domokos' had the lowest height and biomass, with prostrate to semi-prostrate development, whereas all other genotypes exhibited upright growth. Physiological metrics showed differences as well. The genotype "Domokos" had the highest relative chlorophyll content (SPAD value), but the lowest stomatal conductance and photosynthetic rates.

CHAPTER III

MATERIALS AND METHODS

The current study was centered on the genetıc diversity, biochemical characterization, morphological analysis, and product development of common purslane (*portulaca oleracea* L.) Populatıons. This study was completed in the experimental field (greenhouse) and the scientific laboratories of the Plant Production and Technologies Department and the Agricultural Sciences and Technologies Faculty at the Nigde Omer Halisdemir University Nigde, Turkiye (37.943046 North and 34.631736 East) during summer 2021-22.

3.1 Resources for Research

3.1.1 Plant Materials, Growing Conditions, and Sampling Analysis

Seeds of 25 common purslane accessions were obtained from different geographical locations of Turkiye (Table 3.1). Pots (depth × diameter = 22.5 × 16.5 cm) were filled with a mixture of compost and perlite (2:1) (Fig. 3.1). The compost used had a pH of 6, the electrical conductivity of 0.4 dS m^{-1}, and NPK of 1.2 kg m^{-3} of the compost. Seeds of each of the accession were instantly sowed in pots. Four seeds per pot were sown at a depth of 1.5cm (Fig. 3.2). Subsequent to sprouting, pots were rogued to maintain two common purslane plants per pot (Fig. 3.3). Watering was performed consistently (starting on the day of sowing) according to the requirement of the plants (Fig. 3.4). At 10 and 20 days after planting, nitrogen fertilizer (46 kg ha^{-1}) was administered in two equivalent dosages. During the growing process, no pesticides or other agrochemicals were used. Harvesting was carried out after 30 days of sowing (Fig. 3.5).

Figure 3.1. Sowing of common purslane accessions

Figure 3.2. Common purslane seedlings

Figure 3.3. Common purslane plants at different growing stage in greenhouse

Figure 3.4. Applying fertilizer application to common purslane accessions

Figure 3.5. Harvesting of common purslane plants

Table 3.1. List of common purslane accessions seeds from various Turkiye locations used in this study

S/No	Accessions	Locations
1	Agr Tohum	Nigde
2	Agri	Samsun
3	Aktarix	Malatya
4	Arzuman	Antalya
5	Assem	Kirsehir
6	Asya	Bursa
7	Balikesik	Balikesir
8	BT Altinkulak	Nigde
9	Dogal	Ankara
10	Duzce	Duzce
11	ERO-I	Istanbul
12	Ero-II	Alanya
13	Gungurler	Nigde
14	Mercan	Adana
15	Noroz Nazlisi	Adana
16	Pasa	Antalya
17	Rita	Izmir
18	Sari Ferik	Ege
19	Simagro	Konya
20	Sun Agri	Konya
21	Tunc	Konya
22	Yagmur	Bursa
23	Yerli	Nevsehir
24	Yesim-I	Aksaray
25	Yesim-II	Aksaray

3.2 Experimental Site

The studies were conducted under semi-controlled greenhouse conditions (no artificial lighting, temperature ~25°C Day/10°C night, humidity ratio 50%) in Plant Productions and Technologies Department and the Agricultural Sciences and Technologies Faculty at the Nigde Omer Halisdemir University Nigde, Türkiye (37.943046 North and 34.631736 East). The experiment was done four times during the summer season; two experiments over the time (i.e., two repeats of the same experiment) were employed for morphological data collection, whereas two other repetitions were utilized for biochemical analyses.

3.3 Morphological Analysis and Product Development

A fully randomized design (CRD) with five replicas was used to plant the accessions. Details about growing conditions and the site of the experiments are provided in the two previous subsections. Common purslane plants for the 25 accessions were harvested 30 days after sowing; i.e., immediately before initiation of flowering. After harvesting, aerial plant components were separated into stems and leaves and growth parameters were recorded.

From the soil's surface to the uppermost point of each accession's leaf, the height of the plant was determined in centimeters (cm). Plants were cut from the surface of the soil and fresh weights of plant shoots (stem + leaves) and stem only were recorded by using a sensitive weighing balance (ATX24, SHIMADZU). The leaves were detached from each plant and counted manually and were weighed for leaf fresh weight and stored in paper bags for dry weight analysis. Roots were collected, tap water was used to cleanse, and dried with tissue paper to measure the fresh weight of roots from each pot by using a sensitive weighing balance (ATX24, SHIMADZU). Subsequently, the root length for each accession was measured manually using a measuring tape. Finally, leaves stems, and roots were put together and maintained for 48 hours in a 70°C kiln to get plant DW for each accession in the study. Similarly, the below equation was used to determine the moisture levels:

Moisture content (%) = (Initial weight – Final weight) ÷ (Initial weight) × 100 (1)

3.3.1 Product Development

The dried plant leaves of all the accessions were combined and then crushed by a plant grinder (Nutribullet PRO High Speed), and used for product development. This powder was used to prepare three different cookie recipes. Wheat flour (1000 g), table salt (20 g), sugar (200 g), baking powder (1.5 g), and milk (6.6 L) were mixed with either 0, 10, 20, or 30 g of common purslane powder and baked at 120°C in an oven for 15 min. The cookies for each common purslane accession were subjected to sensory evaluation involving 14 different untrained panelists (males and females) recruited from the faculty. Sensory functions like smell, appearance, texture, appearance, taste, as well as general acceptance, were assessed. Every participant was requested to decide on a number between 1 and 10, representing dislike extremely (1) to like extremely (10).

3.4 Biochemical Analysis of the Common Purslane Accessions

Five replicas of a fully randomized design (CRD) were used to plant the accessions. Details about growing conditions and the site of the experiments are provided in the subsections "**Plant material and growth conditions**" and "**Experimental site**" of this article. Common purslane plants for the 25 accessions were harvested 30 days after sowing; i.e., immediately before initiation of flowering. Leaves from each common purslane accession were collected and homogenized in a commercial blender (Nutribullet PRO High Speed) for different biochemical analyses.

3.4.1 Determination of Total Phenolics

The Folin Ciocalteu technique was employed to analyze the number of phenolics (Montedoro et al., 1992). Deionized water was used to dilute the stock solution (100 L) before they were mixed with reduced Folin Ciocalteu reagent (2.5 mL). After 1 minute, a Na_2CO_3 solution was applied. After two hours at ambient temp, the samples were read by spectrophotometer at 765nm (UV 1800, SHIMADZU, JAPAN).

3.4.2 Anthocyanin Contents

It was quantified by following the protocol provided by (Ranganna, 1986). Ten g of leaf tissues were homogenized in 10 mL of acidic ethanol (95.0% ethanol with 1.50 N Hydrochloric acid) and centrifuged at 13000 rpm. The aqueous phase was collected. The final amount was raised to 100 mL of acidified ethanol, and the extract was stored in the refrigerator overnight at 4°C. Finally, utilizing a filter paper (Whatman filter paper No. 42), the extract was processed, and the sample was determined at 515 and 700 nm via a spectrophotometer (UV 1800, SHIMADZU, JAPAN).

3.4.3 Determination of Flavonoids Content

The flavonoid level of the extracts of the common purslane accessions was measured by an aluminum chloride spectrophotometric procedure (Zhishen et al., 1999). 5% $NaNO_3$ and distilled water were added to each sample solution of catechin. 10% aluminum chloride was introduced after five minutes, and the mixture was then maintained for another six minutes. Samples were determined at 510 nm via a spectrophotometer (UV 1800, SHIMADZU, JAPAN).

3.4.4 β-carotene Contents

β-carotene concentration was analyzed as per the description by Nagata and Yamashita (1992). Ten grams of each leaf sample was immersed individually in 100 mL of methanol and at 45°C, each solution was added and stirred in a steam-bath. The sample solution was determined at 663 nm by using a spectrophotometer (UV 1800, SHIMADZU, JAPAN).

3.4.5 Determination of Chlorophyll and Carotenoids Concentration

Carotenoid and chlorophyll (a, b, and total) levels have been evaluated by following the assay mentioned by Sadasivam and Manickam (1992). The leaf sample (5 g) was homogenized into 80.0% acetone and allowed to extract completely for 24 hours at 4 °C.

After that, it was centrifuged at 4°C. The reading aqueous phase was read against the blank (80% acetone) at 663, 645, and 480 nm.

3.4.6 Lycopene Contents

Davis's (2003) method was used to determine the lycopene in common purslane. 0.5 grams of plant sample was used for the determination of lycopene in common purslane. Then 5 ml of 80% acetone was introduced and the samples were put in the shaker (200 rpm) for 2 hours. After this, The samples underwent centrifugation, and the upper phase was collected the volume was raised to 25 mL by adding 80% acetone. Finally, the upper phase was taken in a separate tube and measured at 503 absorbances.

3.4.7 Lutein Contents

Spectrophotometric analysis is accustomed to assessing the lutein amount of leaves of common purslane, as previously described by Bulda et al. (2008). A frozen sample of liquid nitrogen was pulverized in a mortar and pestle using an acetone-to-ethanol ratio of 1:1. KOH (1g mL^{-1}) newly prepared is added to the solution. The sample was allowed to settle after the aliquot was heated to 45°C for 5 mins and then refrigerated on cold. For analysis, the uppermost colored % was chosen. Final lutein content in common purslane leaves was calculated using the following equation after samples were read on a spectrophotometer at 480 and 495 nm (UV 1800, SHIMADZU, JAPAN).

Formula = 11.51 A480 – 20.61 A495 ……..(2)

3.4.8 Reducing Power

The Oyaizu (1986) technique was used to estimate the reducing power. A sample of 25 microliters of common purslane was collected, then 25 microliters of ethanol and 2500 microliters of 0.2M phosphate buffer were added. After the reaction mixture had been incubated, 2500 microliters of TCA 10% was added, and it was vortexed. 2500 microliters of supernatant were collected, combined with 2500 microliters of distilled

water, and 500 microliters of $FeCl_3$ were introduced. The samples were determined at 700 nm (UV 1800, SHIMADZU, JAPAN).

3.4.9 Total Soluble Protein Contents

It was carried out according to AOAC International. A working reagent Albumin (BSA) standards and BCA were prepared. In an independent centrifuge tube with labels, 1.6 mL of the reagent, 0.1 mL of the standards, and 0.1 mL of the extract of leaves were well combined. The vials were then maintained at 37°C for more than half an hour. Absorbance was measured at 562nm (UV 1800, SHIMADZU, JAPAN).

3.4.10 Glycine Betaine

A technique published by Grieve and Grattan in 1983 was used to measure the glycine betaine content. Mixed with H_2SO_4 and the samples were heated in an Eppendorf for 10 minutes at 60ºC. Samples were centrifuged at 14000 rpm at room temperature. Before centrifuging for 30 min at 14,000 revolutions per minute, the vials were stored at 4 °C for 16 hs. The upper phase was meticulously drained, leaving just glycine betaine particles attached to the channel walls and bottom. A spectrophotometer of 550 nm was used to measure the absorbance (UV 1800, SHIMADZU, JAPAN).

3.4.11 Ascorbic Acid

The ascorbic acid was carried out according to Mukherjee and Choudhuri (1985). Ten milliliters of 6% trichloroacetic acid were used to extract around 250 mg from the frozen leaf. Four milliliters of the extract were combined with two milliliters of 2% dinitrophenylhydrazine, then the solution was boiled for 15 min at 100°C in water-bath. Following the addition of five mL of cooled H_2SO_4 (80%), the solutions were read at 530 nm.

3.4.12 Hydrogen Peroxide

Young leaves of common purslane were powdered in liquid nitrogen. Centrifuging the mixture at 10,000 rpm for 20 mins at 4°C as explained by Ruch et al. (1989). The reaction mixture's absorbance value at 230 nm (UV 1800, SHIMADZU, JAPAN) was measured after 40 min.

3.4.13 Total Alkaloids Determination

Total alkaloids analysis was executed out in accordance with to Shamsa et al., 2008. The leaves (100g) were pulverized and extraction with methanol. 5 mL of BCG (Bromocresol green) mixture and 5 mL of phosphate buffer were added to the mixture and were vigorously agitated. Ans sample solutions were at 470 nm (UV 1800, SHIMADZU, JAPAN) against blank.

3.5 Total Antioxidant Activity

Four distinct methods—2,2-Diphenyl-1-picrylhydrazyl (DPPH), cupric reducing antioxidant capacity (CUPRAC), ferric reducing antioxidant power (FRAP), and total antioxidant activity (TEAC)—were used to calculate the total antioxidant capacity.

3.5.1 FRAP

A leaf sample (1g) was obtained and combined with a 10 mL solution of Methanol and HCl (99:1 v/v). The FRAP technique described by Benzie and Strain (1996) was used for one mL of sample solution. Before analysis, an aliquot of the material was combined with a FRAP reagent. After 30 min, the mixture's wavelength was assessed using a spectrophotometer set to 593 nm (UV 1800, SHIMADZU, JAPAN).

3.5.2 CUPRIC

Apak's CUPRAC technique was used (2004). The CUPRAC technique is based on antioxidants reducing copper I to copper II. A 10M Cu (II) solution was made, and test tubes were filled with 1mL Cu (II), and NH4Ac buffer mixture. After 30 minutes of

incubation, the absorbance at 450 nm (UV 1800, SHIMADZU, JAPAN) (A450) was measured against a blank reagent.

3.5.3 TEAC

The sample was created by mixing 0.5 grams of the samples with 15 mL of a 99:1 solution of methanol and Hydrochloric. Following the TEAC technique as detailed by Ozgen and Sekerci (2013), 1 mL of the sample was obtained. Potassium bisulfate was combined with ABTS, which was then mixed and incubated with aliquots of the sample. A spectrophotometer measurement was conducted at a frequency of 734 nm.

3.5.4 DPPH

The approach outlined by Berwal et al. (2021) was used to perform the DPPH quenching experiment. Common purslane leaves were permitted to interact with 2.90 mL of 0.0060% ethanolic DPPH for 10 mins in the dark. In addition, instead of extract, 100 mL of distilled water was used as a control. At 517 nm, the absorbance was measured (UV 1800, SHIMADZU, JAPAN).

3.6 Antioxidant Enzyme Activity

Fresh leaf samples were used to measure antioxidant enzymes activity as follows:

3.6.1 Superoxide Dismutase Activity (SOD)

The Dhindsa et al., 1981 approach was used to calculate total SOD activity. After being frozen in liquid nitrogen, 1 g of leaf extract was crushed in 10 mL of extraction. Riboflavin was applied to 3 mL of the reaction medium to initiate the reaction, and the vials were then exposed to two 15 W fluorescent lamps for 15 mins. At 560 nm, the samples were assessed. (UV 1800, SHIMADZU, JAPAN).

3.6.2 Catalase Activity

Catalase activity was measured utilizing UV-visible spectrophotometry at a wavelength of 275 nm (UV 1800, SHIMADZU, JAPAN), as revised by Lubinsky and Bewley (1979). The leaf extracts were collected into a total of 20 L, which was then diluted 1:20 with 380 L of phosphate buffer (pH 7). 150 mL of sulphuric acid, 2 mL of hydrogen peroxide, and 400.00 mL of phosphate buffer (pH 7) made up the blank.

3.6.3 Ascorbate Peroxidase Activity (APX)

Measurements of APX activity were carried out by Cao et al., 2007. One g of powdered common purslane was combined with 5 mL of phosphate buffer, and 0.001 mL of ascorbic acid. after centrifuging the mixture, the upper phase was collected. Samples were at 290 nm (UV 1800, SHIMADZU, JAPAN) per gram of tissue, and APX activity was measured.

3.6 Genetic Diversity

A total of 25 common purslane accessions in all, obtained from 25 regions of Turkiye, were utilized in our research (Figure 1). A slightly modified version of Doyle and Doyle's cetyltrimethylammonium bromide (CTAB) technique, DNA extraction was carried out from the young leaves of the common purslane populations (Doyle and Doyle, 1987). Each DNA sample's final concentration was diluted with 1X TE buffer to achieve the necessary concentration before being stored at -20 °C for further investigation.

3.6.1 Primers Amplification

After the screening of various articles, a total of 25 Inter Simple Sequence (ISSR) primers (Table 3.2) were selected and employed for genetic diversity among the collected common purslane accessions.

