République Algérienne Démocratique et Populaire
Ministère de l'Enseignement Supérieur et de la Recherche Scientifique
Université A.MIRA-BEJAIA

Faculté : Sciences de la Nature et de la Vie
Département Sciences Alimentaires
Laboratoire ou unité de recherche de rattachement : Laboratoire de biomathématiques, biophysique biochimie et Scientométrie (3BS)

THÈSE

EN VUE DE L'OBTENTION DU DIPLOME DE DOCTORAT

Domaine : Sciences de la Nature et de la Vie Filière : Sciences Alimentaires
Spécialité : Qualité des Produits et Sécurité Alimentaire

Présentée par
TAIBI Abdeslem

Thème

Valorisation des fruits de myrte (*Myrtus communis* L.) : exploration du potentiel bioactif et caractérisation phénolique en vue de développer un aliment fonctionnel

Soutenue le : 14/10/2025 **Devant le Jury composé de :**

Nom et Prénom	Grade		
Mr KATI Djamel Eddine	**Prof.**	Univ. de Bejaia	Président
Mr MOKRANI Abderrahmane	**Prof.**	Univ. de Bejaia	Rapporteur
Mme BRAHMI Fatiha	**Prof.**	Univ. de Bejaia	Examinateur
Mr KADRI Nabil	**Prof.**	Univ. de Bouira	Examinateur

Année Universitaire : **2025/2026**

Acknowledgments

Acknowledgments

First and foremost, I express my deepest gratitude to Allah, the Almighty, for granting me the strength, patience, perseverance, and guidance to undertake and complete this research work.

I would like to express my sincere appreciation to my thesis supervisor, Pr. MOKRANI Abderrahmane, for his constant support, insightful guidance, and invaluable scientific advice throughout this research. His mentorship played a crucial role in the successful completion of this work, and I am truly grateful for his patience and encouragement.

I extend my heartfelt thanks to Professor Kati Djamel-Eddine, President of the jury, as well as to the distinguished jury members, Professor Brahmi Fatiha and Professor Kadri Nabil, for accepting to evaluate this thesis and for their valuable time, feedback, and recommendations.

I also wish to express my special appreciation to Professor Lila Boulekbache-Makhlouf, Director of the laboratory, for providing the research facilities and a stimulating scientific environment.

My sincere thanks and deep gratitude go to Mrs Fatiha Hamitri-Guerfi for her generous help, valuable advice, and continuous support throughout this work. Her guidance and practical tips were of great importance for the successful completion of this thesis.

I gratefully acknowledge all members of the 3BS team—technicians, engineers, and doctoral students—for their collaboration, technical assistance, and the positive atmosphere that made my work experience enjoyable. I am also particularly thankful to the engineer of the Applied Biochemistry Laboratory, Faculty of Natural and Life Sciences, University of Bejaia, for her kind help and support.

To my beloved parents, no words can truly express my gratitude. Your sacrifices, unconditional love, and constant prayers have been the foundation of this accomplishment. May Allah reward you abundantly.

To my loving wife, thank you for your infinite patience, understanding, and unconditional support. Your belief in me and your presence by my side have been essential throughout this path.

To my wonderful children, Sohaib Anes and Asma, your smiles and love gave me the energy and motivation to keep moving forward every day.

To my dear siblings, Saida, Madjid, Fateh, Nabila, Nadia, Rebiha, Farid, Hicham, Sabrina and Houda, thank you for your unwavering support and encouragement. Your presence in my life has been a source of strength and comfort throughout this journey.

Acknowledgments

To my extended family, both near and far, I sincerely thank each one of you for your moral support and encouragement.

To my friends and colleagues, especially Saleh, Ahcene, Mohand, Younes, Lamine and Mounir, thank you for your camaraderie, encouragement, and for making this research journey both memorable and meaningful.

Finally, I express my sincere appreciation to all those who, in one way or another—directly or indirectly—contributed to the realization of this work. May Allah guide us all toward success in our future endeavors.
Abdeslem TAIBI

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Journal: CHEMISTRY & BIODIVERSITY

Date: November 2024

DOI : https://doi.org/10.1002/cbdv.202301675

Scientific article (2): Impact of Thermal and Alternative Processing on Phytochemical Quality and Antioxidant Activity of Algerian *Myrtus communis* L. Fruit

Authors: Abdeslem TAIBI, Abderrahmane MOKRANI, Fatiha HAMITRI-GUERFI, Ahcene KADI, Mohand TEFFANE, Younes ARROUL, Widad SOBHI, Lila BOULEKBACHE-MAKHLOUF and Khodir MADANI.

Journal: Food and Feed Research

Date: June 2025

DOI: https://doi.org/10.5937/ffr0-57499

Communications

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1-National Seminar on Toxicology and Phyto-Aromatherapy, University of Relizane, Department of Biology / Faculty of Science and Technology, in partnership with the Research Laboratory for Environment and Sustainable Development of the University of Relizane, and with the participation of ATRSSV

Communication title: Myrtle (*Myrtus communis*) fruit powder as a Functional Ingredient for Obtaining Value-Added products.

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2- The 5th Conference on Natural and Life Sciences. University of Bejaia/Faculty of Natural and Life Sciences (December 6–7, 2022).

Communication title: Valorization of Myrtle (*Myrtus communis* L.) Fruits Cultivated in the Bejaia Region for the Development of Novel Products and Functional Foods.

Authors: TAIBI A., Mokrani A., Kadi A., Teffane M., Arroul Y.

3-The 7th Conference on Natural and Life Sciences. University of Bejaia/Faculty of Natural and Life Sciences (December 10–11, 2024).

Communication title: Development of a new analytical extraction method for Phenolic Compound Recovery and Antioxidant Potency Bioactive Compounds in Algerian *Myrtus communis* L. Fruits.

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1-The International Congress of Food Sciences: Sustainable Food Security: Innovation and Challenges. University of Constantine 1/ INATAA (October 16-17, 2024)

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Authors: TAIBI Abdeslem**,** MOKRANI Abderrahmane, HAMITRI-GUERFI Fatiha, KADI Ahcene, TEFFANE Mohand and ARROUL Younes.

2-The 3rd International Korkut Ata Scientific Research Conference. Osmaniye Korkut Ata University, Institute of Economic Development and Social Research, Turkey (November 22-24, 2024).

Communication title: Valorization of Algerian *Myrtus communis* L. fruits through their incorporation into a value-added Functional Food Product.

Authors: TAIBI Abdeslem**,** MOKRANI Abderrahmane, HAMITRI-GUERFI Fatiha, KADI Ahcene, TEFFANE Mohand and ARROUL Younes.

3-The 3rd International Conference on Contemporary Academic Research ICCAR 2024 at Konya/Turkey (November 10 – 11 in 2024).

Communication title: Optimization of Conventional Extraction Techniques for Phenolic Compound Recovery and Antioxidant Potency in Algerian *Myrtus communis* L. Fruits: A Valorization Approach for Functional Food Ingredient Development.

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Ahcene, TEFFANE Mohand and ARROUL Younes.

List of Abbreviations

List of abbreviations

AAE: Ascorbic Acid Equivalent
ABTS: 2.2 ′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid diammonium salt
AC: Anthocyanin content
ANOVA: Analyse Of Varience
ATCC: American Type Culture Collection
BC: Before Christ
BD: Before digestion
BHA: Butylated Hydroxyanisole
BHT: Butylated Hydroxytoluene
BSA: Bovine serum albumin.
CE: Catechin equivalents
CFU: Colony Forming Units
CGE: Cyanidin-3-glucoside equivalents
CM: Conventional Methods
CUPRAC: Cupric reducing antioxidant capacity
CV: Coefficient of Variation
D: Digested
DE: Dry extract
DF: Degree of Freedom
DM: Dry Matter
DMSO: Dimethyl sulfoxide.
DNA: Deoxyribonucleic Acid
DNS: Dinitro-Salicylic acid
DOI: Digital Object Identifier
DPPH: 2,2-diphenyl-1-picrylhydrazyl
DW: Dry Weight
DSMZ: Deutsche Sammlung von Mikroorganismen und Zellkulturen **(**German Collection of Microorganisms and Cell Cultures)
EAE: Enzyme-Assisted Extraction
EDTA: Ethylene-Diamine Tetra-Acetic acid
EU: European Union
FAO: Food and Agriculture Organization
FD: Freeze Drying
FIC: Ferrous Ion Chelating
FRAP: Ferric ion Reducing Antioxidant Power
FRP: Ferric Reducing Power
GAE: Gallic acid equivalent
GD: After Gastric Digestion
h: hour
HAA: Hydrogen Atom Abstraction
HCl: Hydro-Chloric acid
IC50: Half-maximal Inhibitory Concentration

List of Abbreviations

ID: After Intestinal Digestion
IFBDO: International Federation of Blood Donor Organizations
IP: Inhibition Percentage
LDL: Low Density Lipoproteins
LLE: Liquid-Liquid Extraction
LPSE: Liquid-Phase Separation Extraction
MAE: Microwave-Assisted Extraction
mBar: Millibar
ME: Maceration Extraction
MEF: Moderate Electric Field
MHz: Megahertz
MRSA: Methicillin-Resistant Staphylococcus aureus
MWD: Microwave Drying
NADPH: Nicotinamide Adenine Dinucleotide Phosphate Hydrogen
NO: Nitric oxide scavenging activity
OD: Optical Density
OD: After Oral Digestion
OD: Oven Drying
ORAC: Oxygen Radical Absorbance Capacity
PAA: Phosphomolybdenum antioxidant activity
PAC: Proanthocyanidins
PEFAE: Pulsed Electric Field Assisted Extraction
pH: Potential of Hydrogen
PLE: Pressurized Liquid Extraction
QE: Quercetin equivalent
RBCs: Red Blood Cells
RE: Rutin equivalent
RNS: Reactive Nitrogen Species
ROS: Reactive Oxygen Species
RSM: Response Surface Methodology
SCFE: Super Critical Fluid Extraction
SD: Sun Drying
SE: Soxhlet Extraction
SED: Single Electron Donation
SET: Single Electron Transfer
SGF: Simulated Gastric Fluid
SIF: Simulated Intestinal Fluid
SLE: Solid-Liquid Extraction
SSF: Simulated Salivary Fluid
SWE: Subcritical Water Extraction
TAA: Total Antioxidant Activity
TAC: Total Antioxidant Capacity
TBARS: Thio-Barbituric Acid Reactive Substances
TEAC: Trolox Equivalence Antioxidant Capacity

List of Abbreviations

TSS: Total Soluble Solid
UAE: Ultrasound-Assisted Extraction
UD: Undigested
UV: Ultraviolet
UV-VIS: Ultraviolet Visible
WHO: World Health Organization
CTC: Total condensed tannins content
AC: Total anthocyanins content

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GENERAL INTRODUCTION

GENERAL INTRODUCTION

In recent years, there has been growing interest in food plants and their bioactive compounds due to increasing consumer awareness of the role of dietary components in promoting health and preventing disease. Oxidative stress, characterized by an imbalance between free radicals and antioxidant defenses, has been implicated in the development of chronic diseases such as cardiovascular disease, diabetes, cancer, and neurodegenerative disorders (Aleksandrova et al., 2021). Numerous epidemiological and clinical studies have demonstrated that regular consumption of fruits and vegetables reduces the risk of these oxidative stress-related diseases, largely due to their high content of antioxidant compounds such as polyphenols, flavonoids, and other secondary metabolites (Arcusa et al., 2021; Devirgiliis et al., 2024; Gariballa et al., 2023; Myhrstad & Wolk, 2023). Polyphenols, in particular, are recognized for their strong antioxidant properties, which stem from their ability to scavenge free radicals, chelate pro-oxidant metal ions, and act as cofactors for antioxidant enzymes (Cherrak et al., 2016; Hussen & Endalew, 2023). Consequently, the search for natural antioxidants and functional ingredients from plant sources has intensified, offering promising alternatives to synthetic antioxidants (Samtiya et al., 2021).

Among the various medicinal and aromatic plants of the Mediterranean basin, Myrtle (*Myrtus communis* L.), as one of the most frequently cited medicinal plants in ancient books on traditional medicine, is an evergreen shrub widely growing in the Mediterranean area but also in America, Australia, and the Himalayas (Gorjian & Khaligh, 2023; Mahboubi, 2017). In Algeria, this plant is called Rihan or Mersin and grows wild in the coastal Tell Atlas region (Quézel & Santa, 1962).

Pharmacological and clinical studies have shown that *Myrtus communis* possesses a wide range of biological activities, including anti-inflammatory, antimicrobial, antioxidant, antidiabetic, anticancer, dermatological, cardiovascular, neuroprotective, and gastrointestinal protective effects (Aykac et al., 2019; Azimi & Hasheminasab, 2020; Bagatin et al., 2023; Talebianpoor et al., 2019).

Myrtle fruits have recently attracted considerable attention as a promising natural resource for

food, pharmaceutical, and agrochemical applications (Aggul et al., 2022; Bouaoudia-Madi et al., 2022; Kordali et al., 2016). This interest stems from their high antioxidant potential and rich phenolic composition (Hacıseferoğulları et al., 2012; Tuberoso et al., 2006). In particular, black myrtle berries are recognized as an excellent source of diverse phenolic compounds (Messaoud & Boussaid, 2011), mainly flavonoids (Viuda-Martos et al., 2011) and anthocyanins (Montoro, Tuberoso, Perrone, et al., 2006; Scorrano et al., 2017a). They also contain other bioactive constituents, including tannins (Medda et al., 2021), essential oils (Bouzabata et al., 2015; Chalchat et al., 1998; Gardeli et al., 2008; Mhamdi & Marzouk, 2007), and fatty acids (Fabio Correddu et al., 2019; Jabri et al., 2017).

However, since *Myrtus communis* is a seasonal fruit, appropriate drying methods are required to preserve its antioxidant properties for year-round use (Dinçer et al., 2022). Drying not only improves the shelf life but can also concentrate phenolic compounds, thereby increasing the functional value of the product (Bassey et al., 2024). Additionally, the efficiency of polyphenol extraction depends on several factors, including solvent type, concentration, pH, temperature, and extraction time (Ćujić et al., 2016; Dent et al., 2013).

In this context, incorporating myrtle berry powder into food formulations represents a promising strategy for developing value-added functional products. Due to their high content of polyphenols, flavonoids, and other bioactives, berries are widely used in the Mediterranean diet as functional foods with anti-inflammatory, antioxidant, and antimicrobial benefits (Battino et al., 2019).

Despite the growing body of research on *Myrtus communis*, further investigation is needed to better understand how processing techniques, such as drying and extraction parameters, influence the retention and bioavailability of its valuable phytochemicals. Careful selection and optimization of these processes are crucial, as they determine the efficacy and stability of bioactive compounds in the final product. Moreover, integrating myrtle berry powder into food systems offers a promising avenue for developing functional products that align with current consumer preferences for natural, health-enhancing ingredients.

Within this context, the objectives of this thesis work were as follows:

(i) To investigate the effect of different drying methods on the phytochemical content and antioxidant activity of myrtle fruit powder, to identify the most effective method for preserving the maximum amount of bioactive compounds in the dried product;

(ii) To optimize the extraction of phenolic compounds (total phenolic content, total flavonoid content, and total proanthocyanidin content) as well as the antioxidant activity from myrtle fruits under varying conditions of solvent type, solvent concentration, solvent acidity, temperature, and time;

(iii) To characterize the phenolic composition of myrtle berry optimized extracts using UHPLC;

(iv) To evaluate their antioxidant activity through various in vitro assays, including DPPH radical scavenging activity (DPPH-RSA), ABTS radical scavenging activity (ABTS-RSA), ferric reducing power (FRP), phosphomolybdenum antioxidant capacity (PAA), cupric reducing antioxidant capacity (CUPRAC), ferrous ion chelating activity (FIC), β-carotene bleaching, and nitric oxide scavenging activity (NO); to assess their antidiabetic, anti-inflammatory, and hemolytic activities; and to investigate the stability and bioaccessibility of phenolic compounds through in vitro digestion.

(v) Finally, to explore the potential application of myrtle fruit powder as a functional ingredient in the formulation of enriched mayonnaise.

BIBLIOGRAPHICAL PART

Chapter I
Overview of the Myrtus communis plant

Chapter I: Overview of the *Myrtus communis* plant

1. History of *Myrtus communis*

Medicinal plants are currently of considerable importance due to their particular attributes as an important source of therapeutic phytochemicals that can lead to the development of new drugs. Most phytochemical compounds of plant origin, including phenols and flavonoids, are believed to promote health and contribute to cancer prevention (Venugopal & Liu, 2012). Among the diverse array of medicinal plants, *Myrtus communis* L., commonly known as common myrtle, is an evergreen shrub of the Myrtaceae family, which encompasses approximately 5,500 species classified into 144 genera and 17 tribes (Migliore et al., 2012). *Myrtus communis* is endemic to the Mediterranean basin, a biogeographical region that includes Southern Europe, North Africa, and Western Asia, where the species is widely distributed and valued for its ecological, medicinal, and cultural significance (Sumbul et al., 2012). Before the widespread introduction of pepper, *Myrtus communis* was commonly used as a culinary spice. The plant also held a prominent role in ancient medicinal literature, being cited by renowned figures such as Hippocrates (Greek doctor, circa 377 BC), Dioscorides and Pliny (Roman doctors of the 1st century AD), Galen, and Avicenna. Galen highlighted the notable *astringent* properties of the leaves, stems, fruits, and juice of myrtle. Pliny described the therapeutic application of myrtle berries in treating dysentery, indolent ulcers, and ocular inflammations. In the *Canon of Medicine*, Avicenna recommended the use of myrtle for managing abnormal uterine bleeding. Additionally, the fruits, whether fresh or dried, were valued for their diuretic properties and efficacy in treating hemoptysis and cystitis, while the seeds were considered tonics for the intestines and urinary bladder, and effective in combating halitosis (Akbar, 2020; Gryc, 1985).

All parts of the plant (leaves, fruits, flowers, and roots) have been used in traditional medicine for their diverse therapeutic properties (Farah et al., 2006).). It is particularly renowned for its anti-hyperglycemic (Sepici-Dincel et al., 2007), analgesic (Twaij et al., 1989), antigenotoxic (Hayder et al., 2004) and antibacterial (Bonjar, 2004) activities**.** Additionally, myrtle is highly aromatic due to its richness in essential oils, particularly 1,8-cineole, myrtenyl acetate, and linalool, found in its leaves, flowers, and fruit glands (Aidi Wannes et al., 2009). These

properties have made it a valuable ingredient in the food, cosmetic, and pharmaceutical industries (Chalchat et al., 1998; Matsehorova & Odyntsova, 2024; Nedjimi, 2024).

In Algeria, *M. communis* is widely distributed in northern regions and known locally as "Al-Rihan" or "El-Halmouche" in other areas. It is traditionally used to manage blood sugar levels, treat digestive and respiratory ailments, and promote general well-being (Berka-Zougali et al., 2012). Algeria is distinguished by its phytogeographical richness, being the only country known to host both *Myrtus communis* in its northern Mediterranean regions and *Myrtus nivellei* in the arid southern zones. This coexistence reflects the country's diverse ecological and climatic landscapes (Bouzabata et al., 2016). Due to its richness in bioactive phytochemical compounds with demonstrated health benefits, *Myrtus communis* has attracted considerable scientific attention. Numerous studies have been conducted to elucidate its biological activities and to substantiate its therapeutic potential (Abdessemed et al., 2024; Hennia et al., 2019; Romani et al., 2004). However, the increasing demand has surpassed the capacity of wild populations, making domestication essential for sustainable industrial development (Mulas et al., 1997). Consequently, various efforts have been undertaken to domesticate this valuable source of bioactive compounds in order to ensure its sustainable use and maximize its health-related benefits (Dessena et al., 2015; Medda & Mulas, 2021; Mulas & Cani, 1999).

2. Botanical study of *Myrtus communis*

2.1. Overview of the Myrtaceae Family

Myrtaceae is an ecologically and economically important family of flowering plants (angiosperms), comprising trees and shrubs. The family derives its name from the genus *Myrtus*, which includes *Myrtus communis*, a shrub native to the Mediterranean region of North Africa, Southern Europe, and parts of the Middle East. Estimates suggest that the family contains approximately 140 to 144 genera and between 3,800 to 5,650 species, classified into 17 tribes. The tribe *Myrteae* alone accounts for nearly half of the family's biodiversity, with 51 genera and about 2,500 species, primarily concentrated in the Neotropics, though around 450 species are found in other continents (Migliore et al., 2012; Mitra et al., 2012). The Myrtaceae family is classified within the following clades: Angiosperms, Eudicots, Rosids, Malvids, and

finally the order Myrtales, according to the APG III system (Group, 2009).

2.2. Systematic classification

According to the traditional Linnaean taxonomy as described by Crété (1965), the Myrtaceae family, which includes the species *Myrtus communis*, is defined based on morphological traits from a botanical perspective, following the divisions outlined in Table 1 below:

Table 1. Traditional botanical classification of *Myrtus communis* L. (Crété, 1965)

Taxonomic Rank	Classification
Kingdom	Plantae
Subkingdom	Eukaryota
Division	Spermatophyta
Subdivision	Angiospermae
Class	Dicotyledonae
Order	Myrtales
Family	Myrtaceae
Subfamily	Myrtoideae
Genus	*Myrtus*
Species	*Myrtus communis* L.

In modern phylogenetic classification of Angiosperm Phylogeny Group IV (Group et al., 2016), the Myrtaceae family, which includes *Myrtus communis*, is placed within the order Myrtales, under the clades Malvids, Rosids, and Eudicots, within the Angiosperms. This phylogenetic classification reflects evolutionary relationships based on molecular data as described in the Table 2 below:

Table 2. Botanical divisions of Myrtaceae (Group et al., 2016)

Taxonomic Rank / Clade	Classification
Domain	Eukaryota
Kingdom	Plantae
Clade	Angiosperms
Clade	Eudicots
Clade	Rosids
Clade	Malvids
Order	Myrtales
Family	Myrtaceae
Subfamily	Myrtoideae
Genus	*Myrtus*
Species	*Myrtus communis* L.

2.3. Botanical description of the plant

Common myrtle (*Myrtus communis*) is an evergreen phanerophyte that can live for over 300 years (Rameau et al., 2008). The plant is characterized by reddish, highly branched stems (Figure 1). Its leaves are opposite, closely spaced, oval-shaped with acute tips, entire, evergreen, glossy, and bright green. Flowering begins in summer, producing solitary, highly fragrant flowers that are white or occasionally pink-spotted, with a diameter of approximately 3 cm. These flowers emerge singly from the leaf axils and are borne on long peduncles. The fruit of *Myrtus communis* is an oval, blue-black berry that reaches full ripeness in November and is known for its bitter and resinous flavor (Giuliani et al., 2022). The seeds exhibit variation in

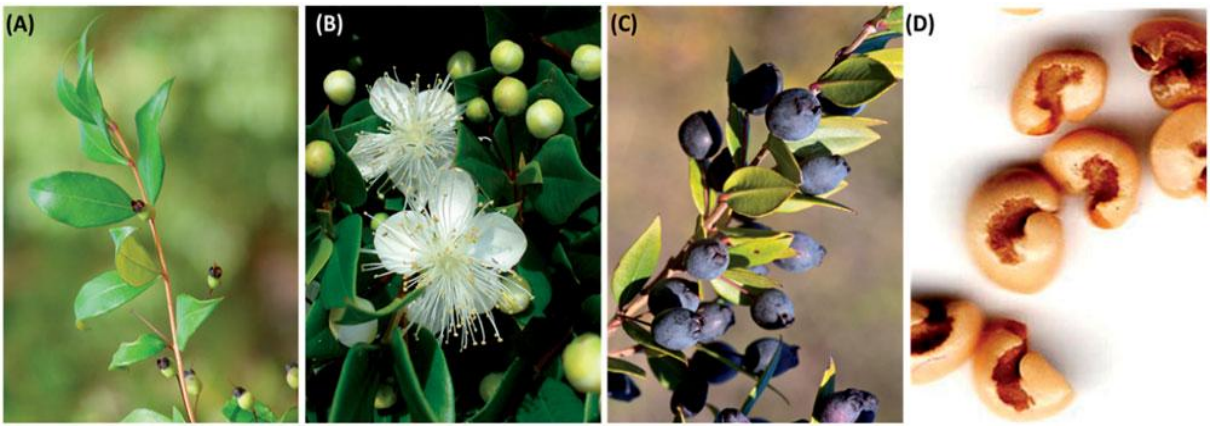

Figure 1. Botanical characteristics of *Myrtus communis*: (A) leaves; (B) young flowers and immature fruits; (C) mature fruits; and (D) seeds (Jabri et al., 2018a).

size and shape, typically appearing curved or snail-shaped, with a thick seed coat and a central elaiosome (Ciccarelli et al., 2005). In addition to *Myrtus communis* L., the *Myrtus* genus includes another species, *Myrtus nivellei*, which exhibits key botanical differences compared to *Myrtus communis* (Bouzabata et al., 2016).

2.3.1. Difference between *Myrtus communis* and *Myrtus nivellei*

Both *Myrtus communis* (common myrtle) and *Myrtus nivellei* (Saharan myrtle) are evergreen shrubs characterized by rough bark, opposite leaves, star-shaped white flowers (typically with 5 to 9 petals), and berries that range in color from white and purple to blue or black. However, they differ in several key morphological traits. The leaves of *M. nivellei* are linear-lanceolate, measuring 4–5 cm in length and 6–8 mm in width, making them narrower than those of *M. communis*, which are oval-lanceolate, 2–5 cm long, and 10–20 mm wide. The fruits of *M. communis* are variable in shape (ellipsoidal, subglobose, pyriform, elongated, or flattened), typically 7–9 mm long, whereas the fruits of *M. nivellei* are smaller (4–5 mm) and globose in shape. In terms of height, *M. communis* generally grows between 0.5 and 3 meters, while *M. nivellei* reaches a height of 1 to 2 meters (Alipour et al., 2014; Melito et al., 2016; Migliore et al., 2012).

3. Geographical location and distribution

3.1. In the world

- **Common Myrtle**

Common myrtle (*Myrtus communis*) is a Mediterranean plant that grows in various regions, including Macaronesia (Madeira and the Azores), as well as parts of Iran and Afghanistan, at altitudes of up to 500 meters above sea level. The Myrtaceae family, to which *Myrtus communis* belongs, comprises over 15 genera and approximately 450 species, which can be found across regions such as Southeast Asia, northeastern Australia, the Pacific Islands, New Caledonia, and New Zealand (Migliore et al., 2012; Vasconcelos et al., 2017).

Common myrtle (*Myrtus communis*) thrives in subhumid to perhumid climates, typically growing on siliceous and calcareous substrates, and prefers warm to temperate conditions. It is the only species of the *Myrtus* genus naturally occurring in the wild in the Mediterranean region, although the Myrtaceae family includes many other wild species globally (Wahid, 2013). Myrtle is commonly found at altitudes ranging from 500 to 600 meters above sea level, particularly in pine forests and along the banks of the Taurus Mountains in Turkey (Aydın & Özcan, 2007).

- **Level Myrtle**

Saharan myrtle (*Myrtus nivellei*) grows in the mountainous regions south of the Sahara, thriving in rocky and sandy gorges at high altitudes, typically above 1400 meters (Migliore et al., 2012).

3.2. In Algeria

Common myrtle (*Myrtus communis*) is found in the Tellian Atlas and the coastal regions of Algiers and Constantine (Quézel & Santa, 1962), as well as in coastal scrublands and forests (Kaddem, 1990).

Saharan myrtle (*Myrtus nivellei*) is native to the desert regions of Algeria, primarily in the Hoggar and Tassili mountain ranges (Tassili N'Ajjer, Tassili N'Immidir, and Tefedest), as well as in Chad (Tibesti), at altitudes approximately 1000 km from the Mediterranean coast (Migliore, 2011). Figure 2 above illustrates the global and Algerian distribution of both species.

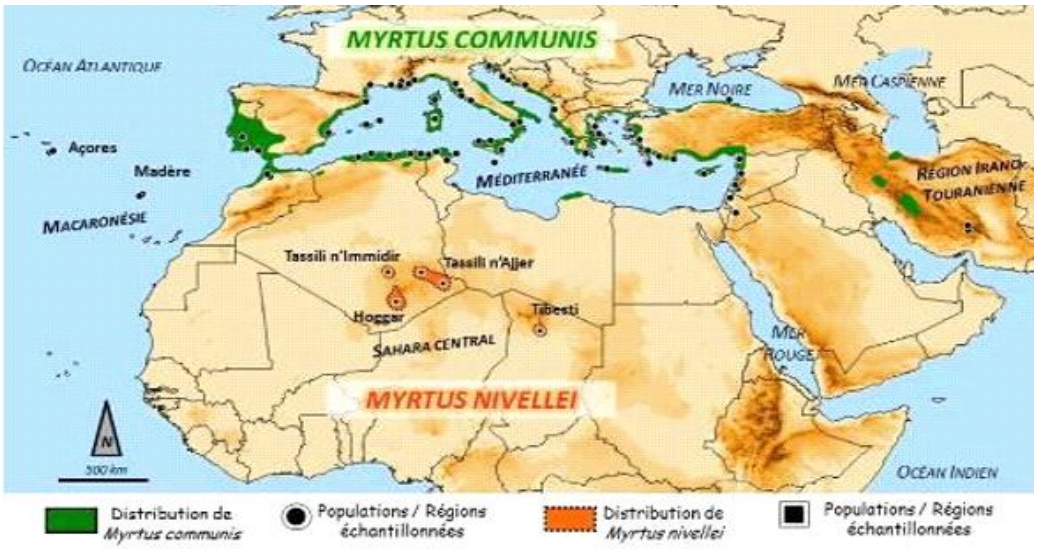

Figure 2. Global distribution of the *Myrtus* genus and its presence in Algeria (Migliore, 2011)

4. Biochemical composition of *Myrtus communis*

Myrtus communis is an aromatic and medicinal plant recognized as a natural source of antioxidants. It is rich in secondary metabolites, including phenolic compounds and essential oils (Amensour et al., 2009; Messaoud & Boussaid, 2011).

Regarding its chemical composition, previous studies have identified a wide range of bioactive compounds in different parts of the plant. The leaves, in particular, are known to contain essential oils, tannins, phenolic acids, and flavonoids such as quercetin, catechin, and myricetin derivatives (Romani et al., 1999; Yarahmadi et al., 2024). Wannes, Mhamdi, Sriti, Jemia, et al. (2010) examined the chemical composition and antioxidant activity of essential oils and methanolic extracts from the leaves, stems, and flowers of *Myrtus communis* L., highlighting the richness of these plant parts in bioactive compounds. In our previous study (Taibi et al., 2024), we reported a high phenolic content in myrtle fruit, reaching 87 mg GAE/g of dry weight.

4.1. Organic compounds

Numerous studies have characterized the organic composition of *Myrtus communis*, highlighting its diverse chemical characteristics. Fadda et al. (2017), reported a total soluble solid content of 19.87 ± 0.25%, a pH value of 5.31 ± 0.01, and a titrable acidity of 0.44 ± 0.02% expressed as malic acid. Similarly, Hacıseferoğulları et al. (2012) found a pH value of 4.39 ± 0.76 and a titrable acidity of 0.10 ± 0.01%. In addition, Şan et al. (2015) reported total soluble

solids ranging from 15.50 ± 0.00% to 24.00 ± 0.00%, titratable acidity from 0.06 ± 0.006% to 0.13 ± 0.003% (malic acid), and pH values from 5.38 ± 0.38 to 5.64 ± 0.63, further supporting the consistency of these chemical parameters across different studies.

As for acidity and soluble solids, myrtle fruits exhibit notable macronutrient composition variability. Protein content can reach 9.02%, while carbohydrate content is approximately 79.78 ± 0.48% (Fabio Correddu et al., 2019). Ash content is reported at 0.725%, crude fiber content at 17.41% (Aydın & Özcan, 2007) and oil concentration around 2.52% (Mohamadi et al., 2021). Unsaturated fatty acids are predominant, particularly linoleic (18:2) and oleic (18:1) acids, whereas palmitic (16:0) and stearic (18:0) acids are the most abundant saturated fatty acids (Akyüz et al., 2019; Asif et al., 1979; Cakir, 2004). Dry matter content is about 24.28 ± 2.34% (Hacıseferoğulları et al., 2012).

Regarding lipid content, as reported by Wannes, Mhamdi, Sriti and Marzouk (2010), myrtle fruit is an important source with a value of 28.97 mg/g in whole fruit, 61.26 mg/g in seeds and 4.14 mg/g in pericarp.

Additionally, the most common organic acids present in myrtle include citric, ascorbic, tartaric, tannic, and malic acids, whose concentrations vary widely (Fadda et al., 2017; Hacıseferoğulları et al., 2012; Şan et al., 2015).

The proximate composition of myrtle fruits is strongly influenced by several factors, including plant genotype, geographical origin, climatic conditions, cultural practices, ripening stage, and fruit maceration period (Hazrati et al., 2022; Tuberoso et al., 2007; Usai et al., 2018).

4.2. Inorganic compounds

The mineral composition of the *Myrtus communis* plant is influenced by several factors, including genotype, plant part, and environmental conditions. Yildirim et al. (2015) reported that, depending on the genotype studied, the main minerals identified were P, K, Ca, Mg, Mn, Fe, Cu, Zn, Na, and B, all of which were detected in the three genotypes examined. In contrast, Ag and Mo were found only in Genotype 1, while Se was detected in Genotypes 1 and 17. Hacıseferoğulları et al. (2012) also noted that the mineral profile of *Myrtus communis* fruits, according to the geographic area studied, included Al, B, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, Se, and Zn, with varying concentrations. Furthermore, Özcan, Al Juhaimi, Ahmed,

Babiker, et al. (2020) observed that mineral content varied according to the plant type and part, with Ca, K, Mg, O, S, and Mn being the predominant minerals across both plant types. Among the different parts, fruits and leaves exhibited the highest mineral concentrations compared to stems.

4.3. Essential oils

Essential oils, also known as volatile or ethereal oils, are aromatic oily liquids extracted from various parts of plants (Burt, 2004). They are complex mixtures composed of numerous distinct compounds, primarily derived from terpenes and their oxygenated derivatives. Each constituent contributes to the overall biological activity, which may be either beneficial or undesirable (Prabuseenivasan et al., 2006). Essential oils have been widely studied for their applications in food preservation (Sandri et al., 2007; Tongnuanchan & Benjakul, 2014) and perfumery (Butnariu, 2021). Due to their relatively safe profile, broad consumer acceptance, and potential for functional uses, essential oils and their components are receiving growing scientific and industrial interest (Hennia et al., 2019; Sawamura, 2000).

In general, hydrocarbon monoterpenes, oxygenated monoterpenes, and sesquiterpenoids are the predominant classes of compounds found in essential oils extracted from myrtle berries and leaves. Among these, 1,8-cineole, α-pinene, limonene, geranyl acetate, linalool, estragole, terpinyl acetate, myrtenyl acetate, bergamotene, and β-caryophyllene are the most representative constituents (Figure 3) (Brada et al., 2012; Hennia et al., 2019; Kordali et al., 2016; Mahmoudvand et al., 2015; Usai et al., 2018)

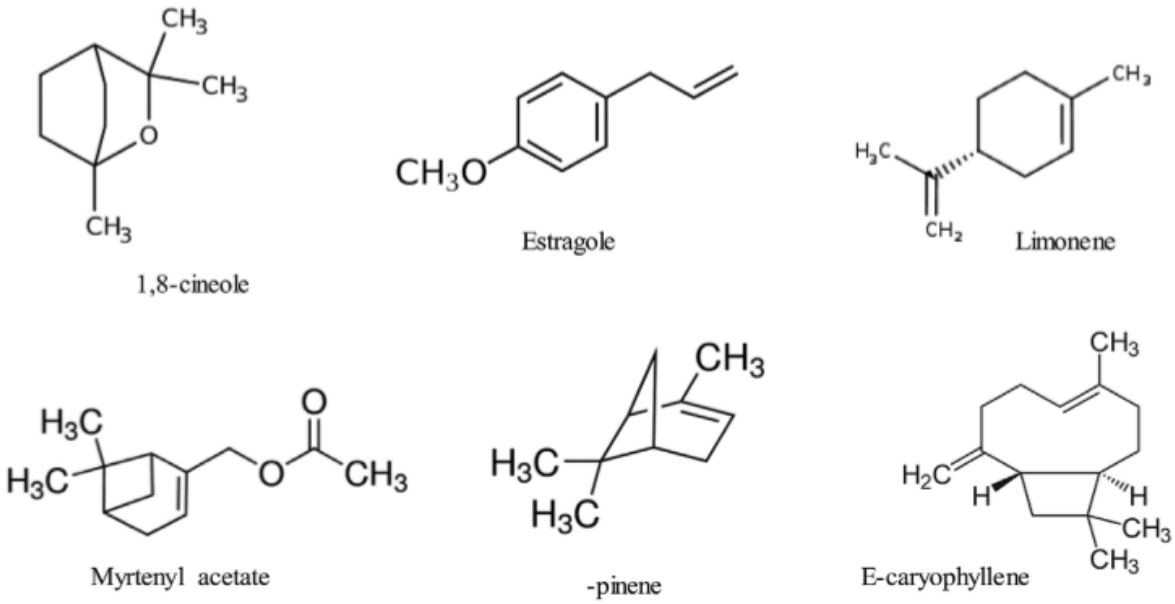

Figure 3. Predominant monoterpenes and sesquiterpenes identified in essential oils of *Myrtus communis* L. (Giampieri et al., 2020)

4.4. Phenolic compounds

Polyphenols are a large class of organic compounds distinguished by the presence of multiple phenol units (a phenyl group bonded to a hydroxyl group). Widely distributed in plants, they encompass over 8,000 different identified compounds (Safer et al., 2013). In terms of phytochemical composition, the fruits and leaves of myrtle are the parts that have been studied the most extensively. In both fruits and leaves, polyphenols are the predominant compounds, playing a crucial role not only in the plant's morphological and physiological functions but also in promoting human health through their diverse biological activities (Babou et al., 2016; Özcan, Al Juhaimi, Ahmed, Babiker, et al., 2020; Pereira et al., 2016)

4.4.1. Fruit phenolic compounds

The content of total polyphenols, flavonoids, and anthocyanins in myrtle fruits varies depending on the ecotype, geographical origin, and extraction method (Barboni, Cannac, et al., 2010; Martín et al., 1999; Taibi et al., 2024).

More specifically, the phytochemical composition of myrtle berries is marked by the dominant presence of anthocyanins, which are responsible for the fruit's dark purple color and contribute

to its health benefits (Montoro, Tuberoso, Piacente, et al., 2006; V. González de Peredo et al., 2019).

In myrtle berries, these bioactive compounds are mainly represented by delphinidin 3-O-glucoside, petunidin 3-O-glucoside, malvidin 3-O-glucoside, and peonidin 3-O-glucoside, followed by cyanidin 3-O-glucoside, delphinidin-pentose, and petunidin-pentose. In addition, phenolic acids (such as gallic acid and its derivatives, caffeic acid, and syringic acid), flavanones (naringin), and flavonols (myricetin, myricetin 3-O-galactoside, myricetin 3-O-rhamnoside, quercetin 3-O-glucoside, and quercetin 3-O-rhamnoside), among others, have been identified in berry extracts (Barboni, Cannac, et al., 2010; Bouaoudia-Madi et al., 2017; Bouaziz et al., 2015; Tuberoso et al., 2010)

4.4.2. Leaf Phenolic compounds

Concerning leaf composition, the concentration of total polyphenols and flavonoids varies depending on the myrtle variety and the methods used for extraction and analysis.

Catechin, epicatechin, isovitexin, cinnamic acid, and quercetin have been identified as the most abundant phenolic compounds in Australian myrtle leaves (Ali et al., 2024). In Tunisian myrtle leaves, flavonoids, along with ellagic acid and its derivatives, and gallic acid and its derivatives, were reported as the major phenolic fractions (Taamalli et al., 2014). Furthermore, in Algerian myrtle leaves, the main phenolic compounds identified were two hydroxybenzoic acids (gallic acid and ellagic acid), six flavonols (myricetin-3-O-rhamnoside, quercetin-3-O-galactoside, quercetin-3-O-rutinoside, myricetin, quercetin, and kaempferol), and two anthocyanins (delphinidin-3-O-glucoside and malvidin-3-O-glucoside) (Babou et al., 2016).

4.4.3. Seed phenolic compounds

Concerning seeds, which are part of the berries, their phytochemical composition has been characterized in only a limited number of studies. As reported by Wannes and Marzouk (2016) and Jabri, Tounsi, et al. (2016), the concentrations of total polyphenols and flavonoids vary considerably depending on the myrtle variety and the methodologies employed for extraction and analysis. Interestingly, myrtle berry seeds exhibit a particularly high tannin content, with levels nearly twice as high as those detected in the whole fruit (Aidi Wannes & Marzouk, 2013), substantially contributing to the characteristic astringency of myrtle berries.

Table 3 provides a summary of the biochemical composition of *Myrtus communis* L.

Table 3. Biochemical (Nutritional and Phytochemical) Composition of *Myrtus communis* L.

Compound class	Specific compound	Approximate amount/range	Plant part	Reference(s)
Nutritional (organic) constituents	Protein	4.17–9.02 %	Berry	(Fabio Correddu et al., 2019; Hacıseferoğulları et al., 2012)
	Carbohydrates	~79.78 %	Berry	(Hacıseferoğulları et al., 2012)
	Cellulose	~23.49 %	Berry	
	Ash	0.73–3.47 %	Berry	
	Oil	2.37–3.48 %	Berry	(Fabio Correddu et al., 2019)
	Crude fiber	~17.41 %	Berry	(Aydın & Özcan, 2007)
	Total lipids	28.97 mg/g (whole fruit); 4.14 mg/g (pericarp); 61.26 mg/g (seed)	Whole berry	(Aydın & Özcan, 2007; Hacıseferoğulları et al., 2012; Wannes, Mhamdi, Sriti, & Marzouk, 2010)
	Neutral glycerolipids	58.45 mg/g (mainly triacylglycerols)	Seed	(Wannes, Mhamdi, Sriti, & Marzouk, 2010)
Fatty acids	Linoleic acid (C18:2n6c)	69 %	Berry/seed	(Jabri et al., 2017; Özcan, Al Juhaimi, Ahmed, Babiker, et al., 2020; Wannes, Mhamdi, Sriti, & Marzouk, 2010)
	Oleic acid (C18:1n9c)	9 %	Berry/seed	
	Palmitic acid (C16:0)	10 %	Berry/seed	
	Stearic acid (C18:0)	4 %	Berry/seed	
Organic acids	Ascorbic acid	2.82 mg/100 g	Berry	(Şan et al., 2015)
	Tannic acid	52.46 µg/g	Berry	
	Citric acid	120–1104.85 mg/100 g	Berry	(Fadda et al., 2017; Hacıseferoğulları et al., 2012)
	Malic acid	0.17–0.30 %	Berry	
	Tartaric acid	0.29–0.30 mg/100 g	Berry	
Minerals	Ca (6,72 mg/kg), K (22,65 mg/kg), P (4,34 mg/kg), Mg (2,14 mg/kg), Na (3,34 mg/kg), Mn (42,09	Major minerals	Leaf and fruit	(Hacıseferoğulları et al., 2012; Özcan, Al Juhaimi, Ahmed, Babiker, et al., 2020; Yildirim et al., 2015)

	mg/kg), Fe (105,31 mg/kg)			
Essential oils (main terpenes)	α-Pinene, 1,8-Cineole, Limonene, Myrtenyl acetate, Linalool, E-caryophyllene	Main EO components	Leaf, berry	(Brada et al., 2012; Hennia et al., 2019; Kordali et al., 2016)
Phenolic compounds (berry)	Total phenolics	14.68–138.21 mg GAE/g	Berry	(Amensour et al., 2010; Fadda & Mulas, 2010; Maldini et al., 2016; Messaoud & Boussaid, 2011; Scorrano et al., 2017b; V. González de Peredo et al., 2018)
	Flavonoids	52.03–158.94 mg/g	Berry	
	Anthocyanins	5.36–60.25 mg/g	Berry	
	Main anthocyanins	Delphinidin-3-O-glucoside, Petunidin-3-O-glucoside, Malvidin-3-O-glucoside, Peonidin-3-O-glucoside	Berry	
	Phenolic acids	Gallic, Caffeic, Syringic acids	Berry	(Barboni, Cannac, et al., 2010)
	Flavonols	Myricetin, Quercetin glycosides	Berry	(Tuberoso et al., 2010)
Phenolic compounds (leaf)	Total phenolics	31.25–298.63 mg GAE/g	Leaf	(Amensour et al., 2010; Özcan, Al Juhaimi, Ahmed, Babiker, et al., 2020)
	Flavonoids	129.96–376.82 mg/g	Leaf	
	Main compounds	Myricetin glycosides, Quercetin derivatives, Tannins, Ellagic acid, Gallomyrtucommulones	Leaf	(Babou et al., 2016; Pereira et al., 2016)
Phenolic compounds (seed)	Total phenolics	25.25–147.56 mg GAE/g DW	Seed	(Aidi Wannes & Marzouk, 2013; Jabri et al., 2017; Wannes & Marzouk, 2016)
	Flavonoids	0.75–85.06 mg/g DW	Seed	
	Tannins	~18.01 mg GAE/g DW	Seed	
	Main tannins	Ellagitannins (eugeniflorin D2, oenothein B, tellimagrandin I)	Seed	(D'Urso et al., 2017)
	Anthocyanins	Peonidin diglu, Petunidin-3-O-glu, Delphinidin-3-O-gal	Seed	(Jabri, Rtibi, Ben-Said, et al., 2016)

5. Therapeutic potential and pharmacological effects of *Myrtus communis*

Human health is increasingly influenced by a variety of environmental and lifestyle factors, including climate, diet, and exposure to harmful chemicals and toxins, which can enter the body through multiple pathways. In 2019, the World Health Organization (WHO) estimated that exposure to toxic chemicals was responsible for 1.6 million deaths globally, while the Centers for Disease Control and Prevention (CDC) announced 3960 deaths linked specifically to natural toxins (Yahyazadeh et al., 2021). *Myrtus communis* exhibits a broad spectrum of pharmacological properties. The richness of its biochemical profile and its therapeutic potential across various parts of the plant highlight its value as a safe and effective candidate for pharmaceutical development (Al-Snafi et al., 2024). This section of Chapter I provides a summary and discussion of the various pharmacological effects of *Myrtus communis* as reported in previous studies.

