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Chemical and Molecular Study of Some Genera of the Cactaceae Family

A Thesis
Submitted to the Council of the College of Science for Women/ University of Babylon in Partial Fulfillment of the Requirements for the Degree of Master in Sciences/ Biology

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿ قَالَ رَبِّ اشْرَحْ لِي صَدْرِي ﴿ 25 ﴾ وَيَسِّرْ لِي أَمْرِي
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Dedications...

** To the Messenger of Allah, Muhammad, peace be upon him, the greatest Savior and the herald of the great ends

** To my good father who used to cover fatigue with a smile full of faith

**To my mother who made me embrace joy every day, To you, O wonderful human being

** To my beloved husband Mohammed Because you are my great certainty, my heart to which I shelter, and my infallibility from the sadness of the world because you are my fascination and my senses and my destinations. Thank you for your smile that gives me strength in the face of life

** To my uncle Haj Abu Amir and my aunt or my mother, thank you for your material and moral support

** To my daughter and the apple of my eyes ‘’Narges’’

** To all my brothers, sisters and friends.

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Summary

The family Cactaceae (Cacti) comprises many genera, having taxonomic controversies at the species level, and have also not been previously studied at the chemical and genetic level in Iraq as diagnostic characters. In the present study, attention will be paid to species of chemical compounds based on the Gas chromatography-mass spectrometry (GC-MS) method and genotyping based on the Random amplification of polymorphic DNA (RAPD) and Sequences methods. The s]x different species such as., *Hylocereus undatus* (Haw.) Britton & Rose, *Aloe vera* (L.) Burm.f., *Opuntia ficus-indica* (L.) Mill., *Espostoa guentheri* (Kupper) Buxb., *Echinocactus grusonii* Hildm, and *Mammillaria elongate* DC., which classified based on phenotypic characters to determine their accurate taxonomic names. Samples were collected from different nurseries and also from the home garden, and work continued in the laboratories of the College of Science for women, University of Babylon, from September (2022) to March (2023).

The chemical study dealt with the study of the effective content of the extracts of the species of leaves studied, as the compounds were identified with GC-MS technology, and by comparing the chemical content, it was found that all the studied species contained 15 compounds in each species, but share together in four active compounds which are Oxime-, methoxy-phenyl- Cyclotetrasiloxane, octamethyl and_ 1H-Trindene, 2,3,4,5,6,7,8,9-octahydro-1,1,4,4,9,9-hexamethyl, and Oleic Acid were recorded according to the available scientific references has antimicrobial and anticancer activity, antibacterial, antifungal, antiviral, antiseptic, hair conditioner, skin moisturizer as well as has antioxidant value.

As for the molecular study, DNA analysis was performed using one of the DNA indicators based on polymerase chain reactions (PCR), which are indicators of polymerase chain reactions. To implement PCR reactions, genome DNA was isolated from the fresh extracts from vegetative part of the studied species, as the highest concentration of

DNA was in the *Mammillaria elongate*, which reached 8.8ng/ µl and the lowest concentration of DNA by species, *Aloe vera*, which reached 5.1ng/ µl.

RAPD reactions were conducted to obtain a fingerprint of the studied species by testing seven primers (OP-V19, OP-R06, OP-V14, OP-V09, OP-L05, OP-M05 and OP-P04) and detecting variations in the polymorphic pieces of each species after dyeing and migrating the replication products of the samples in agarose gel.

The short primers showed the highest polymorphic bands (100%), and no monomorphic correlation among these species. This highly polymorphic relationship indicated that each species has a special identity and unique evolutionary trend. The primers were considered productive by showing highly distinct and sharp bands, while others showed faint bands. The outcome of this research confirmed the efficacy of RAPD primers in measuring polymorphism, comparing genotypes, and identifying Cacti species using specialized RAPD markers and evaluating kinship by constructing the phylogeny tree RAPD-PCR.

The molecular study was also confirmed by studying the genetic sequence of the *rbc*L and *mat*K genes after sending the amplification products to the Korean company and then comparing them with the global identification numbers registered in the Genome Bank (NCBI).

Outcomes from this study confirmed the *rbc*L (at 550 base pairs) and *mat*K (at 600 base pairs) genes were as particularly suited for molecular identification target genes, Variations in the nucleotide structure of the *rbc*L and *mat*K genes were successfully used to detect and diagnose the species in this study.

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No	**Abbreviations**
rbcl	Gene Ribulose biphosphate carboxylase
matk	Gene Megakaryocyte-Associated Tyrosine Kinase
NCBI	National center of biotegnology information
GC- MS	Gas chromomatography mass spectrometry
PCR	Polymerase chain reaction
RAPD	Random amplification of poiymorphic DNA
ITS1	Intenral transcrpted spacer 1
ITS2	Intenral transcrpted spacer 2
UPGMA	Unweighted Pair Group Method with Arithmetic Average

Chapter one

Introduction and LiteratureReview

Chapter One

Introduction and Literature reviews

1-1 : Introduction

The kingdom of plants, which people have been interacting with since creation in order to extract from them the good and the beneficial “food and medicine” and avoid the harmful” is maybe the most familiar to man than anything else around him. Many years ago, man first became aware of the diversity and differences among plant species. Scientists chronicled and depicted these variations, and their efforts eventually led to the development of taxonomy. This research initially depended on phenotypical traits including color, scent, and overall form.

Modern taxonomic studies, however, have broadened and are no longer as narrow-minded as they once were to keep up with scientific progress in all areas of the life sciences. The study of characteristics attracted the attention of many researchers and categorization enthusiasts. Many Taxa's taxonomic positions were determined by anatomical, chemical, and pollen features and other taxonomic data, as well as technological developments, all contributed to the growth of these studies (Singh, 2014).

The family Cactaceae, which belongs to the Caryophyllales order of Angiosperms, is renowned for the wide variety of growth forms it contains. Cacti can take the shape of xerophytic trees or shrubs with prominent, evergreen leaves (Goebel, 1889) or, more commonly, can take the form of columnar to globular succulents with branched or unbranched stems. Cacti can have scandent, epiphytic, or epilithic growth patterns, and their stems can be thin and terete or they can have flattened cladodes that resemble leaves. The following significant alterations to the basic structure of a perennial dicotyledonous flowering plant have resulted to the development of an atypical vegetative morphology in the plant (Gibson &Nobel, 1986).

Cacti are indigenous to the Americas and may be found from Patagonia in the south to some regions in the western portion of Canada in the north. Their range encompasses a wide variety of ecosystems, including arid, scorching deserts, sandy coastal stretches, scrublands, dry deciduous forests, high alpine steppes, and even tropical rainforests (Barthlott, 1983). Cacti are among the most noticeable and recognizable plants that may be found in warm and dry regions of the New World (Altemimi *et al*., 2017).

Since the earliest European botanists, zoologists, and biogeographers ventured to the tropics to find and document the abundance of life, there has been intense scientific interest in tracing and understanding global patterns of biodiversity (Bhattarai *&* Vetaas , 2003). There has been a long history of scientific inquiry into the patterns and mechanisms of various distributions throughout space, but our understanding of these features remains limited. In the context of conservation planning, international politics, and sustainable use of genetic resources, understanding the patterns that shape the global distribution of life is no longer just of academic interest to ecologists and biogeographers (Bachman *et al*., 2004).

Biodiversity is notably high in the arid regions of North and South America, such as the southwestern United States and Mexico, East Brazil, and the eastern and western slopes of the South American Andes. Although (Barthlott and Hunt, 1993) . only focused on a single epiphytic species, *Rhipsalis* Stearn., its native distribution includes the New World as well as southern Africa, Madagascar, and Sri Lanka (Barthlott and Taylor, 1995).

Within succulent plant family tree, the Cactus family is considered to be the most significant branch. Morphologists have historically been responsible for the study of different species of cactus, and throughout the previous century, several taxonomic reorganizations have been suggested. (Edwards and Ogburn, 2012). In recent decades, a great number of evolutionary studies that make use of genetic markers have been published. These studies focus on a group that is both iconic and ecologically significant (Gibson, 1973). Classical molecular markers, on the other hand, often do not provide adequate information in a number of samples to clarify phylogenetic connections (Inglese *et al*., 2017) as well as variations in genetic makeup within the same species. (Morales *et al*., 2012).

The researchers are of the opinion that a tipping point has been reached and that now is the ideal time to conduct a review of the most recent information that has been attained via the use of those molecular markers. This assumption is based on past attempts to collect samples of conventional molecular markers for the family Cactaceae and to promote genomic sampling from it in order to increase access to modern sequencing technology (Altemimi *et al*., 2017).

The use of molecular markers in evolutionary studies has been more common in recent decades as a means of investigating and understanding the natural history of organisms and their relationships with one another such as gene flow, taxonomy and evolutionary linkages. Additionally, target taxa may be valuable as a biological model for the investigation of larger features of their habitats and the geography of their locations, such as changes in the environment and evolutionary processes (Park *et al*., 2008).

In this respect, cacti have the necessary characteristics to be regarded as biological models for the research of evolutionary processes. For instance, given that it is a group that has just recently radiated, numerous clades may be helpful in the investigation of recent and rapid diversification. In addition, many different species can hybridize in the wild Because there are no dividing boundaries between the species and genera of the Cacti family in Iraq(Perfumi and Tacconi, 1996)., The usage of bioactive phytochemicals, which are derived from various plant components and have various impacts on human health, is becoming more popular (Avato and Argentieri, 2018). As noted by (Abdalla *et al*. ,2010) some of these chemicals are anti-diabetic, anti-fungal, anti-inflammatory, anthelmintic, antipyretic, and cholesterol.

1-2 : The Aim of the study

The purpose of this study is to investigate the chemical and molecular characteristics of some species that are members of the Cactaceae family. Specifically, the investigation will focus on new characteristics that are helpful in confirming or altering the current taxonomic status. This can be accomplished by adding or merging some taxonomic ranks and determining the evolutionary relationships between species.

2- Literature Reviews

2-1 : Brief profile of the family Cactaceae

A Cactus is a plant that belongs to the family Cactaceae, which has roughly 300 genera and about 2000 species. Cacti are classified in the order Caryophyllales (The Angiosperm Phylogeny Group. *et al*., 2016). The term "Cactus" comes from the ancient Greek word κάκτος (káktos), which was first used by Theophrastus to refer to a spiky plant whose identity is unknown at this time. The word "Cactus" comes to us via the Latin language. There is a diverse variety of sizes and forms of Cacti that may be discovered. Although there are cactus species that are adapted to quite moist settings, the vast majority of cacti are found in regions that experience at least some degree of drought. The Atacama Desert in Chile is one of the driest regions on Earth, and many people choose to live there because of the dry environment. As a result of this, Cacti have developed a wide variety of strategies to preserve water. Succulents are characterized by having swollen, fleshy sections that are suited to hold water. For instance, practically all cacti are succulents. (Barthlott and Hunt., 1993).

In contrast to many other species of succulents, the process that is essential to the life of most cacti takes place only in the stem. The majority of Cactus species no longer have actual leaves and instead only have spines, which are essentially highly modified leaves. In addition to acting as a deterrent against animals, the spines on a cactus may also reduce the amount of wind that blows through the plant and provide some degree of shade. Cacti's expanded stems are able to carry out photosynthesis although they lack genuine leaves (Inglese *et al.*, 2017)

Cacti are most often found in dry desert or semi-desert locations that have high average daytime temperatures, chilly nights, and high rates of evaporation. Cacti may also be found in areas that have arid climates. It is possible to find Cacti in dry and warm locations of the Americas, such as deserts, sandy coastal stretches, scrublands, dry deciduous woods, high alpine steppes, and tropical rainforests. Their range extends from Canada to Argentina. These are only a few of the many environments in which Cacti may be found (Perfumi and Tacconi, 1996). Mexico and the southwestern United States, the Andean core region, central Brazil, Paraguay, Uruguay, and Argentina are the primary centres of biodiversity. Due to Mexico's status as the world's wealthiest and most biologically diverse area (Ennouri *et al*., 2005).

With 31% of species now threatened with extinction, Cacti are the seventh most endangered major taxonomic category. The cultivation and use of many different species of Cacti are significant sources of revenue for huge populations as well as small communities, the majority of which are located in Latin America; only lately has this trend begun to spread to the United States. Indigenous people in Mexico make use of more than 150 different types of cactus, and at least 50 of those species are farmed, constituting reservoirs of variety for alternative cropping systems (Chang *et al*., 2008). Cacti play an important part in the tradition and culture of the people who live in Mexico and other parts of South America, where their prevalence is more obvious. In the year 9000 B.C., hunter-gatherer societies in the southwestern United States and Mexico were already making use of species of *Opuntia* Mill, and the process of domestication began as early as 6000 B.C. (Salvo *et al*., 2002).

2-2 : Geographical distribution of the cactus family

Because they are so unique to this part of the world, Cacti are considered to be one of the most defining plant families of the Americas. In the Old World, only one epiphytic species, *Rhipsalis baccifera* (J.S.Muell.) Stearn has populations that are geographically separated (Altemimi, 2017). The region known as Latin America is notable for having a large number of distinct landscapes and zones of vegetation.

The fact that the habitats of Cacti reflect such a wide variety of climatic circumstances may come as a surprise to someone who is not an expert on the subject. They may be found growing in coastal and dune areas up to an elevation of around 5000 meters in the high Andes, as well as in the southern regions of Canada and Argentina, encompassing a latitudinal range of more than 100 degrees (Murray *et al*., 2016).

Extreme cold and, in most instances, humid circumstances, along with the short days seen during the winter, are the factors that restrict the distribution range of the family at high latitudes. In addition, the majority of Cacti will avoid the more humid regions of Mediterranean-style winter rainfall zones in northern California and south-central Chile. The effect of repeated fires may be a significant factor that prevents Cactus development in locations that get winter precipitation (Altemimi *et al*., 2017). In many grassland and savannah habitats, fire also inhibits the growth of Cacti and other succulent plants. Even though Cacti are most often associated with the arid regions of the New World, members of this family may be found in a broad variety of environments. Some species of cactus can even be found growing in moist tropical rainforests. (Ghazi *et al.*, 2015).

In general, Cacti are poor competitors when grown in environments that are humid and productive. Therefore, the majority of species are located in habitats that have low water availability at least for part of the year, particularly in edaphic situations such as rock outcrops, saline substrates, gypsum substrates, serpentine substrates, or sand fields. This comprises flora species found in open deserts and semi-deserts, as well as prickly bushes and dry deciduous and semi-deciduous woods. It has been established by several writers that several species of Cactus exhibit greater rates of establishment and survival in semi-arid areas when they are placed in the shade of trees or shrubs, which function as "nurse plants." (Ghazi *et al*., 2013).

On an ecoregional scale, the Mexican pine-oak forests of the Sierra Madre oriental and occidental as well as of the Trans-Mexican Volcanic belt belong to the areas with the highest richness of Cactus species. The same can be said for the Meseta Central matorral and the Chihuahua desert in Mexico, which are included in the Central Andean Puna Ecoregion according to the World Wildlife Fund. (Taoufik *et al*., 2015).

Cacti have been a source of fascination for botanists and plant lovers for ages, and many are kept as houseplants today due to their peculiar growth patterns and huge, eye-catching blossoms (Stintzing *et al.*, 2003). There are around 1400 succulent and non-succulent species that belong to the cactus family, and they are found throughout the whole of the American continent. Nevertheless, Mexico, the Andean area, which includes northern Argentina, Bolivia, and Peru, as well as eastern Brazil, are home to the greatest levels of endemism and richness in their plant and animal populations. (Labuschagne and Hugo, 2010).

2-3 : Characteristics of the Cactaceae family:

According to (Gharby *et al*., 2013), the cactus family is distinguished by the following external characteristics: short shoots that develop into areoles; shoot apical meristems that are organized into four distinct zones; and ovaries that are "sunken" in the receptacles, which are then covered with bracts and areoles. Traditionally, taxonomists have acknowledged cacti to be a distinct group unto itself and placed them in their own order, which is known as Cactales. because of the radically changed vegetative and floral morphology of cacti. Studies of embryology, plant pigment chemistry, and sieve-element plastids showed a close kinship of the family Cactaceae to the core Caryophyllales (Habibi *et al*., 2008).

The cactus family is unique for the wide variety of growth types that may be found within the family. Most cacti are columnar to globular succulents, branching or unbranched, scandent, epiphytic, or epilithic, and they have either thin, terete stems or flattened, leaflike cladodes, however others, like the Pereskia Mill., are xerophytic trees or shrubs with conspicuous permanent leaves. (Altemimi, 2017). The following are examples of major structural changes performed to a perennial dicotyledonous flowering plant, resulting in the evolution of an unusual vegetative morphology (Cardador –Martinez *et al*., 2011):

- the leaves become much smaller or fall off entirely.
- the stems stay green and continue to be photosynthetically active for many years, but the creation of bark is slowed down.
- Areoles, which are clusters of spines, are formed from the modification of shorter side branches.
- Branching is sometimes much decreased.

2-4 : Taxonomical study of Cactaceae

The family is home to around 1,500–2000 species spread over fewer than 100 genera (Zine *et al*., 2013) and includes 1,438 species in 124 genera worldwide (Santos-Díaz and Camarena-Rangel, 2019). The present system of categorization is derived from the most recent findings obtained from studies carried out by an ad hoc Working Party convened and operating under the aegis of the International Organization for the Study of Succulent Plants (Tesoriere *et al*., 2004).

Cactaceae is a plant family native to the New World that is home to a great number of endangered species (Goettsch *et al*., 2015). Almost the whole family is on the endangered list, and. Over the last 15 years, a plethora of new material has been uncovered thanks to studies of molecular phylogeny, which provide the major phylogeny-based taxonomic backbone for the Cactaceae and specify the species and infraspecific levels. This information has been generated as a result of molecular phylogenetic research. It synthesizes the existing published evidence for the present knowledge of species and generic circumscriptions by combining the results of evolutionary investigations with related synoptic treatments, recent revisions and monographs, and regional treatments in floras and checklists from across the world. It is a compilation of the evidence that has been published and is available to the public (Anceschi and Magli, 2018).

To clarify all of the names now used in the Cactaceae family and to avoid the incorrect use of the Global Caryophyllales in the future, (Turland *et al*., 2018) have made an attempt to integrate both officially published scientific names in Cactaceae and the many invalid names identified in the literature. A group of taxonomists are working together to provide a comprehensive online summary of species diversity in the Caryophyllales (Borsch *et al*., 2015).

There are three major subfamilies within the Cactus family, or Cactaceae: the Pereskioideae, the Opuntioideae, and the Cactoideae. *Maihuenia* (F. A. C. Weber) K. Schum., which has historically been classified with *Pereskia* in the subfamily Pereskioideae, has recently been proposed as a subfamily in its own right (Koubaa *et al*., 2017). (Barthlott and Hunt ,1993) are mentioned the abundant Cactoideae, which includes both globular and columnar cacti, the related Opuntioideae, which includes prickly pears and other Cacti with barbed spines and glochids and seeds enclosed by a bony aril, and the rare and species-poor Pereskioideae, which consists of the genera *Maihuenia* and *Pereskia*.

The subfamily Opuntioideae has been very well defined, and the distinguishing characteristics that were employed by early writers such as Schumann (1897–1898) continue to be relevant today. Many other molecular studies provide even more credence to the conclusion that the taxon is monophyletic (Wallace, 1995; Wallace and Dickie, 2002; Griffiths and Porter, 2009). The stems of almost every species are clearly segmented, and many species exhibit the characteristic development shape of a "prickly pear." In addition to the conventional spines, which are often seen in a retrorsely barbed configuration, the areoles can generate a large number of glochids, which may be rather unpleasant. Glochids and spines develop at the same time and share the same ontogeny, however glochids do not get sclerified at the base, therefore they are more likely to break off (Robinson, 1974).

The subfamily Cactoideae has the greatest number and greatest variety of species; it has over 1530 species and accounts for approximately 80% of the total number of cactus species (Anderson, 2001, 2005). The existence of cortical vascular bundles, which create a three-dimensional network across the cortex and are diagnostic for all species with the exception of Blossfeldia, is considered a crucial novelty for this clade (Mauseth, 2006). The current taxonomy of the Cactoideae tribes is primarily based on the work done by Franz Buxbaum, and it has undergone relatively minor changes as a result of the reassignment of a few difficult taxa and the combination of several tribes moved a few genera to new or different tribes (i.e., *Calymmanthium* F. Ritter to Calymmantheae; *Uebelmannia* Buining to Cereeae; *Acanthocereus* (A. Berger) Britton & Rose, *Corryocactus* Britton & Rose, *Echinocereus* Engelm. and *Leptocereus* (A. Berger) Britton &

Rose to Pachycereeae; *Harrisia* Britton to Trichocereeae) based on results from the recent systematic studies but otherwise adhered to the general framework of Buxbaum's tribal classification of Cactoideae (Tesoriere *et al.,* 2004).

Although (Nyffeler and Eggli .,2010) explain the phylogenetic summary tree of the estimated connections between the major species of the Cactaceae and subfamilies transform this hypothesis into a hierarchical suprageneric category device highlighting four four Cactoideae,Opuntioieae,Blossfeldieae,Cacteae,phyllocacteae,Rhipsalideae,and Cereeae.

2-5 : Medicinal and Economical Importance of Cactaceae:

While there are around 1500 different species of Cactus found throughout the globe and belonging to the family Cactaceae, cactus is not often consumed as a food owing to its bitter taste and the high concentration of seeds found inside its fruit. Cacti in their natural state, on the other hand, have been shown in recent research to provide a wide variety of advantages to human health and medical practice (Soel *et al.,* 2007). It has been shown that the *Opuntia dillenii* (Ker Gawl.) Haw. plant may reduce inflammation, alleviate pain, prevent hyperglycemia, and lower cholesterol levels.." (Astiataran and Martinez , 2000). Inorganic nutrients such as calcium, phosphorus, iron, magnesium, copper, and zinc may be found in high concentrations in Cactus, Nevertheless, these minerals are often only needed in low to moderate levels (1 to 2500 mg per day) (Altemimi *et al*., 2017).

Cactaceae is a plant family native to the New World that is home to a great number of endangered species (Goettsch *et al*., 2015). Almost the whole family is on the endangered list, and extinction threatens several species. Over the last 15 years, a plethora of new material has been uncovered thanks to studies of molecular phylogeny, which provide the major phylogeny-based taxonomic backbone for the Cactaceae and specify the species and infraspecific levels. This information has been generated as a result of molecular phylogenetic research. It synthesizes the existing published evidence for the present knowledge of species and generic circumscriptions by combining the results of evolutionary investigations with related synoptic treatments, recent revisions and monographs, and regional treatments in floras and checklists from across the world. It is a compilation of the evidence that has been published and is available to the public (Anceschi and Magli, 2018).

(El-Mostafa *et al*., 2014), (Ibrahim *et al*., 2018) and (Jurca *et al*., 2019) consider studies showing that these plants have antioxidant, anti- inflammatory, antiproliferative, hepatoprotective, antidiabetic, antihyperlipidaemic, antibacterial, anti-ulcerogenic, neuroprotective, and diuretic activity, as well as wound healing activities.

2-6 : Species under study:

2-6-1 : *Hylocereus undatus* (Haw.): Due to the fact that this species has been cultivated for such a long time, its natural distribution is unknown. Although its exact origin cannot be confirmed, it is widely believed to have spread from the West Indies and the Caribbean basin to southern Mexico through Belize, Guatemala, El Salvador, and Costa Rica. It is the most widely cultivated member of its genus, both for personal and commercial use, and it often becomes naturalized in tropical regions across the world. Not only does it have a wide naturalized distribution, but it also spans a wide altitude range, being found everywhere from sea level to a height of 2,750 meters (Coşkuner and Tekin, 2003).

The cactus known as *Hylocereus undatus* may grow in either a terrestrial or epiphytic environment and can either sprawl or vine. They ascend by use of a network of aerial roots and may grow to a height of at least 10 meters by attaching themselves to rocks and trees. They exhibit a high degree of variability and are closely linked to *Selenicereus*. Stems are green with three wings, ranging in length from a few centimeters to five meters (in mature plants), with borders that are undulating and horny and measuring between four and seven and a half centimeters broad, and having wings that are between two and five centimeters wide.

Hylocereus undatus is a lithophyte or hemi-epiphyte that grows in partial shade and may be replicated in the spring by stem cuttings or, less often, through the planting of seeds. The seed has to be put in compost that has good drainage, and it should sprout anywhere from 14 to 28 days later at temperatures between 18 and 21C It is grown primarily for its decorative value (Gharby *et al*., 2011).

Growers from all over India produce the dragon fruit because of its attractive color, mellow mouth-melting pulp with a black color edible seed buried in the pulp, and wonderful nutritional benefits. Secondary metabolites extracted from the ground-up plants are the source of a wide variety of manufactured goods, including flavorings, food additives, medicines, and other synthetic items. (Sathyamurthy *et al*., 2019).

The dragon fruit is native to Mexico, Central America, and South America. Long-day plants like the "Noble Woman" or "Queen of the Night" produce a stunning flower at night (Perween *et al*., 2018). The fruit of this plant goes by many different names, including Strawberry Pear, Dragon Seed, Pithaya, Night Blooming Cereus, Belle of the Night, Cinderella Vine, and Jesus in the Cradle.

Since the peel contains a larger quantity of flavonoids than the flesh, it was predicted that the two might have different antioxidant capacities when extracted using ethanol (Zulkifli *et al*., 2020). The polyphenols, flavonoids, and betanins that are present in *H.undatus* have been proven in several studies to be responsible for the anticancer activities of the plant. Peel of *H.undatus* was extracted using ethanol and water at a ratio of 50:50 by weight. It was shown that the solvent solution had an action that inhibited cell proliferation (Bueno *et al*., 2021). Extracts of *H.undatus* peel were tested for their ability to inhibit the growth of Gram-positive and Gram-negative bacilli using the disc diffusion method in ethanol, chloroform, and hexane (Paśko *et al*., 2021). The ethanolic extract of *H.undatus* flesh included around 85 % of different oligosaccharides mixed together. These oligosaccharides have a higher level of resistance to infection in humans (Attar *et al*., 2022). *Hylocereus undatus* has been used therapeutically for the treatment of gastrointestinal issues and kidney stones (Blanco and Thiagarajan, 2017; Castro *et al.*, 2018).

2-6-2 : *Aloe vera* (L.) Burm.f.: is a member of the Asphodelaceae family and is a succulent plant that can survive in dry conditions for lengthy periods. The word "aloe" comes from two different languages: Arabic "Alloeh" and Hebrew "halal," both of which imply "bitter shiny substance." (Manvitha and Bidya, 2014).

Because of their ability to store large amounts of water in their tissues and their tolerance of drought and fluctuating water conditions, aloes are classified as perennial succulents or xerophytes. They also make use of crassulacean acid metabolism, an adaptation to the photosynthetic pathway that involves the formation of malic acid (Barthlott *et al*., 2015). This plant's leaves are created by a thick epidermis (skin) coated with cuticles encircling the mesophyll, which may be separated into chlorenchyma cells and thinner-walled cells creating the parenchyma. The plant has a turgid green color and is linked at the stem in a rosette pattern, this parenchyma, also known as the inner leaf, inner leaf fillet, or *Aloe* fillet, is part of the leaf that contains the *Aloe vera* gel and makes up the bulk of the leaf in terms of volume (Guo and Mei, 2016). The length of the leaves ranges from 30 to 50cm, and they are green in color (when young, they are speckled with white). There are brilliant yellow tubular flowers that vary in length from 25 to 35cm, and they are grouped on a thin loose spike (WHO, 1999). Water and minerals are transported from the roots to the leaves through vascular bundles, while synthesized components are returned to the roots and latex is stored along the leaf margins. The number of vascular bundles on a plant might vary based on its age, the size of its leaves, and the climate in which it is grown. (Ni *et al*., 2004)

About 98.5%–99.5% of *Aloe vera* gel is made up of water, while the remaining solids comprise more than 200 distinct components, with polysaccharides being the most prevalent molecules (WHO, 1999). Other chemical components, including soluble sugars, glycoproteins, phenolic anthraquinones, flavonoids, flavonols, enzymes, minerals, essential and non-essential amino acids, sterols, saponins, and vitamins, have also been found (Rodriguez and Loeb., 2018).

Since the fourth century B.C., when ancient Greek physicians got aloe from the island of Socotra in the Indian Ocean, the *Aloe* plant and its derivative products have played a role in the field of medicine and health care. This history may be traced all the way down to modern times. The first time that aloe vera is mentioned in human history, it is referred to as a laxative in certain Sumerian hieroglyphics that were inscribed on clay tablets during the Mesopotamian civilisation about 2200 BC. There is evidence that people in ancient Egypt, Greece, and China used aloe vera as a medicinal remedy (Park *et al*, 2006).