Table 3.2. List of ISSR markers used for genetic variation analysis

S/No	Sequence
1	5’-ACA CAC ACA CAC ACA CG- 3’
2	5’-ACA CAC ACA CAC ACA CT-3’
3	5’-ACA CAC ACA CAC ACA CC-3’
4	5-AGA GAG AGA GAG AGA GC-3’
5	5’-AGA GAG AGA GAG AGA GT-3’
6	5’-GAG AGA GAG AGA GAG-3’
7	5’-GAG AGA GAG AGA GAG AT-3’
8	5’-CTC TCT CTC TCT CTC TG-3’
9	5’-AGA GAG AGA GAG AGA GGT-3’
10	5’-ACA CAC ACA CAC ACA CGG-3’
11	5’-ACA CAC ACA CAC ACA CGA-3’
12	5’-TAG AGA GAG AGA GAG AGA G-3’
13	5’-GAC ACA CAC ACA CAC ACA C-3’
14	5’-CCA CTC TCT CTC TCT CTC T-3’
15	5’-AGA GAG AGA GAG AGA GG-3’
16	5’-GGA TGG ATG GAT GGA T-3’
17	5’-AGA GAG AGA GAG AGA GCT T-3’
18	5’-TCT CTC TCT CTC TCT CG-3’
19	5’-CAC ACA CAC ACA CAC AG-3’
20	5’-ACA CAC ACA CAC ACA CGT-3’
21	5’-AGA GAG AGA GAG AGA GGC-3’
22	5’-TCC TCCTCCTCCTCC TG-3’
23	5’-ACA CAC ACA CAC ACA CGA-3’
24	5’-CAC ACA CAC ACA CAC ATA-3’
25	5’ -GGG TGG GGT GGG GTG- 3’

To amplify ISSR segments, a polymerase chain reaction (PCR) has been accomplished in an 8µL volume which included 0.80µL of DNA sample, 1.20µL of ISSR primers, 3.60µL of master mix, and 2.40µL of nuclease-free water. The three-phase Touchdown PCR technique was used for PCR amplification in a thermocycler (Don et al., 1991). The

baseline temp was raised to 95.0°C for 5 min, the denaturation was done at 95.0°C temp was set at 94.0°C for 45 seconds, the early annealing temp was 52°C for 45 seconds, the preliminary temp was 72.0°C for 2 mins. In the second stage, the annealing temp was determined at Tm = 52°C for 45 seconds, and the extension temp was 72.0°C for 2 minutes, similarly, the last phase's extension temp was 72°C for 8 min. The first and second stages were run respectively nine and thirty times. One mL of 1X TBE buffer was added to 1% molecular grade Agarose gel for electrophoresis.

3.6.2 Data Scoring

To create a binary data grid, the amplicons for ISSR marker loci in a particular location in a gel image are graded optically as "1" = existence and "0" = missing of a band.

3.6.3 Data Analysis

Applying Jaccard's identity factors, genetic proximity and resemblance factor were computed based on the numerical score results of the gel image (Rohlf, 2002) (Fig. 3.6). based on the similarities grid dataset, cluster analysis was conducted using UPGMA and the NTSYS-pc 2.10 program to create a phylogenetic tree. According to Anderson et al., (1993), polymorphism information content (PIC) scores have been computed to assess varying aspects of every ISSR primer.

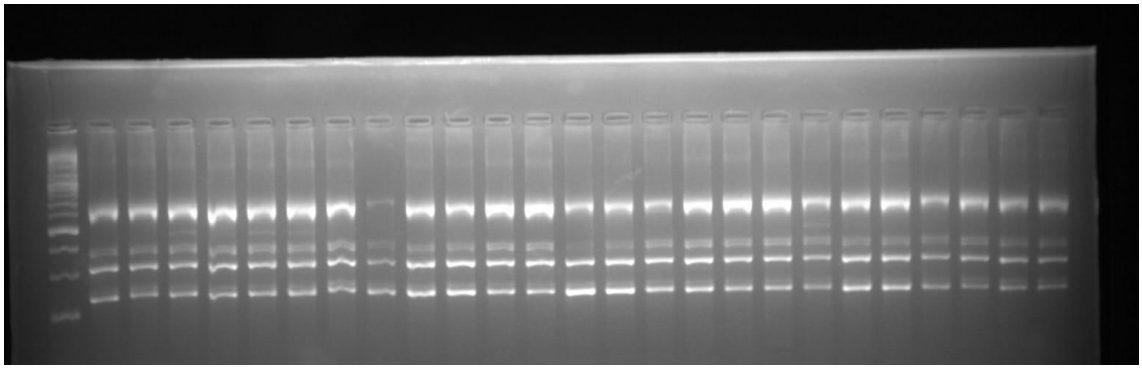

Figure 3.6. The polymorphic banding pattern of gel image

3.7 Product Development

3.7.1 Plant Material

The collected fresh common purslane was cleaned, After dehydrating at 60°C in an oven, the samples were ground into powdery using a plant grinder. The powder was then placed in polyethylene bags, where it would be kept for subsequent analysis while being kept at room temperature.

3.7.2 Preparation of Cookies

Wheat flour, salt, sugar, baking powder, and milk in amounts of 1000, 200, 20, 66, 1.5, and 200 g were the main ingredients utilized in the preparation of the biscuits. Wheat flour was substituted with 0, 10, 20, and 30 g of common purslane powder for four various degrees of enrichment (Fig. 3.7). The dough was divided into sections of about 8 g each, formed into flat, round biscuits, and cooked in an electric oven at 180°C for 15 minutes, or until a brown hue emerged on the exterior. The biscuits were then allowed to cool at room temperature for an additional hour (Fig. 3.8).

Figure 3.7. Making of dough for cookies preparation

3.7.2.1 Sensory Evaluation

A random sampling of cookies was performed for sensory attributes. Each person was instructed to choose a number between 1 and 10, with 1 being the most disliked and 10 being the most liked. The sensory attributes evaluated were color, odor, taste, texture, appearance, and overall acceptability (Fig. 3.8).

Figure 3.8. Sensory evaluvation of prepaared cookies

3.8 Statistical Analysis

Statistix 8.1 was used to assess the data. The data collected over two studies were analyzed using analysis of variance (ANOVA) taking studies as a factor. The Least Significant Difference (LSD) test ($p \leq 0.01$) was employed to establish the distinction between the treatments. Principle component analysis (PCA) was performed by Origin Pro (2020) software. Genetic analysis calculation was carried out by NTSYS-pc 2.11e version software.

CHAPTER IV

RESULTS

Using plant-based naturally occurring substances has thus been a focus of research over the past few decades. Plants are a rich source of phytochemicals that can be exploited as a substantial source of nutraceutical compounds (Palaniswamy et al., 2001, Dkhil et al., 2011). Additionally, these plants include non-toxic biological components that are useful in combating different oxidative stress. Recently, the nutritional and biochemical content of common purslane, one of these rarely eaten vegetables, has attracted the attention of nutritionists worldwide. This chapter describes the findings that were made throughout the entire process of the investigation.

4.1 Morphological Analysis

The outcomes of the statistical analysis of variance showed that the research accessions had a significant variation regarding their morphological characteristics (Tables 4.1 and 4.2). Although all the accessions were of erect type (except Dogal, which had a prostrate growth habit; data not shown), they differed significantly in their plant height (Table 4.1). Accession BT-Altinkulak collected from the Niğde province of Türkiye had the greatest plant height (34.18 cm), while the accessions Dogal and Ero-I collected from the Ankara and Istanbul regions showed the lowest plant height i.e., 22.3, and 27.75 cm, respectively. Similarly, the number of leaves also varied considerably for different accessions. Accession Pasa showed a maximum number of leaves, while accession Gungurler had the least number of leaves of all accessions (Table 4.1).

Root length analysis is an important parameter in the morphological analysis of plant growth and development. Root length analysis provides a quantitative measure of plant growth and can be used to compare different plant genotypes or treatments. For example, root length has been used to study the effects of heavy metals on plant growth in maize (Li et al., 2018) and to compare the growth of different rice cultivars (Xu et al., 2017). Root length analysis can be used as a screening tool to identify plant traits that are desirable for specific agricultural or horticultural purposes. For example, root length has

been used to identify rice cultivars with improved drought tolerance (Shimizu et al., 2018) and to screen for Arabidopsis mutants with altered root growth (Xu et al., 2018).

The root morphology also showed significant variation among the accessions. The highest root length was found in the accession Assem (30 cm), while most of the accessions had a root length in the range of 23 to 25 cm (Table 4.1). The accessions also varied for their biomass, and the accessions Yerli and Sun Agri respectively had the highest fresh and dry weights of 111.8 g and 7.38 g. In terms of plant fresh weight, most of the accessions ranged from 50 to 100 g fresh weight while nine accessions were found in the range of 40 to 50 g fresh weight (Table 4.1). Similarly, the moisture content among the common purslane accessions showed weakly significant variation across the accessions. The accession Yerli showed the highest moisture content of 95.14% whereas the accession Dogal was observed to have the lowest moisture content. Most of the accessions ranged from 90 to 94% in terms of moisture content (Table 4.1). Moreover, the grown accessions also showed a considerable variation in leaf fresh and dry weights. The highest leaf fresh and dry weights (39.18 g and 2.71 g) were achieved in the accession Yerli collected from Nevsehir, and the BT-Altinkulak (2.83 g) also had the highest leaf dry weight (Table 4.2). In terms of stem fresh weight, significant variability was noted among the accessions. The highest stem fresh weight was achieved by the accession BT-Altinkulak (74.62 g) while the lowest stem fresh weight was by Gungurler (1.35 g) (Table 4.2). The fresh root weight of most of the accessions ranged from 4 to 5 g. Similarly, the dry root weight of most of the accessions ranged between 0.4 to 0.5 g (Table 4.2).

4.1.1 Correlation Analysis

Correlation analysis was performed for several morphological features to identify the significant trait link (Fig. 4.1a). The correlation between the major morphological features was significant and positive. Highly correlated parameters showing a significant positive association were leaf fresh weight and plant fresh weight ($r = 0.93$), stem fresh weight and leaf fresh weight ($r = 0.90$), and plant fresh weight and stem fresh weight ($r = 0.97$). Root fresh weight and root dry weight also showed a favorable association with each other ($r= 0.79$) (Fig. 4.1a).

4.1.2 Principal Component Analysis (PCA)

The PCA biplot of the morphological features' first two components, PC1 (50.7%) and PC2 (15.8%) are shown in Fig. 4.1b. The PCA produced three distinct groups of accessions. The green dots reflect accessions that outperformed the other groups in terms of morphological performance, with higher plant height, number of leaves, plant fresh weight, stem fresh weight, and moisture contents. The black dots reflect the accessions' average performance, whereas the red ones are classified as poor accessions based on their physical characteristics (Fig. 4.1b).

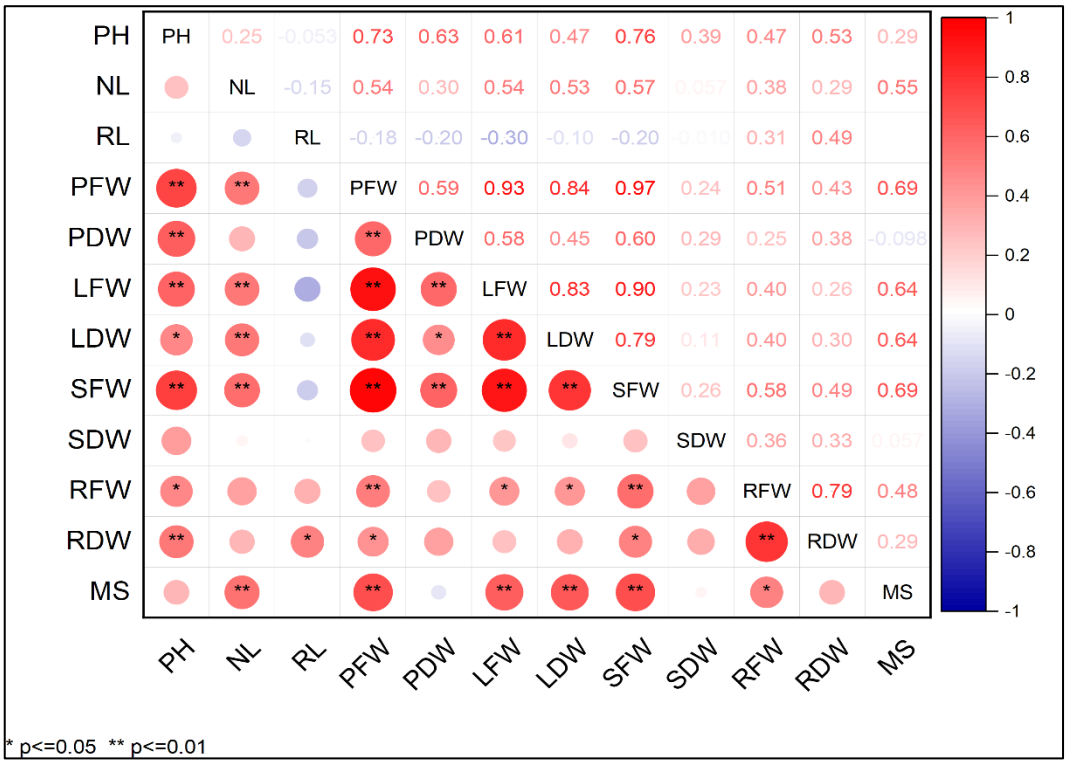

Figure 4.1. (a) Correlation analysis showing the distribution of 25 common purslane accessions using various morphological traits

* = significant at p<0.05; ** = significant at p<0.01; PH = plant height; NL= number of leaves; RL= root length; PFW= plant fresh weight; PDW= plant dry weight, LFW= leaf fresh weight; LDW= leaf dry weight, SFW= stem fresh weight; SDW= stem dry weight; RFW= root fresh weight; RDW= root dry weight, and MS= moisture content

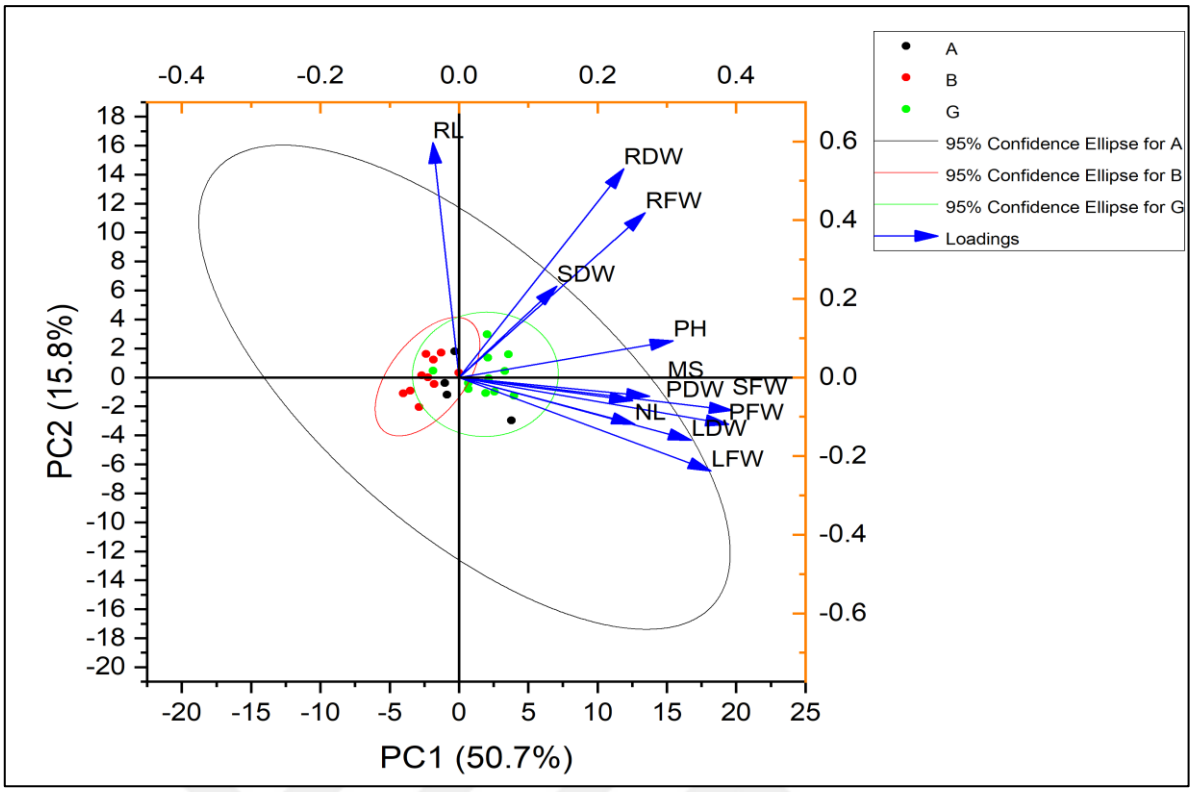

Figure 4.1. (b) principal component bi-plot showing the distribution of 25 common purslane accessions using various morphological traits

PH = plant height; NL= number of leaves; RL= root length; PFW= plant fresh weight; PDW= plant dry weight, LFW= leaf fresh weight; LDW= leaf dry weight, SFW= stem fresh weight; SDW= stem dry weight; RFW= root fresh weight; RDW= root dry weight, and MS= moisture content

Table 4.1. Growth parameters of different common purslane accessions grown under greenhouse conditions