5.1. Antimicrobial properties

5.1.1. Antibacterial properties

Among the various biological properties attributed to myrtle essential oils, antimicrobial activity, both antibacterial (against Gram-negative and Gram-positive bacteria) and antifungal (against yeasts and fungi), is the most studied (Hennia et al., 2019). According to Amensour et al. (2010), myrtle extracts and essential oils exhibit antibacterial activity by increasing the permeability of the bacterial cell wall and membrane, leading to the leakage of intracellular contents and impairing essential membrane functions such as nutrient uptake, enzymatic activity, and electron transport. Additionally, Mansouri et al. (2001) reported that the methanolic crude extract of *Myrtus communis* L. exhibited antibacterial activity against a broad spectrum of laboratory strains, inhibiting six Gram-positive (*Staphylococcus aureus, Micrococcus luteus, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus agalactiae, Listeria monocytogenes*) and three Gram-negative bacteria (*Escherichia coli, Proteus vulgaris,* and *Pseudomonas aeruginosa*), with inhibition zones ranging from 8 to 18 mm. Similarly, Cvitković et al. (2025) investigated the antibacterial activity of three myrtle-based formulations (essential oil, aqueous leaf extract, and supercritical fruit extract) and observed significant inhibitory

effects against both Gram-positive (*Staphylococcus aureus, Bacillus subtilis, Enterococcus faecium, Listeria monocytogenes*) and Gram-negative bacteria (*Pseudomonas aeruginosa, Escherichia coli,* and *Salmonella enterica* subsp. *Typhimurium*), with inhibition zones ranging from 6 to 35.25 mm. In the study by Polat et al. (2014), methanol and acetone extracts of *Myrtus communis* demonstrated higher efficacy against six pathogenic bacteria, including *Bacillus cereus, Escherichia coli, Listeria monocytogenes, Staphylococcus aureus, Salmonella Typhimurium,* and *Yersinia enterocolitica*. The essential oil from Myrtus communis leaves collected in Northern Cyprus, as demonstrated in the study conducted by Akin et al. (2010), exhibited notable antibacterial activity against various pathogens, including *Staphylococcus aureus*, *Listeria monocytogenes*, *Enterococcus durans*, *Salmonella*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus subtilis.*

5.1.2. Antifungal properties

Regarding the antifungal activity of *Myrtus communis*, Brahmi et al. (2023) reported that its essential oil exhibited strong antifungal effects against *Penicillium digitatum* and *Aspergillus niger*, with inhibition zone diameters of 53.67 mm and 49 mm, respectively. This antifungal activity is primarily attributed to oxygenated monoterpenes, which irreversibly disrupt fungal cell membranes, causing leakage of intracellular contents and leading to cell death (Giampieri et al., 2020). Similarly, Kordali et al. (2016) confirmed that oxygenated monoterpenes, accounting for 73.02–83.83% of the total oil composition, exhibited inhibitory effects against the growth of 19 phytopathogenic fungi, in particular, *Cladosporium herbarum*, *Sclerotinia minor*, *Rhizoctonia solani*, *Botrytis cinerea*, and *Sclerotinia sclerotiorum* were the most sensitive fungi, with inhibition percentages reaching up to 100% for certain strains. In the study conducted by Alyousef (2021), which evaluated the antifungal activity of *Myrtus communis* aerial parts against five *Candida* strains, the methanolic extracts of roots and leaves showed the strongest activity against *C. glabrata*, with inhibition zones of 14.5 ± 0.61 mm and 20.7 ± 0.22 mm, respectively, while other plant parts exhibited minimal or no activity.

5.2. Antioxidant properties

Antioxidants are molecules that, even at low concentrations, neutralize free radicals and delay the oxidation of biomolecules such as proteins, lipids, carbohydrates, and DNA, thereby

reducing oxidative stress and protecting cells from damage (Sindhi et al., 2013).

Myrtle is recognized as a valuable source of phenolic compounds, offering numerous health benefits (D'Urso et al., 2019; Taibi et al., 2024). Various studies have demonstrated the antioxidant activity of different parts of *Myrtus communis.* Snoussi et al. (2021) reported that the leaf extract exhibited strong antioxidant capacity, and its incorporation enhanced the oxidative stability of soybean oil. Similarly, Medda et al. (2021) demonstrated the antioxidant potential of myrtle leaves and berries using FRAP, ABTS, DPPH, and β-carotene assays. Furthermore, Guzelmeric et al. (2022) evaluated the antioxidant activities of hydroalcoholic extracts from the floral buds, flowers, leaves, and fruits of *Myrtus communis* using DPPH, ABTS, FRAP, and superoxide radical scavenging activity (SRSA) assays, the latter based on an enzymatic inhibition mechanism. Their findings consistently confirmed strong antioxidant activity across all tested extracts.

5.3. Anti-inflammatory properties

Inflammation, alongside oxidative stress, plays a key role in the onset and progression of major human diseases, including metabolic disorders, cardiovascular diseases, and cancer (Joseph et al., 2014). The use of natural antioxidants that neutralize free radicals and mitigate both inflammation and oxidative stress may serve as an effective strategy for preventing various human diseases (Hosseinzadeh et al., 2011). Soomro et al. (2019), isolated Myrtucommuacetalone-1 (MCA-1) from *Myrtus communis* and confirmed its potent anti-inflammatory effects *in vivo* by inhibiting nitric oxide production in LPS-stimulated macrophages (IC_{50} < 1 µg/mL). MCA-1 also reduces reactive oxygen species, decreasing superoxide and hydrogen peroxide levels by 48% and 53%, respectively, and modulates inflammation by blocking NF-κB activation without affecting p38 MAP kinase. Another study by Jabri et al. (2018b), observed that the seed water extract, administered to adult male Wistar rats at doses of 25, 50, and 100 mg/kg for 2 months, resulted in decreased plasma inflammatory cytokines, reduced erythrocyte ROS and lipid peroxidation, and increased antioxidant enzyme activity.

5.4. Anticancer properties

Cancer is among the deadliest diseases of modern times, causing a significant number of

deaths annually. In 2022, there were 19.3 million new cancer diagnoses, with the number expected to rise to 22.1 million by 2030 (Chhikara & Parang, 2023; Miller et al., 2019). Myrtle leaf essential oils exhibited antimutagenic activity in *E. coli* by reducing the rate of spontaneous mutations (Mimica-Dukić et al., 2010). Whereas, the hydroalcoholic extract prepared from the Myrtle leaves exhibited the highest anticancer activity against the human colorectal cancer cell line (Guzelmeric et al., 2022). The polyphenol-enriched fraction from *Myrtus communis* exhibited potent anti-cancer activity against leukemia cells (HL60 and K562) while remaining non-toxic to normal cells and safe in mice (Mechchate et al., 2022). Similarly, compounds from *Myrtus communis* leaves caused cell death in several cancer cell lines by triggering apoptosis through caspase activation and DNA damage. Cells lacking caspase-9 were resistant, suggesting that the effect primarily involves the mitochondrial apoptosis pathway while having a limited impact on normal cells (Tretiakova et al., 2008).

5.5. Effect on the cardiovascular system

The role of dietary bioactive compounds in supporting cardiovascular health has attracted considerable scientific attention. The protective effects of *Myrtus communis* L. extract against high-fat diet-induced cardiovascular damage were demonstrated through its ability to reduce oxidative stress and modulate nitric oxide metabolism, thereby alleviating heart and aorta injury in vivo (Yay et al., 2023). Additionally, myrtle berry extract (50 mg/kg, administered for 30 and 45 days) exhibited hypolipidemic and antithrombotic effects by lowering serum triglycerides, low-density lipoprotein, and cholesterol levels in cholesterol-fed rabbits (Khan et al., 2014).

5.6. Neuroprotective properties

Dessì et al. (2025) investigated the neuroprotective potential of *Myrtus* berry by-product extract in a cellular model of neurodegeneration using PC12 cells exposed to 6-hydroxydopamine (6-OHDA). The results demonstrated that *Myrtus* extract effectively protected against 6-OHDA-induced cytotoxicity, reduced reactive oxygen species (ROS) levels, and modulated the expression of key stress-related genes, highlighting its potential as a neuroprotective agent. Additionally, Tumen et al. (2012) demonstrated that *Myrtus communis* berry extracts exhibited stronger inhibitory effects than leaf extracts against acetylcholinesterase, butyrylcholinesterase, and tyrosinase, key enzymes implicated in

neurodegenerative disorders such as Alzheimer's disease.

5.7. Effect on diabetes

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels. According to global statistics from the International Diabetes Federation in 2019, approximately 463 million adults aged 20 to 79 are affected worldwide, with this number projected to increase by 10.2% by 2030 and 10.9% by 2045 (Liu et al., 2023). As reported by Olfert and Wattick (2018), a vegetarian diet, primarily consisting of whole grains, legumes, fruits, and vegetables, along with polyphenols and other bioactive antioxidants, may help reduce the risk of developing diabetes by improving associated metabolic risk factors.

Several studies using experimental models have highlighted the potential of *Myrtus communis* berry and leaf extracts, as well as its essential oil, in managing diabetes. Talebianpoor et al. (2019) demonstrated that the administration of a hydroalcoholic extract of myrtle fruits at doses of 250 mg/kg/day and 500 mg/kg/day to diabetic rats (for 45 days in the case of type I diabetes and 10 days for type II diabetes) resulted in a significant improvement in metabolic and renal complications in diabetic rats. Similarly, the study by Panjeshahin et al. (2016), the authors found that the hydroalcoholic, water, and ethanolic extracts of *Myrtus communis* leaves significantly lowered blood glucose levels in diabetic rats. The essential oil of *Myrtus communis* significantly reduced blood glucose levels, serum triglycerides, and hepatic nitrite, while increasing hepatic glucokinase activity and glycogen concentration, following both acute (50 and 100 mg/kg, once daily for 1 week) and chronic (2.5-5 drops at 50-100 mg/kg for 4 hours up to 21 days) administration. However, it did not affect serum insulin levels (Sepici et al., 2004)

5.8. Effect on the digestive system

Myrtus communis is effective in managing various digestive disorders, including diarrhea, gastroduodenal ulcers, and esophagitis. In the study by Sumbul et al. (2010), aqueous and methanolic extracts from myrtle berries, administered at doses of 105 and 175 mg/kg (aqueous) and 93 and 154 mg/kg (methanolic), demonstrated significant ulcer-protective effects, as confirmed by histological examination of gastric tissues in indomethacin- and pyloric ligation-induced ulcer models in Wistar rats. The antidiarrheal effect of the leaf extract was

demonstrated by Sisay et al. (2017), where administration of the methanolic extract (200 and 400 mg/kg), along with the chloroform and methanol fractions (400 mg/kg), significantly delayed the onset of diarrhea. In the case of myrtle berry extract, Jabri, Rtibi, Ben-Said, et al. (2016) demonstrated that in vivo administration of myrtle berry seed extracts provided significant protection against diarrhea and intestinal fluid accumulation. In an in vivo study, Jabri, Rtibi, Sakly, et al. (2016) revealed that administration of myrtle berry juice at doses of 5 and 10 mL/kg significantly inhibited intestinal motility and gastric emptying in adult male Wistar rats.

6. Uses of *Myrtus communis*

6.1. Traditional use

Myrtus communis was recognized as an important analgesic and anti-inflammatory agent in Avicenna's Canon of Medicine (980–1037). It was administered topically, orally, and by inhalation to manage pain and inflammation associated with conditions such as testicular inflammation, headache, arthritis, ear infections, chronic eye diseases, gingivitis, and hemorrhoids (Mahboubi, 2017). The leaves of *Myrtus communis* were traditionally consumed as a beverage, while its fresh or dried berries were used to treat mouth ulcers. Additionally, crushed leaves were mixed with butter or oil to formulate ointments for treating skin conditions and for hair and body care. A decoction of the leaves, combined with goat's milk and warmed over charcoal, was employed by Algerian nomads in the Tassili region to manage liver disorders (Gorjian & Khaligh, 2023). In Algeria, various parts of *Myrtus communis* were traditionally applied to treat a wide range of ailments. For example, a decoction of the leaf powder served to manage hypertension, eczema, other skin conditions, respiratory disorders, and hemorrhoids (Beloued, 1998). *Myrtus communis* was also used to treat hypertension and diabetes (Ziyyat et al., 1997).

6.2. Medicinal use

A clinical study assessed the effects of freeze-dried myrtle berry capsules (1000 mg/day for 4 weeks) in patients with gastroesophageal reflux disease, showing that myrtle supplementation reduced reflux and dyspepsia symptoms and demonstrated efficacy comparable to omeprazole, a standard anti-acid medication (Zohalinezhad et al., 2016).

The therapeutic potential of *Myrtus communis* fruit was also investigated in clinically suspected COVID-19 patients aged 18–65 years with mild to moderate symptoms and no respiratory distress. All participants received conventional therapy, while the intervention group additionally received a *Myrtus communis* preparation for 5 days. Clinical status, mortality rate, and adverse effects were monitored over 14 days. The authors suggested that the antiviral properties of *Myrtus communis* may offer benefits in the early stages of the disease; however, the study results have not yet been published (Azimi & Hasheminasab, 2020). In a double-blind clinical trial, the effect of *M. communis* cream on episiotomy wound healing and pain reduction was evaluated in 100 nulliparous women. Participants applied either *M. communis* cream or a placebo twice daily for 10 days. Pain severity and wound healing were assessed at 2 hours, and on the 5th and 10th postpartum days. Although no differences were observed immediately after delivery, the *Myrtus communis* group showed significantly lower pain levels and enhanced wound healing on the 5th and 10th days compared to the placebo group, indicating that *M. communis* cream effectively accelerates episiotomy healing and relieves pain (Mirzaee et al., 2019). Additionally, in women with grade I and II hemorrhoids, the application of a myrtle-based ointment twice daily for 4 to 8 weeks significantly alleviated symptoms, notably reducing anal itching and improving patient satisfaction (Malekuti et al., 2019).

6.3. Food use

Functional foods provide health benefits beyond basic nutrition by promoting well-being and reducing disease risk. Popularly known as "nutraceuticals" or "designer foods," the concept originated in Japan in the 1980s and later expanded to North America and global markets (Essa et al., 2023). In this context, *Myrtus communis* has gained increasing attention for its potential as a functional ingredient due to its rich phytochemical composition and bioactive properties. Various parts of the plant are used in the food industry, particularly for flavoring meats and sauces (Chalchat et al., 1998). Beyond its traditional culinary uses, recent studies have explored innovative applications of *Myrtus communis* to improve both the nutritional and functional quality of food products. For example, the potential of *Myrtus communis* essential oil (MEO) to enhance carcass traits and meat quality in goats was evaluated by supplementing diets with 0.3% or 0.6% MEO. This supplementation improved the oxidative stability of the meat by

reducing lipid oxidation during storage, without altering the fatty acid profile (Smeti et al., 2021). These findings suggest that *Myrtus communis* essential oil can be used not only as a flavoring agent but also as a natural preservative to enhance meat quality. In addition to meat applications, polyphenolic extracts from *Myrtus communis* fruits have been incorporated into dairy-based products. For instance, when these extracts were added to whey in either liquid or powder form, the resulting formulations demonstrated antioxidant potential, prebiotic benefits, and improved shelf stability. These characteristics offer innovative opportunities for the development of functional whey-based beverages (Detti et al., 2025). Similarly, the incorporation of *Myrtus communis* fruit jelly into stirred yogurt has been shown to enhance both physicochemical and textural properties. The addition of fruit jelly increased viscosity, consistency, and protein content while reducing pH and microbial counts. Furthermore, it enriched the yogurt with bioactive compounds, significantly boosting its antioxidant capacity. Sensory evaluations revealed high consumer acceptability, particularly at incorporation levels of 9–12%, supporting the use of *Myrtus communis* fruit jelly to improve both the nutritional quality and sensory appeal of yogurt and other functional dairy products (Bouacida et al., 2022). Expanding its application to frozen desserts, the incorporation of *Myrtus communis* fruit pulp into probiotic goat milk ice cream containing *Lactobacillus casei* has also been investigated. The pulp exhibited prebiotic potential by supporting *L. casei* viability, despite a slight reduction (0.80–1.32 log CFU/g) after freezing. Its addition increased the total phenolic content, while antioxidant activity remained stable during storage. These results further highlight the potential of *Myrtus communis* fruit to enhance the functional and nutritional qualities of probiotic ice cream (Öztürk et al., 2018).

Overall, these diverse applications underscore the remarkable versatility of *Myrtus communis* as a functional food ingredient, capable of enhancing a wide range of food matrices with bioactive compounds that offer both nutritional value and technological advantages.

6.4. Other uses

Extracts and derivatives of *Myrtus communis* have shown promising applications beyond food. An extract from *Myrtus communis* leaves was tested as a natural surfactant to improve oil recovery. It reduced the tension between oil and water and performed even better when

combined with salt and alkali. In experiments, it increased oil recovery by 14.3% on its own and by 16.4% when used with other chemicals like alkali and polymer, highlighting its potential as an eco-friendly and effective option for enhanced oil recovery (Nowrouzi et al., 2022). In addition to industrial applications, *Myrtus communis* essential oils extracted from the bark, leaves, and flowers are widely used in perfumery, soaps, skincare, and other cosmetic products (Lim, 2011). Furthermore, exhausted myrtle berries (EMB), rich in polyphenols, have been studied as a feed additive for sheep to improve digestion and rumen microbial balance. In this study, sheep received either 50 g or 100 g of EMB per day. EMB did not affect rumen pH or methane production but lowered ammonia levels, improving nitrogen use. The lower dose increased beneficial compounds like conjugated linoleic acid (CLA) and raised fungal populations, while both doses reduced bacteria that break down proteins. Overall, EMB may help sheep use protein more efficiently and support better gut health (F Correddu et al., 2019).

Chapter II
Phenolic compounds

Chapter II. Phenolic compounds: Structure, classification, extraction and biological properties

1. Definition

Phenolic compounds are secondary plant metabolites, characterized as polyhydroxylated phytochemicals found in a wide variety of plants, including fruits, vegetables, spices, nuts, and cereals (Mocanu et al., 2015). They are one of the most abundant and widely distributed natural compounds in the human diet, valued for their notable biological properties (Rasouli et al., 2016).

2. Structure and classification of phenolic compounds

Polyphenolic compounds are defined by structures containing at least one aromatic ring with one or more hydroxyl groups (Crozier et al., 2009). Their structural diversity arises from variations in the number of phenolic rings, the nature of the linkages between these rings, and the polarity and types of functional groups attached (Chen et al., 2024). Based on these structural characteristics, phenolic compounds are broadly classified into flavonoids and non-flavonoids. They can be further subdivided into three main groups: (i) simple phenolics, including phenolic acids (hydroxybenzoic and hydroxycinnamic acids) and coumarins; (ii) polyphenols, comprising flavonoids and tannins; and (iii) other phenolic compounds such as stilbenes, lignans, and lignins (Da Silva et al., 2023).

2.1. Phenolic acids

Phenolic acids, a major class of plant-derived polyphenols, are characterized by the presence of a single carboxylic acid group. They are abundant in various plant-based foods, particularly in seeds, fruit skins, and vegetable leaves, where they predominantly occur in bound forms such as amides, esters, or glycosides, and only rarely in free form. Phenolic acids are commonly categorized into two subgroups: hydroxybenzoic acids and hydroxycinnamic acids (Kumar & Goel, 2019).

2.1.1. Hydroxybenzoic acids

Hydroxybenzoic acids are derivatives of benzoic acid with a C6–C1 structure (figure 4,

table 3). They are found either in conjugated forms with sugars or organic acids, or bound to cell wall components. Common examples include gallic acid, p-hydroxybenzoic acid, salicylic acid, ellagic acid, gentisic acid, protocatechuic acid, syringic acid, and vanillic acid, which differ in their aromatic ring substitutions (Da Silva et al., 2023; Rashmi & Negi, 2020)

2.1.2. Hydroxycinnamic acids

Hydroxycinnamic acids are derivatives of cinnamic acid characterized by a C6–C3 structure (Figure 4, Table 4). Common examples include p-coumaric acid, cinnamic acid, caffeic acid, ferulic acid, sinapic acid, isoferulic acid, and p-hydroxycinnamic acid. These compounds are more abundant in nature than hydroxybenzoic acids and typically occur in various conjugated forms (Da Silva et al., 2023; Rashmi & Negi, 2020)

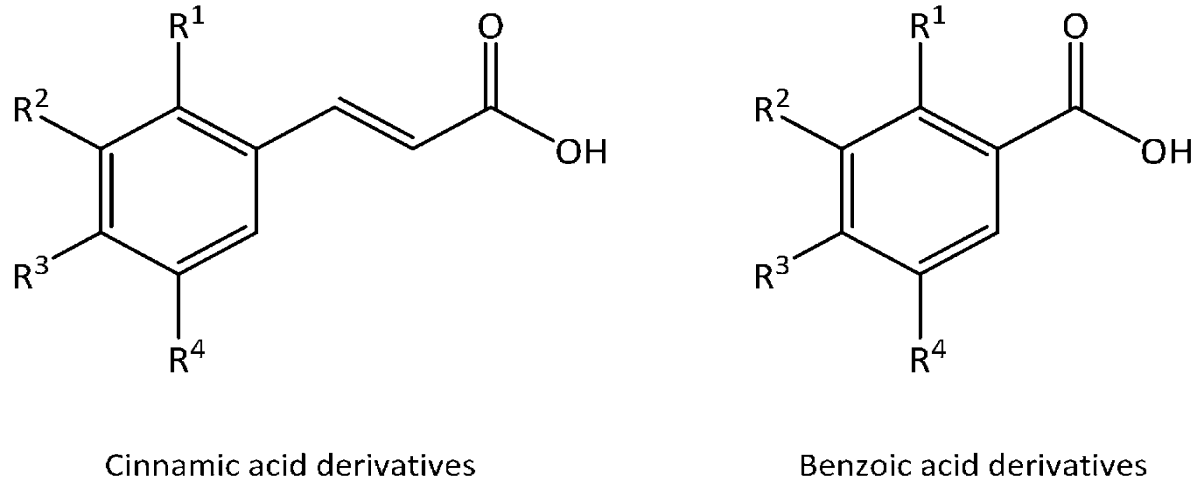

Figure 4. Chemical structures of principal hydroxycinnamic and hydroxybenzoic acid derivatives occurring in vegetables (Kaushik et al., 2015).

Table 4. Substitution patterns of major cinnamic and benzoic acid derivatives (Kaushik et al., 2015).

Substitution	Cinnamic Acid Derivatives	Benzoic Acid Derivatives
$R^3 = OH$	*p*-Coumaric acid	*p*-Hydroxybenzoic acid
$R^3 = R^4 = OH$	Caffeic acid	Protocatechuic acid
$R^2 = OCH_3$, $R^3 = OH$	Ferulic acid	Vanillic acid
$R^2 = R^3 = R^4 = OH$		Gallic acid
$R^2 = R^4 = OCH_3$, $R^3 = OH$	Sinapic acid	Syringic acid
$R^2 = R^3 = OH$ [plus the carboxylic group being esterified with quinic acid]	5-*O*-caffeoylquinic acid	

2.2. Flavonoids

Flavonoids (figure 5) are polyphenolic compounds made of two aromatic rings (A and B) connected by a three-carbon bridge forming a heterocyclic ring (C). Rings A and C usually have one to three hydroxyl groups. The heterocyclic ring is commonly a pyrone, as in luteolin, or a pyrylium, as in delphinidin. Flavones, flavonols, anthocyanins, and flavanones have ring C attached at C2, while isoflavones attach at C3. Chalcones differ by having an open three-carbon chain between rings A and B. The C2–C3 bond is usually double, but it is single in flavanones (Al Mamari, 2021). Flavonoids are the largest and most diverse subgroup of polyphenols, produced as secondary metabolites in plants. They are widely present in fruits, vegetables, and medicinal plants, and contribute to plant growth, development, and defense (Ravishankar et al., 2013). Over 6,000 flavonoids have been identified in plants and are classified into flavonols, flavones, flavanols, isoflavones, flavanones, and anthocyanidins based on their structural characteristics (Xiao, 2017).

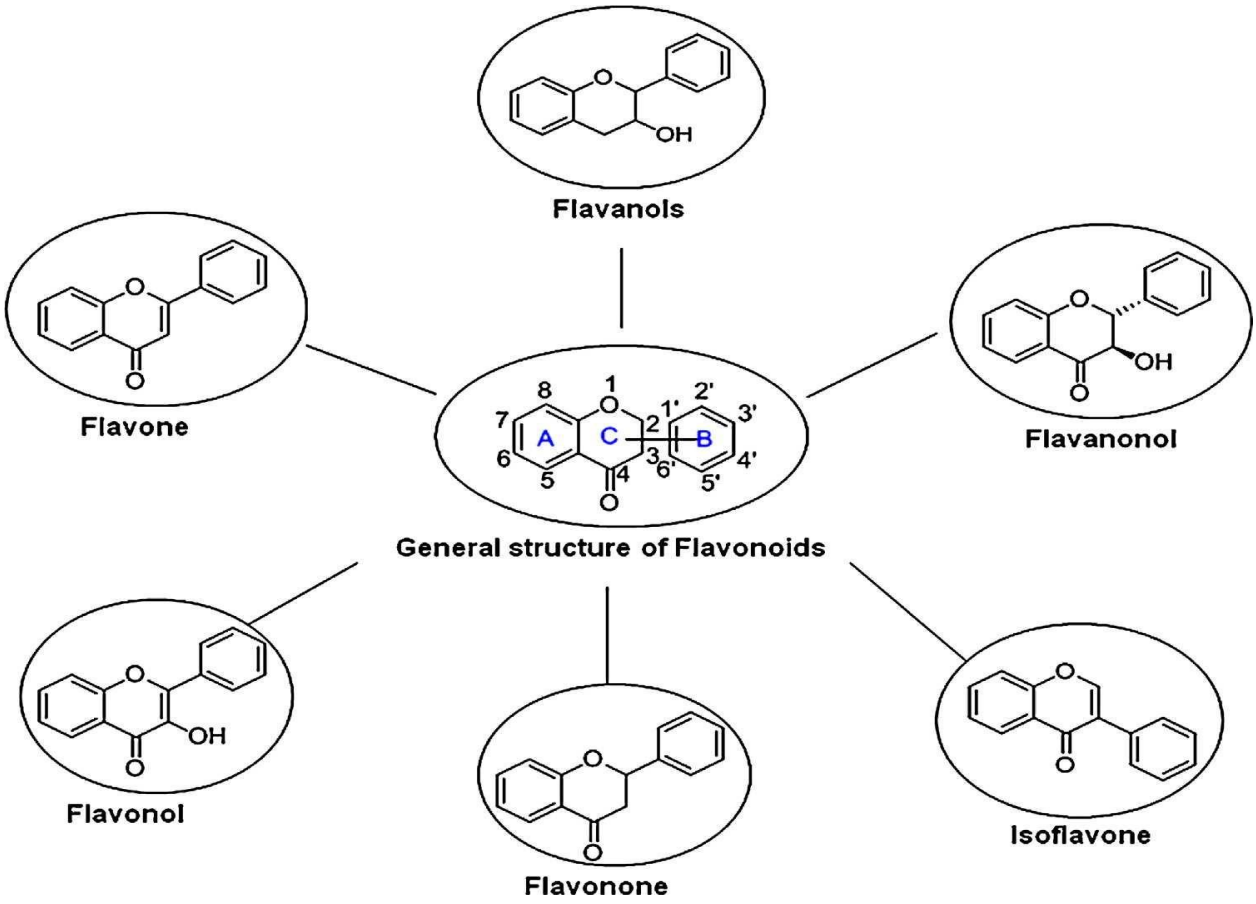

Figure 5. Basic chemical structure of flavonoids and their derivatives (Ravishankar et al., 2013).

Among these subclasses, flavonols (Figure 6) are the most widespread. In myrtle fruits, analysis by HPLC-UV at 280 nm identified flavonols glycosides as the predominant phenolics, accounting for 58% of the total quantified polyphenols (Barboni, Cannac, et al., 2010). Flavonols are commonly found in a variety of plant-based sources, including onions, kale, apples, olives, beans, and leafy greens (Leo & Woodman, 2015). The principal flavonols are quercetin, kaempferol, myricetin, isorhamnetin, and rutin. They are distinguished by a hydroxyl group at the 3-position of the C ring, typically present in plants as glycosides conjugated with sugars such as glucose or rhamnose (Fantini et al., 2015). Flavonols exhibit biological activities that contribute to cancer prevention, mainly through their antiproliferative, antioxidant, and pro-apoptotic effects demonstrated in various cancer cell lines (Lea, 2015).

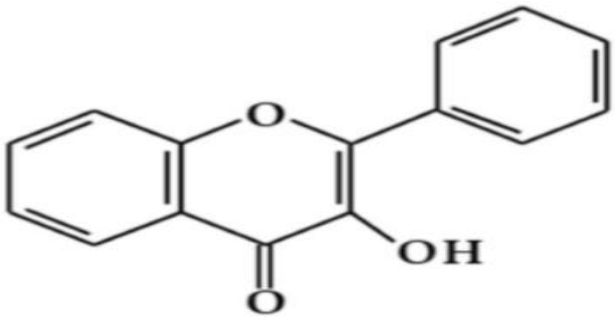

Figure 6. Flavonols structure (Graf et al., 2005)

Another important subclass is anthocyanins. Anthocyanins (Figure 7) are water-soluble flavonoid pigments responsible for the red, blue, and purple colors in plant tissues such as flowers, fruits, and tubers. Their color is pH-dependent, appearing red in acidic conditions and blue in alkaline environments. Structurally, anthocyanins are characterized by a flavylium ion (2-phenylchromenylium), which carries a positive charge on the oxygen atom of the C-ring. Their stability is influenced by factors such as pH, light, temperature, and molecular structure (Khoo et al., 2017). In recent years, anthocyanins have gained attention for their potential health benefits, including protective effects on the brain, liver, and kidneys, as well as roles in preventing cardiovascular diseases, managing obesity, and supporting cancer therapy (Bendokas et al., 2020).

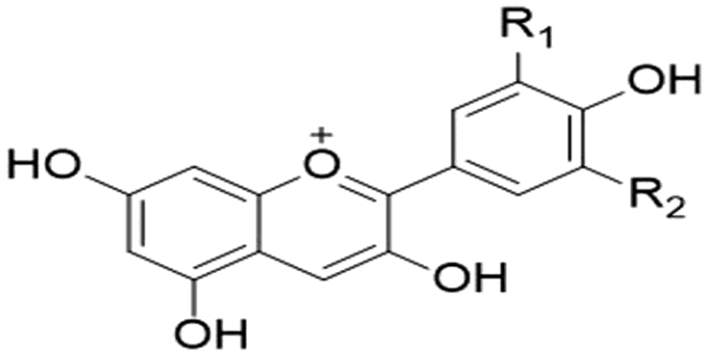

Figure 7. Common chemical structures of anthocyanins (Qi et al., 2023).

2.3. Tannins

Tannins (Figure 8) are polyphenolic compounds found in cereals, legumes, fruits, vegetables, and seaweeds, known for their antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory effects. They are classified into hydrolyzable tannins, composed of gallic or ellagic acid esters bound to glucose, and condensed tannins (proanthocyanidins), which are polymers of flavan-3-ol units. Hydrolyzable tannins release glucose, gallic acid, or ellagic acid upon hydrolysis, while condensed tannins, such as procyanidins and prodelphinidins, vary in size and structure and are abundant in foods like fruits, cocoa, grains, and legumes (Zhang et al., 2022)

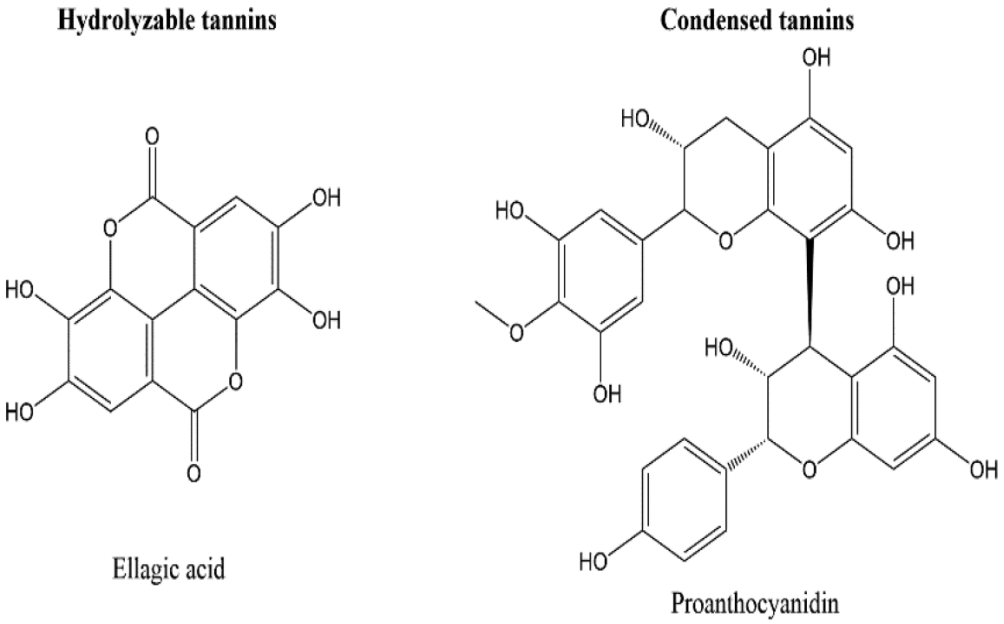

Figure 8. Chemical structures of hydrolysable and condensed tannins (Zhang et al., 2022)

2.4. Stilbenes

Stilbenes (Figure 9) are plant-derived phenolic compounds with a 1,2-diphenylethylene structure, produced to protect against biotic and abiotic stress. They exist as aglycones or glycosides and are valued for their potential health benefits. Resveratrol is the most studied stilbene, but interest in other stilbenes like pterostilbene and piceatannol is growing. Despite their promising bioactivities, information on their physicochemical properties and limitations remains limited, highlighting the need for further research to improve their application in food,

cosmetics, and pharmaceuticals (Navarro-Orcajada et al., 2023).

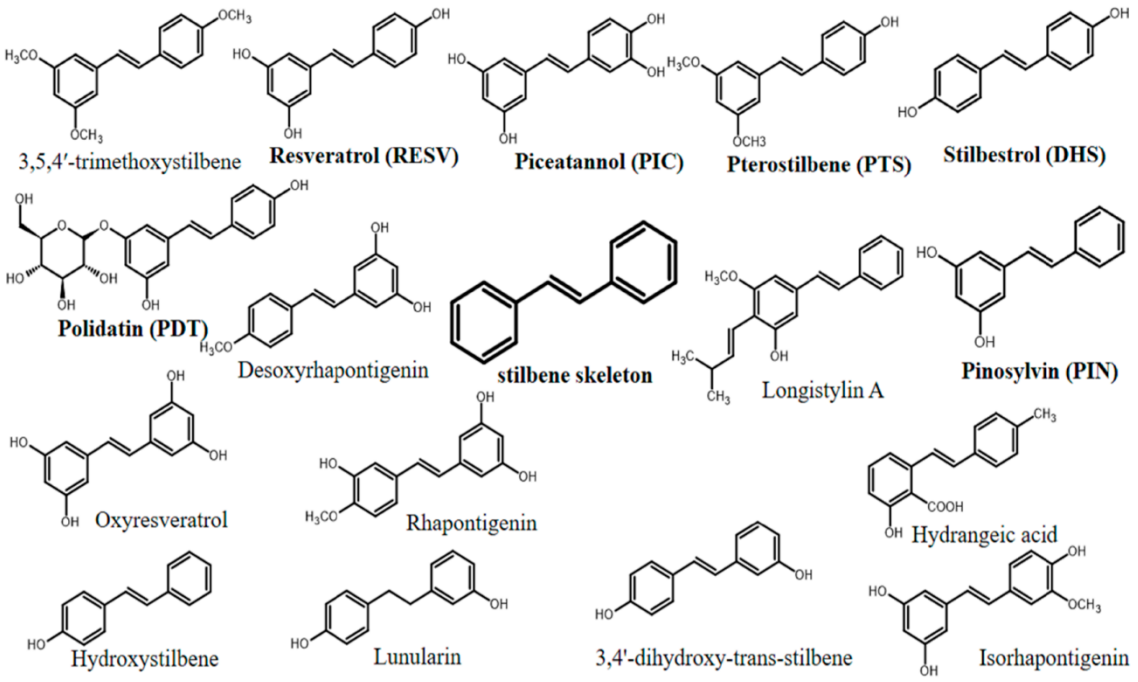

Figure 9. Chemical Structures of Stilbenes (Mendonça et al., 2024).

2.5. Lignans

Lignans (Figure 10) are phenolic compounds formed by the dimerization of two phenylpropane units. They are found in linseed, vegetables, fruits, nuts, oilseeds, and beverages such as wine, tea, and coffee. Lignans exhibit various biological activities, including antioxidant, antitumor, anti-inflammatory, and antiviral effects, making them valuable in both traditional and modern medicine. Recently, their structural diversity and potential for further biological applications have been highlighted (Zhang et al., 2022). They are promising compounds for managing various diseases, especially those linked to lifestyle. For example, podophyllotoxin shows anticancer effects by blocking tubulin formation, which stops cell division and limits cancer cell growth (Chhillar et al., 2021). Figure 10 below illustrates the chemical structures of several selected lignans.

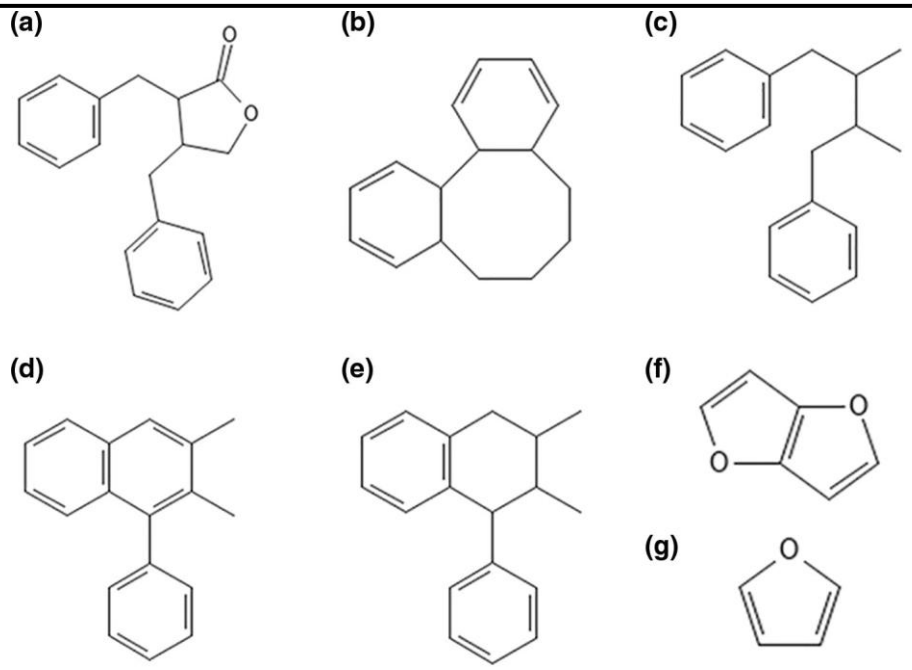

Figure 10. Representative chemical structures of major lignan classes: (a)dibenzylbutyrolactone, (b) dibenzocyclooctadiene, (c) dibenzylbutane, (d) aryltetralin, (e) arylnaphthalene, (f) furofuran, and (g) furan (Singh et al., 2024).

3. Mechanisms of action of polyphenols

Studies show that free radicals accumulate with age, accelerating aging and increasing the risk of diseases such as cancer and tumors. Polyphenols, widely present in plants, are low in toxicity and exhibit broad pharmacological effects. Their multiple hydroxyl groups allow them to neutralize free radicals, converting them into stable compounds, which helps prevent oxidative chain reactions, slow aging, and reduce disease risk. These properties make polyphenols valuable for new drug development (Lv et al., 2021). Their mechanisms of action can include (1) inhibiting the formation of reactive oxygen species (ROS) by blocking enzymes or chelating trace metals involved in free radical generation; (2) directly scavenging ROS; and (3) strengthening or preserving the body's antioxidant defenses (Pietta, 2000).

3.1. Free radical scavenging

Numerous studies have demonstrated that the antioxidant capacity of polyphenols is closely influenced by their chemical structure, particularly the number, position, and arrangement of hydroxyl groups, which directly affect their ability to scavenge free radicals (Platzer et al., 2022; Silva et al., 2002). The contribution of polyphenols to antioxidant activity

has been widely investigated through their ability to reduce various reactive radicals. Owing to their low redox potential, polyphenols (Ar–OH) can efficiently donate hydrogen atoms to neutralize oxidizing free radicals, including superoxide ($O_2^{\bullet -}$), peroxyl (ROO•), alkoxyl (RO•), and hydroxyl (•OH) radicals, thereby terminating radical chain reactions (Andrés et al., 2023; Caruso et al., 2022) (Figure 11).

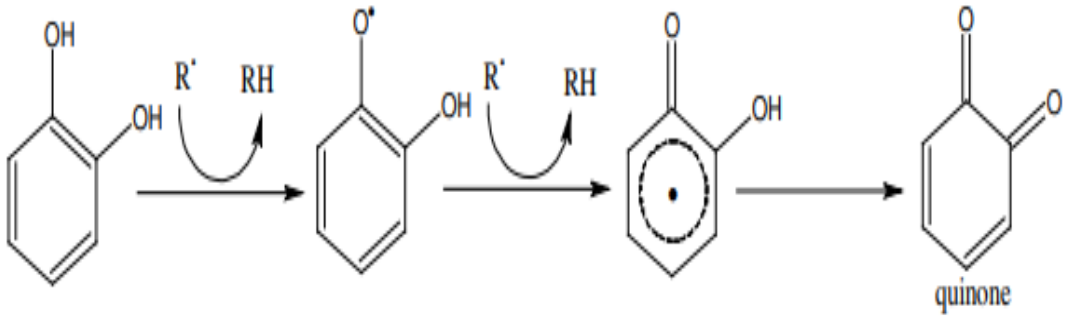

Figure 11. Reactive oxygen species (ROS) scavenging activity of polyphenols (Geng et al., 2023).

3.2. Chelation of metal ions

Transition metal ions in their reduced (low oxidation) states, such as Fe^{2+} and Cu^{+}, can catalyze redox reactions that generate free radicals.

A notable example is the Fenton reaction, in which hydroxyl radicals (•OH), among the most reactive oxygen species, are produced from hydrogen peroxide in the presence of a reduced metal ion: $H_2O_2 + M^{n+} (Fe^{2+}, Cu^{+}) \longrightarrow OH^- + \cdot OH + M^{(n+1)+}$

This reaction can occur under physiological conditions, particularly in biological environments where hydrogen peroxide accumulates. For instance, within dopaminergic neurons of the nervous system, normal dopamine catabolism leads to endogenous production of hydrogen peroxide, providing a potential substrate for Fenton chemistry and contributing to oxidative stress (Fischbacher et al., 2017; Pham et al., 2013; Wang et al., 2021). Polyphenols possess multiple functional groups, such as hydroxyl and carbonyl moieties, that can serve as potential chelation sites for metal ions (Scarano et al., 2023). Among polyphenols, flavonoids are particularly notable for their metal-chelating capacity due to their characteristic structural

features. In flavonoids containing a 4-carbonyl group and hydroxyl substituents at positions 3′, 4′, 3, and 5, three distinct chelation sites can be identified. For instance, quercetin possesses three primary metal-binding domains: the catechol moiety on ring B, the 3-hydroxyl and 4-oxo group on ring C, and the 5-hydroxyl and 4-oxo group between rings A and C (Figure 12). These sites enable effective chelation of transition metal ions such as Fe^{2+}, Fe^{3+}, Cu^{2+}, and Zn^{2+}, thereby contributing to the antioxidant potential of quercetin and related flavonoids (Cherrak et al., 2016; Kejík et al., 2021; Scarano et al., 2023).

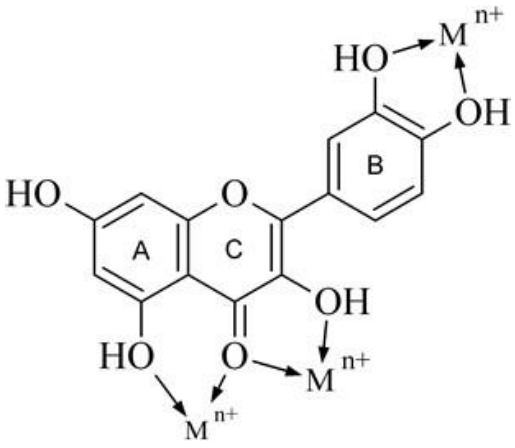

Figure 12. Chelation metal ions by potential flavonoid sites (Kasprzak et al., 2015).

3.3. Enzyme inhibition

The inhibition of enzymes responsible for generating free radicals in biological systems is a key antioxidant mechanism of polyphenols. Numerous studies have highlighted that flavonoids are the primary compounds involved in this activity (Li et al., 2021; Majumder et al., 2017; Nile et al., 2018), acting through inhibitor-enzyme complex formation and/or direct ROS scavenging. Flavonoids are known to suppress enzymes that contribute to the production of superoxide anions, such as xanthine oxidase and protein kinase C. They also inhibit a variety of other enzymes involved in reactive oxygen species (ROS) production, including cyclooxygenase, lipoxygenase, microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase, and NADH oxidase (Iglesias et al., 2019; Salehi et al., 2020; Sroka et al., 2017; Zhou et al., 2024).

4. Extraction methods of polyphenols

Extracting polyphenols from plant matrices is a critical step for their study and

characterization. Both conventional and non-conventional methods are employed for this purpose. Conventional extraction techniques typically include maceration, Soxhlet extraction, liquid–liquid extraction (LLE), and solid–liquid extraction (SLE). In contrast, non-conventional methods encompass advanced techniques such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), and pressurized liquid extraction (PLE) (Ajila et al., 2011).

4.1. Conventional extraction methods

Extracting polyphenols from plants is important because they are usually present in small amounts. Conventional methods, often preceded by drying, grinding, or crushing, include maceration, Soxhlet extraction, percolation, decoction, and heat reflux extraction. These techniques use solvents to separate compounds from the plant material. Percolation slowly passes solvent through the sample over one to two days. Decoction boils the sample to extract water-soluble compounds but is not suitable for heat-sensitive ones. Heat reflux extraction uses controlled heating to speed up extraction. Soxhlet extraction is effective for complete extraction but it takes a long time and uses a lot of solvent and energy. Maceration is simpler, uses lower temperatures, and can give higher yields in less time, but it still takes time to reach full extraction and may have batch variations. Overall, conventional methods are easy to use but can be time-consuming, use large amounts of solvent, and risk degrading some compounds (Sridhar et al., 2021).

4.2. Unconventional Extraction Techniques

Conventional extraction methods (maceration, percolation, Soxhlet) are widely used for their simplicity and low cost but suffer from low efficiency, high solvent consumption, and long extraction times. To overcome these limitations, innovative techniques such as microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), pressurized liquid extraction (PLE), supercritical CO_2 extraction (SC–CO_2), and enzyme-assisted extraction (EAE) have been developed. These methods offer higher yields, reduced solvent use, shorter extraction times, and improved selectivity (Alara et al., 2021).

4.2.1. Microwave-Assisted Extraction (MAE)

MAE uses microwave energy to heat solvents and enhance compound diffusion from plant

matrices. It reduces extraction time and solvent volume but may degrade heat-sensitive compounds like anthocyanins. Extraction efficiency depends on solvent type (e.g., ethanol, water), microwave power, time, and sample characteristics (Bagade & Patil, 2021).

4.2.2. Ultrasound-Assisted Extraction (UAE)

UAE applies ultrasonic waves (20–2000 kHz) to induce cavitation, disrupting plant cells and accelerating extraction. It is simple, cost-effective, and efficient for polyphenol extraction at low temperatures and short times. Prolonged sonication or high energy may degrade sensitive compounds (Wen et al., 2018).

4.2.3. Pressurized Liquid Extraction (PLE)

Also known as accelerated solvent extraction, PLE uses high pressure (3.3–20.3 MPa) and temperature (40–200 °C) to enhance solubility and desorption of compounds. It allows rapid extraction with minimal solvents and can use subcritical water. Solvent selection and process parameters (temperature, time, flow rate) are critical for optimizing phenolic extraction (Alvarez-Rivera et al., 2020).

4.2.4. Supercritical Carbon Dioxide (CO_2) Extraction (SC–CO_2)

This technique uses CO_2 at supercritical conditions (≥31.6 °C, 7.386 MPa) as a solvent. It preserves thermolabile compounds, is eco-friendly, and offers tunable extraction conditions. To extract polar compounds, such as polyphenols, polar co-solvents (e.g., ethanol) are added. Optimization of co-solvent type, percentage, pressure, and temperature is essential (Chañi-Paucar et al., 2022).

4.2.5. Enzyme-Assisted Extraction (EAE)

EAE employs enzymes (cellulases, pectinases, hemicellulases) to degrade plant cell walls and release bound bioactives. It improves polyphenol extraction from lignin-rich matrices. Enzymatic pretreatment enhances extraction efficiency and is particularly effective for recovering phenolics from plant wastes (Łubek-Nguyen et al., 2022).