Consuming *Aloe vera* juice either on its own or mixed with another beverage of any sort provides treatment for a wide variety of gastrointestinal conditions, including irritable bowel syndrome, reflux disease, indigestion, heartburn, and a great number of other issues. It has a calming effect on the stomach since it assists in maintaining the proper balance of the acids that are found in the stomach. The anti-bacterial and anti-inflammatory qualities of *Aloe vera* contribute to its effectiveness as a treatment for a variety of oral and gum conditions, including severe gum diseases. Massaging the gums may be applied to the teeth in the form of gel or toothpaste. In addition to this, it has the ability to heal skin conditions such as eczema, burns, and wounds (Ajayi and Ajayi, 2009) and orally as a laxative (Steenkamp and Stewart, 2007). Many anthraquinone C-glycosides, anthrones, and free anthraquinones have been identified from Aloe vera latex, which is mostly phenolic. Aloe vera latex contains anthraquinone C-glycosides at varying quantities, up to 30% of its dry weight. (Boudreau and Lakhani., 2013).

2-6-3 : *Opuntia ficus-indica* (L.) Mill: The prickly pear cactus, or Nepal cactus, is a member of the cactus family, is the kind of cactus that has been domesticated for a longest time and is found in most regions of the globe. It has been and will continue to be the most conspicuous and typical feature of dry and semiarid areas (Suja *et al.,* 2005). Due to its CO2 fixation (Crassulacean Acid Metabolism), *Opuntia ficus-indica* is a unique plant that thrives in severely dry regions. Cacti are recognized for their spines and green succulent stalks with chlorophyll. Opuntia fruits are fleshy, elongated berries with firm seeds in orange, yellow, red, purple, green, and white. 80–140 g, 54.18% edible. (Pezzuto and Park, 2002).

O. ficus-indica was revered by ancient Middle American cultures for its ability to provide sustenance and alleviate thirst, as shown by historical accounts and archaeological finds (Ramrez *et al*., 2017). Its sensitive cladodes are used as fresh green vegetables and salads, and its fruit is pleasant and nutritious (Reyes-Agüero *et al*., 2005). Prickly pear is grown for the purpose of harvesting its fruit, which may be eaten; however, certain components of the plant are also used in some nations in the food and cosmetics industries. (Pareek *et al*., 2003).

The fruits and stems of prickly pears have a long history of usage in traditional medicine, both in a number of different nations and for a variety of different medical conditions. A sizable number of researchers, however, have focused on the genus *Opuntia* in an effort to identify characteristics of the plant that could form the basis for its use in the prevention and treatment of chronic disorders. It was discovered that *O.ficus-indica* has many different kinds of bioactive chemicals (Griffith *et al*., 2004).

Prickly pears have been shown to contain between 80 and 90 mg/100g of dry weight of the flavonoids myricetin, orientin, and certain derivatives of pyrone, including kaempferol, rutin, aromadendrin, taxifolin or dihydroquercetin, isorhamnetin, vitexin, quercetin, betalains, betacyanins, and isorhamnetin. Essential oils from plants are well recognized for their health benefits, including antibacterial and antioxidant effects (Zhao *et al.*, 2007)

2-6-4 : *Espostoa guentheri* (Kupper) Buxb.: (syn: *Vatricania guentheri*) is a shrubby columnar cactus with elegant golden-spined stems that display variable densities of fox-red lateral cephalia. This refers to beds of woolly hairs produced on the sides of the branches from which emerge flowers and fruits. In the past, the term "cephalium" referred to a portion of a cactus shoot that developed straight from the shoot apical meristem. This part of the shoot was thought to be made of confluent areoles, which are the origin of flowers, and to contain a large number of spines and trichomes. Additionally, it was believed that the cephalium was covered by a thick periderm rather than an even thicker cortex. According to (Pezzuto and Park's ,2002) the bristles and hairs on blooming sections are much longer than those on non-flowering parts.

Cephalia is chlorenchyma and stomata-free. Cephalia has epiphyllotactic phyllotaxy. Lateral cephalia lean at the shoot apical meristem, but new ribs straighten the shoot. Cephaliums must meet these requirements. Cephalia may be restricted to the Browningieae-Cereeae-Trichocereeae clade of Core Cactoideae II or independently evolved in the Pachycereinae (Singh *et al*., 2015).

Espostoa guentheri (Kupper) Buxbaum seems to have a cephalium at or below the shoot apical meristem under this updated description. Their flowers exclusively come from cephalia, which contain an amazing density of confluent or almost confluent spines and long thin flexible spines (bristles) that hide the epidermis, preventing photosynthesis (Gorelick *et al*., 2014).

2-6-5 : *Echinocactus grusonii* Hildm.: Exclusively found in central Mexico between the states of San Luis Potosi and Hidalgo, this Cactaceae family member is endeamic to that region. the well-known species of cactus known as *Echinocactus grusonii*. It was first described by Heinrich Hildmann in the year 1891, however its common name has changed to "Golden Ball" or "Golden Barrel Cactus" in recent years. The golden barrel cactus is one of the most widely grown cacti in Thailand right now (Leo *et al*., 2010), and it belongs to the little genus *Echinocactus* (Pezzuto and Park, 2002), which is related to the genus *Ferocactus* and is often known as barrel cacti.

Even though it is one of the most famous cacti to grow in gardens, it is rare and in danger of extinction in the wild.(Oonsivilai *et al*., 2010) say that after many years, it may grow into a big, roughly circular globe that is more than a meter tall. There may be up to 35 clear ribs on a grown plant, but they may not be noticeable on a young plant, which may look like a stick. Golden Barrels that are younger don't look the same as ones that are older. The long, sharp spines can be straight or slightly curled, and they can be different shades of yellow or sometimes even white. Small yellow flowers bloom around the plant's top in the summer, but they don't show up for about twenty years (Oonsivilai *et al*., 2010).

2-6-6 : *Mammillaria elongate* DC: The gold lace cactus, often known as the ladyfinger cactus, is a commercially significant cactus with elegant, freely brandling elongated branches and tubercles topped with spreading spines. The rising need for these plants calls for more efficient means of propagation. Research on the potential of axillary meristems as explants for the tissue-cultured propagation of numerous Cactaceae species has recently been conducted. One of its benefits is that uniform plants may be developed swiftly from structured tissue in each axil.

According to (Oonsivilai *et al*,. 2010), *Mammillaria elongata* is an endemic species that can only be found in Mexico, specifically in the state of Guanajuato.

This species prefers calcareous soils and may be found growing at elevations ranging from around 1,300 to 2,300 meters above sea level in matorral. However, mining, land use change for agriculture, and habitat degradation owing to grazing by goats are having an influence on certain subpopulations of this species (Ramrez-Moreno *et al.,* 2017). Although this species is prolific and common across its restricted territory, it is still threatened by these human activities.

Growing *M.elongate* is as simple as growing any other classic cactus; all it needs is plenty of indirect sunlight without scorching the plant to promote thick, strong spines. It's a fantastic plant for the cactus gardener since it quickly forms a clump of stems adorned with thick golden spines and ringed with yellow blossoms. The growth rate of this species is above average. It's simple to cultivate in clumps, although the offsetting pattern may be erratic. Offsets are common among plants, and clumps may be grown from them in only a few years (Turland *et al.,* 2018).

Cacti belonging to the genus *Mammillaria* are typically small in size, globoid, and have a stem that has a unique tuberculate appearance. Plants may exist on their own or cluster together to produce large mounds. This trait, along with the existence of dimorphic areoles, is shared by other members of the "Mammilloid clade" (Butterworth *et al*., 2002). Blooming areoles are located in the axils of the tubercles, whereas the vegetative (spine-bearing) areole is borne on the apex. In contrast to the four taxa named above (*Coryphantha*, *Escobaria*, *Pelecyphora*, and *Ortegocactus*), *Mammillaria* does not have an adaxial groove (Huelsenbeck *et al*., 2001).

Without a doubt, *Mammillaria* is the most species-rich and morphologically diverse genus in existence. Previous infrageneric investigations have shown conflicting findings about the confines of the generic scope, and there is considerable controversy as to whether or not the genus is monophyletic. Chloroplast DNA sequence data from the rpl16 intron and the psbA-trnH intergenic spacer were used to answer phylogenetic issues concerning *Mammillaria* for a total of 125 species, 113 of which belonged to this genus (Butterworth *et al*., 2002).

2-7 : Chemical study: According to research conducted by (Cragg and Newman,2013), several plant species have been used in a variety of medicinal preparations throughout history, most often in the form of infusions. The existence of a variety of bioactive compounds, which have piqued the interest of the pharmaceutical sector due to the favorable effects on the health of consumers, has been related to the presence of these compounds. However, bioactive chemicals are also of interest to the nutraceutical and cosmetics sectors for use in improving the functionality of their products. This has led to the characterisation of bioactive compounds in several traditionally used plants. Phenolic compounds have been used as natural ingredients and additives in the food, cosmetic, and pharmaceutical industries in recent decades due to their wide range of beneficial biological effects and lack of toxicity (Petropoulos *et al*. 2019).

To achieve a high degree of purity in the alcoholic extracts, a Gas chromatography-mass spectrometry (GC-MS) machine was utilized to separate contaminants and fatty compounds.

Secondary metabolites are tiny organic compounds that originate during the development of plants from primary metabolites. The chemical nature and makeup of metabolites in plants varies from species to species. The interesting nature of secondary metabolites may be attributed to a wide range of factors, including their one-of-a-kind structural makeup and the fact that they may have applications in the medical or antioxidant fields (Guerrero *et al*., 2019)

Due to their complexity, industry cannot replicate the wide range of chemical structures found in plant metabolites. Metabolites from plants have been utilized for various reasons for at least 4,000 years; this usage dates back to 2600 BC. These metabolites have been put to use in a variety of ways, including as food, medicine, and even poison. More than 30% of medical drugs are generated directly or indirectly from natural products, demonstrating the important role that plant secondary metabolites have played (Lvarez-Carretero *et al*., 2022).

Because of advances in technology, plant metabolite study has exploded in popularity over the last century. The low quantities of metabolites in plants meant that their biological activities were largely unknown, and for a long time they were only seen as metabolic waste or detoxifying products. Over the last four decades, our understanding of secondary metabolites has expanded from its historical focus on their harmful effects on animal cells to its current focus on their ecological value and plethora of other advantages (Martin., 2011).

Methylation, glycosylation, and hydroxylation biosynthetic changes create secondary metabolites from parent metabolites. Secondary metabolites have more complicated side chains and structures than primary metabolites. Phenolic groups (simple sugars and benzene rings), carbon and hydrogen-containing terpenes and steroids, and nitrogen-containing compounds are the three main types of plant metabolites based on the biosynthetic pathway (Petropoulos *et al*., 2019).

Betalains (red betacyanins and yellow betaxanthins), phenolic compounds, triterpenes, sterols, fatty acids, mucilage, vitamins, minerals, and fiber are among the most common chemical groups found in both *Hylocereus* and *Opuntia* species (Wybraniec *et al*., 2010; Wright and Setzer, 2013).

There are numerous recent researes that corroborate the study of chemical compounds using the GC-MS tech(nique for many varieties of the cactus family. One such study is the study that (Czigle *et al.* ,2020) conducted on aromatic compounds in three genera of cactus plants like *Epiphyllum*, *Hylocereus*, and *Opuntia* . In addition (El-Beltagi *et al.*, 2019) detected numerous chemicals that were present in the ethanolic extract of pulp and peel *O.ficus-indica* and established its prophylactic value in the process of creating and producing pharmacological medications with fewer adverse effects. Investigations conducted in vitro as part of the current research give considerable evidence that cactus peel, an inedible waste product, is a rich source of antioxidants, antibacterial agents, and anticancer activity. This suggests that its usage as a value-added component for functional purposes should be pursued.

2-8 : Historical tendencies of molecular markers in Cactaceae:

Molecular techniques have emerged as an important link between agricultural and systems biology research to facilitate the development of improved plant breeding methods and enhance our knowledge of previously poorly understood plant genomes.

In the most recent few decades, a significant number of investigations with an emphasis on evolutionary theory have been carried out on cacti. Molecular markers, phylogenetics, biogeography, species delimitation, hybridization, and genome assemblies have all been useful in plant classification, but they have been especially helpful in the case of the Cactaceae. Integrating the cactologist community, especially those from low-income countries with high cactus diversity, and discussing the methodological challenges involved in genomic research are necessary for a proper understanding of family evolution and alternative methods for data analysis (Unver *et al.,* 2017). In order to stress the need for an integrated strategy in the future of cactus research, this study illustrates the various breakthroughs, limits, and biases in the field at the present time (Suja *et al*., 2005).

Buxbaum hypothesized that the development of diverse characteristics of stems, flowers, fruits, and seeds followed a regular pattern of greater specialization and morphological reduction through time. This concept of directionality, or trend, in the development of cacti, informed many future concepts regarding the evolutionary connections between different species (Yong and Salimon, 2006). However, a precise phylogenetic framework is required for a comprehensive evaluation of these oversimplifications on the evolutionary history of cacti. For example, Using sequencing data and an explicit phylogenetic framework, (Porter *et al.* ,2000) investigated the homoplasy of morphological characteristics and the emergence of paedomorphosis in Sclerocactus Britton & Rose (Singh *et al.*, 2014).

The comparative assessments of growth from evolution are what reveal the most important connections within the Cactaceae genus utilizing genetic sequences from the chloroplast genome. The *mat*K gene together with its two surrounding introns make up what is known as the *trn*K intron (sometimes written as *trn*K/*mat*K). Within the Cactaceae, this area has close to 2,600 base pairs. Although the *mat*K gene, which produces a protein that is physically linked to the maturases, has been employed in various infrafamilial investigations (Keys *et al.,* 1957), the surrounding non-coding introns of 1050bp have just recently been exploited for

1- Random amplification of polymorphic DNA (RAPD):

Called "rapid" is a form of PCR that amplifies random DNA fragments. RAPD researchers use a large genomic DNA template and multiple random, short primers (10–12 nucleotides) to amplify fragments. RAPD reactions may provide a semi-unique profile by resolving the patterns. No knowledge of the targeted genome's DNA sequence is needed since the primers will bind anywhere in the sequence (Leger *et al*., 2021).

This method is helpful for comparing DNA in biological systems that have received little attention from the scientific community or that have a limited number of DNA sequences available for comparison (Anderson *et al*., 2018). Degraded DNA samples are limited by their reliance on a large, undamaged DNA template sequence. It has poor resolving power. After PCR amplification, samples are put onto agarose or polyacrylamide gels for electrophoresis. Depending on the sample source, random amplification's different sizes will split along the gel. This provides a unique DNA fingerprint (Valette *et al.,* 2003).

RAPD does not need knowledge of the target organism's DNA sequence since identical 10-mer primers will or will not amplify a segment of DNA depending on complementary locations. No fragment is formed if primers are annealed too widely apart or if their 3' ends are not facing each other. If the template DNA has mutated at the spot that was complementary to the primer, a PCR product will not be created, resulting in a distinct pattern of amplified DNA segments on the gel. Species-specific DNA comparison approaches like short tandem repeats. In recent years, RAPD has been employed to describe and track the phylogeny of numerous plant and animal taxa (Bellesia *et al*. 1996).

Decamer DNA fragments from polymerase chain reaction amplification of random genomic DNA using a single primer of arbitrary nucleotide sequence (RAPD markers) have been shown to discriminate between persons of different ancestry, albeit this distinction is not always reproducible. One decamer oligonucleotide generates numerous DNA products from various genetic loci (Mohr *et al.* 2014). Mutations or rearrangements at or between primer binding sites cause polymorphisms, which appear as RAPD bands. As dominant markers, RAPDs cannot detect heterozygotes (Singh *et al*., 2014).

2- Ribulose biphosphate carboxylase Large (*rbc*L):

Because of its universality and the ease with which it can be amplified and analyzed, the *rbcL* gene, which is part of the DNA sequence that is found in cpDNA, has the potential to be utilized as a DNA barcode (Hasebe *et al.*, 1994). This coding region is present in cpDNA. Because its complete length is around 1400 base pairs, this gene offers a large number of features that may be used to research phylogenetics (CBOL, 2009).

Within the context of taxonomy, the use of DNA barcoding is suggested as an instrument for identifying and validating species. The *rbcL* gene is the DNA barcode that plants use to identify themselves. In the context of photosynthetic carbon metabolism, (Buchanan *et al.* ,2000) provide an overview of the role and functioning of the protein complex known as Rubisco.

According to (Bafeel *et al*. 2011), primers of *rbc*L and *mat*K can amplify the DNA of a large number of plant species, but they may not work in other situations. However, they suggested the creation of a way to construct fresh universal primers to cover barcoding for a greater variety of plant species. This would be a step in the right direction, therefore, (Guo *et al*. 2011) research has shown that the *rbc*L gene may function as an efficient and reliable DNA barcode to identify the species of medicinal plants.

3. The Chloroplast maturase gene (*mat*K):

Is one of the genes that code for proteins in angiosperms that has the largest variety, and it has been postulated that it may act as a "barcode" for land plants because of this variance. The limited universality of presently available primers and the mononucleotide repeat DNA barcoding both contribute to the fact that *mat*K's amplification and sequencing rates are rather low. This is a theory that has only lately gained a lot of attention, and it is differentiated by the exploitation of one or a few DNA fragments in order to differentiate between various species (CBOL, 2009).

The Consortium for the Barcode of Life (CBOL) Plant Working Group has recommended *mat*K and *rbc*L as the gene components that should be used for a plant DNA barcode; however, a consensus has not yet been established on this matter (CBOL, 2009). The imperfection of any gene derived from the chloroplast, mitochondrial, or nuclear genomes contributes to the challenge associated with picking specific genes to be used as a plant barcode. Because the mitochondrial genes in plants develop so slowly, this kind of genetic analysis is not useful for discriminating between various plant species. According to (Taoufik *et al*., 2015), the creation of universal primers is challenging to achieve because plant nuclear genes often appear in multiple copies and are extremely variable.

Since the invention of DNA barcoding of plants in 2008 (Yu *et al*., 2021) there have been several genes that have been reported to be beneficial. In prior research, the plastid *mat*K and *rbc*L genes as well as the nuclear ITS1 and ITS2 genes were shown to be the most prevalent indicators. In addition, the combination of these markers is essential for accurately conferring species identification (Kress, 2017). In phylogenetic and phylogeographic studies, plastid DNA is often used as a marker of choice. Despite its widespread usage, however, very little is known about the use of plasmid DNA in assessing the connections between closely related species. Taxonomic studies at lower levels, particularly those conducted on populations, are hampered by the glacial pace at which cpDNA-specific evolution occurs. Studies have shown that the utility in phylogenetic analyses of different cpDNA non-coding regions within a given taxonomic group can vary enormously (Hamilton, 2003; Sakai *et al*., 2003). In addition, there is sometimes a dearth of knowledge on the rate of evolution across distinct non-coding cpDNA areas, making it difficult to identify the suitable cpDNA region.

Taxonomic studies at lower levels, particularly those conducted on populations, are hampered by the glacial pace at which cpDNA-specific evolution occurs. Studies have shown that the utility in phylogenetic analyses of different cpDNA non-coding regions within a given taxonomic group can vary enormously (Hamilton, 2003; Sakai *et al*., 2003).

Chapter Two

Materials and Methods

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2.1 : Collection of Plant Samples.

All plants were obtained from private cactus farms located in Babil Governorate, where the current study relied on fresh samples in nurseries if six species of the cactus family were selected, and the collection period was from 17/9/2022 to 27/9/2022. These nurseries were in the different areas of the city of hilla. On each specimen, information was written down, including the name of the collector, the name of the sample, the date it was taken, and the name of the location from which the plant was obtained. Then, these samples were put in the College of Science for Women at the University of Babylon's advanced plant laboratory. (Table 2-1 and Figure 2-1).

Table 2-1: Cactus species under study and their collection areas.

Name of cactus species	Code Number	location of Plant Samples
Hylocereus undatus	1	Alsahha district
Aloe vera	2	Aljamiyia area
Opuntia ficus-indica	3	Alsahha district
Espostoa guentheri	4	Aljazaer district
Echinocactus grusonii	5	Aljazaer district
Mammillaria elongate	6	Aljamiyia area

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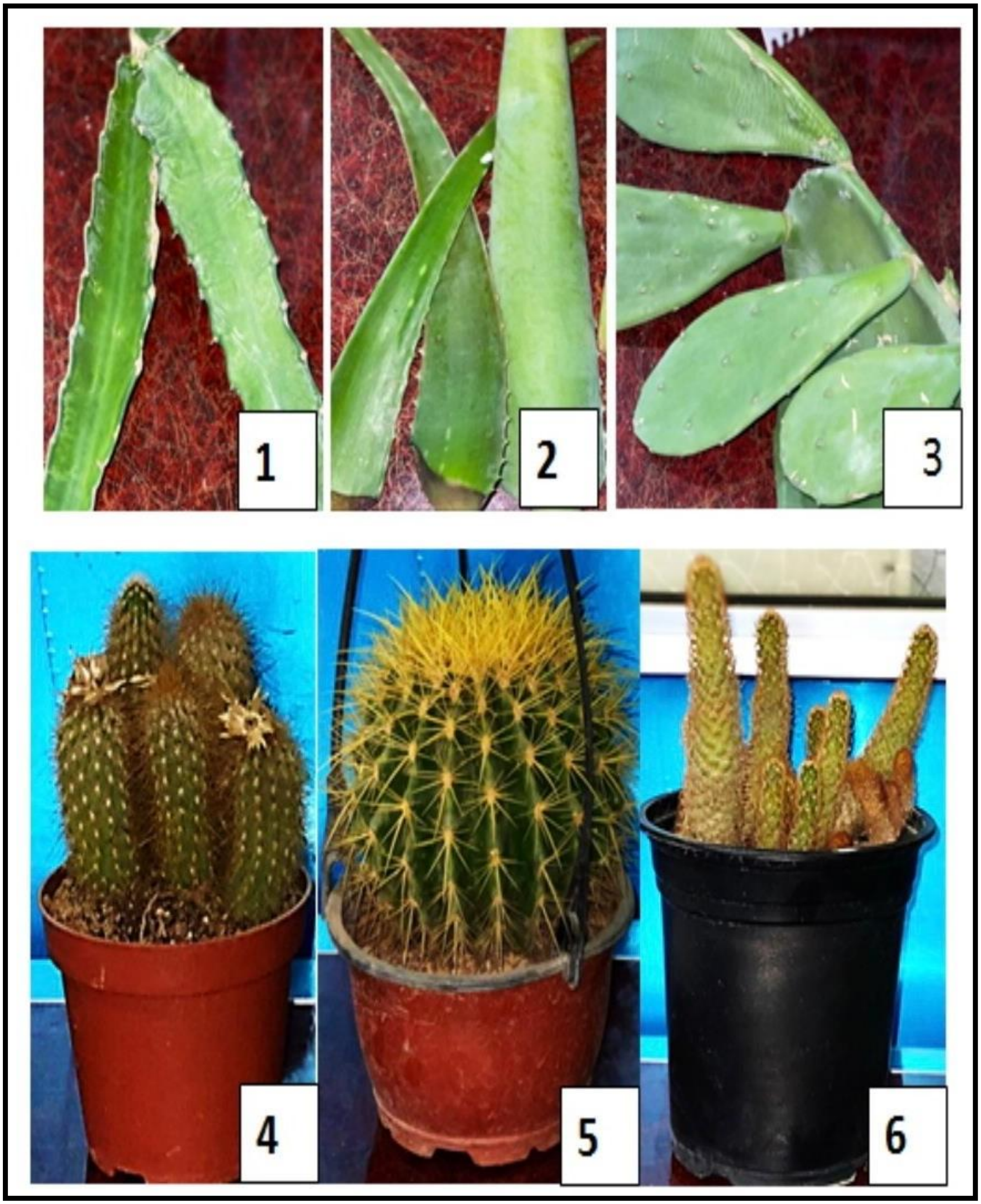

Figure 2-1: Samples of the plant under study (1- *Hylocereus undatus*, 2-*Aloe vera*, 3-*Opuntia ficus-indica*, 4-*Espostoa guentheri*, 5-*Echinocactus grusonii*, 6-*Mammillaria elongate)*

2.2 : Preparation of plant samples for chemical and molecular studies

Parts of the whole cactus plant that is not infected with any bacterial, fungal, or viral diseases were selected, and it was cleaned and washed using running water several times to remove any dirt or dust after the thorns were removed from it. After making sure that the pieces taken were clean, they were cut into very small pieces to be ready for chemical and molecular studies.

2.3 : Chemical Study

The chemical properties of the extract prepared from the whole plant were studied after removing the thorns to separate the chemical compounds using the GC-MS technology

2.3.1 : Phytochemical screening

The phytochemical analysis of the plant extracts was by standard methods, Specifically, the extracts were screened for phytochemicals (saponins, glycosides, alkaloids, flavonoids, tannins, terpenoids) as thus explained.

2.3.1.1 :Test for alkaloids by Dragendorff's reagent:

To make Solution (A), combine 0.85 g of Bismuth Subnitrate with 10 ml of acetic acid and 40 ml of distilled water.

As a Plan B: To get ready, mix 8 grams of potassium iodide with 20 ml of distilled water.

Prepare Dragendorff's reagent by combining (a) and (b) in a 1:1 ratio to make a stock solution, then adding (2 ml) acetic acid and (10 ml) distilled water. Mix 1 ml of the plant extract with 1 ml of Dragendorff's reagent. Brown deposits suggest the existence of alkaloids (Stahl,1969).

2.3.1.2 : Test for Tannins (Lead acetate reagent)

Four drops of ferric chloride were added to 2ml of the aqueous solution of each extract. A preliminary test for the tannins ingredient in the plant was the appearance of green precipitation (Harborne, 1998).

2.3.1.3 : Test for flavonoids (Alcoholic potassium hydroxide reagent)

Mix 1 ml of the extract with 1 ml of 0.5 M potassium hydroxide and shake the mixture well. The presence of flavonoids is shown in the yellow hue (Al-Khazragi, 1991).

2.3.1.4 : Test for saponin (Mercury Chloride Reagent)

The presence of saponins may be confirmed by adding 1 ml of a reagent containing mercuric chloride to 1.5 ml of the extract in a test tube (Al-Khazragi, 1991). This will result in the formation of a white precipitate.

2.3.1.5 : Test for Terpenoids

Mix 1 ml of plant extract with 2 ml of chloroform and add a drop of anhydrous acetic acid and a drop of concentrated sulfuric acid. If the light brown color indicates the presence of terpenoids (Al-Maisry, 1999).

2.3.1.6 : Test for phenol by Ferric chloride reagent

Ferric chloride (FeCl3) may be made by dissolving 1 g into 100 ml of water. The plant extract was used to wet filter paper, which then had drops of Follen's reagent or ferric chloride added to it before being subjected to ammonia vapor. The presence of phenols may be seen as a bluish tint (Adedayo *et al.*, 2001).

2.3.1.7 : Test for glycosides by Keller-Killani’s Test:

About 2 ml of each extract was treated with 1 ml of concentrated Sulfuric acid (H_2SO_4) and 1 ml of glacial acetic acid solution (1 drop of ferric chloride solution). The presence of glycoside in the plant extract was inferred from the appearance of a reddish brown color at the interface of the two layers (Harborne, 1998).