Populations	Plant height (cm)	Number of leaves	Root length (cm)	Plant FW (g)	Plant DW (g)	Moisture content (%)
Agr Tohum	33.93 ab	173 abcd	23.9 bcde	98.65 abc	5.241 abcd	94.97 ab
Agri	29.08 defg	162 bcde	26.9 abcd	55.98 ghi	3.6 d	93.18 abc
Aktarix	27.8 g	131 de	23.15 bcdefg	51.84 ghi	4.79 bcd	90.03 abcd
Arzuman	28.6 efg	128 de	27.55 abc	51.55 ghi	3.67 d	92.33 abc
Assem	30.75 def	145 bcde	30 a	83.96 cdef	5.46 abcd	93.04 abc
Asya	33.48 abc	169 abcde	26.2 abcde	93.16 abcd	6.93 ab	92.27 abc
Balikesik	27.9 fg	184 abc	24.87 bcde	53.83 ghi	5.58 abcd	89.43 abcd
BT-Altinkulak	34.18 a	171 abcde	22.77 cdefgh	108.13 ab	6.32 abc	93.84 ab
Dogal	22.3 h	143 bcde	22.3 defgh	56.82 ghi	3.49 d	93.59 ab
Duzce	29.68 defg	139 bcde	25.82 abcde	51.58 ghi	3.79 d	86.3 d
Ero-I	27.75 g	128 de	23.45 bcdef	49.04 hi	4.32 cd	91.13 abcd
Ero-II	31.05 abcdefg	122 de	23.07 bcdefgh	68.84 efgh	6.03 abc	89.17 bcd
Gungurler	29.05 defg	121 e	18.75 fgh	43.68 i	5.54 abcd	87.28 cd
Mercan	31.8 abcde	190 ab	22.45 defgh	80.09 cdef	5.05 bcd	93.37 ab
Noroz Nazlisi	28.7 efg	150 bcde	27.9 ab	54.8 ghi	4.57 cd	91.40 abcd
Pasa	30.23 cdefg	217 a	23 cdefgh	90.26 abcde	5.45 abcd	93.75 ab
Rita	29.78 defg	165 bcde	25 bcde	86.36 bcdef	5.68 abcd	92.89 abc
Sari Farik	32.18 abcd	131 de	24.02 bcde	83.62 cdef	5.55 abcd	93.3 ab
Simagro	29.05 defg	139 bcde	21.9 efgh	68.43 efghi	4.8 bcd	92.85 abc
Sun Agri	29.88 defg	143 bcde	24.5 bcde	63.84 fghi	7.38 a	92.93 abc
Tunc	31.06 abcdefg	165 bcde	22.85 cdefgh	87.72 bcde	5.2 abcd	93.53 ab
Yagmur	29.18 defg	141 bcde	25.6 abcde	54.8 ghi	4.61 cd	90.7 abcd
Yerli	33.68 ab	160 bcde	18.5 gh	111.81 a	5.19 abcd	95.14 a
Yesim-1	31.25 abcdef	136 cde	23.6 bcdef	73.57 defg	4.73 bcd	93.46 ab
Yesim-II	30.64 bcdefg	176 abcde	18.25 h	70.89 defgh	5.24 cdefg	92.46 abc
LSD (0.01)	3.4021	52.436	4.8768	22.611	2.209	5.8998

This means in columns with different letters are significantly different from each other at $p \leq 0.01$ according to the Least Significant Difference (LSD) test
FW= fresh weight, DW= dry weight

Table 4.2. Growth parameters of different common purslane accessions grown under greenhouse conditions

Populations	Leaf FW (g)	Leaf DW (g)	Stem FW (g)	Stem DW (g)	Root FW (g)	Root DW (g)
Agr Tohum	31.78 abc	2.13 bcdef	67.72 ab	3.05 defg	7.19 a	0.64 abcd
Agri	17.85 hij	1.74 fghi	37.03 ghi	2.81 efghij	5.87 abcd	0.57 bcde
Aktarix	19.23 fghij	2.16 bcdef	33.15 hi	3.18 cdefg	4.09 defgh	0.5 defg
Arzuman	17.97 ghij	1.69 fghi	34.38 ghi	2.32 ijk	5.57 abcdef	0.5 defg
Assem	25.41 cdefgh	2.33 abcde	57.93 bcd	3.41bcdef	7.25 a	0.76 a
Asya	30.82 abcd	2.36 abcd	63.36 abc	4.42 a	7.12 ab	0.73 ab
Balikesik	19.29 fghij	1.84 efghi	34.35 ghi	3.45 bcde	3.46 gh	0.46 efgh
BT-Altinkulak	36.22 ab	2.83 a	74.62 a	3.67 bc	4.72 defgh	0.55 defg
Dogal	24.29 cdefghij	2.12 bcdefg	32.24 hi	1.35 i	3.74 abcdef	0.33 i
Duzce	18.87 ghij	1.58 hij	32.93 hi	1.81 kl	3.72 fgh	0.46 efgh
Ero-I	15.55 ij	1.56 ij	31.7 hi	2.19 jk	2.43 h	0.48 defgh
Ero-II	27.37bcdefg	2.04 defghi	41.11 fghi	3.8 abc	3.80 efgh	0.48 defgh
Gungurler	15.23 j	1.12 j	28.33 i	2.77 fghij	3.10 gh	0.41 gh
Mercan	26.19 cdefgh	2.55 abc	56.15 bcdef	3.45 bcde	6.83 abc	0.59 bcde
Noroz Nazlisi	18.46 ghj	1.63ghi	37.37 ghi	2.42 hijk	4.87 cdefg	0.50 defg
Pasa	32.8 abc	2.35 abcd	57.38 bcde	2.85 efghij	4.84 cdefg	0.58 bcdef
Rita	28.96 bcde	1.98 defghi	58.21 bcd	3.92 abc	7.13 ab	0.72 abc
Sari Farik	28.45 bcdef	1.81 fghijk	55.94 bcdef	3.13 cdefg	4.43 defgh	0.49 defg
Simagro	19.96 efghij	2.08 cdefgh	49.17 cdefg	2.64 ghij	4.08 defgh	0.42 fgh
Sun Agri	21.48 defghij	1.67 fgi	42.19 efghi	2.95 efgh	5.85 abcde	0.56 defg
Tunc	32.34 abc	2.6 ab	55.89 bcdef	3.35 bcdef	4.32 defgh	0.51 defgh
Yagmur	19.53 efghij	1.91 defghi	34.9 ghi	3.08 defg	4.02 defgh	0.48 defgh
Yerli	39.18 a	2.71 a	70.64 ab	3.36 bcdef	4.92 cdefg	0.44 efgh
Yesim-1	21.23 efghij	2.14 cdef	46.5 defgh	2.8 efghij	5.08 bcdefh	0.6 bcde
Yesim-II	24.86 cdefghi	1.92 defghi	57.58 bcd	3.26 bcdefg	5.85 abcd	0.57 cdefg
LSD (0.01)	9.5188	0.4992	15.228	0.6742	2.0523	0.1588

This means in columns with different letters are significantly different from each other at $p \leq 0.01$ according to the Least Significant Difference (LSD) test
FW= fresh weight, DW= dry weight

4.2 Preparation of Cookies

The analysis of sensory attribution of cookies is illustrated in Fig. 4.2. Smell, color, texture, appearance, taste, and overall acceptability for all cookies with varying amounts (10 g, 20 g, and 30 g) was rated from 3 to 10 points.

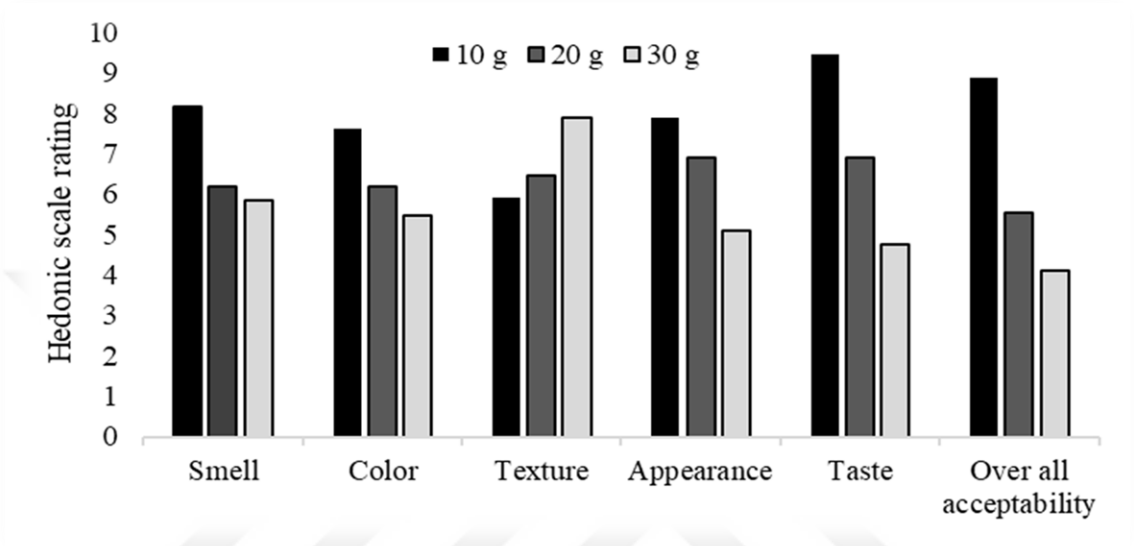

Figure 4.2. Sensory characteristic of cookies prepared from common purslane

4.3 Biochemical Analysis of Common Purslane

The accession Yagmur has the good total phenolic content (685.4 mg AEE 100^{-1}), followed by Mercan and BT-Altinkulak accessions, while the accession Arzuman showed the lowest total phenolic content (343.18 mg AEE 100^{-1}) (Fig. 4.3a). The highest total flavonoid contents were observed in accession Asya (0.569 mg mL^{-1}) followed by Agri Tohum and Agri, while Yerli accession showed the lowest 0.476 mg mL^{-1} (Fig. 4.3b). The accessions (Agr Tohum and BT-Altinkulak) showed the highest anthocyanin concentration (1.09 and 1.06 µg mL^{-1}), while the accessions Gungurler and Yesim-I showed the lowest anthocyanin contents (0.68 µg mL^{-1}) (Fig. 4.4a). Similarly, accessions Yesim-II, Tunc, and Mercan were noted for the highest total antioxidant content (Fig. 4.3c), chlorophyll a and b, respectively (Fig. 4.5a, b), while accession Gungurler showed the lowest in total antioxidant content, chlorophyll a, and b. Accessions Yerli and Sari Farik showed the highest total chlorophyll contents (13.6 and 13.2 mg mL^{-1}), while accessions Noroz Nazlisi and Gungurler showed the lowest total chlorophyll contents

(Fig. 4.5c). Similarly, the highest total carotenoid contents (1.56, 1.49, 1.4, and 1.39 µg mL^{-1}) were observed for accessions BT-Altinkulak, Yagmur, Tunc, and Yesim-II, respectively, followed by Mercan and Agr Tohum, while the lowest total carotenoid contents were noted for accessions Sun Agri and Duzce (0.75 µg mL^{-1}) (Fig. 4.4b). Moreover, the highest β-carotene contents were recorded for the accessions Simagro and Mercan (5.14 and 5.11 µg mL^{-1}) followed by Agr Tohum, while the lowest β-carotene contents were noted from the accession Balikesik (4.03 µg mL^{-1}) (Fig. 4.4c).

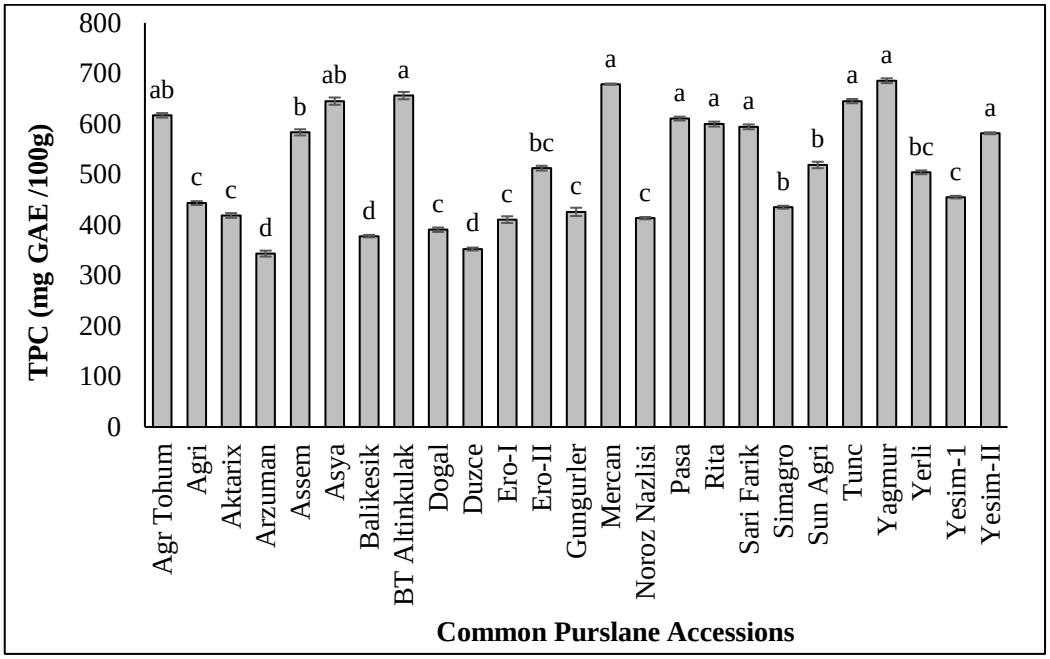

Figure 4.3. (a) Total phenolic contents of different common purslane accessions
TPC= Total phenolic contents; The vertical bars on the histograms are SE while the histograms with different letters are significantly different from each other at $p \leq 0.01$ according to the Least Significant Difference (LSD) test.

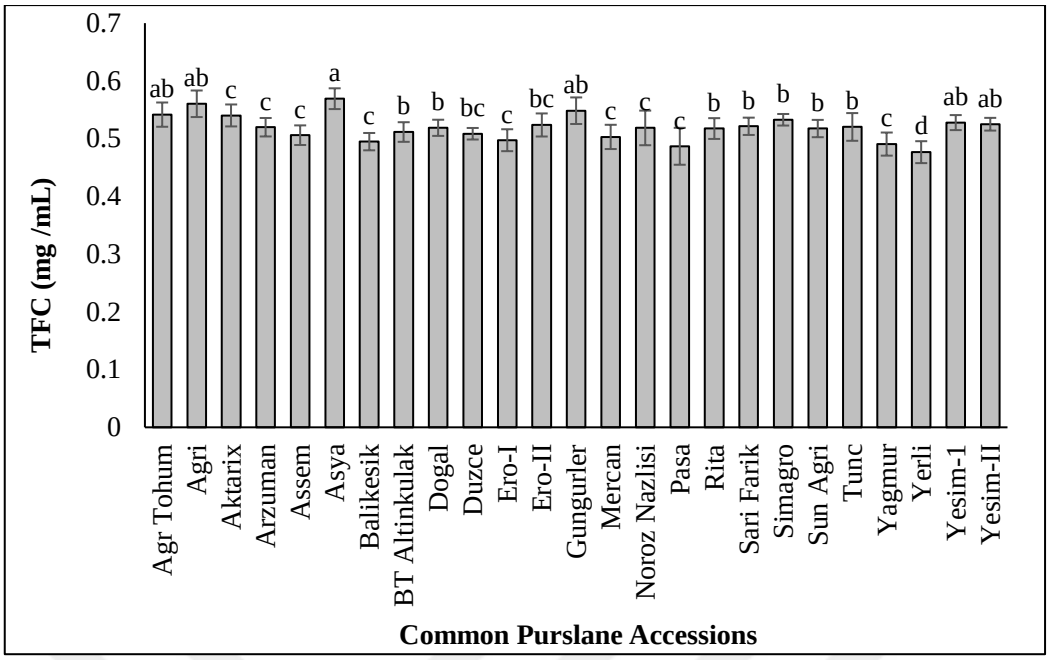

Figure 4.3. (b) Total flavonoid contents of different common purslane accessions

TFC= Total flavonoid contents; The vertical bars on the histograms are SE while the histograms with different letters are significantly different from each other at $p \leq 0.01$ according to the Least Significant Difference (LSD) test.

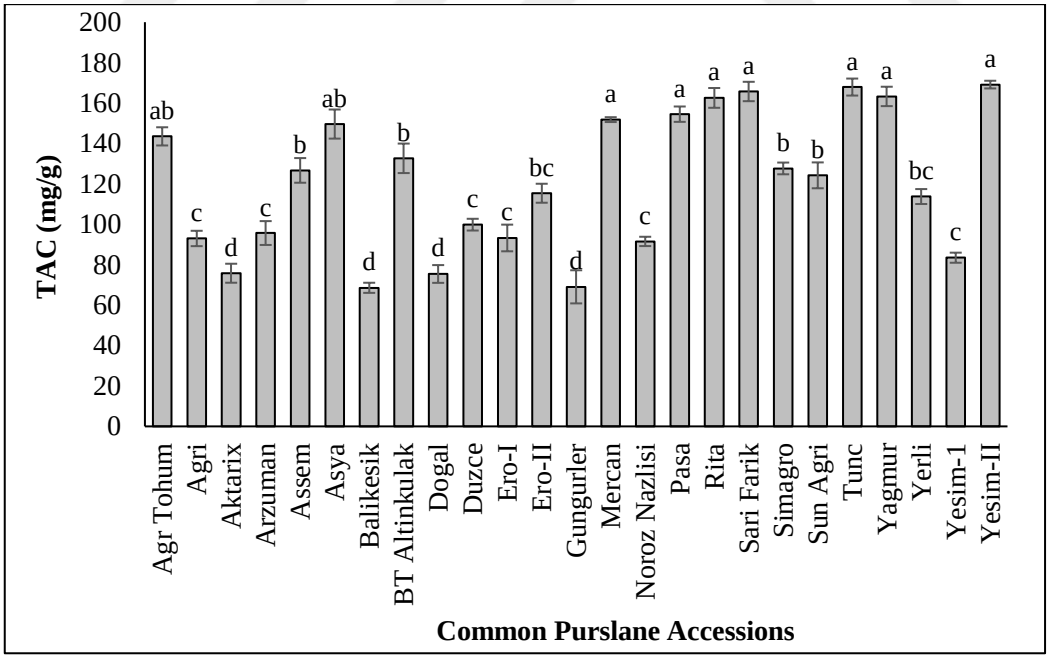

Figure 4.3. (c) Total antioxidant contents of different common purslane accessions

TAC= Total antioxidant contents; The vertical bars on the histograms are SE while the histograms with different letters are significantly different from each other at $p \leq 0.01$ according to the Least Significant Difference (LSD) test.