5. Biological properties

The concept of food as a means to promote health dates back to Hippocrates over 2000 years ago. Phenolic compounds, natural phytochemicals predominantly found in fruits and vegetables, have attracted growing attention for their role in the prevention and management of

chronic diseases such as cardiovascular disorders, cancer, obesity, diabetes, and infections. Characterized by one or more benzene rings and often occurring as glycosides, phenolic compounds are associated with a reduced risk of various diseases (Rasouli et al., 2017).

5.1. Antioxidant properties

The antioxidant properties of phenolic compounds are largely determined by the number and position of their hydroxyl groups. These compounds act as effective free radical scavengers by donating electrons, thereby stabilizing reactive species and halting lipid peroxidation chain reactions. In addition to their radical scavenging activity, polyphenols exhibit metal-chelating properties, binding transition metals such as Fe^{2} and Cu^{2}, which catalyze free radical formation via Fenton reactions. By sequestering these metals, polyphenols prevent oxidative damage to biomolecules, including DNA, and contribute to overall antioxidant protection (Rathod et al., 2023). Previous studies have strongly highlighted the antioxidant properties of phenolic compounds, which have been extensively evaluated using various in vitro assays such as DPPH radical scavenging, ABTS radical cation decolorization, ORAC, FIC, FRAP, FIKFIC, PAA, β-Carotene bleaching assay, NO and others (Ali et al., 2024; Deseo et al., 2020; George et al., 2022; Loucif et al., 2020; Moldoveanu & Oden, 2021).

5.2. Anticancer properties

Cancer remains a major global health concern, with its incidence continuing to rise and effective therapies still limited. Plant-derived functional foods have attracted considerable attention due to their safety and therapeutic potential. Over the past 10–15 years, the relationship between polyphenol consumption and cancer risk has been extensively investigated (Bhosale et al., 2020). Numerous studies have emphasized the significance of polyphenols as promising anticancer agents, as well as their potential to alleviate the side effects of anticancer chemical treatments like chemotherapy (Guzelmeric et al., 2022; Hashemzaei et al., 2017; Jakobušić Brala et al., 2023).

5.3. Anti-inflammatory properties

Inflammation is involved in many diseases such as cancer, type II diabetes, obesity, arthritis, neurodegenerative, and cardiovascular disorders. Polyphenols from plants have shown anti-inflammatory and antioxidant activities, making them promising therapeutic agents. They

help regulate inflammation by modulating pro-inflammatory genes (cytokines, lipoxygenase, nitric oxide synthases, cyclooxygenase) and scavenging reactive oxygen species (ROS). These effects have been widely investigated in both experimental and epidemiological studies (Yahfoufi et al., 2018). Many clinical studies have explored the anti-inflammatory effects of dietary polyphenols. A recent meta-analysis reviewed 47 randomized controlled trials with 3852 participants, covering 15 types of polyphenols such as curcumin, quercetin, resveratrol, and tea polyphenols. These studies showed that polyphenols can reduce disease activity, lower inflammation markers (C-reactive protein and erythrocyte sedimentation rate), and decrease oxidative stress, without causing more side effects. Overall, these results support the role of polyphenols in managing inflammation and chronic diseases (Long et al., 2023).

5.4. Cardioprotective properties

The World Health Organization (WHO) reports that cardiovascular diseases are the main cause of death worldwide. In recent years, unhealthy eating habits, less physical activity, and lifestyle changes have increased the number of people affected. The risk is even higher for those who consume alcohol, tobacco, or drugs. These diseases mainly affect the heart and blood vessels and include problems like heart attacks, strokes, blocked arteries, and other heart and blood circulation issues (Alotaibi et al., 2021). Several studies have demonstrated a link between the intake of polyphenolic compounds and a reduced risk of cardiovascular diseases (Balea et al., 2018; Ganapathy et al., 2020; Sergazy et al., 2020; Tesfaye et al., 2021).

5.5. Antimicrobial properties

The increase in antibiotic resistance against human pathogenic microorganisms is a major public health challenge. Routine antibiotic use has led to resistance and even multidrug resistance in many bacterial populations. Additionally, concerns about residues of antimicrobial agents in the environment have increased among consumers. Accordingly, the identification of natural antimicrobials effective against both pathogenic and spoilage microorganisms has become a priority (Bouarab Chibane et al., 2019). Polyphenols exhibit significant antioxidant potential, one of the key mechanisms by which natural extracts inhibit the growth of pathogenic microorganisms (Fei et al., 2018). The antimicrobial activity of these compounds has been widely studied, with numerous investigations demonstrating their ability to inhibit a broad

range of pathogenic microorganisms (Prevete et al., 2024; Shehata et al., 2021; Sun et al., 2021).

5.6. Other biological properties

Studies highlighted the relationship between the consumption of polyphenols and bone health. Polyphenols play a crucial role in managing osteoporosis and improving bone mass (Wang & Hu, 2023), especially in postmenopausal women (Salvio et al., 2023). In addition to their effects on bone health, dietary flavonoids have been shown to contribute significantly to the prevention and alleviation of cognitive disorders (Godos et al., 2024). Furthermore, polyphenols enhance and prolong the physiological actions of catecholamines in animals by protecting these agents from enzymatic degradation, which contributes to their neuroprotective effects (Rebas et al., 2020). Beyond these benefits, polyphenols also exhibit hepatoprotective properties by shielding liver cells from toxins, reducing liver inflammation, and improving lipid metabolism, making them particularly valuable in managing liver diseases such as non-alcoholic fatty liver disease (Yamamoto et al., 2022). Moreover, polyphenols have been extensively studied for their potential as antidiabetic agents, with research spanning cell models, animal studies, human trials, and clinical studies (C. Sun et al., 2020).

EXPERIMENAL PART

Material and methods

I. Material and methods

1. Chemicals and reagents

Analytical grade chemicals and reagents were used throughout the experimental study. They were purchased from Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO, USA), Biochem Chemopharma (Montreal, Canada), VWR Chemicals (AnalaR NORMAPUR, France), and Alfa Aesar (Thermo Fisher Scientific, USA).

β-Carotene, ascorbic acid, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), ethylenediaminetetraacetic acid (EDTA), α-amylase (from *Aspergillus oryzae*), diclofenac sodium, acarbose, DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), dimethyl sulfoxide (DMSO), ferrozine, bovine serum albumin (BSA), starch, potassium–sodium tartrate, 3,5-dinitrosalicylic acid (DNS), linoleic acid, Tween 20, potassium phosphate monobasic (KH_2PO_4), potassium phosphate dibasic (K_2HPO_4), trichloroacetic acid (TCA), dipotassium hydrogen orthophosphate anhydrous, potassium ferricyanide, iron(III) chloride ($FeCl_3$), potassium persulfate, acetone, chloroform, ethanol, methanol, hexane, sodium hydroxide (NaOH), phenolphthalein, silver nitrate ($AgNO_3$), potassium chromate (K_2CrO_4), potassium iodide (KI), acetic acid, sodium thiosulfate, starch flour, sodium carbonate, Folin–Ciocalteu reagent, and distilled water.

2. Plant material

Fresh, mature fruits of *Myrtus communis* L. were harvested in November 2019 from the Amizour region, Béjaïa Province, in northeastern Algeria (36.642° N, 4.903° E; altitude: 96 m). The fruits were collected in sufficient quantity to perform all planned analyses. Upon delivery to the Laboratory of Biomathematics, Biophysics, Biochemistry, and Scientometry, the samples were carefully washed with distilled water to remove surface contaminants. They were subsequently prepared for a series of investigations.

3. Effect of drying methods on black myrtle (*Myrtus communis* L.) fruit samples

A quantity of cleaned and prepared fresh fruits was sliced into quarters using a knife, then further divided into equal-sized pieces.100 grams of each sample were dried using four selected methods: freeze drying (FD), sun drying (SD), oven drying (OD), and microwave drying

(MWD), until their weight remained constant over repeated measurements.

Freeze Drying (FD): The freeze-drying process was conducted using a laboratory-scale freeze dryer (D-37520, Christ, Germany). Sliced fresh *Myrtus communis* L. fruits were initially frozen at –55°C to solidify their water content. They were then subjected to primary drying under a vacuum pressure of –0.07 mBar, which facilitated the direct sublimation of ice into vapor. The entire drying cycle lasted 20 hours, during which samples were weighed at regular intervals until a stable weight was achieved, indicating complete moisture removal. Secondary drying further reduced residual moisture to minimal levels, enhancing the stability and shelf life of the freeze-dried product.

Sun Drying (SD): The drying process involved exposing fresh *Myrtus communis* L. fruits to natural sunlight under ambient conditions, with temperatures ranging from 25 to 27°C. The fruits were evenly arranged in a single layer on clean drying trays to promote adequate air circulation and uniform drying. This process lasted for 120 hours (5 days), during which the samples were periodically turned to ensure consistent moisture removal and minimize the risk of microbial growth. Drying continued until the samples reached a stable weight, indicating complete moisture loss.

Oven Drying (OD): Fresh *Myrtus communis* L. fruits were washed, sliced, and uniformly arranged in a single layer on glass petri dishes for consistent drying. The samples were placed inside a ventilated drying oven (UF 55, Memmert GmbH, Germany) with controlled airflow to promote even heat distribution and moisture removal. Drying was conducted at a constant temperature of 50°C for 9 hours, a condition selected to balance efficient moisture evaporation while minimizing thermal degradation of sensitive bioactive compounds. The fruits were weighed at regular intervals until a constant weight was reached, indicating the completion of drying.

Microwave Drying (MWD): Microwave drying was carried out using a domestic microwave oven (Samsung ME6124T-1, Malaysia) operating at a constant power of 300 W. Fresh *Myrtus communis* L. fruits were sliced and evenly spread in a microwave-safe glass container, positioned at the center of the oven to ensure uniform exposure to microwave radiation (2450 MHz). The drying process lasted 30 minutes, during which internal moisture

was rapidly removed through volumetric heating. This technique significantly reduced drying time compared to conventional methods by directly exciting water molecules within the sample. Table 5 below summarizes the conditions applied in the different drying methods.

Table 5. Drying methods, systems and parameters used in the study.

Drying method	Drying system and drying parameters	Drying time
Freeze drying (FD)	Freeze dryer (D-37520, Christ, Germany), -55°C, -0,07 mBar pressure	20 h
Sun drying (SD)	Room temperature (range from 25 to 27°C)	120 h
Oven drying (OD)	Drying oven (UF 55, Memmert, GmbH, Germany), drying temperature: 50°C	09 h
Microwave drying (MWD)	Microwave apparatus (ME6124T-1, Samsung, Malaysia), power: 300 W	0.5 h

3.1. Extraction procedure

After drying and grinding, the obtained powders and fresh myrtle fruits were subjected to the extraction of phenolic compounds. The extraction parameters used in this section were selected based on the previous optimization by Mokrani and Madani (2016), with some modifications. Briefly, 500 mg of each sample was mixed with 20 mL of solvent (acetone 50%) and extracted in a shaking water bath at 40°C for 180 min. The extracts were then centrifuged at 5000 rpm for 10 min, filtered in Whatman paper, and kept at -20°C before analyses.

3.2. Evaluation of phenolic compounds content

Before evaluating the phenolic compounds content, the moisture content in fresh fruits was determined in order to calculate phenolic concentrations on a dry weight basis. Briefly, in triplicate, each fresh fruit sample (10 g) was sliced into tiny pieces and was dried in an oven at 105°C (Memmert, Germany) until weight stabilization (Fabio Correddu et al., 2019).

3.2.1. Total phenolic content (TPC)

The method recommended by Singleton and Rossi (1965) was adopted to assess the phenolic compounds content of fresh and dried samples**.** The Folin-Ciocalteu reagent, a mixture

of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$), is reduced in the presence of polyphenols to form a mixture of blue tungsten (W_8O_{23}) and molybdenum (Mo_8O_{23}) oxides. The blue coloration produced is directly proportional to the amount of phenolic compounds present in the reaction mixture (Plaza et al., 2012). Briefly, 200 µL of myrtle extract were mixed with 1.0 mL of Folin-Ciocalteu's reagent (10%, v/v) and 0.8 mL of sodium carbonate (7.5%, w/v). The mixtures were vortexed and incubated in the dark at room temperature for 30 min. The absorbance was then read at 765 nm using a UV-VIS spectrophotometer (Shimadzu UV mini1240, Suzhou Jiangsu, China) against a blank. The value of TPC was calculated as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW) using the linear equation of the gallic acid standard curve as follows: $y = 8.985 x + 0.0068$ ($R^2 = 0.9984$). All measurements were carried out in triplicate.

3.2.2 Total flavonoids content (TFC)

Total flavonoids content was measured using the aluminum chloride colorimetric method according to the protocol of Santas et al. (2008) with some modifications. The quantification of flavonoids was based on the formation of the complex flavonoid-aluminum chloride. This reaction causes a shift in the color absorbed by the complex towards longer wavelengths and an increase in the amount of light absorbed at the new wavelength. The rate of light absorbed at this new wavelength can be measured spectrophotometrically to determine the amount of flavonoids present in the sample (Fernandes et al., 2012). To perform the assay, 1 mL of the sample extract was mixed with an equal volume of 2% $AlCl_3$. After incubation in the dark for 10 min at room temperature, the absorbance was measured at 410 nm against a blank. The value of TFC was determined as milligrams of quercetin equivalents per gram of dry weight (mg QE/100 g DW) using the linear equation of the quercetin standard curve as follows: $y = 19.43 x – 0.0095$ ($R^2 = 0.9956$). All tests were performed in triplicate.

3.2.3. Total flavonols content

Total flavonols content in dried and fresh fruits was estimated according to the method of Yermakov et al. (1987). To each 2 mL of extract, 2 mL of 2% aluminum trichloride and 3 mL of 50 mg/mL sodium acetate were added. After incubation for 2.5 h at 20°C, the absorbance was measured at 440 nm. A calibration curve with rutin as standard was employed and total

flavonols content was calculated as milligrams of rutin equivalent per gram of dry weight (mg RE/g of DW).

3.2.4. Total condensed tannins content (CTC)

The approach of Sun et al. (1998) was adopted to assess the amount of total CTC. The vanillin-hydrochloric acid assay is simple, quick, and widely used to quantify proanthocyanidins in plant material. Hence, in an acidic environment, monomeric or polymeric flavan-3-ols associate with vanillin to give a red color complex detectable at 500 nm. The intensity of the red color is proportional to the amount of proanthocyanidins present in the sample (Mitsunaga et al., 1998). An aliquot of 200 µl from each extract was combined with 500 µl of 1% vanillin in methanol and 500 µl of HCl (9M in methanol). The mixtures were incubated at 30°C for 20 min. The absorbance was measured at 500 nm against a blank. The TPAC was calculated as milligrams of catechin equivalents per gram of dry weight (mg CE/g DW), referring to the linear equation of catechin standard curve as follows: $y = 1.344x - 0.0034$ ($R^2 = 0.9986$). Quantifications were assessed in triplicate.

3.2.5. Total anthocyanins content (AC)

Total anthocyanins content in fresh and dried myrtle samples was determined using pH differential method described by Wang and Xu (2007) with two buffer systems: a potassium chloride buffer with pH 1.0 (0.025 M) and a sodium acetate buffer with pH 4.5 (0.4 M). Briefly, 1 mL of each sample was mixed with 4 mL of the corresponding buffer solution. The absorbance was then measured at wavelengths of 510 nm and 700 nm. The following equation was used:

$$TA = \frac{A \times MW \times DF \times 1000 \times V}{\varepsilon \times \iota \times m}$$

Where $A = (A_{520nm} - A_{700nm})\ pH_{1.0} - (A_{520nm} - A_{700nm})\ pH_{4.5}$

MW refers to the molecular weight of cyanidin-3-glucoside (449.2 g/mol), DF is the dilution factor, l is the path length of the cuvette (in cm), **ε** denotes the molar extinction coefficient of cyanidin-3-glucoside (26,900 $L \cdot mol^{-1} \cdot cm^{-1}$), 1000 is the conversion factor from grams to milligrams, V represents the total volume of extract (in mL), and m is the mass of the sample used (in g). The anthocyanin content was expressed as milligrams cyanidin-3-glucoside equivalents (CGE) per gram of each sample (fresh and dry fruits).

3.3. Determination of antioxidant capacity

Several antioxidant tests were used to assess the extracts' antioxidant potential, including DPPH radical scavenging, ABTS radical scavenging, ferric reducing power (FRP), and phosphomolybdenum antioxidant activity (PAA) assays. These tests involve different mechanisms of action by which antioxidants counteract harmful free radicals.

3.3.1. DPPH assay

The DPPH radical neutralization activity was determined using the proposed method of Blois (1958). This method is commonly applied to estimating the antioxidant activity of single compounds and plant extracts. In this assay, the amount of disappeared DPPH° in the examined system, determined spectrophotometrically, measures the compounds' antioxidative (hydrogen-donating) activity (Abd Rashed et al., 2017). This approach relies on neutralizing DPPH by introducing an antioxidant, which leads to the discoloration of the DPPH solution. The evaluation of antioxidant activity is subsequently determined by the reduction in absorption at 517 nm. Briefly, to 50 µl of each sample extract 950 µl of DPPH solution (0.04% in methanol) was added. The mixtures were placed in the dark at room temperature for 20 min and their changes in absorbance were read at 517 nm against a blank. The DPPH-RSA was determined as milligrams trolox equivalents per gram of dry weight (mg TE/ g DW) exploiting the linear equation of the trolox standard curve as follows: $y = -0.0889\ x + 0.8097$ ($R^2 = 0.9989$). All measurements were done in triplicate.

3.3.2. ABTS assay

The capacity of myrtle fruit extracts for scavenging the ABTS°+ free radical was determined using the 2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS) decolorization assay according to the method of Re et al. (1999) based on the reduction of $ABTS^{\bullet+}$ radical generated by oxidizing ABTS with potassium persulfate. This reduction occurs in the presence of an antioxidant inducing the proportional decolorization of the solution in correlation with the antioxidant concentration. Briefly, a concentrated ABTS solution of 7 mM in 10 mL distilled water was prepared, and then mixed in equal volume with potassium persulfate solution (2.45mM) to generate the ABTS·+ radical after incubation in the dark at room temperature for 16 h. The resulting ABTS solution was then diluted with ethanol to have an absorbance of 0.700 ± 0.02 at 734 nm. Myrtle extract or trolox standard (50 µl) was allowed

to react with 1.95 mL of diluted ABTS solution, and the absorbance was read at 734 nm after 6 min using a UV-VIS spectrophotometer. The ABTS-RSA was expressed as milligrams trolox equivalent per gram of dry weight (mg TE/g DW) ($y = 4.0357 x + 0.6573$ ($R^2 = 0.9981$). All tests were performed in triplicate.

3.3.3. Ferric reducing power (FRP) assay

The ability of myrtle fruit extracts to reduce Fe3+ to Fe2+ was determined according to the potassium ferricyanide-ferric chloride method of Oyaizu (1986). Compounds exhibiting reduction potential undergo a reaction with potassium ferricyanide, leading to the formation of potassium ferrocyanide. Subsequently, this compound interacts with ferric chloride to generate a colored ferric-ferrous complex, characterized by its peak absorption at 700 nm (Gupta et al., 2016). Briefly, 1 mL of each myrtle extract was mixed with 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide (w/v). The obtained solutions were incubated in a water bath at 50°C for 20 min, followed by the addition of 2.5 mL of 10% Journal Pre-proof 10 trichloroacetic acid (w/v). A fraction of 2.5 mL of the resulting solution was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% FeCl3 (w/v) and vortexed. The absorbance of the colored complex was read at 700 nm against a blank. Ferric reducing power was determined as milligrams of ascorbic acid equivalents per gram of dry weight (mg AAE/ g DW) using the linear equation of the ascorbic acid standard curve as follows: $y = 9.91 x + 0.003$ ($R^2 = 0.999$). The assays were carried out in triplicate.

3.3.4. Phosphomolybdenum antioxidant activity (PAA) assay

The method validated by Prieto et al. (1999) was used to estimate the phosphomolybdenum antioxidant activity of the extracts. This assay relies on the conversion of Mo (VI) to Mo (V) by the antioxidants present in the sample, followed by the creation of a green phosphate/Mo (V) complex under acidic conditions. Briefly, 0.1 mL of the extract was combined with 1 mL of reagent solution (consisting of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) in a test tube. The tube was then vortexed, sealed and placed in a water bath at 95°C for 90 min. Upon cooling to room temperature, the absorbance of each extract was measured at 695 nm against a blank. The phosphomolybdenum antioxidant activity was donated as milligrams of Trolox equivalents per gram of dry weight (mg TE/g DW) using the linear

equation of the Trolox standard curve as follows: y = 1,6x - 0,0247 (R^2 = 0,9998). The assays were carried out in triplicate.

4. Optimization of extraction conditions of phenolic compounds and antioxidant activity from Myrtle (*Myrtus communis* L.) Fruit

4.1. Extract preparation

Freeze-dried myrtle fruit powder (13.5 mg), obtained using the most suitable drying method identified in preliminary tests, was weighed into a glass vial and added to 20 mL of extracting solvent. Extraction was performed using a stirring water bath at different temperatures and extraction times. The mixture was centrifuged at 5000 rpm at 4°C for 15 min and then filtered through Whatman paper. The extraction process was conducted in duplicate. The extracts were stored at 4°C until analysis. These extracts were used to determine the total phenolic content (TPC), total flavonoid content (TFC), total proanthocyanidins content (TPAC) and the antioxidant activity including DPPH-radical scavenging activity (DPPH-RSA), ABTS-radical scavenging activity (ABTS-RSA), and ferric reducing power (FRP).

4.2. Experimental Design

In this work, a single-factor experiment approach was adopted to determine the optimal conditions for obtaining the highest yields of phenolic compounds and antioxidant activity from myrtle berries. Five extraction parameters were studied including solvent type (50% acetone, 50% methanol, 50% ethanol and water), acetone concentration (30, 50, 70 and 100%; v/v), solvent acidity (0, 0.001, 0.005, 0.01, 0.05, and 0.1 N), extraction time (30, 90, 180, 270 and 360 min), and extraction temperature (20, 25, 30, 35 and 40°C). One parameter was varied at a time while other parameters were kept constant at the optimal level previously determined. The optimal extraction conditions were selected based on two criteria: firstly, by referring to the TPC; secondly, by considering other antioxidant assays (TFC, TPAC, DPPH-RSA, ABTS-RSA, and FRP).

4.2.1. Effect of solvent type extraction

Solvent extraction is a process designed to separate soluble phenolic compounds by diffusion from a solid matrix (plant tissue) using a liquid matrix (solvent). This process is widely employed for phenolic extraction from various plant materials (Lapornik et al., 2005; Li

et al., 2006). Fixing the extraction time at 180 min and the extraction temperature at 40°C, powdered myrtle berries were extracted using different types of solvents, including 50% acetone, 50% ethanol, 50% methanol, and water.

4.2.2. Effect of solvent concentration

Based on the previously identified best extraction solvent, myrtle fruits were macerated with different concentrations of solvent (30, 50, 70, and 100%, v/v) at an extraction time of 180 min and an extraction temperature of 40°C.

4.2.3. Effect of solvent acidity

The powdered myrtle berries were extracted using the best solvent type and gradient as confirmed in the previous steps at a ratio of 85% of the best solvent type and 15% of HCl (85:15, v/v). Different concentrations of HCl (0.001, 0.005, 0.01, 0.05, and 0.1 N) were used, while keeping the extraction time and temperature at 180 min and 40°C, respectively.

4.2.4. Effect of extraction temperature

Myrtle fruits were extracted using the best solvent concentration and acidity determined in the previous steps. However, the extraction temperature was ranged from 20 to 40°C (20, 25, 30, 35, and 40°C). The extraction time was set to 180 min.

4.2.5. Effect of extraction time

Applying the best conditions determined previously (solvent type, solvent gradient, solvent acidity, and extraction temperature), powdered myrtle berries were extracted at different times ranging from 30 to 360 min (30, 90, 180, 270, and 360 min).

The extract obtained under each parameter was analyzed to quantify TPC, TFC, and proanthocyanidins, as well as to evaluate antioxidant activity (DPPH radical scavenging, ABTS radical scavenging, and FRP), using the same analytical protocols employed to investigate the impact of drying methods on the phenolic compounds of myrtle fruits.

5. Phytochemical characterization of myrtle (*Myrtus communis* L.) fruit polyphenols

5.1. Extraction of phenolic compounds

Phenolic compounds were extracted from freeze-dried myrtle fruit samples using solid/liquid extraction. Briefly, 10 g of myrtle fruit powder were added to 100 mL of 50% acetone. The mixture was extracted in a shaking water bath under the optimized conditions

described by Taibi et al. (2024), using 50% acetone at 40 °C for 180 minutes. The resulting extracts were centrifuged at 5000 rpm for 10 min. Supernatants were collected and stored at 4°C, while the pellets were subjected to a second extraction under the same conditions. The extracts from both extractions were combined and evaporated in a ventilated oven at 40°C, followed by lyophilization. The obtained dry extracts were stored at 4°C for further analysis.

5.2. Evaluation of phenolic compounds content

Before performing chromatographic analysis of the myrtle fruit extract, a comprehensive assessment of its phenolic profile was conducted. This included the quantification of total phenolic content, total flavonoid content, condensed tannin content, and anthocyanin content.

5.2.1. Total phenolic content (TPC)

The total phenolic content (TPC) was determined using the colorimetric method of Singleton and Rossi (1965). The dry extract was dissolved in methanol at a concentration of 1mg/mL, and an aliquot of 200 µL was added to 1 mL of 10% Folin-Ciocalteu reagent and 0.8 mL of 7.5% sodium carbonate solution. The absorbance was then read at 765 nm after incubation in the dark for 30 min. The results were expressed as mg equivalent gallic acid per gram of dray extract (mg GAE/g).

5.2.2. Total flavonoids content (TFC)

The total flavonoid content was measured utilizing the modified protocol of Santas et al. (2008). Briefly, 1 mL of the sample extract was mixed with an equal volume of 2% $AlCl_3$. After 10 min of incubation in the dark at room temperature, the absorbance was recorded at 410 nm. Quercetin served as a positive standard for the calibration curve, and the flavonoid content was expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g).

5.2.3. Condensed tannins content (CTC)

Total condensed tannin content (CTC) was quantified using the vanillin-hydrochloric acid method as described by Sun et al. (1998). Briefly, 200 µL of sample extract was incubated with 500 µL of 1% vanillin in methanol and 500 µL of 9 M HCl in methanol at 30°C for 20 min. the absorbance was taken at 500 nm against a blank. Total CTC was quantified using the linear equation from the catechin standard calibration curve.

5.2.4. Anthocyanins content (AC)

Total anthocyanin contents in fresh and dried myrtle samples were determined using the pH differential method described by Wang and Xu (2007), with two buffer systems: a potassium chloride buffer with pH 1.0 (0.025 M) and a sodium acetate buffer with pH 4.5 (0.4 M). Briefly, 1 mL of the dry extract, reconstituted in methanol at a concentration of 1 mg/mL, was mixed with 4 mL of the corresponding buffer solution. The absorbance was then measured at wavelengths of 510 nm and 700 nm. The following equation was used:

$$TA = \frac{A \times MW \times DF \times 1000 \times \mathrm{V}}{\varepsilon \times \iota \times \mathrm{m}}$$

Where $A = (A_{520nm} - A_{700nm})\ pH_{1.0} - (A_{520nm} - A_{700nm})\ pH_{4.5}$

MW denotes the molecular weight of cyanidin-3-glucoside (449.2 g/mol), DF is the dilution factor applied during sample preparation, l represents the path length of the cuvette in centimeters (cm), ε is the molar extinction coefficient of cyanidin-3-glucoside (26,900 $L \cdot mol^{-1} \cdot cm^{-1}$), and 1000 is the conversion factor from grams to milligrams. V refers to the total volume of the extract in milliliters (mL), and m is the mass of the sample used, expressed in grams (g). The anthocyanin content was expressed as milligrams of cyanidin-3-glucoside equivalents (CGE) per gram of dry extract.

5.3. Identification of *Myrtus communis* fruit Phenolic Compounds by UHPLC-QqQ-MS

The identification of phenolic compounds in *Myrtus communis* berry extract were carried out using ultra-high-performance liquid chromatography (UHPLC) coupled with a triple quadrupole mass spectrometer (UPLC-QqQ-MS), following the protocol outlined by Benbouguerra et al. (2021) and Loupit et al. (2020). Analyses were performed on an Agilent 1260 Infinity system equipped with a Poroshell 120 EC-C18 column (150 × 2.1 mm, 2.7 µm), maintained at 35 °C. The mobile phases consisted of water with 0.1% formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B), with a flow rate of 0.3 mL/min. The gradient program was: 5–17.5% B (0–5 min), 17.5–33% B (5–7.5 min), 33% B (7.5–10 min), 33–40% B (10–15 min), 40–95% B (15–16 min), 95% B (16–19 min), and 5% B (19–21 min). Detection was performed using an Agilent 6430 triple quadrupole detector in electrospray ionization (ESI) mode. Multiple reaction monitoring (MRM) was applied using compound-specific transitions in either positive or negative mode. Source parameters were set as follows: gas temperature 350 °C, nitrogen flow 11 L/min, nebulizer pressure 15 psi, and capillary voltage 3000 V.

Table 6 below summarizes the retention time and corresponding percentage of each phase in the UHPLC gradient program.

Table 6. Elution Gradient for UHPLC-QqQ-MS analysis of phenolic compounds.

Time (min)	Solvent A	Solvent B
0	95 %	5%
5.00	82.5 %	17.5%
7.50	67%	33%
10.00	67%	33%
15.00	60%	40%
16.00	5%	95%
19.00	5%	95%
21.00	95%	5%

6. Study of biological properties

6.1. Study of antioxidant properties

Various analytical methods have been employed to assess the antioxidant capacity of natural substances in vitro. Nevertheless, due to the complex phytochemical composition of plant extracts and the diverse mechanisms of antioxidant action, a single assay is insufficient for comprehensive evaluation. The antioxidant activity may involve multiple mechanisms, including prevention of chain initiation, decomposition of peroxides, inhibition of hydrogen abstraction, free radical scavenging, reducing capacity, and metal chelation (Hamrouni-Sellami et al., 2013).

All analyses of the different antioxidant tests were conducted in three independent experiments, with each experiment performed in triplicate.

6.1.1. DPPH Radical scavenging activity

The antioxidant capacity of the extracts was assessed by measuring their ability to scavenge the DPPH radical (the electron-donating ability), as described by Blois (1958). Briefly, 50 µl of extract (0.25 mg/mL in methanol) were added to 950 µl of DPPH solution (0.04% in methanol).

The incubation conditions and expression of antioxidant capacity were conducted as detailed previously.

Different concentrations of both extract (0.5, 0.25, 0.125, 0.0625 mg/mL in methanol) and standard (Trolox: 0.02-0,140 mg/mL) were prepared for calculating IC50 (mg/mL).

The DPPH$^{\bullet}$ radical scavenging ability (RSA) was calculated as inhibition percentage (%) using the following equation:

$$\text{DPPH-RSA (\%)} = \frac{A0 - A1}{A0} \; x \; 100$$

where A_0 is the absorbance of the blank (solvent + DPPH solution) and A_1 is the absorbance of the sample (extract or standard + DPPH solution). All tests were conducted in triplicate. The results were expressed as IC50.

6.1.2. ABTS Radical Scavenging Activity

The antioxidant capacity of the extracts was determined using the ABTS radical cation decolorization assay adapted from Re et al. (1999). Briefly, 50 µl of extract (0.25mg/mL in methanol) were added to 1950 µl of $ABTS^{+}$ solution. The incubation conditions and the assessment of antioxidant capacity were performed as previously described.

The IC50 values of the extract and standard were determined as described in the DPPH assay.

6.1.3. Ferric reducing power assay (FRP)

The antioxidant capacity of bioactive compounds can be assessed through their ability to donate electrons, as evidenced by the reduction of ferric ion (Fe^{3+})-ferricyanide complexes to ferrous ion (Fe^{2+}). The resulting ferrous ions react with ferric chloride to form the Prussian blue complex, which exhibits a characteristic absorbance at 700 nm. The extent of reduction is indicated by a color change in the test solution from yellow to green or blue. An increase in absorbance directly correlates with enhanced reducing power and, consequently, greater antioxidant activity (Hamrouni-Sellami et al., 2013).

The protocol of Oyaizu (1986) was involved in assessing the ferric reducing power. Briefly, A solution with a concentration ranging from 0.25 to 0.75 mg/mL of extract was prepared in methanol and the remaining steps followed the procedure outlined previously. Ascorbic acid was used as standard. The IC50 value represents the concentration at which absorbance is 0.5.

6.1.4. Ferrous ion chelating activity (FIC)

In this assay, Ferrozine quantitatively reacts with free and unbound ferrous ions (Fe^{2+}) to form a stable red-colored complex. This reaction is inhibited in the presence of chelating agents, such as antioxidants, leading to a decrease in the red color intensity. By quantifying this color reduction, the chelating capacity of the sample can be determined. This method is based on the principle that chelating agents compete with ferrozine for ferrous ions (Chelliah & Oh, 2022). The ferrous ion chelating capacity was evaluated using the method described by Adusei et al. (2019), with some modifications. Briefly, 0.1 mL of different extract concentrations (0.1, 0.5, 1.0, 1.5 and 2.0 mg/mL) in methanol were mixed with 0.05 mL of $FeCl_2$ (2 mM) and incubated in the dark for 5 min. Afterward, the reaction was initiated by adding 0.1 mL of 5 mM ferrozine, followed by dilution to a final volume of 3 mL with deionized water. After shaking and incubating at room temperature for 10 min of the resulting mixture, the absorbance was read at 562 nm. The chelating activity of the extract was calculated as:

Metal chelating effect (%) = [(A0 - A1)/A0] x 100

where: A0 represents the absorbance of the ferrozine-Fe^{2+} complex and A1 is the absorbance of the extract or standard.

Results were expressed as IC50 for both the extract and the positive control, representing the concentration required for 50% ferrous ion chelation. EDTA served as the positive control, and all assays were conducted in triplicate.

6.1.5. β-Carotene bleaching assay

The β-Carotene linolic acid system is based on the principle of β-Carotene rapid bleaching in the absence of antioxidants resulting of the coupled oxidation of β-carotene and linoleic acid, leading to free radical formation. The linoleic acid free radical targets the highly unsaturated β-carotene molecules inducing the formation of characteristic orange color, which is monitored spectrophotometrically. The introduction of an antioxidant inhibits β-carotene degradation (Kulisic et al., 2004).

The antioxidant activity of extract and standard (BHA) was measured as described by Dawidowicz and Olszowy (2015) with modifications. Briefly, a solution of chloroform and β-carotene (0.5 mg/mL) was prepared and 3 mL of this solution was transferred to a flask containing 40 mg linoleic acid and 400 mg tween 40. The chloroform was then evaporated in

an oven at 40°C and 100 mL of hydrogen peroxide (30%) was incorporated. After mixing, 3 mL of the solution formed was added to 0,5 mL of extract (50-200 µg/mL) /standard (50-250 µg/mL) and incubated for 2 h at 50°C. The absorbance at 470 nm was read both before and after the incubation period.

The antioxidant activity (AA) was calculated using the following formula:

$AA\ (\%) = [(A_{120} - C_{120}) / (C_0 - C_{120})] \times 100,$

where A_{120} and C_{120} represent the absorbance values of the sample and control, respectively, measured at 120 minutes, and C_0 represents the absorbance of the control at 0 minutes.

6.1.6. Cupric reducing antioxidant capacity (CUPRAC) assay

In this assay, in the presence of antioxidants, which act as electron donors, cupric ions (Cu^{2+}) are reduced to cuprous ions (Cu^{+}). These cuprous ions then react with a chromogenic reagent, typically Neocuproine, to form a colored complex. The intensity of the color, measured at 450 nm, is directly proportional to the amount of Cu^{+} produced, which in turn reflects the antioxidant capacity of the sample (Apak et al., 2008).

The cupric ion reducing antioxidant capacity assay was conducted following the method described by Apak et al. (2004). Briefly, 0,200 mL of extract (50-250 µg/mL) or BHA (20-100 µg/mL) were mixed with 0,250 mL of $CuCl_2$ (10 mM), 0,250 mL of Neocuproine (7.5 mM in ethanol) and 0,300 mL of CH_3COONH_4 buffer (1 M, pH = 7.0). The absorbance was taken at 450 nm after 40 min. The $A_{0.5}$ values (concentration required to achieve 50% metal chelation) were calculated by interpolating the absorbance versus extract concentration curves obtained in the ferrous ion chelating assay.

6.1.7. Nitric oxide scavenging activity

The Nitric Oxide (NO) scavenging assay was performed to assess the ability of samples, such as phenolic compounds, to neutralize NO radicals. In this assay, sodium nitroprusside (SNP) acts as a NO donor, releasing NO in an aqueous solution under physiological conditions (pH 7.4, 25–37°C). The released NO reacts with oxygen to form nitrite (NO_2^-), which is quantified using the Griess reagent, producing a pink azo dye. Antioxidants in the sample compete with oxygen for NO, reducing nitrite formation. A lower absorbance indicates greater NO scavenging activity, reflecting the sample's antioxidant potential (Apak et al., 2022).

The antiradical activity of extracts/standards in this assay was measured following the protocol outlined by Oliveira et al. (2009) with modifications. Briefly, A 100 mM potassium phosphate buffer solution (pH 7,4) was prepared and subsequently used to prepare a 1.66 mM sodium nitroprusside solution, along with varying concentrations of extract/standard (ranging from 0.0625 to 1 mg/mL), with ascorbic acid serving as the reference standard. The Griess reagent was freshly prepared by mixing equal volumes of Griess A and Griess B. Griess A consisted of 1% sulfanilamide in 2,5% phosphoric acid, while Griess B contained 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride in water. After the preparation of reagents, the assay was conducted as outlined in the following Table 7:

Table 7. Incubation protocol for Nitric oxide scavenging activity assay.

Reagents	C	E	E′
Buffer	500 µl	-	-
Extract/standard	-	500 µl	500 µl
SNP	500 µl	500 µl	500 µl
Incubation under a fluorescent lamp for 15 min			
Griess	500 µl	500 µl	-
Phosphoric acid (2,5%)	-	-	500 µl
Incubated in the dark for 10 min			
Absorbance read at 562 nm			

Where C represents the control, E refers to the extract/standard, and E′ serves as the color control. The absorbance of the color was subtracted to account for background interference.

$$\text{Nitric oxide radical scavenging activity (\%)} = \frac{AC - AE}{AC} \; x \; 100$$

Where AC represents the absorbance of the negative control, AE is the absorbance of the extract/standard. The results were expressed as IC50 values.

6.1.8. Phosphomolybdenum antioxidant activity (PAA) assay

The method outlined by Prieto et al. (1999) was applied to estimate the phosphomolybdenum antioxidant activity of the extracts. This assay relies on the conversion of Mo (VI) to Mo (V) by the antioxidants present in the sample, followed by the creation of a

green phosphate/Mo (V) complex under acidic conditions. Briefly, 0.1 mL of the extracts (10-1000 µg/mL) was combined with 1 mL of reagent solution (consisting of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) in a test tube. The tube was then vortexed, sealed and placed in a water bath at 95°C for 90 min. Upon cooling to room temperature, the absorbance of each extract was measured at 695 nm against a blank. The phosphomolybdenum antioxidant activity was determined as described in the previous section 2.9.1. IC50 of the extract and standard (trolox) were evaluated as A0.5.

6.2. Study of anti-inflammatory activity

The anti-inflammatory capacity of the extracts/standard was assessed by the protocol established by Truong et al. (2019). Various concentrations of the dry extract (0.1 to 1 mg/mL) were prepared in DMSO. Diclofenac (0.1 to 1 mg/mL), a standard anti-inflammatory agent, served as the positive control, while DMSO was used as the negative control. Equal volumes of a 1% aqueous solution of bovine serum albumin (BSA) and the extract or standard were mixed, and the pH was adjusted to 6,3. The reaction mixture was incubated at 37°C for 20 minutes, followed by heating at 51°C for 30 minutes. Upon cooling to room temperature, absorbance was measured at 660 nm using a UV-Vis spectrophotometer. The percentage inhibition of protein denaturation was calculated using the following equation:

$$BSA\ denaturation\ inhibition\ (\%) = \frac{Acontrol - A(\text{sample/standard})}{Acontrol} x\ 100$$

where Acontrol and A(sample/standard) are the absorbances of the control (BSA + DMSO) and the sample/standard (BSA + extract/standard), respectively. The results were expressed as IC50 values.

6.3. In vitro assessment of cytotoxic effects on erythrocytes

The antihemolytic activity was measured following the protocol of Malagoli (2007) with slight modifications. Erythrocytes were isolated from the blood of healthy A+ Rh donors in accordance with the International Federation of Blood Donor Organization (IFBDO) standard operating procedures. Blood samples were centrifuged at 3000 rpm for 10 minutes, and the erythrocytes were washed three to four times with 0.9% NaCl solution until the supernatant became colorless. The erythrocytes were then resuspended in phosphate-buffered saline (PBS: 137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4).

The hemolytic effect of the extract at concentrations ranging from 0,2 to 1 mg/mL was assessed by incubating 500 µL of 2% erythrocyte suspensions with an equal volume of test compounds or a positive control at 37°C for 1 hour. After incubation, the samples were centrifuged, and the absorbance of the supernatant was measured at 540 nm. Hemolysis was calculated using the formula:

$$\text{Hemolysis (\%)} = \frac{\text{Abs}_{extract}}{\text{Abs}_{positive\ control}} x100$$

6.4. Antidiabetic activity

- **alpha-amylase inhibition**

Diabetes mellitus is a cluster of metabolic disorders characterized by chronically elevated blood glucose levels, resulting from a deficiency in insulin production, impaired insulin action, or a combination of both factors. Insufficient insulin and resistance in target tissues lead to dysfunctions in the metabolic pathways of carbohydrates, lipids, and proteins (Galicia-Garcia et al., 2020). Acarbose and Voglibose are widely utilized as medications for managing type 2 diabetes; however, their use is often associated with gastrointestinal adverse effects and elevated costs. Consequently, exploring phytochemical inhibitors with minimal or no side effects represents a promising alternative for glycemic control. This approach is substantiated by scientific validation of the antidiabetic properties of medicinal plants and their bioactive constituents (Vichayanrat et al., 2002). The effect of extract/standard was evaluated as presented by Thilagam et al. (2013) with slight modifications. A volume of 0.2 mL of either the extract or a standard solution was added to 0.5 mL of potassium phosphate buffer (0.02 M, pH 6.9, containing 0.006 M NaCl) with α-amylase (0.5 mg/mL). The mixture was pre-incubated at 25°C for 10 minutes. Subsequently, 0,5 mL of a starch solution (1% in the same buffer) was added, and the reaction mixture was incubated at 25°C for an additional 10 minutes. The enzymatic reaction was terminated by the addition of 1 mL of DNS (dinitro-salicylic acid) reagent (a solution of 1 g of DNS was prepared by dissolving it in 20 mL of sodium hydroxide, followed by the gradual addition of 30 g of potassium sodium tartrate). The mixtures were incubated in a boiling water bath for 5 minutes, then cooled to room temperature. After dilution with 10 mL of distilled water, the absorbance was measured at 540 nm. Acarbose served as the positive control, and the percentage inhibition of α-amylase was calculated as follows:

$$\alpha - amylase\ inhibition(\%) = \frac{Acontrol - A(sample/standard)}{Acontrol} x\ 100$$

$Acontrol$ represents the absorbance of the negative control (DMSO in place of the extract), while $A(sample/standard)$ denotes the absorbance of the sample/acarbose. The antidiabetic activity of the extract was expressed in milligrams of acarbose equivalents per gram of dry extract (mg AE/g DE). IC50 values were calculated.

6.5. Determination of the antimicrobial activity

The rise of antimicrobial resistance, due to the excessive use of antibiotics and the adverse effects associated with synthetic antimicrobial agents, has encouraged researchers to explore new lead compounds with antimicrobial properties (Woo et al., 2023). Consequently, there is a growing interest in developing new antimicrobial agents that can reduce reliance on conventional antibiotics and combat the emergence of resistance. This need has led researchers to focus on isolating and identifying novel bioactive compounds from plants as an alternative approach to effectively address microbial resistance (Wong & Chow, 2024).

The antibacterial activity of the extracts was evaluated using the agar well diffusion method, where the antimicrobial effect was quantified by measuring the diameter of the inhibition zones surrounding the wells containing the test samples. The assay was performed against a panel of eleven bacterial strains and three fungal strains, and the results are presented in Table 8 below:

Table 8. Microbial strains tested.

Strain	Origin	Gram
Staphylococcus aureus (Foodborne)	ATCC 6538	+
Methicillin-resistant *Staphylococcus aureus* (MRSA)	ATCC 43300	+
Enterococcus faecalis	ATCC 29212	+
Bacillus subtilis	ATCC 6633	+
Bacillus cereus	ATCC 10876	+
Pseudomonas aeruginosa	DSMZ 1117	-
Acinetobacter baumannii	ATCC 19606	-
Klebsiella pneumoniae	ATCC 13883	-
Escherichia coli	ATCC 25922	-
Salmonella typhi	ATCC 14028	-
Morganella morganii	ATCC 25830	-
Aspergillus niger	ATCC 16404	Mold
Aspergillus flavus	ATCC 22546	Mold
Aspergillus ochraceous	ATCC 11028	Mold

6.5.1. Investigation of antibacterial activity

- **Verification of Bacterial Purity**

The bacterial strains were purified on Mueller-Hinton agar using the quadrant streaking method under aseptic conditions. Incubation was carried out at the optimal temperature for each strain, typically at 37°C for 24 hours.

- **Preparation of the inoculum**

Bacterial strains were streaked onto Mueller-Hinton agar plates and incubated at 37°C for 24 hours to optimize growth conditions. A Pasteur pipette was then used to collect a few well-isolated, morphologically identical colonies of each bacterial strain. The Pasteur pipette was subsequently immersed in 5 mL of nutrient broth (9 mL of sterile physiological water containing

9g NaCl per liter of distilled water) to prepare the bacterial suspension. The suspension was homogenized and incubated for 10-24 hours at 37°C. The optical density (OD) of 1 mL of the inoculum was measured using a spectrophotometer at 625 nm. The OD should be adjusted to achieve a turbidity equivalent to a 0.5 McFarland standard, or an OD ranging from 0.08 to 0.10 at 625 nm. If necessary, the inoculum concentration can be adjusted by adding additional cell culture to increase turbidity, or sterile physiological saline to dilute the suspension (CASFM, 2018).

- **Performing antibacterial susceptibility assays**

According to the solid diffusion method (CASFM, 2018), the interaction between bacterial cells and the test sample must occur on an agar medium. In this study, Mueller-Hinton agar, sterilized by autoclaving at 121°C for 20 minutes, was used as the solid medium. Bacterial inoculation was performed using the swabbing technique to ensure uniform distribution across the agar surface. A sterile swab was dipped into the bacterial suspension and then excess liquid was removed by pressing the swab firmly against the inner wall of the tube. The inoculation was carried out by streaking the swab over the entire agar surface in tight, parallel striations, covering the plate from top to bottom. This process was repeated twice, rotating the plate by 60° each time to ensure even distribution. The swab was reloaded as needed to inoculate multiple plates with the same bacterial strain.

Antimicrobial susceptibility testing was conducted following the method described by Holder and Boyce (1994), using the agar well diffusion assay. Sterile wells (8 mm in diameter) were aseptically punched into the inoculated agar medium with a sterile cork borer and filled with 50 µL of the extract at two concentrations (50 mg/mL and 100 mg/mL in DMSO). In parallel, wells containing antibiotic (amoxicillin) solutions (positive control) and DMSO (negative control) were included for comparison. Petri dishes were refrigerated at 4°C for 2 hours to facilitate the diffusion of test compounds before incubation at 37°C for 24 hours. All experiments were performed in triplicate, with three plates per extract concentration and bacterial strain, to ensure reproducibility. After incubation, the diameters of the inhibition zones, including the well diameter, were accurately measured using calipers.