2.3.2 : Preparation of crude chemical compound extracts

The Markham approach (1982) was modified as follows at the College of Science for Women's advanced plant laboratory in order to extract the chemicals:

1- Pieces of the plant sample are put in a beaker100ml. with a capacity of 1000ml and soaked in a concentration of 70% ethanol alcohol for 24hr.
2- After that, each sample is filtered with clean gauze and put it in the numbered tubes according to the sample number
3- Then put it in the centrifuge to get rid of all the deposits until getting a pure sample of impurities for 10 min at 2000 rpm
4- After the completion of the centrifuge put 5ml of the sample and put it in a tube and put 2ml of solvent hexane with it and shake them. The impurities and the fatty layer will separate.
5- withdraw fatty layer and the impunities to obtain a pure sample
6- After that, the filter is prepared for analysis using GC-MS (Gas Chromatography-Mass Spectrometry), as in (Figure 2-2)

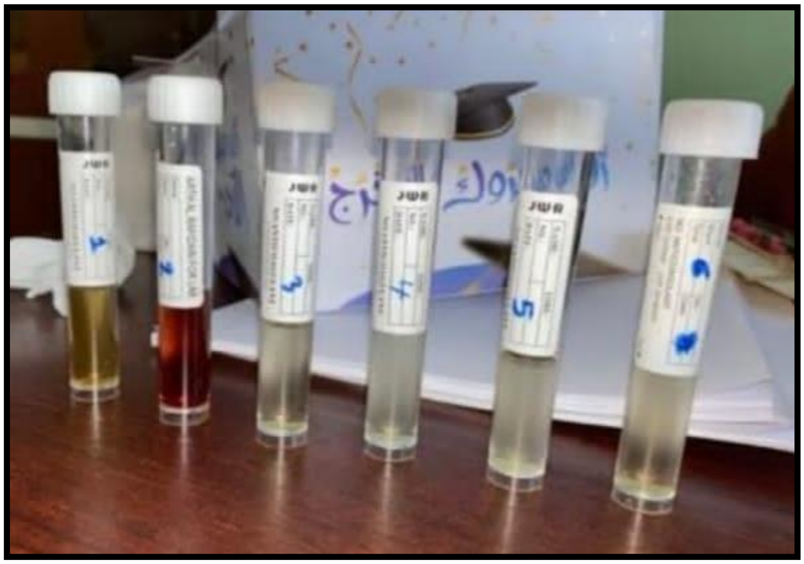

Figure 2-2: Samples of plant extracts before examination by GC-MS analysis

2.3.3 Separation and identification of the active compounds from the crude compounds extract of the species under study using the GC-MS device

Whole plant extracts were used for the analysis, which was done at the GC-MS unit at the Ibn Al-Bitar Center in Baghdad using an Agilent System (7820A) USA GC Mass Spectrometer and depicted in (Figure 2-3) using a conductive gas chromatography-mass spectrometer (GC-MS):

1- The approved conditions for the GC-MS analysis method

- Gas Chromatograph Type: Agilent (7820A) USA GC- Spectrometer MS
- Analysis and separation column (stationary phase): (30 m long x 250 µm diameter x 0.25 µm inner diameter)
- Use 99.999% helium (mobile phase) as carrier gas with a constant flow of 1 ml/min.
- The volume of the injected fluid is 1 microliter.
- The pressure is 11.933 psi
- Programming a temperature of 250 °C for the injector, and 310 °C for the ionic source.

- Injection type: Spitless
- • The temperature of the oven is set to rise at a rate of 8 °C per minute from 80°C to 136 °C, from 180 °C to 244 °C, and from 280 °C to 298 °C.
- The total time to run the GC is 1.30 minutes

2- Characterization of compounds

Using the National Institute of Standards and Technology (NIST) database, the interpretation was done on the GC-MS mass spectra and then compared the mass spectra of unknown chemicals to those of recognized substances kept in the NIST collection. It was confirmed that the materials' constituent parts' names, molecular weights, and structures were accurate.

Figure (2-3): Gas chromatography (GC-MS) apparatus Ibn Al-Bitar Center in Baghdad.

2.4 : Molecular study

Following the completion of the molecular investigation in the labs of the department of biology between 25 October 2022 and 25 January 2023, the DNA sequence was evaluated and carried out at the Macrogen Center in South Korea. Tables (2-2 and 2-3) present the results of the molecular investigation's usage of various types of materials and laboratory apparatus.

Table 2-1: Materials used in the molecular study.

No.	Material	Company
1	Agarose	Canada / USA
2	Red safe staining solution	Intron / Korea
3	DNA Loading dye	Canada/ BioBasic
4	Ladder 1000 plus	Intron / Korea
5	Pre-mix PCR	Intron / Korea
6	Tri-Borate-EDTA Buffer (TBE buffer) 10X	USA/ Promega
7	Primer	Integrated DNA technologies /USA
8	ZR Plant/Seed DNA MiniPrep™	Zymo / USA
9	Liquid nitrogen	Iraq / AID
10	Deionized Distilled Water	Sigma/ USA

Table 2-2: laboratory equipment and apparatus used in the molecular study.

N0.	Apparatus	Origin \Company
1	AURA TM PCR Cabinet	Italy\Euro clone
2	Micro spin 12, High-speed Mini-centrifuge	Germany\Bio san
3	Pipette Variable Volume	Germany\ Labnet
4	Mini-Power Supply 300V, 2200V	Chain\ Supplier
5	Multi Gene Opti Max Gradient Thermal Cycler	USA\ Labnet
6	Electrophoreses	USA\ CBS scientific
7	Gel Document system	UK \ Cleaver Scientific
8	UV- Trans illuminator	UK \ LAB- LINE
9	Micro spin	Lativa\ Biosan
10	Combi-spin	Lative \Biosan
11	Sensitive balance	Switzerland \ Sartorius
12	Incubator	England \ Memmert
13	Microwave	China\ Gosonic
14	Water distillation	China\ Rovsun
15	Spectrophotometer (Nanodrop)	England \ Cleaver Scientific
16	Vortex mixer	USA\ Tomy
17	Liquid Nitrogen container	USA\Native Industrialization
18	Isolation)Hood chamber)	Iraq\Native Industrialization
19	Eppendorf tubes	Belgium\ BioBasic Inc
20	Digital camera	Japan\ Sony
21	Ceramic crucible	-
22	Tips Variable Volume	-

2.4.1:DNA Extracted from Plant Samples

A simple and efficient method for isolating DNA from plant material, including genomic, mitochondrial, and chloroplast DNA, is made available by the ZR Plant/Seed DNA Plasmid Collection Kit. As per the included leaflet, the DNA was taken from the entire plant used in the study as follows:

The working method for extracting and purifying DNA was followed with the extraction kit using ZR plant/seed DNA MiniPrep™ and before starting extraction, to dilute the genomic lysis buffer solution to 0.5% (or 250ml/50ml), as instructed in the extraction kit's booklet, ethanol (supplied by the user) was added.

1- Fresh plant samples were cut into very small pieces and pulverized with liquid nitrogen continuously until they turned into a very fine powder.

2- Weighing 150 mg and transferring it to the ZR BashingBead^{TM} Lysis Tube.

3- Put 750µl of Lysis Solution in the tube, cap it securely, and give it a thorough mixing with a vortex for 5 min., then put it in an ultracentrifuge at 10,000 rpm for 1 min.

4- After centrifuging the supernatant at 8000 rpm for 1 min., up to 400µl was moved to a Zymo-Spin^{TM} IV spinning filter tube fitted in a collecting tube. The filtrate was discarded after the process.

5- In order to separate the DNA from the RNA, 1200µl of genomic lysis buffer was added to the filter in the collecting tube before being swirled at 8,000 rpm for 1 min.

6- The solution from Step 5 is poured into a collecting tube containing a Zymo-Spin^{TM} IIC column, centrifuged at 10,000 rpm for 1 minute, and the supernatant is removed before the procedure is repeated.

7- Drop 200µl of DNA Pre-Wash Buffer solution into a new collection tube with this column in it, and spin the tube at 10,000 rpm for 1min, then adding 500µl Wash Buffer of DNA to the Column, and spin the mixture for 1 min. at 10,000 rpm.

8- Place the Zymo-Spin™ IIC Column into a sterile microcentrifuge tube with a capacity of 1.5ml, and then add 50–100µl Elution Buffer of DNA to the column matrix, with a minimum volume of 25µl. The DNA may be extracted by centrifuging the sample at 10,000 rpm for 30 seconds.

9- Put the DNA that was eluted in Step 10 to a Zymo-Spin™ IV-HRC Spin Filter that has been prepared and placed in a micro centrifuge tube that is clean. Next, centrifuge the mixture at exactly 8,000 rpm for one minute. The DNA that has been screened is now appropriate for use in PCR and other downstream processes.

2.4.1 : Analysis of DNA purity and concentration:

A spectrophotometer (Nano drop) was employed for this, with a drop of 1 µl of DNA extract placed on its sensitive lens following calibration with a drop of elution buffer. Next, at wavelengths 260 and 280, the DNA concentration values of each plant extract were recorded.

2.4.2 : Agarose gel electrophoresis of DNA

Following the procedures outlined by (Sambrook and Russel ,.2001), genome electrophoresis was performed:

1- A glass plate was prepared, the edges of which were surrounded by strong adhesive tape, and the special comb for drilling was fixed on it at one end of the gel.

2- Preparing a 1% agarose gel required dissolving 1 gram of agarose in 10 milliliters of 10x TBE buffer and then adding enough distilled water to bring the total amount to 100 milliliters.

3- Heating the mixture in a microwave until the agarose powder melts, and removing the solution from the oven before it reaches the boiling point.

4- Leaving the agarose gel to cool to a temperature of 50°C.

5- Adding 5µl of Red Safe Nucleic acid dye solution 10 mg/ml after the mixture has cooled, and mix gently.

6- Pouring the agarose gel slowly into the tip, taking care not to form bubbles in the solution, so that the gel does not deform and then leave for 30 min to cool.

7- The comb and adhesive tape were carefully removed from the frozen agarose after 30 minutes. The plate was then attached to its support in the horizontal electrophoresis unit, submerged in TBE buffer solution, and connected to the electricity source so that the negative pole is with its counterpart and the positive with its counterpart.

8- Adding 5µl of DNA Ladder Size Guide to the first hole and use that to find out the size of the separated DNA pieces.

9- 3µl of DNA sample was placed on Parafilm, mixed with 2µl of loading dye, and mixed with a pipette.

10- The electrical relay was closed, and then the electric current was passed with a voltage of 70 volts and left until the loading dye from the pits to the other side volts for 45 min.

11- Screening of the gel using a UV trans-illuminator at wavelengths (240 and 360) nm, and then photography using a digital camera.

2.4.3 : The interaction's primers

Bioneer lyophilized seven primers, which were employed in the reaction. The primers were diluted by adding double-distilled water (ddH_2O) to obtain a concentration of 100 pmol/µl in accordance with the attached leaflet from the company that supplied them. After that, the required concentration was prepared by taking 10 pmol/µl of the stock solution and completing the volume to 100 µl.

having been prepared for use by adding water that has undergone double distillation (Table 2-4).

Table 2-3: The name of the primer and the sequence of nitrogenous bases for the RAPD indicator

NO.	Primer	Sequence (5 to 3)	References
1	OP-V19	GGGTGTGCAG	(Ismail *et al*., 2020)
2	OP-R06	GTCTACGGCA	(Ismail et al., 2020)
3	OP-V14	AGATCCCGCC	(Ismail et al., 2020)
4	OP-V09	TGTACCCGTC	(Habeeb *et al*., 2022
5	OP-L05	ACGCAGGCAC	(Habeeb *et al*.,2022)
6	OP-M05	GGGAACGTGT	(Habeeb *et al*.,2022)
7	OP-P04	GTGTCTCAGG	(Habeeb *et al*.,2022)

2.4.4 : Polymerase Chain Reaction (PCR)

The RAPD molecular indicator used in this study was employed in the PCR method by adding 5µl DNA template and 2µl primer into a prepared master mix reaction tube. The reaction was then finished by adding deionized distilled water to the reaction to create a volume of the solution of 25µl. (Table 2-5). As stated in table, the thermocycler was put into a special primers schedule (2-6)

Table 2-4: Mixture of the specific interaction for diagnosis gene

Components	Volume µl
Taq PCR PreMix	5
primer	10 picomols/ (2)
DNA	2
Distill water	16
Final volume	25

Table 2-5: Thermal cycle stage of RAPD molecular indicator Polymerase Chain Reaction technology.

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	94	5 min.	1
2-	Denaturation -2	94	30 sec	40
3-	Annealing	36	45sec	
4-	Extension-1	72	45 sec	
5-	Extension -2	72	7 min.	1

2.4.5 : Detection of PCR products

Following the completion of the reaction period, the tubes were taken out of the thermopolymer, and 10 µl were taken from each and loaded, with the DNA ladder loaded on one side, onto the agarose gel etch that had been previously made with a concentration of 2%. The PCR results were then subjected to ultraviolet light using the gel documentation system for imaging at 70 volts (Bani Hassan, 2021).

2.4.6 : Detection of *mat*k and *rbc*L genes using (PCR) technique

Primers for amplification were used to carry out the process of detecting the particular primers of the genes *mat*k and *rbc*L. A portion of the *matk* and *rbcL* genes were amplified by employing a forward primer and a reverse primer, as indicated in the nucleotide sequences of these primers in Table (2-7). These primers are made available by IDT (Technology Company). Integrated DNA, Canada). Integrated DNA, Canada). A PCR amplification was carried out using a total volume of 25µl, which included 2µl of DNA, 5µl of Taq PCR PreMix, and 1µl of each primer (10pmol). Following this, distilled water was added to the tube to bring the volume up to a total of 25µl. (table 2-8).

Table 2-6: Specialized primers for genes *mat*k and *rbc*L.

Primers name		Sequence 5′ to 3′	Tm (°C)	Product size
*mat*K	Forward	ACTGTATCGCACTATGTATCA	50.6	400-650 base pair
	Reverse	GCATCTTTTACCCARTAGCGAAG	55	
*rbc*L	Forward	ATGTCACCACAAACAGAGACTAAAGC	57.2	500-650 base pair
	Reverse	GTAAAATCAAGTCCACCACG	52	

Table 2-7: Mixture of the specific interaction for diagnosis gene

Components	Volume µl
Taq PCR PreMix	5
Forward primer	10 picomols/ (1)
Reverse primer	10 picomols/ (1)
DNA	2
Distill water	16
Final volume	25

Temperature optimization conditions were carried out using a thermocycler, the *mat*k gene was denatured by heating it to 95°C for three minutes, then putting it through 35 cycles heating it to 95°C and then 50°C for one minute each, and then on 72°C for one minute and then an additional seven minutes as a final incubation (Table 2-9). The *rbc*L gene was heated for three minutes at 94°C, and then cycled through 35 cycles of 94°C and 52°C for one minute each and then at 72°C for one minute, subsequent to seven minutes as a last stage of incubation (Table 2-10). After this stage, the PCR products were separated using electrophoresis on an agarose gel at 2%, and after that, they were dyed with a red dye before being subjected to UV radiation with a wavelength of 302 nm.

Table 2-8: The optimum condition of detection of the *mat*k gene

No.	Phase	Tm (^{o}C)	Time	No. of cycle
1-	Initial Denaturation	95	3 min.	1
2-	Denaturation -2	95	45sec	35
3-	Annealing	50	45sec	
4-	Extension-1	72	1 min	
5-	Extension -2	72	7 min.	1

Table 2-9: The optimum condition of detection of the *rbc*L gene

No.	Phase	Tm (^{o}C)	Time min.	No. of cycle
1-	Initial Denaturation	94	3	1
2-	Denaturation -2	94	1	35
3-	Annealing	55	1	
4-	Extension-1	72	1	
5-	Extension -2	72	7	1

2.4.8. : Data analysis

Using the UV band program, the number of multiplying bands and their molecular sizes were known. The characterization data was turned into tables that showed whether or not the band was present in each of the studied samples by putting the number 1 when the band was present and the number 0 when it wasn't. Similarity values between a pair of samples were calculated in several ways, but the most important ones are:

1- Similarity index: It can be calculated in two ways, the most common of which is the method (Bani Hassan, 2021), as it is possible to estimate similarity values as follows:

Similarity = 2nxy\nx+ny

Where nxy is the number of bands shared by both the A and B models, and nx and ny are the full sets of bands for the A and B models, respectively.

2- The amount of dissimilarity was measured by subtracting the similarity value from the number 1, that is, it is equal to (1-S), and it was applied to the similarity data to find the genetic distance between each of the two samples based on the similarity values between those two samples under study.

2.4.9: Dendrogram Genetic Relationship Tree

The Unweighted Pair Group Method with Arithmetic Average (UPGMA) program was adopted to obtain the genetic relationship tree (evolution and phylogeny) using the vital statistical program PAST version 62.1 (Hammer *et al*., 2001)). By using the aforementioned method, the genetic dimension was derived, and a genetic relationship tree was created.

The discriminatory power, morphological heterogeneity, and efficiency of each primer were calculated using the following equations, according to the method of (Grundmann *et al*., 1995).

$$\textbf{Percentage }(\%)\textbf{Polymorphism per primer} = \frac{\textbf{Number of variant bundles in the primer}}{\textbf{total number of primer bands}} \times \mathbf{100}$$

Each primer's percentage of discriminating power was determined using the following formula:

$$\textbf{Discrimination power per primer }(\%) = \frac{\textbf{Number of differential bands of the primer}}{\textbf{number of differential bands of all primers}} \times \mathbf{100}$$

It was determined using the following equation how much of a percentage each primer contributed to the overall efficiency of the reaction:

$$\textbf{The efficiency of each primer }(\%) = \frac{\textbf{Total number of primer bands}}{\textbf{total number of all primer bands}} \times \mathbf{100}$$

2.4.9: Analysis of sequence data for comparison and genetic drawing of the studied species

After the PCR test was finished, the product of the PCR reaction was sent to the Korean company Macrogen for DNA sequencing. During this process, the company used the Basic Local Alignment Search Tool (BLAST) and National Center for Biotechnology Information (NCBI) software, the Gen database, and the BioEdit program. Following this, it was saved on the bases (that is, each rule was set against it) with spaces left for mutations, and the results were then analyzed. if there is any, to initiate decomposition through three programs of maximum similarity, which are then sent to Cipres for the analysis of results related to the nucleotide sequences of the plant species that are being researched, while the analysis by the programs UPGAM and Neighbor-Joining is performed directly by the program PAUP in a computer in order to draw a polygenetic analysis tree.

Chapter Three

Results and Discussion

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3.1 Chemical Study

Using the chemical content of the whole plant of each species, the findings of the GC-MS chemical investigation in this study have helped to distinguish between the species, with applications in both medical and non-taxonomic research.

3.1.1: Preliminary Phytochemical Analysis of Plant Extracts.

Using the chemical content of the whole plant of each species, the findings of the GC-MS chemical investigation in this study have helped to distinguish between the species, with applications in both medical and non-taxonomic research.

The results of the preliminary analysis of the plant extracts showed the presence of some active compounds in the various extracts used in the current study Table 3-1, saponine are found in all species expect *Echinocactus* ,alkaloids are found in all species under study expect *Echinocactus*,glycoside are found in *Hylocereus* ,*Aloe vera* , *Opuntia ficus- indica* while abscent in vest of the species,but the presence of terpenoids and tannins in all species except *Espostoa guntheri.*

There is a diverse array of chemical components in cactus plants, all of which are generated in response to environmental conditions. Polyphenols, alkaloids, betalains, terpenes, and fatty acids, many of which have medicinal or culinary uses, are among the most abundant chemicals obtained (Das *et al*., 2020).

Cactus plants have been shown to contain at least 50 different phenethylamines and almost 80 different isoquinolines. According to (Santos-Diaz and Camarena-Rangel,2019), some of the most frequent alkaloids in these plants are mescaline, hordenine, N-methyltyramine, tyramine, and macromerine. *Opuntia* species, namely *O. ficus-indica*, have been documented as containing polyphenols, while the *Hylocereus*, *Pereskia*, *Ariocarpus*, and *Coryphantha* have all been linked to polyphenolic content (Das *et al*., 2020). (Kakuta and coworkers 2012), (Salazar and Céspedes ,2013) and report finding a wide variety of substances in cactus plants, including the classes of compounds terpenes, saponins, and sterols. Several variables, including the environment, harvest time, plant age, and extraction technique, contribute to discernible variations in essential oil content (Souza *et al*., 2016).

Table 3-1: The presence of the active compounds in each methanol plant extract.

Plant extracts	Phenol	Alkaloids	Glycoside	Flavonoids	Saponins	Terpenoids	Tannins
Hylocereus undatus	+	+	+	+	+	+	+
Aloe vera	+	+	+	+	+	+	+
Opuntia ficus-Indica	+	+	+	+	+	+	+
Espostoa guentheri	+	+	-	+	+	-	-
Echinocactus Grusonii	+	-	-	+	-	+	+
Mammillaria Elongate	+	+	-	+	+	+	+

A sign (+) indicates the presence of the compound while a sign (-) indicates the absence of the compound in the extract

3.1.1 : Analysis of plant extracts using GC-MS

The present research was successful in identifying 15 significant chemical compounds in each plant that were investigated using GC-MS technology. These chemicals included alkaloids, terpenes, glycosides, flavonoids, saponins, phenols, and tannins, all of which function as active molecules in these extracts.

Through the use of GC-MS, it was discovered that there are variations in the kinds of chemical compounds, their peaks, and the times at which they manifest themselves. Additionally, the examination of the compounds demonstrated in the process of extracting the studied species in the form of peaks of varying heights and areas according to the various compounds that were identified.

3.1.1.1 **:** ***Hylocereus undatus***

Hylocereus undatus plant extract chromatogram showed 15 peaks, the highest peak area being 44.90% for Oxime-, methoxy-phenyl at a retention time of 4.083 min and the lowest area being 1.06% for Melamine, tris(trimethylsilyl) derivative at a retention time of 11.570 min (Table 3-2 and Figure 3-1). *Hylocereus undatus*, when compared to other studies, was found to have eight peaks (Sudha *et al.*, 2017).

Table 3-2 : GC–MS chromatogram of the alcohol extract of *Hylocereus undatus* of the whole plant

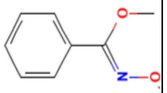
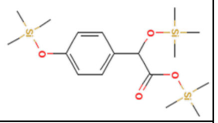
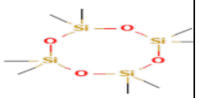
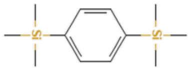
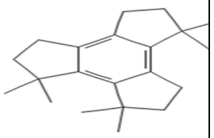
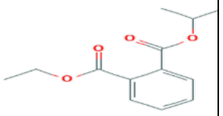
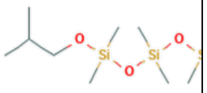
No.	Name of compound	Retention time (min)	Area %	Molecular weight (g/mol)	Molecular formula	Chemical formula
1	Oxime-, methoxyphenyl	4.083	44.90	151.16	$C_8H_9NO_2$	
2	4-Trimethylsilyloxybenzoic acid, - (trifluoroacetyl oxycarbonyl) phenyl ester	4.321	6.90	384.7	$C_{17}H_{32}O_4Si_3$	
3	Cyclotetrasiloxane,octametyl	4.763	2.28	296.61	$C_8H_{24}O_4Si_4$	
4	1,4-Bis(trimethylsilyl)benzene	6.381	14.70	222.47	$C_{12}H_{22}Si_2$	
5	1H-Trindene, 2,3,4,5,6,7,8,9-octahydro-1,1,4,4,9,9-hexamethyl	6.683	7.15	282.5	$C_{21}H_{30}$	
6	Phthalic acid, ethyl 2-isopropoxyphenyl ester	7.223	2.01	328.4	$C_{19}H_{20}O_5$	
7	1,1,3,3,5,5,7,7-Octamethyl-7-(2-methylpropoxy)tetrasiloxan-1-ol	8.841	2.87	370.74	$C_{12}H_{34}O_5Si_4$	

Table 3-2 : GC–MS chromatogram of the alcohol extract of *Hylocereus undatus* of the whole plant

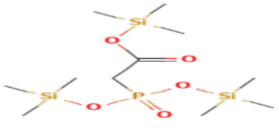
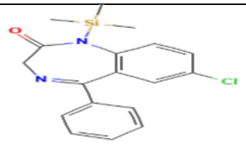
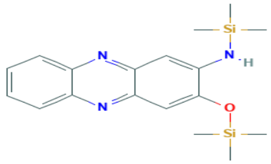
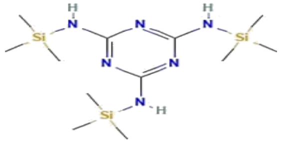
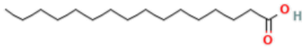
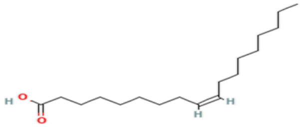
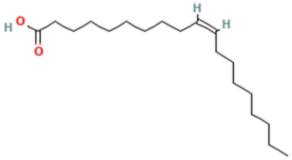
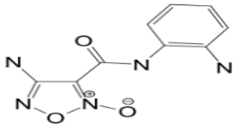
No.	**Name of compound**	**Retention time (min)**	**Area %**	**Molecular weight (g/mol)**	**Molecular formula**	**Chemical formula**
8	Acetic acid, [bis[(trimethylsilyl) oxy] phosphinyl]-, trimethylsilyl ester	9.024	2.21	356.57	$C_{11}H_{29}O_5PSi_3$	
9	2H-1,4-Benzodiazepin-2-one, 7-chloro-1,3-dihydro-5-phenyl-1-(trimethylsilyl)-	9.251	1.68	431.1	$C_21H_{27}ClN_2O_2 Si_2$	
10	3-Amino-2-phenazinolditms	11.322	1.67	355.6	$C_{18}H_{25}N_3OSi_2$	
11	Melamine,tris(trimethylsilyl) derivative	11.570	1.06	278.38	$C_9H_{27}BO_3Si_3$	
12	n-Hexadecanoic acid (Palmitic Acid)	20.094	2.16	256.42	$C_{16}H_{32}O_2$	
13	Oleic Acid	22.219	5.45	282.5	$C_{18}H_{34}O_2$	

Table 3-2 : GC–MS chromatogram of the alcohol extract of *Hylocereus undatus* of the whole plant

No.	Name of compound	Retention time (min)	Area %	Molecular weight (g/mol)	Molecular formula	Chemical formula
14	cis-10-Nonadecenoic acid	22.478	3.13	296.5	$C_{19}H_{36}O_2$	
15	4-Amino-2-oxy-furazan-3-carboxylic acid	22.812	1.84	235.2	$C_9H_9N_5O_3$	

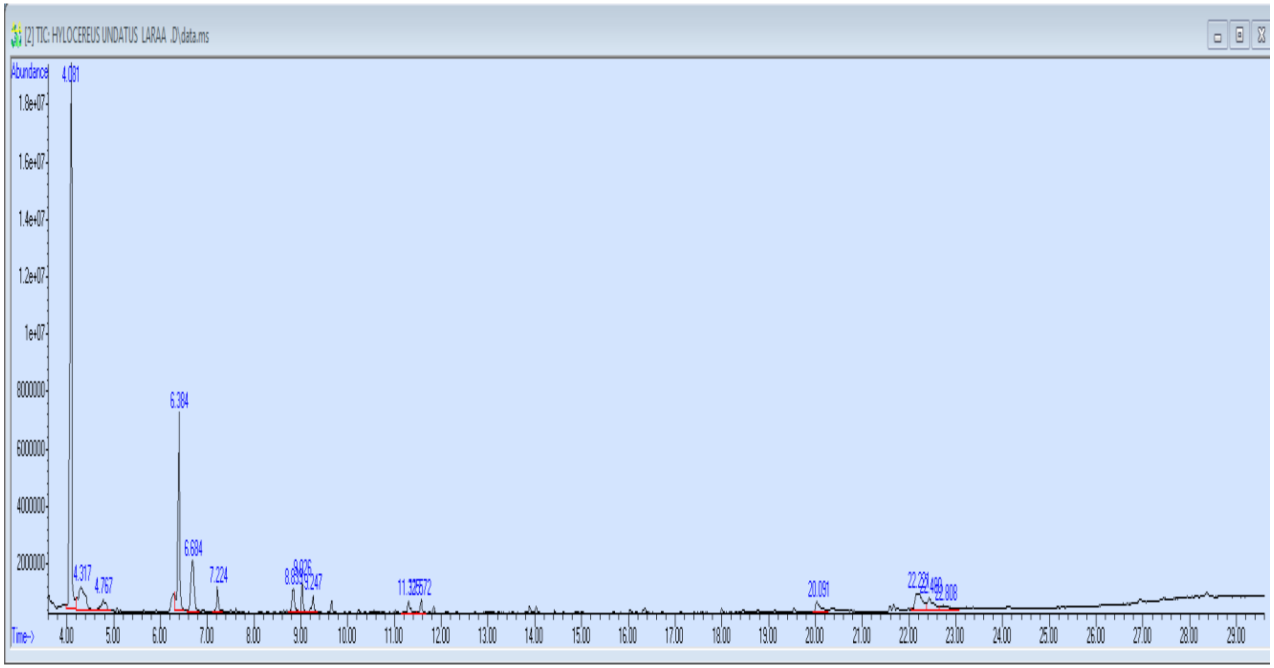


Figure 3-1: Chromatogram profile for GC-MS for extract of *Hylocereus undatus*

3.1.1.2 **:** ***Aloe vera***

In *Aloe vera* ethanolic extract for the whole plant, the chromatogram shows 15 peaks (Figure 3-2). The retention time of the first peak was 4.062 min, which represented the compound Oxime-, methoxy-phenyl with a peak area of 40.02% as the highest peak, While the Cyclopentasiloxane, decamethyl has a lowerest area of 1.40% in retention time of 11.333 min, and the other chemical compounds recorded different retention times and with different areas, as shown in Table (3-3). The analysis of GC mas performed on *Aloe vera* in the previous years proved that there were 18 compounds, di(2-propylpentyl) ester and hexadecenoic acid ethyl ester of phthalic acid were the most abundant compounds in the *A.vera* extract (Alghamdi *et al.*, 2023)