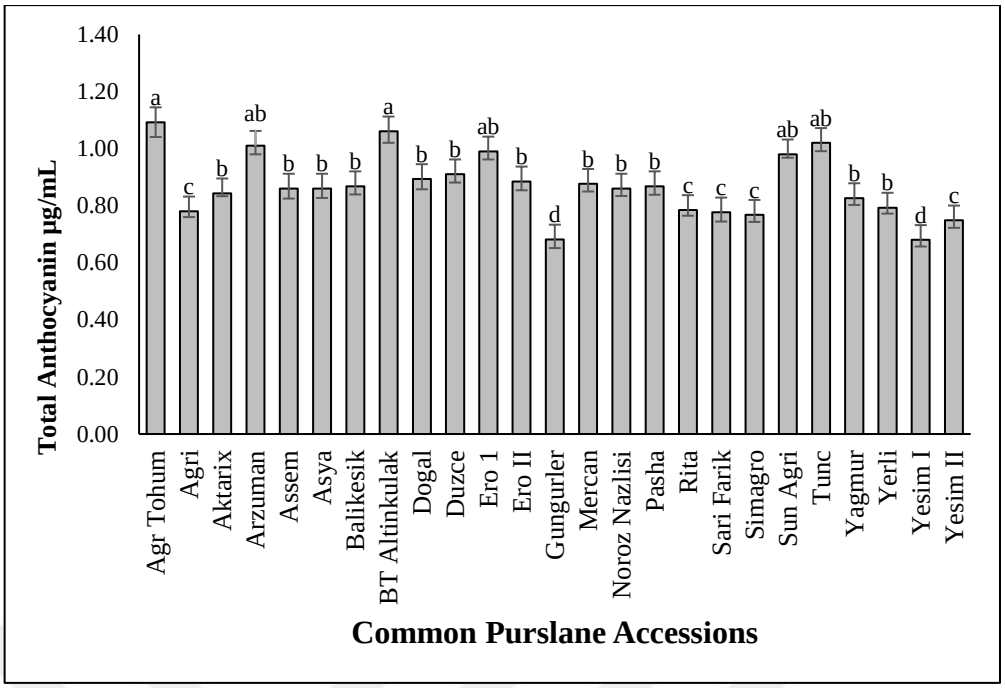

Figure 4.4. (a) Total anthocyanin contents of different common purslane accessions

The vertical bars on the histograms are SE while the histograms with different letters are significantly different from each other at $p \leq 0.01$ according to the Least Significant Difference (LSD) test.

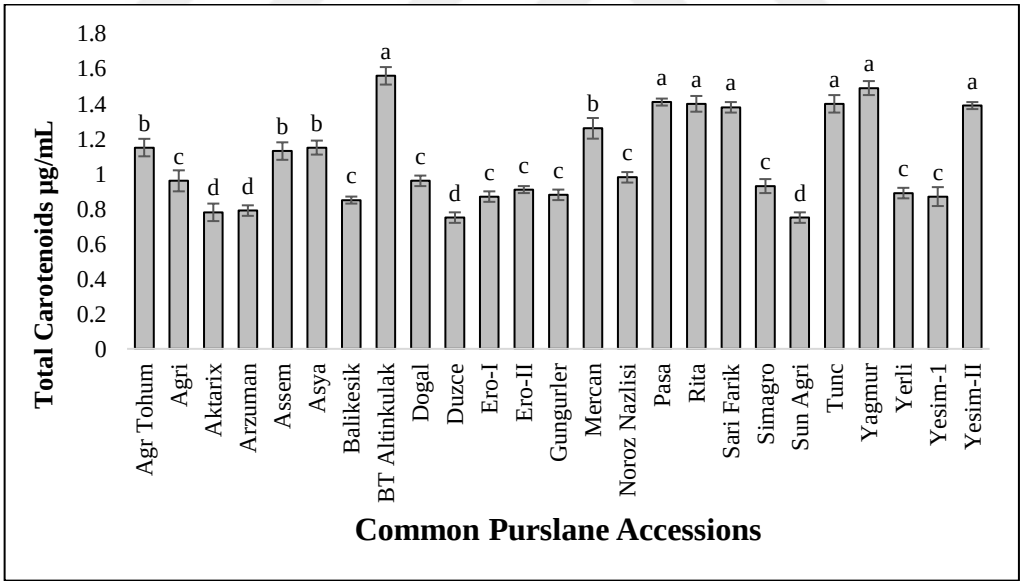

Figure 4.4. (b) Total carotenoids contents of different common purslane accessions

The vertical bars on the histograms are SE while the histograms with different letters are significantly different from each other at $p \leq 0.01$ according to the Least Significant Difference (LSD) test.

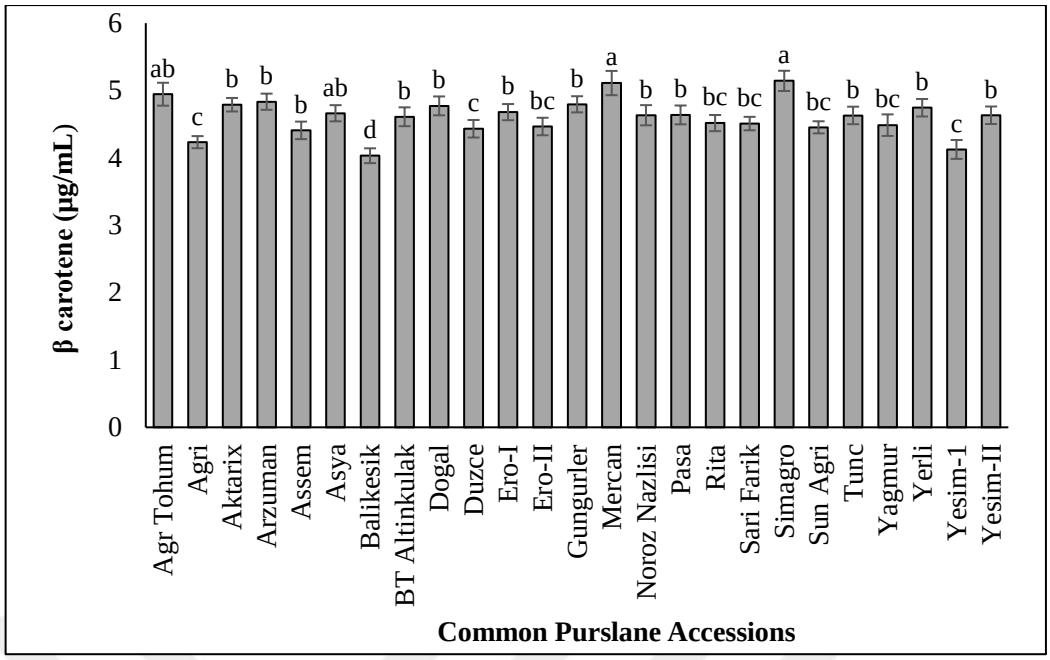

Figure 4.4. (c) β-carotene contents of different common purslane accessions

The vertical bars on the histograms are SE while the histograms with different letters are significantly different from each other at $p \leq 0.01$ according to the Least Significant Difference (LSD) test.

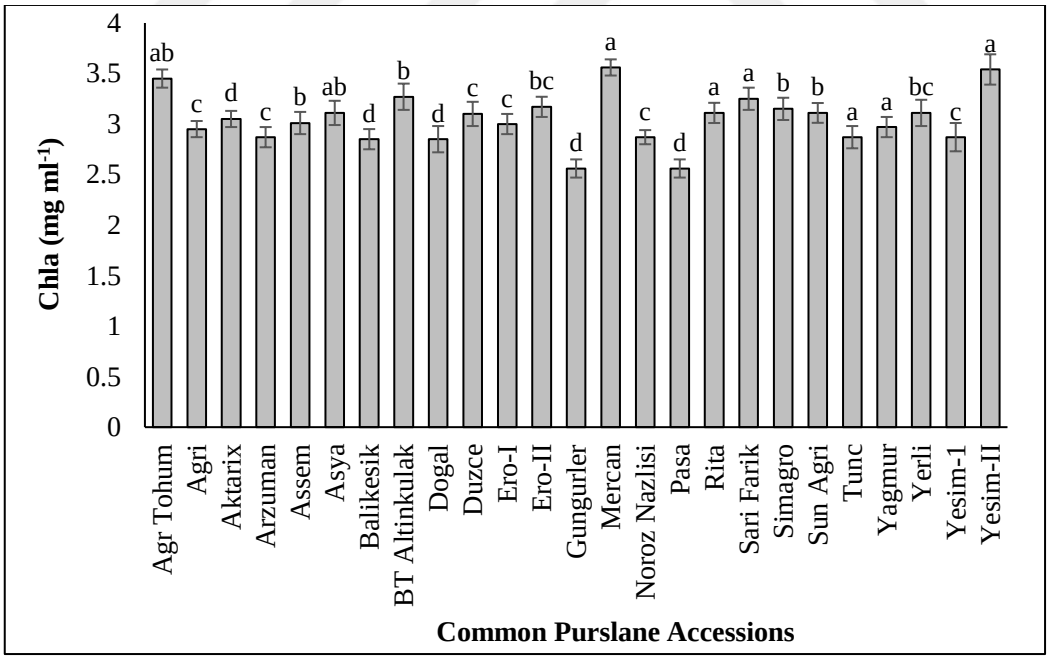

Figure 4.5. (a) Chlorophyll a content of different common purslane accessions

Chl a= Chlorophyll a; The vertical bars on the histograms are SE while the histograms with different letters are significantly different from each other at $p \leq 0.01$ according to the Least Significant Difference (LSD) test.

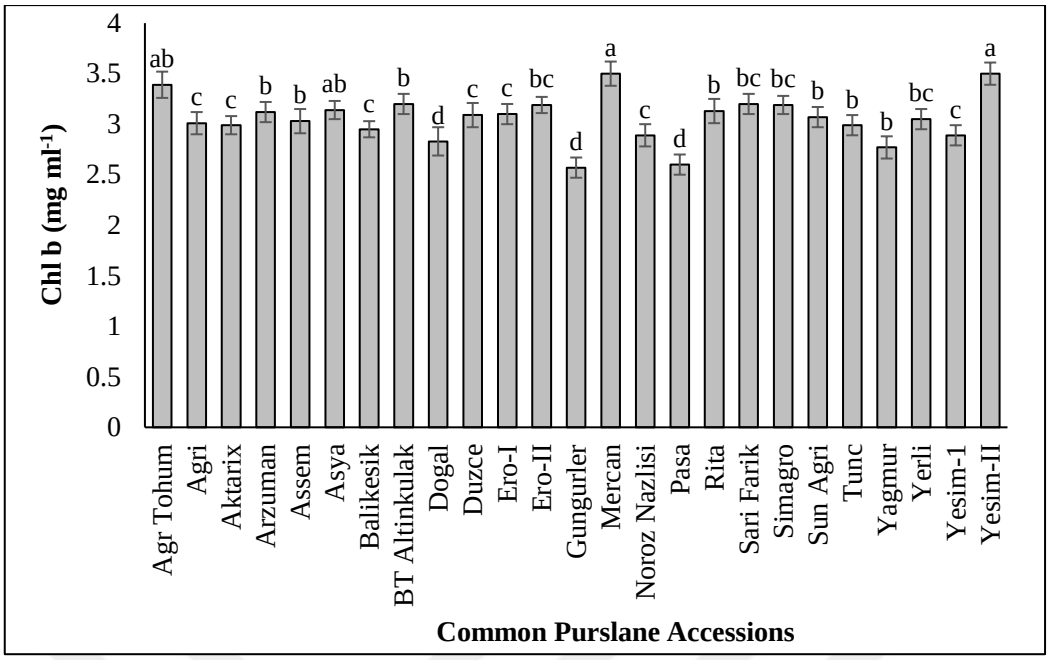

Figure 4.5. (b) chlorophyll b contents of different common purslane accessions

Chl b= Chlorophyll; The vertical bars on the histograms are SE while the histograms with different letters are significantly different from each other at $p \leq 0.01$ according to the Least Significant Difference (LSD) test.

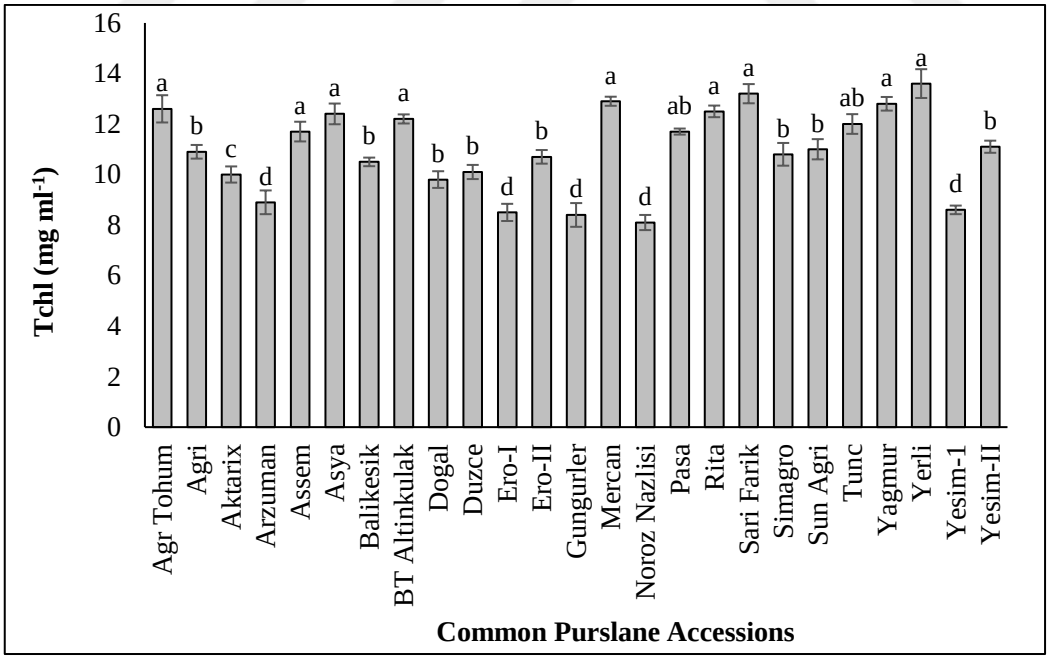

Figure 4.5. (c) Total chlorophyll contents of different common purslane accessions

Tchl= Total chlorophyll; The vertical bars on the histograms are SE while the histograms with different letters are significantly different from each other at $p \leq 0.01$ according to the Least Significant Difference (LSD) test.

The mean values of lycopene contents from various common purslane accessions are shown in Figure 4.6. The level of lycopene was substantially greater in accession Assem 52.95 mg g^{-1} FW followed by Balikesik and Pasa. Whereas the accessions of Arzuman and Gungurler were statistically similar to Balikesik and Pasa accessions. The lowest lycopene concentration was recorded in Yagmur accession 10.52 mg g^{-1} FW which is statistically similar to accession Sari Farik (Fig. 4.6). The highest lutein content was recorded in accession Assem 37.33 mg g^{-1} FW followed by Agr Tohum and Yesim-I accessions. The lowest lutein content was recorded for the accession Arzuman with a statistical value of 12.63 mg g^{-1} FW (Fig.4.7).