6.5.2. Investigation of antifungal activity

- **Preparation of the inoculum**

The fungal isolates were subcultured from stock water suspensions onto potato dextrose agar slants and incubated at 35°C for 48 to 72 hours to obtain young fungal cultures. The inoculum was standardized by microscopic examination, adjusting the concentration to a range of 1.0×10^6 to 5.0×10^6 conidia/mL using a Malassez counting chamber (Rodriguez-Tudela et al., 2003). Four to five well-isolated colonies were carefully picked and transferred to a sterile tube containing 9 mL of physiological water using a sterile cotton swab. The resulting suspension was homogenized for 15 seconds using a vortex mixer and its optical density was measured at 530 nm, following the same procedure as for bacterial strains.

- **Performing antibacterial susceptibility assays**

The method used follows the same procedure as for bacterial strains, with the Sabouraud medium replacing the Mueller-Hinton medium. The incubation period was set between 48 and 72 hours at 35°C.

6.6. In vitro gastrointestinal digestion

The approach involves simulating human digestion using artificial digestive fluids, including salivary (SSF), gastric (SGF), and intestinal (SIF) fluids as described by Minekus et al. (2014) .

During the oral digestion phase, 5 mL of the extract was blended with 3.5 mL of SSF, 0.5 mL of α-amylase solution, 25 µl of $CaCl_2$, and 0.975 mL of deionized water. The mixture was maintained at 37°C for 2 minutes. In the gastric digestion phase, the entire oral phase mixture was combined with 6 mL of SGF, 1.28 mL of pepsin, and 5 µl of $CaCl_2$. The pH was lowered to 3 using 1M HCl, followed by incubation at 37°C for 2 hours. The remaining gastric phase mixture was supplemented with 7.7 mL of SIF, 3.5 mL of pancreatin, 1.75 mL of fresh bile, and 28 µl of $CaCl_2$ for the intestinal phase. The pH was adjusted to 7 using 1M NaOH, and the mixture was incubated at 37°C for an additional 2 hours. All incubation steps were carried out in a shaking water bath. Table 9 summarizes the volume of each constituent used.

Table 9. Composition of simulated digestion fluids: SSF, SGF, and SIF (Minekus et al., 2014).

Constituent	SSF (pH 7)	SGF (pH 3)	SIF (pH 7)
Volume of constituents (mL)			
KCl (37.3 g/l)	15.1	6.9	6.8
KH_2PO_4 (68 g/l)	3.7	0.9	0.8
$NaHCO_3$ (84 g/l)	6.8	12.5	42.5
NaCl (117 g/l)	—	11.8	9.6
$MgCl_2(H_2O)_6$ (30.5 g/l)	0.5	0.4	1.1
$(NH_4)_2CO_3$ (48 g/l)	0.06	0.5	—

7. Use of Myrtle (*Myrtus communis* L.) fruit powder as a functional ingredient for obtaining value-added mayonnaise

Today, one of the main areas of research in food science and technology is the development of new functional foods containing natural ingredients with biological activities that can contribute to consumer well-being (Plaza et al., 2012).

7.1. Formulation of mayonnaise

The mayonnaise was prepared in the Research and Development Laboratory of the Cevital Agro-Industry group, following the standard mayonnaise production process (company standard) and the modified protocol outlined by Raikos et al. (2016). The recipe included the following ingredients (expressed as percentages): rapeseed oil (70%), liquid egg yolk (5%), sugar (0.5%), water (18%), vinegar (3.5%), mustard (2%), and salt (1%).

Freeze-dried myrtle fruit powder (DMFP) was incorporated into the formulation. A total of two kilograms of each mayonnaise sample was produced.

To evaluate the effect of myrtle fruit powder as a functional ingredient on mayonnaise quality, four samples were prepared:

- Control sample (CS): Commercial full-fat mayonnaise without any enrichment.
- Sample (A): Mayonnaise supplemented with 0.3% DMFP.
- Sample (B): Mayonnaise supplemented with 0.6% DMFPL.
- Sample (C): Mayonnaise supplemented with 1 g of DMFP.

Each sample was placed in airtight glass jars and stored at room temperature (20°C ± 5) for one month. Physicochemical and sensory analyses were conducted on all mayonnaise samples at 0, 14, and 28 days.

7.2. Physicochemical analyses

The physicochemical analyses were conducted in the sauce production unit laboratory of the Cevital Group. The key parameters analyzed in the mayonnaise samples included pH, acid index, moisture content, salt content, consistency, and viscosity. In addition, phytochemical analyses, including total phenolic content (TPC), DPPH radical scavenging activity, ABTS assay, and ferric reducing power (FRP), were performed at the Research Laboratory of Biomathematics, Biophysics, Biochemistry, and Scientometry at the University of Bejaia. All formulations were prepared in triplicate for each powder concentration level including the control sample. Furthermore, physicochemical measurements were conducted in triplicate to ensure accuracy and reproducibility.

7.2.1. pH measurement

The pH is measured using an electronic pH meter by immersing the electrode directly into the mayonnaise sample (Khalid et al., 2021). The pH value is recorded once the reading stabilizes on the device's display.

7.2.2. Acidity level measurement

The acidity of a product was assessed as described by Alizadeh et al. (2019), which primarily corresponds to the presence of organic and mineral acids used in its formulation. This is determined using an acid-base titration, where acetic acid is neutralized by a 0.1N sodium hydroxide (NaOH) solution in the presence of phenolphthalein as a pH indicator.

- **Procedure**

Titratable acidity was determined by weighing 1 g of the sample into an Erlenmeyer flask and diluting it with 100 mL of distilled water. Phenolphthalein (2–3 drops) was added as an indicator, and the mixture was titrated with 0.1 N NaOH until a persistent light pink endpoint was observed. The volume of NaOH required was recorded for further calculations.

- **Expression of results :**

Acidity is conventionally expressed as grams of acetic acid per gram of product and is

calculated using the following formula:

$$A\% = \frac{V(mL) \times N \times M}{SM}$$

Where :

- **V** = Volume of NaOH used for titration (mL)
- **N** = Normality of NaOH (0.1N)
- **M** = Molar mass of acetic acid (60 g/mol)
- **SM** = Sample mass (g)

7.2.3 Moisture Content

Moisture content, determined according to AOAC (2008), represents the amount of water present in mayonnaise. It is determined by drying the sample in a desiccator equipped with an infrared-based electronic system, which calculates the total dry extract.

➢ **Procedure :**

Moisture content was determined using a moisture analyzer. An empty dish was weighed inside the desiccator and tared. Subsequently, 1 g of mayonnaise was evenly spread on the dish, which was then placed back into the desiccator and securely sealed. The analysis was initiated, and the result was recorded automatically once the instrument signaled completion.

➢ **Expression of results:**

The moisture percentage is calculated based on the total dry extract using the formula:

$$M\% = 100\% - TDE$$

where TDE represents the total dry extract.

7.2.4. Determination of Salt Content

After dissolving the mayonnaise by adding boiling water, the chloride content is titrated with a standardized silver nitrate ($AgNO_3$) solution using potassium chromate (K_2CrO_4) as a color indicator, following Mohr's method (Sheen & Kahler, 1938). This method is based on the reaction between silver ions (Ag^+) and chloride ions (Cl^-), forming a precipitate of silver chloride:

$$Na^+ Cl^- + AgNO_3 \longrightarrow AgCl + NaNO_3$$

At the equivalence point, a low concentration of Ag^+ ions causes the potassium chromate

(K_2CrO_4) indicator to turn brick red:

$$K_2CrO_4 + 2AgNO_3 \longrightarrow 2\,KNO_3 + Ag_2CrO_2$$

➢ **Procedure:**

Chloride content was determined by the Mohr titration method. Briefly, 1 g of the sample was weighed into an Erlenmeyer flask and diluted with 100 mL of boiling distilled water. Under continuous stirring, 2 mL of potassium chromate solution was added as an indicator. The mixture was titrated with a standard silver nitrate solution until the appearance of a persistent brick-red endpoint lasting at least 30 seconds. The volume of silver nitrate consumed was recorded for further calculations.

➢ **Expression of results:**

The salt content is calculated using the following formula:

$$\text{NaCl}\ \% = \frac{\text{V(mL)} \times \text{N} \times 5.85}{\text{SW}}$$

Where :

- **V** = Volume of silver nitrate solution used (mL).
- **N** = Normality of $AgNO_3$ (0.1N).
- **SW** = Sample weight (g).
- **5.85** = a conversion factor used to express the chloride concentration as a percentage of NaCl in the analyzed sample.

7.2.5. Assessment of the consistency

Consistency is represented by the distance in centimeters (cm) covered by the sample in the 30s along a channel under the effect of gravity (Elsebaie et al., 2022).

➢ **Procedure:**

The consistency of the sample was measured using a Bostwick consistometer. Prior to analysis, the instrument was leveled by aligning the bubble of the spirit level to the center. The trough was then filled with the sample, ensuring uniform distribution. The gate was released simultaneously with the start of a stopwatch, and after 30 seconds the distance traveled by the sample along the Bostwick scale was recorded. (Figure 13).

Figure 13. Flow through the Bostwick consistometer.

7.2.6. Measurement of viscosity

Viscosity is defined as the coefficient of internal molecular friction. It is determined by the frictional forces of a solid moving in a liquid, following the International Union of Pure and Applied Chemistry recommendations (Lehmann et al., 1996). Viscosity (given in centipoise) is measured using a torsion viscometer connected to a thermostated bath at 20°C.

- **Procedure:**

Viscosity was determined using a rotational viscometer. The viscometer mounting rod was inserted into a vial containing 100 g of the sample, and the instrument was adjusted until the bubble level was centered. The analysis was initiated by pressing the 'Motor On' button, and Probe 64 was set to rotate at the desired speed (50, 12, or 6 rpm). The viscosity value was recorded once a stable reading was obtained.

7.2.7. Evaluation of phenolic compounds content of Mayonnaise

- **Extraction of Polyphenols**

Mayonnaise samples were subjected to the extraction method described by Romeo et al. (2021), with slight modifications. In brief, 1 g of mayonnaise was dissolved in 10 mL of 50% acetone, vortexed thoroughly, and then centrifuged at 5000 rpm for 10 minutes. The supernatant was subsequently filtered through filter paper.

The resulting extracts were used to determine the total polyphenol content and assess the antioxidant activity (DPPH radical scavenging, ABTS radical scavenging and FRP) according to the previously described methods.

8. Statistical analysis

The results were expressed as means ± standard deviation (SD). One-way analysis of variance (ANOVA) completed by Fisher's least significant difference (LSD) test was conducted to determine the significant differences between means at $p < 0.05$ as the level of significance. Correlations between phenolics and antioxidant assays were evaluated using Pearson's correlation coefficient test. All these statistical analyses were performed using Statistica 12 software (Stat Soft Inc., Oklahoma, USA). Principal component analysis (PCA) was conducted using HJ-biplot methods included in the JMP software (JMP Pro Version 14.0, SAS, USA).

Results and discussion

II. Results and discussion

1. Effect of drying methods on phenolic compounds and antioxidant activity of *Myrtus communis* L. fruit

Introduction

Since *Myrtus communis* is a seasonal fruit, drying methods should be employed to take advantage of its therapeutic properties and richness in antioxidants year-round (Dinçer et al., 2022). Drying is the oldest and the most popular storage and preservation method, which reduces the water content, restricts microbiological activity and increases the shelf life of food products (Lee & Kim, 2009). In addition, drying leads to the concentration of phenolic compounds, enhancing the fruit's value as a healthy product (Bassey et al., 2024). Several drying methods have been proposed for drying fruits and vegetables. Freeze drying (FD) has emerged as an excellent method for drying products with heat-sensitive compounds, as it preserves the initial functional properties of these components nearly intact, resulting in a product with high aroma quality. However, its high cost, especially for large-scale commercial production, needs to be considered (Abouelenein et al., 2021; López-Parra et al., 2024). Sun drying is still widely employed as a drying process due to its low cost, produces a product with rich color and a translucent appearance, but comes with limitations. It is time-consuming, weather-dependent, labor-intensive, and highly exposed to potential environmental contamination (Arslan & Özcan, 2010). Oven drying is commonly used as a traditional method for post-harvest processing and storage of various plant products due to its simplicity and efficiency. Nevertheless, the prolonged drying time and high temperatures often degrade product quality by diminishing nutritional and nutraceutical compounds, as well as affecting color and flavor. Additionally, the OD process is known for its high energy consumption, and low productivity (Garcìa et al., 2021). Microwave drying (MWD) has emerged as a promising alternative drying method for various food products due to its numerous advantages, including faster drying times, lower costs, and high energy efficiency. Indeed, the volumetric heating penetrates the entire sample, unlike conventional methods. However, improper heat control and mass transfer can cause product damage. Therefore, combining microwave drying with

pretreatment techniques is necessary to prevent product quality degradation (Calín-Sánchez et al., 2020).

Despite their nutritional and health benefits, myrtle fruits are seasonal and perishable. Therefore, proper preservation is essential to ensure year-round availability and to minimize significant post-harvest losses. The drying process is the most common and easiest way of food preservation. Many studies have been conducted for the profiling of phytochemical and antioxidant potential in myrtle fruits but limited knowledge is present regarding the effects of different drying methods on these fruits. Thus, this section aimed to assess the impact of different drying methods, namely freeze drying (FD), sun drying (SD), oven drying (OD) and microwave drying (MWD) on phenolic compound content and antioxidant activity of Algerian *Myrtus communis* L. fruits growing wild in Bejaia province, Algeria.

1.1. Drying experiments

In this study, according to the drying method used, the drying time differed significantly (Table V). The MWD process yielded the fastest drying time (30 min), contrasted with the other three methods. Conversely, the SD method resulted in the longest drying time (120 h). Alkaltham et al. (2021) dried black myrtle fruits with different methods and reported a similar finding that the drying time depended on the drying method. They reported that MWD recorded the lowest drying time, while the longest drying time was recorded with sun drying.

1.2. Effect of drying method on the phenolic compounds and the antioxidant activity of the samples

1.2.1. Total phenolic content (TPC)

The TPC of fresh myrtle was measured at 252.38 ± 3.09 mg GAE/g of DW. The TPC of dried samples varied significantly depending on the drying method employed. The freeze-dried (FD) sample recorded the highest value (88.12 ± 2.70 mg GAE/g of DW) followed by SD (51.30±1.58 mg GAE/g of DW), OD (52.74±0.96 mg GAE/g of DW) and MWD (53.22±2.36 mg GAE/g of DW) dried samples without any significant difference observed among the latter three methods (Figure 14(A)). Previous studies have reported that drying methods significantly affect the TPC in plant materials (Bassey et al., 2024; Pashazadeh et al., 2024; Yue et al., 2021). Our findings align with those reported in the literature. In the research conducted by Bakar et

al. (2021), it was found that the biochemical content of dried myrtle fruits was higher with freeze drying (FD) compared to sun and microwave (MW) drying methods**.** Saifullah et al. (2019) confirmed that the FD method registered the highest value of TPC, TFC, proanthocyanidins, gallic acid, hesperetin and antioxidant activity compared to other drying methods used (hot air drying, vacuum drying, microwave drying, sun drying and shade drying) in lemon myrtle (*Backhousia citriodora*) leaves. Yue et al. (2021) who studied the effect of hot-air drying (HD), microwave drying (MD), vacuum drying (VD), vacuum micro wave drying (VMD), and vacuum freeze-drying (VFD) on purple cabbage and reported that the VFD cabbage showed the greatest retention of TPC, TFC, anthocyanins and antioxidant ability. Additionally, Bi et al. (2024) reported that FD and pulsed vacuum drying (PVD) were possible strategies for effectively minimizing the degradation of rape bee pollen quality during the drying process.

It is important to note that precision in selecting drying conditions is crucial for achieving reliable comparisons and accurate results, as variations in these conditions can significantly impact outcomes. Some studies have confirmed that prolonged drying processes and high temperatures negatively affect TPC values, leading to decreased levels in dried samples (Alean et al., 2016; Kayacan et al., 2020; Snoussi et al., 2022).

1.2.2. Total flavonoids content (TFC)

As with polyphenols, the flavonoid content is also influenced by the drying method applied (Figure. 14(B)). The highest value was observed in the sample dried using the FD method, which recorded 12.05 mg QE/g of DW. This was followed by the SD sample at 11.68 mg QE/g of DW, the OD sample at 11.27 mg QE/g of DW, and the MWD sample at 7.15 mg QE/g of DW. The TFC of fresh myrtle was measured at 28.98 ± 1.45 mg QE/g of DW.

Our results corroborate with previous studies reported in the literature. Hamrouni-Sellami et al. (2013), investigated the effect of different drying methods on total phenolics, flavonoids, and antioxidant activity of Sage (*Salvia officinalis* L.) and remarked that the flavonoids content increased with drying method compared with fresh sample. Same findings for the work of Periche et al. (2016), they concluded that freeze drying increased the concentrations of most flavonoids and phenolic acids. However, Mohd Zainol et al. (2009) reported that drying

methods led to flavonoid degradation, with air-oven treatment resulting in the highest total flavonoid degradation, while freeze drying resulted in the lowest degradation. This degradation is likely related to the extended drying times, which can compromise the stability of the compounds. The low degradation observed in the freeze-drying process may also be attributed to the lower temperature used compared to other drying methods. Catechin and rutin were identified as the most stable flavonoids.

1.2.3. Total flavonols content

Flavonols are a subgroup within the vast family of flavonoids exhibiting a wide range of chemical structures and characteristics (Aherne & O'Brien, 2002). Analysis by HPLC-UV at 280 nm identified flavonols glycosides as the predominant phenolics in myrtle fruit extracts, accounting for 58% of the total quantified polyphenols (Barboni, Cannac, et al., 2010). In the best of our knowledge, there is no data about the effect of drying methods on the flavonols content of *Myrtus communis* fruits. As with TPC and TFC, the flavonol content also showed variation depending on the drying method used (Figure 14(C)). The highest value (29.99±1.81 mg RE/g of DW) was observed in the freeze-dried sample, followed by both oven-dried (19.20±0.62 mg RE/g of DW) and sun-dried (18.05±0.54 mg RE/g of DW) samples, which did not exhibit significant differences. Finally, the microwave-dried sample (14.10±1.01 mg RE/g of DW) showed the lowest content. The flavonol content of fresh myrtle fruit was measured at 59.28 ± 1.70 mg RE/g of DW.

1.2.4. Total condensed tannins content (CTC)

Similarly to the TPC, TFC and flavonols, the content of CTC varied depending on the type of drying method used (Figure 14(D)). The highest value was observed in the FD sample at 75.40±3.25 mg GAE/g of DW, while the MWD sample showed the lowest value at 15,40±4.45. Both the OD sample (58.18±1.05 mg GAE/g of DW) and the SD sample (51.49±1.85 mg GAE/g of DW) were significantly lower than the FD sample, with $p < 0.05$ for all comparisons. The CTC of the fresh sample was measured at 220.56 ± 2.02 mg CE/g of DW.

Our findings were in line with those reported by Turkiewicz et al. (2019), who studied the influence of different drying methods on the quality of Japanese quince fruit. They concluded that MWD method caused significant reduction in flavan-3-ol content by 30% compared to

freeze-drying. While drying with convective-vacuum-microwave at 70°C best preserved both flavan-3-ols and polymeric proanthocyanidins, showing levels closest to those found in freeze-dried Japanese quince fruit. Additionally, Bouaoudia-Madi et al. (2022) investigated the impact of ultrasound as a pre-treatment in microwave-drying (MD) on the dehydration of myrtle (*Myrtus communis*) fruits, on their phytochemical content, and their antioxidant activity. They exhibited that microwave drying alone my led to the degradation of phenolic compounds in fruits due to high temperatures and long drying times. Therefore, it is essential to combine microwave drying with a pretreatment such as ultrasonic pretreatment is a promising approach to minimize product quality degradation.

1.2.5. Total anthocyanins content (AC)

Anthocyanins naturally occur as pigments found in myrtle fruits (Maldini et al., 2016; Scorrano et al., 2017b). Similar to TPC, TFC, flavonols, and CTC, the concentration of anthocyanins also varied depending on the specific drying method employed (Figure 14(E)). Among the drying methods, the freeze-dried (FD) sample exhibited the highest anthocyanins content (AC) (04.96 ± 0.10 mg CGE/g of DW). Conversely, the MWD sample displayed the lowest AC with a value of 0.65 ± 0.20 mg CGE/g of DW. The two remaining methods, SD and OD, showed intermediate AC values of 3.82 ± 0.36 mg CGE/g of DW and 4.00 ± 0.37 mg CGE/g of DW, respectively, with no significant difference between them ($p > 0.05$), both lower than the freeze-dried sample. The fresh sample contained 5.23 ± 0.25 mg CGE/g of DW.

Our results are consistent with those reported in the literature. Wu et al. (2010) assessed the effects of refrigerated storage and processing technologies on the bioactive compounds and antioxidant capacities of Marion and Evergreen blackberries and noted that freeze-dried Evergreen had higher anthocyanin content. Nemzer et al. (2018) demonstrated that freeze-dried blueberries, tart cherries, and strawberries retained significantly more anthocyanins compared to those subjected to convection and refractance window drying methods.

The low anthocyanin content observed in the MWD samples is likely due to two mechanisms. Firstly, the high temperatures and long irradiation times during microwaving directly degrade anthocyanins (De la Fuente-Blanco et al., 2006). Secondly, furfural compounds generated by the thermal degradation of sugars during MWD further promote anthocyanin breakdown (Y.

Sun et al., 2020). Additionally, Charmongkolpradit et al. (2021) indicated that high temperatures significantly reduce anthocyanin levels, particularly at 80°C, suggesting that lower temperatures may be more suitable for preserving these compounds.

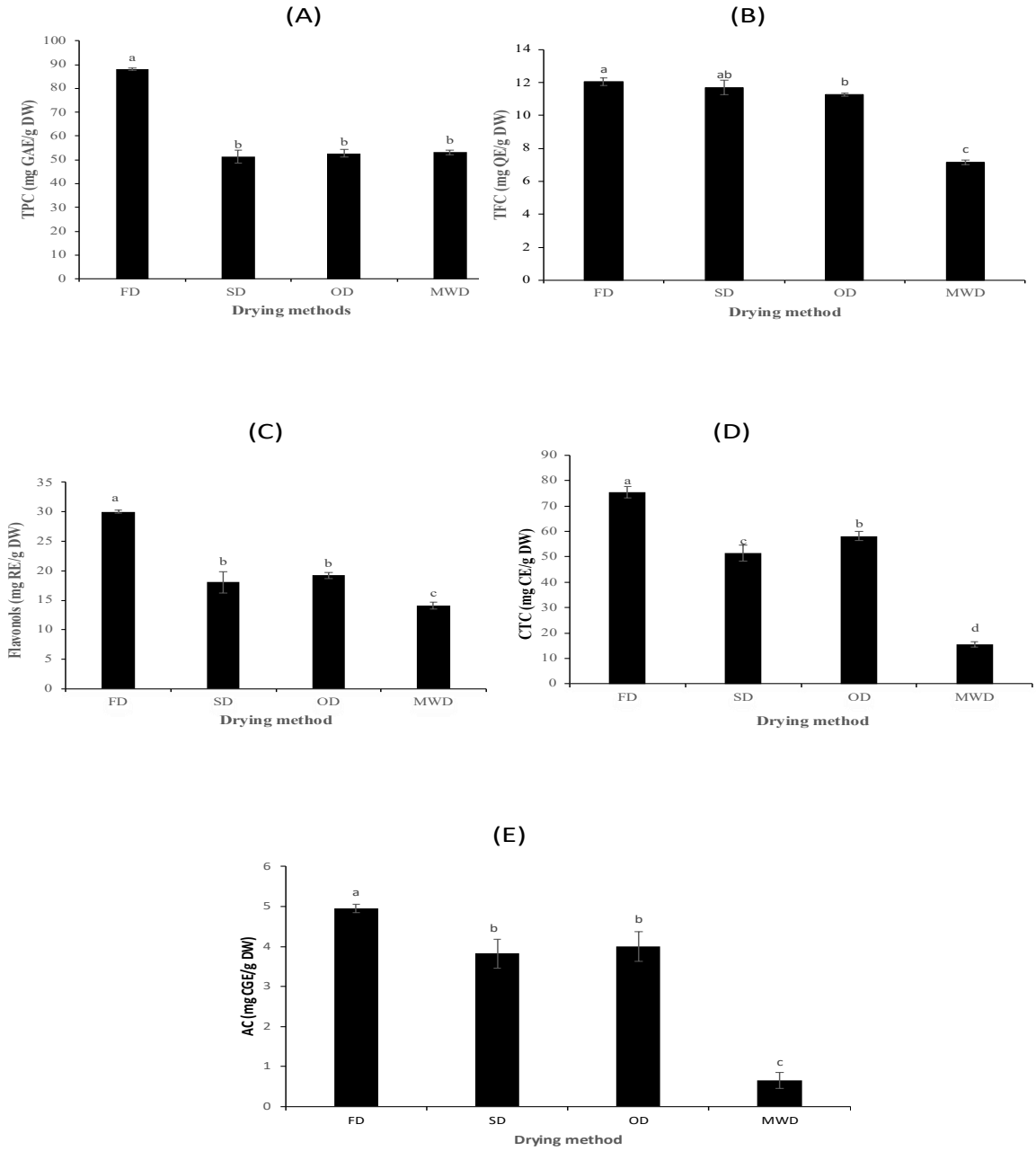

Figure 14. Influence of drying method (FD: freeze drying, SD: sun drying, OD: oven drying and MWD: microwave drying) on the extraction of total phenolic content (A), total flavonoid content (B), flavonols (C), condensed tannins content (D), and anthocyanins content (E) from myrtle (*Myrtus communis* L.) fruit (n=2)*. Values are presented as means ± SD of six measurements. Values identified by different lowercase letters (a-d) show significant differences ($p<0.05$). *Repeat extractions

1.2.6. Antioxidant activity

Regardless of the method used, the antioxidant capacity of *Myrtus communis* fruits varied depending on the drying technique applied.

The DPPH-RSA recorded the highest antioxidant activity in the FD sample, with a value of 143.37 ± 4.70 mg TE/g of DW. This was followed by the OD sample at 125.30 ± 0.58 mg TE/g of DW, the MWD sample at 124.01 ± 2.10 mg TE/g of DW, and the SD sample at 122.57 ± 1.68 mg TE/g of DW. No significant differences were observed among the latter three drying methods (Figure 15(A)). The DPPH-RSA of the fresh sample was 383.36 ± 10.14 mg TE/g of DW.

Similar results were obtained for the ABTS-RSA, where the highest value was also recorded in the FD sample at 154.31 ± 03.23 mg TE/g of DW. The OD and MWD samples displayed intermediate values of 100.88 ± 02.28 mg TE/g of DW and 99.05 ± 02.69 mg TE/g of DW, respectively, with no significant difference between them (Figure 15(B)). The SD sample exhibited the lowest antioxidant activity at 89.24 ± 06.42 mg TE/g of DW. The ABTS-RSA of fresh myrtle fruits was 310.91 ± 13.29 mg TE/g of DW.

Consistent with the DPPH-RSA and ABTS-RSA, the FRP also varied with the drying method employed. The FD sample exhibited the highest value at 89.25 ± 05.31 mg AAE/g of DW. The MWD, OD, and SD samples showed intermediate values of 49.96 ± 01.69 mg AAE/g of DW, 47.11 ± 02.10 mg AAE/g of DW, and 41.51 ± 03.16 mg AAE/g of DW, respectively (Figure 15(C)). The FRP of fresh myrtle fruits was 230.58 ± 6.76 mg AAE/g of DW.

For the PAA, a similar trend was observed; the amounts varied depending on the drying method used (Figure 15(D)). The FD sample again exhibited the highest PAA at 354.58 ± 5.47 mg TE/g of DW, followed by the OD sample at 262.21 ± 04.82 mg TE/g of DW, the SD sample at 235.65 ± 04.16 mg TE/g of DW, and the MWD sample at 152.21 ± 04.51 mg TE/g of DW. the PAA of fresh myrtle fruits was 441.06 ± 13.09 mg TE/g of DW.

Our findings align with those reported in previous studies of various plants. Das et al. (2012), studied the effects of freeze drying and oven drying on the antioxidant properties of fresh wheatgrass and concluded that FD yielded the highest values in both DPPH-RSA and FRP assay. Valadez-Carmona et al. (2017) investigated the impact of microwave, hot air (HAD), and

freeze-drying on the phenolic compounds, antioxidant capacity, enzyme activity, and microstructure of cacao pod husks (*Theobroma cacao* L.). They found that FD resulted in the greatest increase in antioxidant capacity, followed by MWD and HAD methods. The ABTS assay showed a two-fold increase after HAD, a three-fold increase after MWD, and a four-fold increase after FD. Similarly, the DPPH assay demonstrated increases of two, four, and five-fold after HAD, MWD, and FD, respectively. The observed increase in antioxidant capacity may be attributed to enhanced extraction of phenolic compounds during the drying processes.

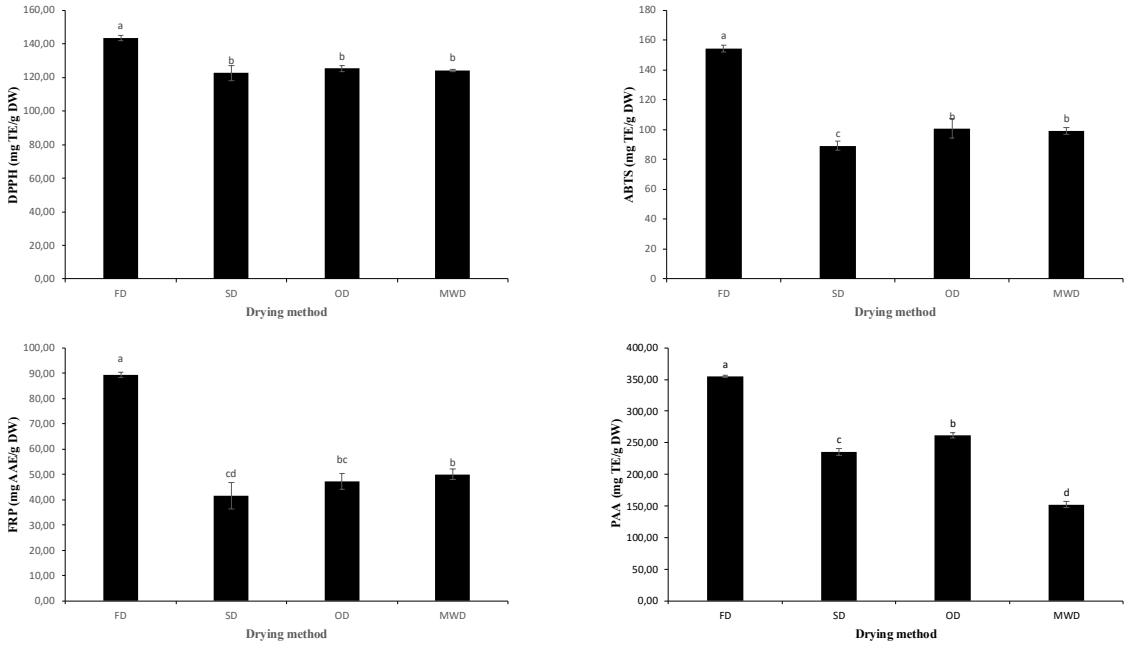

Figure 15. Influence of drying method (FD: freeze drying, SD: sun drying, OD: oven drying and MWD: microwave drying) on the antioxidant activity (DPPH (A), ABTS (B), ferric reducing power (C) and phosphomolybdenum antioxidant activity (D)) from myrtle (*Myrtus communis* L.) fruit (n=2)*. Values are presented as means ± SD of six measurements. Values identified by different lowercase letters (a-d) show significant differences ($p<0.05$). *Repeat extractions**.**

1.3. Principal component analysis

Figure 16 illustrates the results of the principal component analysis (PCA) examining the effects of different drying methods (freeze drying [FD], microwave drying [MWD], oven drying [OD], and sun drying [SD]) on the phenolic compounds and antioxidant capacity of myrtle fruits. Two principal components (PCs) characterized the total phenolic content (TPC), total flavonoid content (TFC), flavonols, condensed tannins (CTC), and antioxidant capacities

(DPPH-RSA, ABTS-RSA, FRP, and PAA) of myrtle fruits. The first principal component (PC1) had the highest eigenvalue of 7.43 and accounted for 82.6% of the variability in the dataset. The second principal component (PC2) had an eigenvalue of 0.95 and accounted for 10.6% of the variance. Together, these components explained a total of 93.2% of the variability in the plotted data.

In the biplot, the angles between the parameter vectors indicate their correlations: acute angles (less than 90°) represent positive correlations, obtuse angles (greater than 90°) or straight angles (180°) indicate negative correlations, and right angles (90°) suggest no correlation (Özcan, Al Juhaimi, Ahmed, Uslu, et al., 2020). The biplot (Figure 16) shows acute angles between the vectors of bioactive compounds (TPC, TFC, flavonols, CTC, and antioxidant capacities) and the various antioxidant assays (DPPH-RSA, ABTS-RSA, FRAP, and PAA), suggesting a strong positive correlation.

Furthermore, the freeze-dried (FD) samples are positioned on the far right, close to the variable arrows, indicating their high effectiveness in preserving these compounds. In contrast, sun-dried (SD) and oven-dried (OD) samples are positioned to the right, near the origin, reflecting moderate effectiveness in retaining antioxidants. Microwave-dried (MWD) samples, located on the left, suggest negative correlations with bioactive compounds and antioxidant activity.

These findings indicate that both the levels of antioxidants and their interactions with other plant constituents can significantly impact the antioxidant capacity of plant extracts. This aligns with observations by Terpinc et al. (2012), who noted that samples with similar total phenolic concentrations can exhibit substantial differences in antioxidant activity due to synergistic and antagonistic interactions among antioxidants.

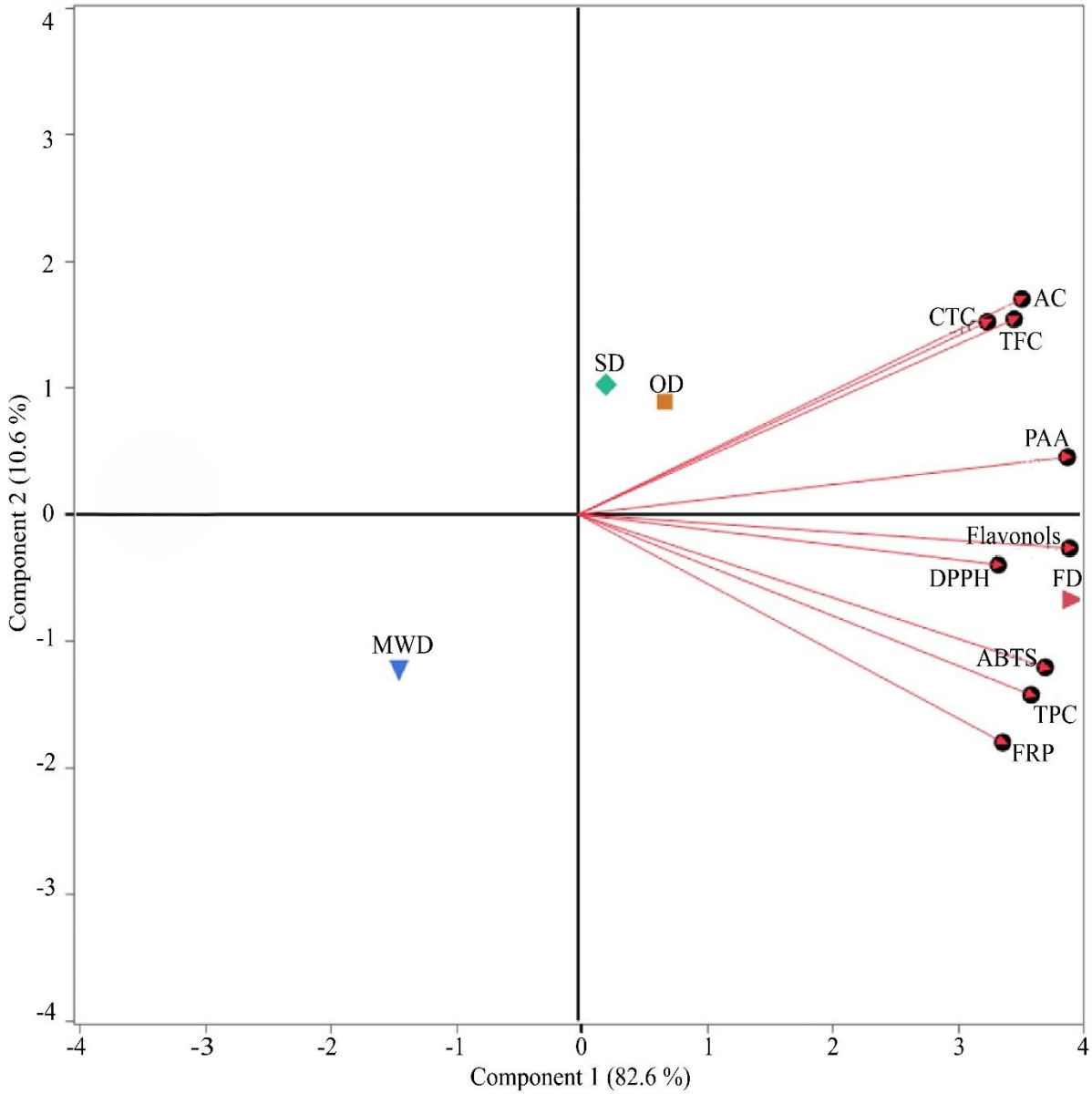

Figure 16. Principal component analysis biplot of phenolic compounds (TPC: total phenolic content, TFC: total flavonoids content, flavonols, CTC: total condensed tannins content, AC: total anthocyanins content) and antioxidant capacity (DPPH, ABTS, FRP: ferric reducing power and PAA: phosphomolybdenum antioxidant activity) in fresh and dried myrtle fruit samples. (FD: freeze drying, SD: sun drying, OD: oven drying and MWD: microwave drying)

Conclusion

Myrtus communis L. fruits are an important source of bioactive compounds but are prone to spoilage due to their high moisture content. Drying offers an effective solution for preserving these valuable fruits. In this study, we investigated the influence of different drying methods, including FD, SD, OD, and MWD on the content of phenolic compounds (TPC, TFC, flavonols,

CTC, and AC) as well as antioxidant capacity (DPPH-RSA, ABTS-RSA, FRP, and PAA) in myrtle fruits growing wild in Bejaia province, Algeria. Among the four drying methods assessed, freeze drying emerged as the most effective for preserving both phytochemicals and antioxidant properties of myrtle fruits. However, while freeze drying offers superior retention of bioactive compounds, it also has significant disadvantages. This method is typically more costly and energy-intensive compared to other drying techniques. Additionally, the complex equipment required for freeze drying may not be accessible in all settings, limiting its practicality for large-scale applications. In contrast, sun drying, oven drying, and microwave drying generally preserve good levels of most phenolic compounds and antioxidant capacity in myrtle fruits. However, microwave drying resulted in lower levels of condensed tannins and anthocyanins, likely due to the degradation of these bioactive molecules. Additionally, the correlation test and Principal Component Analysis (PCA) confirmed the effectiveness of FD method in preserving the bioactive compounds and antioxidant activities of myrtle fruits. These findings indicate that myrtle fruit is an excellent source for recovering bioactive compounds with beneficial functional properties, making it suitable for the development of functional foods and nutraceuticals. Ultimately, while freeze drying is effective for conserving healthy bioactive components, its practical limitations should be considered when selecting a drying method for commercial applications.

2. Optimization of extraction conditions of phenolic compounds and antioxidant activity from Myrtle (*Myrtus communis* L.) Fruit

Introduction

The extraction of phenolic compounds and their antioxidant capacity are influenced by the extraction method, the type of solvent, the temperature as well as the contact time (Ćujić et al., 2016; Dent et al., 2013).

The most common method for isolating plant antioxidant compounds is solvent extraction. Due to the presence of various antioxidant compounds with varying chemical characteristics and polarities that may or may not be soluble in a particular solvent, the extract yields and resulting antioxidant activities of plant materials are highly dependent on the nature of the extracting solvent. Polyphenols are frequently recovered from plant matrices using polar solvents. Aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate (hot or cold) are the most suitable of these solvents (Peschel et al., 2006). Type of solvent, pH, temperature and time are among the essential factors contributing to the effectiveness of the extraction solvent process (Escribano-Bailon, 2003; Gunathilake et al., 2018). Researchers have been dissecting different aspects of this plant, and from the last century to this day studies are still emerging (Alkaltham et al., 2021; Barboni, Venturini, et al., 2010; Montoro, Tuberoso, Piacente, et al., 2006; V González de Peredo et al., 2019). Despite the important number of scientific works, several aspects remain to be still elucidated. It is significant to evaluate the extraction materials and procedures, because extracting with different solvents can enhance or eliminate different essential chemicals, resulting in varied pharmacological and biological consequences.

The objective of this section is to evaluate the effect of diverse factors namely: solvent type (acetone, methanol, ethanol and water), solvent at different gradients (30%, 50%, 70% and 100%), at different acidities (0, 0.001, 0.005, 0.01, 0.05, and 0.1N), different temperature (20, 25, 30, 35 and 40° C) and at all the proposed times (30, 90, 180, 270 and 360) min; on the extraction efficiency of phenolic compounds (TPC, TFC and TPAC) and antioxidant capacity (DPPH, ABTS and FRP) from myrtle berries applying single factor methodology.

Phytochemicals were obtained from plants through several steps like milling, grinding, homogenization and extraction. However, extraction is the principal step for recovering and

isolating phytochemicals from plant samples, prior to analysis. Several conditions may influence the extraction efficiency of bioactive phytochemicals such as their chemical nature, the extraction method applied, the size of the sample particles, the solvent utilized and the presence of interfering molecules. Additional steps may be required if the removal of undesirable phenolics and non-phenolic compounds like waxes, fats, terpenes and chlorophylls is of interest (Stalikas, 2007). In this study, optimization of phenolic compounds extraction from myrtle fruits was carried out using solid-liquid execration by testing five extraction conditions including solvent type (50% acetone, 50% methanol, 50% ethanol, and water), acetone concentration (30, 50, 70, and 100%; v/v), solvent acidity (0, 0.001, 0.005, 0.01, 0.05, and 0.1N), extraction time (30, 90, 180, 270, and 360 min), and extraction temperature (20, 25, 30, 35, and 40°C).

2.1. Solvent type

Solvents such as methanol, ethanol and acetone or their mixtures with water are frequently used in the extraction of phenolic compounds from plant materials (Liu & Yao, 2007). Therefore, the choice of an appropriate solvent system is one of the most pertinent steps in optimizing the recovery of TPC, TFC and other antioxidant substances from a sample(Ghasemzadeh et al., 2010). The extraction yield depends on the polarity of the solvent, pH, temperature, extraction time, and sample composition. Under the same time and temperature conditions, the solvent and sample composition are considered among the most essential parameters (Do et al., 2014).

In this study, myrtle extracts were obtained by using 50% of aqueous acetone, methanol, ethanol, and water. The results showed that solvent type significantly affected(p<0.05) the extraction of TPC, TFC, TPAC and the antioxidant activity (DPPH-RSA, ABTS-RSA, and FRP) (figure 17). Among the solvents used, 50% acetone was the most efficient for extracting TPC with a maximum value of 87 mg GAE/g DW followed by methanol and ethanol (with no significant differences values of 59 and 58 mg GAE/g DW, respectively), and water which recorded the lowest TPC (39 mg GAE/g DW). This phenomenon could be caused by the fact that phenolic compounds are usually more soluble in organic solvents less polar than water (Kim & Lee, 2002). Liu et al. (2000) noted that solvents with elevated polarity, like water, or

low solvent strength, like chloroform and hexane, did not provide good extraction results. Solvents with moderate polarity (water-ethanol, ethanol, ethanol-acetone, and acetone) had more universal extraction capacities.

Additionally, 50% acetone was the best efficient solvent for extracting TFC (12 mg QE/g DW) followed by 50% ethanol (10 mg QE/g DW), 50% methanol (9 mg QE/g DW), and water (7 mg QE/g DW). The same thing was observed for TPAC where 50% acetone extracted the highest yield of these compounds (76 mg CE/g DW) followed by 50% methanol (59 mg CE/g DW), 50% ethanol (58 mg CE/g DW), and water (24 mg CE/g DW). These differences were attributed to the different polarities of the solvents used. As is known, the phenolic compounds extraction was dependent on the type of solvent used and its polarity, the solubility of phenolic compounds in the extraction solvents (Dent et al., 2013) and their degree of polymerization as well as their interaction with other food constituents and formation of insoluble complexes (Antolovich et al., 2000). However, the solvent polarity has a primary role in increasing the solubility of phenolic compounds (Naczk & Shahidi, 2006). Consequently, there is no uniform or completely satisfactory procedure for extracting all phenolic compounds or specific classes of phenolics from plant materials (Antolovich et al., 2000). In general, the higher the polarity of the solvent, the more polar phenolic compounds will be extracted. Thus, different types of solvents should be tested in investigations in which the phenolic composition is analyzed (Polat et al., 2014). From the results of this study, it can be suggested that less polar phenolic compounds were dominant in myrtle berries.

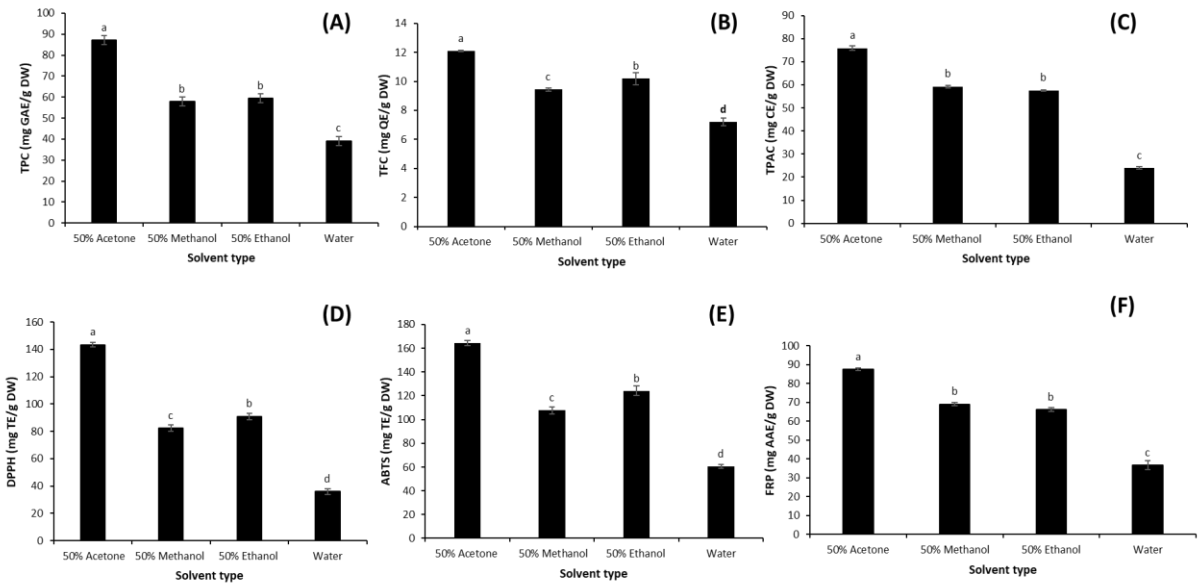

Figure 17. Effect of solvent type on the extraction of TPC (A), TFC (B), TPAC (C), DPPH-RSA (D), ABTS-RSA (E) and FRP (F) from myrtle berries (n=2) *. Values are presented as means ± SD of six measurements. Values marked with the different lower case letters (a-d) are significantly ($p< 0.05$) different. *Replication of extractions. The error bars represent the standard deviation.