Table 3-3: GC–MS chromatogram of the alcohol extract of *Aloe vera* of the whole plant

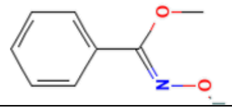
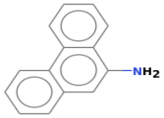
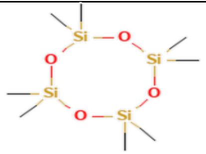
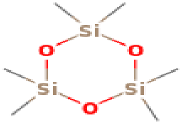
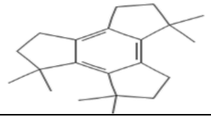
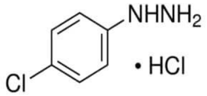
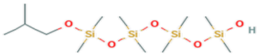
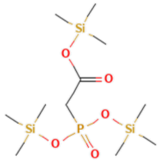
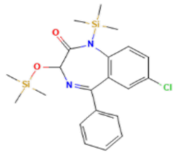
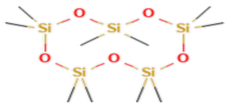
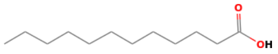
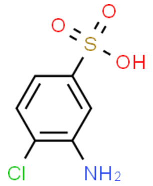
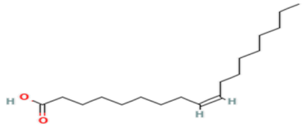
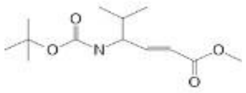
No.	**Name Of Compound**	**Retention time (min)**	**Area%**	**Molecular weight (g/mol)**	**Molecular formula**	**Chemical formula**
1	Oxime-, methoxy-phenyl-	4.062	40.02	151.16	$C_8H_9NO_2$	
2	9-Phenanthrenamine	4.288	10.33	193.2438	$C_{14}H_{11}N$	
3	Cyclotetrasiloxane, octamethyl	4.763	2.89	296.61	$C_8H_{24}O_4Si_4$	
4	Cyclotrisiloxane, hexamethyl	6.327	13.05	222.4618	$C_6H_{18}O_3Si_3$	
5	1H-Trindene, 2,3,4,5,6,7,8,9-octahydro-1,1,4,4,9,9-hexamethyl	6.673	9.44	282.5	$C_{21}H_{30}$	
6	4-(4-Chlorophenyl)-2,6-diphenylpyridine	7.223	1.61	224.64	$C_{11}H_9ClO_3$	
7	1,1,3,3,5,5,7,7-Octamethyl-7-(2-methylpropoxy)tetrasiloxan-1-ol	8.830	2.40	370.74	$C_{12}H_{34}O_5Si_4$	
8	Acetic acid, [bis[(trimethylsilyl) oxy]phosphinyl]-, trimethylsilyl ester	9.025	2.78	372.57	$C_{11}H_{29}O_6PSi_3$	

Table 3-3: GC–MS chromatogram of the alcohol extract of *Aloe vera* of the whole plant

No.	Name Of Compound	Retention time (min)	Area%	Molecular weight (g/mol)	Molecular formula	Chemical formula
9	2H-1,4-Benzodiazepin-2-one, 7-chloro-1,3-dihydro-5-phenyl-1-(trimethylsilyl)-	9.240	2.39	431.1	$C_{21}H_{27}ClN_2O_2Si_2$	
10	Cyclopentasiloxane, decamethyl	11.333	1.40	370.77	$C_{10}H_{30}O_5Si_5$	
11	Dodecanoic acid	20.104	2.38	200.3178	$C_{12}H_{24}O_2$	
12	2-Chloroaniline-5-sulfonic acid	20.450	1.49	60181	$C_6H_6ClNO_3S$	
13	9-Octadecenoic acid, (E)-	22.230	4.94	282.46	$C_{18}H_{34}O_2$	
14	Oleic Acid	22.478	3.29	282.5	$C_{18}H_{34}O_2$	
15	2-(E)-Pentenoic acid, (4S)-4-[(t-butoxycarbonyl-(S)alanyl)amino], ethyl ester	22.791	1.61	257.33	$C_{13}H_{23}NO_4$	

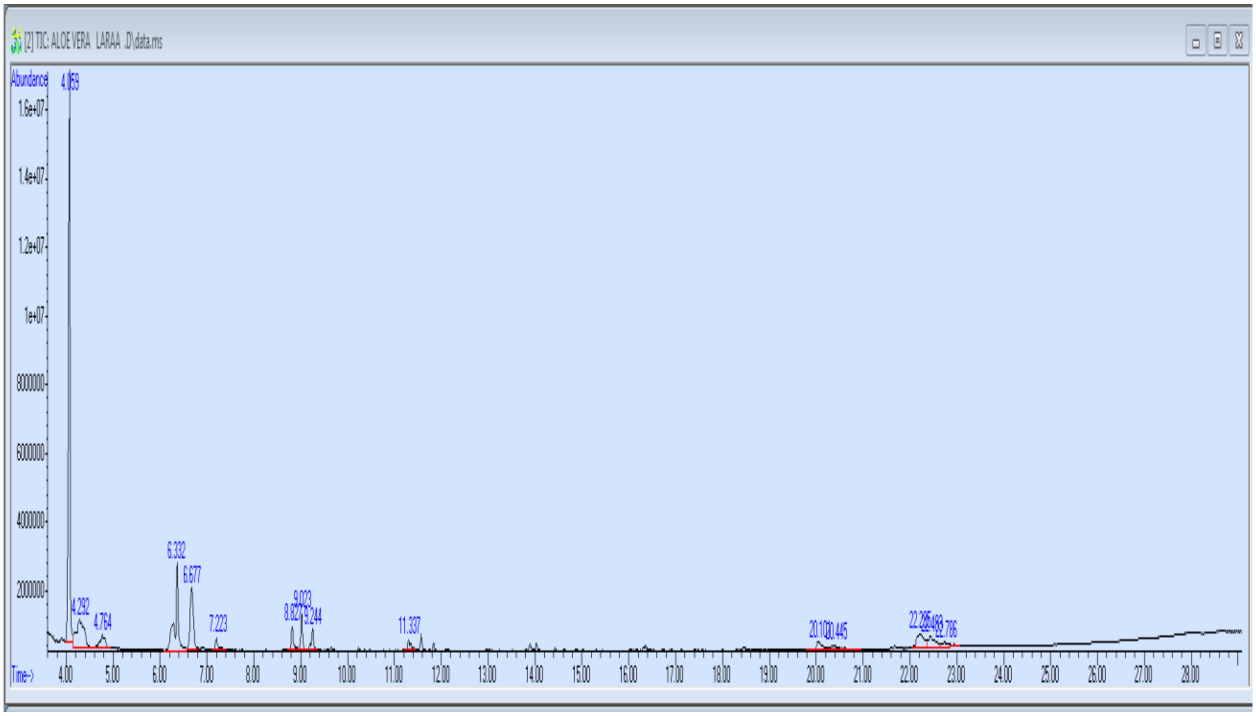


Figure 3-2: Chromatogram profile for GC-MS of *Aloe vera*

: *Opuntia ficus indica*

In the *Opuntia ficus indica* extract, the first peak belonged to an Oxime-, methoxy-phenyl compound with a retention time of 3.98 min with a peak area of 36.84% as the highest peak. Also, the Benzoic acid, 3-pentafluoropropionyloxy-, tert.-butyldimethylsilyl ester had its lowest peak in retention time of 7.223 and a peak area of 1.47%. While the other chemical compounds area recorded different times of appearance as shown in Table (3-4), Figure (3-3). In previous study, GC-FID and GC-MS were used to identify 14 new compounds in the n-hexane extract. Hexadecanoic acid, heptacosane, methyl linoleat, camphor, borneol, verbenone, pentacosane, and α-terpineol were the main components (Karadağ *et al*., 2018).

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Table 3-4: GC–MS chromatogram of the alcohol extract of *Opuntia- ficus- indica* of the whole plant

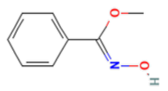
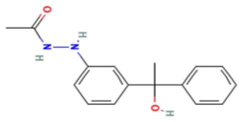
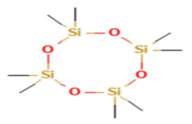
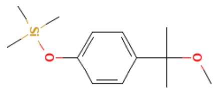
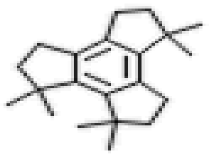
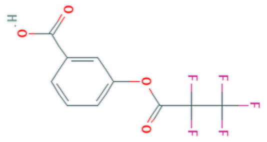
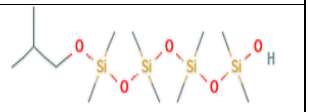
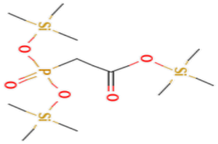
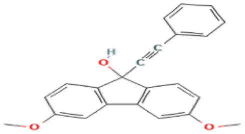
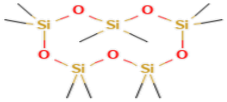
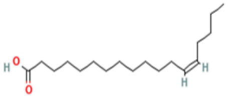
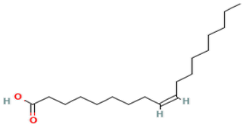
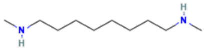
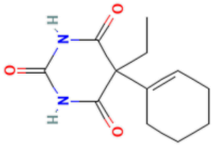
No.	Name of compound	Retention time (min)	Area%	Molecular weight (g/mol)	Molecular formula	Chemical formula
1	Oxime-, methoxy-phenyl	3.986	36.84	151.16	$C_8H_9NO_2$	
2	Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl)phenyl] hydrazide	4.310	8.90	270.33	$C_{16}H_{18}N_2O_2$	
3	Cyclotetrasiloxane, octamethyl	4.741	3.98	296.61	$C_8H_{24}O_4Si_4$	
4	Trimethyl[4-(1-methyl-1-methoxyethyl)phenoxy] silane	6.306	10.74	238.40	$C_{13}H_{22}O_2Si$	
5	1H-Trindene, 2,3,4,5,6,7,8,9-octahydro-1,1,4,4,9,9-hexamethyl	6.683	10.48	282.5	$C_{21}H_{30}$	
6	Benzoic acid, 3-pentafluoropropionyloxy-, tert-butyldimethylsilyl ester	7.223	1.47	398.40	$C_{16}H_{19}F_5O_4Si$	
7	1,1,3,3,5,5,7,7-Octamethyl-7-(2-methylpropoxy)tetrasiloxan-1-ol	8.830	3.00	370.74	$C_{12}H_{34}O_5Si_4$	

Table 3-4: GC–MS chromatogram of the alcohol extract of *Opuntia- ficus- indica* of the whole plant

No.	Name of compound	Retention time (min)	Area%	Molecular weight (g/mol)	Molecular formula	Chemical formula
8	Acetic acid, [bis[(trimethylsilyl) oxy]phosphinyl]-, trimethylsilyl ester	9.024	3.19	356.57	$C_{11}H_{29}O_5PSi_3$	
9	Fluoren-9-ol, 3,6-dimethoxy-9-(2-phenylethynyl)-	9.251	2.44	342.4	$C_{23}H_{18}O_3$	
10	Cyclopentasiloxane, decamethyl	11.333	1.98	370.77	$C_{10}H_{30}O_5Si_5$	
11	Tetradecanoic acid	20.104	2.53	228.371	$C_{14}H_{28}O_2$	
12	cis-13-Octadecenoic acid	22.240	5.19	282.5	$C_{18}H_{34}O_2$	
13	Oleic Acid	22.489	3.71	282.5	$C_{18}H_{34}O_2$	
14	1,8-Octanediamine, N,N'-dimethyl	22.812	2.11	172.31	$C_{10}H_{24}N_2$	
15	Cyclobarbital	26.470	3.45	236.27	$C_{12}H_{16}N_2O_3$	

3.1.1.3 : *Espostoa guentheri*

Plant extract chromatogram of *Espostoa guentheri* showed 15 peaks too, the highest peak area being 49.26 % for Oxime-, methoxy-phenyl at a retention time of 4.029 min and the lowest area being 1.29% for Hydrazinecarboxamide at a retention time of 7.234 min. The rest included the area percentages of the chemical compounds between those values above and at different times (Table 3-5 and Figure 3-4).

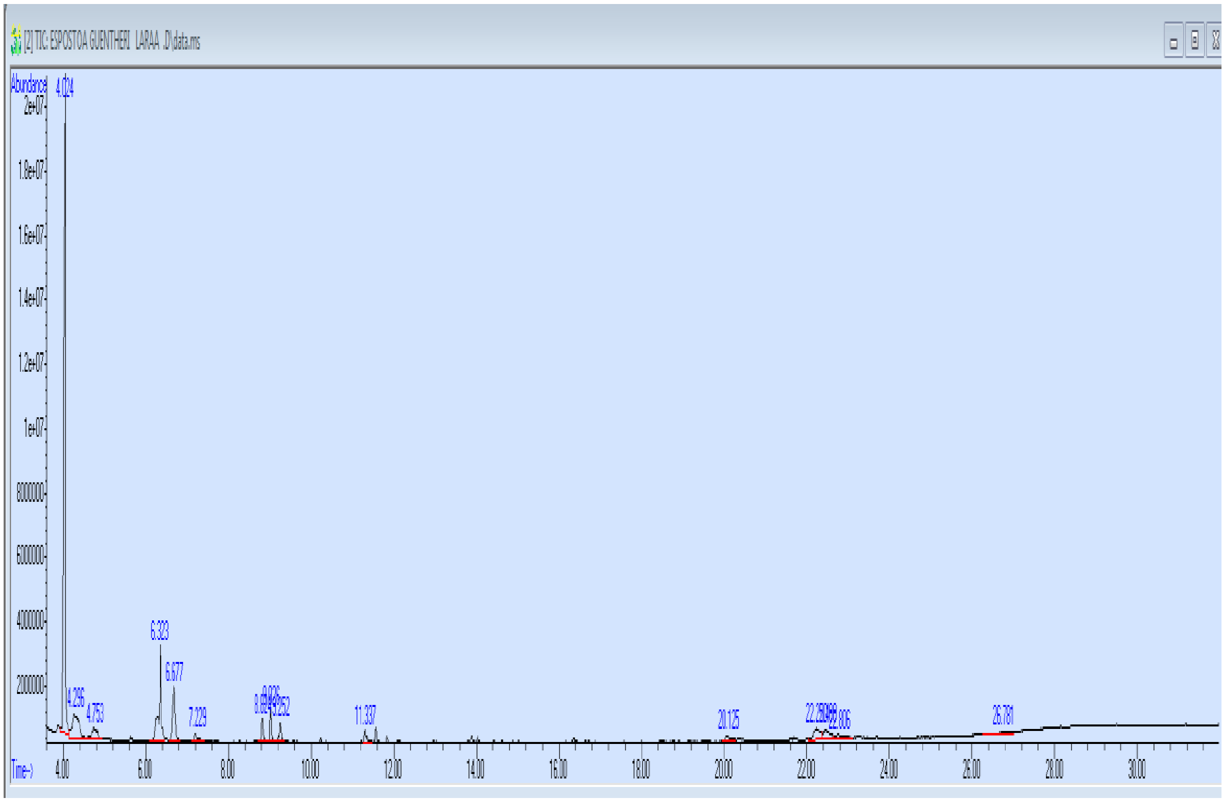


Figure 3-4: Chromatogram profile for GC-MS of *Espostoa guentheri*

Table 3-5: GC–MS chromatogram of the alcohol extract of *Espostoa guentheri* of the whole plant

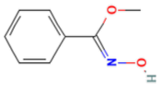
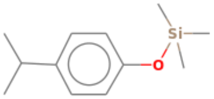
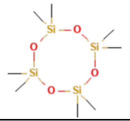
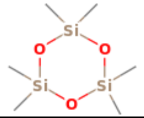
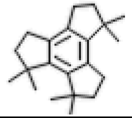
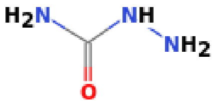
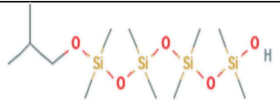
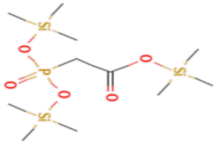
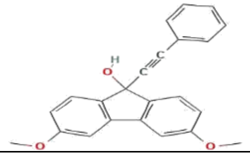
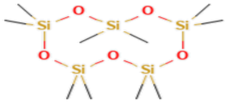
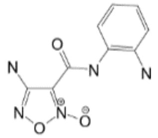
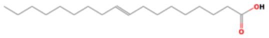
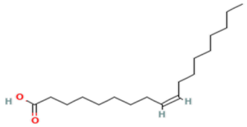
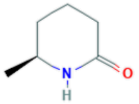
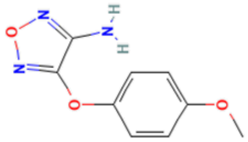
No.	Name of compound	Retention time (min)	Area%	Molecular weight (g/mol)	Molecular formula	Chemical formula
1	Oxime-, methoxy-phenyl	4.029	49.26	151.16	$C_8H_9NO_2$	
2	4-Isopropylphenol, trimethylsilyl ether	4.299	8.10	208.3721	$C_{12}H_{20}OSi$	
3	Cyclotetrasiloxane, octamethyl	4.752	2.68	296.61	$C_8H_{24}O_4Si_4$	
4	Cyclotrisiloxane, hexamethyl	6.327	11.36	222.4618	$C_6H_{18}O_3Si_3$	
5	1H-Trindene, 2,3,4,5,6,7,8,9-octahydro-1,1,4,4,9,9-hexamethyl	6.673	7.39	282.5	$C_{21}H_{30}$	
6	Hydrazinecarboxamide	7.234	1.29	75.0699	CH_5N_3O	
7	1,1,3,3,5,5,7,7-Octamethyl-7-(2-me thylpropoxy) tetrasiloxan-1-ol	8.819	2.20	370.74	$C_{12}H_{34}O_5Si_4$	
8	Acetic acid, [bis[(trimethylsilyloxy]phosphinyl]-, trimethylsilyl ester	9.024	2.35	356.57	$C_{11}H_{29}O_5PSi_3$	

Table 3-5: GC–MS chromatogram of the alcohol extract of *Espostoa guentheri* of the whole plant

No.	Name of compound	Retention time (min)	Area%	Molecular weight (g/mol)	Molecular formula	Chemical formula
9	Fluoren-9-ol, 3,6-dimethoxy-9-(2-phenylethynyl)-	9.251	1.95	342.4	$C_{23}H_{18}O_3$	
10	Cyclopentasiloxane, decamethyl	11.333	1.54	370.77	$C_{10}H_{30}O_5Si_5$	
11	4-Amino-2-oxy-furazan-3-carboxylic acid	20.126	1.64	235.2	$C_9H_9N_5O_3$	
12	9-Octadecenoic acid, (E)-	22.251	3.69	282.4614	$C_{18}H_{34}O_2$	
13	Oleic Acid	22.489	3.02	282.5	$C_{18}H_{34}O_2$	
14	2-Piperidinone, 6-methyl	22.802	1.65	113.16	$C_6H_{11}NO$	
15	1,2,5-Oxadiazol-3-amine, 4-(4-methoxyphenoxy)-	26.783	1.88	207.19	$C_9H_9N_3O_3$	

3.1.1.4 : *Echinocactus grusonii*

Plant extract chromatogram of *Echinocactus grusonii* showed 15 peaks too, the highest peak area being 26.01% for Oxime-, methoxy-phenyl at a retention time of 4.148 min and the lowest area being 0.84% for N-Dimethylaminomethyl-N-methylformamide at a retention time of 22.823min. The rest included the area percentages of the chemical compounds between those values above and at different times (Table 3-6 and Figure 3-5). In a chemical study by Oonsivilai *et al.* (2010), it was proved that the age of the plant and the type of solvent used in the extraction have an effect on the quality of the chemical compounds produced by chromatography. In their study, they indicated that *Echinocactus grusonii* age 3 years crude extracts showed total chlorophylls contents higher than at the age of 6 years.

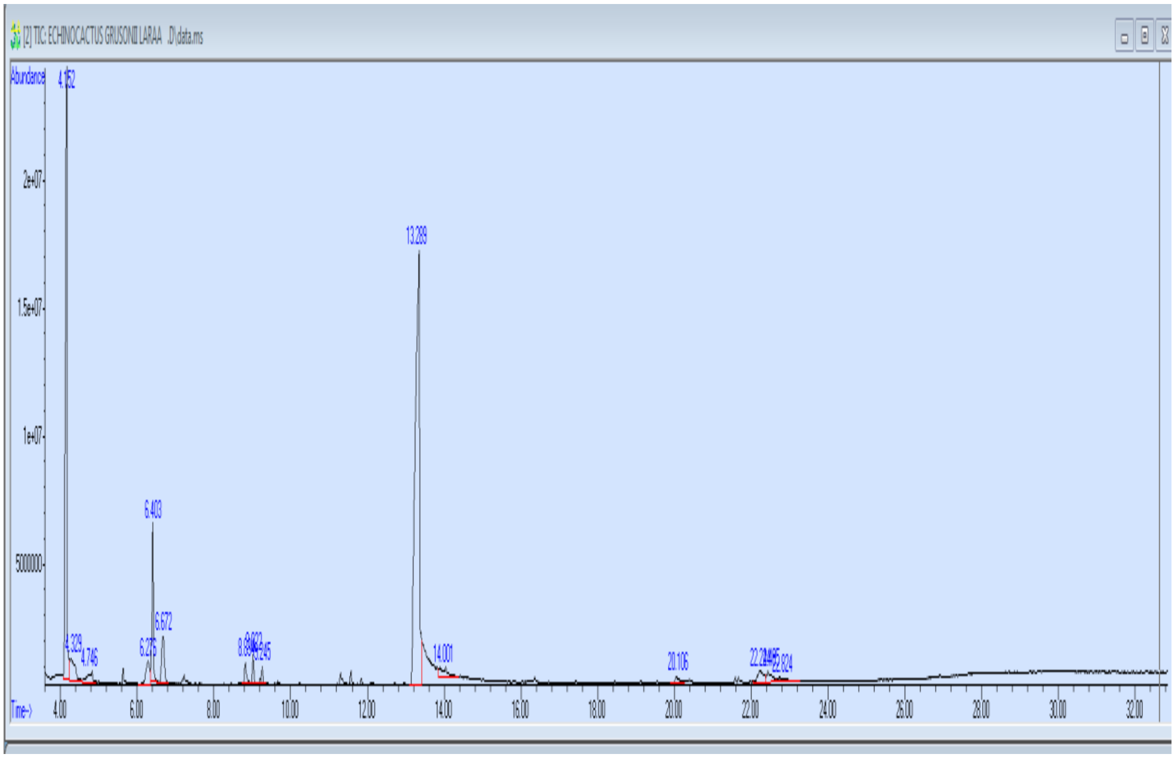


Figure 3-5: Chromatogram profile for GC-MS of *Echinocactus grusonii*

Table 3-6: GC–MS chromatogram of the alcohol extract of *Echinocactus grusonii* of the whole plant

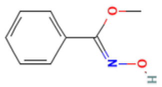
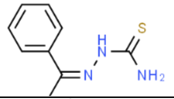
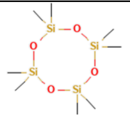
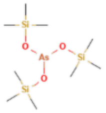
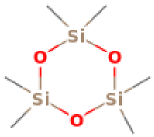
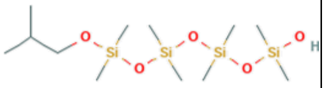
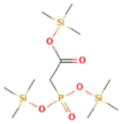
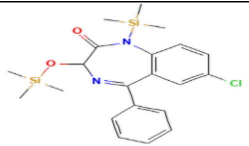
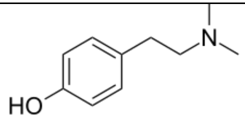
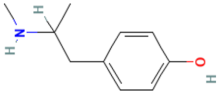
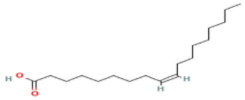
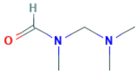
No.	Name of compound	Retention time (min)	Area%	Molecular weight (g/mol)	Molecular formula	Chemical formula
1	Oxime-, methoxy-phenyl-_	4.148	26.01	151.16	$C_8H_9NO_2$	
2	Hydrazinecarbothioamide, 2-(1-phen ylethylidene)-	4.331	2.62	4514357	$C_9H_{11}N_3S$	
3	Cyclotetrasiloxane, octamethyl	4.741	1.36	296.61	$C_8H_{24}O_4Si_4$	
4	Arsenous acid, tris(trimethylsilyl) ester	6.273	2.36	342.49	$C_9H_{27}AsO_3Si_3$	
5	Cyclotrisiloxane, hexamethyl-	6.403	5.23	222.4618	$C_6H_{18}O_3Si_3$	
6	1H-Trindene, 2,3,4,5,6,7,8,9-octah ydro-1,1,4,4,9,9-hexamethyl	6.673	3.50	282.462	$C_{21}H_{30}$	
7	1,1,3,3,5,5,7,7-octamethyl-7-(2-methylpropoxy)tetrasiloxan-1-ol	8.830	1.19	370.74	$C_{12}H_{34}O_5Si_4$	
8	Acetic acid, [bis[(trimethylsilyl oxy]phosphinyl]-, trimethylsilyl ester	9.025	1.02	356.57	$C_{11}H_{29}O_5PSi_3$	

Table 3-6: GC–MS chromatogram of the alcohol extract of *Echinocactus grusonii* of the whole plant

No.	Name of compound	Retention time (min)	Area%	Molecular weight (g/mol)	Molecular formula	Chemical formula
9	2H-1,4-Benzodiazepin-2-one, 7-chloro-1,3-dihydro-5-phenyl-1-(trimethylsilyl)	9.240	0.94	431.1	$C_{21}H_{27}ClN_2O_2Si_2$	
10	Hordenine	13.286	48.99	165.236	$C_{10}H_{15}NO$	
11	Pholedrine	13.998	1.81	165.23	$C_{10}H_{15}NO$	
12	Ethanamine, N-ethyl-	20.104	0.86	171.22	$C_4H_{13}NO_4S$	
13	Oleic Acid	22.241	1.83	282.5	$C_{18}H_{34}O_2$	
14	3-Eicosene, (E)-	22.489	1.44	280.5	$C_{20}H_{40}$	
15	N-Dimethylaminomethyl-N-methylformamide	22.823	0.84	116.16	$C_5H_{12}N_2O$	

3.1.1.5 **: *Mammillaria elongate***

Plant extract chromatogram of *Mammillaria elongate* showed 15 peaks, the highest peak area being 48.15% for Oxime-, methoxy-phenyl at a retention time of 4.202 min and the lowest area being 0.83% for Octadecanoic acid, 2- [(trimethylsi lyl) oxy]-, methyl ester at a retention time of 11.570 min. The rest included the area percentages of the chemical compounds between those values above and at different times (Table 3-7 and Figure 3-6).