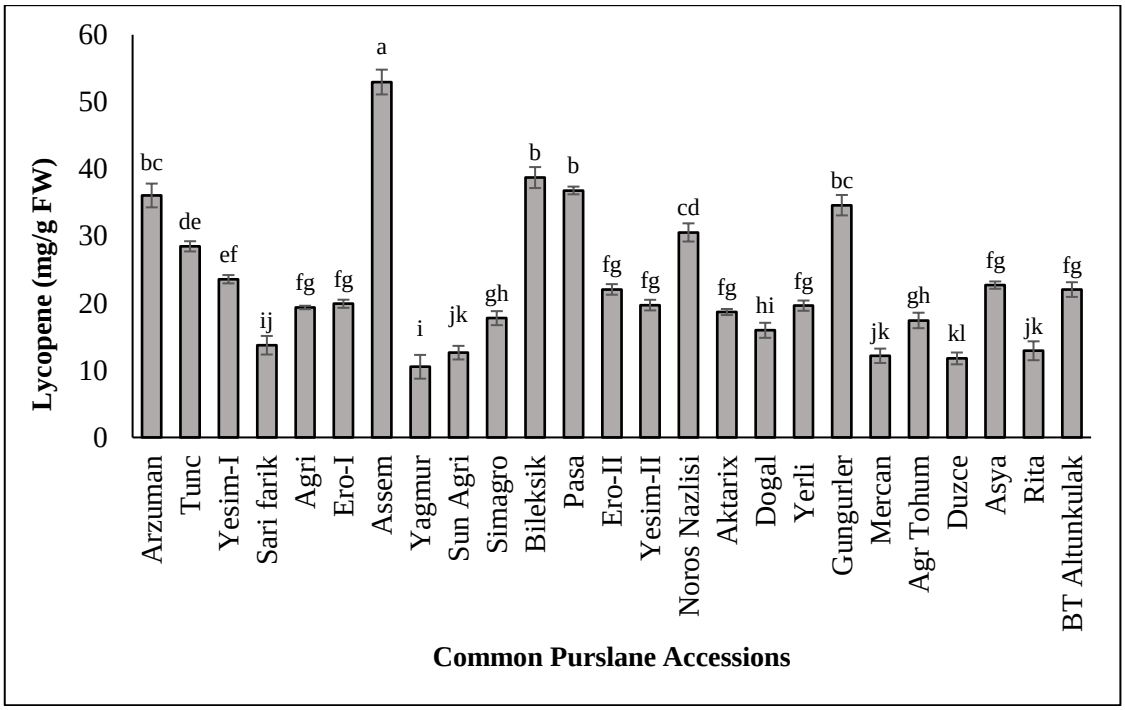

Figure 4.6. Lycopene analysis of common purslane

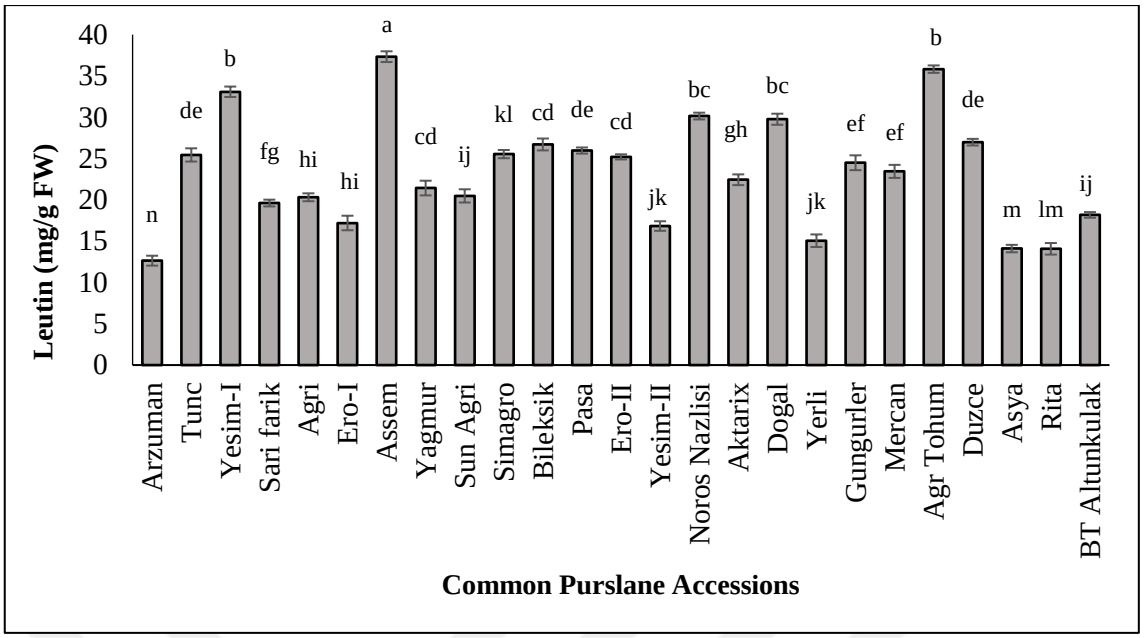

Figure 4.7. Lutein analysis of common purslane

Our evaluation explored the antioxidant activities of the collected common purslane accessions. The FRAP values were found significantly different in the collected common purslane accessions (Fig. 4.8). The highest FRAP concentration was found in BT Altinkulak 8.23 mg g^{-1} FW, while the Rita accession was recorded for the lowest FRAP concentration of 3.00 mg g^{-1} FW. The accession BT Altinkulak was also significantly similar to the accessions Yesim-I and Sari Farik. The CUPRAC assay statistical analysis shows a significant variation in the collected common purslane accessions. The highest CUPRAC was observed in BT Altinkulak 123.44 mg g^{-1} FW, while the lowest was recorded in Tunc 24.17 mg g^{-1} FW (Fig. 4.9).

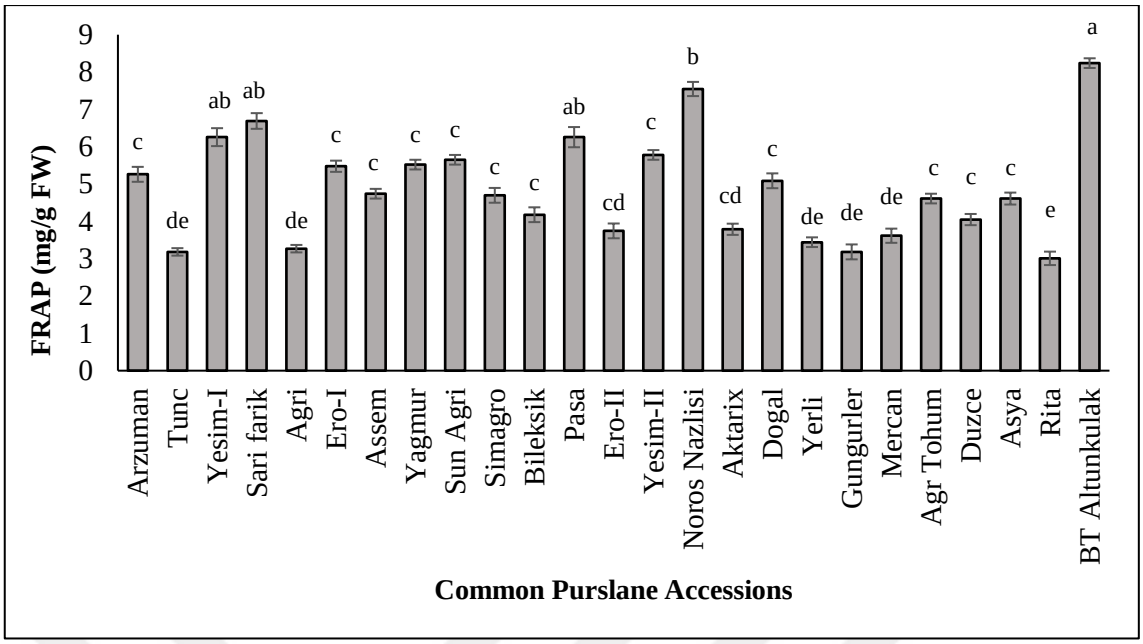

Figure 4.8. FRAP analysis of common purslane

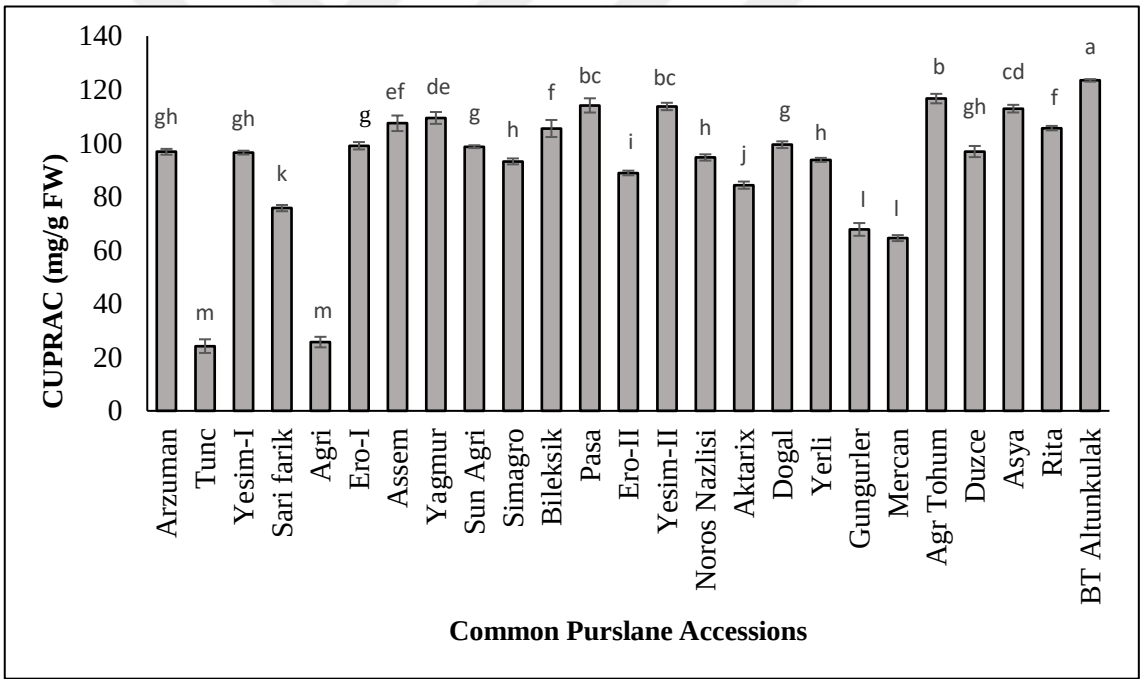

Figure 4.9. CUPRAC analysis of common purslane

The antioxidant activity of collected common purslane was measured by DPPH assay is presented in (Fig. 4.10). The highest antioxidant activity was shown by the accession Sun Agri 0.79 % scavenging DPPH, while the accession Agri showed the lowest 0.39% scavenging DPPH. The analyzed data was almost similar for almost accessions. The statistically analyzed data of the collected common purslane demonstrated that the results

are not distinguishable from one another. The highest TEAC values were recorded for the accession Yagmur 135.33 mg g^{-1} FW, while the lowest was found in accession Duzce 66.25 mg g^{-1} FW (Fig. 4.11).

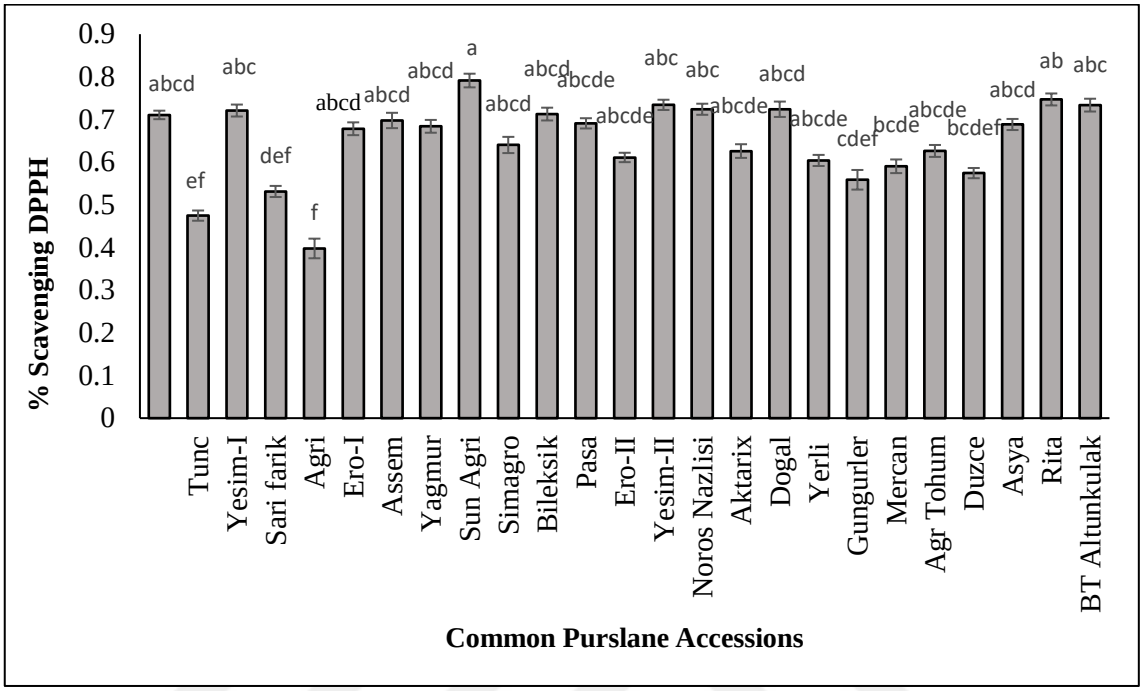

Figure 4.10. DPPH analysis of common purslane

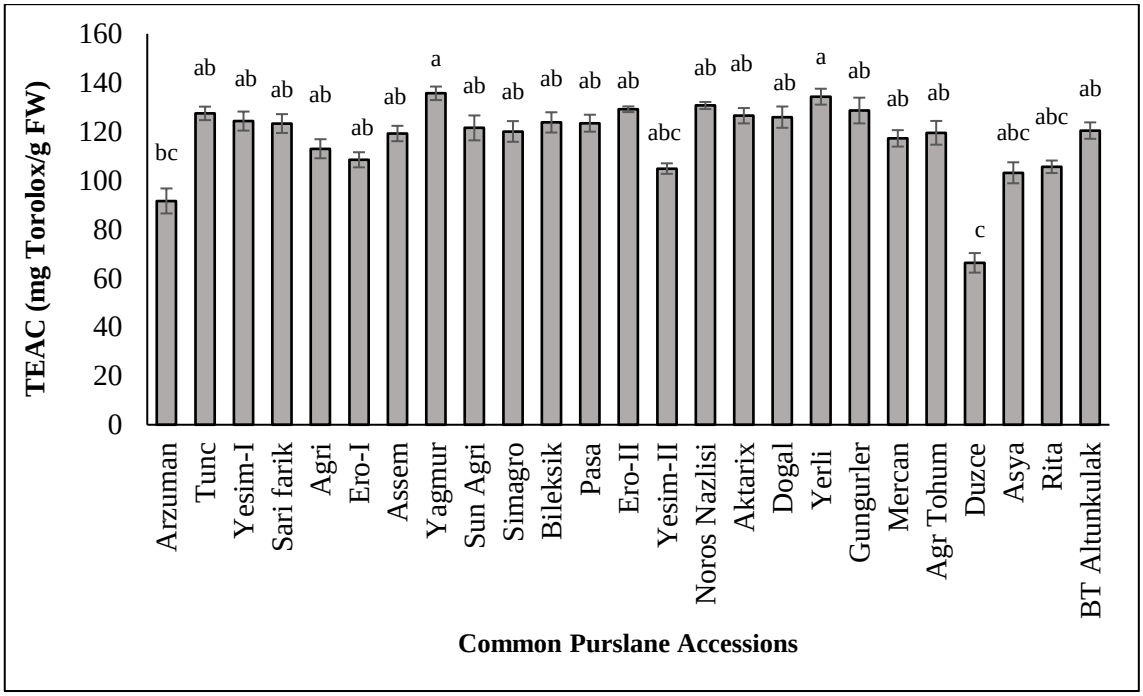

Figure 4.11. TEAC analysis of common purslane

As seen in (Fig.4.12) the reducing power of the collected common purslane accessions were significantly different from each other ranging from 0.701 to 0.316 Trolox mg g^{-1} FW. Where the accession Ero-I was recorded for the highest reducing power expressing the highest antioxidant power, while the accession sari Farik was revealed for the lowest reducing power with the minimum antioxidant and scavenging properties.

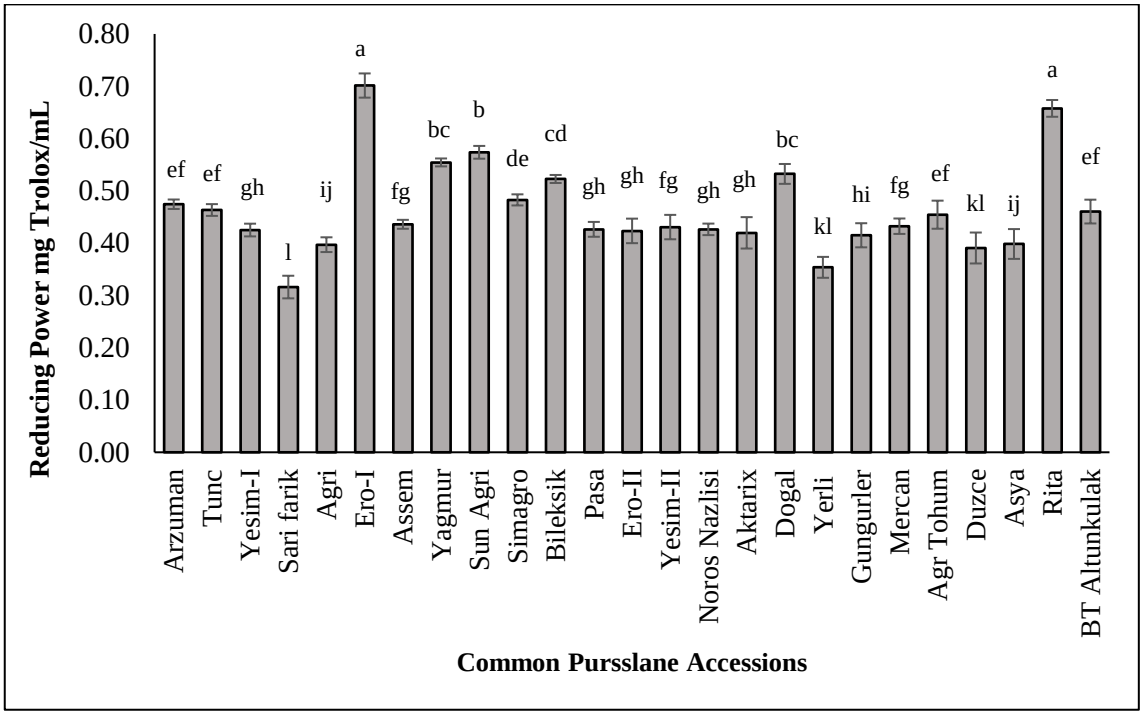

Figure 4.12. Reducing power analysis of common purslane

In our current research, the concentration of TSP ranged from 14.00 to 8.2 mg g^{-1} FW (Fig. 4.13), which showed notable variation amongst the common purslane accessions. The highest concentration of TSP was found in accession Pasa, while the lowest concentration was observed in Agri accession. Our results also indicated a significant variation in the GB concentration. The GB concentration ranged from 4.8 to 1.30 mg g^{-1} FW (Fig.4.14).