Our results are in accordance with those of Polat et al. (2014), who studied the effect of four extraction solvents (70% acetone, 80% ethanol, 80% methanol, and water) on the TPC and the biological activities of extracts of myrtle fruits harvested from different locations in Turkey and found that acetone was a more appropriate solvent for extracting TPC (114-205 mg/g dry extract) for the most myrtle samples. The lowest TPC values were noted in the aqueous extract samples (52-170 mg/g dry extract).

Acetone/water mixture with different concentrations was also reported to be more efficient in the extraction of phenolic compounds comparing to other solvents in several previous published studies. Mokrani and Madani (2016) found that 60% acetone was the most efficient solvent for extracting polyphenols from peach fruits. Bhebhe et al. (2016) reported in their study that 50% acetone was the best solvent to extract more phenolic compounds with higher antioxidant activity from *C. sinensis,* L. *javanica* and *I. paraguariensis*. Musa et al. (2011) recommended 50% acetone as a solvent for extracting antioxidant compounds from

pink-flesh guava fruit. Mansouri et al. (2021) demonstrated that 70% acetone gave the highest TPC from barley seed extracts. Furthermore, extraction with 80% acetone led to the highest polyphenol, flavonoid and tannin contents for Cumin (*Cuminum cyminum* L.) seeds (Iness et al., 2012).

The antioxidant capacity of myrtle berries extracts was determined by various methods widely used to evaluate the antioxidant activity of plant extracts, including DPPH-RSA, ABTS-RSA, and FRP.

The antioxidant activity values of the myrtle fruit extracts (figure 17) showed that solvent extraction significantly affected ($p< 0.05$) these activities. Among the solvents tested, 50% acetone exhibited the highest antioxidant activity (DPPH-RSA, ABTS-RSA, and FRP). For the both anti-radical scavenging assays (DPPH-RSA and ABTS-RSA), 50% acetone manifested the strongest anti-radical activity (143 and 164 mg TE/g DW, respectively) followed by 50% ethanol (91 and 124 mg TE/g DW, respectively), 50% methanol (82 and 108 mg TE/g DW, respectively) and finally water which exhibited the weakest DPPH-RSA and ABTS-RSA (36 and 60 mg TE/g DW, respectively).

Similarly to the anti-radical scavenging assays, the 50% acetone solvent presented the highest FRP (87 mg AAE/g DW), more than two times the one of water (37 mg AAE/g DW). While, 50% methanol and 50% ethanol showed intermediate values of 69 and 66 mg AAE/g DW, respectively, with no significant differences ($p<0.05$).

Since 50% acetone gave the highest yield of TPC, TFC, TPAC and manifested the strongest anti-radical scavenging activity (DPPH-RSA, ABTS-RSA, and FRP) for myrtle fruits extracts, it is selected as the best solvent for the optimization of the next extraction parameters.

2.2. Solvent concentration

Different concentrations of acetone (30%, 50%, 70%, and 100%, v/v) were tested for recovering polyphenols from myrtle berries (figure 18). Results showed that phenolics (TPC and TFC) of myrtle extracts increased with increasing acetone concentration from 30% acetone (58and 10 mg QE/g DW for TPC and TFC, respectively) to 50% acetone where they reached their maximum values (87and 12 mg QE/g DW, respectively). However, extracting phenolics with acetone concentrations greater than 50% induced a decrease in TPC and TFC from 70%

acetone (75 and 10 mg QE/g DW for TPC and TFC, respectively) to 100% acetone (37 and 6 mg QE/g DW, respectively, representing the lowest values). The same thing was observed for TPAC which reached its maximum values at 50% and 70% acetone with no significant (p<0.05) difference in contents of 76 and 80 mg EC/g DW, respectively.

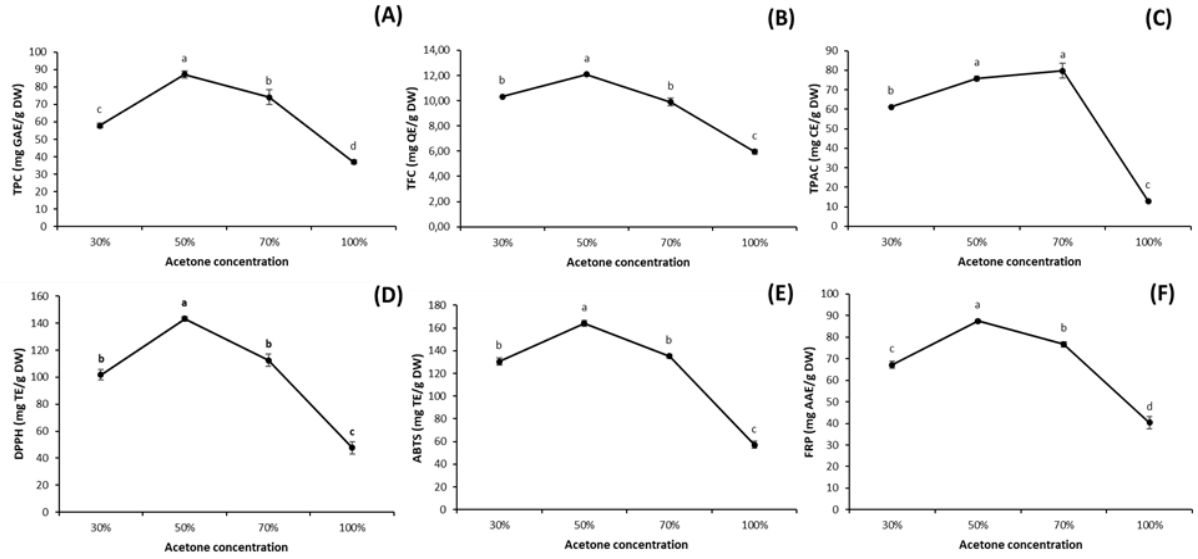

Figure 18. Effect of acetone concentration on the extraction of TPC (A), TFC (B), TPAC (C), DPPH-RSA (D), ABTS-RSA (E) and FRP(F) from myrtle berries (n=2) *. Values are presented as means ± SD of six measurements. Values marked with the different lowercase letters (a-d) are significantly (p< 0.05) different. *Replication of extractions. The error bars represent the standard deviation.

In the same way, antioxidant activity (DPPH-RSA, ABTS-RSA, and FRP) of myrtle extracts increased with the increase of acetone concentration from 30% acetone (5086 mg TE/g DW, 131 mg TE/g DW and 68 mg AAE/g DW for DPPH-RSA, ABTS-RSA and FRP, respectively) to 50% acetone where they reached their maximum values (143 mg TE/g DW,164 mg TE/g DW and 87 mg AAE/g DW for DPPH-RSA, ABTS-RSA and FRP, respectively). Using acetone concentrations greater than 50% induced a decrease in antioxidant activity (DPPH-RSA, ABTS-RSA, and FRP) from 70% acetone (113 mg TE/g DW, 135 TE/g DW and 77 mg AAE/g DW for DPPH-RSA, ABTS-RSA and FRP, respectively) to 100 % acetone (48 mg TE/g DW, 57 TE/g DW and 40 mg AAE/g DW, respectively, representing the lowest values). The strongest antioxidant activity of acetonic myrtle extracts may be explained by their highest phenolic content. Chew et al. (2011) demonstrated that extracting phenolics from plant samples using binary solvent system was more efficient compared to the single solvent system.

As suggested by Spigno et al. (2007), adding some water to an organic solvent generally produces a more polar medium, which facilitates the recovery of polyphenols. By increasing the water/acetone ratio, the polarity of the solvent will also increase. In this case, the solvent system can extract phenolics from the polar ends (high and low polarity substances) and substances of medium polarity (Zhang et al., 2007). Additionally, using mixtures of organic solvents and water has a higher efficiency in the recovery of antioxidant compounds comparing to pure organic solvents (Boeing et al., 2014) because water can facilitate the penetration of the solvent into the solid matrix by swelling the plant material which increases the extractability (Horax et al., 2010).

Our results are in agreement with previous studies reporting that solvent-water mixtures are more efficient for the extraction of phenolic compounds than mono solvents (water or acetone). Do et al. (2014) obtained the highest extraction yield of TPC from *Limnophila aromatic* by using 50% aqueous acetone. Zhou and Yu (2004) recommended 50% acetone as solvent for extracting phenolic antioxidants from wheat bran.Wijekoon et al. (2011) registered that 50% acetone extract showed highest amount of total phenols from bungakantan inflorescence. Aqueous acetone (50%) extract contained the highest content of polyphenols from Ajwa date fruit as confirmed by Nematallah et al. (2018). Assefa and Keum (2017) reported that mixed solvents exhibited better results compared to the absolute solvents and 50% acetone exhibited the highest TPC value in the seeds of yuzu fruits. It was reported that acetone/water (7:3) is more effective than acetone absolute in recovering the maximum amount of condensed tannins from different varieties of peas, whether or not acid is added (Chavan et al., 2001).

The acetone with a gradient of 50% was chosen for testing the effect of solvent acidity, temperature and time.

2.3. Solvent acidity

The effect of the acidity on the extraction of myrtle phenolics and its antioxidant activity was evaluated using different concentrations of HCl (0.001, 0.005, 0.01, 0.05 and 0.1 N) mixed with the best solvent extraction (50% acetone) at the ratio of 85:15 (50% acetone: HCl/85:15, v/v). Results showed that 50% acetone without acidification gave the most yield of TPC (87mg

GA/g DW), TFC (12 mg QE/g DW), and TPAC (76 mg CE/g DW) (figure 24). Furthermore, 50% acetone extract exhibited the strongest DPPH-RSA (143 mg TE/g DW), ABTS-RSA (164 mg TE/g DW), and FRP (87 AAE/g DW). A decrease in the recovery of phenolic compounds from myrtle berries was observed when the concentration of HCl was higher than 0.001 N. The same observation was noted for the antioxidant activity (DPPH-RSA, ABTS-RSA, and FRP) of myrtle. However, the application of high concentrations of HCl (0.1N) induced an increase in TPC (86 mg GAE/g DW) and antioxidant activity of myrtle berries.

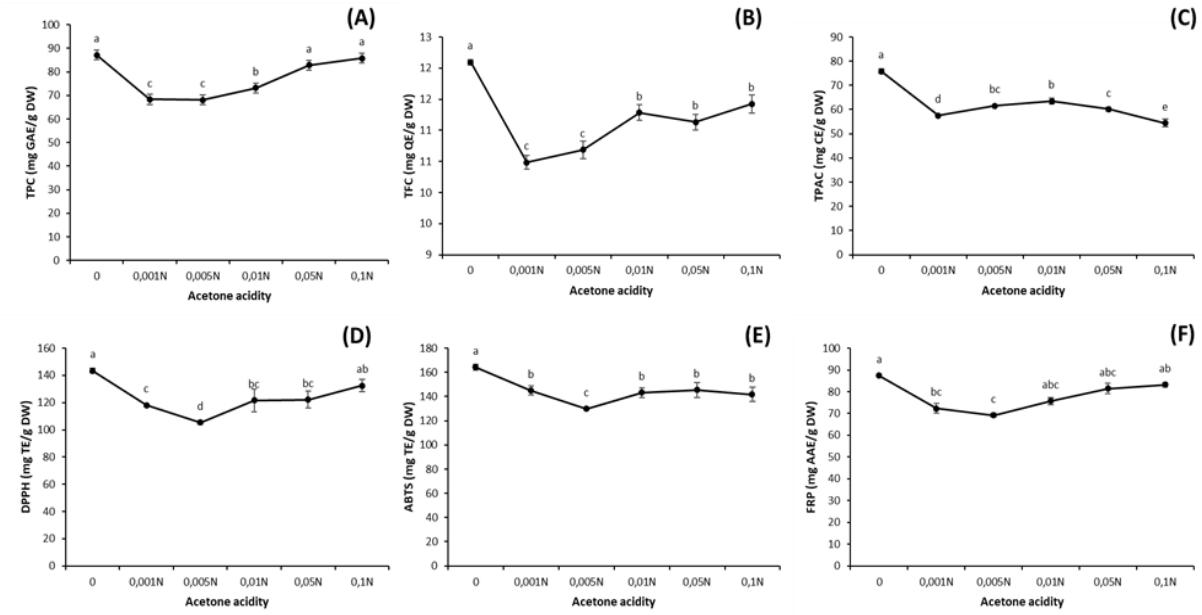

Figure 19. Effect of acetone acidification on the extraction of TPC (A), TFC (B), TPAC (C), DPPH-RSA (D), ABTS-RSA (E) and FRP(F) from myrtle berries (n=2) *. Values are presented as means ± SD of six measurements. Values marked with the different lowercase letters (a-e) are significantly ($p< 0.05$) different. *Replication of extractions. The error bars represent the standard deviation.

Acidification of the solvent increases the ability to extract phenolic compounds, especially when protic polar solvents are utilized. By acidifying the medium, the phenol-phenolate equilibrium shifts toward the less polar phenyl form, thus facilitating extraction with organic solvents. The addition of acid in the extraction solvent could have acted in different ways. First, it may have increased the stability of the phenolic compounds such as anthocyanins(Escríbano-Bailón & Santos-Buelga, 2003). Second, it may have favored the dissolution of polyphenols, which are initially part of polymers or bound to other cell wall constituents, via a hydrolysis mechanism. This is the case for hydroxycinnamic acids and procyanidins (Naczk & Shahidi,

2004; Tsao & Deng, 2004). Third, it may have improved the disintegration of cell walls, thus facilitating the solubilization and diffusion of phenolic compounds from the plant material. However, too low pH levels or HCl concentrations higher than 0.1% could produce partial hydrolysis and decomposition of some phenolics such as anthocyanins (Revilla et al., 1998; Rodriguez-Saona & Wrolstad, 2001).

Since 50% acetone without the addition of acid gave the best yield of TPC, TFC, TPAC and had the strongest antioxidant activity (DPPH-RSA, ABTS-RSA, and FRP), and in order to avoid any possible degradation or change of the native forms of the phenolic compounds, 50% acetone without acidification was chosen as the best solvent extraction system for the next experiments.

2.4. Extraction temperature

Among the factors affecting the efficiency of the extraction of phenolic compounds, the temperature. In the present study, the effect of temperature on the extraction efficacy of phenolics from myrtle berries was performed at temperatures ranging from 20 to 40°C (figure 20). Statistical analysis indicated that temperature highly influenced antioxidants yield for all the measured compounds, with higher yield at 40°C except for TFC. As shown in figure 25, increasing the extraction temperature from 20 to 40°C induced an increase in TPC, TFC, TPAC and antioxidant activity. The temperature of 40°C was the best temperature for extracting TPC, TPAC with values of 87 mg GAE/g DW and 76 mg CE/g DW, respectively, and for the DPPH-RSA, ABTS-RSA and FRP with values of 143 mg TE/g DW, 164 mg TE/g DW and 87 mg AAE/g DW, respectively. However, for TFC, the best yield was recorded at 25°C and 30°C with no significant difference in the value of 13 mg QE/g DW.

Our results are in accordance with of those of Marques et al. (2016) who found that 40°C was the best temperature for the extraction of phenolic compounds from Guaraná seeds, because this low temperature decreases the likelihood of inactivation of substances in the extract. In the study conducted by Zhang et al. (2007), the extraction temperatures ranging from 20 to 40°C were selected as the extraction temperatures for the optimization of the extraction of lignans from flaxseed because very high extraction temperatures will decrease the activity of lignans and increase the consumption of solvent sharply. Uma et al. (2010) noted that applying

temperature extraction higher than 45°C decreased drastically the TPC in Henna (*Lawsonia inermis*) leaves extracts. de Simón et al. (1990) reported that the extraction temperature should always be below 40°C since above this value various reactions, such as hydrolysis, internal redox and polymerizations take place that alter the composition of the sample. Temperatures below 20°C are not recommended because they greatly increase extraction time. Dai and Mumper (2010) showed that extraction at high temperatures would increase the risk of phenolic oxidation, thus reducing the yield of polyphenols in the extract.

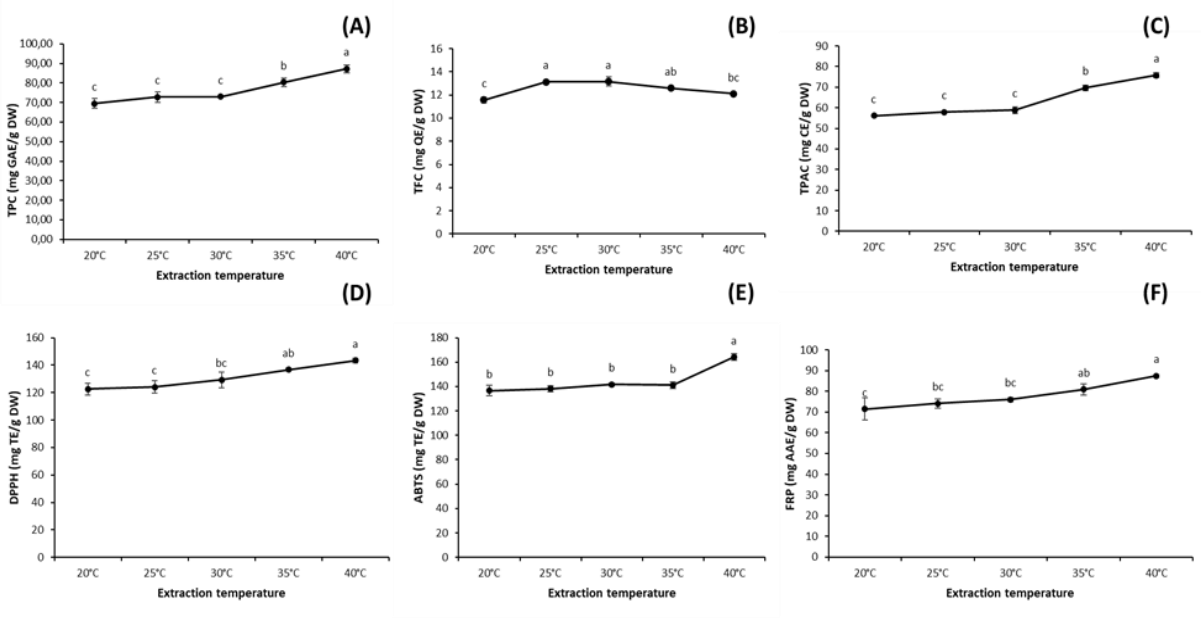

Figure 20. Effect of temperature on the extraction of TPC (A), TFC (B), TPAC (C), DPPH-RSA (D), ABTS-RSA (E) and FRP(F) from myrtle berries (n=2) *. Values are presented as means ± SD of six measurements. Values marked with the different lowercase letters (a-c) are significantly ($p< 0.05$) different. *Replication of extractions. The error bars represent the standard deviation.

In general, increasing temperature will favor extraction by enhancing the solubility and diffusion coefficient of the solute (Pinelo et al., 2005; Spigno et al., 2007). However, increasing the temperature beyond certain values may encourage possible concurrent decomposition of phenolic compounds which were already mobilized at lower temperature or even the breakdown of phenolics that are still remained in the plant matrix. Furthermore, high temperature may encourage solvent loss through vaporization and increase the cost for extraction process from the industrialization point of view (Hismath et al., 2011). Vaporization will create a more concentrated solvent extraction system, where a high concentration will increase the organic

solvent content, reducing polarity and eventually disturbing the phenolic extraction process, as a higher concentration yields lowers the extraction of phenolic compounds (Uma et al., 2010).

Taking into account all these considerations and since 40°C gave the best yield of TPC, TPAC and antioxidant activity (DPPH-RSA, ABTS-RSA, and FRP), the temperature of 40°C was chosen as the best extraction temperature.

2.5. Extraction time

The selection of an appropriate extraction time was the final step in a series of single-factor experiments. Extraction time represents a key parameter for the bioactive compounds to reach equilibrium concentration during the extraction process. It affects not only the extraction efficiency, but also the biological activity of the extracts (Fan et al., 2016; Gong et al., 2015; Spigno et al., 2007).

Using the best extraction conditions determined before (50% acetone without acidification at 40°C), phenolic compounds were extracted from myrtle berries at different extraction times ranging from 30 to 360 min. The results (Figure 21) showed that increasing extraction time from 30 to 180 min was accompanied by a significant (p<0.05) increment in TPC, TPAC, DPPH-RSA, ABTS-RSA, and FRP. After 180 min, further increase in process duration induced a significant (p<0.05) decrease in TPC, TPAC, DPPH-RSA, ABTS-RSA, and FRP.

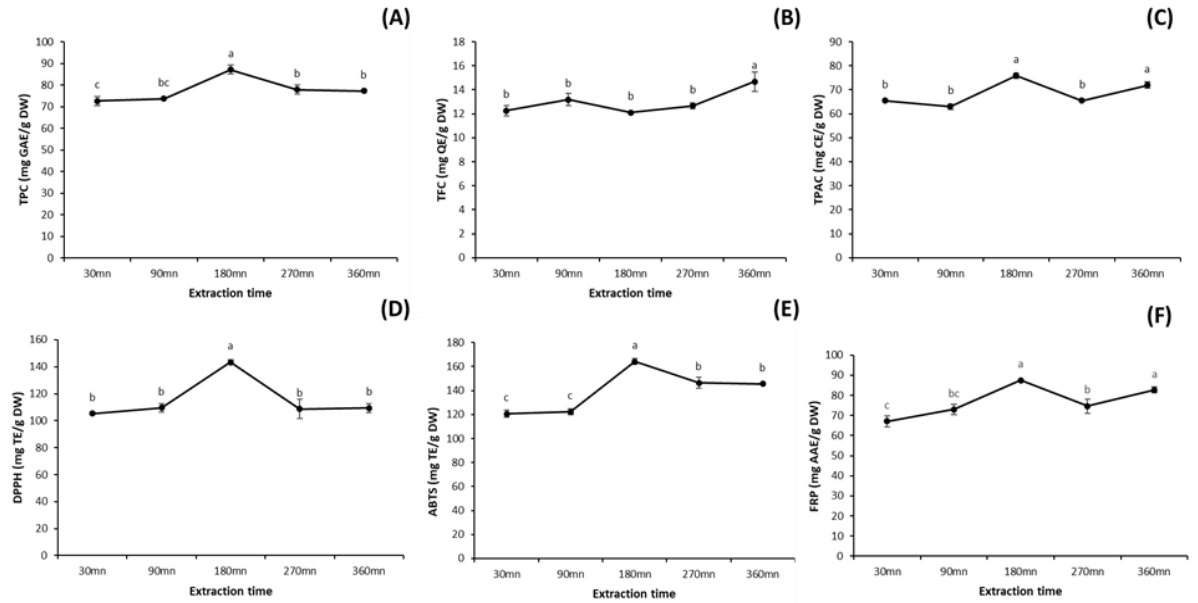

Figure 21. Effect of time on the extraction of TPC (A), TFC (B), TPAC (C), DPPH-RSA (D), ABTS-RSA (E) and FRP(F) from myrtle berries (n=2) *. Values are presented as means ± SD of six measurements. Values marked with the different lowercase letters (a-c) are significantly ($p < 0.05$) different. *Replication of extractions. The error bars represent the standard deviation.

Our results are in accordance with other studies that found that 180 min was the best time for polyphenols extraction. Durling et al. (2007) highly recommended that the extraction time should not exceed 3 h for the extraction of TPC from *Salvia officinalis* leaves. In another study, Adjé et al. (2010) observed an increase in the anthocyanin and flavonol contents until 180 min maceration time, then decreased slowly for anthocyanins and drastically for flavonols. Lafka et al. (2011) noted the time of 180 min as optimum for the extraction of polyphenols from olive oil mill wastes. Latoui et al. (2012) obtained the highest total polyphenol contents from *Vitex agnus-castus* L leaves at 180 min.

This observation was well explained by Fick's second law of diffusion, which stated that final equilibrium would be achieved between the solute concentrations in the solid matrix (plant matrix) and in the bulk solution (solvent) after a certain time, hence, an excessive extraction time was not useful to extract more phenolic antioxidants (Silva et al., 2007). Furthermore, increasing the extraction time prolongs the risk of decomposition and oxidation of phenolic compounds because they are exposed to unfavorable environmental factors such as

temperature, light and oxygen for a long time (Naczk & Shahidi, 2004). On the other hand, increasing the extraction time is not economical and may increase the loss of solvent due to evaporation, which directly affects the solvent/solid ratio (Tan et al., 2013).

Since the highest TPC, TPAC and the strongest antioxidant activity (DPPH-RSA, ABTS-RSA, and FRP) were recorded at the time of 180 min, this time was chosen as the best time for extracting phenolics from myrtle berries.

2.6. Pearson correlation analysis

Correlations between TPC, TFC, TPAC and all antioxidant assays under different extraction conditions were investigated in order to better understand the links between the antioxidant activity and phenolic contents of myrtle fruit extracts (Table 10).

Table 10. Pearson correlation coefficient between phenolic contents and antioxidant activities of the extraction parameters.

Correlation coefficient (r)	**TFC**	**TPAC**	**DPPH**	**ABTS**	**FRP**
Solvent type					
TPC	$0,97^{***}$	$0,93^{**}$	$0,99^{***}$	$0,98^{***}$	$0,96^{***}$
TFC		$0,96^{***}$	$0,99^{***}$	$0,99^{***}$	$0,97^{***}$
TPAC			$0,96^{***}$	$0,97^{***}$	$0,99^{***}$
DPPH				$0,99^{***}$	$0,97^{***}$
ABTS					$0,97^{***}$
Solvent concentration					
TPC	$0,91^{**}$	$0,92^{**}$	$0,97^{***}$	$0,95^{***}$	$0,97^{***}$
TFC		$0,91^{**}$	$0,97^{***}$	$0,99^{***}$	$0,96^{***}$
TPAC			$0,93^{**}$	$0,95^{***}$	$0,95^{***}$
DPPH				$0,99^{***}$	$0,99^{***}$
ABTS					$0,98^{***}$
Solvent acidity					
TPC	$0,82^{**}$	$0,31^{ns}$	$0,82^{**}$	$0,62^{*}$	$0,97^{***}$
TFC		$0,66^{*}$	$0,86^{***}$	$0,72^{**}$	$0,85^{***}$
TPAC			$0,48^{ns}$	$0,70^{*}$	$0,42^{ns}$
DPPH				$0,88^{***}$	$0,87^{***}$
ABTS					$0,73^{**}$
Extraction temperature					
TPC	$-0,13^{ns}$	$0,97^{***}$	$0,91^{***}$	$0,82^{**}$	$0,94^{***}$
TFC		$-0,16^{ns}$	$-0,10^{ns}$	$-0,20^{ns}$	$-0,13^{ns}$
TPAC			$0,91^{***}$	$0,82^{**}$	$0,92^{***}$

DPPH				0,81**	0,82**
ABTS					0,79**
Extraction time					
TPC	-0,26ns	0,85**	0,92***	0,92***	0,87**
TFC		0,02ns	-0,35ns	-0,04ns	0,16ns
TPAC			0,77**	0,84**	0,89**
DPPH				0,77**	0,76*
ABTS					0,88**

ns Not significant

* Significant at $p<0,05$

** Significant at $p<0,01$

*** Significant at $p<0,001$

Under the influence of solvent type and solvent concentration parameters, strong positive correlations were observed between TPC, TFC, TPAC and all antioxidant assays: DPPH, ABTS and FRP ($0.91 < r < 0.99$) with *p*-value at least < 0.01 (table 10). These findings suggested that total phenolics, flavonoids, and proanthocyanidins may play an important role in the antioxidant activity of myrtle extracts under solvent type and solvent concentration conditions.

With regards to solvent acidity concentration, high significant correlations were found between TPC and all antioxidant assays ($0.62 < r < 0.97$) with *p* value at least < 0.05, and between TFC and all antioxidant tests ($0.72 < r < 0.85$) with *p* value at least < 0.01 (table 10). Furthermore, a significant correlation was found between TFC and all antioxidant assays ($0.72 < r < 0.86$) with *p* value at least < 0.01. However, no significant correlation was found between TPAC and antioxidant tests (DPPH and FRP). TPAC were weakly correlated with ABTS ($r = 0.70$, $p< 0.05$). These correlations suggested that TPC and TFC contribute to the antioxidant activity of myrtle berries more than TPAC, under the influence of solvent acidity. These findings may be explained by the effect of acid treatment on the behavior of proanthocyanidins which not only decompose into monomeric anthocyanidins but also undergo a gradual polymerization process, leading to the formation of amorphous phlobaphens (Strumeyer & Malin, 1975).

Under the effect of time and temperature extraction parameters, all antioxidant assays (DPPH, ABTS and FRP) were highly positively correlated with TPC ($0.82< r < 0.97$) with *p* value at least < 0.01 and with TPAC ($0.77< r < 0.92$) with p value at least < 0.01 (table 10). However, no significant correlation was observed between TFC and all antioxidant tests probably due to the decomposition of TFC (phenolic compounds of low-molecular weight)

during the prolonged extraction time and extended exposure to temperature (Do et al., 2020). These correlations suggested that TFC may be more sensitive to heat and oxidation comparing to the other phenolic compounds present in myrtle extracts.

Generally, antioxidant activities correlate well with phenolic compound contents. However, it was observed that the correlations of antioxidant properties across different tests are also influenced by the solvent system used. The results of the correlation analyses suggested that the unique phenolic compounds extracted by the selected solvent systems contributed variably to the overall antioxidant activities of myrtle fruit. Thus, for antioxidant or physiological investigations of this fruit, it is crucial to choose extraction solvents carefully.

Conclusion

The single factor methodology was used to assess the influence of various extraction parameters, including solvent (type, gradient and acidity), temperature and time on phenolic compounds and antioxidant capacity of myrtle berries extract. The phenolic compounds (TPC, TFC and TPAC) extracted of myrtle berries and their antioxidant activity (DPPH, ABTS and FRP) tested were significantly affected by different parameters studied (solvent type, solvent concentration, solvent acidity, temperature and time). Referring to the polyphenols yield and antioxidant activity assays, the best conditions of extraction were 50% acetone without acidification for 180 min at 40 °C. TPC value obtained under these conditions was 87 mg GAE/g with a best antioxidant activity (DPPH: 143 mg TE/g, ABTS: 164 mg TE/g, FRP: 69 mg AAE/g). Pearson correlation coefficients showed a good and positive correlation between polyphenols and antioxidant activity (DPPH, ABTS and FRP), particularly under the effect of solvent type and solvent gradient. Bougiote (Algeria) myrtle berries seem a good source of bioactive molecules due to its high antioxidant power and can be used in the development of functional foods and nutraceuticals. Evaluation of the interaction of factors using Response Surface Methodology (RSM) is strongly recommended to maximize the extraction yield of polyphenols.

3. Identification of phenolic compounds

Introduction

The wide range of biological activities observed in various parts of the myrtle plant, particularly in berries, can be attributed to the presence of numerous bioactive compounds. These include volatile compounds; a wide range of flavonoids, including derivatives of quercetin, catechin, and myricetin, as well as anthocyanins; and various phenolic compounds such as coumarins, ellagitannins, galloyl-glucosides, galloyl-quinic acid derivatives, and phenolic acids like caffeic, gallic, and ellagic acids (Giampieri et al., 2020). The comprehensive identification and quantification of these phenolic constituents are crucial not only for understanding the biological activities associated with myrtle fruit extract but also for validating its potential as a natural ingredient in functional food, nutraceutical, and pharmaceutical applications (Detti et al., 2025). Therefore, the use of advanced analytical tools, particularly ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS), enables accurate separation, detection, and structural elucidation of individual phenolic compounds, even at trace levels (González-Domínguez et al., 2022; Taamalli et al., 2014).

This section aims to identify the phenolic compounds present in the acetone extract of *Myrtus communis* fruits using UHPLC-QqQ-MS, with the goal of understanding the key molecules that may contribute to its biological activities.

3.1. Phytochemical characterization of myrtle (*Myrtus communis* L.) fruit polyphenols

3.1.1. Evaluation of Phytochemical content

Plants are rich sources of phenolic compounds such as flavonoids, tannins and phenolic acids. These substances have attracted growing interest because of their ability to act as antioxidants, neutralize free radicals, and bind metals. Such properties could contribute to various health-promoting effects (Wannes & Marzouk, 2016).

The polyphenol content of myrtle fruits was determined using the Folin-Ciocalteu method. This method was selected due to its simplicity, sensitivity, and rapidity for TPC quantification compared to other available assays. The Folin-Ciocalteu reagent preferentially reacts with phenolic compounds, forming a complex with phosphomolybdic-tungstic acids, which induces

a color change from yellow to blue. The intensity of this color is measured at 765 nm. However, the Folin-Ciocalteu reagent can also be reduced by certain non-phenolic compounds, such as copper (II) ions and vitamin C (Gull et al., 2012).

Table 11 presents the TPC, TFC, PAC, and AC values of *Myrtus communis* fruits analyzed in this study. These values are expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g DE) for total phenolic content (TPC), milligrams of quercetin equivalents per gram (mg QE/g) for total flavonoid content (TFC), milligrams of catechin equivalents per gram (mg CE/g) for proanthocyanidin content (PAC), and milligrams of cyanidin-3-glucoside equivalents per gram (mg CGE/g) for anthocyanin content (AC), respectively.

Table 11. Polyphenols, Flavonoids, Condensed tannins and Anthocyanins of Myrtus communis fruits (the results were expressed in dry extract DE).

TPC(mg GAE/g)	TFC(mg QE/g)	PAC(mg CE/g)	AC(mg CGE/g)
247,75±7,79	31,14±0,37	158,58±4,55	5,67±0,13

The antioxidant potential of natural sources is largely attributed to their total phenolic content. The TP levels in fruit extracts highlight their richness in phenolic compounds. Plant genotype, environmental conditions, and the harvesting period may influence variations among different accessions. Phenolic compounds are key contributors to the strong antioxidant capacity observed in edible plants (Serce et al., 2010).

A value of total phenolic compounds of 247,75 mg/g was found in our sample, which is higher than the TPC found in myrtle fruits of previous studies ranging from 5,31 to 190,23 mg/g GAE (Çelik & Şan, 2023; Fernández-Ruiz et al., 2016; Serce et al., 2010; Vega et al., 2025). In Turkish myrtle fruits, this value was 207.4 mg GAE/g, which is approximately close to our result (Polat et al., 2014). Additionally, close values (250.29 mg GAE/g in ethanolic extract and 242.39 mg GAE/g in methanolic extract) were found in the study of Tumen et al. (2012). This variation in phenolic compound content can be attributed to multiple factors. Barboni, Cannac, et al. (2010), studied the polyphenol composition of *Myrtus communis* L. berries from different localities in Corsica over multiple years and concluded that their polyphenol concentration

varies significantly due to environmental factors such as soil conditions and harvest year. Medda et al. (2022), revealed that phenolic compound accumulation in *Myrtus communis* L. cultivars is significantly influenced by both the climatic conditions of their growing sites and their genetic background. Stambouli-Essassi et al. (2025) and Taibi et al. (2024), demonstrated that extraction conditions, particularly the choice of solvent, significantly influence the TPC yield of myrtle fruits. Additionally, as stated by Prior et al. (2005), these differences could also be attributed to the non-specificity of the Folin-Ciocalteu reagent, which reacts not only with phenolic compounds but also with proteins (tyrosine and tryptophan), reducing sugars, tartaric acid, ascorbic acid, and sulfur compounds.

Similar to total phenolic content, the total flavonoid content (TFC) in myrtle fruits was notably high (31,14±0,37 mg QE/g DE). Several studies have highlighted the significant health benefits associated with these bioactive compounds (Aleksic & Knezevic, 2014; Babou et al., 2016; Hayder et al., 2008). As shown in the table, the flavonoid content in our sample was significantly higher than that reported by Aidi Wannes and Marzouk (2013), who recorded a total flavonoid content (TFC) of 1.21 mg QE/g in Tunisian myrtle fruits. In comparison, our results are approximately 30 times higher. Similarly, the study by Yeğin et al. (2022), reported TFC values ranging from 1.30 to 3.42 mg CE/g, which are also considerably lower. However, Tumen et al. (2012), reported a TFC of 42.95 mg QE/g in the dichloromethane extract, a value comparable to that obtained in our study.

Regarding the proanthocyanidin content, the measured value was 158.58 ± 4.55 mg CE/g (table 11). In the study by Kanoun et al. (2014), the authors analyzed the secondary metabolites of the stem, leaf, and berry. They found, similar to our results, that the fruits contained higher condensed tannin levels. Similarly, Kanoun et al. (2014), confirmed that myrtle berries contained the highest concentration of condensed tannins (27.20 mg CE/g DM) compared to other parts of the plant. These molecules offer various health benefits. They can help inhibit lipid oxidation, reduce the mutagenic effects of carcinogens, and slow down tumor development (Bladé et al., 2016; Higdon & Frei, 2003; Okuda, 2005; Okuda et al., 1992).

Anthocyanins, like flavonoids, are among the main phytochemicals in myrtle berries, as outlined by Tuberoso et al. (2010) and Messaoud and Boussaid (2011). The phytochemical

composition of myrtle fruits is largely dominated by a high anthocyanin content, which characterizes their deep purple color. These compounds are crucial for health benefits, contributing to antioxidant, anti-tumor, anti-inflammatory, and lipid-lowering effects, among others (Giampieri et al., 2020). The amount of anthocyanins in our sample was 5,67±0,13mg CGE/g DE. In the study of Özcan, Al Juhaimi, Ahmed, Babiker, et al. (2020) the anthocyanins content value was 1.07±0.06 mg/g CGE/g (2.38 ± 0.13 µmol/g), which was lower than in our sample. The concentration of anthocyanins is proportionally linked to health benefits, including positive effects on various diseases, such as antimutagenic, anticancer, and cardioprotective properties (de Pascual-Teresa & Sanchez-Ballesta, 2008).

3.1.2. Chromatographic Identification of Polyphenols in Myrtle (*Myrtus communis* L.) Fruit Extract using UHPLC-QqQ-MS

The optimized myrtle fruit extract was analyzed using UHPLC-QqQ-MS to characterize and identify its phenolic constituents. The identification was based on retention times, precursor ions, and specific fragmentation patterns obtained from the mass spectral data, allowing for the tentative assignment of major polyphenolic compounds as summarized in the corresponding Table 12:

Table 12. Identified compounds in *Myrtus communis* L. fruit using UHPLC-QqQ-MS.

RT	Parent ion	MS^2: relative abundance of fragments	Compound
0.58	[M-H]- 191.0558	71.0126; 85.0281; 127.0391; 173.0448; **191.0557**	(D)-quinic acid
0.87	[M+FA-H]-169.0133	97.02816; **125.02345**	Gallic acid
3.33	[M-H]- 495.0790	70.5668; 109.4739; 143.0341; **169.0135**; 285.9591; 325.0570	3,4-di-O-galloylquinic acid
4.63	[M-H]- 631.0958	58.0287; 151.0025; 227.0344; 299.0209; **316.0230**; 479.0844	Myricetin 3-(6"-galloylgalactoside)
4.82	[M-H]- 479.0839	137.0232; 242.0217; 270.0180; **287.0200**	Myricetin-3-O-galactoside
5.27	[M-H]- 463.0892	151.0027; 242.0215; 270.0178; 288.0270; **316.0229**	Myricitrin
5.35	[M-H]- 300.9993	185.02367:76611;201.01872:61405 283.99625:81606; **300.9995**	Ellagic acid

The phenolic composition of the optimized *Myrtus communis* fruit extract was characterized using UHPLC-QqQ-MS (Table 12). Several major compounds were tentatively identified based on their retention times, deprotonated molecular ions, and MS/MS fragmentation patterns, in agreement with literature reports.

(D)-Quinic acid was detected at RT 0.58 min with a $[M–H]^-$ ion at *m/z* 191.0558. Its typical fragmentation into *m/z* 127, 173, and 85 corresponds to successive losses of hydroxyl and carboxylic groups. Quinic acid is commonly found in *Myrtus communis* and other Mediterranean plants (Bouaoudia-Madi et al., 2019; D'Urso et al., 2017).

Gallic acid appeared at RT 0.87 min as a formic acid adduct $[M+FA–H]^-$ at *m/z* 169.01, with fragments at *m/z* 125 and 97, characteristic of decarboxylation and dehydroxylation reactions. This hydroxybenzoic acid derivative is a major phenolic compound in *Myrtus communis* fruits and leaves (Bounaas et al., 2024; Mërtiri et al., 2025).

3,4-di-O-galloylquinic acid, observed at RT 3.33 min with $[M–H]^-$ at *m/z* 495.08, yielded fragment ions such as *m/z* 325 and 169, corresponding to galloyl and quinic acid derivatives. This compound has also been reported in other galloyl-rich plants, though its presence in myrtle is less commonly documented, suggesting it may contribute to its distinctive bioactivity (Okaiyeto et al., 2023; Taamalli et al., 2014).

Myricetin 3-(6"-galloylgalactoside) was identified at RT 4.63 min with $[M–H]^-$ at *m/z* 631.10. The presence of key fragments at *m/z* 479 (loss of galloyl moiety) and *m/z* 316 (myricetin aglycone) supports its structure. This compound has been rarely reported in myrtle berries and could be responsible for enhanced antioxidant properties (Bouaoudia-Madi et al., 2019; Taheri et al., 2020).

Myricetin-3-O-galactoside, found at RT 4.82 min ($[M–H]^-$ at *m/z* 479.08), fragmented to *m/z* 287 and 270, confirming the glycosylated form of myricetin. This flavonol glycoside is frequently found in *Myrtus communis* and contributes to its strong radical scavenging activity (Babou et al., 2016).

Myricitrin (myricetin-3-O-rhamnoside) was observed at RT 5.27 min with a $[M–H]^-$ at *m/z* 463.09. It fragmented to ions at *m/z* 316 and 288, indicating the loss of rhamnose and confirming the identity of the myricetin core. This flavonoid is commonly reported in the myrtle

genus (Pereira et al., 2017).

Ellagic acid was detected at RT 5.35 min, with a molecular ion at *m/z* 301.00. Its MS^2 spectrum showed characteristic fragments at *m/z* 283, 201, and 185. Ellagic acid is a known constituent in many *Myrtaceae* species, contributing to their anti-inflammatory and antioxidant potential (Ali et al., 2024; Tuberoso et al., 2010).

Conclusion

The phytochemical profiling and UHPLC-QqQ-MS analysis of *Myrtus communis* fruit extract revealed a rich and diverse composition of phenolic compounds, including high levels of total phenolics, flavonoids, proanthocyanidins, and anthocyanins. These compounds are well known for their potent antioxidant properties and potential health benefits. The chromatographic identification confirmed the presence of key bioactive molecules such as quinic acid, gallic acid, ellagic acid, and various glycosylated forms of myricetin, as well as the less commonly reported 3,4-di-O-galloylquinic acid and myricetin 3-(6"-galloylgalactoside). These findings provide strong evidence of the functional potential of *Myrtus communis* fruits as a valuable source of polyphenols, supporting their use in the development of nutraceutical, functional food, and therapeutic formulations. The presence of both commonly known and rare phenolics also suggests a possible synergistic contribution to the biological activities traditionally associated with myrtle fruit consumption.

4. Biological activities of Myrtle fruit extract

Introduction

Myrtus communis L., commonly known as myrtle, is an aromatic evergreen shrub native to Southern Europe, North Africa, and Western Asia. It has since been introduced to South America, the Northwestern Himalayas, and Australia, and is widely distributed throughout the Mediterranean region. Myrtle is cultivated for its ornamental and aromatic qualities, particularly in Northwestern India. In traditional medicine, myrtle was used to treat conditions such as fever, headaches, and diseases affecting the skin, bones, heart, lungs, and reproductive system. However, scientific validation of these traditional uses remains limited (Hussein et al., 2023).

Myrtus communis berries are widely recognized as a rich source of bioactive compounds, particularly phenolics, flavonoids, and anthocyanins, which are largely responsible for their potent antioxidant, antimicrobial, antidiabetic, and anti-inflammatory activities. In the study by Al-Maharik et al. (2023), myrtle essential oil and extracts exhibited significant antioxidant activity, attributed to their high total phenolic and flavonoid contents. Furthermore, the extracts showed notable antimicrobial effects against various bacterial strains, highlighting their potential as natural preservatives or therapeutic agents. The study also reported promising antidiabetic and anticancer activities. Additionally, Mërtiri et al. (2025) also confirmed the antioxidant, antimicrobial, and antidiabetic properties of myrtle berry extracts, supporting their potential use as functional ingredients in food formulation.

The objective of this section was to evaluate the biological activities of *Myrtus communis* fruit extract, including its antioxidant, anti-inflammatory, anti-hemolytic, and antidiabetic properties, as well as the behavior of its phenolic compounds during in vitro digestion.

4.1. Evaluation of antioxidant activity

Since phenolic compounds interact with free radicals in various ways, including single electron transfer, single oxygen atom transfer, and metal chelation, assessing their antioxidant activity requires diverse assays to account for the complexity of antioxidant mechanisms (Bibi Sadeer et al., 2020). Moreover, no single, universal, and reliable method currently exists for determining antioxidant capacity. Therefore, to accurately assess the overall antioxidant effect

of a plant extract, food resource, or food product, multiple activity tests are necessary (Cao & Prior, 1998).

The antioxidant activity of the dried extract was evaluated using eight complementary in vitro assays, namely DPPH-RSA, ABTS-RSA, FRP, FIC, β-carotene bleaching, CUPRAC, NO scavenging, and PAA. The results were expressed as IC_{50} values. The detailed results are summarized in Table 13.

Table 13. Antioxidant, anti-inflammatory, antihemolytic and antidiabetic activities of *Myrtus communis* L. fruit dry extract.

Biological activity	**Test**		**Value**
			IC50 (µg/mL)
Antioxidant activity	DPPH	Dry extract (DE)	6.42±0.005
		Trolox	2,85±2,12
	ABTS	Dry extract	14,22±1,44
		Trolox	3,09±0,16
	FRP	Dry extract	157,80±3,37
		Ascorbic acid	50,15±1,75
	FIC	Dry extract	26,06±0,62
		EDTA	1,43±0,05
	β-carotene bleaching	Dry extract	24,44±1,99
		BHA	20,08±1,96
	CUPRAC	Dry extract	14,88±0,34
		Trolox	10,70±0,005
	NO scavenging	Dry extract	86,38±2,17
		Ascorbic acid	110,22±4,70
	PPA	Dry extract	143,47±0,91
		Trolox	32,87±0,072

The evaluated assays can be classified into three categories according to their mechanisms: DPPH, ABTS, FRP, CUPRAC, and PPA measure the ability of antioxidants to transfer a single

electron to reduce a radical or metal ion. The β-carotene bleaching assay and nitric oxide (NO) scavenging assay assess the ability of antioxidants to donate an oxygen atom or prevent oxidation processes. The ferrous ion chelating (FIC) assay evaluates the ability of antioxidants to bind metal ions, preventing their participation in oxidation reactions (Apak et al., 2007; Chelliah et al., 2022; Gulcin & Alwasel, 2022; Jan et al., 2013; Ueno et al., 2014).

The dry extract of myrtle fruit tested exhibited a good effect against all antioxidant assays.

4.1.1. DPPH scavenging activity

For the DPPH assay, the results presented in Table 13 show an IC_{50} value of 6.42 ± 0.005 µg/mL, indicating a strong radical scavenging activity, although lower than that of Trolox (2.85 ± 2.12 µg/mL), which was used as a positive control. In comparison, Yegin and Güder (2021), who investigated the hypoglycemic and antioxidant activities of *Myrtus communis* L., reported a significantly higher IC_{50} value of 63.52 µg/mL. Similarly, the berry extracts analyzed by Amensour et al. (2009) showed an inhibition percentage of 47.1% at 50 µg/mL, indicating an IC_{50} value greater than 50 µg/mL. This value is higher than the IC_{50} obtained for our sample. Furthermore, our result is lower than those reported by Babou et al. (2016), who observed IC_{50} values ranging from 23.70 to 26.51 µg/mL for berries harvested in December.