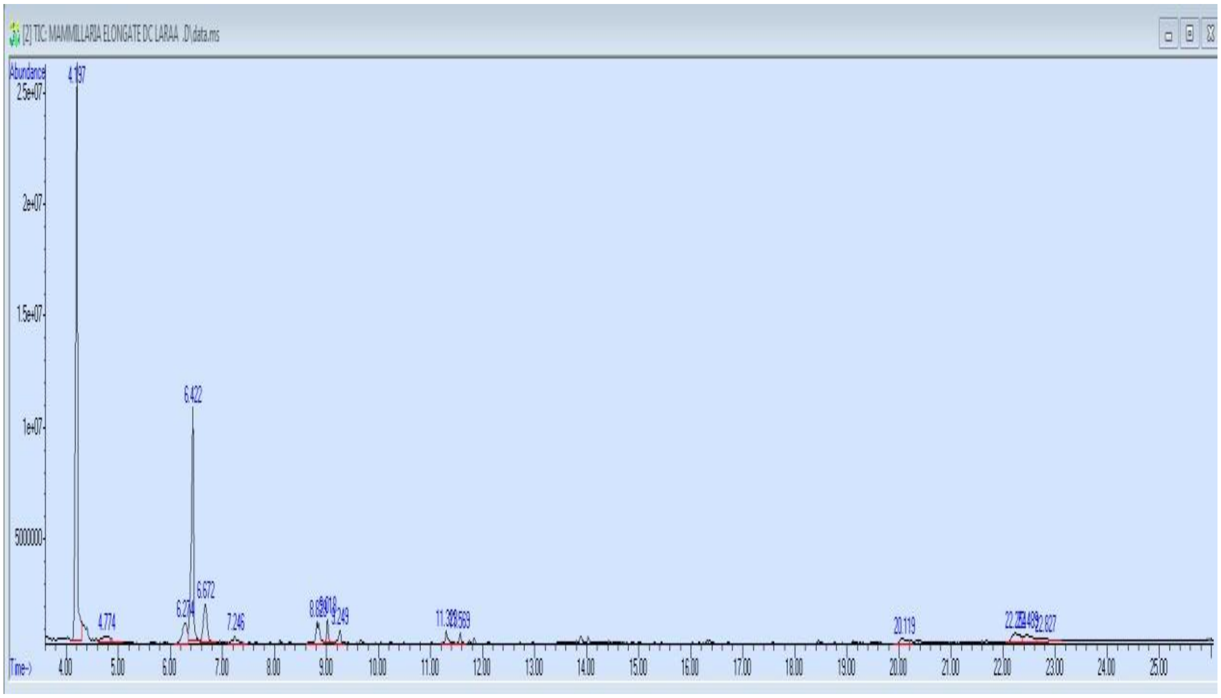


Figure 3-6: Chromatogram profile for GC-MS of *Mammillaria elongate*

Table 3-7: GC–MS chromatogram of the alcohol extract of *Mammillaria elongate* of the whole plant

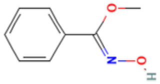
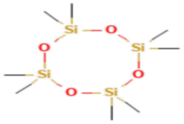
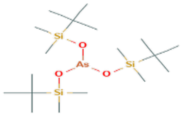
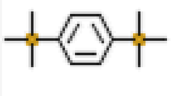
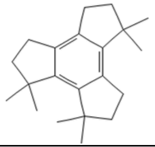
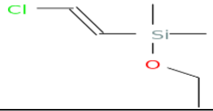
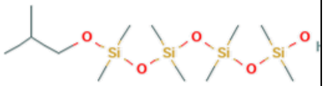
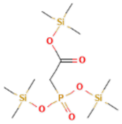
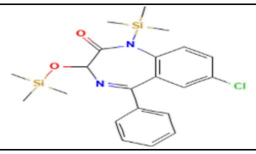
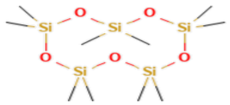
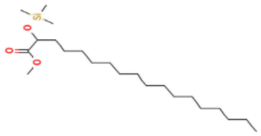
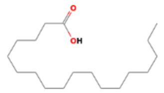
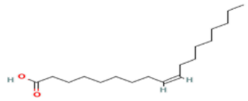
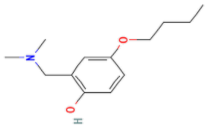
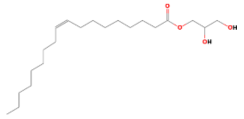
No.	Name of compound	Retention time (min)	Area%	Molecular weight (g/mol)	Molecular formula	Chemical formula
1	Oxime-, methoxy-phenyl	4.202	48.15	151.16	$C_8H_9NO_2$	
2	Cyclotetrasiloxane, octamethyl	4.774	2.51	296.61	$C_8H_{24}O_4Si_4$	
3	Tris(tert-butyldimethylsilyloxy)arsane	6.273	4.38	468.7	$C_{18}H_{45}AsO_3Si_3$	
4	1,4-Bis(trimethylsilyl)benzene	6.424	20.85	222.47	$C_{12}H_{22}Si_2$	
5	1H-Trindene, 2,3,4,5,6,7,8,9-octahydro-1,1,4,4,9,9-hexamethyl	6.672	5.82	282.5	$C_{21}H_{30}$	
6	trans-(2-Chlorovinyl) dimethylethoxysilane	7.244	1.29	164.70	$C_6H_{13}ClOSi$	
7	1,1,3,3,5,5,7,7-Octamethyl-7-(2-me thylpropoxy)tetrasiloxan-1-ol	8.830	3.58	370.74	$C_{12}H_{34}O_5Si_4$	
8	Acetic acid, [bis[(trimethylsilyl) oxy]phosphinyl]-,trimethylsilyl ester	9.013	1.71	356.57	$C_{11}H_{29}O_5PSi_3$	

Table 3-7: GC–MS chromatogram of the alcohol extract of *Mammillaria elongate* of the whole plant

No.	Name of compound	Retention time (min)	Area%	Molecular weight (g/mol)	Molecular formula	Chemical formula
9	2H-1,4-Benzodiazepin-2-one, 7-chloro-1,3-dihydro-5-phenyl-1-(trimethylsilyl)-	9.251	1.37	431.1	$C_{21}H_{27}ClN_2O_2Si_2$	
10	Cyclopentasiloxane, decamethyl	11.322	1.26	370.77	$C_{10}H_{30}O_5Si_5$	
11	Octadecanoic acid, 2-[(trimethylsi lyl)oxy]-, methyl ester	11.570	0.83	386.7	$C_{22}H_{46}O_3Si$	
12	Octadecanoic acid	20.115	1.40	284.477	$C_{18}H_{36}O_2$	
13	Oleic Acid	22.251	2.81	282.5	$C_{18}H_{34}O_2$	
14	Phenol, 4-butoxy-2[(dimethylamino)methyl]-	22.488	2.42	223.31	$C_{13}H_{21}NO_2$	
15	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	22.823	1.62	356.54	$C_{21}H_{40}O_4$	

The results of the analysis showed that there are four compounds common in all the studied species as Oxime-, methoxy-phenyl- Cyclotetrasiloxane, octamethyl and 1H-Trindene, 2,3,4,5,6,7,8,9-octahydro-1,1,4,4,9,9-hexamethyl and Oleic Acid at a rate ranging from 1.62 to 44.90 min.

Table 3-8 shows the importance of the chemical compounds that were recorded through GC-MS analysis and according to the available scientific sources. (Banday *et al.* ,2014) mentioned that the compound Oxime-, methoxy-phenyl- that was present in all the studied species has antimicrobial and anticancer activity. (Mary and Giri ,2016) referred to the activity of the compound Cyclotetrasiloxane, octamethyl which was found in all species, to its importance in anti-microbial, antiseptic, hair conditioning agent, skin conditioning agent- emollient as well as the compound 1H-Trindene, 2,3,4,5,6,7,8,9-octahydro-1,1,4,4,9,9-hexamethyl has antioxidant value (Cedeño-Pinos *et al*., 2022) and the compound had antibacterial properties according to (Dilika *et al.* ,2000) which was also found in all the species studied, and from that the good importance of the plants selected in the study was shown through the presence of secondary compounds in the plant extracts.

As well as it became evident through Table 3-2 the participation of the compounds diagnosed with one or two of studied species, which show their medical importance through the research carried out, most of which were anti-bacterial, anti-fungal, oxidative and anti-cancer, each according to its references.

(Jayapriya and Shoba ,2015) pointed out that the separation of chemical compounds using MS-GC technology can clarify the relationship between the biological activities of the compounds and the chemical activity of each compound, and that such studies also show us information about the active compounds if it's found in the plant.

Table 3-8: Compounds recorded in the studied species with their biological importance

No.	Name of chemical compound	Species	Biological activity	References
1	Oxime-, methoxy-phenyl-	*Hylocereus undatus, Aloe vera, Opuntia ficus- indica, Espostoa guentheri, Echinocactus grusonii Mammillaria elongate*	Antimicrobial activity, Anticancer activity	(Banday *et al*., 2014)
2	Cyclotetrasiloxane, octamethyl		Anti-microbial, antiseptic, hair conditioning agent, skin conditioning agent- emollient	(Mary and Giri , 2016)
3	1H-Trindene, 2,3,4,5,6,7,8,9-octahydro-1,1,4,4,9,9-hexamethyl		Antioxidant	(Cedeño-Pinos *et al*., 2022)
4	Oleic Acid		Antibacterial activity	(Dilika *et al*., 2000)
5	4-Trimethylsilyloxybenzoic acid, -(trifluoroacetyloxycarbonyl) phenyl ester	*Hylocereus undatus,*	Fighting nosocomial antibiotic-resistant microbes.	(Amrati, *et al*., 2021)
6	1,4-Bis(trimethylsilyl)benzene	*Hylocereus undatus, Mammillaria elongate*	Antibiotic	(Slassi, *et al*., 2019)
7	Phthalic acid, ethyl 2-isopropoxyphenyl ester	*Hylocereus undatus*	inhibitory effect on Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*) and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*	(Huang *et al*.,2021)
8	1,1,3,3,5,5,7,7-Octamethyl-7-(2-methylpropoxy)tetrasiloxan-1-ol	*Hylocereu undatus Aloe vera, Opuntia ficus indica, Espostoa guentheri, Echinocactus grusonii*	The antifungal and antibacterial activities	*(*Roy *et al*., 2007)

9	Acetic acid, [bis[(trimethylsilyl) oxy] phosphinyl]-, trimethylsilyl ester	*Hylocereus undatus, Aloe vera, Opuntia ficus- indica , Espostoa guentheri*	antibacterial activity against micro-organisms such as *Pseudomonas aeruginosa*.	(Fraise *et al*., 2013)
10	2H-1,4-Benzodiazepin-2-one, 7-chloro-1,3-dihydro-5-phenyl-1-(trimethylsilyl)-	*Hylocereus undatus, Aloe vera Echinocactus grusonii, Mammillaria elongate*	attacked the DNA of *E. coli* strains and damaged it, antimicrobial, anticancer	(Hamed *et al*., 2020) (Verma *et al*., 2019).
11	Melamine, tris(trimethylsilyl) derivative	*Hylocereus undatus*	antibacterial activity against *Staphylococcus aureus, Staphylococcus warneri* and *Enterococcus faecium*	(Abd-El-Aziz *et al*., 2016).
12	n-Hexadecanoic acid	*Hylocereus undatus*	Antibacterial activities	(Shaaban *et al*., 2021).
13	cis-10- decenoic acid	*Hylocereus undatus*	bactericide and anti-inflammatory activity in human colon cancer cells	(Yang *et al*., 2018)
14	4-Amino-2-oxy-furazan-3-carboxylic acid	*Hylocereus undatus*	Antibacterial activity *against Pseudomonas aeruginosa*	(Letha *et al*., 2020).
15	9-Phenanthrenamine	*Aloe vera*	antimicrobial activities	(Zhao *et al*., 2018)
16	Cyclotrisiloxane, hexamethyl	*Aloe vera, Espostoa guentheri, Echinocactus grusonii*	Antibacterial activity, antioxidant	(Papitha *et al*., 2017) (Juliet *et al*., 2018)
17	4-(4-Chlorophenyl)-2,6-Diphenylpyridine	*Aloe vera*	antitumor and antimicrobial activities	(El-Sayed *et al*., 2011).
18	Dodecanoic acid (Lauric acid)	*Aloe vera*	Inhibitor of *Clostridium difficile* Growth	(Yang *et al*., 2018)
19	2-Chloroaniline-5-sulfonic acid	*Aloe vera*	Synthesis Nanocomposites	(Linganathan *et al*., 2014).
20	9-Octadecenoic acid, (E)-	*Aloe vera, Espostoa guentheri*	Antimicrobial activities	(Rahman *et al*., 2014)
21	2-(E)-Pentenoic acid, (4S)-4-[(t-b utoxycarbonyl-(S) alanyl)	*Aloe vera*	against Gram-positive and Gram-negative bacteria	(Sujarit *et al*., 2020)

	amino]-, ethyl ester			
22	Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl)phenyl]hydrazide	*Aloe vera*	against Gram-positive bacteria and *Candida* spp.	(Popiolek *et al*.,2022)
23	Trimethyl[4-(1-methyl-1-methoxyeth yl)phenoxy]silane	*Opuntia ficus indica*	Antibacterial Activity	(Popiołek *et al*., 2021)
24	Benzoic acid, 3-pentafluoropropionyloxy-, tert.butyldimethylsilyl ester	*Opuntia ficus- indica*	strongest antibacterial activity	(Synowiec *et al*., 2021)
25	Fluoren-9-ol, 3,6-dimethoxy-9-(2-phenylethynyl)-	*Opuntia ficus- indica, Espostoa guentheri*	Antibacterial antifungal	(Turan-Zitouni *et al*. 2007).
26	Tetradecanoic acid (Myristic acid).	*Opuntia ficus- indica*	antibacterial activity against *S. aureus* and antifungal	(Okukawa *et al*. 2021).
27	cis-13-Octadecenoic acid	*Opuntia ficus- indica*	antimicrobial activity against *Staphylococcus aureus*	(Zahara *et al*., 2022)
28	Cyclobarbital	*Opuntia ficus indica*	Antimicrobial	(Joshi *et al*., 2020)
29	4-Isopropylphenol, trimethylsilyl ether	*Espostoa guentheri*	Antibacterial Activity	(Suneetha *et al*., 2017).
30	Hydrazinecarboxamide	*Espostoa guentheri, Echinocactus grusonii*	against gram negative bacteria of *Escherichia coli* and gram positive bacteria of *Streptococcus epidermidis*	(Kumar *et al*.,2016)
31	2-Piperidinone, 6-methyl	*Espostoa guentheri*	Antimicrobial activity against *P. aeruginosa, P.mirabilis*, and *C. Albicans*	(Al-Salman, 2019).
32	1,2,5-Oxadiazol-3-amine, 4-(4-methoxyphenoxy	*Espostoa guentheri*	activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* than the reference drugs (ciprofloxacin and amoxicillin)	(Glomb and Świątek, 2021).

33	Hydrazinecarbothioamide, 2-(1-phen ylethylidene)-	*Echinocactus grusonii*	Antimicrobial Activity Screening Of Some Hydrazinecarbothioamides And Heterocyclic Compounds	(Bărbuceanu *et al*., 2016).
34	Arsenous acid, tris(trimethylsilyl) ester	*Echinocactus grusonii*	Arsenic toxicity in natural and humanly impacted environments, where arsenic poisoning is known as 'Black foot' because of the necrotic destruction of tissue	(Ahsan *et al*., 2000)
35	Hordenine	*Echinocactus grusonii*	Antibiofilm Agent against *Pseudomonas aeruginosa*	(Zhou *et al*., 2018)
36	Pholedrine	*Echinocactus grusonii*	Antimicrobial, Antioxidant, Anticancer	(Wei *et al*., 2011).
37	3-Eicosene, (E)-	*Echinocactus grusonii*	antimicrobial activities of *Photorhabdus* sp. strain ETL, symbiotically associated to an insect pathogenic nematode, *Heterorhabditis zealandica*, against human pathogenic bacteria and toxigenic fungi,	(Lulamba *et al*., 2021).
38	N-Dimethylaminomethyl-N-methylformamide	*Echinocactus grusonii*	against Gram-positive *Staphylococcus aureus* and Gram-negative *E.coli* by disk diffusion method	(Mushtaq *et al*. 2021).
39	trans-(2-Chlorovinyl) dimethylethoxysilane	*Mammillaria elongate*	Antimicrobial agents, anticarsogenic bacteria, against *Streptococcus mutans*	(De Castilho *et al*., 2019).
40	Phenol, 4-butoxy-2-[(dimethylamino) methyl]-	*Mammillaria elongate*	Antibacterial and antifungal	(Ecevit *et al*., 2022).
41	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	*Mammillaria elongate*	strong antibacterial activities against all the bacterial strains tested with MIC value being in the range of 125–1000 μg/ml	(Sohn *et al*., 2013).

3.2 : Molecular study

environmental factors may occasionally have an impact on even the most helpful classical phenotypic parameters, such as physical qualities. This is why molecular tools have been increasingly integrated into the traditional approaches to assessing genetic diversity during the last decade. Among them are the characterisation of macromolecules and the study of chemical components (such as plant secondary metabolites). Research in several fields, including taxonomy, phylogeny, ecology, genetics, and plant breeding, has benefited enormously from the introduction of molecular markers, which are based on polymorphisms discovered in proteins or DNA (Weising *et al.* 1995). These five distinguishing features of molecular markers over morphological markers account for their enhanced value.

However, environmental factors may occasionally have an impact on even the most helpful classical phenotypic parameters, such as physical qualities. This is why molecular tools have been increasingly integrated into the traditional approaches to assessing genetic diversity during the last decade. Among them are the characterisation of macromolecules and the study of chemical components (such as plant secondary metabolites). Research in several fields, including taxonomy, phylogeny, ecology, genetics, and plant breeding, has benefited enormously from the introduction of molecular markers, which are based on polymorphisms discovered in proteins or DNA (Weising *et al.* 1995).

3.2.1 : Genomic DNA Isolation

The results of the extracted DNA from six species of plants by using the ZR Plant/Seed DNA MiniPrep kit appeared in Figure (3-7) and Table (3-9). The results presented the highest responsible concentration of DNA in *Mammillaria elongate* (8.8 ng/µl), while the lowest concentration was observed in the species *Aloe vera* (5.1 ng/µl). The purity of DNA was high in the majority of the species under interest ranged 1.8-2. The fluctuation in DNA concentration may be correlated with the chemical compound that constitutes each species. However, sometimes carbohydrates may cause a reduction of DNA available during extraction methods, and handling can induce fluctuations in DNA concentration, and the age and environmental conditions of the plant material impact the effectiveness of high-quality DNA isolation (Ramos *et al*., 2014). Isolating DNA from cactus species is a challenging task since these plants have a significant concentration of polysaccharides and secondary metabolites, which may form insoluble complexes with nucleic acids during the process of DNA extraction (Guillemaut and Maréchal-Drouard, 1992). However, these secondary metabolites and polysaccharides have been observed to inhibit enzyme activity in cactus species plants (Porebski *et al*., 1997).

Table 3-9: An illustration of measuring DNA concentration and purity.

No.	Species	Nucleic acid Conc. (ng/µl)	260/280 purity
1	*Hylocereus undatus*	6.8	1.8
2	*Aloe vera*	5.1	1.81
3	*Opuntia ficus-indica*	5.9	1.8
4	*Espostoa guentheri*	7.2	1.96
5	*Echinocactus grusonii*	8.5	1.83
6	*Mammillaria elongate*	8.8	2.0

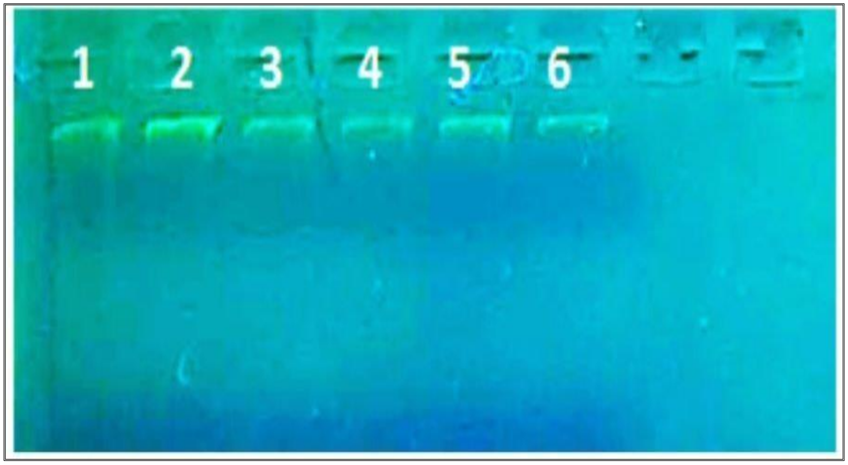


Figure 3-7: Genomic DNA from six plant species was electrophoresed on a 1% agarose gel at 70 volts for 30 minutes. (number of species according to table 3-9)

3.2.2: RAPD indices-dependent DNA replication

This study involved the use of 7 primers for RAPD-PCR analysis of all tested genetic DNA, and the current results using RAPD indicators showed variation between the studied genotypes through the presence of heterogeneous, multiplicated and individual bundles as the primers gave OP-V19, OP-R06, OP-V14, OP-V09, OP-L05, OP-M05, and OP-P04 Individual fingerprints for each genotype, but this did not apply to the other primers, these primers gave amplified products that could be detected and therefore were useful, these primers were listed and sequenced and the results of the fingerprints of plant species were summarized in the table (3-10) RAPD marker helps compare DNA in biological systems that have received little attention from the scientific community or that have a limited number of DNA sequences available for comparison (Anderson *et al*., 2018).

The most popular DNA fingerprinting approach, RAPD, uses low-stringency annealing of short (10-base) primers of the arbitrary sequence to promote the amplification of products of unknown sequences. In contrast to other PCR-based methods, just a single primer is required. Primers may bind to a wide variety of genomic locations due to their short sequences, and effective amplification of DNA fragments may occur when two primer binding sites occur close together (Williams *et al*., 1990). Small amounts of genomic DNA (about 10 ng) are used in RAPD analysis, and a single primer is used to amplify a large number of short DNA sequences (roughly 300 bp to 2000 bp). Different forms of this method include AP-PCR (arbitrary primed PCR; Welsh and McClelland, 1990) and DAF (DNA amplification fingerprinting; Caetano-Anolles *et al*., 1991).

Researchers' acceptance of the RAPD method may be attributed to its technical simplicity, efficiency, and capacity to tolerate large throughput (Deragon and Landry 1992). An essentially infinite number of useful genetic polymorphisms may be obtained using PCR amplification of genomic areas using short oligonucleotide primers of arbitrary sequence (Williams *et al*., 1990; Welsh and McClelland, 1990). As a bonus, genomic DNA may be amplified using just a little amount of tissue because of the specificity of PCR (Saiki *et al*. 1988). In comparison to morphological assessments and isozyme analysis, RAPD has the benefit that environmental factors do not influence the molecular genetic profiles of individuals. For example, RAPD has been used in the fields of conservation biology (Rossetto *et al*., 1999), marker-assisted selection (Kelly and Miklas, 1998), and linkage mapping (Rajapakse *et al*., 1995; Lacou *et al*., 1998).

Many studies emphasized using the efficacy of RAPD in diagnosis such as (Zoghlami *et al*. , 2007) have assessed the genetic diversity of *Opuntia- ficus-indica* in Tunisia; (El-Kharrassi *et al*. ,2018) investigated to assess the genetic diversity within and among *Opuntia* spp. from different regions of Morocco, using morphological descriptors as well as ISSR and RAPD markers and (Rabeh *et al*., 2020) study point out that RAPD markers as an effective tool to authenticate eight *Opuntia* species in the Eygpt.

3.2.2.1 : Primer OP-V19

This primer gave 10 main bands with only 4 bands represented as unique bands that were all different and their molecular sizes ranged between (400-1500) base pairs, and the number of bands recorded 40 bands in 15 levels, the highest number of bands reached 10 in the species *Aloe vera*, and The lowest number appeared as 5 bands in the species *Opuntia ficus- indica*, *Espostoa guentheri* and *Mammillaria elongate* and gave this primer a discriminative ability reached and efficiency of 13.3% while the polymorphism variation of this primer reached 100% (Table 3-10 and Figure 3-8).

Table 3-10: The number of bands in the studied species according to their molecular sizes of the primer OP-V19. *1.Hylocereus undatus, 2.Aloe vera, 3.Opuntia ficus- indica, 4.Espostoa guentheri, 5.Echinocactus grusonii, 6. Mammillaria elongate*

Molecular size (bp)	Studied species					
	1	2	3	4	5	6
1500	0	1	0	0	0	0
1250	0	0	0	0	1	0
1200	0	1	0	0	0	0
1150	1	1	1	0	0	0
1100	1	1	0	1	1	0
1050	1	0	0	0	1	1
950	0	1	0	0	1	1
800	1	1	1	1	0	0
775	0	1	1	1	0	0
700	1	1	1	0	1	1
650	1	1	0	1	1	0
600	0	0	1	0	0	0
525	1	0	0	1	0	1
450	1	1	0	0	0	0
400	0	0	0	0	1	1
Sum	8	10	5	5	7	5

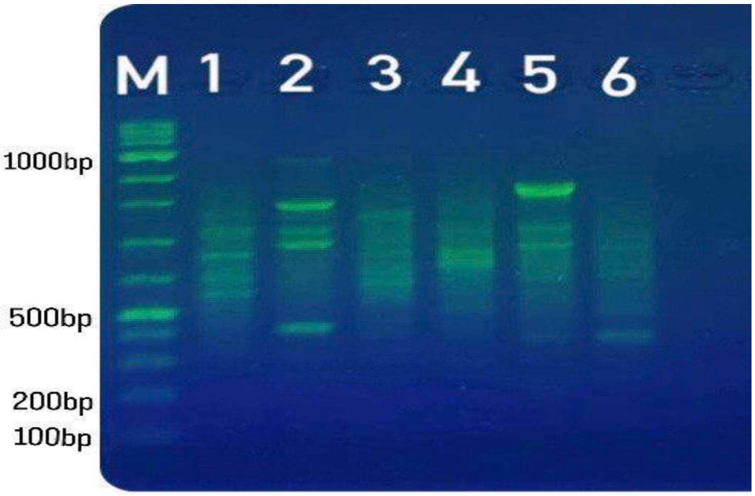


Figure 3- 8: The OP-V19 primer yielded RAPD-PCR results. Electrophoresis on agarose gel 2% at 70 volts was the end result. A 1:30-hour 1x TBE buffer. M: upper (10kbp) and first (100 bp) band of a ladder. *1.Hylocereus undatus, 2.Aloe vera, 3.Opuntia ficus-indica, 4.Espostoa guentheri, 5.Echinocactus grusonii, 6. Mammillaria elongate*

3.2.2.2 : Primer OP-R06

The total number of duplicate bands at this primer was 36, with 10 bands represented as unique bands, the highest number of duplicate bands was 8 in *Opuntia ficus-indica*, while the lowest number was 4 bands in *Aloe vera* and *Mammillaria elongate*. Its molecular sizes ranged between (250-1500) base pairs, and this primer gave a discriminatory capacity and an efficiency of 16.8, while the morphological heterogeneity of this primer was 100% (Table 3-11 and Figure 3-9).

Table 3-11: The number of bands in the studied species according to their molecular sizes of the primer OP-R06*1.Hylocereus undatus, 2.Aloe vera, 3.Opuntia ficus-indica, 4.Espostoa guentheri, 5.Echinocactus grusonii, 6. Mammillaria elongate*

Molecular size (bp)	Studied species					
	1	2	3	4	5	6
1500	0	1	1	0	0	0
1250	0	0	1	1	1	1
1150	0	1	1	0	0	0
1100	1	0	0	0	0	0
1000	0	0	0	1	0	0
900	1	0	0	0	1	0
825	0	0	1	0	0	0
775	1	1	0	1	1	1
725	1	1	1	1	0	0
950	1	0	0	0	1	0
800	1	0	1	0	0	0
750	0	0	1	0	0	1
725	0	0	0	0	1	0
700	1	0	0	1	1	0
650	1	0	0	0	0	1
550	0	0	0	1	0	0
500	0	0	0	0	0	1
450	0	0	1	0	0	0
350	0	0	1	0	1	0
325	0	0	0	1	0	0
300	0	0	1	0	0	0
250	1	0	0	0	0	0
Sum	7	4	8	7	6	4

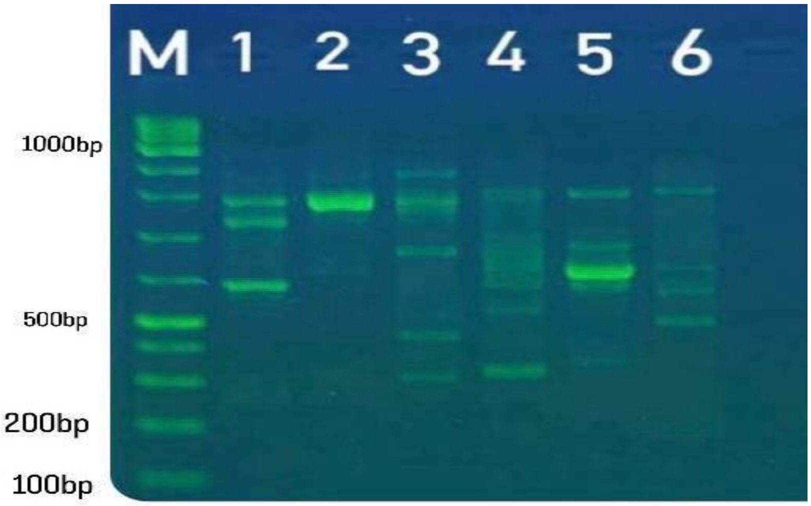


Figure 3- 9: The OP-R06 primer yielded RAPD-PCR results. Electrophoresis on agarose gel 2% at 70 volts was the end result. A 1:30-hour 1x TBE buffer. M: upper (10kbp) and first (100 bp) band of a ladder. *1.Hylocereus undatus, 2.Aloe vera, 3.Opuntia ficus-indica, 4.Espostoa guentheri, 5.Echinocactus grusonii, 6. Mammillaria elongate*

3.2.2.3 : Primer OP-V14

This primer produced 21 main bands and the number of unique bands was only six, and their molecular sizes ranged between (300- 1700) base pairs. The total multiples bands are 53, the highest number of multiple bands was 11 in the species *Echinocactus grusonii*, while the lowest number was 7 in the species *Hylocereus undatus*, and gave a discrimination of primer and efficiency of 18.6 while the polymorphism variation of this primer reached 100% (Table 3-12 and Figure 3-10).