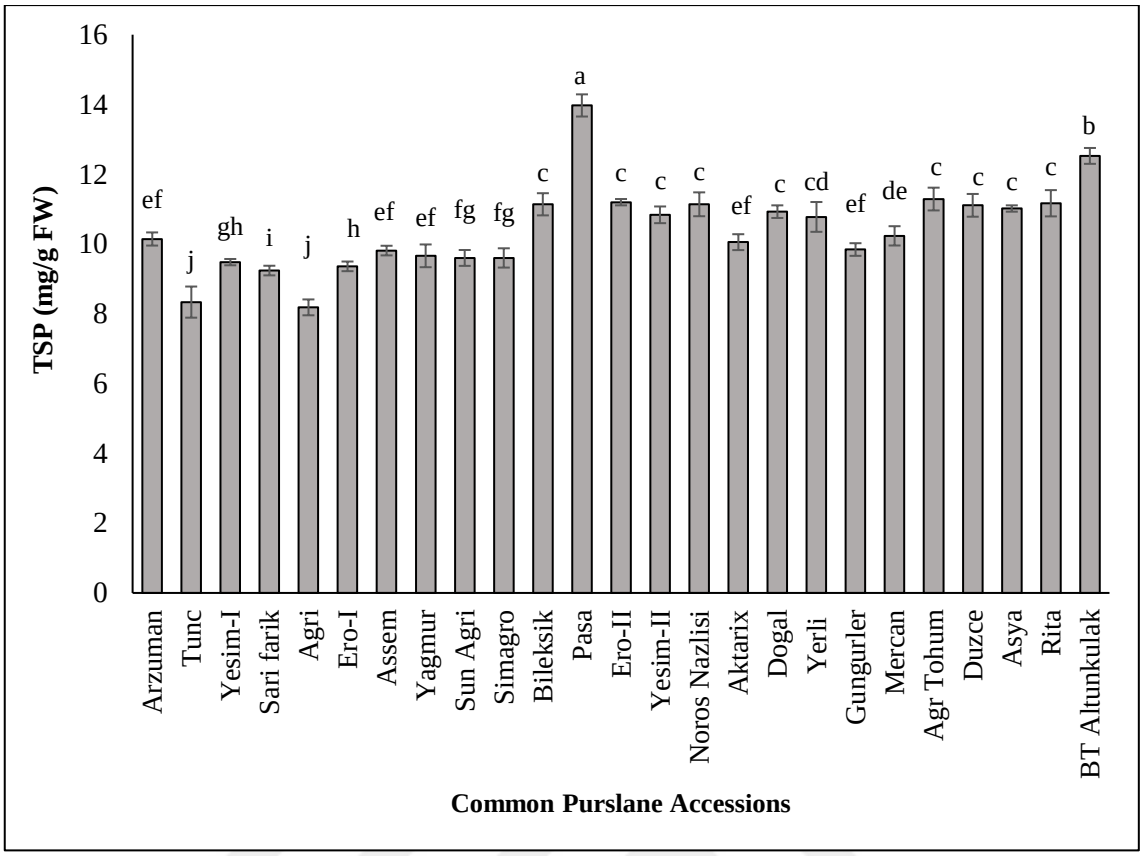

Figure 4.13. TSP analysis of common purslane

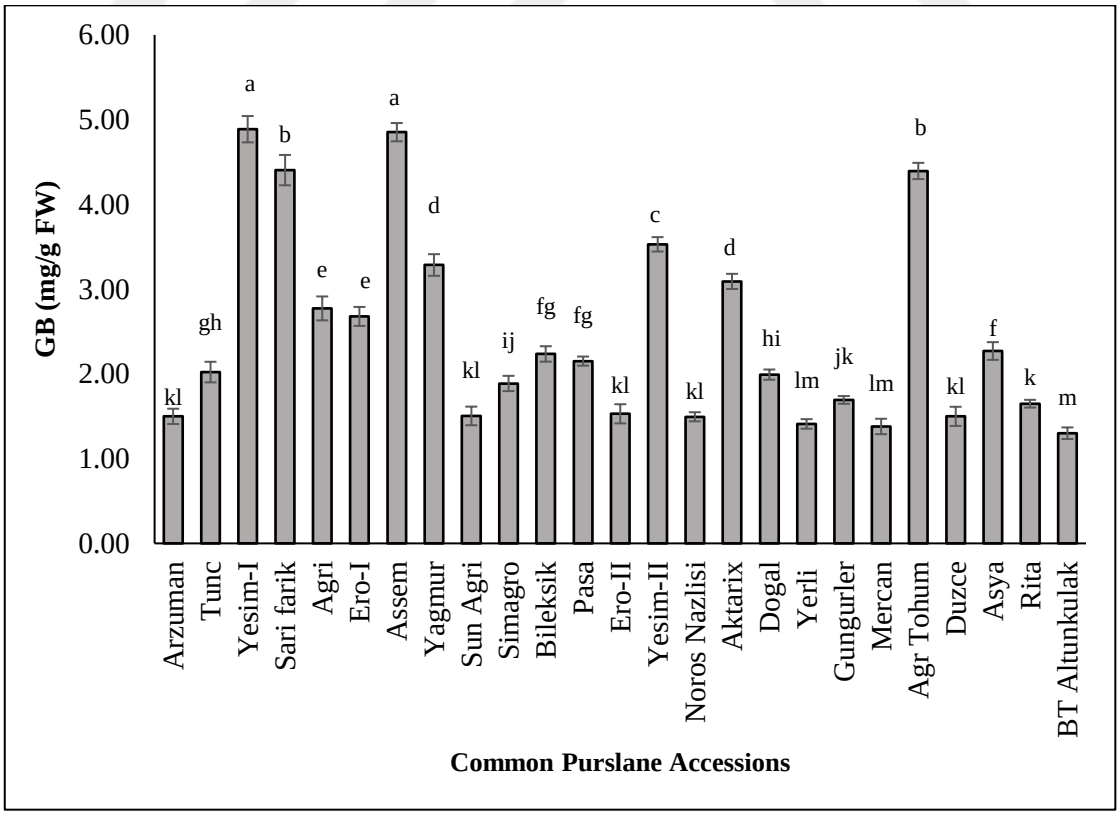

Figure 4.14. GB analysis of common purslane

The total ascorbic acid content of 25 different common purslane studied differed significantly are shown in Figure 4.15. The ascorbic acid content of 25 different common purslane populations ranged from 93.65 to 53.25 mg kg^{-1}. Surprisingly, common purslane accession collected from the Nigde province of Turkiye had much higher total ascorbic acid than other accessions.

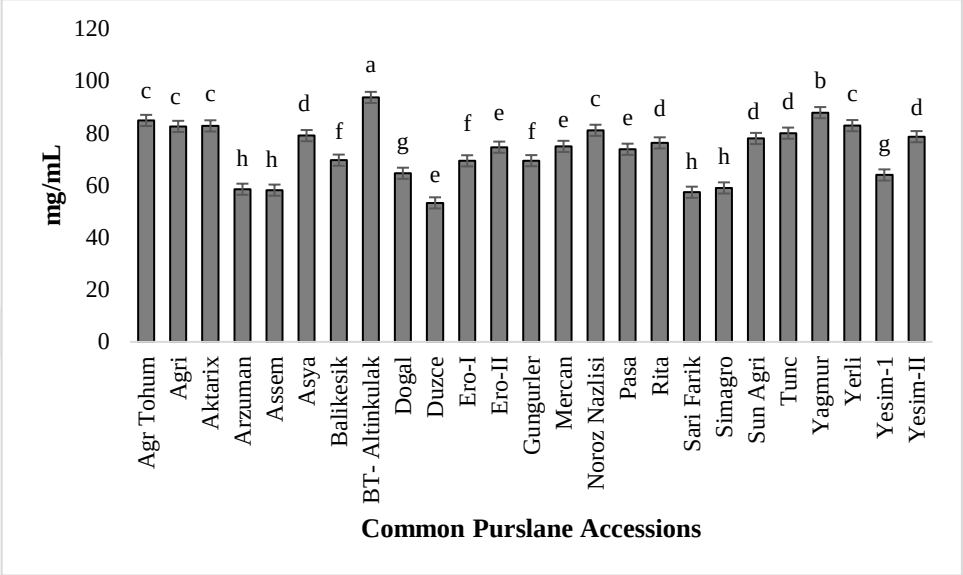

Figure 4.15. Ascorbic Acid concentration in collected common purslane accessions

The total alkaloid concentration of the collected common purslane accessions ranged from 18.47 to 11.23 mg g^{-1}. The highest alkaloid concentration was recorded in the Agr Tohum accession (Fig. 4.16).

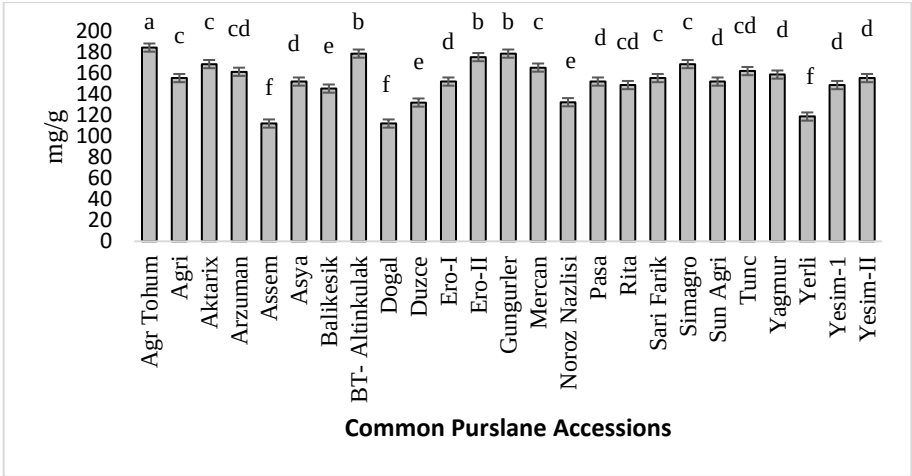

Figure 4.16. Total alkaloid concentration in collected common purslane accessions

The hydrogen peroxide concentration ranged from 556.77 to 307.51 mol g^{-1} (Fig. 4.17). The highest CAT activity was recorded in BT-Altinkulak (Fig. 4.18). As shown in figure 5 and 6 the highest SOD (1004.33 mmol min^{-1} mg^{-1}) (Fig. 4.19) and APX (17.60 mmol min^{-1} mg^{-1}) activity was exhibited in BT-Altinkulak accession collected from Nigde region of Turkiye (Fig. 4.20). A huge significant difference of SOD and APX activities were recorded in the collected common purslane accessions.

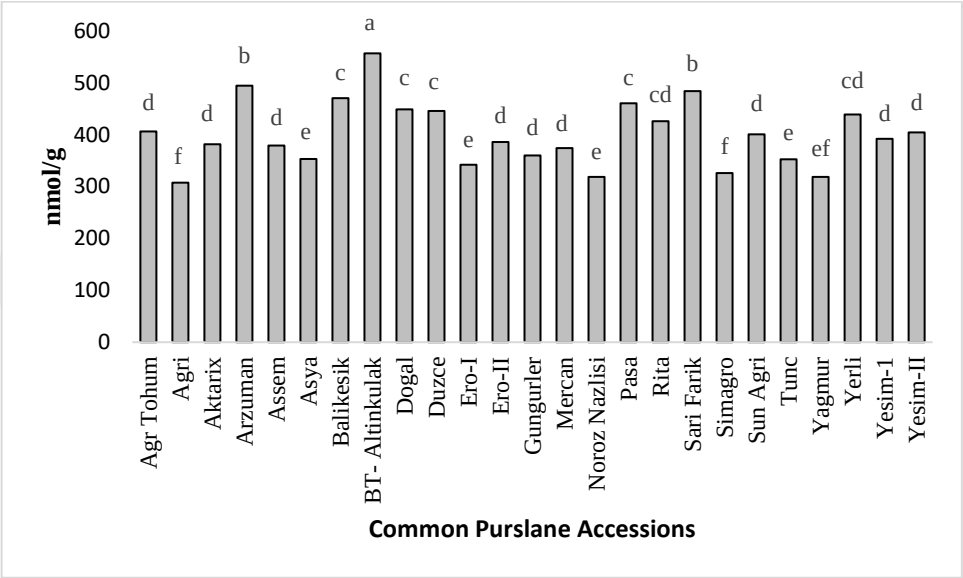

Figure 4.17. Hydrogen peroxide activity in collected common purslane accessions

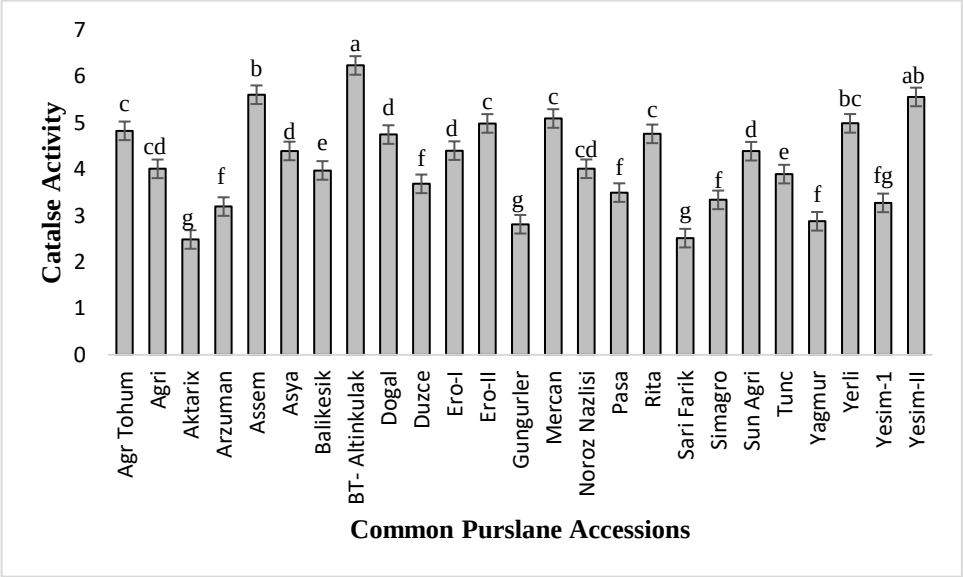

Figure 4.18. CAT antioxidant activity in collected common purslane accessions

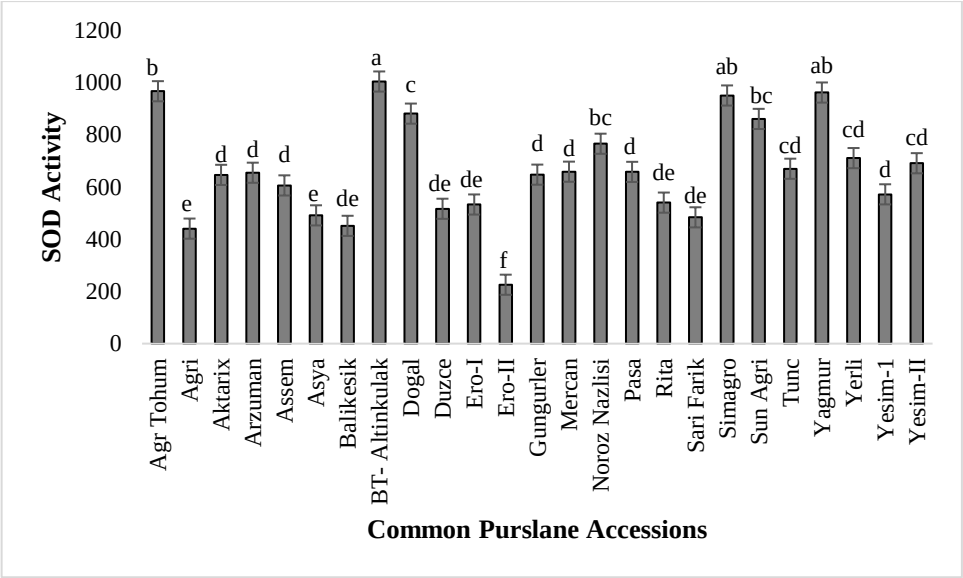

Figure 4.19. SOD antioxidant activity in collected common purslane accessions

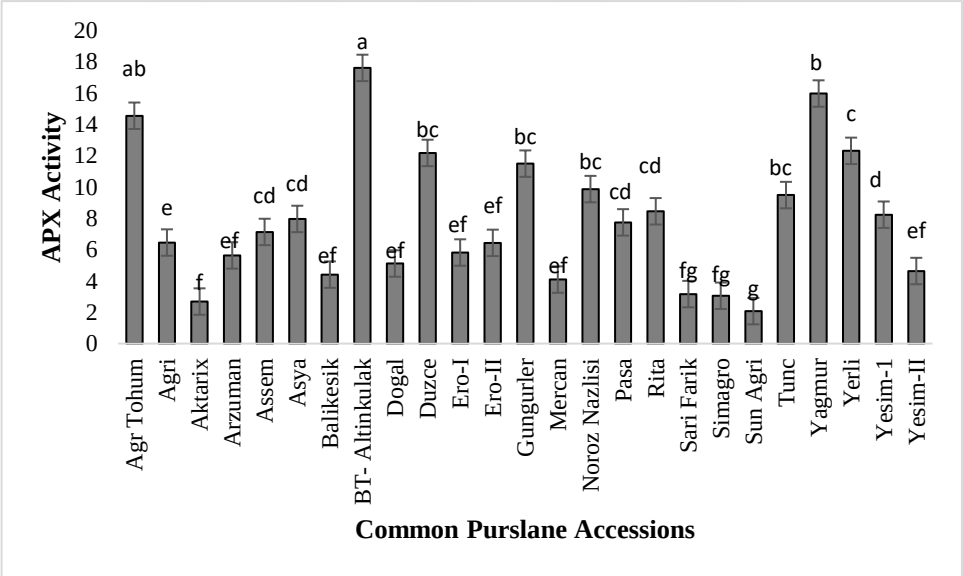

Figure 4.20. APX antioxidant activity in collected common purslane accessions

4.4 Genetic Diversity

To calculate the genetic variation of the collected common purslane accessions, 25 ISSRs were applied. The ISSR primers that were examined had PIC values varying between 0.03 to 0.43, with a weighted mean of 0.24, demonstrating the effectiveness and variety of ISSRs used in our research (Table 4.3). The average for the number of alleles was observed 5.4 (Table 4.3). Whereas the average of no of the polymorphic band was recorded at 4.4 (Table 4.3).