4.1.2. ABTS scavenging activity

As shown in the DPPH scavenging assay, the results obtained for the ABTS radical scavenging effect demonstrated strong activity, with an IC_{50} value of 14.22 ± 1.44 µg/mL, which is higher than the IC_{50} of the positive control (3.09 ± 0.16 µg/mL).

Our results agree with those reported by Akyüz et al. (2019), which showed that the scavenging effects of the fruit on the ABTS radical increased with increasing concentrations. However, the fruit exhibited lower ABTS scavenging activity than Trolox. In the study conducted by Jabri, Tounsi, et al. (2016) on myrtle berry seed extract, they found that the extract exhibited a good radical scavenging effect with an IC_{50} value of 184.34 µg/mL, which is higher than the value obtained for our sample of whole fruit extract.

4.1.3. Ferric reducing power

For ferric reducing power activity, as shown in Table 13, the reducing power of *Myrtus communis* fruit extract increased with concentration, demonstrating a dose-dependent activity.

Our results align with those obtained by Aidi Wannes and Marzouk (2013), who assessed the antioxidant activity of different fruit parts from *Myrtus communis* L. They reported reducing power values close to our results for whole fruit, with an IC_{50} value of 130 ± 0.21 µg/mL, which is higher than that of ascorbic acid used as a positive control (40 ± 0.13 µg/mL). In our case, the IC_{50} value was 157.80 ± 3.37 µg/mL, while the positive control (ascorbic acid) had an IC_{50} value of 50.15 ± 1.76 µg/mL. In the study by Amensour et al. (2009), the authors reported that the methanol extract of myrtle berries exhibited the highest reducing power ($P<0.05$). At concentrations of 500 and 1000 µg/mL, they recorded that the activity of extracts was comparable to that of BHT ($P>0.05$). Additionally, ethanol extracts demonstrated significantly higher reducing power than water extracts across all tested concentrations ($P<0.05$). These variations may be attributed to the physiological diversity of *Myrtus communis* leaves and berries, as well as differences in their phytochemical composition. Tuberoso et al. (2013), investigated the antioxidant properties and in vitro vasodilatory activity of three representative food products from the Mediterranean region, correlating these effects with their phenolic content. Their findings confirmed that liqueur derived from myrtle berries exhibited strong ferric reducing power.

4.1.4. Ferrous ion chelating activity

The results obtained are presented in Table 13. In this assay, both the myrtle fruit extract and the standard compound inhibited the formation of the ferrous-ferrozine complex, demonstrating their chelating activity. This suggests that they effectively sequester ferrous ions, preventing their interaction with ferrozine. As shown in the table 13, the presence of myrtle fruit extract impedes the complete formation of the Fe^{2+}-ferrozine complex, confirming its iron-chelating ability, with an IC_{50} value of 26.06 ± 0.62 µg/mL.

Iron can both catalyze peroxidation through the Fenton reaction and enhance its progression by decomposing lipid hydroperoxides into highly reactive peroxyl and alkoxyl radicals. These radicals facilitate hydrogen abstraction, thereby propagating the chain reaction of lipid peroxidation (Elmastaş et al., 2006). Consequently, metal ion chelation plays a crucial role in antioxidant mechanisms by reducing the concentration of transition metals that catalyze lipid peroxidation (Jomova & Valko, 2011). Our results align with those of Tumen et al. (2012), who

reported that myrtle berries exhibit strong ferric iron-chelating activity. They observed an inhibition percentage of 79.29 ± 1.14% with the dichloromethane extract at a concentration of 2 mg/mL.

4.1.5. β-carotene bleaching activity

The results obtained for both the extract and the standard in the β-carotene bleaching test are presented in Table 13.

In our study, *Myrtus communis* fruit extracts effectively inhibited lipid peroxidation, likely due to their phenolic content. Bouaziz et al. (2015) analyzed *Myrtus communis* chemically and evaluated the hypotensive effects and antioxidant properties of methanol, chloroform, ethyl acetate, and aqueous extracts from its leaves. They suggested that *M. communis* extracts exhibit antioxidant activity at both the early and later stages of lipid peroxidation by scavenging free radicals and decomposing peroxides. In the study conducted by Serce et al. (2010), which investigated the antioxidant activities and fatty acid composition of eight *Myrtus communis* L. accessions from the Mediterranean region of Turkey, the findings indicated that in the β-carotene/linoleic acid assay, myrtle fruit extract effectively inhibited linoleic acid oxidation. This suggests that myrtle fruits possess strong antioxidant potential, making them valuable for both human nutrition and food preservation when incorporated into various food products.

Our findings demonstrated strong β-carotene bleaching activity, with an IC_{50} value of 24.44 ± 1.19 µg/mL, indicating a higher antioxidant potential compared to the 78 µg/mL IC_{50} value reported by Aidi Wannes and Marzouk (2013) for Tunisian *Myrtus communis* fruit. Wannes and Marzouk (2016) characterized myrtle seeds (*Myrtus communis* var. baetica) as a rich source of lipids, phenolic compounds, and antioxidant activity. Their study revealed that the seed extract exhibited a strong ability to prevent β-carotene bleaching, with an IC_{50} value of 40 mg/mL.

4.1.6. CUPRAC assay

The results of the CUPRAC assay are presented in Table 13, showing an IC_{50} value (calculated as the concentration corresponding to the absorbance of 0.5 (A0.5)) of 14.88 ± 0.34 µg/mL.

The ability to donate electrons plays a key role in antioxidant activity, as it enables compounds to restore oxidized molecules or disrupt free radical chain reactions, thereby preventing

oxidative damage (Jayaprakasha et al., 2001). The CUPRAC assay measures antioxidant capacity by evaluating the reduction of the neocuproine-copper complex, leading to the formation of a chromophore that absorbs at 450 nm (Mazouz et al., 2020). The results obtained highlight the extract's strong antioxidant capacity, as assessed by the CUPRAC assay. The findings confirm a correlation between the quantity and quality of phenolic compounds and the extract's ability to reduce copper ions (Bhosale et al., 2025; Hasdemir et al., 2016).

4.1.7. NO radical scavenging

The results obtained from the nitric oxide (NO) scavenging assay, as presented in Table 13, demonstrate a strong activity of the myrtle fruit extract (IC_{50} of 86.38 ± 2.17 µg/mL), compared to ascorbic acid employed as a positive control (IC_{50} value of 110.22 ± 4.70 µg/mL), with a significant difference (p<0,05).

Nitric oxide (NO) is a short-lived free radical with a small dipole moment due to the similar electronegativity of oxygen and nitrogen, making it hydrophobic and freely diffusible across membranes. It is generated from sodium nitroprusside, an inorganic complex used as a NO donor, which releases NO spontaneously under light irradiation in aqueous solution at physiological pH. In the presence of molecular oxygen, NO rapidly autoxidizes to form nitrogen oxides, such as nitrogen dioxide, dinitrogen trioxide, and nitrite, with nitrite being the only stable product. The level of nitrite can be quantified using the Greiss reagent, and NO scavengers compete with oxygen to reduce nitrite production in this assay (Sousa et al., 2008). Nitric oxide (NO), beyond its role as a reactive species, is critically involved in the development of several health disorders, particularly in the progression of inflammation, cancer, and other pathological conditions. Bioactive compounds derived from plants may possess the ability to regulate NO production, thus offering potential to alleviate the detrimental effects associated with its excessive generation in the human body (Mahmoudi et al., 2010). Moreover, protection against these reactive species may represent a promising strategy for managing inflammatory diseases (Nakagawa & Yokozawa, 2002; Shen et al., 2002).

Our results are close to the findings of Babou et al. (2016), assessing the antioxidant and anticholinesterase activity of *M. communis* as a function of the maturation stage of different organs (leaves and fruits), different parts of the fruit (pericarp, seeds and whole fruit) and

different extraction techniques (aqueous and hydromethanolic) and reported for Berries harvested in December an IC50 value of 93,98 0,38 µg/mL.

4.1.8. PPA activity

The total antioxidant capacity of *Myrtus communis* L. fruit extracts was determined spectrophotometrically using the phosphomolybdenum method, which is based on the reduction of Mo(VI) to Mo(V) in the presence of antioxidants (Abdille et al., 2005). The IC_{50} was calculated as the concentration corresponding to the absorbance of 0.5 (A0.5), as shown in Table 13. The results obtained in this study (with IC_{50} 143,47±0,91µg/mL) show a strong positive correlation between the PPA and TPC. Several studies (CASFM, 2018; Milošević et al., 2016; Taibi et al., 2024; Zam et al., 2017) have reported similar findings, indicating that antioxidant capacity is proportionally correlated with the phenolic compounds of the extract. The phosphomolybdate assay evaluates an extract's ability to neutralize free radicals by donating an electron. Khan et al. (2012) demonstrated that numerous flavonoids and polyphenols play a significant role in the molybdate-reducing activity of medicinal plants.

In this study, our findings highlight the strong antioxidant properties of extracts derived from myrtle fruits. The differences observed across all tests, compared to the results of other researchers, were mainly attributed to variations in the phenolic composition of the sample (Cheung et al., 2003)

4.2. Antihemolytic effect

To assess the potential correlation between cytotoxicity, cell membrane destabilization, and lytic activity, the in vitro hemolytic activity of the dry extract of myrtle fruit was evaluated using an erythrocyte model. This model is widely employed due to the ease of erythrocyte isolation from blood and the structural similarity of their cytoplasmic membrane to other cell membranes. The impact of the extract on erythrocyte membrane integrity and resistance is presented in the following Table 14:

Table 14. Hemolytic effect of myrtle fruit extract.

Concentration of extract µg/mL	200	400	600	800	1000
% Hemolysis	0,42±0,36	1,99±0,06	3,52±0,42	4,35±0,15	4,71±0,06

Tukey's post-hoc test confirmed significant differences ($p < 0.05$) between all concentrations, except between 800 and 1000 µg/mL, where no significant difference was observed. Hemolysis appeared to plateau at 800 µg/mL.

The results presented in Table 14 indicate a low percentage of hemolysis, ranging from 0.42% to 4.71%, across concentrations from 200 to 1000 µg/mL. This minimal hemolysis in the red blood cell (RBC) model can be attributed to the antioxidant properties of the phenolic compounds in our extract, which play a protective role against oxidative stress that could otherwise damage RBCs (Kaźmierczak et al., 2023).

Assessing hemolytic activity and inhibition percentages represents a key approach in toxicological, medical, and related research fields, as it provides valuable insight into how substances affect red blood cell stability, an important factor with significant implications for human health (El Kamari et al., 2024). Erythrocytes, due to their high content of polyunsaturated fatty acids such as linoleic acid and arachidonic acid, are particularly vulnerable to free radicals (Jabri et al., 2018b). Previous studies align with our results, demonstrating that phenolic compounds exert a protective effect against free radicals, preventing damage to erythrocyte membranes (Boutaoui et al., 2025; Dai et al., 2006; Meenakshi et al., 2013).

Regarding the results gathered in our study, the extracts appear to be a safe source of polyphenols with minimal effects on human cells, as indicated by a hemolysis rate below 5% at various concentrations evaluated using a direct contact method. This complies with the safety standards specified for biomedical materials (Liu et al., 2015), making them a suitable alternative for use in pharmaceutical and cosmetic applications, as well as a functional ingredient in value-added food product formulations.

4.3. Anti-inflammatory effect

Inflammation plays a crucial role in the development of numerous chronic diseases

(Mueller et al., 2010). Denatured proteins, which function as antigens, are associated with Type III hypersensitivity reactions and can lead to diseases such as serum sickness, glomerulonephritis, rheumatoid arthritis, and systemic lupus erythematosus. Heat-denatured proteins can stimulate delayed hypersensitivity, much like native proteins. It has also been confirmed that conventional non-steroidal anti-inflammatory drugs work by preventing protein denaturation. As a result, the anti-denaturation assay is an effective in vitro method for evaluating anti-inflammatory activity (Hazra et al., 2020).

In our work, we investigated the in vitro anti-inflammatory effect of the phenolic extract using the protein denaturation of bovine serum albumin (BSA) test, which assesses the ability of the extract to prevent the thermal denaturation of the protein. The results obtained are presented in Figure 22 as µg/mL, with an IC_{50} value of 366.64 ± 9.10 µg/mL, which was comparable to that of the reference anti-inflammatory drug diclofenac used as the positive control (IC_{50} of 329.23 ± 5.32 µg/mL), with a statistically significant difference ($p < 0.001$).

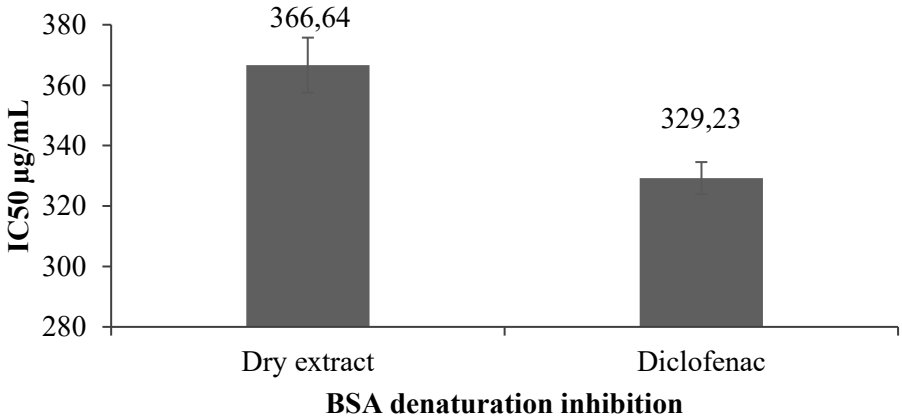

Figure 22. IC_{50} values of *Myrtus communis* fruit dry extract and diclofenac in the BSA protein denaturation assay.

A study conducted by Cruciani et al. (2019) on the biological properties of *Myrtus communis* highlighted its notable anti-inflammatory potential, primarily attributed to its high polyphenol content. The study demonstrated that phenolic extracts from myrtle fruit can significantly reduce oxidative stress and inflammatory responses *in vitro*. In particular, when applied to human intestinal epithelial cells (Caco-2), the extract, mainly in combination with vitamin D_3, was shown to modulate key inflammatory pathways by downregulating pro-inflammatory gene expression and reducing the production of reactive oxygen species (ROS). Additionally,

Hosseinzadeh et al. (2011), in their *in vivo* study using a mouse model, investigated the anti-inflammatory effects of *Myrtus communis* L. aerial part extracts through xylene-induced ear edema and the cotton pellet-induced granuloma test. They observed that both the ethanolic extract (0.05 g/kg) and the aqueous extracts (0.005, 0.015, and 0.03 g/kg) exhibited significant anti-inflammatory activity against chronic inflammation. These findings, together with our results, indicate that polyphenols from *Myrtus communis* L. spice exert protective effects against inflammation, emphasizing their potential as natural therapeutic agents for managing inflammation-related disorders.

4.4. Antidiabetic effect (alpha amylase inhibition)

Diabetes has become one of the most critical global health concerns due to its profound impact on both public health and the economy, as evidenced by the continuously rising number of reported cases (Abd Rashed et al., 2017). According to the International Diabetes Federation (IDF), an estimated 536.6 million people were affected by diabetes worldwide in 2021, and this number is projected to rise to 783.2 million by 2045 (Sun et al., 2022). Among the most effective therapeutic strategies for managing diabetes, the inhibition of α-amylase is one of the most important for controlling postprandial blood glucose levels in diabetic individuals (Remok et al., 2023). As antioxidant-rich diets play a key role in the prevention and management of various diseases, numerous studies have demonstrated a strong correlation between dietary antioxidant intake and enhanced protection against diabetes (Al-Maharik et al., 2023).

The inhibitory effect of myrtle fruit dry extract against α-amylase was evaluated and compared with acarbose as a reference inhibitor. The IC_{50} values are presented in Table 15.

Table 15. Inhibitory effect of myrtle fruit dry extract on α-amylase compared to acarbose (IC_{50}, µg/mL)

Biological activity	**Test**		**Value**
			IC50 (µg/mL)
Antidiabetic activity	Alpha amylase inhibition	Dry extract	28,31±2,95
		Acarbose	2,24±0,09

Our results are consistent with those reported by Yegin and Güder (2021), regarding α-amylase inhibition, where the positive control (acarbose) exhibited an IC_{50} value of

94.89 µg/mL, lower than that of myrtle fruit extract (571.84 µg/mL). However, our findings revealed a significantly lower IC_{50} value of 28.31 ± 2.95 µg/mL, indicating a stronger inhibitory effect. In the study by Mërtiri et al. (2025), an IC_{50} value of 27.27 ± 1.31 µg/mL was reported for an extract obtained by supercritical fluid extraction from myrtle berries, which is close to our result. Nevertheless, the same authors recorded an IC_{50} value of 8.37 ± 0.52 µg/mL for the ultrasound-assisted extract of myrtle berries. As reported in our previous study (Taibi et al., 2024), these variations in phytochemical composition are likely attributable to the influence of several factors, particularly the extraction conditions. In addition to our in vitro findings demonstrating the potential antidiabetic properties of myrtle fruit extract, several in vivo studies have further supported its therapeutic effects. Talebianpoor et al. (2019), confirmed that the administration of hydroalcoholic myrtle fruit extract at doses of 250 mg/kg/day and 500 mg/kg/day to diabetic rats (for 45 days in type I diabetes and 10 days in type II diabetes) has been shown to improve metabolic and renal complications significantly. Elfellah et al. (1984), investigated the hypoglycemic effects of *Myrtus communis* fruit extract in streptozotocin-induced diabetic mice. Intragastric administration of an ethanol–water extract (2 g/kg), given 30 minutes before streptozotocin injection, effectively inhibited the initial onset of hyperglycemia observed 2 hours post-administration. Moreover, when the extract was administered before streptozotocin and repeated at 24 and 30 hours, it delayed the onset of hyperglycemia during the first 48 hours. Notably, administration of the extract 48 hours after streptozotocin significantly reduced hyperglycemia, and this effect was maintained with repeated dosing.

4.5. Antimicrobial Activity

Table 16 presents a summary of the antimicrobial activity of the samples against both Gram-positive and Gram-negative bacteria, as well as fungal strains. Two concentrations (50 mg/mL and 75 mg/mL) were tested, and the diameters of the inhibition zones were measured in millimeters, expressed as the mean ± standard deviation from triplicate tests.

Table 16. Antimicrobial effect of *Myrtus communis* fruit extract.

Bacterial and fungal strains	Origin	Gram	Inhibition zone (mm)			
			50 mg/mL	75 mg/mL	Amoxicillin	Nitronidazol
Staphylococcus aureus (Foodborne)	ATCC 6538	+	$14,75\pm1,06^{c,d}$	$17,00\pm0,71^{c,d}$	$26,25\pm1,06^{c,d,e}$	NT
Methicillin-resistant *Staphylococcus aureus* (MRSA)	ATCC 43300	+	$16,25\pm1,77^{b,c}$	$20,00\pm0,71^{b,c}$	$15,5\pm0,71^{g}$	NT
Enterococcus faecalis	ATCC 29212	+	$17,05\pm0,07^{a,b}$	$21,25\pm0,35^{a,b}$	$17,05\pm0,07^{f,g}$	NT
Bacillus subtilis	ATCC 6633	+	$19,15\pm0,92^{a}$	$22,50\pm0,71^{a}$	$27,5\pm1,41^{b,c}$	NT
Bacillus cereus	ATCC 10876	+	$07,00\pm1,41^{g}$	NT	$27,5\pm1,41^{b,c}$	NT
Pseudomonas aeruginosa	DSMZ 1117	-	$16,25\pm0,35^{b,c}$	NT	$35,5\pm0,71^{a}$	NT
Acinetobacter baumannii	ATCC 19606	-	$10,50\pm0,71^{f,g}$	NT	$26,5\pm0,71^{b,c,d}$	NT
Klebsiella pneumoniae	ATCC 13883	-	$10,40\pm0,57^{f,g}$	$13,40\pm0,71^{f}$	$20,00\pm1,41^{e,f}$	NT
Escherichia coli	ATCC 25922	-	$12,25\pm0,35^{e,f}$	$14,75\pm0,71^{e,f}$	$28,5\pm0,71^{b}$	NT
Salmonella typhi	ATCC 14028	-	$12,06\pm0,85^{e,f}$	$15,20\pm0,14^{d,e}$	$25,5\pm2,12^{d,e}$	NT
Morganella morganii	ATCC 25830	-	$12,95\pm0,04^{d,e}$	$15,60\pm0,57^{d,e}$	R	NT
Aspergillus niger	ATCC 16404	Mold	$13,95\pm0,07^{a}$	NT	NT	R
Aspergillus flavus	ATCC 22546	Mold	$8,50\pm2,12^{c}$	NT	NT	12,75±1,06
Aspergillus ochraceous	ATCC 11028	Mold	$11,00\pm0,00^{b}$	NT	NT	R

Key: NT=Not Tested; R=Resistant

The challenges associated with conventional antibiotics, such as antimicrobial resistance, environmental concerns, carcinogenic potential, side effects, and high costs, have strengthened the shift toward using natural alternatives as antimicrobial agents (Gortzi et al., 2006).

4.5.1. Antibacterial activity

Regarding the antibacterial effect of myrtle fruit extract, as shown in Table 16 above, all bacterial strains tested in this study exhibited inhibition zones. The most sensitive strains were *Bacillus subtilis* and *Enterococcus faecalis*, with inhibition zones of 19.15 ± 0.92^{a} mm and $17.05 \pm 0.07^{a,b}$ mm at 50 mg/mL, and 22.50 ± 0.71^{a} mm and $21.25 \pm 0.35^{a,b}$ mm at 75 mg/mL, respectively. In contrast, the least sensitive strain was *Bacillus cereus*, showing an inhibition zone of 7.00 ± 1.41^{g} mm at 50 mg/mL. The remaining bacterial strains demonstrated moderate sensitivity to the extract at the tested concentrations, with inhibition zones ranging from $10.40 \pm 0.57^{f,g}$ to $16.25 \pm 1.77^{b,c}$ mm at 50 mg/mL. A key observation from the results is that the strains exhibiting the largest inhibition zones were predominantly Gram-positive, while Gram-negative bacteria generally showed only moderate to low susceptibility to the extract. These observations may be attributed to the differences in the cell envelope structures of Gram-negative and Gram-positive bacteria (Shan et al., 2007).

Polat et al. (2014) studied the antibacterial effect of phenolic extracts of myrtle fruits obtained using different solvents and from various regions of Turkey on six bacterial strains, including *Bacillus cereus* FMC 19, *Escherichia coli* O157:H7 RS932, *Listeria monocytogenes* 1/2B, *Staphylococcus aureus* ATCC 28213, *Salmonella Typhimurium* NRRLE 4463, and *Yersinia enterocolitica* ATCC 1501. They confirmed that the acetone extract, similar to our findings with inhibition values falling within a comparable range, exhibited antibacterial activity against *Salmonella*, *S. aureus*, *Bacillus cereus*, and *Escherichia coli*, with inhibition zones ranging from 3.58 to 22.68 mm for *B. cereus*, 8.09 to 30.31 mm for *E. coli*, 7.81 to 23.98 mm for *S. Typhimurium*, and 7.34 to 28.22 mm for *S. aureus*, depending on the locality and solvent dilution used. Mërtiri et al. (2025), investigated the antibacterial activity of myrtle berry extracts obtained via ultrasound-assisted extraction (UAE) and supercritical fluid extraction (SFE) against *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), and *Bacillus* spp. The results demonstrated the effectiveness of myrtle berry extracts against the tested bacterial strains, as indicated by the diameters of the inhibition zones (DIZ, mm). For the ultrasound-assisted extraction (UAE), the inhibition zones were 9.00 ± 0.02 mm for *Bacillus* spp., 10.50 ± 0.71 mm for *Escherichia coli*, and 12.00 ± 2.82 mm for *Staphylococcus aureus*.

In the case of the supercritical fluid extract (SFE), the inhibition zones were 11.50 ± 0.71 mm for *Bacillus* spp., 13.00 ± 0.03 mm for *E. coli*, and 12.00 ± 1.41 mm for *S. aureus*. These results confirm the antibacterial potential of myrtle berry extracts, with variations depending on both the bacterial strain and the extraction method used. Amensour et al. (2010), evaluated the antibacterial activity of myrtle leaf and berry extracts against food spoilage bacteria, including *Pseudomonas aeruginosa* IH, *P. aeruginosa* CECT 118, *P. aeruginosa* CECT 110T, *Pseudomonas fluorescens* CECT 378, and *Bacillus subtilis* DCM 3366 and food-borne pathogenic bacteria such as *Escherichia coli* K12, *Listeria innocua* CECT 4030, *Listeria monocytogenes* CECT 4032, *Enterococcus faecium* CECT 410, *Staphylococcus aureus* MBLA, *S. aureus* CECT 976, *S. aureus* CECT 794, and *Proteus vulgaris* CECT 484. All tested extracts significantly inhibited the growth of at least some of the bacterial strains. Notably, *E. coli* K12 was the only strain that was not inhibited by any of the extracts. Methanolic and ethanolic extracts from both leaves and berries demonstrated considerable antibacterial activity. Inhibition zones for berry extracts ranged from 8.00 ± 0.00 mm to 35.00 ± 1.41 mm, indicating a generally higher efficacy against Gram-positive strains compared to Gram-negative ones, as signaled in the majority of studies. The greater susceptibility of Gram-positive bacteria is thought to be associated with differences in cell wall structure, cellular physiology, metabolic pathways, or the extent of interaction with the antimicrobial agents (Keller & Dörr, 2023).

Amoxicillin was used as the positive control due to its broad spectrum of activity, which explains the large inhibition zones observed for all the bacterial strains tested, ranging from 15.5 ± 0.71 mm to 35.5 ± 0.71 mm, except for *Morganella morganii*, which exhibited resistance.

4.5.2. Antifungal activity

In this study, the antifungal activity of phenolic extracts obtained from myrtle fruits was assessed against three fungal strains: *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus ochraceus*. The extracts were applied at a concentration of 50 mg/mL. After 48 hours of incubation at 30 °C, the results (Table 16) showed that all tested strains were sensitive to the phenolic extract, with inhibition zones of 13.95 ± 0.07 mm for *A. niger*, 8.50 ± 2.12 mm for *A. flavus*, and 11.00 ± 0.00 mm for *A. ochraceus*.

Fungal infections remain difficult to treat due to the limited availability of antifungal drugs,

their associated toxicity, the emergence of resistant strains, frequent relapses, and high treatment costs. These challenges underscore the urgent need for novel antifungal agents. Among natural alternatives, *Myrtus communis*, particularly its essential oils and extracts, has demonstrated promising therapeutic potential (Aleksic & Knezevic, 2014). Certain fungal species, such as *Aspergillus flavus*, are known to produce harmful mycotoxins that can contaminate food. Phenolic compounds have demonstrated significant antifungal properties against these pathogens. Due to their structural characteristics, phenolics can diffuse through microbial membranes and penetrate fungal cells, where they disrupt key metabolic pathways. Specifically, they interfere with the biosynthesis of essential components such as ergosterol, glucans, chitin, proteins, and glucosamine, thereby inhibiting fungal growth and viability (Ansari et al., 2013). To the best of our knowledge and based on a review of the literature, there is a lack, or even absence, of research specifically investigating the antifungal activity of phenolic extracts from myrtle berries. Most studies have primarily focused on the effects of essential oils derived from myrtle leaves and fruits, with only a few addressing the phenolic extracts from myrtle leaves. Alyousef (2021) examined the antifungal activity of *Myrtus communis* aerial parts (stems, flowers, leaves, and roots) against five fungal strains: *Candida albicans* (ATCC 10213), *Candida glabrata* (ATCC 2001), *Candida kefyr* (ATCC 66028), *Candida parapsilosis* (ATCC 22019), and *Candida tropicalis* (ATCC 750). The methanolic extracts of the roots and leaves showed the highest effectiveness against *C. glabrata*, with inhibition zones of 14.5 ± 0.61 mm and 20.7 ± 0.22 mm, respectively. In contrast, the other plant parts exhibited little to no antifungal activity. Cannas et al. (2013), investigated the antifungal activity of essential oil derived from myrtle berries against various *Candida* species, including strains known for their resistance to conventional antifungal drugs, such as *Candida glabrata* and *Candida krusei*, which are often difficult to treat. The results demonstrated notable antifungal effects, particularly against *Candida albicans* and *Candida tropicalis* after 24 to 48 hours of incubation, and against *Candida parapsilosis* after 24 hours. Additionally, significant activity was observed after 48 hours against *C. glabrata*, *C. krusei*, and *C. parapsilosis*. Kordali et al. (2016) confirmed the antifungal activity of essential oil extracted from myrtle fruits of four Turkish genotypes against various fungal strains. Their findings demonstrated that the oils

effectively inhibited the growth of 19 phytopathogenic fungi with inhibition zones ranging from 7,2 ± 1,3 to 33,5 ± 4,6 mm. In addition to other studies that have investigated the antifungal effect of essential oil extracts from myrtle leaves, as well as their phenolic extracts, against various fungi (Brahmi et al., 2023; Erdogan et al., 2014; Fani et al., 2014; Muhanna Al-Abdali et al., 2019).

4.6. In vitro gastrointestinal digestion of myrtle fruit phenolic compounds

Current findings, supported by previous literature, confirm that *Myrtus communis* fruits are a rich source of phenolic compounds (Babou et al., 2016; Messaoud & Boussaid, 2011; Tuberoso et al., 2010). Polyphenols are among the most studied plant-derived antioxidants, widely recognized for their health-promoting properties. However, the intake of phenolic-rich foods does not necessarily guarantee high bioavailability, as the absorption, metabolism, and systemic utilization of these bioactive compounds in the human body may be limited (Palafox-Carlos et al., 2011). This limitation is largely attributed to variations in bioaccessibility, which refers to the proportion of phenolics released from the food matrix during digestion and available for intestinal absorption. Bioaccessibility, typically expressed as a percentage of the total phenolic content, plays a crucial role in determining the in vivo biological activity of these compounds (Zhao et al., 2017).

Furthermore, once ingested, polyphenols undergo various chemical transformations influenced by the physiological conditions of the gastrointestinal tract, including pH, enzymatic activity, and interactions with other dietary components. To exert their systemic antioxidant effects, they must first demonstrate stability during digestion, be efficiently absorbed in the small intestine, and reach the bloodstream for distribution to target tissues and organs (Ozdal et al., 2016)

In this study, the phenolic extract of myrtle fruit was subjected to a simulated oral-gastrointestinal digestion prior to its incorporation into a food system. Total phenolic content (TPC) and DPPH radical scavenging activity were assessed before and after digestion to evaluate their stability under these conditions (Table 17).

Table 17. Total phenolic content (TPC) and antioxidant activity (DPPH) of myrtle fruit phenolic extract before and after in vitro simulated digestion.

Sample	TPC (mg GAE/g)				DPPH [IC50 (µg/mL)]			
	BD	OD	GD	ID	BD	OD	GD	ID
Myrtle fruit Phenolic extract	247.75 ±7.79 [a]	127.51 ±13.91 [b]	126.09 ±10.45 [b]	93.92 ±5.95 [c]	6.42± 0.005 [d]	13.5± 0.99 [c]	17.3± 0.85 [b]	48.98± 1.45 [a]

TPC: Total phenolic extract; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **IC50:** concentration of the phenolic extract required to reduce 50% of the DPPH radical activity; **BD:** Before digestion; **OD:** After oral digestion; **GD:** After gastric digestion; **ID:** After intestinal digestion.

[a,b]: Different superscripted letters (a>b>c) indicate that values (for each independent variable) are statistically different at $p<0.05$ (ANOVA Tukey HSD post-hoc test).

The results of TPC and DPPH assays for both undigested and digested samples are presented in Table 17. Following the first digestion phase (oral digestion), a significant reduction was observed in both total phenolic content (TPC) and DPPH radical scavenging activity. The TPC decreased from 247.75 ± 7.79 mg GAE/g DE to 127.51 ± 13.91 mg GAE/g DE. Similarly, the IC_{50} value of the DPPH assay nearly doubled, indicating a decrease in antioxidant capacity. After the second phase (gastric digestion), no significant changes were observed in total phenolic content (TPC); however, a significant decrease in antioxidant activity was detected. Following the third phase (intestinal digestion), a further significant reduction in TPC was recorded, reaching 93.92 ± 5.95 mg GAE/g DE, while the DPPH radical scavenging activity showed a marked decline, with IC_{50} increasing to 48.98 ± 1.45 µg/mL.

The initial decrease in total phenolic content and antioxidant activity after oral digestion may be attributed to enzymatic activity (e.g., α-amylase) and pH changes in the oral cavity, which can lead to partial degradation or transformation of sensitive phenolic compounds. During gastric digestion, the acidic pH and presence of pepsin may help stabilize certain phenolics, preventing further degradation and thus maintaining relatively stable TPC and antioxidant activity (Odriozola-Serrano et al., 2023). However, the significant reduction observed after intestinal digestion is likely due to the alkaline pH, presence of bile salts, and pancreatic enzymes, which can further degrade or modify phenolic compounds, reducing their availability and antioxidant potential (Bouayed & Bohn, 2010).

These findings suggest that while some phenolic compounds retain their bioactivity after

digestion, others are susceptible to degradation, affecting their overall efficacy (Hamed et al., 2024). This supports the use of phenolic extract from myrtle fruit in food system preparations.

Conclusion

The findings obtained highlight the rich phytochemical composition and remarkable biological potential of *Myrtus communis* fruit dry extract. LC-MS analysis enabled the identification of a diverse range of phenolic compounds, while spectrophotometric assays confirmed high levels of total phenolics, flavonoids, proanthocyanidins, and anthocyanins. These bioactive constituents were closely linked to a broad spectrum of biological activities.

The extract demonstrated strong antioxidant properties across multiple assays (DPPH, ABTS, FRP, FIC, β-carotene bleaching, CUPRAC, nitric oxide scavenging, and phosphomolybdenum assay), confirming its ability to neutralize various reactive species. Additionally, the extract demonstrated promising antihemolytic, anti-inflammatory, and antidiabetic properties, underscoring its therapeutic potential.

Furthermore, simulated in vitro digestion revealed a partial release and transformation of phenolic compounds, indicating moderate bioaccessibility, which is a crucial factor for their physiological effectiveness.

These findings support the use of *Myrtus communis* fruit extract as a valuable source of natural antioxidants and bioactive compounds, with potential applications in functional food development and preventive health strategies.

5. Use of Myrtle (*Myrtus communis*) fruit powder as a functional ingredient for obtaining value-added mayonnaise

Introduction

"Let food be thy medicine and medicine be thy food," a principle attributed to Hippocrates over 2,000 years ago, reflects the long-standing recognition of the health-promoting properties of natural food products since ancient times (Rasouli et al., 2017).

Mayonnaise is among the most widely consumed sauces globally, appreciated for its creamy texture and distinctive flavor (Mirzanajafi-Zanjani et al., 2019). It is commonly incorporated into a wide range of main and side dishes. Technologically, mayonnaise is a semi-solid oil-in-water emulsion, traditionally formulated with egg yolk, refined vegetable oils, stabilizers, flavorings, and spices (Harrison & Cunningham, 1985). Mayonnaise holds a prominent position in the global food market, with its demand steadily increasing (Alvarez-Sabatel et al., 2018; Lee & Kim, 2025).

However, the high content of polyunsaturated fatty acids in vegetable oils makes them particularly susceptible to lipid oxidation, which can negatively affect their sensory and nutritional properties and reduce their stability during storage (Islam et al., 2023). Lipid oxidation leads to the formation of secondary compounds responsible for off-flavors and may even generate potentially toxic substances, thereby compromising food safety.

To prevent these effects, the food industry has long relied on synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) (Xu et al., 2021). However, growing concerns about their potential health risks have prompted manufacturers to explore natural alternatives that are more acceptable to consumers (Nieto et al., 2023).

In this context, plant extracts rich in phenolic compounds, are attracting increasing interest due to their potent antioxidant properties. These compounds are extensively studied in the pharmaceutical, cosmetic, and food industries for their health-promoting effects (Chatterjee & Bhattacharjee, 2015b). Numerous studies have demonstrated that natural ingredients derived from phenol-rich plants, such as berries, grape seeds, sesame germ, and beetroot, can enhance emulsion stability and effectively delay lipid oxidation (Alizadeh et al., 2019; Altunkaya et al., 2013; Raikos et al., 2016).

This section presents the main results obtained from our work. These results are discussed and contrasted with those obtained in other research studies.

5.1. Evaluation of physico-chemical properties

5.1.1. Acidity and pH

Physico-chemical analyses were conducted to evaluate the pH and acidity in mayonnaise over a 28-day storage period (Figures 23 and 24).

The results indicate a gradual decrease in pH values over time.

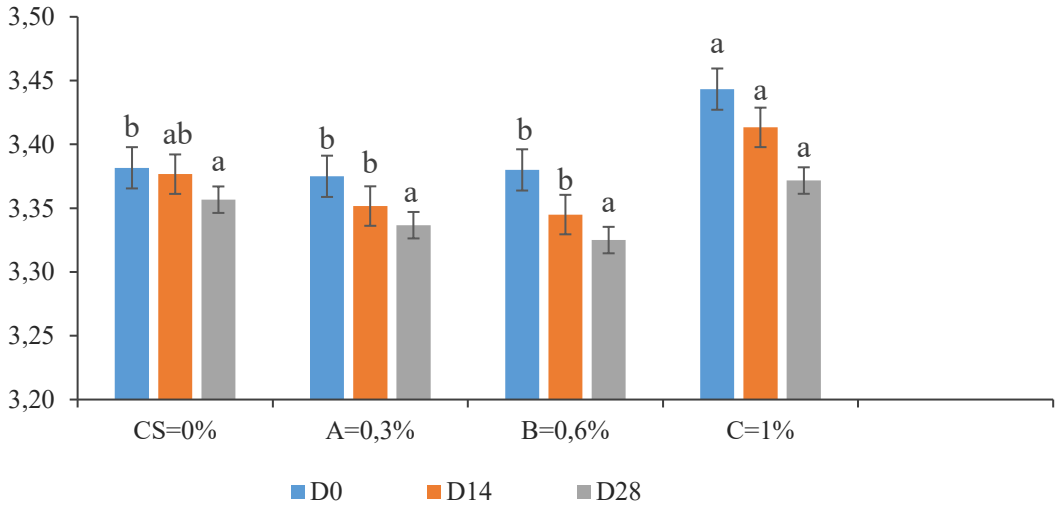

Figure 23. Influence of Storage Duration on pH

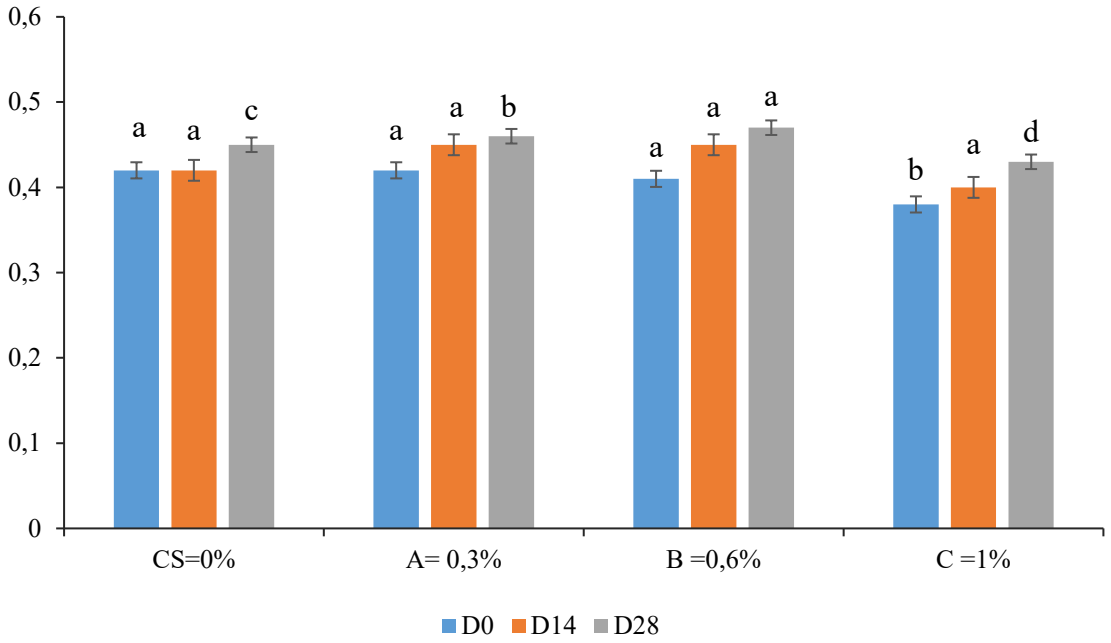

Figure 24. Effect of storage on acidity.

Mayonnaise is classified as an acidic food product. In mayonnaise formulations, pH and acidity are two critical parameters that influence both microbial stability and sensory quality (Yolmeh et al., 2014). The initial pH values of the control mayonnaise (CS) and sample C were significantly different ($p < 0.05$), while no significant difference was observed between the control and samples A and B ($p > 0.05$). A significant difference was also recorded between sample C and both samples A and B ($p < 0.05$). This may be attributed to the initial pH value (5.64) of myrtle fruits (Şan et al., 2015) and the amount of myrtle fruit powder added for the sample C (1%).

At day 14 (D14), a significant difference was noted between the pH of the unenriched sample CS and the enriched samples A, B, and C. However, by the end of the storage period (D28), no significant differences were observed between the samples ($p > 0.05$). Throughout the storage period, sample C consistently showed the highest pH value among all formulations containing myrtle fruit powder.

These findings are in agreement with those of Lee and Kim (2009), who reported that the addition of sesame germ powder increased the average pH of mayonnaise, with the highest pH observed in the most enriched sample. Similarly, Turker et al. (2024), observed that although

the initial pH values of all samples were comparable, those containing fenugreek seed extracts exhibited slightly higher pH levels. Likewise, Lapornik et al. (2005), also found that mayonnaise enriched with ginger powder had a higher pH compared to the control during 20 weeks of storage, while also noting a gradual decline in pH over time.

In a broader context, studies by Worrasinchai et al. (2006) and El-Bostany et al. (2011), also reported a continuous decrease in pH values in mayonnaise samples during storage. This reduction in pH is likely due to the growth of acid-tolerant microorganisms, particularly lactic acid bacteria. Their metabolic activity leads to the production of organic acids, resulting in a gradual pH decline throughout the storage period (Marinescu et al., 2011). Moreover, according to Gmach et al. (2019) and Puligundla et al. (2015), the type of oil used in the formulation of the emulsion has a direct impact on the pH value, influencing the overall stability and behavior of the product.

Regarding titratable acidity, the results observed over the storage period confirmed the trend seen in pH measurements, as acidity and pH are inversely proportional. A significant difference ($p < 0.05$) was recorded at day 0 (D0) between sample C and the other three samples (CS, A, and B), whereas no significant difference ($p > 0.05$) was found between the control sample (CS) and samples A and B. At day 14 (D14), no significant differences in titratable acidity were noted among the samples. However, by the end of the storage period (D28), a significant difference ($p < 0.05$) was observed between all samples. The increase in acidity over time may be primarily attributed to the metabolic activity of acid-tolerant microorganisms, such as lactic acid bacteria, which are likely to proliferate in the aqueous phase of the mayonnaise (El-Waseif et al., 2022; Pourkomailian, 2000). Moreover, these increases are probably due to the activity of hydrolytic and oxidative enzymes present in eggs (Alves Gomes et al., 2017; Zhao et al., 2022)

5.1.2. Salt content

The salinity of the samples showed a slight increase during the storage period, with the exception of sample A, where a decrease was observed, but with no significant difference between all samples (Figure 25).

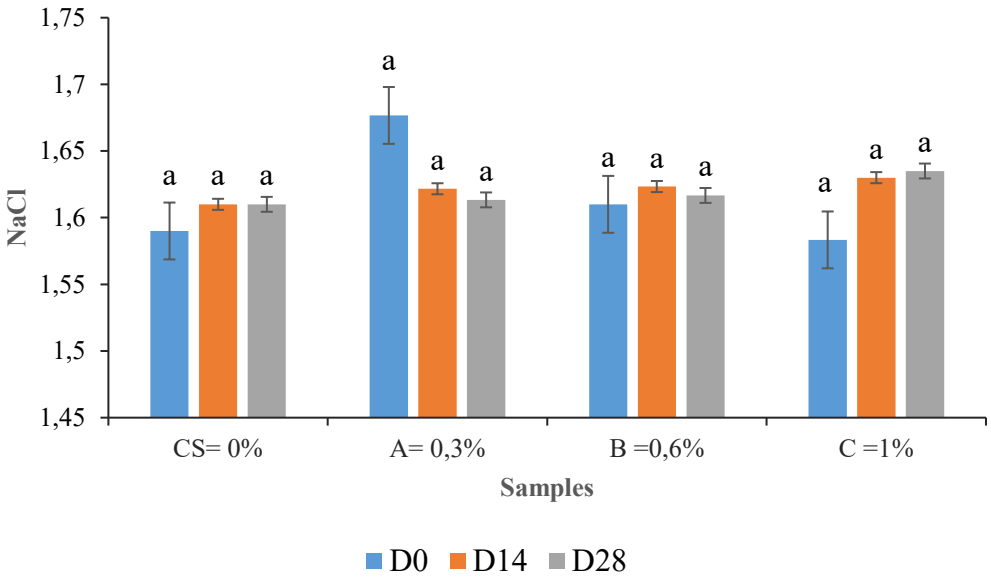

Figure 25. NaCl evolution during storage.

All the analyzed samples had salt contents ranging from 1.58% to 1.68%. Salt is incorporated into mayonnaise to enhance its palatability, inhibit the growth of certain microorganisms, and extend its shelf life. Adding salt enhances the properties and improves the stability of mayonnaise by facilitating the dispersion of egg yolk granules, thereby increasing the availability of surface-active components. It also contributes to neutralizing protein charges, promoting their adsorption and reinforcing the interfacial layer around oil droplets. Moreover, by reducing electrostatic repulsion, salt enables adjacent oil droplets to interact more effectively, leading to improved emulsion stability (Depree & Savage, 2001).

It is used in a precise dose according to the weight of the mayonnaise. At high doses, mayonnaise becomes too salty, and consumption in this state causes hypertension and cardiovascular disease. On the other hand, at low doses, the taste becomes unpleasant and the mayonnaise becomes unpalatable (de Oliveira Lopes et al., 2014) .

5.1.3. Moisture content

The moisture content of the control mayonnaise and the enriched samples (A, B, and C) did not show statistically significant differences during storage (D0, D14, and D28), indicating that the addition of myrtle fruit powder did not markedly alter the overall moisture stability (Figure26).

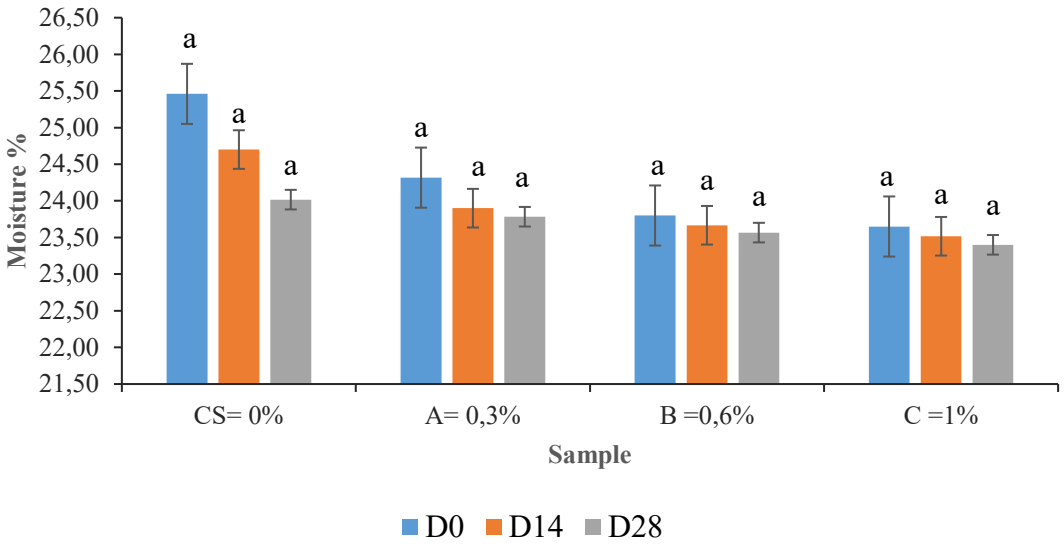

Figure 26. Variation in moisture content as a function of storage time.