Table 3-12: The number of bands in the studied species according to their molecular sizes of the primer OP-V14. *1.Hylocereus undatus, 2.Aloe vera, 3.Opuntia ficus- indica, 4.Espostoa guentheri, 5.Echinocactus grusonii, 6. Mammillaria elongate*

Molecular size (bp)	Studied species					
	1	2	3	4	5	6
1700	0	0	0	0	1	0
1600	0	1	0	0	1	0
1350	0	1	0	0	0	0
1250	0	0	0	1	1	1
1225	1	0	1	1	1	1
1150	0	0	1	0	1	1
1100	1	1	1	1	0	0
1050	1	0	0	0	1	0
1000	1	1	1	1	0	0
950	1	0	0	0	1	0
800	1	0	1	0	0	0
750	0	0	1	0	0	1
725	0	1	0	1	0	1
700	0	1	1	0	0	0
600	0	1	0	1	1	1
550	0	1	1	0	1	1
500	0	0	1	0	1	1
425	1	1	0	1	0	0
350	0	0	0	0	1	0
325	0	0	0	0	0	1
300	0	0	0	1	0	0
Sum	7	9	9	8	11	9

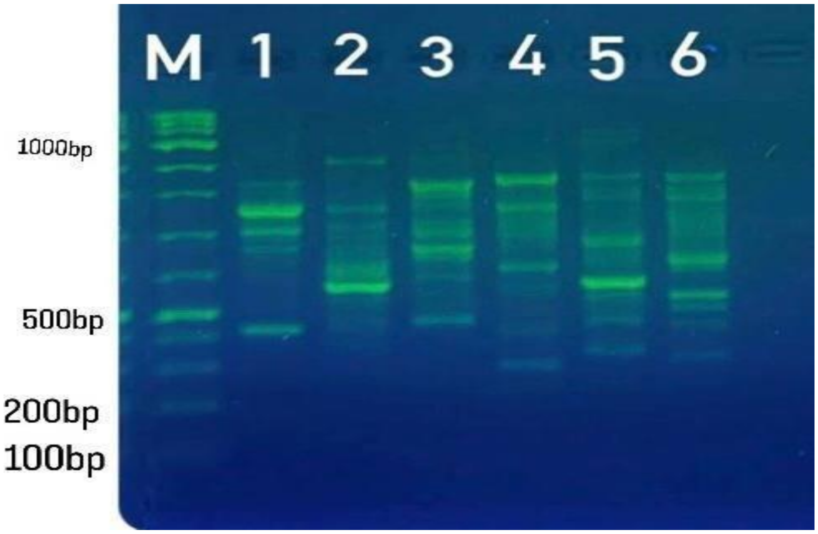


Figure 3- 10: The OP-V14 primer yielded RAPD-PCR results. Electrophoresis on agarose gel 2% at 70 volts was the end result. A 1:30-hour 1x TBE buffer. M: upper (10kbp) and first (100 bp) band of a ladder.*1.Hylocereus undatus, 2.Aloe vera, 3.Opuntia ficus-indica, 4.Espostoa guentheri, 5.Echinocactus grusonii, 6. Mammillaria elongate.*

3.2.2.4 : Primer OP-V09

This primer gave 13 main bands, the number of uniqe bands was only five that were all different and their molecular sizes ranged between (450-1400) base pairs, and the number of multiple bands recorded 25, the highest number of bands reached 7 in the species *Opuntia ficus-indica* but the lowest number was only one band in the species *Aloe vera*, and this primer gave a discriminative ability and efficiency reached 11.5 while the polymorphic variation of this primer reached 100% (Table 3-13 and Figure 3-11).

Table 3-13: The number of bands in the studied species according to their molecular sizes of the primer OP-V09. *1.Hylocereus undatus, 2.Aloe vera, 3.Opuntia ficus-indica, 4.Espostoa guentheri, 5.Echinocactus grusonii, 6. Mammillaria elongate.*

Molecular size (bp)	Studied species					
	1	2	3	4	5	6
1400	0	0	1	1	1	0
1300	1	0	0	0	0	0
1200	1	0	1	0	0	0
1125	1	0	0	0	1	0
1100	1	0	1	0	0	1
1050	0	0	0	1	1	1
950	0	0	1	0	1	0
800	0	0	0	1	0	0
775	0	1	1	0	1	0
675	1	0	1	0	0	0
525	0	0	1	0	0	0
500	0	0	0	0	0	1
450	0	0	0	0	1	0
Sum	5	1	7	3	6	3

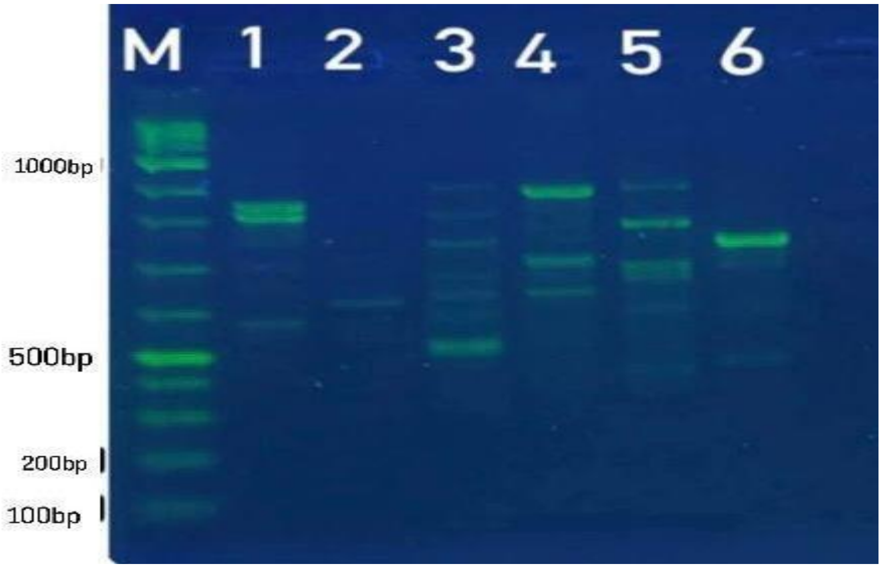


Figure 3- 11: The OP-V09 primer yielded RAPD-PCR results. Electrophoresis on agarose gel 2% at 70 volts was the end result. A 1:30-hour 1x TBE buffer. M: upper (10kbp) and first (100 bp) band of a ladder. *1.Hylocereus undatus, 2.Aloe vera, 3.Opuntia ficus- indica, 4.Espostoa guentheri, 5.Echinocactus grusonii, 6. Mammillaria elongate.*

3.2.2.5 : Primer OP-L05

This primer gave 16 main bands, the number of unique bands was 8 and their molecular sizes ranged between (375-1200) base pairs, thus the discriminatory ability and an efficiency primer equal to 14.2, while the morphological heterogeneity of this primer reached 100%. The number of multiple bands reached 31 bands, the highest number of bands was 7 in *Opuntia ficus- indica*, while the lowest number was 2 in *Aloe vera* (Table 3-14 and Figure 3-11).

Table 3-14: The number of bands in the studied species according to their molecular sizes of the primer OP-L05. *1.Hylocereus undatus, 2.Aloe vera, 3.Opuntia ficus- indica, 4.Espostoa guentheri, 5.Echinocactus grusonii, 6. Mammillaria elongate.*

Molecular size (bp)	Studied species					
	1	2	3	4	5	6
1200	1	0	0	0	0	0
1175	0	0	1	0	0	0
1100	0	0	0	0	0	1
1050	0	0	1	1	1	0
1000	1	0	1	1	0	0
950	0	0	1	1	1	1
800	1	0	0	0	0	0
750	0	1	0	0	0	1
700	1	0	1	0	0	1
675	0	0	1	0	0	0
600	0	0	0	1	1	0
575	0	0	0	0	1	0
525	1	0	0	0	1	1
500	0	1	0	1	0	1
425	0	0	0	0	1	0
375	0	0	1	0	0	0
Sum	5	2	7	5	6	6

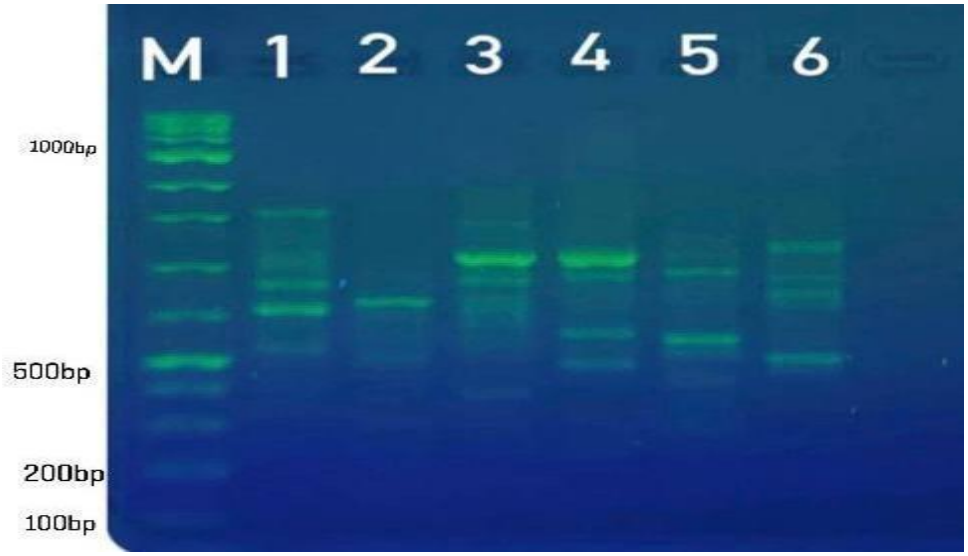


Figure 3- 12: The OP-L05 primer yielded RAPD-PCR results. Electrophoresis on agarose gel 2% at 70 volts was the end result. A 1:30-hour 1x TBE buffer. M: upper (10kbp) and first (100 bp) band of a ladder. *1.Hylocereus undatus*, *2.Aloe vera, 3.Opuntia ficus- indica, 4.Espostoa guentheri, 5.Echinocactus grusonii, 6. Mammillaria elongate.*

3.2.2.6 : Primer OP-M05

This primer recorded 18 main bands, with only 4 bands represented as unique bands, and their molecular sizes ranged between (225-1500) base pairs, and the total number of multiple bands reached 40 bands. The highest number of bands was 12 in *Hylocereus undatus*, while the lowest number was 3 in *Mammillaria elongate* and it resulted in a discriminative ability and an efficiency that reached 15.9, while the morphological heterogeneity of this starter reached 100% (Table 3-15 and Figure 3-12).

Table 3-15: The number of bands in the studied species according to their molecular sizes of the primer OP-M05. *1.Hylocereus undatus, 2.Aloe vera, 3.Opuntia -ficus -indica, 4.Espostoa guentheri, 5.Echinocactus grusonii, 6. Mammillaria elongate.*

Molecular size (bp)	Studied species					
	1	2	3	4	5	6
1500	0	1	0	0	0	0
1350	0	0	0	1	0	0
1300	1	0	0	1	0	0
1200	1	1	1	0	0	0
1050	1	0	1	1	0	0
1000	1	1	1	0	1	0
950	0	1	0	0	1	0
800	1	0	0	1	1	0
700	1	0	1	0	0	0
650	1	1	1	1	0	0
600	1	0	0	0	0	1
500	1	0	0	0	0	0
450	1	0	0	1	0	0
375	0	0	0	1	1	1
325	0	0	0	1	1	0
300	0	0	1	0	0	1
275	1	0	1	0	0	0
225	1	0	0	0	0	0
Sum	12	5	7	8	5	3

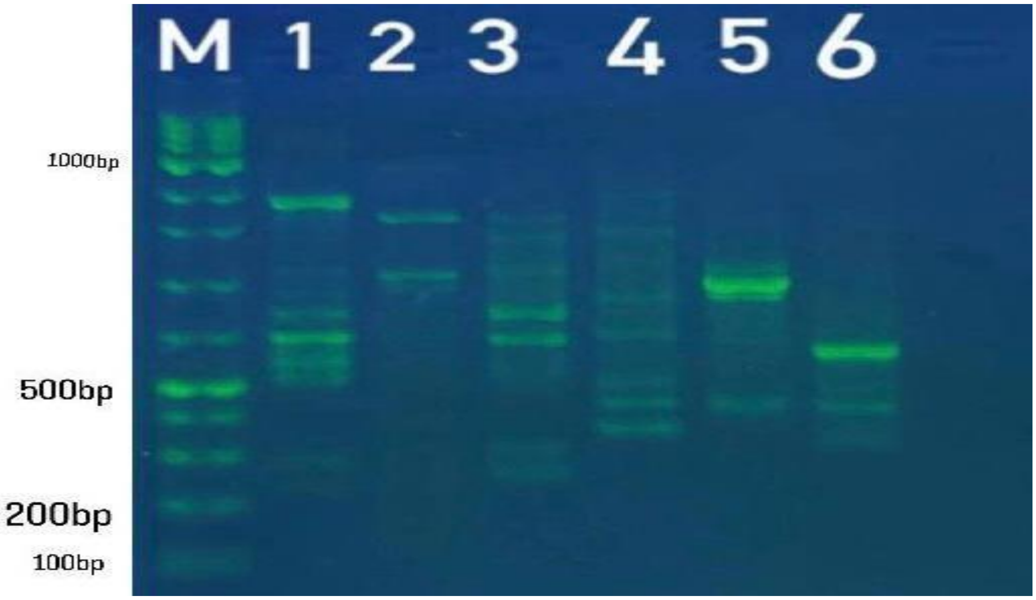


Figure 3- 13: The OP-M05 primer yielded RAPD-PCR results. Electrophoresis on agarose gel 2% at 70 volts was the end result. A 1:30-hour 1x TBE buffer. M: upper (10kbp) and first (100 bp) band of a ladder. *1.Hylocereus undatus, 2.Aloe vera, 3.Opuntia ficus- indica, 4.Espostoa guentheri, 5.Echinocactus grusonii, 6. Mammillaria elongate.*

3.2.2.7 : Primer OP-P04

This primer gave 11 main bands that were all different and their molecular sizes ranged between (300-1250) base pairs, as the number of unique bands was 2 only and the total number of multiple bands recorded 25 bands, the highest number of bands reached 7 in *Opuntia ficus- indica*, the lowest number was 2 in *Aloe vera*, and gave this primer a discriminative ability and efficiency of primer 9.73, while the morphological variation of this primer reached 100% (Table 3-16 and Figure 3-14).

Table 3-16: The number of bands in the studied species according to their molecular sizes of the primer OP-P04. *1.Hylocerus undatus, 2.Aloe vera, 3.Opuntia ficus-indica, 4.Espostoa guentheri, 5.Echinocactus grusonii, 6. Mammillaria elongate.*

Molecular size (bp)	Studied species					
	1	2	3	4	5	6
1250	0	0	1	0	0	0
1175	1	0	1	1	0	0
1100	1	0	0	0	1	1
1000	1	0	1	0	0	0
800	0	0	1	0	0	1
750	1	1	0	0	0	0
700	0	0	1	1	1	0
600	1	0	1	1	0	0
450	1	0	0	0	0	1
350	0	1	1	0	0	1
300	0	0	0	0	1	0
Sum	6	2	7	3	3	4

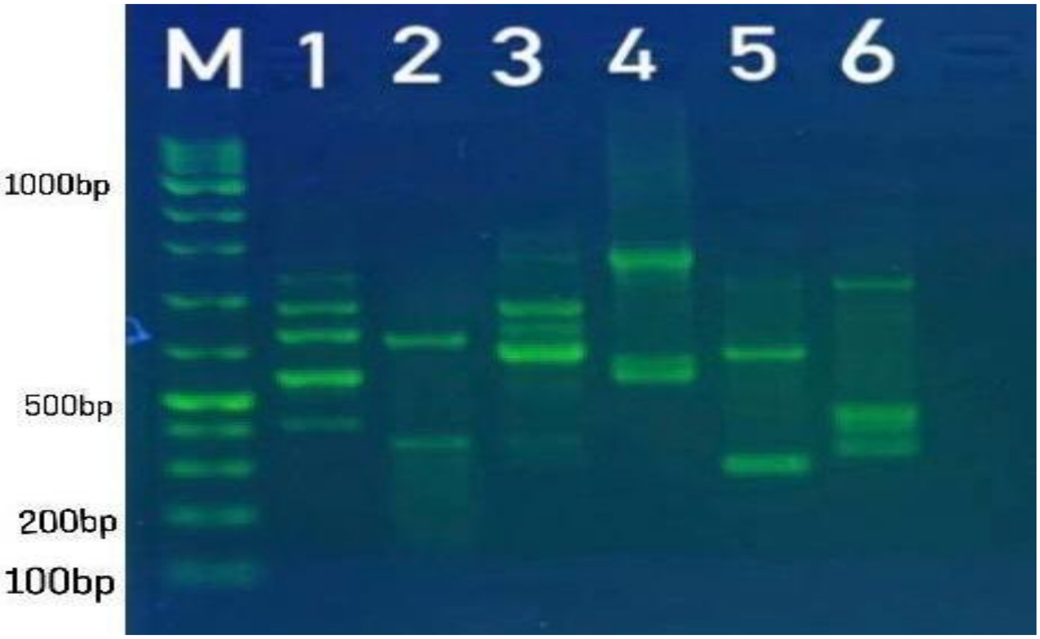


Figure 3- 14: The OP-P04 primer yielded RAPD-PCR results. Electrophoresis on agarose gel 2% at 70 volts was the end result. A 1:30-hour 1x TBE buffer. M: upper (10kbp) and first (100 bp) band of a ladder. *1.Hylocereus undatus, 2.Aloe vera, 3.Opuntia- ficus -indica, 4.Espostoa guentheri, 5.Echinocactus grusonii, 6. Mammillaria elongate.*

3.2.3 : RAPD Data Analysis:

By reviewing the results obtained from the use of 7 primers in this study, many data can be highlighted and concluded, and these data were important to reflect the usefulness of the RAPD-PCR indicators in analyzing the genome of the species under study at the molecular level. DNA polymorphism observed by RAPD can be explained in several ways, including the deletion or insertion of one or both opposing pairs of primer binding sites required to produce a PCR product, or the presence of nucleotide changes (e.g. mutation point) at the annealing primer fusion site in genome DNA, which prevents amplification by inserting a mismatch at only one end of a fragment of DNA.

The study showed that the molecular weight of the studied species ranged between (225-1700) base pairs, as the primer OP-V14 recorded the highest molecular weight, which amounted to 1700 base pairs, while the lowest molecular weight was recorded by the primer OP-M05, which amounted to 225 base pairs. Also, As shown in Table 3-17, the highest number of multiplexed bands appeared in the primer OP-V14, which amounted to 53 bands, while the lowest number of packages estimated at 25 appeared in the primer OP-V09 and OP-P04. Thus, the efficiency of the primer was achieved to become 18.6 in the primer OP-V14 as a maximum and 9.73 in the primer OP-P04 as a minimum efficiency. The OP-R06 primer was also distinguished by having the highest number of individual bands, which is estimated at 11, while the minimum, which is two bands, appeared in the OP-P04 primer As for the rest of the primers, they were confined between the two values. This variation may be due to several factors, such as the combination of primers, template quantity, or the small number of binding sites in the genome (Kernolde *et al.*, 1993). (Al-Marzouk ,2019) showed that the efficiency of the primer is the ability to give a greater percentage of polymorphic bands according to a number of amplification ranges. Thus, the efficiency of the primer does not mean the prefix that gave the largest number of multiplying bands, but rather its ability to show differences between the studied species.

Table 3-17: Plant species genetic polymorphism based on a technique of RAPD-PCR by seven primers, total bands productivity, Heterogenetic, primer efficiency %, and discrimination.

Primer name	Molecular weight	Total number of bands	Heteromorphic band number	number of multiple bands	unique bands	Polymorph ism per primer (%)	Primer efficiency (%)	Discrimination of primer (%)
OP-V19	400-1500	15	15	40	4	100	13.3	13.3
OP-R06	250-1500	19	19	36	10	100	16.8	16.8
OP-V14	300-1700	21	21	53	6	100	18.6	18.6
OP-V09	450-1400	13	13	25	5	100	11.5	11.5
OP-L05	375-1200	16	16	31	8	100	14.2	14.2
OP-M05	225-1500	18	18	40	4	100	15.9	15.9
OP-P04	300-1250	11	11	25	2	100	9.73	9.73
		113	113				100	100

3.2.4 : Genetic dimension values based on RAPD Markers

In this study, the genetic distances and genetic diversity (evolutionary history) of the species under study were also assessed and determined using RAPD indicators. Estimating or calculating genetic distance or variation using RAPD-PCR markers usually requires the results of 7 primers that produce complete and well-defined amplifications (Habeeb *et al.*, 2022), as the maximum genetic similarity between the two species *Hylocereus undatus* and *Opuntia ficus -indica* was 28.2, while the lowest similarity was 15.5 between the two species *Aloe vera* and *Mammillaria elongate* (Table 3-18). The existence of a percentage of genetic closeness between species is most likely due to their sharing the same alleles that descended from a common ancestor, and on this basis, the genetic relationship was built (Verma *et al.,* 2014). The genetic dimension between genotypes and identifying parents is important for selecting suitable parents and reaching the maximum strength of the hybrid in crossbreeding programs, and thus it was found that this method is useful and can be used to determine the phylogenetic relationships between the studied species and that it is likely to contain the most distinctive DNA features on the largest number of the new genes (Ashraf *et al.*, 2003).

Table 3-18: Similarity Matrix computed with Jaccard coefficient among various species.

Species	1	2	3	4	5	6
1	100	22.1	28.2	27.1	19	16.7
2		100	23.9	22	16.7	15.5
3			100	21.9	17.5	18.3
4				100	25.8	17.7
5					100	27.9
6						100

1-*Hylocereus undatus* 2 -*Aloe vera* 3 -*Opuntia ficus –indica* 4 -*Espostoa guentheri* 5 -*Echinocactus grusonii* ,6 -*Mammillaria elongate.*

The phylogenetic tree was another important issue to be determined on the basis of the RAPD profiles obtained in this study. Cluster analysis using the UPGMA technique (Sneath and Sokal, 1973) was used to create the phylogenetic tree for 6 species in the cactus family; the resulting kinship tree or genetic distance was obtained with the help of the pre-made application NTSYS-pc (Numerical Taxonomy System), this program is based on the formula Factories (Nei and Li, 1979). to discover and estimate genetic variation and relatedness; So the results of seven prefixes were entered into a matrix computed by the computer program. Thus, the study of the genetic relationships between the 6 types of genotypes of the studied species based on RAPD can be seen in the dendritic diagram (Fig 3-15). Draw a better visualization that represents the phenotypic (general similarity) or phylogenetic (evolutionary dating) relationships between a diverse group, individuals, or species.

The phylogeny tree of six plant species was based on the coefficient distance among the species. Based on RAPD-PCR, the tree construction for various plant species showed that three species were clustered in clade-1, two clustered in clade-2, while the species *loe vera* was diverging from these five species. The clades clustering indicated that five species belonged to the family Cactaceae, while at the same time, the clustering showed the doubt that *Aloe vera* might belong to the family Asphodelaceae, and not to Cactaceae. The present results also got support from the past findings of *Aloe vera* var. Chinensis (Wang *et al*., 2004). Several variables, like the primer design, the template amount, and the small number of linkage sites in the genome also contribute to this variability (Figure 3-15).

The five species belonging to the family Cactaceae were compared in terms of genetic affinity with one of the succulent plants, *Aloe vera*, to find the relationship of affinity among them. It was proven by the evolutionary tree among the six species using the RAPD-PCR method based on the coefficient distance between species. The existence of three different clades showed that the first clade combines the two species i.e., *Echinocactus grusonii* and *Mammillaria elongate* with a ratio of approximately 27.9 (Figure 3-15 and Table 3-18). This convergence was due to the joining of both species to the subfamily Cactoidae and the tribe Cacteae.

Based on various morphological and molecular traits, the sub-family Cactoideae is a well-known monophyletic group. Cortical vascular bundles, which create a three-dimensional network across the cortex, are used as diagnostic tools for all the taxa except *Blossfeldia*, making up the majority of the family's 1530 species of cacti (Mauseth and Sajeva, 1992; Anderson, 2005). The globular was depressed to short columnar cacti that make up the Cacteae tribe ranging in size from miniature to colossal. *Echinocactus* stems can have ribs, as those of *Coryphantha* (Engelmann) Lemaire, which can have tubercles (Charles *et al*., 2002).

The second Clade includes the two species i.e., *Hylocereus undatus* and *Opuntia- ficus-indica* at a ratio of approximately 28.2, which were associated with the species *Espostoa guentheri*, and this association illustrates the similarity in some phenotypical characteristics of the two tribes Phyllocacteae and Opuntieae belonging to the subfamily Cactoideae and Opuntioideae, respectively. Several molecular investigations further indicate the monophyly of the taxon and the extremely well-circumscribed sub-family Opuntioideae (Wallace and Dickie, 2002; Griffith and Porter, 2009). Overall, all the species have distinct segments in their stems, and many species exhibited the classic "prickly pear" development shape. In addition to regular, retrorsely barbed spines, the areoles also frequently generate large amounts of unpleasant glochids. Although glochids and spines develop at the same time, however, glochids were not sclerotic at the base, making them more brittle (Robinson, 1974).

In the case of the third clade, it confirms the isolation of the *Aloe vera* species from the cactus group, as it belongs to the family Asphodelaceae and the sub-family Asphodeloideae. The genotyping-based RAPD-PCR revealed that *Aloe vera* does not belong to the family Cactaceae, and these results ceased the ambiguity of *A. vera* relationship to the family Cactaceae but it belongs to the family

Asphodelaceae. All cactus plants are succulent, but not all succulents are cacti. The cactus plant can be distinguished from the group of succulent plants, through the presence of thorns arranged in a halo shape, in the middle of which is a central thorn, and around it is a smooth area devoid of hairs and spines. These results were also consistent with the past findings as mentioned in dividing the cactus family into subfamilies and tribes (Wallace, 1995; Wallace and Dickie, 2002; Griffith and Porter, 2009; Nyffeler and Eggli, 2010).

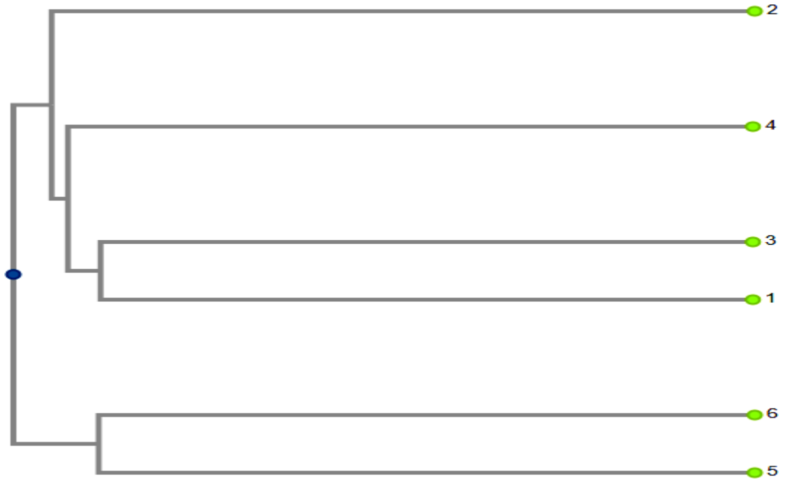


Figure 3-15: Phylogenetic tree dendrogram (similarity coefficient %) among various plant species based on the RAPD–PCR primers. 1-*Hylocereus undatus* 2 -*Aloe vera* 3 -*Opuntia ficus –indica* 4 -*Espostoa guentheri* 5 -*Echinocactus grusonii* ,6 -*Mammillaria elongate*.