Table 4.3. Genetic variability of collected common purslane accessions

Markers	No. of alleles observed	No. polymorphic bands	% of polymorphic bands	PIC values
1	6	4	66.67	0.24
2	4	4	100.00	0.25
3	3	3	100.00	0.12
4	5	5	100.00	0.41
5	5	4	80.00	0.33
6	6	5	83.33	0.28
7	6	5	83.33	0.31
8	7	6	85.71	0.20
9	5	4	80.00	0.21
10	6	6	100.00	0.31
11	4	1	25.00	0.05
12	4	1	25.00	0.03
13	6	5	83.33	0.34
14	5	5	100.00	0.38
15	6	6	100.00	0.37
16	7	7	100.00	0.43
17	8	5	62.50	0.14
18	7	7	100.00	0.39
19	5	3	60.00	0.12
20	5	4	80.00	0.29
21	6	4	66.67	0.29
22	5	2	40.00	0.05
23	5	5	100.00	0.19
24	5	5	100.00	0.21
25	5	4	80.00	0.23

4.4.1 Cluster Analysis

The NTSYS application was utilized to perform a cluster analysis on the ISSR marker values. The UPGMA coefficient approach was used to build the resemblance. To organize the common purslane accessions into a phylogenetic tree, cluster analysis was performed. The 25 common purslane accessions were divided into primary clusters from this phylogenetic tree with a coefficient of 0.80 and a similar coefficient ranging from 0.72 to 0.88. Where common purslane accessions s1 and s3 were closely related to each other on the similarity index. While s5 and s6 were observed similar to each other having the least genetic difference (Fig. 4.21) (Table 4.4).

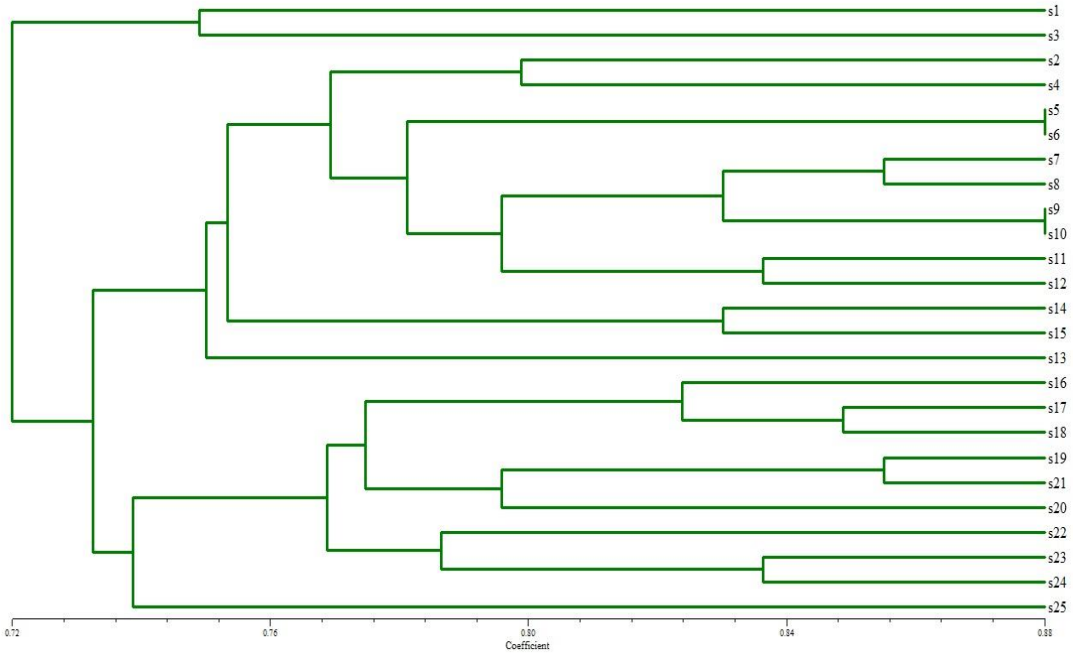

Figure 4.21. Dendrogram tree describing the genetic relationship among the common purslane accessions

Table 4.4. Similarity index genetic relationship among the common purslane accessions

	s1	s2	s3	s4	s5	s6	s7	s8
s1	1.0000							
s2	0.7188	1.0000						
s3	0.7500	0.7438	1.0000					
s4	0.7562	0.8000	0.7813	1.0000				
s5	0.7188	0.7500	0.7937	0.8000	1.0000			
s6	0.7125	0.7813	0.7500	0.7813	0.8812	1.0000		
s7	0.7250	0.7688	0.7625	0.7937	0.8313	0.8250	1.0000	
s8	0.7063	0.7750	0.7312	0.7625	0.7875	0.7937	0.8562	1.0000
s9	0.6937	0.7500	0.7188	0.7625	0.7750	0.7688	0.8438	0.8250
s10	0.6875	0.7813	0.7125	0.7688	0.7937	0.7875	0.8250	0.8313
s11	0.7125	0.7813	0.7125	0.7688	0.7688	0.7875	0.8125	0.8438
s12	0.7125	0.7438	0.7125	0.7562	0.7312	0.7375	0.7625	0.7937
s13	0.6813	0.7125	0.7188	0.7625	0.7125	0.7063	0.7813	0.7750
s14	0.6813	0.7250	0.7438	0.7500	0.7500	0.7438	0.7438	0.8000
s15	0.7250	0.7688	0.7625	0.7937	0.7438	0.7750	0.7375	0.7937
s16	0.6750	0.7688	0.7000	0.7312	0.7188	0.7625	0.7250	0.8063
s17	0.7562	0.7750	0.7188	0.7625	0.7625	0.8063	0.7438	0.8125
s18	0.7312	0.7750	0.7312	0.7625	0.7500	0.7562	0.7562	0.8125
s19	0.7188	0.7500	0.7688	0.7625	0.7750	0.7438	0.7562	0.7750
s20	0.7625	0.6687	0.6875	0.7063	0.6563	0.6500	0.6500	0.6687
s21	0.7000	0.6813	0.7375	0.7438	0.7063	0.7000	0.7250	0.7188
s22	0.7625	0.7438	0.7375	0.7937	0.7312	0.7125	0.7000	0.7438
s23	0.7000	0.7312	0.6750	0.7312	0.6687	0.7000	0.6500	0.7188
s24	0.7000	0.7562	0.7375	0.7188	0.7312	0.7375	0.7250	0.7813
s25	0.6375	0.7438	0.6500	0.6937	0.6813	0.7375	0.6750	0.7188

	s9	s10	s11	s12	s13	s14	s15	s16
s9	1.0000							

s10	0.8812	1.0000						
s11	0.8063	0.7875	1.0000					
s12	0.8063	0.7625	0.8375	1.0000				
s13	0.7375	0.7438	0.7688	0.8063	1.0000			
s14	0.7500	0.7188	0.7562	0.7438	0.7250	1.0000		
s15	0.7188	0.7250	0.7750	0.7750	0.7813	0.8313	1.0000	
s16	0.7438	0.7375	0.7500	0.7750	0.7188	0.7562	0.8000	1.0000
s17	0.7625	0.7688	0.7937	0.7688	0.7500	0.7625	0.7813	0.8313
s18	0.7500	0.7813	0.7562	0.7312	0.7500	0.7500	0.7813	0.8187
s19	0.8125	0.7937	0.7312	0.7562	0.7250	0.7250	0.7312	0.7813
s20	0.6937	0.6875	0.6875	0.7000	0.6813	0.6813	0.6875	0.7125
s21	0.7438	0.7250	0.7125	0.7000	0.6813	0.7438	0.7125	0.7250
s22	0.7188	0.7625	0.7250	0.7125	0.7063	0.7312	0.7750	0.7750
s23	0.6937	0.7250	0.7125	0.7000	0.6687	0.7312	0.7375	0.7375
s24	0.7562	0.7375	0.8000	0.7750	0.7188	0.7813	0.7500	0.7875
s25	0.6687	0.7000	0.7500	0.7000	0.6563	0.6813	0.7375	0.7500

	s17	s18	s19	s20	s21	s22	s23	s24
s17	1.0000							
s18	0.8500	1.0000						
s19	0.8125	0.8375	1.0000					
s20	0.7813	0.7562	0.8063	1.0000				
s21	0.7813	0.7937	0.8562	0.7875	1.0000			
s22	0.7813	0.7813	0.7688	0.7625	0.7750	1.0000		
s23	0.7562	0.7688	0.7813	0.7750	0.7875	0.8000	1.0000	
s24	0.7813	0.7688	0.7937	0.7375	0.7375	0.7750	0.8375	1.0000
s25	0.7562	0.7562	0.7312	0.6750	0.7000	0.7375	0.7625	0.7875

	s25
s25	1.0000

CHAPTER V

DİSCUSSİON

5.1 Discussion

We assessed the various morphological analysis, phenolic compounds, antioxidant activities, and enzyme activity of common purslane accessions to explore their biochemical makeup. A significant variation was observed across the common purslane population's bio-active components.

5.2 Morphological Analysis

There was substantial variation across all 25 common purslane accessions and assessed attributes in our research (Table 4.1 and 4.2), which demonstrates distinct phenotypic diversity among the materials obtained from various sites in Turkiye. This is an important feature for breeders to choose better parents from bulk materials to develop superior lines, besides this, it will also help in product development for food and pharamcuitecual industries as more plant yield in terms of plant height and the highest number of leaves.

In this study, a good degree of morphological diversity was observed in several of the common purslane accessions. The agro-morphological research is the simplest and most straightforward method for plant taxonomy description (Ebadi-Segheloo et al., 2014). Qualitative and quantitative characteristics are known as major indicators in characterizing a plant's physical appearance and for the natural selection process of plants, and socioeconomic configuration (Rashid et al., 2020). Accessions in this study varied in terms of plant height. The difference in their plant height exhibited genetic differences among the accessions. It was also corroborated by the results of this study that Dogal accession showed different growing nature (prostrate) compared to other accessions (erect) (data not shown). This variation in morphology with substantial variances indicates that our accessions have high genetic variability. Salah and Chemli (2004) also discovered that certain common purslane populations grow straight while others grow supine. The plant's foundation is its roots which provide anchorage and necessary nutrients during the development phase (Den Herder et al., 2010). In this study, the

accession Assem collected from the Kirsehir region of Türkiye showed better root characteristics compared to other accessions. It might have a higher potential to uptake and accumulate nutrients in leaves. The accession Yerli from Nevsehir had the highest plant fresh weight and it was also found to have the highest moisture content. Hence, it was confirmed that the fresh weight of the plant is responsible to retain moisture content (Alam et al., 2014). This morphological heterogeneity has been studied in earlier studies (Sdouga et al., 2019; Sdouga et al., 2020), which demonstrated that this plant (common purslane) has significant morphological adaptability, which might be due to its genetic variability (Ocampo and Columbus, 2012). Moreover, the accessions also showed a significant variation in stem fresh weight. Accession obtained from the Niğde region showed the highest stem fresh weight. Furthermore, the obtained results of our study, in terms of morphological characteristics are in agreement with previous studies (Alam et al., 2014; Egea-Gilabert et al., 2014; Lokhande et al., 2009) also observed that common purslane is a very heterogeneous and variable plant, particularly morphologically.

The association of many economically relevant qualities might lead to a better knowledge of the interdependence of characters, finally delivering an enhanced approach for any crop. Therefore, a stronger character correlation might be utilized to assess other features (Eivazi et al., 2008; Fereidoonfar et al., 2018). Mohebodini et al. (2016) worked on 20 different common purslane accessions and found that there was a significant positive relationship between various morphological traits. A positive correlation of qualities indicates that improving one can help to enhance the other (Yücel et al., 2009) (Fig. 4.1a). Principle component analysis (PCA) is a dependable multivariate approach for converting a big data set into a few components including multiple inter-correlated variables (Pachauri et al., 2017). By minimizing the quantity of data, this technique can indicate the magnitude of diversity among a large number of populations (Fereidoonfar et al., 2018). Several studies (Khadivi, 2018; Pachauri et al., 2017; Loko et al., 2018) have demonstrated the significance of this technique for complete crop evaluation similar to our present study (Fig. 4.1a, b).

5.3 Product Development

Color is recognized as a crucial physical attribute of foodstuffs and agricultural goods (Abdullah et al., 2004). The customers' review of the baked cookies in terms of smell,

color, texture, appearance, taste, and overall acceptability for 10 g quantities revealed that they were excellent (Fig.4.2). When evaluating the color findings, we cannot conclude that the participants are primarily interested in light-colored items because certain ratings are higher for darker ones (20 g quantity). At 10 g quantity, cookies had a lower hardness. The texture is an important quality criterion in the fresh and processed food industries for determining customer desirability (de Assis et al., 2009). In terms of overall acceptability, the cookies with a 10 g quantity showed the highest results than the 20 g and 30 g quantities. A sensory grade of at least 7 (like moderately) is normally necessary to debut a product on the market (Hobbs et al., 2014).

5.4 Biochemical Analysis

There are several medicinal plants across the world, that have been used for centuries in the prevention and treatment of various serious illnesses including cancer. Herbalists have recently focused their attention on common purslane, a medicinal herb that contains anti-cancerous as well as anti-oxidant properties in addition to vast nutritional and therapeutic benefits. As a folk remedy, For millennia, it has been employed to treat a variety of illnesses, like stranguries, coughs, burns, skin disorders, stings, stomach pains, migraines, dyspepsia, and corneal disorders (Nugraha et al., 2020). This plant is high in vitamins, minerals, fatty acids, phenols, and various other antioxidants.

Phenolic compounds are a broad range of biologically important metabolites that have a significant role in the prevention of oxidation processes and can act as free radical scavengers (El Kashef et al., 2018). The biochemical analysis of this plant was explored and quantified by investigating total phenol, flavonoid, anthocyanin, carotenoid, β-carotene, antioxidant, and chlorophyll (a and b), and total chlorophyll contents from their different portions of grown common purslane accessions. Total phenolic concentration is linked to antioxidative activity in a variety of plants (López-Vélez et al., 2003). The total phenolic content of fresh common purslane is shown in (Fig. 4.3a). Common purslane's phenolic content differs according to species, geographical location, season, and time of harvest. The results are supported by Lim and Quah (2007), and El Kashef et al. (2018). Moreover, Chowdhary et al. (2013) indicated that common purslane's total phenolic content was also influenced by other factors, such as the plant's maturity and harvesting stage, etc.

Polyphenols are a broad class of chemicals that include simple phenols, flavonoids (flavonols, flavones, flavanones, isoflavones, and anthocyanins), hydroxybenzoic acid, hydroxycinnamic acid, chalcones, aurones, hydroxycoumarins, and hydroxy stilbenes (Lawag et al., 2022). Flavonoids are one of these phenolic chemicals that are extensively dispersed and found in almost every plant. Nutritional flavonoids have antioxidant and antidiabetic properties in humans, and anti-proliferative characteristics, that might defend the skin against different oxidative stress-related illnesses (Mutha et al., 2021).

Flavonoid analysis in plant foods is a trendy field of study to truly comprehend the relationship between flavonoid consumption and health consequences. A lot of research has been done on flavonoids in popular vegetables, but very little has been done on flavonoids in less often used plants, such as common purslane. As a result, the total flavonoid concentration of common purslane extracts was determined on a fresh weight basis in the current study. The concentration of flavonoid contents presented significant variation in the common purslane accessions (Fig. 4.3b). The flavonoid contents were found almost similar in the grown accessions, while the accession Asya obtained from the Bursa region showed the highest flavonoid contents. The accession Yerli collected from the Nevsehir region showed the lowest flavonoid content. Previously, common purslane flavonoid contents have been reported in the same range as observed in our studies (Lim and Quah, 2007; Uddin et al., 2012). Flavonoid concentration may also fluctuate as a result of other major variables such as genetic divergence and rain-fed circumstances (Zhou et al., 2021).