Nevertheless, a slight decreasing trend was observed across all formulations over the storage period, with values ranging from 25.46% to 23.4%. This minor variation may be related to the incorporation level of myrtle fruit powder, but it remained within the acceptable limits established by the Cevital unit for dry extract (77 ± 4%). These findings are consistent with those reported by Wong and Chow (2024), who observed a reduction in the moisture content of mayonnaise during five weeks of refrigerated storage following the addition of moringa leaf extract.

5.1.4. Viscosity and consistency

Figures 27 and 28 present the effects of myrtle fruit powder incorporation and storage duration on the viscosity and consistency of mayonnaise samples.

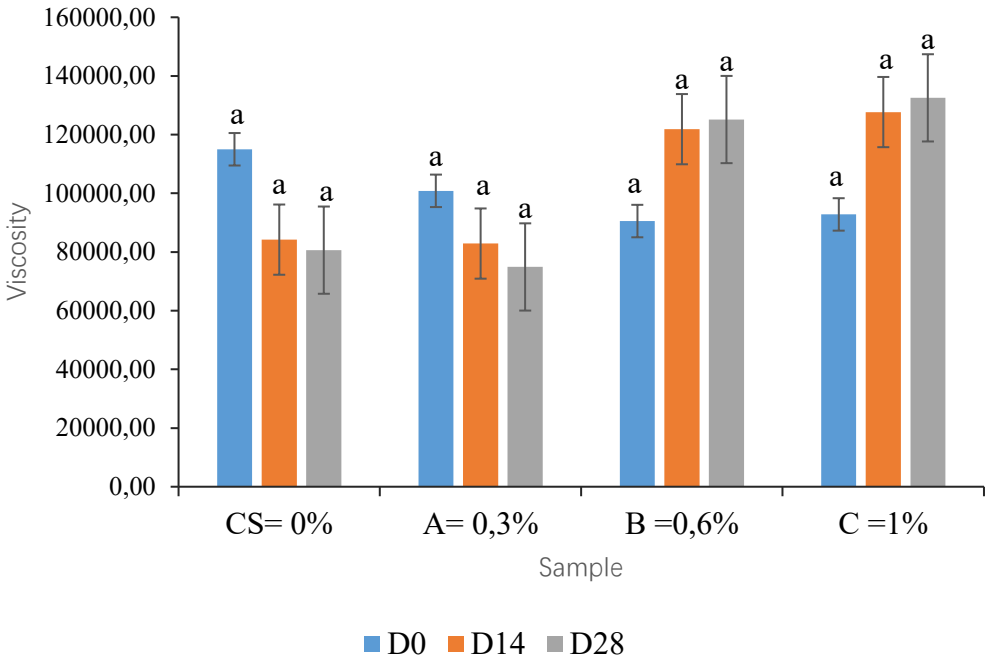

Figure 27. Viscosity variation during storage.

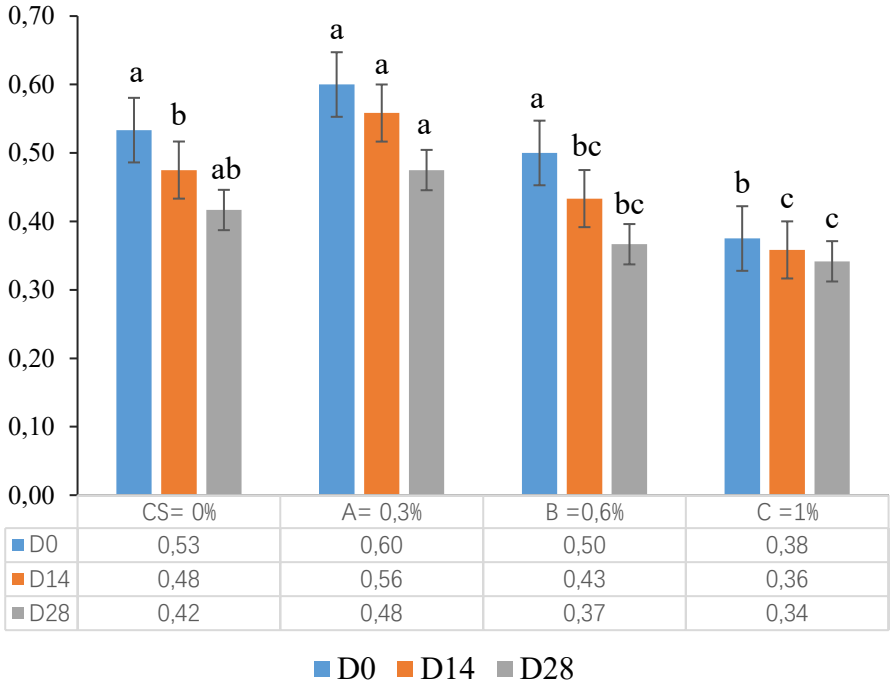

Figure 28. Changes in mayonnaise consistency during storage.

Statistical analysis showed that different concentrations of lyophilized myrtle fruit powder (LMFP) did not significantly affect the viscosity of mayonnaise samples stored at 0, 14 and D28. Viscosity values ranged from [74905-132538 CP] for all samples. A reduction in viscosity

was observed for samples CS and A (0.3%) over D28, while samples B (0.6%) and C (1%) showed an increase.

Changes in viscosity could depend on the sample analyzed, the enrichment rate and the preparation method as signaled in previous studies. According to Gull et al. (2012), the addition of beetroot peels powder to mayonnaise formulations significantly increases viscosity, thereby enhancing the creamy texture of the products. On the other hand, the decrease in viscosity during storage observed in the control and the least enriched samples may be attributed to a reduction in oil droplet size, which increases the distance between droplets and weakens their intermolecular interactions (Katsaros et al., 2020).

No significant differences were observed in consistency values at day 0 (D0) among mayonnaise samples containing different concentrations of LMFP, except for the most enriched sample (1%), which showed a significant difference ($p < 0.05$). Additionally, consistency values measured after 14 and 28 days were significantly affected ($p < 0.05$) when compared to those at D0 for each sample.

As shown in Figure 28, the consistency values of all samples decreased over time. The flow distance of sample C (0.34 cm) was lower than that of samples CS, A, and B (0.42 cm, 0.48 cm, and 0.37 cm, respectively). This indicates that sample C flows more slowly, suggesting higher consistency. All samples exhibited pseudoplastic behavior. These results could be due to the increased amount of LMFP enrichment (Fernández-Ruiz et al., 2016).

These findings are consistent with those reported by Serce et al. (2010), who studied the effect of eugenol clove extract on mayonnaise characteristics and observed a decrease in the consistency index during storage. A similar trend was noted by Lapornik et al. (2005), who investigated the impact of ginger addition on mayonnaise over a 20-week storage period and found that consistency decreased with increasing amounts of added powder.

5.2. Total polyphenol content (TPC)

Figure 29 presents the total polyphenol content of the formulated mayonnaise samples, expressed in milligrams of gallic acid equivalents (mg GAE) per 100 grams of mayonnaise.

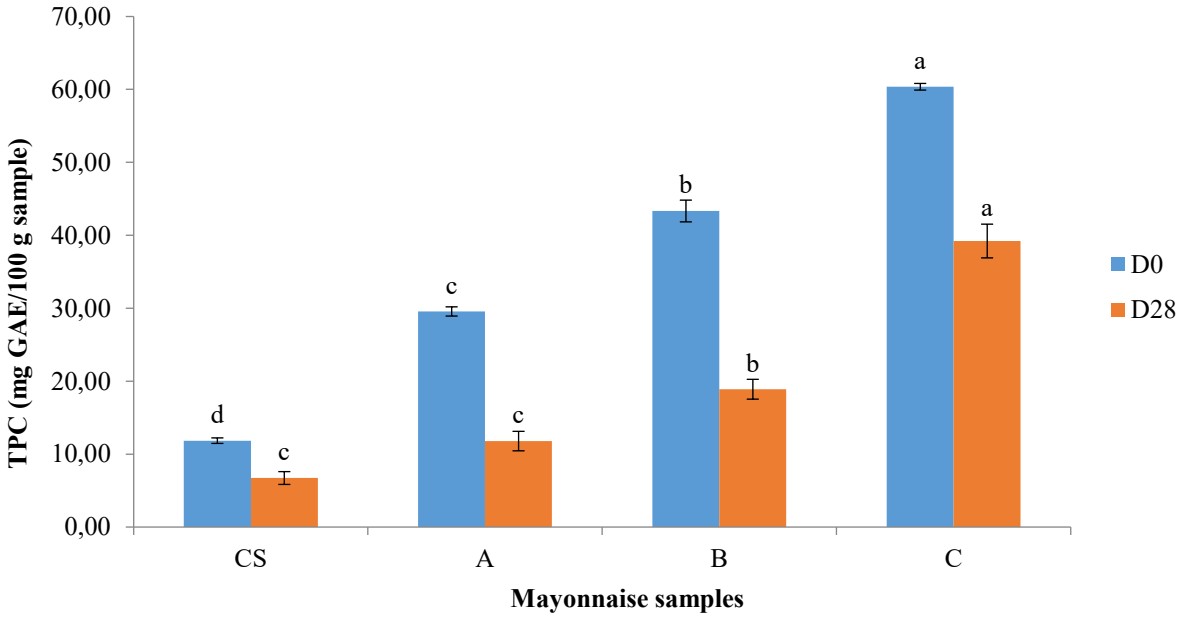

Figure 29. Total polyphenol content in mayonnaise samples (mg (GAE/100g).

The analysis of variance (ANOVA) revealed a significant difference ($p < 0.05$) in the initial total polyphenol content (D0) between all samples. By the end of the storage period (D28), significant differences ($p < 0.05$) were observed between the control sample (CS, without enrichment) and the two enriched samples B and C, as well as between sample A and samples B and C. However, no significant difference ($p > 0.05$) was found between sample CS and sample A at D28.

The total polyphenol content increased with the incorporation of myrtle fruit powder, both at the beginning and at the end of storage. Initial polyphenol values (D0) for samples CS, A, B, and C were 11.84, 29.56, 43.33, and 60.37 mg GAE/100 g, respectively, all of which decreased slightly by day 28. Overall, phenolic content increased proportionally with the level of myrtle fruit powder added, ranging from 29.56 to 60.37 mg GAE/100 g for the most enriched samples. Furthermore, samples B and C exhibited significantly higher levels of bioactive compounds ($p < 0.05$) compared to the control (CS), while sample A showed only a slight, non-significant increase relative to the control at the end of storage (D28).

Similar results were reported by Çelik and Şan (2023), who demonstrated that mayonnaise samples enriched with processed (microwaved) beetroot extract contained significantly higher levels of bioactive compounds (399.6 mg GAE/mL of total polyphenols) compared to the control mayonnaise without beetroot extract. in the same hand, Gull et al. (2012), investigated

the effect of beetroot peel powder (BPP) supplementation at different concentrations (1.5%, 3%, 5%, and 7%) on the bioactive compound content of mayonnaise. Their results showed a significant increase ($p < 0.05$) in total phenolic content, which rose from 197.10 ± 1.91 to 325.9 ± 5.61 mg GAE/100 g with increasing BPP concentration. Similarly, Chatterjee and Bhattacharjee (2015a), found that mayonnaise formulated with eugenol-free clove extract had a significantly higher phenolic content (1.89 mg GAE/g) compared to a commercial mayonnaise sample. Moreover, they observed that the phytochemical content of the control mayonnaise declined after 30 days of storage, while the enriched sample maintained its stability for up to 90 days. As a result, the authors concluded that the addition of clove extract not only enhances the nutritional profile but also extends the shelf life of conventional mayonnaise by approximately 60 days.

5.3. Antioxidant activity

5.3.1. DPPH/ABTS free radical scavenging activity

The antioxidant activity of the extracts was assessed by DPPH and ABTS assays, the radical scavenging capacity being based on the determination of the decrease in absorbance of a methanolic solution of DPPH at 517nm, and of an ethanolic solution of ABTS at 734nm.

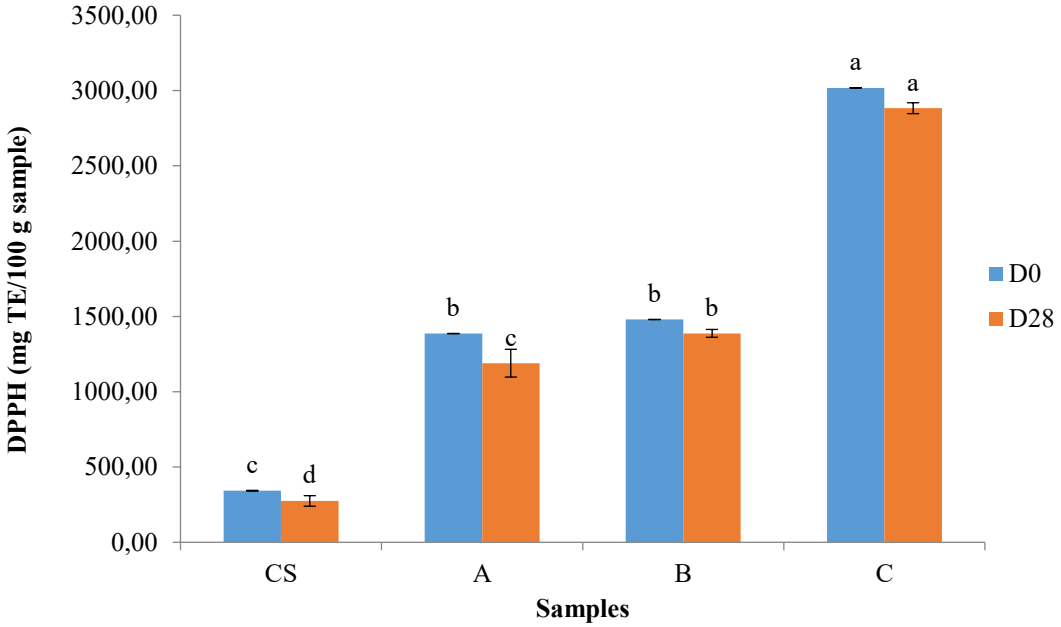

Figure 30. DPPH radical scavenging activity of mayonnaise samples during storage.

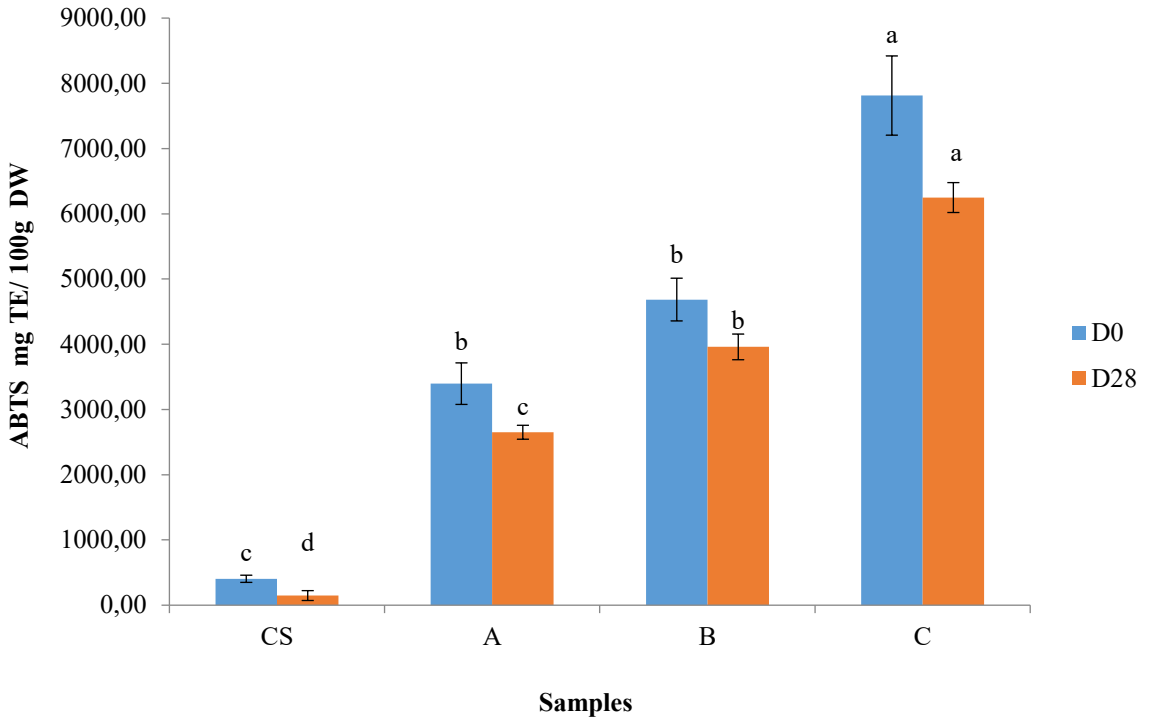

Figure 31. ABTS radical scavenging activity of mayonnaise samples during storage.

According to the results presented in Figures 30 and 31, a notable increase in both DPPH and ABTS radical scavenging activity was observed in all mayonnaise samples during storage. Mayonnaise formulations enriched with freshly prepared myrtle fruit powder exhibited significantly higher antioxidant activities ($p < 0.05$) compared to the control sample. Specifically, DPPH activity values were 1386.28, 1479.60, and 3018.22 mg TE/100 g for samples A, B, and C, respectively, compared to 342.44 mg TE/100 g in the control. Similarly, ABTS activity values reached 3395.83, 4685.18, and 7812.50 mg TE/100 g for samples A, B, and C, respectively, while the control sample showed only 405.09 mg TE/100 g. These findings confirm the strong antioxidant potential of myrtle fruit powder when incorporated into mayonnaise formulations. However, the storage study revealed a general decline in antioxidant capacity after 28 days. This decrease was statistically significant ($p < 0.05$) for all samples at the end of the storage period. At day 28, DPPH radical scavenging activity in enriched mayonnaise samples A, B, and C was 1189.67, 1387.58, and 2882.81 mg TE/100 g, respectively, compared to 274.74 mg TE/100 g in the control sample (T). Similarly, ABTS radical scavenging activity in samples A, B, and C was 2651.23, 3959.87, and 6248.97 mg TE/100 g, respectively, while the control sample showed only 147.63 mg TE/100 g. These

results further support the effectiveness of myrtle fruit powder in enhancing and maintaining antioxidant activity in mayonnaise during storage.

According to Vega et al. (2025), antioxidant activity measured by both DPPH and ABTS assays showed significant differences among the samples ($p < 0.01$). The DPPH assay revealed that all formulations exhibited higher free radical scavenging activity on the day of production, with inhibition values ranging from 27.5% to 70.33%, proportionally correlated with total polyphenol content. In contrast, ABTS values ranged from 5.96 to 6.34 mmol TE/g, showing limited variability. Although the ABTS assay is considered more suitable for assessing both hydrophilic and lipophilic antioxidants, the results did not demonstrate a clear dose-dependent relationship with the extract concentration. Nonetheless, variability in antioxidant activity was generally influenced by the level of phenolic enrichment.

Additionally, mayonnaise samples enriched with beetroot peel powder (BPP) exhibited higher antioxidant activity compared to the control, which can be attributed to the elevated levels of bioactive compounds provided by the BPP (Gull et al., 2012)**.**

In another study, Stambouli-Essassi et al. (2025) reported that the incorporation of watermelon rind flour into reduced-fat mayonnaise had a highly significant effect on enhancing antioxidant capacity ($p < 0.01$). The antioxidant capacity increased proportionally with the amount of powder added, with mean values ranging from 51.64% to 74.02%. The control sample, which contained no watermelon rind flour, exhibited the lowest antioxidant activity.

5.3.2. Ferric reducing power activity

Figure 32 presents the ferric reducing antioxidant power (FRAP) results of mayonnaise formulations enriched with varying concentrations of myrtle fruit powder.

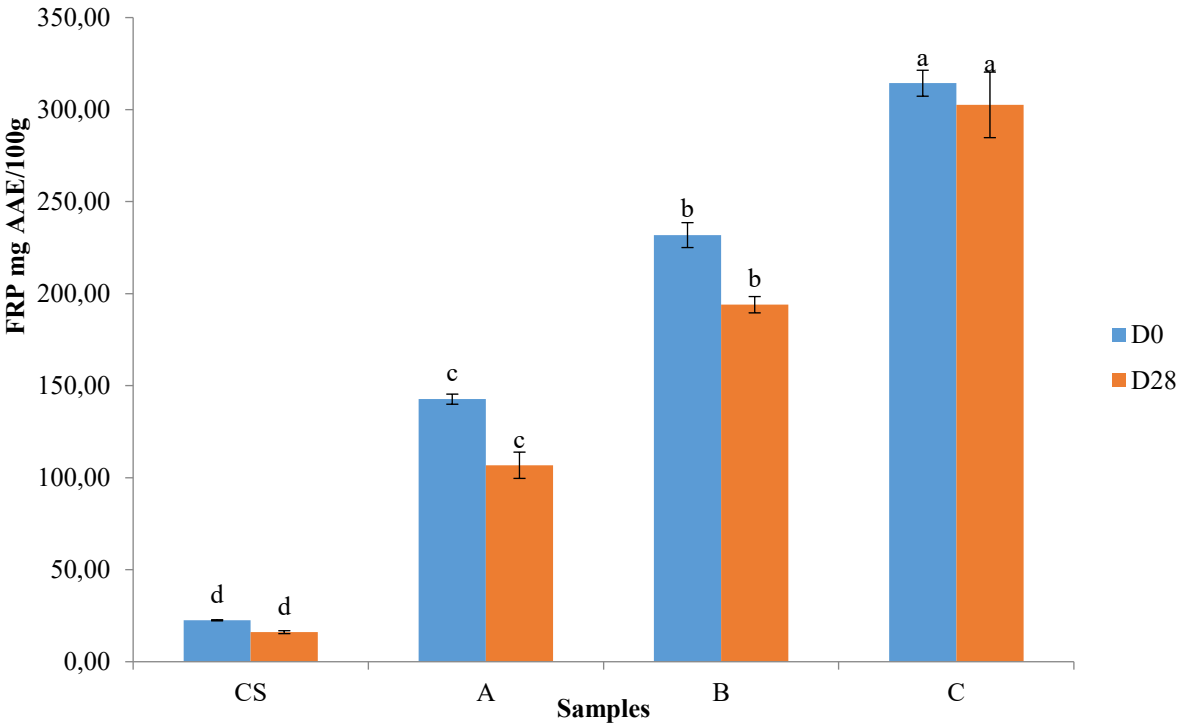

Figure 32. Changes in the reducing power content of mayonnaise.

The reducing power results, assessed by the FRAP assay, revealed significant differences among the mayonnaise samples throughout the storage period. On day 0 (D0), all formulations exhibited their highest ferric reducing antioxidant power. The control sample (CS) recorded the lowest value at 22.51 mg AAE/100 g, while the enriched samples demonstrated markedly higher values, sample C showing the highest (314.33 mg AAE/100 g), followed by sample B (231.81 mg AAE/100 g) and sample A (142.67 mg AAE/100 g). Over the 28-day storage period, a gradual decline in FRAP values was observed in all samples. By day 28 (D28), the control sample again had the lowest value (16.06 mg AAE/100 g), while sample C retained the highest reducing power (302.57 mg AAE/100 g).

Lee and Kim (2009), reported comparable findings, where mayonnaise samples enriched with sesame sprout powder exhibited significantly higher FRAP values than the control (SSP0), which showed the lowest antioxidant activity (30.07 to 8.96 µmol TE/mL over 45 days). Enriched samples, particularly those containing 1.0–1.25% powder, outperformed synthetic antioxidants (EDTA and BHT), likely due to the presence of phenolic compounds that effectively scavenged ferrous ions and delayed oxidative degradation. According to Serce et al. (2010), mayonnaise formulated with eugenol-lean clove extract exhibited significantly higher

ferric reducing antioxidant power (FRAP) on day 0 (D0) compared to commercial mayonnaise. However, this antioxidant capacity declined markedly with prolonged storage.

5.4. Sensory analysis evaluation

Mayonnaise samples prepared with varying concentrations of myrtle fruit powder were assessed for sensory attributes, including color, appearance, taste, odor, texture, and overall acceptability. The results are presented in Table 18.

Statistical analysis of the sensory data revealed that all evaluated parameters were significantly affected ($p < 0.05$) by the incorporation of myrtle berry powder at different concentrations, even within a few hours of preparation.

The highest color and appearance scores were recorded for sample C, followed by sample B, while samples A and the control (CS) received comparatively lower scores. Regarding odor, sample C again received the highest rating, whereas the control had the lowest; samples A and B received intermediate scores.

In terms of taste, all samples were generally well accepted, although sample C received a slightly lower score compared to the others. For texture, the control sample (CS) achieved the highest score, while sample C had the lowest.

Overall acceptability scores indicated a decline in consumer preference as the concentration of myrtle fruit powder increased. The control sample (CS) remained the most preferred among the panelists, suggesting that while myrtle fruit powder may enhance certain functional and sensory characteristics, higher concentrations might compromise overall consumer acceptability.

Comparable findings were reported by Wong and Chow (2024) who evaluated the sensory properties of mayonnaise enriched with Moringa leaf extract. Their study demonstrated that the addition of the extract resulted in lower sensory scores across evaluated parameters compared to the control sample.

Table 18. Sensory evaluation of mayonnaise samples prepared with different concentrations of myrtle fruit powder.

Mayonnaise samples	Sensory evaluation parameters					
	Color	Aspect	Odor	Taste	Texture	Preference
CS	1±0^{b}	1,25±0,375^{a}	1±0^{b}	5±0^{a}	4,13±0,22^{a}	7,75±0,56^{a}
A	2±0^{c}	2,5±0,75^{b}	2±0,5^{c}	2,375±1,03^{b}	3±0.75^{ab}	6,25±0,94^{b}
B	2,25±0,38^{c}	2.25±0.94^{ab}	2,63±0,47^{c}	1.375±0.56^{bc}	2,63±0,813^{b}	5,75±1^{b}
C	3,75±0,38^{a}	2,75±0,375^{b}	3,875±0,22^{a}	1,25±0,38^{c}	2,63±0,719^{b}	5,5±1^{b}

Conclusion

In this study, we formulated a mayonnaise enriched with freeze-dried *Myrtus communis* (myrtle) berry powder, aiming to replace synthetic antioxidants commonly used in the food industry with natural alternatives. This substitution represents an innovative and promising approach to developing functional foods with potential nutritional and therapeutic benefits.

Mayonnaise samples enriched with different concentrations of myrtle powder, A (0.3%), B (0.6%), and C (1%), were evaluated over a 28-day storage period. Physicochemical and sensory analyses were conducted to assess product quality and stability.

The physicochemical results demonstrated compliance with standard quality criteria, particularly in terms of pH (relatively low), salt content ($1.58 \pm 0.01\%$ to $1.68 \pm 0.01\%$), moisture content ($23.4 \pm 0.2\%$ to $25.46 \pm 0.4\%$), and consistency (0.34 ± 0.02 to 0.60 ± 0.01), confirming the overall stability of the product. Additionally, the enriched mayonnaise samples exhibited a marked increase in total polyphenol content (11.84 ± 0.1 to 60.37 ± 0.8 mg/100 g GAE), along with enhanced antioxidant activity, as confirmed by ABTS, DPPH, and FRP assays. These results support the efficacy of myrtle powder as a natural source of bioactive compounds.

Tukey's statistical analysis revealed significant differences ($P < 0.05$) among the enriched samples with respect to polyphenol content and antioxidant capacity. However, no significant differences were observed in pH, salt content, moisture, or viscosity across the different formulations.

Sensory evaluation further confirmed the acceptability of the enriched products at all concentrations (0.3%, 0.6%, and 1%) by the expert panel, despite some variation in individual assessments of taste and texture.

GENERAL CONCLUSION AND PERSPECTIVES

GENERAL CONCLUSION AND PERSPECTIVES

The present thesis aimed to valorize *Myrtus communis* L. fruits by extracting and characterizing their phenolic compounds and evaluating their biological activities, with a view toward their incorporation into functional food products.

The first part of the study investigated the impact of different drying methods (freeze-drying (FD), sun drying (SD), oven drying (OD), and microwave drying (MWD)) on the phytochemical content and antioxidant activity of myrtle fruit extracts. Among the four methods, freeze-drying proved to be the most effective in preserving bioactive compounds. The freeze-dried samples exhibited the highest levels of total phenolic content (88.12 ± 2.70 mg GAE/g DW), total flavonoid content (12.05 ± 0.44 mg QE/g DW), flavonols (29.99 ± 1.81 RE/g DW), condensed tannins (75.40 ± 3.25 mg CE/g DW), and anthocyanins (4.96 ± 0.1 mg CGE/g DW). In terms of antioxidant activity, freeze-dried extracts showed superior performances in DPPH radical scavenging (143.37 ± 4.70 mg TE/g DW), ABTS-RSA (154.31 ± 3.23 mg TE/g DW), ferric reducing power (89.25 ± 5.31 mg AAE/g DW), and phosphomolybdenum activity (354.58 ± 5.47 mg TE/g DW).

Other drying methods (SD, OD, MWD) showed relatively higher TPC and antioxidant activity values. However, microwave drying led to lower levels of condensed tannins (15.40 ± 4.45 mg CE/g DW) and anthocyanins (0.65 ± 0.20 mg CGE/g DW), likely due to degradation of heat-sensitive compounds.

Based on these findings, freeze-dried myrtle fruit powder was selected for optimizing extraction conditions. The optimal parameters (50% acetone, 180 minutes, 40°C) yielded an extract with TPC of 87.19 ± 2.08 mg GAE/g DW, TFC of 12.09 ± 0.04 mg QE/g DW, and condensed tannins of 75.83 ± 1.01 mg CE/g DW.

The optimized phenolic-rich extract, obtained under these conditions, was characterized by LC-MS, revealing a rich phenolic profile with major compounds such as quinic acid, gallic acid, 3,4-di-O-galloylquinic acid, myricetin-3-O-galactoside, and ellagic acid. The extract displayed potent antioxidant effects across eight assays:

– DPPH-RSA: IC50 = 6.42 ± 0.005 µg/mL

– ABTS-RSA: IC50 = 14.22 ± 1.44 µg/mL

– FRP: IC50 (corresponding à A05) = 157.80 ± 3.37 µg/mL

– Phosphomolybdenum: IC50 (corresponding à A05) = 143.47 ± 0.09 µg/mL

– β-carotene bleaching inhibition: IC50 = 24.44 ± 1.99 µg/mL

– Ferric chelation: IC50 = 26.06 ± 0.62 µg/mL

– NO scavenging: IC50 = 86.38 ± 2.17 µg/mL

– CUPRAC: IC50 (corresponding à A05) = 14.88 ± 0.34 µg/mL

In addition to their antioxidant properties, the extracts exhibited low hemolysis rates (<5%) across concentrations ranging from 200 to 1000 µg/mL, confirming good biocompatibility and supporting their safe use in pharmaceutical, cosmetic, and functional food applications. They also showed promising anti-inflammatory activity (IC_{50} = 366.64 ± 9.10 µg/mL) and significant antidiabetic potential, as indicated by strong α-amylase inhibition (IC_{50} = 28.31 ± 2.95 µg/mL). Moreover, the phenolic extract demonstrated broad-spectrum antimicrobial activity. It effectively inhibited the growth of both Gram-positive bacteria (*Staphylococcus aureus*, MRSA, *Enterococcus faecalis*, *Bacillus subtilis*) and Gram-negative strains (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*). In addition, antifungal activity was observed against *Aspergillus niger*, *A. flavus*, and *A. ochraceus*. These results suggest that the extract may serve as a natural antimicrobial agent, particularly valuable for food preservation, nutraceuticals, and pharmaceutical formulations.

Finally, freeze-dried myrtle powder was incorporated into mayonnaise at three concentrations (0.3%, 0.6%, and 1%), and the resulting formulations were compared with a control commercial mayonnaise (without myrtle). The most enriched sample (1%) exhibited a significantly higher total phenolic content (TPC: 39.22 ± 3.20 mg GAE/100 g) and enhanced antioxidant activity, as evidenced by DPPH-RSA (2882.81 ± 20.87 mg TE/100 g), ABTS-RSA (6248.97 ± 258.98 mg TE/100 g), and ferric reducing power (302.57 ± 8.05 mg AAE/100 g). Furthermore, the addition of myrtle powder contributed to improved and stable antioxidant and compositional properties throughout 28 days of storage, without adversely affecting key physicochemical parameters, including pH, titratable acidity, viscosity, and consistency.

This research highlights *Myrtus communis* fruits as a remarkably rich and promising natural resource, endowed with a wide spectrum of bioactive compounds including flavonoids, tannins, and anthocyanins. Their multifunctional biological activities (antioxidant, antimicrobial,

antihemolytic, anti-inflammatory, and antidiabetic) underline their potential as a genuine "medicinal food" and a natural alternative to synthetic additives. Far beyond a simple fruit, *Myrtus communis* embodies both scientific value and cultural heritage of the Mediterranean region, offering a unique opportunity to bridge tradition and innovation. By providing strong scientific evidence, this work encourages greater attention and investment in the valorization of this underexploited plant, paving the way for its incorporation into functional foods, nutraceuticals, pharmaceuticals, and cosmetics. In this context, *Myrtus communis* should not only be seen as a subject of academic study but as a strategic resource capable of contributing to human health promotion, sustainable development, and the creation of high-value products. Based on these findings, which open the way to new opportunities, several perspectives for future research can be proposed.

Perspectives

Building on the promising results of this study, several future directions are proposed to enhance the valorization of *Myrtus communis* L. fruits:

1. **Adoption of Green Extraction Methods**

   Investigate eco-friendly techniques, such as ultrasound-assisted, and ultrafiltration-based extraction, to reduce solvent use and energy consumption while maintaining phenolic integrity.

2. **In Vivo and Clinical Evaluation**

   Conduct animal and human studies to confirm the efficacy, safety, and bioavailability of myrtle phenolic extracts.

3. **Bioavailability and Mechanistic Insights**

   Investigate intestinal absorption and metabolic fate of major phenolic compounds using advanced models (e.g., Caco-2 cells), complemented by mechanistic studies (molecular docking, cell-based assays) to clarify their modes of action.

4. **Encapsulation and Delivery Systems**

   Apply modern delivery strategies (nanoemulsions, liposomes, biopolymers) to improve the stability, controlled release, and bioavailability of bioactive compounds.

5. **Functional Food Development and Valorization**

Myrtle fruit powder can be incorporated into a variety of food products such as dairy (yogurts, cheeses), beverages (juices, smoothies, teas), and baked goods (biscuits, breads, energy bars), where it can act as both a natural preservative and a functional ingredient. Its use could enhance nutritional quality, extend shelf-life, and reduce reliance on synthetic additives. In addition, valorizing other plant parts (leaves, seeds, and by-products) would support a sustainable, zero-waste approach. This strategy aligns with the growing demand for clean-label, health-promoting foods and reinforces the industrial and commercial potential of *Myrtus communis* as a versatile Mediterranean bioresource.

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composition, total phenols and favonoids

contents, antimicrobial, antioxidant, anticancer,

and α-amylase inhibitory activity

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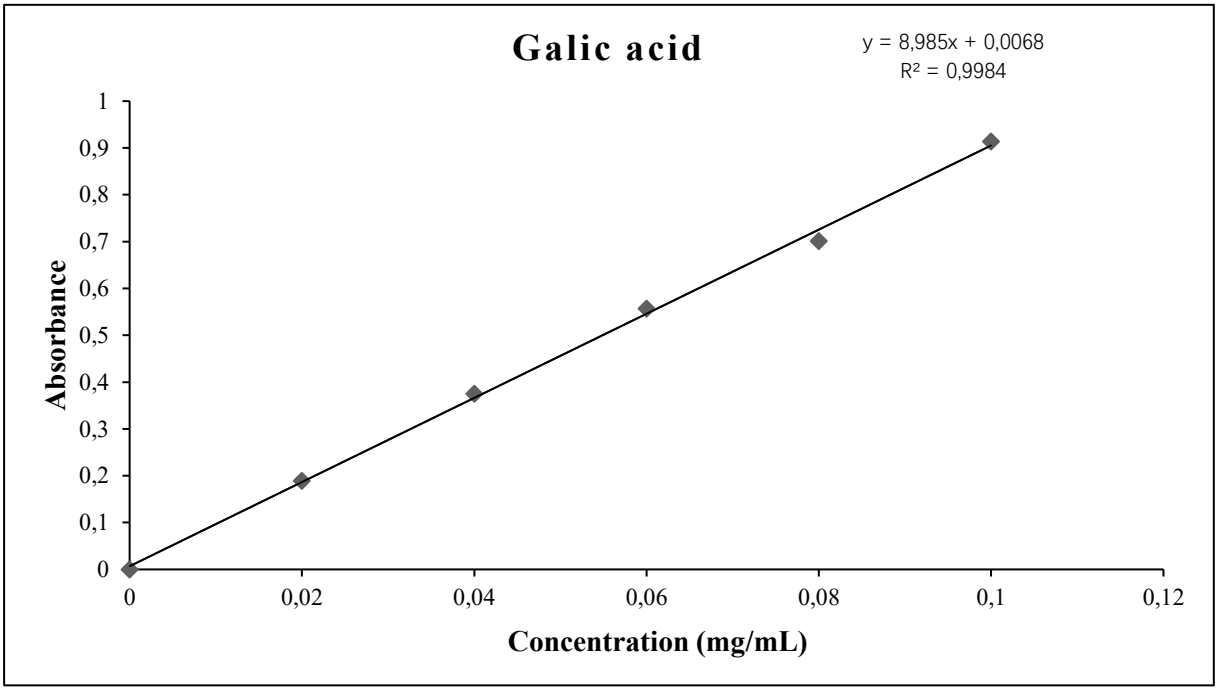
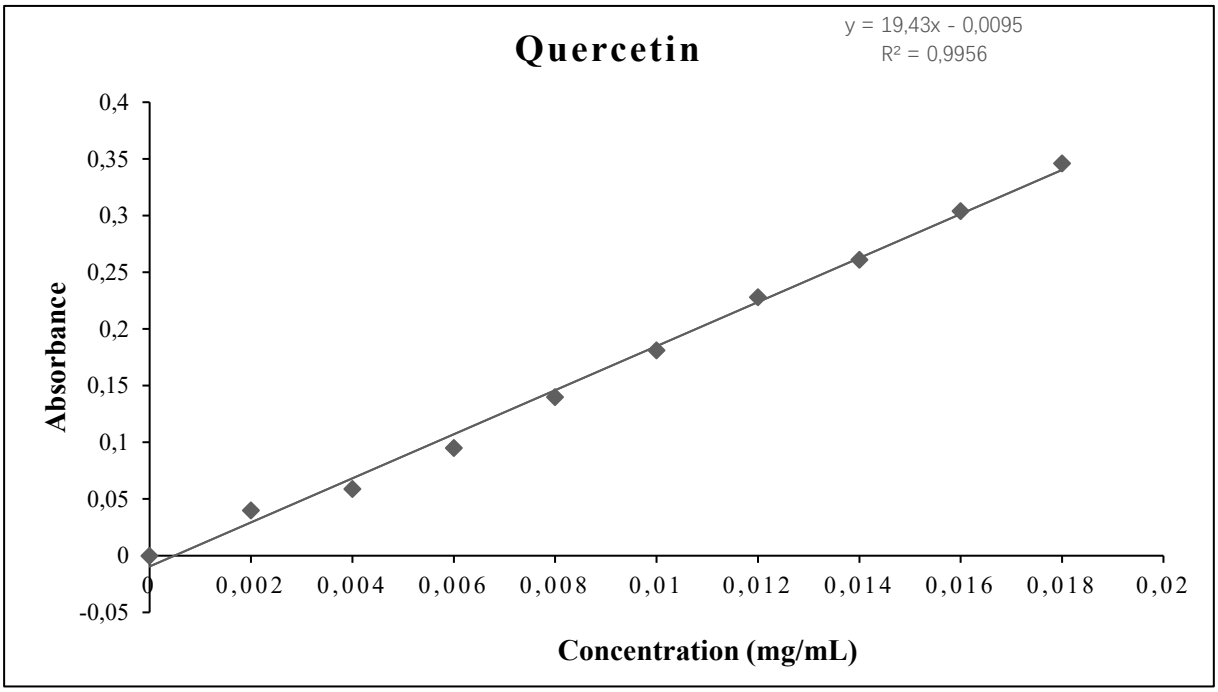
Appendix