The observed high degree of variation might be explained by the fact that the study of DNA variability was based on RAPD markers (Singh and Grange, 2006). The lowest similarity among the studied species arose due to genotyping of different species. In general, RAPD-PCR by arbiter primer was used to differentiate among the closed related biotypes or some strains in the same species. However, these RAPD-PCR genotyping results differ from other patterns of genotyping in various past studies (Abdul et al., 2014; Al-Tamimi, 2019; Anis and Al-Dulaimi, 2020).

The genotyping pattern also revealed that each plant species under interest was a dependent taxonomic unit and has a special evolutionary line (Greenberg and Donoghue, 2011). These conclusions also accord with (Wang *et al*., 2004) that this variability was produced by a variety of variables, including primer structure, template quantity, and the limited number of binding sites in the genome.

3.2.5 : Amplify the gene using the *rbc*L and *mat*K primers

Results of amplification of the primer *rbc*L at pb550 molecular size for all studied series are shown in Figure 3-16 and at pb600 molecular size in *mat*K primer in Figure 3-17.

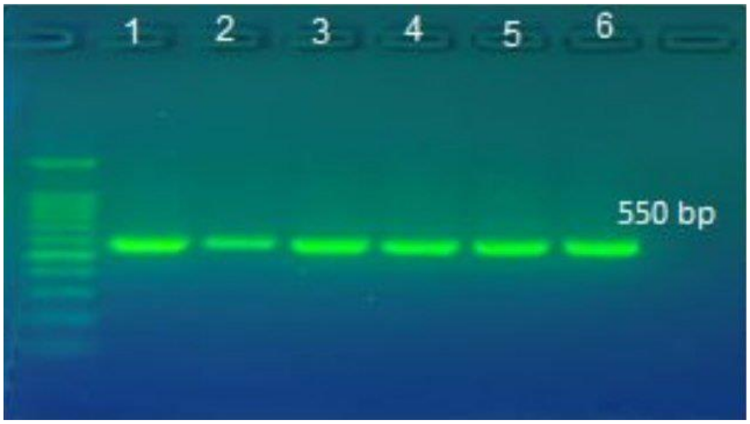


Figure 3-16: Polymerase chain reaction product with 550 bases run on 2% agarose at 5 V/cm2 electrophoresis. The *rbc*L gene may be seen under UV light with red-safe staining after being incubated in 1x TBE buffer for 1:30 hours. L: DNA ladder (1000).

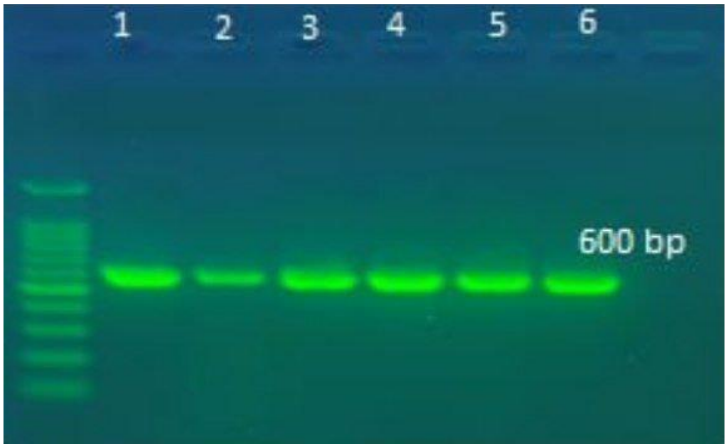


Figure 3-17: Polymerase chain reaction product with 600 bases run on 2% agarose at 5 V/cm2 electrophoresis. The *mat*K gene may be seen under UV light with red-safe staining after being incubated in 1x TBE buffer for 1:30 hours. M: DNA ladder (1000).

3.2.6 : Sequence analysis of the *rbc*L gene in the studied species

The polymerase chain reaction (PCR) technique is the approved basis for molecular classification and thus knowledge of the sequencing of plant species. It depends mainly on the purity of the DNA extracted from the smallest amount of plant tissue from plant samples, and this is what the results of the current study reached by obtaining good polymerization products and thus reaching knowledge The sequence for most of the species under study and for the *rbc*L and *mat*K genes, which was confirmed by (Al-Adhari, 2022). The process of finding out the DNA sequences of a specific region in the plastids, nucleus, or mitochondria was used to track and find evolutionary molecular phylogensis relationships in plant species belonging to different families, and in particular the use of chloroplast genes.

The DNA replication products were sent using PCR technology to the Korean company Macrogene to sequence the nitrogenous bases of the samples under study, and the results were compared with the Basic Local Alignment program ((BLAST Search Tool) and the BioEdit program with the genes registered in the NCBI Gen Bank, as the results proved the success of 6 species and the results were matched The sequence of the species under study with the sequences of the nitrogenous bases found in the NCBI GenBank.

After obtaining the sequence of the genetic bases of the DNA Sequence of the molecular polymerization products, it was analyzed and compared with the data obtained from the NCBI Gen Bank. Outcomes from this research, the *rbc*L and *mat*K genes was confirmed as particularly suited for molecular identification target genes, Variations in the nucleotide structure of the *rbc*L and *mat*K genes were successfully used to detect the species in the study.

After obtaining the sequence of the genetic bases of the DNA Sequence of the molecular polymerization products of the *rbc*L gene, it was analyzed and compared with the data obtained from the NCBI Gen Bank, and the Aloe vera species was registered within the Gen Bank under the identification number (KX377524.1) as in (Table 3-19) and Figure (3-18, 3-19) and a similarity rate of 85%, which confirmed that the global classification of the species is **Eukaryota; Viridiplantae; streptophyta; Embryophyta; Tracheophyta; Spermatophyta; magnoliopsida; Liliopsid; asparagales; Asphodelaceae; Asphodeloidae; *Aloe*.**

As for the species *Opuntia ficus-indica*, it was recorded in the Genome Bank under the identification number (KR737121.1) as in Figure (3-20, 3-21), with a similarity rate of 100%, which confirmed that the global classification of the species is

Eukaryota; Viridiplantae; streptophyta; Embryophyta; Tracheophyta; Spermatophyta; magnoliopsida; eudiocotyledons; Gunneridae; Pentapetalae; Caryophyllales; Cactineae; Cactaceae; Opuntioideae; *Opuntia*.

The same is the case with the type Echinocactus grusonii. It was recorded in the Genome Bank under the identification number (MK449095.1) as in Figure (3-

22, 3-23), with a similarity rate of 99%, which confirmed that the global classification of the species is

Eukaryota; Viridiplantae; streptophyta; Embryophyta; Tracheophyta; Spermatophyta; magnoliopsida; eudiocotyledons; Gunneridae; Pentapetalae; Caryophyllales; Cactineae; Cactaceae; Cactoidae; Cacteae; *Echinocactus*

As for the rest of the studied species, the genetic sequence was not relied upon. They have scattered and unclear genetic hyperplasia, so they were excluded

Table 3-19: Comparative nucleotide substitution at the sequence of Iraqi sample of the *rbc*L gene with globle species under study from NCBI.

No.	Type of substitution	Location	Nucleotide	Sequence ID with compare	Source	Identities
1	**Multiple mutations**			**ID:KX377524.1**	***Aloe vera* chloroplast;**	**85%**
2				**ID: KR737121.1**	***Opuntia ficus-indica* ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) gene,**	**100%**
3	**Transition/ Transversion**	**150**	**(A,G)R/A**	**ID:MK449095.1**	***Echinocactus grusonii* ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) gene, partial cds;**	**99%**
	Transition/ Transversion	**232**	**(A,G)R/A**			
	Transition/ Transversion	**294**	**(C,T)Y/T**			
	Transversion	**357**	**(G,T)K/G**			
	Transversion	**390**	**(A,C)M/A**			

Score	Expect	Identities	Gaps	Strand
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Query  71    CYGATATCTTGGCASCATTCCGAGTAACYCCTCAACCTGGAGTTCCGCCWGAAKAAGCWG 130
             | |||||||||||| ||||||||||||| |||||||| |||||||| || ||| |||| |
Sbjct  54646 CTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGAGTTCCCCCTGAAGAAGCAG  54705

Query  131   GMGCYGCRCTAKCTGCCGAATCTTCTACTGGTACMTGGACGWCTGGACGTACCGAYGGAC 190
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Query  191   TTACCAGTCTTGATCGTTACAAAGGACGRTGCTACCACATCGATGCCGTTCCTGGAGAAG 250
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Sbjct  54766 TTACCAGTCTTGATCGTTACAAAGGAAGGTGCTACCACATTGAGGCTGTTGCTGGGGAAG  54825

Query  251   ACAATCAATATATTTGTTATGTAGCTTACCCATTAGACCTTTTTGAAGAAGGTTCTGTTA 310
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Query  311   CTAATATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAGGCCCTGCGTGCTCTAC 370
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Sbjct  54886 CTAACATGTTTACTTCCATTGTAGGTAATGTATTTGGTTTCAAAGCCCTACGAGCTCTAC  54945

Query  371   GTTTGGAGGATTTGCGAATCCCTGTTGCTTATATAAAAACTTTCCAAGGCCCGCCCCACG 430
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Query  491   TTAARCCSAAATTGGGRTTATCYGCTAAAAACTAYGGTMGAGCRGTTTATGAATGTCTTC 550
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Sbjct  55066 TTAAACCCAAATTGGGATTATCTGCAAAAAACTACGGTAGAGCGGTTTATGAATGTCTAC  55125

Query  551   GYGGTGGAYTTGATKKKAGCaaa  573
             | |||||| |||||   | ||||
Sbjct  55126 GCGGTGGACTTGATTTTACCAAA  55148
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Figure (3-18): Alignment of the DNA sequence of *Aloe vera* chloroplast, complete genome (Sequence ID: KX377524.1 Length: 152875 Number of Matches: 1)

GenBank: KX377524.1

```
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VERSION     KX377524.1
KEYWORDS    .
SOURCE      chloroplast Aloe vera
  ORGANISM  Aloe vera
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
            Spermatophyta; Magnoliopsida; Liliopsida; Asparagales;
            Asphodelaceae; Asphodeloideae; Aloe.
REFERENCE   1 (bases 1 to 563)
  AUTHORS   Lee,Y.S. and Yang,T.-J.
  TITLE     The complete chloroplast genome sequence of Aloe vera and Aloe
            saponaria (Xanthorrhoeaceae)
  JOURNAL   Unpublished
REFERENCE   2 (bases 1 to 563)
  AUTHORS   Lee,Y.S. and Yang,T.-J.
  TITLE     Direct Submission
  JOURNAL   Submitted (12-JUN-2016) Department of Plant Science, Seoul National
            University, Gwanak 599, Gwanak-ro, Gwanak-gu, Seoul, Seoul 08826,
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                     IIREAAKWSPELAAACEIWKAIKFEFEPVDKIDK"
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      301 ctaacatgtt tacttccatt gtaggtaatg tatttggttt caaagcccta cgagctctac
      361 gtctggagga tctacgaatt ccccctgctt attccaaaac tttccaaggt ccgccccacg
      421 gtatccaagt tgaaagagat aaattgaaca agtatggtcg tcccctattg ggatgtacta
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      541 gcggtggact tgattttacc aaa
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Figure (3-19): *Aloe vera* species registration document in the NCBI Genbank

Score	Expect	Identities	Gaps	Strand
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Query 163 TACATGGACAACTGTATGGACCGACGGACTTACCAGTCTTGATCGTTACAAAGGACGATG 222
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Query 283 ATTAGACCTTTTTGAAGAAGGTTCTGTTACTAATATGTTTACTTCCATTGTGGGTAATGT 342
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Figure (3-20): Alignment of the DNA sequence of *Opuntia ficus-indica* ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) gene, partial cds; chloroplast (Sequence ID: KR737121.1 Length: 415 Number of Matches: 1)

GenBank: KR737121.1

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ACCESSION   KR737121 REGION: 1..402
VERSION     KR737121.1
KEYWORDS    BARCODE.
SOURCE      chloroplast Opuntia ficus-indica
  ORGANISM Opuntia ficus-indica
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
            Spermatophyta; Magnoliopsida; eudicotyledons; Gunneridae;
            Pentapetalae; Caryophyllales; Cactineae; Cactaceae; Opuntioideae;
            Opuntia.
REFERENCE   1 (bases 1 to 402)
  AUTHORS   Kartzinel,T.R., Chen,P.A., Coverdale,T.C., Erickson,D.L.,
            Kress,W.J., Kuzmina,M.L., Rubenstein,D.I., Wang,W. and Pringle,R.M.
  TITLE     DNA metabarcoding illuminates dietary niche partitioning by African
            large herbivores
  JOURNAL   Proc. Natl. Acad. Sci. U.S.A. (2015) In press
   PUBMED   26034267
  REMARK    Publication Status: Available-Online prior to print
REFERENCE   2 (bases 1 to 402)
  AUTHORS   Kartzinel,T.R., Chen,P.A., Coverdale,T.C., Erickson,D.L.,
            Kress,W.J., Kuzmina,M.L., Rubenstein,D.I., Wang,W. and Pringle,R.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (14-MAY-2015) Department of Ecology and Evolutionary
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      241 atttgggttc aaggccctgc gtgctctacg tttggaggat ttgcgaatcc ctgttgctta
      301 tataaaaact ttccaaggcc cgccccacgg tatccaagtt gagagagata aattgaacaa
      361 gtatggccgt cctctactgg gatgcactat taagccgaaa tt
```

Figure (3-21): *Opuntia ficus-indica* species registration document in the NCBI Genbank

Score	Expect	Identities	Gaps	Strand
730 bits(395)	0.0	400/405(99%)	0/ 405 (0%)	Plus/ Plus

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Query1     TCCTCAACCTGGAGTTCCGTCAGAAGAAGCAGGAGCCGCAGTAGCTGCCGAATCTTCTAC  60
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Sbjct84    TCCTCAACCTGGAGTTCCGTCAGAAGAAGCAGGAGCCGCAGTAGCTGCCGAATCTTCTAC 143

Query61    TGGTACRTGGACAACTGTATGGACCGACGGACTTACCAGTCTTGATCGTTACAAAGGACG 120
           |||||| |||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct144   TGGTACATGGACAACTGTATGGACCGACGGACTTACCAGTCTTGATCGTTACAAAGGACG 203

Query121   ATGCTACCACATCGATGCCGTTCCTGGARAAGACAATCAATATATTTGTTATGTAGCTTA 180
           |||||||||||||||||||||||||||| |||||||||||||||||||||||||||||||
Sbjct204   ATGCTACCACATCGATGCCGTTCCTGGAAAAGACAATCAATATATTTGTTATGTAGCTTA 263

Query181   CCCCTTAGACCTTTTTGAAGAAGGTTCTGTYACTAATATGTTTACTTCCATTGTGGGTAA 240
           |||||||||||||||||||||||||||||| |||||||||||||||||||||||||||||
Sbjct264   CCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAATATGTTTACTTCCATTGTGGGTAA 323

Query241   TGTATTTGGGTTCAAAGCCCTGCGTGCTCTACGKTTGGAGGATTTGCGAATCCCTGTTGC 300
           ||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||
Sbjct324   TGTATTTGGGTTCAAAGCCCTGCGTGCTCTACGGTTGGAGGATTTGCGAATCCCTGTTGC 383

Query301   TTATATMAAAACTTTCCAAGGCCCGCCTCACGGTATCCAAGTTGAGAGAGATAAATTGAA 360
           |||||| |||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct384   TTATATAAAAACTTTCCAAGGCCCGCCTCACGGTATCCAAGTTGAGAGAGATAAATTGAA 443

Query361   CAAGTATGGCCGTCCTCTACTGGGATGCACTATTAAGCCGAAATT  405
           |||||||||||||||||||||||||||||||||||||||||||||
Sbjct444   CAAGTATGGCCGTCCTCTACTGGGATGCACTATTAAGCCGAAATT  488
```

Figure (3-22): Alignment of the DNA sequence of *Echinocactus grusonii* ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) gene, partial cds; chloroplast (Sequence ID: MK449095.1 Length: 539 Number of Matches: 1)

GenBank: MK449095.1

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DEFINITION Echinocactus grusonii ribulose-1,5-bisphosphate
            carboxylase/oxygenase large subunit (rbcL) gene, partial cds;
            chloroplast.
ACCESSION   MK449095
VERSION     MK449095.1
KEYWORDS    .
SOURCE      chloroplast Echinocactus grusonii
  ORGANISM Echinocactus grusonii
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
            Spermatophyta; Magnoliopsida; eudicotyledons; Gunneridae;
            Pentapetalae; Caryophyllales; Cactineae; Cactaceae; Cactoideae;
            Cacteae; Echinocactus.
REFERENCE   1 (bases 1 to 539)
  AUTHORS   Vazquez-Sanchez,M., Sanchez,D., Terrazas,T., De La Rosa-Tilapa,A.
            and Arias,S.
  TITLE     Polyphyly of the iconic cactus genus Turbinicarpus (Cactaceae) and
            its generic circumscription
  JOURNAL   Bot. J. Linn. Soc. 190 (4), 405-420 (2019)
  REMARK    DOI: 10.1093/botlinnean/boz027
REFERENCE   2 (bases 1 to 539)
  AUTHORS   Vazquez-Sanchez,M.
  TITLE     Direct Submission
  JOURNAL   Submitted (25-JAN-2019) Jardin Botanico, Instituto de Biologia,
            Universidad Nacional Autonoma de Mexico, Av. Universidad 3000,
            Mexico, Distrito Federal 04510, Mexico
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            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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                     /gene="rbcL"
     CDS             <1..>539
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                     /transl_table=11
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                     TSIVGNVFGFKALRALRLEDLRIPVAYIKTFQGPPHGIQVERDKLNKYGRPLLGCTIK
                     PKLGLSAKNYGRAVYECLRG"
ORIGIN
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       61 atcttggcag cattccgagt aagtcctcaa cctggagttc cgtcagaaga agcaggagcc
      121 gcagtagctg ccgaatcttc tactggtaca tggacaactg tatggaccga cggacttacc
      181 agtcttgatc gttacaaagg acgatgctac cacatcgatg ccgttcctgg aaaagacaat
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      301 atgtttactt ccattgtggg taatgtattt gggttcaaag ccctgcgtgc tctacggttg
      361 gaggatttgc gaatccctgt tgcttatata aaaactttcc aaggcccgcc tcacggtatc
      421 caagttgaga gagataaatt gaacaagtat ggccgtcctc tactgggatg cactattaag
      481 ccgaaattgg ggttatctgc taaaaactat ggtcgagcag tttatgaatg tcttcgcgg
```

Figure (3-23): *Echinocactus grusonii* species registration document in the NCBI Genbank

As for the rest of the species, using the matK gene, the similarity rates were about 99%, 99%, 98%, 98%, and 100% for *Hylocereus undatus, Opuntia ficus-indica*, *Espostoa guentheri*, *Echinocactus grusonii*, and *Mammillaria elongata*, respectively, with the exception of *Aloe vera*, which was recorded The similarity rate is 69%, which confirms the change in the genetic sequence of the species because it does not belong to the Cactus family Table (3-20) and Figures (3-24 to 3- 35).

Table 3-20: Comparative nucleotide substitution at the sequence of Iraqi sample of the *mat*K gene with globle species under study from NCBI.

No.	Type of substitution	Location	Nucleotide	Sequence ID with compare	Source	Identities
1	**Transition**	**980**	**A/G**	ID: LT745695.1	***Hylocereus undatus*** **chloroplast *mat*K gene for maturase K and partial *trn*K gene intron,**	**99%**
	Transversion	**982**	**C/A**			
2	**Multiple mutations**			ID: JQ276402.1	***Aloe vera*** **maturase K (*mat*K) gene, complete cds;**	**69%**
3	**Transversion**	**835**	**R/G**	ID: FN997314.1	***Opuntia ficus-indica*** **chloroplast *mat*K gene and partial tRNA-Lys intron, isolate JARA2035**	**99%**
	Gap	**886**	**R/G**			
4	**Transition**	**796**	**G/A**	ID: JX683871.1	***Espostoa guentheri*** **isolate EspgueE639 tRNA-Lys (*trn*K) gene, partial sequence; and maturase K (*mat*K) gene, complete cds; chloroplast**	**98%**
	Transversion	**812**	**C/G**			
	Transversion	**903**	**C/A**			
	Transition	**932**	**C/T**			
	Transition	**981**	**A/G**			
	Transversion	**983**	**C/A**			
	Transition	**999**	**A/G**			
	Transition	**1017**	**T/C**			
	Transversion	**1059**	**(G,T)K/G**			
	Transversion	**1061**	**A/C**			
	Transition/ Transversion	**1107**	**(A,G)R/G**			
5	**Transition/ Transversion**	**798**	**(A,G)R/A**	ID: FN997554.1	***Echinocactus grusonii*** **chloroplast *mat*K gene and partial tRNA-Lys intron**	**98%**
	Transversion	**814**	**(C,G) S/G**			
	Transition/ Transversion	**847**	**(C,T)Y/T**			
	Transition/ Transversion	**925**	**(C,T)y/T**			
	Transversion	**926**	**(A,C)m/A**			
	Transition/ Transversion	**983**	**(A,G)R/G**			
	Transversion	**985**	**(A,C)M/A**			
	Transition/ Transversion	**1001**	**(A,G)R/G**			
	Transversion	**1007**	**(A,T)W/A**			
	Transition/ Transversion	**1019**	**(C,T)Y/C**			
6				ID: FN997474.1	***Mammillaria elongata*** **chloroplast *mat*K gene and partial tRNA-Lys intron,**	**100%**