Anthocyanins, another category of plant pigments, are intracellularly hues that are dissolved in water. Plants use them as antioxidants against reactive oxygen species generated by abiotic stressors such as excessive exposure to UV radiation and high temperatures (Cirillo et al., 2021). Plants use them as antioxidants against reactive oxygen species generated by abiotic stresses such as excessive exposure to UV radiation and high temperatures (Breusegem et al., 2006; Qiu et al., 2016). The highest anthocyanin contents were found in the accessions Agr Tohum and BT-Altinkulak collected from the Niğde region of Turkiye (Fig. 4.4a). According to the study by Silva et al. (2014), the leaves of the common purslane were poor in total anthocyanin which is in contradiction to our

findings; high anthocyanin concentrations in several of the common purslane accessions were observed in this study.

Leafy vegetables are also high in photosynthetic pigments, including chlorophylls and carotenoids. The mixture of these pigments results in the unique color of the foodstuff, and its quantity matches well with the plants' photosynthetic activity (Priyadharshana et al., 2022). They have been shown to protect against coronary heart disease (Anand et al., 2022). As a result, the chlorophyll content of common purslane was analyzed. Leafy vegetables are also high in photosynthetic pigments, including chlorophyll a and b (Thoma et al., 2020). They have been shown to protect against several diseases, including malignancy and coronary heart disease (Znidarcic et al., 2011). In our study, the leaves of common purslane accessions had significant amounts of total chlorophyll, chlorophyll a, and chlorophyll b, which might have positive effects against the illnesses (Youssef et al., 2014). This is also confirmed by Bessrour et al. (2018) that common purslane is an efficient photosynthetic plant.

In the current research, pro-vitamin A (ß-carotene) is a vital and significant nutrient with a series of health-encouraging properties. It is involved in the growth and development of various cells in the human body, especially the eyes (Saini et al., 2022). Pro-vitamin A (β-carotene) is a vital and significant nutrient with a series of health-encouraging properties. From this study, it was observed that β-carotene content varied significantly among the common purslane accessions. A high concentration of β-carotene contents in one of the accessions (Simagro) in this study shows that common purslane can act as a good source of β-carotene (Joshi and Mehta, 2010). Moreover, Sarpong et al. (2019) mentioned that ß-carotene can be decreased on drying because of oxidative degradation.

Common purslane is broadly disturbed around the globe and possesses medicinally important mucilaginous compounds. The present study also revealed a significant amount of antioxidant features and other important biochemicals in this plant. The antioxidant and biochemical analysis profiling of each accession based on their solvent extraction technique and their total concentration values are presented in various figures in this study. The antioxidant properties of the plant may be evaluated using a variety of in vitro tests, each of which is dependent on at least one aspect of antioxidant activity. However, due to the complex structure of phytochemicals, the total antioxidant capabilities of plants cannot be assessed using a single approach (Abeysinghe et al., 2021). As a result, two or

more methodologies must be used to assess the impacts of phytoconstituents from green leafy vegetables, which are often antioxidative. The antioxidant activity of the collected common purslane accessions evaluated in this research varied considerably.

Different plants include bioactive components that may be identified and which help to lessen oxidative stress, which makes it possibly beneficial in treating a variety of severe illnesses. (Ketnawa et al., 2021; Kario et al., 2021). Boosting fruit and vegetable consumption is commonly promoted to prevent or cure chronic diseases. Lycopene and lutein are common carotenoids that are frequently present in our foods and plant tissues (Mapelli-Brahm et al., 2020). Lycopene an important carotenoid is one of such bioactive components that help in various diseases (Khan et al., 2021). As a dietary supplement, lycopene is a natural fiber that may be used in great dosages without endangering the health or metabolism of people. In light of these results, lycopene has drawn considerable attention as a potential nutraceutical for the treatment of many diseases, particularly for enhancing vascular function and reducing blood pressure (Hedayati et al., 2021). The collected common purslane accessions also exhibited a significant amount of lycopene in their edible plant body. Our results are in agreement with Sakil et al. (2018) found a considerable amount of lycopene in Indian spinach. It is also mentioned that plants and vegetables with dark green leaves have a significant amount of lycopene content.

Green leafy vegetables are an essential part of healthy eating regimens and a significant lutein source (Li et al., 2021). Green leafy foods such as spinach contain lutein (kale, broccoli, spinach, lettuce, peas, and common purslane (Mitra et al., 2021; Vijay et al., 2018). Our results also showed a high amount of lutein in common purslane which is found similar (34mg $100g^{-1}$) by the Vietnam National University of Agriculture. Ejoh et al. (2021) researched various green leafy vegetables he also exhibited almost a similar range of lutein concentration in *Basella alba.* According to Mrowicka et al. (2022), kale and other green leafy veggies like spinach, lettuce, broccoli, and cabbage have lutein in their edible parts as 39.55, 11.93, 2.63, 2.44, 0.31 mg g^{-1}, respectively, which shows that the common purslane has the highest nutritive value (lutein) except kale which is almost similar lutein concentration to that of common purslane.

A variety of techniques can be used to study the antioxidant activity of the plant sample but no single standard is proposed because of the complexity of the extracts. In the present

study, four different methods namely FRAP, CUPRAC, TEAC, and DPPH radical scavenging activity were employed to evaluate the anti-oxidant properties of collected common purslane. The accession BT Altinkulak has the highest FRAP and CUPRAC values, while the accessions Sun Agri and Yagmur had the superior TEAC and DPPH radical scavenging activity respectively. Correlation analysis also exhibited a positive correlation relation among the FRAP, CUPRAC, TEAC, and DPPH radical scavenging activity.

In essence, the FRAP assay assesses the capacity of antioxidants to reduce in a hydrophilic condition, and typically, lipid-soluble molecules have lower FRAP values than water-soluble ones (Apak et al., 2004). This outcome is in line with what Lim and Quah determined (2007). This result has some similarities to that of Uddin et al. 2014 found. Alam et al. (2014) also showed similar lowest results for FRAP concentration. The CUPRAC technique is an optical technique that is widely used, and it works by spectrophotometric analysis of the Cu (I)-Nc complex produced by the reduction process of Cu (II)-Nc. It determines the efficacy of bioactive chemicals for detecting oxidative damage and evaluating their antioxidant activity in biological samples or food (Uzunboy et al., 2017; Bayarsaikhan et al., 2019). Our findings in terms of CUPRAC are in agreement with Banerjee et al., 2013. We revealed DPPH scavenging activity Analysis shows some level of consensus with the findings of Uddin et al., 2014. Alam et al., 2014 also found the same antioxidant activity of the common purslane. In terms of TEAC analysis Mihaylova et al., 2021 also found the same TEAC values. Gunathilake and Ranaweera (2016) studied various leafy vegetables for their antioxidant activities and also revealed the reducing power in the same range, which shows that our findings are in agreement with those (Gunathilake and Ranaweera, 2016). Several in vitro assays may be used to determine the antioxidative capacity of plant extracts, and each assay is based on at least one characteristic of antioxidant activity. Nevertheless, due to the complexity of its phytochemicals, plants' overall antioxidant capabilities cannot be assessed by a single approach (Chu et al., 2000). Our findings are consistent with Mihaylova et al. (2021). The antioxidant impact rises sharply as the reducing power develops, demonstrating the rise of reduction. Because of this, they can serve as both primary and secondary antioxidants (Amarowicz et al., 2004).

Recent research brought attention to how crucial plant-based foods are to food sources. One may argue that plant-based protein blends can provide a complete, necessary, and regulated source of amino acids that effectively meets normal metabolic demands (Hertzler et al., 2020). Our findings are consistent with the research that was done by Sarkar et al. (2020) on protein content determination in *Marsilea quadrifolia*. The current experimental plants possess an adequate quantity of proteins, and these leaves may be employed as a protein-enriched food source for nutritional reasons based on the comparative study findings of protein estimates of leaves from different types of plants. Glycine betaine is an osmoprotectant that is produced by many plants in response to abiotic stresses such as drought, salinity, and extreme temperatures. However, glycine betaine can also be present in plants even in the absence of stress. Glycine betaine has been found to play several roles in plants, including in normal growth and development, as well as in stress tolerance (Li et al., 2019). Zhou et al. (2019) studied glycine betaine under salt stress and also found similar results in control analysis which are in agreement with our results. In plants, GB retained a greater photosynthetic rate. When plants are subjected to various environmental stressors, such as salt, GB is biosynthesized (Xu et al., 2018; Li et al., 2019). In addition, measuring glycine betaine levels in plants under non-stress conditions can provide a baseline for comparison with plants exposed to stress. By comparing glycine betaine levels in plants before and after exposure to stress, researchers can determine the extent to which plants can produce or accumulate glycine betaine in response to stress, and whether this response is correlated with stress tolerance (Zhang et al., 1996). In summary, glycine betaine is measured in plants even under non-stress conditions to understand its role in normal plant growth and development, to identify natural variations in its accumulation, and to provide a baseline for comparison with plants exposed to stress (Munns and Tester, 2008).

According to Huang and Johnson (1995) accumulation of glycine betaine and the expression of genes involved in its synthesis in creeping bentgrass under low-temperature stress. The authors found that glycine betaine accumulation was associated with increased activity of antioxidant enzymes, such as superoxide dismutase and peroxidase. The authors suggested that glycine betaine and antioxidant enzymes may work together to protect plants against low-temperature stress.

Islam et al. (2009) investigated the effects of exogenous proline and glycine betaine on antioxidant enzyme activities and cadmium tolerance in tobacco cells. The authors found that both proline and glycine betaine increased the activities of antioxidant enzymes, such as superoxide dismutase, catalase, and ascorbate peroxidase, and conferred greater tolerance to cadmium stress. The authors suggested that this osmoprotectant may enhance plant stress tolerance by increasing antioxidant defense systems. Wang and his co-workers 2016 investigated the role of glycine betaine in regulating UV-B signaling in Arabidopsis. The authors found that glycine betaine accumulation was associated with reduced levels of reactive oxygen species (ROS) and increased activity of antioxidant enzymes, such as catalase and ascorbate peroxidase. The authors suggested that glycine betaine may regulate UV-B signaling by modulating ROS levels and antioxidant defense systems.

Overall, these studies suggest that there may be interactions between glycine betaine and antioxidant defense systems in plants. Glycine betaine accumulation may increase the activity of antioxidant enzymes and help protect plants against various stresses, including low temperature, cadmium toxicity, and UV-B radiation.Due to its role as a cofactor for various enzymes involved in both plant and human metabolism, vitamin C is not merely an antioxidant. Vitamin C shields DNA, proteins, and lipids from oxidative damage by scavenging free radicals (Paciolla et al., 2019). All cell divisions, such as the apoplast (the cell wall and extracellular space), contained vitamin C, with chloroplasts containing the highest quantity at 20 mM. However, the amount of vitamin C in various plant species and cultivars of the same species varies greatly (Smirnoff, 2018). The correct operation of photosynthesis depends on vitamin C, which serves a variety of roles in chloroplasts (Paciolla et al., 2019). Significant variations in vitamin C concentration in horticultural crops have been linked to factors including analytical technique, sample handling before analysis, and pre-and post-harvest factors such as climate variability, maturity, farming techniques, and storage (Joshi and Bains, 2021). Our results are in agreement with Dewanti et al., 2021 who also found a similar range of ascorbic acid in common purslane in Indonesia. Their utility in medicine and other sectors is increased by the fact in addition to protecting plants against predators, they also stop bacterial and fungal development (Adamski et al., 2020).

Alkaloids from plants have been found to exhibit anti-inflammatory properties by inhibiting a variety of pro-inflammatory protein complexes associated with inflammatory signaling pathways (Aryal et al., 2022). In this regard, the neglected plant common purslane has a highest alkaloid concentration than other cultivated vegetables. A study carried out by Habibian et al., 2020 also showed similar results for common purslane alkaloid concentration. Plants typically create hydrogen peroxide during regular physiological processes and in response to stress circumstances (Musa and Öztürk, 2020). Our results are in agreement with Junglee et al. (2014) also recorded in the same range.

Reactive oxygen species (ROS) are formed when oxygen is not completely reduced (Khalid et al., 2020). Among the ROS molecules, H_2O_2 has gained the greatest recognition from the scientific community in the latest period. H_2O_2 content in plant cells must be tightly regulated due to its several functions on the one hand, and the risk it poses at high concentrations on the other (Anjum et al., 2022). Enzymes are vital in decreasing ROS levels and avoiding oxidative damage. Superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase are all basic antioxidant enzymes. Researchers may examine and comprehend an essential element of oxidative stress defense by measuring this enzyme activity spectrophotometrically (Sarker and Oba, 2018). Findings of the antioxidant enzyme activity comparison showed a significant variation among the collected common purslane accessions (Rosa et al., 2021). The SOD is one of the enzymes that, in living organisms, catalyzes the conversion of dangerous hydrogen peroxide which is produced by most living species, whether aerobic or anaerobic and requires both water and oxygen to survive. Ascorbic acid is employed as a reduction substrate, and the APX typically serves as the primary predator found in plants' cytoplasm and chloroplasts (Akram et al., 2017). Catalase is an important antioxidant enzyme that converts H_2O_2 to water and molecular oxygen. Our results showed that common purslane has good antioxidant enzymatic activity (Glorieux et al., 2015). The antioxidant enzymes, which demonstrate the significant activity to eliminate destructive free radicals, can prevent and inhibit a variety of diseases and aging (Dumanović et al., 2021).

5.5 Genetic Diversity

To optimize the selection of progenitors in a crop eugenics program, the research of genetic variability is a crucial part of practical plant breeding. To identify progenitors or

suppliers for hybrid or recombinant breeding, as well as to breed for certain agricultural production settings and scenarios, a comprehensive germline evaluation is necessary (Kumar et al., 2012). The most effective and speedy approach to determining the links and/or variations among the genotypes is through phylogenetic studies at the molecular level utilizing PCR-based markers (Schulman, 2007). Molecular markers are prominent PCR-based markers that are suited for randomized trials, both for genetic variation and reproductive studies (Arolu et al., 2012). The choice and implementation of primer pairs that will provide the clear, visually distinguishable, efficient, and adequate data required for assessment of the differentiation which happens in the interior of the plant are necessary for the efficient and coherent usage of microsatellite tools like ISSR for genetics crop or tree crop evolution study (Alam et al., 2015). In our study, the frequency of polymorphic loci found for each set of primers varied depending on the primer. The 25 common purslane accessions' unique band patterns were amplified using ISSR markers.

PIC values showed a similar outcome (Table 4.3). The investigated PIC scores had a mean of 0.03 to 0.43, with an average set of 0.24. In this current study, the ISSR markers also revealed that there are fewer degrees of genetic variation observed in the collected common purslane accessions. The UPGMA cluster analysis employing ISSR markers offered a higher resolution of the relationships between the collected common purslane accessions. Comparability factor values of 0.80, accessions of common purslane were densely packed and the join together ranged from 0.72 to 0.88, which discover significant groups in our study.

CHAPTER V

CONCLUSION

The current study demonstrated that there was a great variation of morphological and biochemical properties among different common purslane accessions collected from various locations in Türkiye. The accessions which had the best morphological characters were BT-Altinkulak (Niğde), Pasa (Antalya), Assem (Kirsehir), and Yerli (Nevsehir), and the accessions with the best biochemical properties were Yagmur (Bursa), Asya (Bursa), Agr Tohum (Niğde), and BT-Altinkulak (Niğde). The product (cookies) development exhibited that a 10 g quantity had the highest sensory appeal. The results of this study will be useful for plant breeders to develop common purslane as a new leafy vegetable. Similarly, the results will also be useful in the food and pharmaceutical industries to develop food products with high nutritional and antioxidant properties.

Any crop's genetic heterogeneity is crucial for enhancing phenotypic and genotypic crop genotypes over the current ones. Numerous cultivars have effectively used the approachable characteristics of ISSR markers to fully understand genetic variability, the adaptation process, regional variation, and dispersion. However, this is the 1st approach to the use of ISSR markers to investigate the genetic variability of common purslane collected from different various geographical locations of Turkiye. No specific genetic variation was observed in the collected common purslane accessions whereas the similarity ratio was higher in the collected accessions than the variation ratio, which shows that all these accessions collected from various locations of Turkiye have almost the same parents and spread out to various locations by different sources.

Common purslane has previously been shown to be moderate to highly resistant to salt and drought, as well as being abundant in vitamins, minerals, and antioxidants, making it a special contender for prospective vegetable investigation. Accessions that are widely distant relying on respective geographical components might be chosen as parents for additional breeding programs after taking into account all of those factors and the findings

from the marker aided no specific diversity analysis. This will lead to an improvement in production and overall performance, which will lead to an elevated index value.

Based on our research, it is highly recommended that specific populations of common purslane that have superior traits, such as higher yield and good biochemical and antioxidant properties, be considered for use by farmers. Examples of such populations are BT-Altinkulak (Niğde), Agr Tohum (Niğde), Pasa (Antalya), and Assem (Kirsehir), which are effective.

By selecting and planting these accessions, farmers can increase their chances of success and improve the quality of their leafy vegetables, leading to greater profitability and a more sustainable farming system.

It is also recommended that farmers consider sharing their knowledge and experience with other farmers in their community and collaborating with agricultural experts and researchers to continue to identify and develop new populations that can benefit both farmers and consumers.

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PUBLICATIONS PRODUCED FROM PhD RESEARCH

From this thesis 1 (one) international article was published, and this research work has been presented at 1 (one) international conference. The detail is presented below.

(1) Assessment of morphological and biochemical characteristics of common purslane (*Portulaca oleracea* L.) accessions Pakistan Journal of Agricultural Sciences Faisalabad, Pakistan

(2) International Conference of the Turkish Journal of Agriculture-Food Science and Technology (25-29 October 2021).