Score	Expect	Identities	Gaps	Strand
987 bits(534)	0.0	538/540(99%)	0/540(0%)	Plus/ Plus

```
Query 1     AGAAACTCCCTGCttttttttAGTTTTCGGTCTAATTTGAAATGGAAGAATTCCAAAGAT  60
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Sbjct 651   AGAAACTCCCTGCTTTTTTTTAGTTTTCGGTCTAATTTGAAATGGAAGAATTCCAAAGAT  710

Query 61    ATATAGAACTAGATAGGTCTTGGCAACACAACTTTTTCTATCCACTTATCTTTCAGGAAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 711   ATATAGAACTAGATAGGTCTTGGCAACACAACTTTTTCTATCCACTTATCTTTCAGGAAT  770

Query 121   ATATTTATGGATTTGCATATGATCGTGGTTTAAATAAATCCATTTTGTTGGAAAATGCAG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 771   ATATTTATGGATTTGCATATGATCGTGGTTTAAATAAATCCATTTTGTTGGAAAATGCAG  830

Query 181   GCGACAAGAAATACAGTTTACTGATTGTAAAACGTTTAATTAACCGAATGTATCAACAGA  240
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Sbjct 831   GCGACAAGAAATACAGTTTACTGATTGTAAAACGTTTAATTAACCGAATGTATCAACAGA  890

Query 241   CTCATTTGATTCTTTCTGCTAATCATTCTAACCAAAATGACTTTTTTGGGCACAAGCACA  300
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Sbjct 891   CTCATTTGATTCTTTCTGCTAATCATTCTAACCAAAATGACTTTTTTGGGCACAAGCACA  950

Query 301   AGAAGAATTTGTATTATCAAATAATATCAAACGGATTTGCAGTCATTATGGAAATTCCAT  360
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Sbjct 951   AGAAGAATTTGTATTATCAAATAATATCAGAAGGATTTGCAGTCATTATGGAAATTCCAT 1010

Query 361   TTTCCTTACTGTTAATATCTTCCCTAGAGGCAAAAGaaaaaaaaaTAGTAAAATCCCATA  420
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Sbjct 1011  TTTCCTTACTGTTAATATCTTCCCTAGAGGCAAAAGAAAAAAAAATAGTAAAATCCCATA 1070

Query 421   ATTTGCGATCAATTCATTCAATATTTCCTTTTTTCGAGGACAAATTCTTACATTTAAATT  480
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Sbjct 1071  ATTTGCGATCAATTCATTCAATATTTCCTTTTTTCGAGGACAAATTCTTACATTTAAATT 1130

Query 481   ATGTGTTAGAAATATTAATACCTTACCCCATCCATCTGGAAATTTTGGTTCAAACTCTTC  540
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Sbjct 1131  ATGTGTTAGAAATATTAATACCTTACCCCATCCATCTGGAAATTTTGGTTCAAACTCTTC  1190
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Figure (3-24): Alignment of the DNA sequence of *Hylocereus undatus* chloroplast *mat*K gene for maturase K and partial *trn*K gene intron, specimen voucher B GH 18378, 18378a, isolate CA226 (Sequence ID: LT745695.1 Length: 2465 Number of Matches: 1)

GenBank: LT745695.1

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LOCUS       LT745695     555 bp    DNA     linear   PLN 20-DEC-2017
DEFINITION  Hylocereus undatus chloroplast matK gene for maturase K and partial
            trnK gene intron, specimen voucher B GH 18378, 18378a, isolate
            CA226.
ACCESSION   LT745695 REGION: 651..1205
VERSION     LT745695.1
KEYWORDS    .
SOURCE      chloroplast Selenicereus undatus
  ORGANISM  Selenicereus undatus
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
            Spermatophyta; Magnoliopsida; eudicotyledons; Gunneridae;
            Pentapetalae; Caryophyllales; Cactineae; Cactaceae; Cactoideae;
            Hylocereeae; Selenicereus.
REFERENCE   1
  AUTHORS   Korotkova,N., Borsch,T. and Arias,S.
  TITLE     A phylogenetic framework for the Hylocereeae (Cactaceae) and
            implications for the circumscription of the genera
  JOURNAL   Phytotaxa 327 (1), 1-46 (2017)
REFERENCE   2 (bases 1 to 555)
  AUTHORS   Korotkova,N.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-FEB-2017) Freie Universitaet Berlin, Botanisches
            Museum, Koenigin-Luise-Strasse 6-8, 14195 Berlin, GERMANY
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                     /isolate="CA226"
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                     FLHYVRYRGKSILASKGTSLLMHKWKYYLFNFWQCHFSLWSPPRRIYINRLSKHSLDF
                     MGFFSSVRLNSSVVRSQMVENSFLIDNPIKKFDTIVRIIPLVGSLAKAQFCNVLGHPI
                     SKSVWTDLLDSDIIDRFGRICRNLSHYYSGSSRKKSLYRIKYILRLSCARTLARKHKS
                     TVRAFLKRLGSEFLEEFFTEEEKVLSLILPRDSSISRGLYRGPFWYLDIICIHDLAND
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481 atgtgttaga aatattaata ccttacccca tccatctgga aattttggtt caaactcttc
541 gttactgggt gaaag
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Figure (3-25): *Hylocereus undatus* species registration document in the NCBI Genbank

Score	**Expect**	**Identities**	**Gaps**	**Strand**
279 bits(308)	3e-76	356/516(69%)	26/516(5%)	Plus/ Plus

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Query  44   ggaarawtyccaaaratwtwtaramctarataGGTCTKGGCAMCMCAACTTTTTCTATCC  103
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Sbjct  3    GGAAGAATTACAACGATATTTAGAAAAAGATAGATCTCGGCAACAACACTTTCTATATCC  62

Query  104  ACTTATCTTTCAGGAATATWTTTATGGATTTGCWTATGATCATGGTTTAAATAAATCTAT  163
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Sbjct  63   GCTTCTCTTTCAAGAGTATATTTATGCACTTGCTCATGATCATGGTTTAAATAGTTCGAT  122

Query  164  TTTGT---------KGGAAAATGCAGGCCA---CAARAAATACAGTTTACTGATTGTAAA  211
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Sbjct  123  TTTTTACGAACCCGTGGAAATTCTTGGTTATGACAATAAATCTAGTTTAGCACTTGTAAA  182

Query  212  ACGTTTAWTTACCCRAATGTATCAMCARAWTCWTTTGATYCTTTCTGCTAATCATTCTAA  271
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Sbjct  183  ACGTTTAATTATTCGAATCTATCAACAGAATTATTTGATTTCTTCAGTTAATGATTTTAA  242

Query  272  CCAAAATGACTTTTTTGGGCACAASCACa ------ araaraaTTTGTWTTATCAAATAA  324
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Sbjct  243  CCAAAATAGATTCCTTGGGCACAA-CACGTTTTTTTATTCTCATTTTTATTCTCAAATTA  301

Query  325  TATCARAAGGATTTGCAKTCATTGKGGAAATYCCATTTTCCCTACTGTTAATATCTTCCC  384
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Query  445  TTCCYTTTTTCRAGGACAAWTTYTTMCWTTTAAATTATGKGTTAAAAATATWAATACCTT  504
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Sbjct  416  TTCCCTTTTTAGAGGACAAATTCTCACATTTAAATTATGTTTCAGATATACTAATACCTT  475

Query  505  ACCCCWTTCATCTARAAATCTKGGTTCAAACTCTTC  540
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Sbjct  476  ATCCCATCCATATGGAAATCTTGGTTCAAATCCTTC  511
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Figure (3-26): Alignment of the DNA sequence of *Aloe vera* maturase K (*mat*K) gene, complete cds; chloroplast (Sequence ID: JQ276402.1 Length: 1557 Number of Matches: 1)

GenBank: JQ276402.1

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DEFINITION Aloe vera maturase K (matK) gene, complete cds; chloroplast.
ACCESSION   JQ276402 REGION: 3..511
VERSION     JQ276402.1
KEYWORDS    .
SOURCE      chloroplast Aloe vera
  ORGANISM Aloe vera
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
            Spermatophyta; Magnoliopsida; Liliopsida; Asparagales;
            Asphodelaceae; Asphodeloideae; Aloe.
REFERENCE   1 (bases 1 to 509)
  AUTHORS   Steele,P.R., Hertweck,K.L., Mayfield,D., McKain,M.R.,
            Leebens-Mack,J. and Pires,J.C.
  TITLE     Quality and quantity of data recovered from massively parallel
            sequencing: Examples in Asparagales and Poaceae
  JOURNAL   Am. J. Bot. 99 (2), 330-348 (2012)
   PUBMED   22291168
REFERENCE   2 (bases 1 to 509)
  AUTHORS   Steele,P.R., Hertweck,K.L., Mayfield,D., McKain,M., Leebens-Mack,J.
            and Pires,J.C.
  TITLE     Direct Submission
  JOURNAL   Submitted (29-NOV-2011) Division of Biological Sciences, University
            of Missouri, 371 B Bond Life Sciences Center, Columbia, MO
            65211-7310, USA
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     CDS             <1..>509
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                     IHMEILVQILQCRIQDVPFLHLLRFFLHEYHNCNSLLITQNKSIYVFSNENKRLFQLL
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      481 ccatatggaa atcttggttc aaatccttc
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Figure (3-27): *Aloe vera* species registration document in the NCBI Genbank

Score	Expect	Identities	Gaps	Strand
821 bits(444)	0.0	446/448(99%)	1/448 (0%)	Plus/ Plus

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Sbjct  655  CCTGCTTTTTTTTTAGTTTTCGGTCTAATTTTAAATGGAAGAATTCCAAAGATATATAGA 714

Query  61   ACTAGATAGGTCTTGGCAACACAACTTTTTCTATCCACTTATCTTTCAGGAATATATTTA 120
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Sbjct  715  ACTAGATAGGTCTTGGCAACACAACTTTTTCTATCCACTTATCTTTCAGGAATATATTTA 774

Query  121  TGGATTTGCATATGATCATGGTTTAAATAAATCTATTTTGTTGGAAAATGCAGGCCACAA 180
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Sbjct  775  TGGATTTGCATATGATCATGGTTTAAATAAATCTATTTTGTTGGAAAATGCAGGCCACAA 834

Query  181  RAAATACAGTTTACTGATTGTAAAACGTTTAATTACCCGAATGTATCAACARAATCATTT 240
             |||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||
Sbjct  835  GAAATACAGTTTACTGATTGTAAAACGTTTAATTACCCGAATGTATCAACAGAATCATTT  894

Query  241  GATTCTTTCTGCTAATCATTCTAACCAAAATGACTTTTTTGGGCACAAGCACAAGAAGAA 300
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Sbjct  895  GATTCTTTCTGCTAATCATTCTAACCAAAATGACTTTTTTGGGCACAAGCACAAGAAGAA 954

Query  301  TTTGTATTATCAAATAATATCAGAAGGATTTGCAGTCATTGTGGAAATTCCATTTTCCCT 360
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Sbjct  955  TTTGTATTATCAAATAATATCAGAAGGATTTGCAGTCATTGTGGAAATTCCATTTTCCCT 1014

Query  361  ACTGTTAATATCTTCCCTAGGGGCAAAAGaaaaaaaaaTAGTAAAATCTCATAATTTGCG 420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 1015  ACTGTTAATATCTTCCCTAGGGGCAAAAGAAAAAAAAATAGTAAAATCTCATAATTTGCG 1074

Query  421  ATCAATTCATTCAATATTTCCCTTTTTC  448
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Sbjct 1075  ATCAATTCATTCAATATTTCCCTTTTTC  1102
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Figure (3-28): Alignment of the DNA sequence of *Opuntia ficus-indica* chloroplast matK gene and partial tRNA-Lys intron, isolate JARA2035 (Sequence ID: FN997314.1 Length: 2386 Number of Matches: 1)

GenBank: FN997314.1

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LOCUS       FN997314     554 bp    DNA     linear   PLN 30-JAN-2012
DEFINITION Opuntia ficus-indica chloroplast matK gene and partial tRNA-Lys
            intron, isolate JARA2035.
ACCESSION   FN997314 REGION: 650..1203
VERSION     FN997314.1
KEYWORDS    .
SOURCE      chloroplast Opuntia ficus-indica
  ORGANISM Opuntia ficus-indica
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
            Spermatophyta; Magnoliopsida; eudicotyledons; Gunneridae;
            Pentapetalae; Caryophyllales; Cactineae; Cactaceae; Opuntioideae;
            Opuntia.
REFERENCE   1
  AUTHORS   Barcenas,R.T., Yesson,C.J. and Hawkins,J.A.
  TITLE     Molecular systematics of the Cactaceae
  JOURNAL   Unpublished
REFERENCE   2 (bases 1 to 554)
  AUTHORS   Barcenas,R.T.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-JUN-2010) to the INSDC. Barcenas R.T., Darwin
            Laboratorium of Molecular Systematics and Evolution, Universidad
            Autonoma de Queretaro, Av. de la Ciencia s/n, 76230, MEXICO
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                     /transl_table=11
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                     VEIPFSLLLISSLGAKEKKIVKSHNLRSIHSIFPFFEDKFLHLNYVLKILIPYPIHLE
                     ILVQTLRYWVKDASSLHLLRFFLYEYRNWNSLITPQKSISIFSKKNRRLFLFLYNFHV
                     CEYESIFVFLCNQSSHLRSTSFGALLERIYFYGKLEYLVKVKTFTKDFRLILWLFKDP
                     FLHYVRYRGKSILASKGTSLLMYKWKYYLINFWQCHFSLWSQPRRIYINRLSKHSLDF
                     MRIPY"
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      121 atttatggat ttgcatatga tcatggttta aataaatcta ttttgttgga aaatgcaggc
      181 cacaagaaat acagtttact gattgtaaaa cgtttaatta cccgaatgta tcaacagaat
      241 catttgattc tttctgctaa tcattctaac caaaatgact tttttgggca caagcacaag
      301 aagaatttgt attatcaaat aatatcagaa ggatttgcag tcattgtgga aattccattt
      361 tccctactgt taatatcttc cctaggggca aaagaaaaaa aaatagtaaa atctcataat
      421 ttgcgatcaa ttcattcaat atttcccttt ttcgaggaca aattcttaca tttaaattat
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      541 tactgggtga aaga
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Figure (3-29): *Opuntia ficus-indica* species registration document in the NCBI Genbank

Score	Expect	Identities	Gaps	Strand
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Query61     TATCCACTTATCTTTCAGGAATATATTTATGGATTTGCATATGATCGTGGTTTAAATAAA  120
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Sbjct750    TATCCACTTATCTTTCAGGAATATATTTATGGATTTGCATATGATCATGGTTTAAATAAA  809

Query121    TCCATTTTGTTGGAAAATGCAGGCGACAAGAAATACAGTTTACTGATTGTAAAACGTTTA  180
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Sbjct810    TCGATTTTGTTGGAAAATGCAGGCGACAAGAAATACAGTTTACTGATTGTAAAACGTTTA  869

Query181    ATTAACCGAATGTATCAACAGACTCATTTGATTCTTTCTGCTAATCATTCTAACCAAAAT  240
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Sbjct870    ATTAACCGAATGTATCAACAGACTCATTTGATTATTTCTGCTAATCATTCTAACCAAAAT  929

Query241    GACTTTTTTGGGCACAAGCACAAGAAGAATTTGTATTATCAAATAATATCAAACGGATTT  300
            || |||||||||||||||||||||||||||||||||||||||||||||||| | ||||||
Sbjct930    GATTTTTTTGGGCACAAGCACAAGAAGAATTTGTATTATCAAATAATATCAGAAGGATTT  989

Query301    GCAGTCATTATGGAAATTCCATTTTCCTTACTGTTAATATCTTCCCTAGAGGCAAAAGaa  360
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Sbjct990    GCAGTCATTGTGGAAATTCCATTTTCCCTACTGTTAATATCTTCCCTAGAGGCAAAAGAA  1049

Query361    aaaaaaaTAKTAAAATCCCATAATTTGCGATCAATTCATTCAATATTTCCTTTTTTCRAG  420
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Query421    GACAAATTCTTACATTTAAATTATGTGTTAGAAATATTAATACCTTACCCCATCCATCTG  480
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Sbjct1110   GACAAATTCTTACATTTAAATTATGTGTTAGAAATATTAATACCTTACCCCATCCATCTG  1169

Query481    GAAAT  485
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Sbjct1170   GAAAT  1174
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Figure (3-30): Alignment of the DNA sequence of *Espostoa guentheri* isolate EspgueE639 tRNA-Lys (trnK) gene, partial sequence; and maturase K (*mat*K) gene, complete cds; chloroplast (Sequence ID: JX683871.1 Length: 2456 Number of Matches: 1)

GenBank: JX683871.1

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DEFINITION Espostoa guentheri isolate EspgueE639 tRNA-Lys (trnK) gene, partial
            sequence; and maturase K (matK) gene, complete cds; chloroplast.
ACCESSION   JX683871 REGION: 655..1205
VERSION     JX683871.1
KEYWORDS    .
SOURCE      chloroplast Espostoa guentheri
  ORGANISM Espostoa guentheri
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
            Spermatophyta; Magnoliopsida; eudicotyledons; Gunneridae;
            Pentapetalae; Caryophyllales; Cactineae; Cactaceae; Cactoideae;
            Trichocereeae; Espostoa.
REFERENCE   1 (bases 1 to 551)
  AUTHORS   Lendel,A., Eggli,U. and Nyffeler,R.
  TITLE     Molecular phylogenetics of the tribe Cereeae (Cactaceae)
  JOURNAL   Unpublished
REFERENCE   2 (bases 1 to 551)
  AUTHORS   Lendel,A., Eggli,U. and Nyffeler,R.
  TITLE     Direct Submission
  JOURNAL   Submitted (12-SEP-2012) Institute of Systematic Botany, University
            of Zurich, Zollikerstrasse 107, Zurich CH-8008, Switzerland
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                     ILVQTLRYWVKDASSLHLLRFFLYEYRNWNSLITPQKSISIFSNQRLFLFLYNFYVCE
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                     HYVRYRGKSILASKGTSLLMHKWKYYLFHFWQCHFSLWSPPRRIYINRLSKHSLDFMG
                     FFSSVRLNSSVVRSQMVENSFLIDNPINKFDTIVRPLVGSLAKAKFCNVLGHPISKSV
                     WTDLLDSDIIDRFGRICRNLSHYYSGSSRKKSLYRIKYILRLSCARTLARKHKSTVRA
                     FLKRLGSEFLEEFFTEEEKVLSLILPRDSSISRGLYRGPFWYLDIICIHDLANDE"
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      121 tttatggatt tgcatatgat catggtttaa ataaatcgat tttgttggaa aatgcaggcg
      181 acaagaaata cagtttactg attgtaaaac gtttaattaa ccgaatgtat caacagactc
      241 atttgattat ttctgctaat cattctaacc aaaatgattt ttttgggcac aagcacaaga
      301 agaatttgta ttatcaaata atatcagaag gatttgcagt cattgtggaa attccatttt
      361 ccctactgtt aatatcttcc ctagaggcaa aagaaaaaaa aatagtcaaa tcccataatt
      421 tgcgatcaat tcattcaata tttccttttt tcgaggacaa attcttacat ttaaattatg
      481 tgttagaaat attaatacct taccccatcc atctggaaat cttggttcaa actcttcgtt
      541 actgggtgaa a
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Figure (3-31): *Espostoa guentheri* species registration document in the NCBI Genbank.

Score	Expect	Identities	Gaps	Strand
839 bits(454)	0.0	474/484 (98%)	0/ 484 (0%)	Plus/ Plus

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Query1      CGGTCTAATTTGAAATGGAAGAATTCCAAAGATATATAGAACTAGATAGGTCTTGGCAAC  60
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Query61     ACAACTTTTTCTATCCACTTATCTTTCAGGAATATATTTATGGATTTGCATATGATCRTG  120
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Sbjct741    ACAACTTTTTCTATCCACTTATCTTTCAGGAATATATTTATGGATTTGCATATGATCATG  800

Query121    GTTTAAATAAATCSATTTTGTTGGAAAATGCAGGCGACAAGAAATAYAGTTTACTGATTG  180
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Query181    TAAAACGTTTAATTAACCGAATGTATCAACAGACTCATTTGATTCTTTCTGCTAATCATT  240
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Sbjct861    TAAAACGTTTAATTAACCGAATGTATCAACAGACTCATTTGATTCTTTCTGCTAATCATT  920

Query241    CTaaymaaaaTGACTTTTTTGGGCACAAGCACAAGAAGAATTTGTATTATCAAATAATAT  300
            |||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct921    CTAATAAAAATGACTTTTTTGGGCACAAGCACAAGAAGAATTTGTATTATCAAATAATAT  980

Query301    CARAMGGATTTGCAGTCATTRTGGAAWTTCCATTTTCCYTACTGTTAATATCTTCCCTAG  360
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Sbjct981    CAGAAGGATTTGCAGTCATTGTGGAAATTCCATTTTCCCTACTGTTAATATCTTCCCTAG  1040

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Query421    CTTTTTTCGAGGACAAATTCTTACATTTAAATTATGTGTTAGAAATATTAATACCTTACC  480
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Query481    CCAT  484
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Sbjct1161   CCAT  1164
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Figure (3-32): Alignment of the DNA sequence of *Echinocactus grusonii* chloroplast *mat*K gene and partial tRNA-Lys intron, isolate V39_3 (Sequence ID: FN997554.1 Length: 2472 Number of Matches: 1)

GenBank: FN997554.1

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DEFINITION Echinocactus grusonii chloroplast matK gene and partial tRNA-Lys
            intron, isolate V39_3.
ACCESSION   FN997554 REGION: 658..1203
VERSION     FN997554.1
KEYWORDS    .
SOURCE      chloroplast Echinocactus grusonii
  ORGANISM Echinocactus grusonii
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
            Spermatophyta; Magnoliopsida; eudicotyledons; Gunneridae;
            Pentapetalae; Caryophyllales; Cactineae; Cactaceae; Cactoideae;
            Cacteae; Echinocactus.
REFERENCE   1
  AUTHORS   Barcenas,R.T., Yesson,C.J. and Hawkins,J.A.
  TITLE     Molecular systematics of the Cactaceae
  JOURNAL   Unpublished
REFERENCE   2 (bases 1 to 546)
  AUTHORS   Barcenas,R.T.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-JUN-2010) to the INSDC. Barcenas R.T., Darwin
            Laboratorium of Molecular Systematics and Evolution, Universidad
            Autonoma de Queretaro, Av. de la Ciencia s/n, 76230, MEXICO
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      241 tttgattctt tctgctaatc attctaataa aaatgacttt tttgggcaca agcacaagaa
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      421 gcgatcaatt cattcaatat ttccttttt cgaggacaaa ttcttacatt taaattatgt
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      541 ctgggt
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Figure (3-33): *Echinocactus grusonii* species registration document in the NCBI Genbank

Score	**Expect**	**Identities**	**Gaps**	**Strand**
939 bits(508)	0.0	508/508(100%)	0/ 508 (0%)	Plus/ Plus

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Query61    TAGAACTAGATAGGTCTTGGCAACACAACTTTTTATATCCACTTATCTTTCAGGAATATA  120
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Sbjct718   TAGAACTAGATAGGTCTTGGCAACACAACTTTTTATATCCACTTATCTTTCAGGAATATA  777

Query121   TTTATGGATTTGCATATGATCATGGTTTCAATAAATCGATTTTGTTGGAAAATGCAGGCG  180
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Sbjct778   TTTATGGATTTGCATATGATCATGGTTTCAATAAATCGATTTTGTTGGAAAATGCAGGCG  837

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Sbjct838   ACAAGAAATATAGTTTACTGATTGTAAAACGTTTAATTAACCGAATGTATCAACAGACTC  897

Query241   ATTTGATTCTTTCTCTTTCTGCTAATCATTCTAATCAAAATGACTTTTTTGGGCACAAGC  300
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Query301   ACAAGAAGAATTTGTATTATCAAATAATATCAGAAGGGTTTGCAGTCATTGTGGAAATTC  360
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Sbjct1078  ATAATTTGCGATCAATTCATTCAATCTTTCCTTTTTTCGAGGACAAATTCTTACATTTAA  1137

Query481   ATTATGTGTTAGAAATATTAATACCTTA  508
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Sbjct1138  ATTATGTGTTAGAAATATTAATACCTTA  1165
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Figure (3-34): Alignment of the DNA sequence of *Mammillaria elongata* chloroplast *mat*K gene and partial tRNA-Lys intron, isolate T1597 (Sequence ID: FN997474.1 Length: 1847 Number of Matches: 1)

GenBank: FN997474.1

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DEFINITION Mammillaria elongata chloroplast matK gene and partial tRNA-Lys
            intron, isolate T1597.
ACCESSION   FN997474 REGION: 655..1201
VERSION     FN997474.1
KEYWORDS    .
SOURCE      chloroplast Mammillaria elongata
  ORGANISM Mammillaria elongata
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            Spermatophyta; Magnoliopsida; eudicotyledons; Gunneridae;
            Pentapetalae; Caryophyllales; Cactineae; Cactaceae; Cactoideae;
            Cacteae; Mammillaria.
REFERENCE   1
  AUTHORS   Barcenas,R.T., Yesson,C.J. and Hawkins,J.A.
  TITLE     Molecular systematics of the Cactaceae
  JOURNAL   Unpublished
REFERENCE   2 (bases 1 to 547)
  AUTHORS   Barcenas,R.T.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-JUN-2010) to the INSDC. Barcenas R.T., Darwin
            Laboratorium of Molecular Systematics and Evolution, Universidad
            Autonoma de Queretaro, Av. de la Ciencia s/n, 76230, MEXICO
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                     /country="Mexico"
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                     /gene="tRNA-Lys"
     intron          <1..>547
                     /gene="tRNA-Lys"
                     /number=1
     gene            42..>547
                     /gene="matK"
     CDS             42..>547
                     /gene="matK"
                     /codon_start=1
                     /transl_table=11
                     /product="maturase K"
                     /protein_id="CBV65723.1"
                     /translation="MEEFKRYIELDRSWQHNFLYPLIFQEYIYGFAYDHGFNKSILLE
                     NAGDKKYSLLIVKRLINRMYQQTHLILSLSANHSNQNDFFGHKHKKNLYYQIISEGFA
                     VIVEIPFSLLLISSPEAEENQIVKSHNLRSIHSIFPFFEDKFLHLNYVLEILIPYPIH
                     LEILVQTLRYWVKDASSLHLLRFFLYEYRNWNSLITPIKESTIILVPL"
ORIGIN
        1 agaaaccccc tgcttttttt tagttttcgg tctaatttga aatggaagaa ttcaaaagat
       61 atatagaact agataggtct tggcaacaca actttttata tccacttatc tttcaggaat
      121 atatttatgg atttgcatat gatcatggtt tcaataaatc gattttgttg gaaaatgcag
      181 gcgacaagaa atatagttta ctgattgtaa aacgtttaat taaccgaatg tatcaacaga
      241 ctcatttgat tctttctctt tctgctaatc attctaatca aaatgacttt tttgggcaca
      301 agcacaagaa gaatttgtat tatcaaataa tatcagaagg gtttgcagtc attgtggaaa
      361 ttccattttc cctactgtta atatcttccc cggaggcaga agaaaatcaa atagtaaaat
      421 cccataattt gcgatcaatt cattcaatct ttccttttt cgaggacaaa ttcttacatt
      481 taaattatgt gttagaaata ttaatacctt accccattca tctggaaatc ttggttcaaa
      541 ctcttcg
```

Figure (3-35): *Mammillaria elongate* species registration document in the NCBI Genbank.

In the current study, the *mat*K gene was used as a barcode to distinguish between several species of *Opuntia*, *Hylocereus*, *Aloe vera*, *Espostoa*, *Echinocactus*, and *Mammillaria*. These findings provide additional data to the existing body of knowledge about the sequencing analysis of these species. The results appears that no previous research has been conducted on these species of interest, with the exception of a study by(Nyffel, 2002) that used the *mat*K gene sequence for many plants belonging to the Cactoideae, and to which *trnL-trnF* sequences from members of these clades were added for a combined analysis.

Many plant molecular taxonomies use the *mat*K gene as a barcode region (Kim *et al*., 2014; Xu *et al*., 2015; Rajaram *et al*., 2019). Although DNA barcoding has gained in popularity since(Kress *et al*., 2005), and many other genes are considered molecular tools in plant molecular taxonomy (Zhu *et al*., 2018), using the ITS region in plants can yield questionable results due to the region's historical use as a barcoding marker for fungi. Fungal DNA may be present in the extracted DNA of the target plants if these fungi have attacked the plants as diseases, symbionts, saprophytes, or inhabitants of stomata. The sequencing data also started to be meaningless.

The findings of this analysis, in which certain genera were classified as distant genera based on sequencing data while others showed connections by far the most varied clade of Cactaceae, are what the research is primarily concerned with. This molecular study has found results that are at odds with more conventional wisdom. This finding further supports the use of the *mat*K gene as a genetic barcode. Multiple previously confusing species names have been clarified thanks to the current genetic investigation. A novel sequencing analysis of the five species in the genus Cactoideae in Iraq is also presented in this work for the first time. Several new issues in cactus systematics were also uncovered by the current investigation

Conclusion and Recommendation

Conclusions

1- The results of the study of the chemical content in plant extracts belonging to the cactus family by gas chromatography technique GC-MS showed great importance in differentiation between species, and the studied species participated in some compounds, which led to an increase in the kinship between them.

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2- The chemical study proved that there are four compounds common in all the studied species as Oxime-, methoxy-phenyl-; Cyclotetrasiloxane, octamethyl; 1H-Trindene, 2,3,4,5,6,7,8,9-octahydro-1,1,4,4,9,9-hexamethyl and Oleic Acid

3- A comprehensive study of the phylogenetic connections among various species of the family Cactaceae by molecular phylogenetic investigations such as RAPD-PCR methodologies.

4- The RAPD technique was an authenticated way to figure out how genetically close and far apart plants are from different places, species, and genera of the family Cactaceae. By comparing the RAPD data with the botanical data, accurate conclusions can be made. The dendrogram made by the computer processing of the RAPD data explained how some populations of cacti were found related phylogenetically and how genetically different from this family as a whole.

5- Genotyping-based RAPD-PCR explained that *Aloe vera* is a Asphodelaceae liliaceae family, not a Cactaceae.

6- This study supports the use of the *mat*K gene as a genetic barcode better than the *rbc*L gene.

Recommendations

1. Because of the large number of species belonging to the Cactus family in Iraq, we need many studies to diagnose their species and provide the flora of Iraq with the most important diagnostic characteristics.
2. Given the large number of active compounds in the studied species, it was suggested to form nanoparticles from plant extracts and compare them with antibiotics and test them against pathogenic microorganisms.
3. Measurement of antioxidants in plant extracts of the studied species
4. Reliance on the chemical and molecular study in diagnosing plant species as supporting phenotypic traits
5. Researchers are urged to resume their work on the comparative sequencing of members of this family.

6.Used HPCL for identified some secondary metabolics as phenols,alkaloids, glycoside& teripens etc. .

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الخلاصة

تتكون عائلة الصبارية (الصبار) من لبعض من الأجناس، ولها اراء تصنيفية على مستوى الأنواع، ولم يتم دراستها سابقًا على المستوى الكيميائي والجيني في العراق كصفات تشخيصية. في هذه الدراسة، تم إيلاء الاهتمام لأنواع المركبات الكيميائية المتواجدة باستخدام طريقة اللوني للغاز – مقياس الطيف الكتلي (GC–MS) والتنميط الجيني على أساس التضخيم العشوائي للحمض النووي متعدد الأشكال (RAPD) وطرق التسلسل الجيني للقواعد النيتروجينية لستة أنواع مختلفة، وهي *Hylocereus undatus* (Haw.) Britton & Rose، *Aloe vera* (L.) Burm.f.، *Opuntia ficus-indica* (L.) Mill.، *Espostoa guentheri* (Kupper) Buxb.، *Echinocactus grusonii* Hildm ، و*Mammillaria elongate* DC ، والتي صنفت بناءً على الصفات المظهرية لتحديد أسمائها التصنيفية الدقيقة. تم جمع العينات من مشاتل مختلفة وكذلك من حديقة المنزل، واستمر العمل في مختبرات كلية العلوم للبنات، جامعة بابل، من سبتمبر (2022) إلى مارس (2023).

تناولت الدراسة الكيميائية دراسة المحتوى الفعال لمستخلصات أنواع النباتات المدروسة، حيث تم تشخيص المركبات بتقنية GC-MS، ومن خلال مقارنة المحتوى الكيميائي وجد أن جميع الأنواع المدروسة تحتوي على 15 مركبًا. في كل نوع، ولكن يتشاركون معًا في أربعة مركبات نشطة وهي Oxime-, methoxy-phenyl- و Cyclotetrasiloxane, octamethyl و1H-Trindene, 2,3,4,5,6,7,8,9-octahydro-1,1,4,4,9,9-hexamethyl, وOleic acid، وفقًا للمراجع العلمية المتاحة له نشاط مضاد للجراثيم، مضاد للفطريات، مضاد للفيروسات ، مضاد للميكروبات، مطهر، مكيف للشعر، مرطب للبشرة وكذلك له قيمة مضادة للأكسدة.

أما بالنسبة للدراسة الجزيئية، فقد تم إجراء تحليل الحمض النووي باستخدام أحد مؤشرات الحمض النووي على أساس تفاعلات البلمرة المتسلسلة (PCR)، وهي مؤشرات لتفاعلات سلسلة البلمرة. لتنفيذ تفاعلات PCR، تم عزل DNA الجينوم من المستخلصات الطازجة للأنواع المدروسة، حيث كان أعلى تركيز للحمض النووي في *Mammillaria elongate*، والذي بلغ 8.8 نانوغرام / ميكرولتر وأقل تركيز للحمض النووي حسب الأنواع وجد في النوع *Aloe vera*، والذي بلغ 5.1 نانوغرام / ميكرولتر.م

أظهرت البادئات القصيرة أعلى نطاقات متعددة الأشكال (100٪)، ولا يوجد ارتباط أحادي الشكل بين هذه الأنواع. تشير هذه العلاقة متعددة الأشكال إلى أن كل نوع له هوية خاصة واتجاه تطوري فريد. اعتبرت البادئات منتجة من خلال إظهار فرق حادة ومتميزة للغاية، بينما أظهر البعض الآخر فرقًا باهتة. أكدت نتائج هذا البحث فاعلية البادئات RAPD في قياس تعدد الأشكال ومقارنة الأنماط الجينية وتحديد أنواع الصبار باستخدام علامات RAPD المتخصصة وتقييم القرابة من خلال بناء شجرة التطورية .RAPD-PCR

كما تم تأكيد الدراسة الجزيئية من خلال دراسة التسلسل الجيني للجينات *rbcl* و *Matk* بعد إرسال منتجات التضخيم إلى الشركة الكورية ومن ثم مقارنتها بأرقام التعريف العالمية المسجلة في بنك الجينات (NCBI).

كدت نتائج هذه الدراسة أن جينات *rbcl* (عند 550 زوجًا أساسيًا) وجينات *matk* (عند 600 زوج أساسي) كانت مناسبة بشكل خاص للجينات المستهدفة للتعريف الجزيئي، واستخدمت الاختلافات في بنية النيوكليوتيدات لجينات *rbcl* و *matk* بنجاح للكشف والتشخيص الأنواع في هذه الدراسة.

جمهوريه العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل /كلية العلوم للبنات قسم علوم الحياة

دراسة كيميائية وجزيئية لبعض الاجناس من العائلة الصبارية

رسالة مقدمة الى

مجلس كلية العلوم للبنات، جامعة بابل

وهي جزء من متطلبات نيل درجة ماجستير علوم/ في علوم الحياة

من قبل

لارا عامر نعمة

(بكالوريوس علوم الحياة/ جامعة بابل، 2020)

يإشراف

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1444هـ 2023م