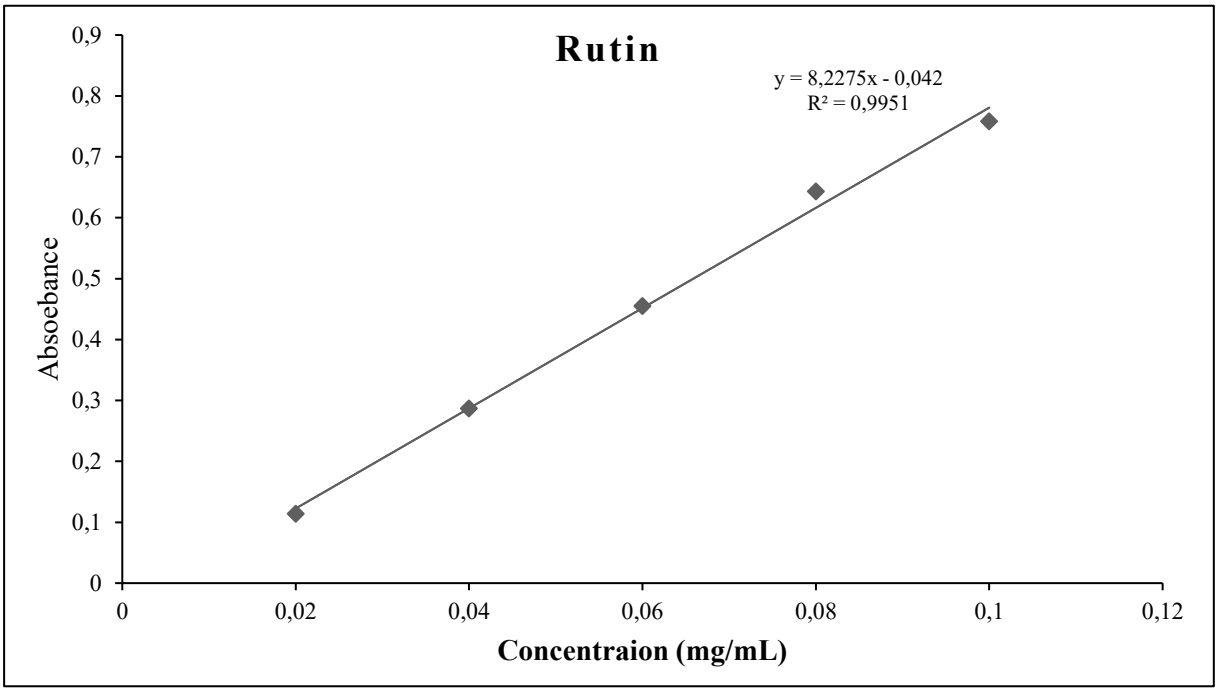
Appendix

Appendix 1. Calibration curves

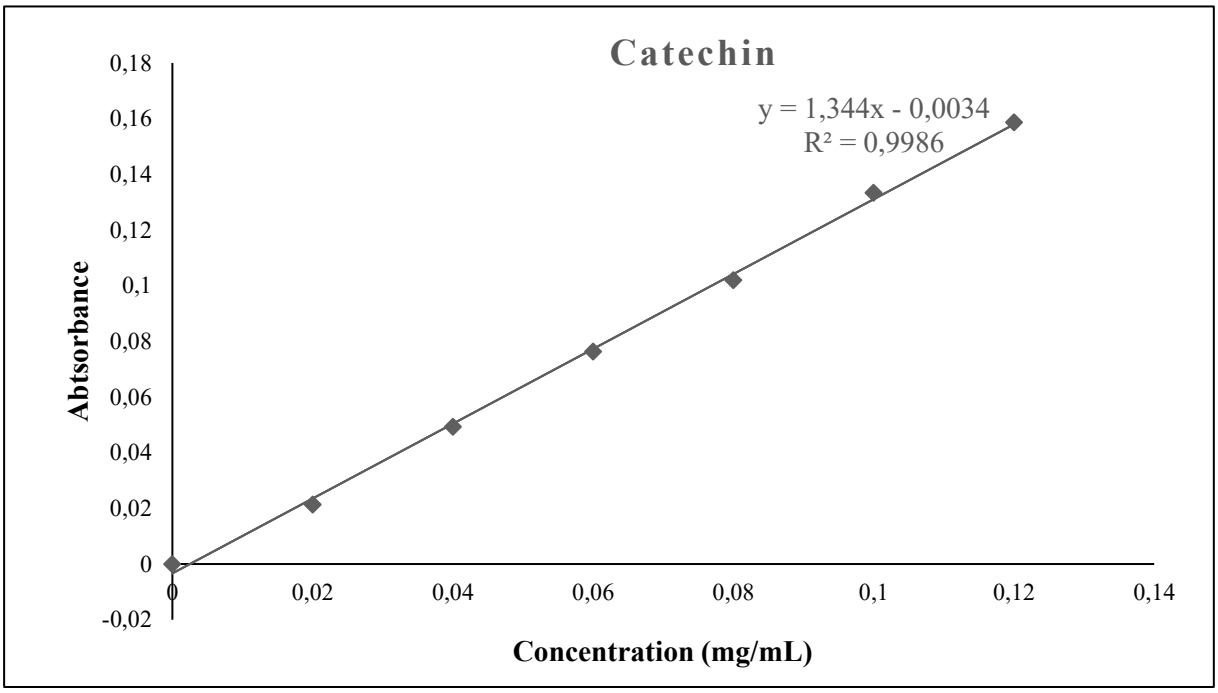




Quercetin calibration curve for TFC content

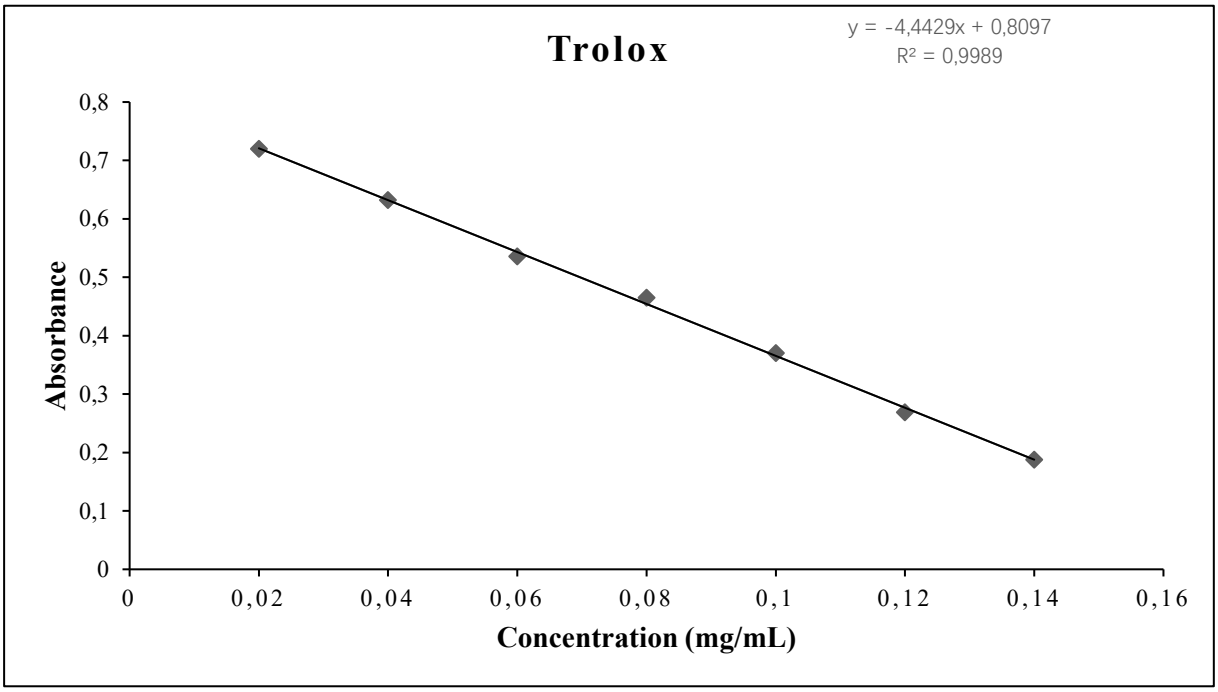


Rutin calibration curve for Flavonols content

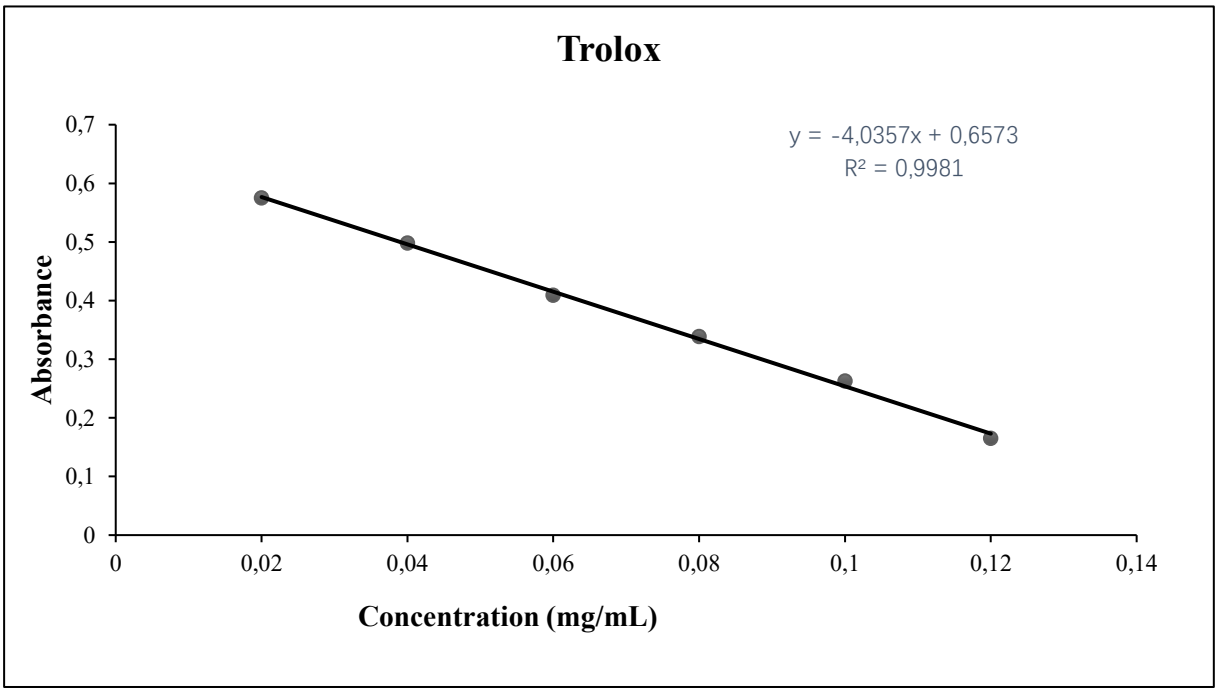


Catechin calibration curve for PAC

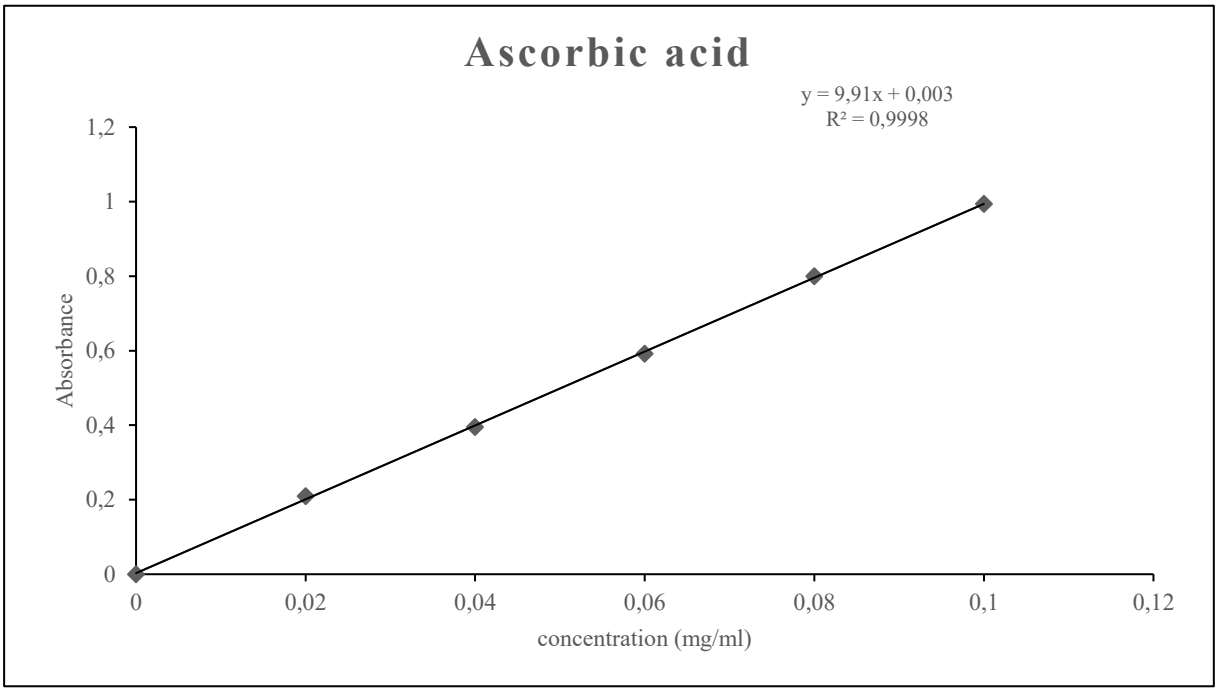
Appendix



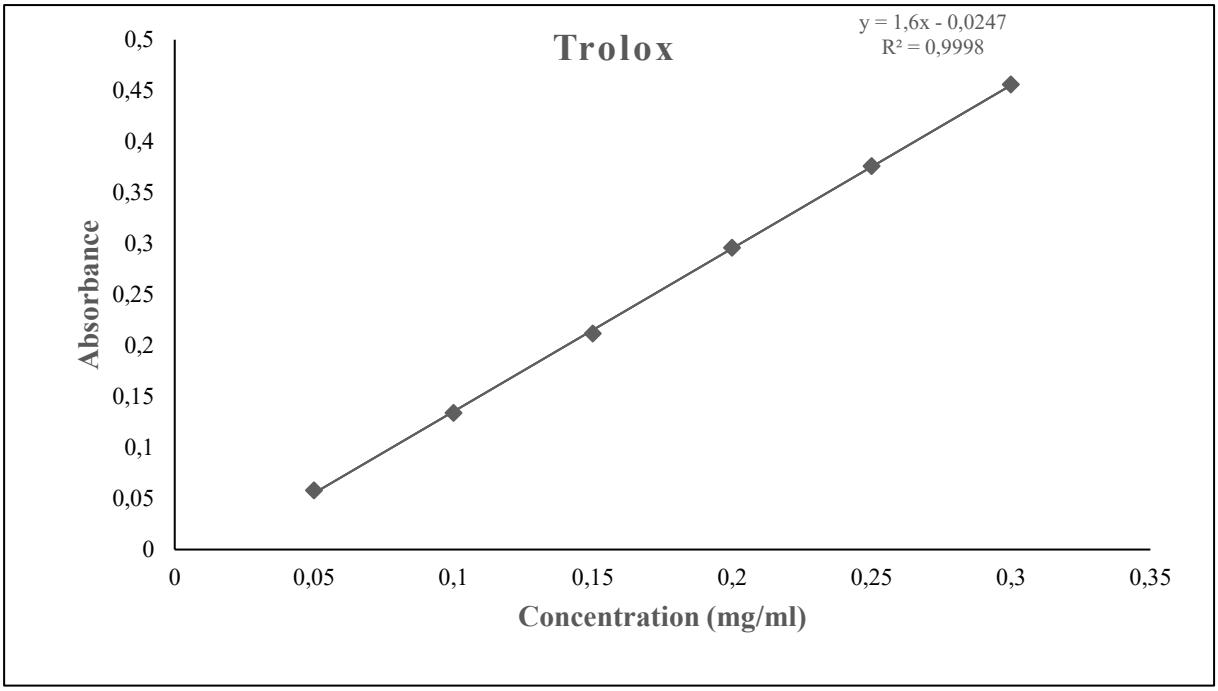
Trolox calibration curve for DPPH test



Trolox calibration curve for ABTS test

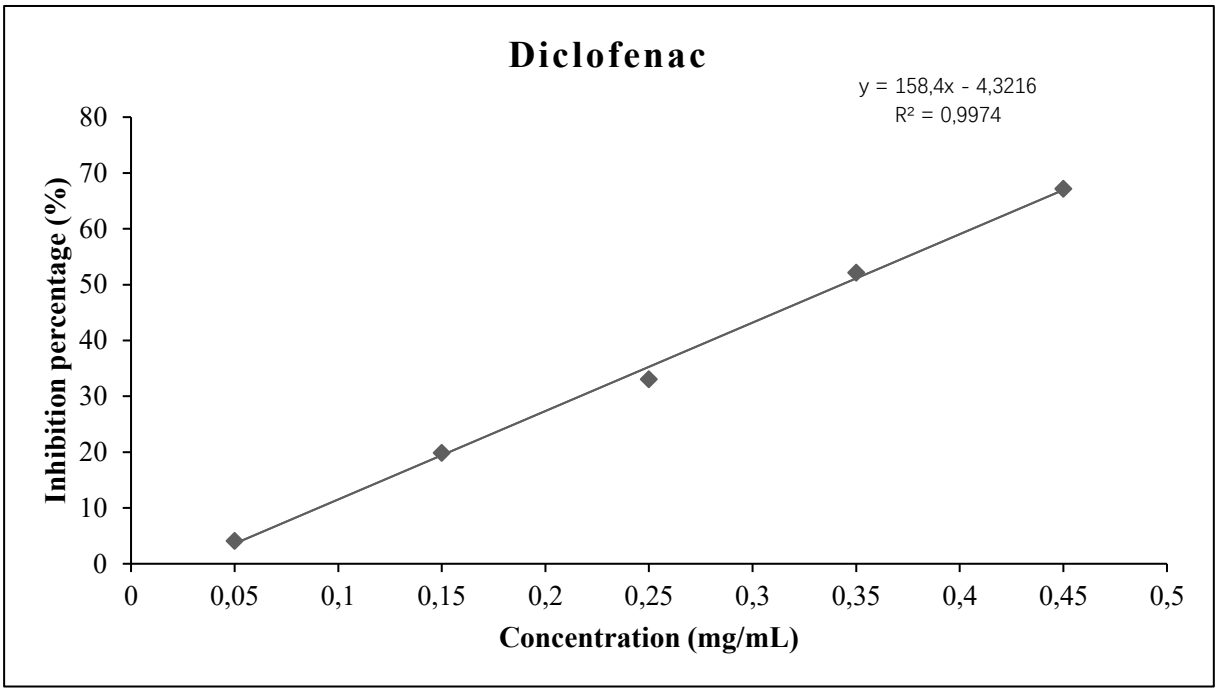


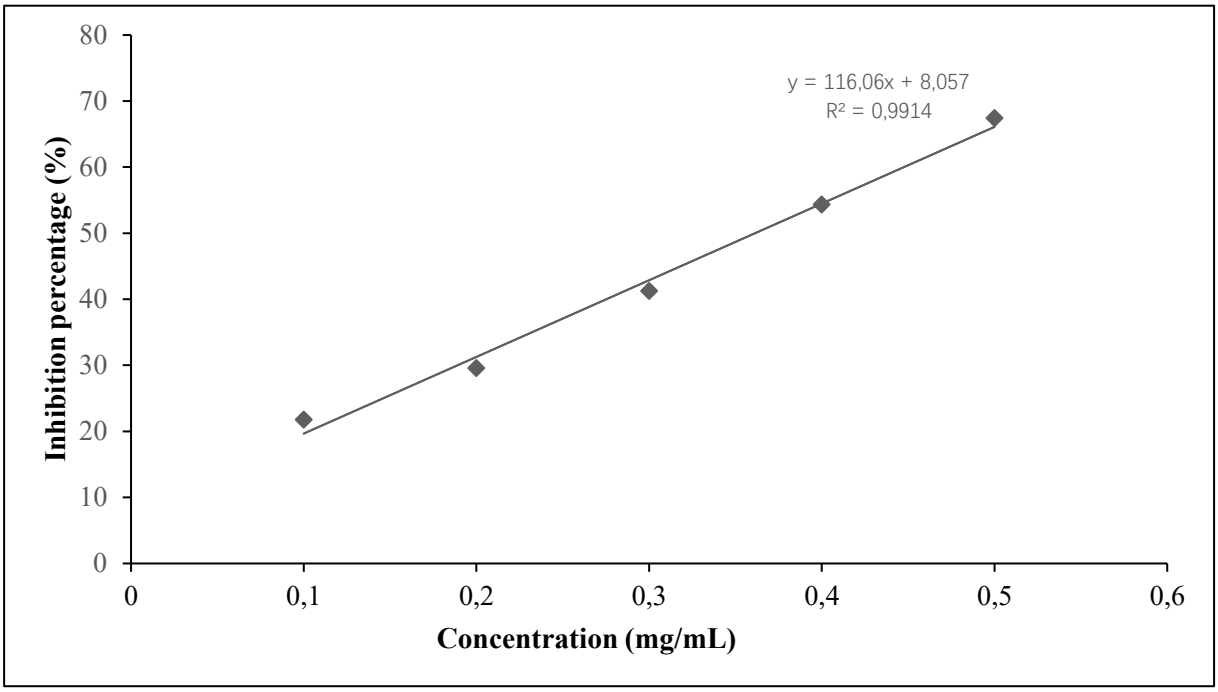
Ascorbic acid calibration curve for FRP test



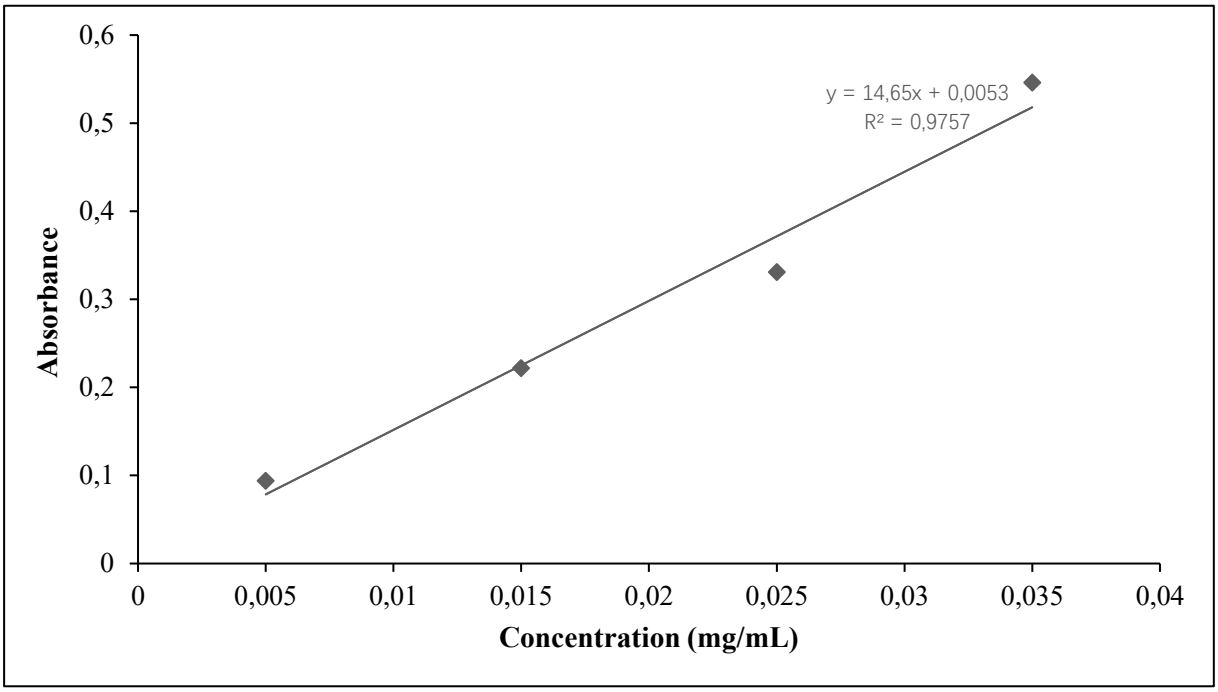
Trolox calibration curve for PAA test

Appendix 2. Calibration Curves for IC_{50} determination

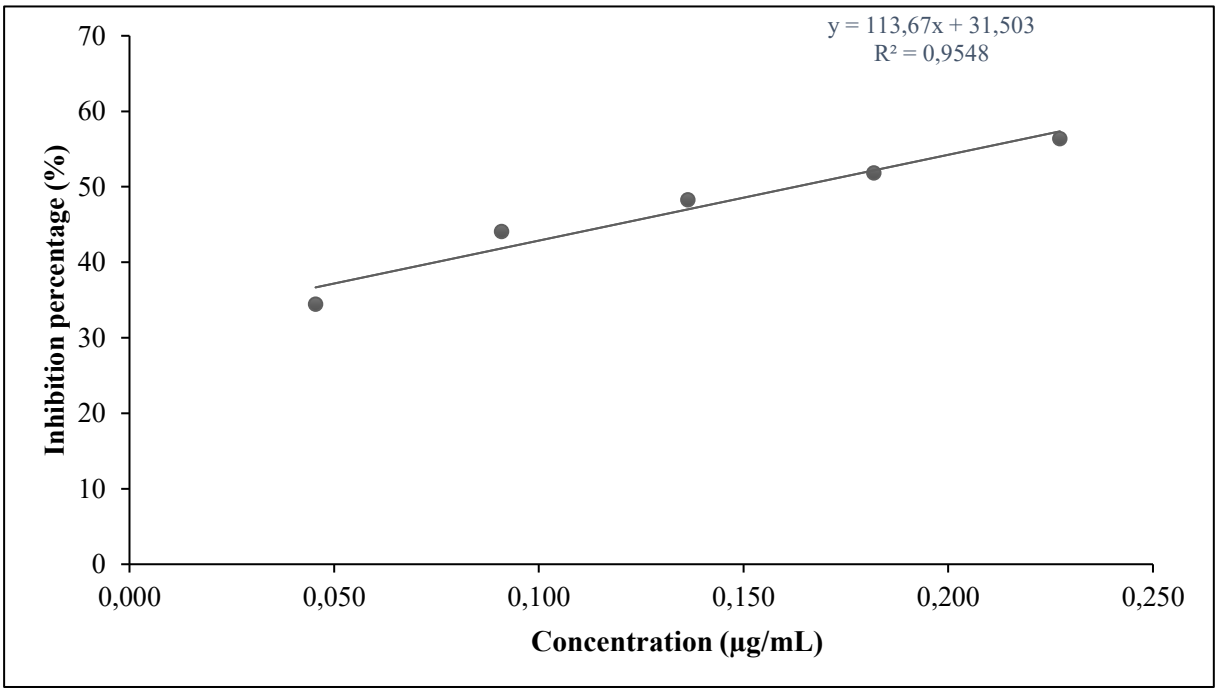




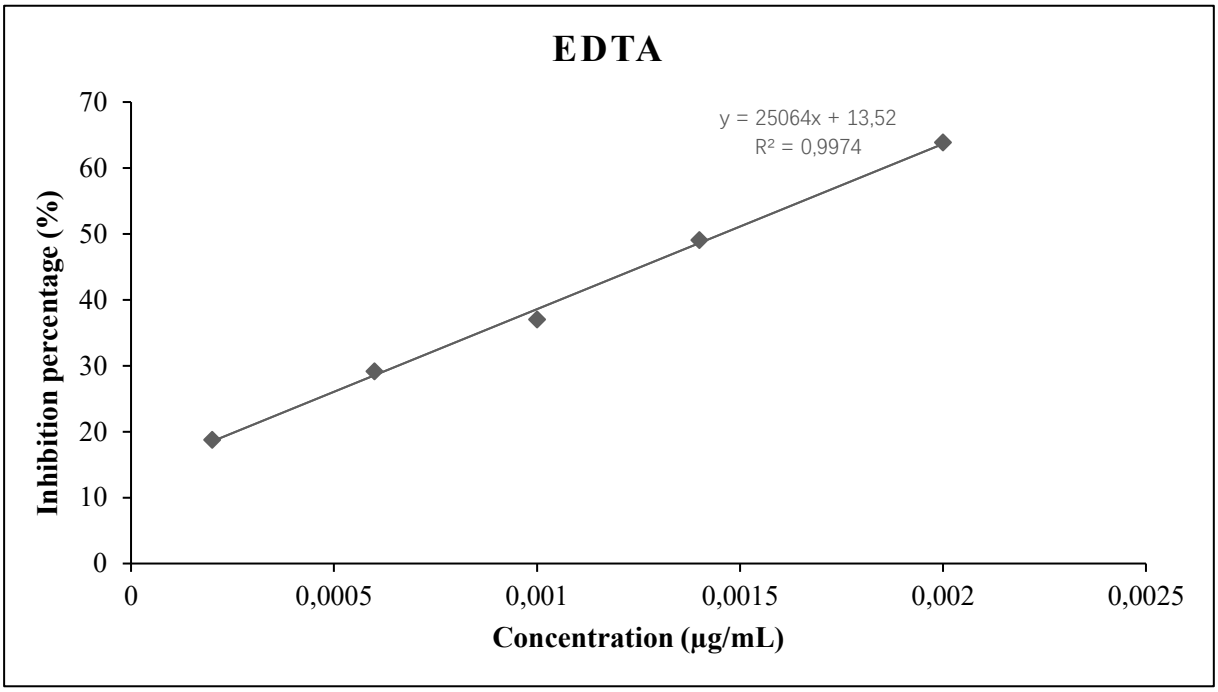
Inhibition percentage of BSA by myrtle extract in the anti-inflammatory test

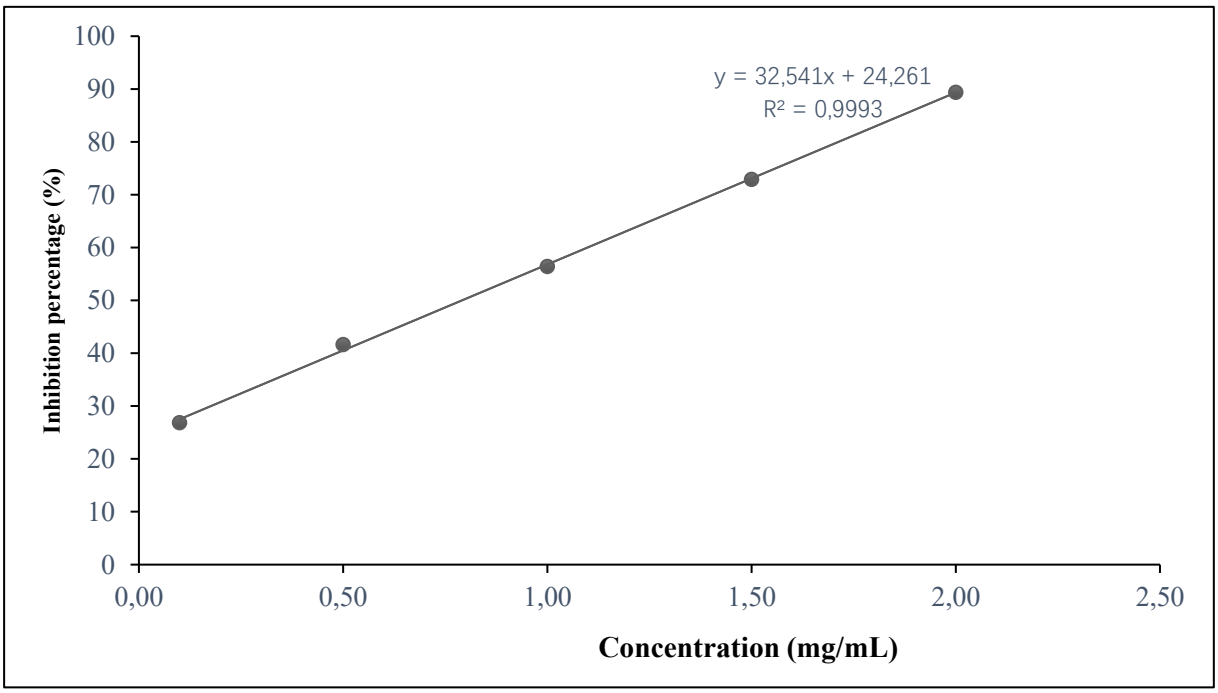


Calibration curve for IC50 determination of PAA test (myrtle extract)

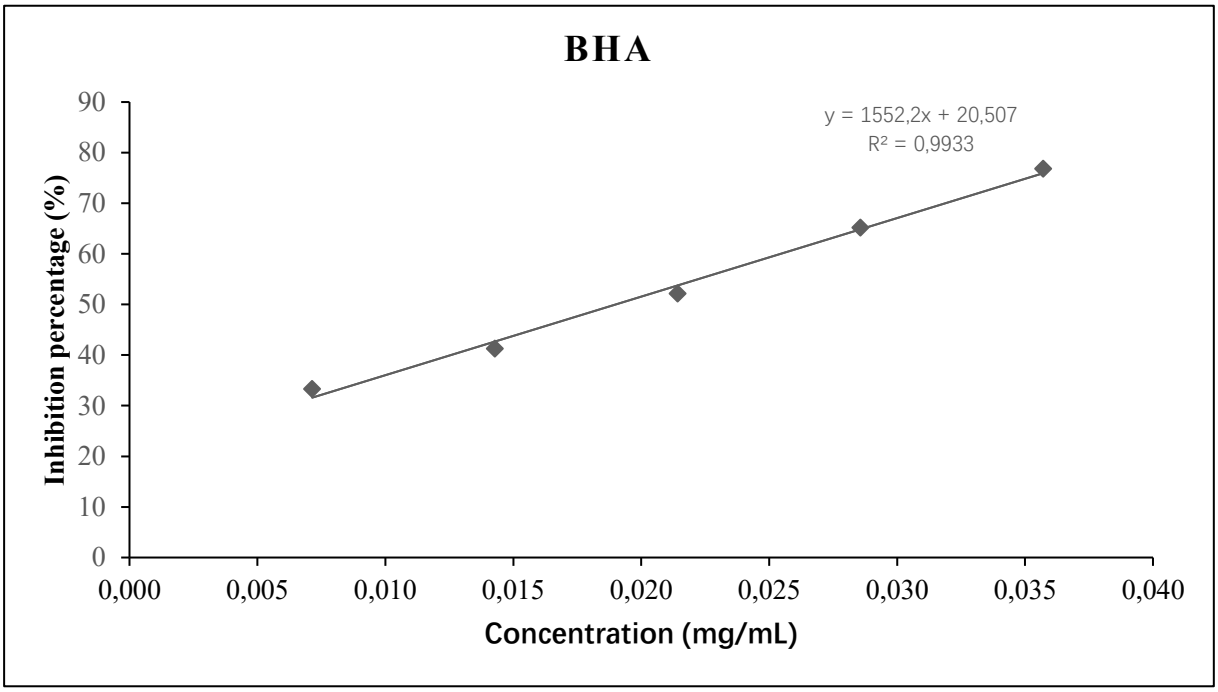


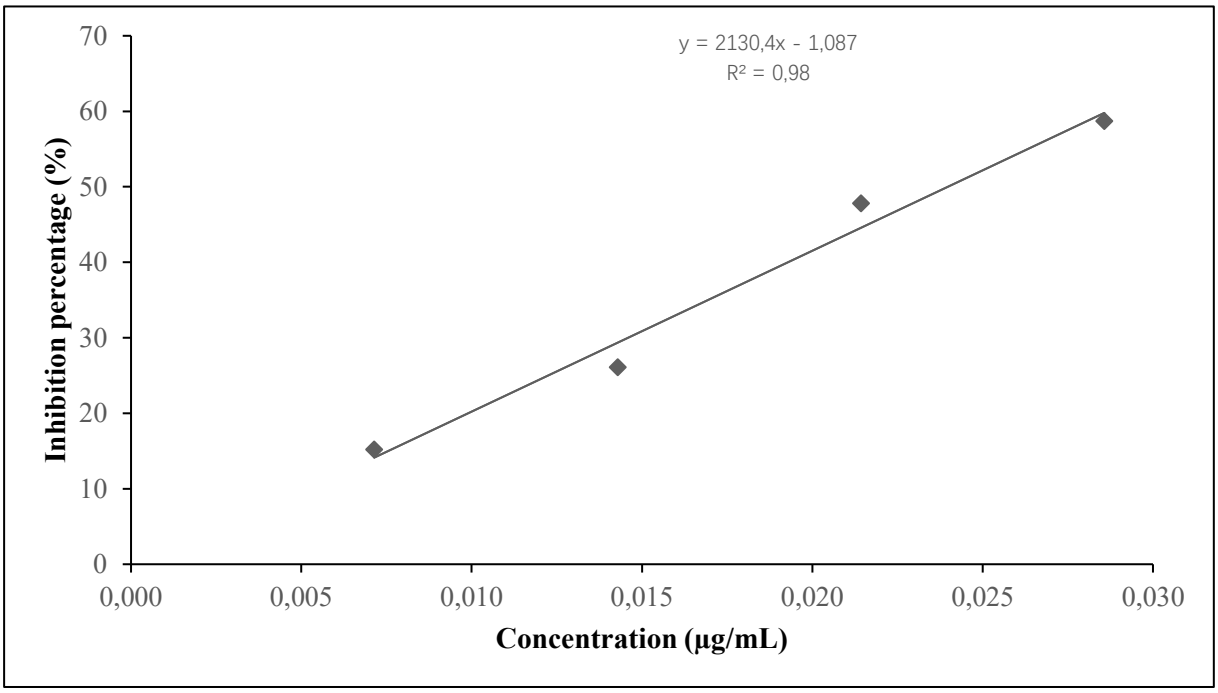
Inhibition percentage of alpha amylase by myrtle extract



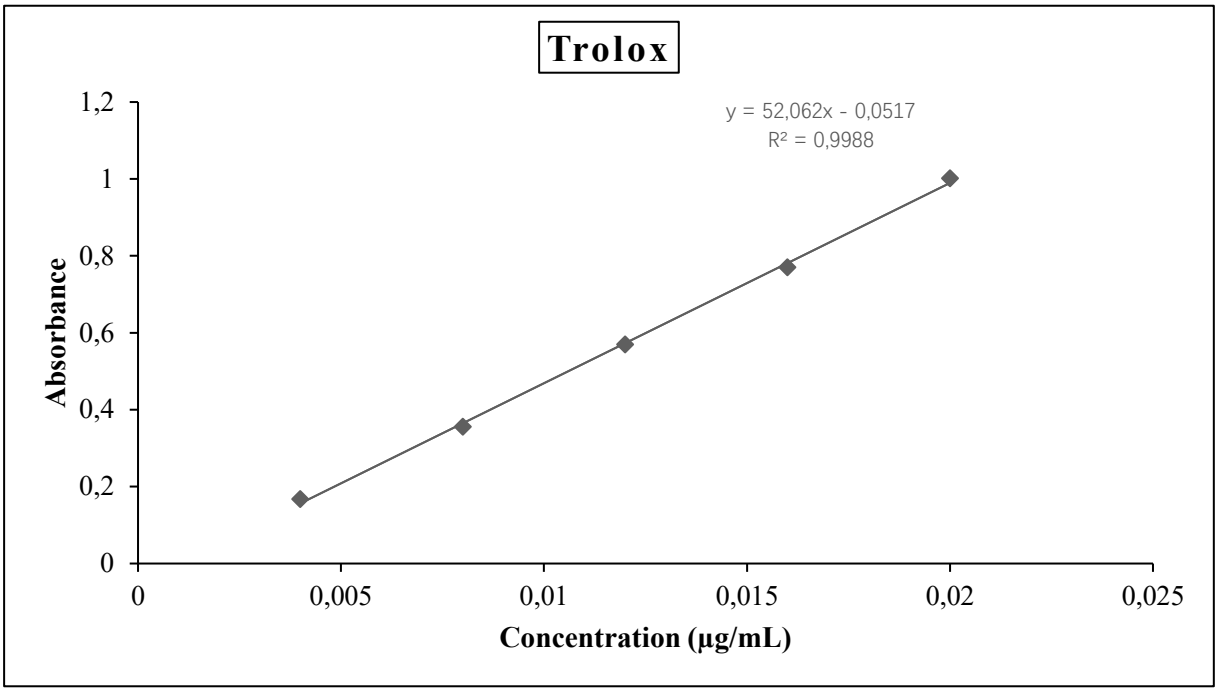


Inhibition percentage by myrtle extract in FIC test

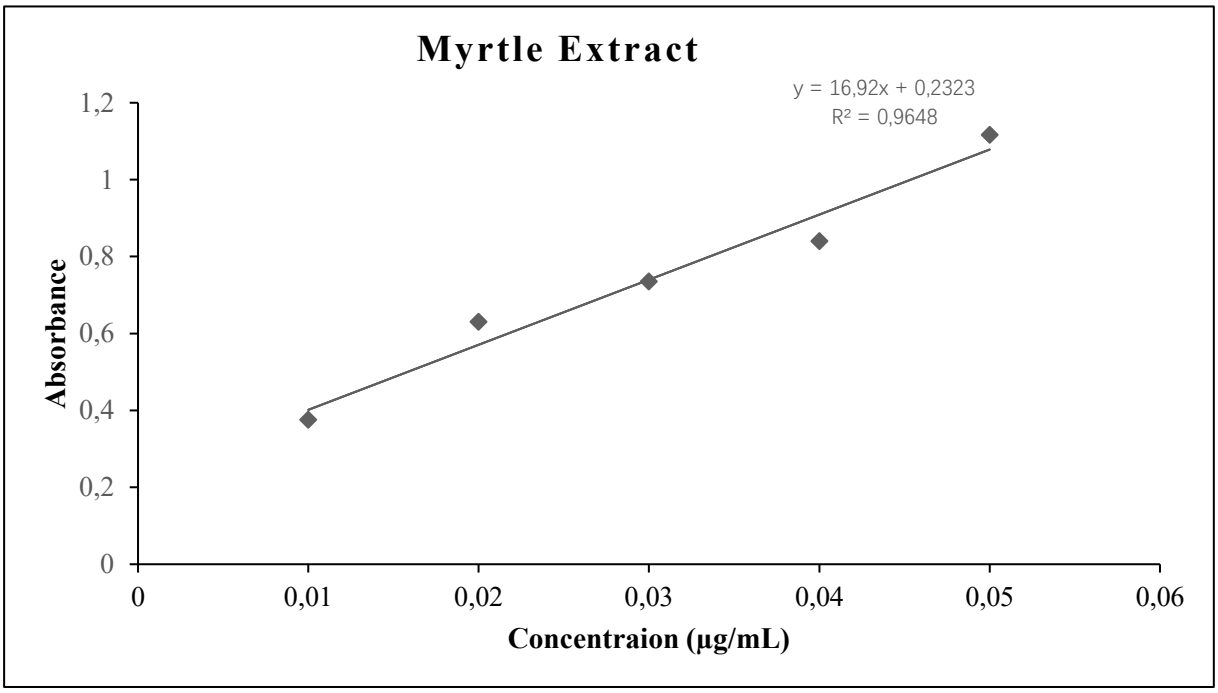




Inhibition percentage in the β-carotene bleaching test by the extract



CUPRAC test



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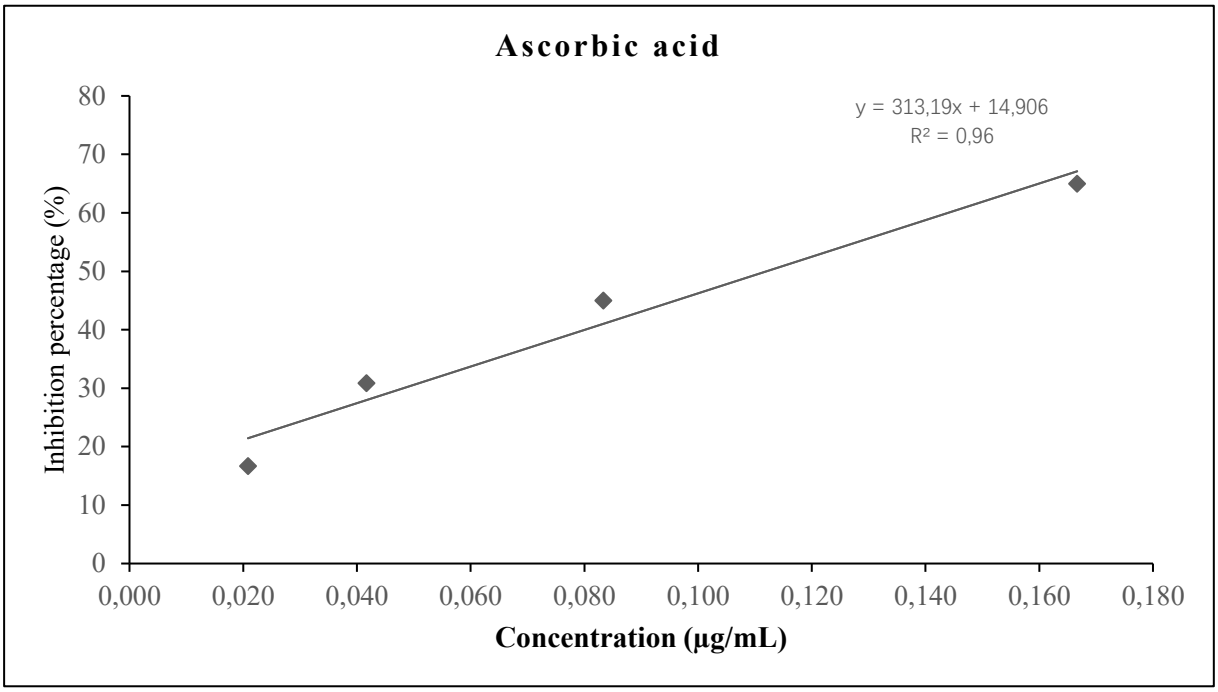
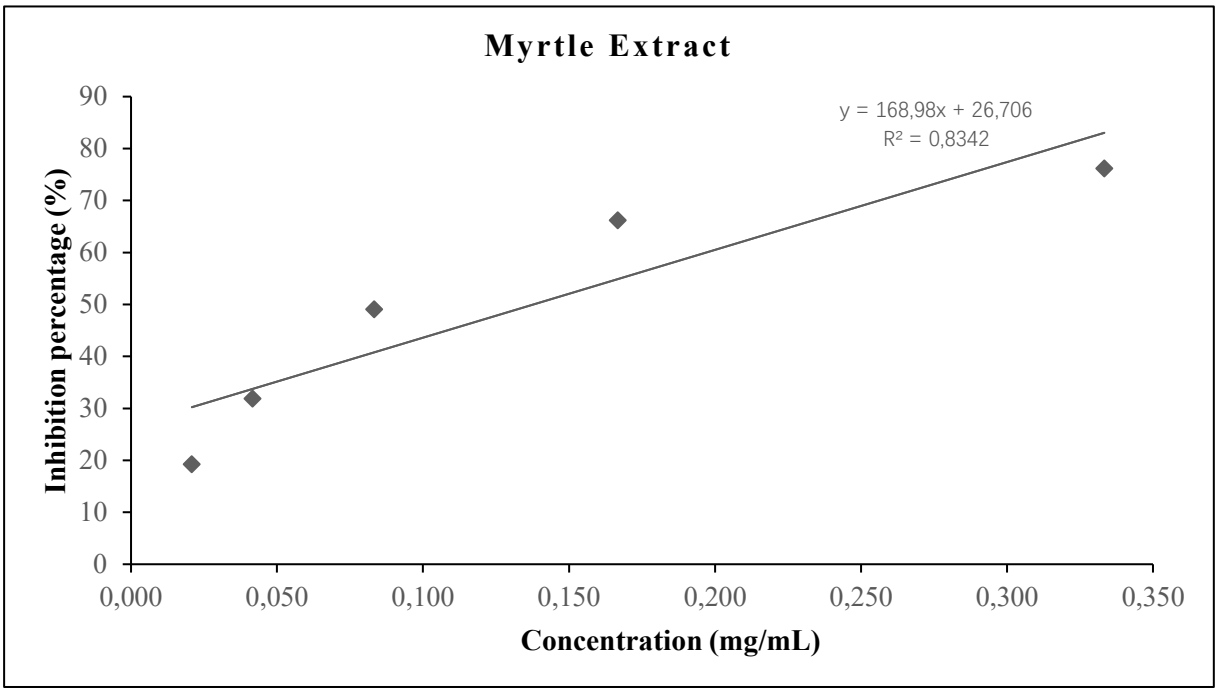
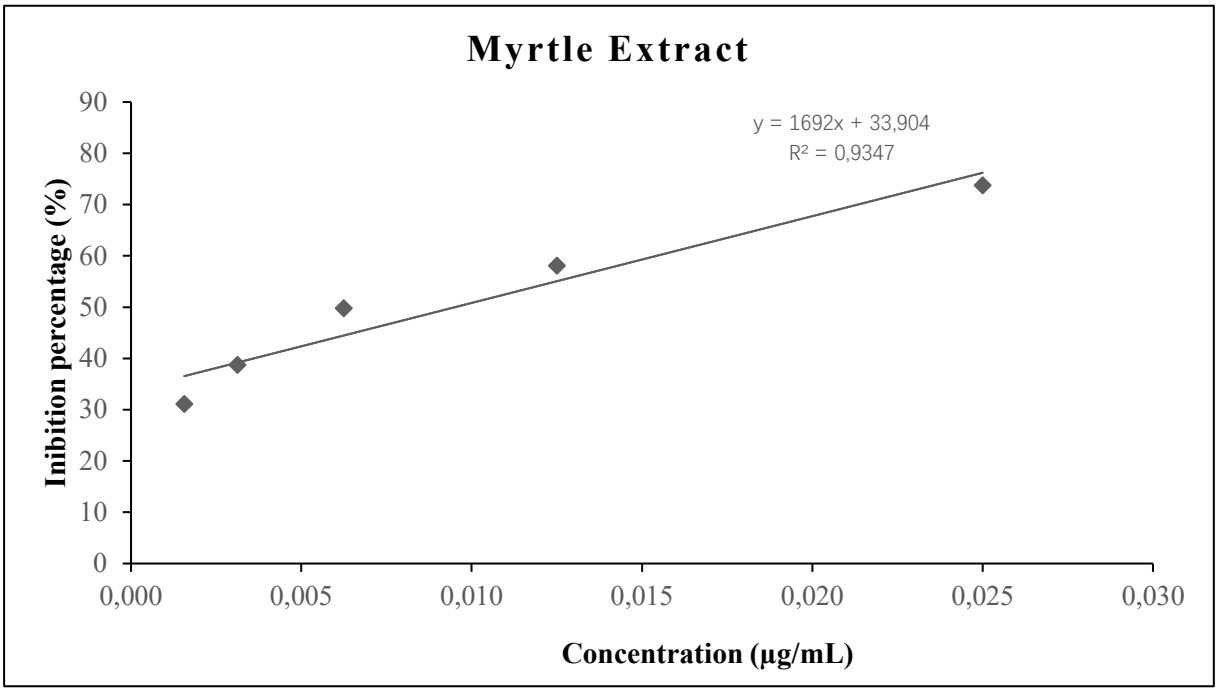


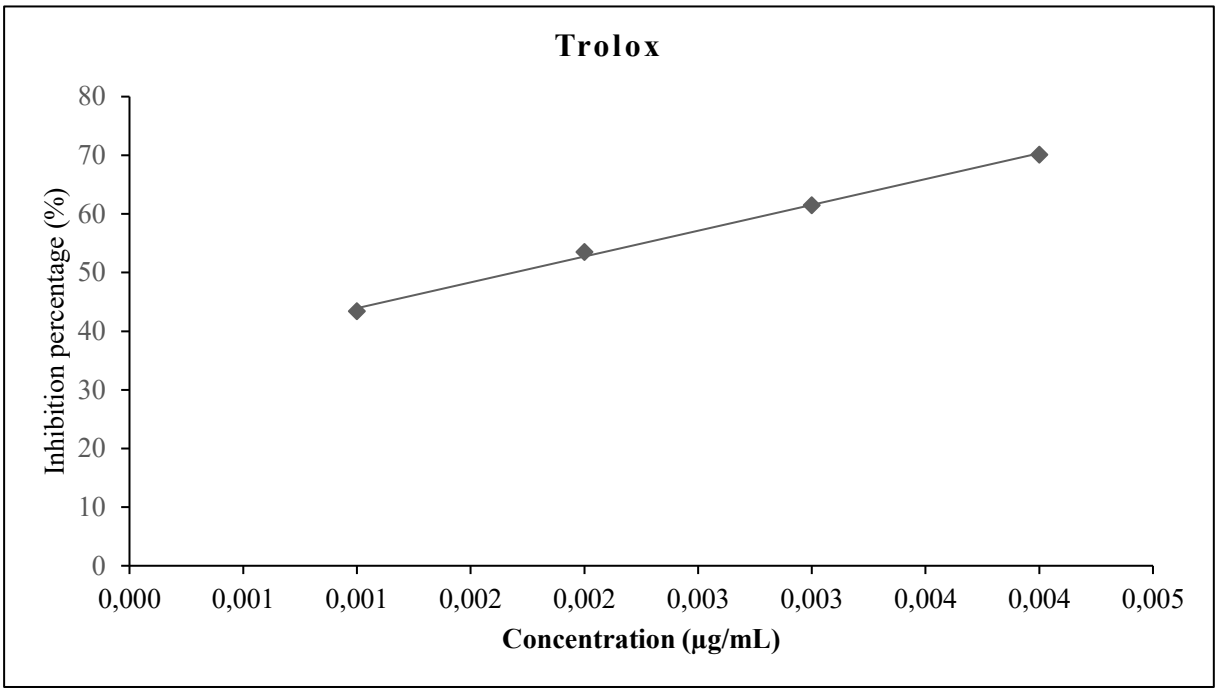
Ascorbic acid standard for the NO scavenging test



NO scavenging test



Inhibition percentage of DPPH before digestion



Inhibition percentage of DPPH

Abstract
The growing demand for natural bioactive ingredients has heightened interest in medicinal plants like Myrtus communis L., traditional Mediterranean specie known for its richness in phenolic compounds. This study aimed to valorize myrtle fruits by characterizing their phenolic profile, assessing their biological activities, and exploring their potential in functional food
applications. The impact of various drying methods on the preservation of bioactive compounds was evaluated, with freeze- drying proving to be the most effective in preserving phenolic compounds, including flavonoids and anthocyanins, as well as antioxidant activity. Optimized extraction conditions (50% acetone, 40°C, 180 minutes) resulted in a phenolic-rich extract with strong antioxidant properties, as demonstrated by multiple assays, including DPPH-RSA, ABTS-RSA, and ferric reducing power. The extract exhibited potent α-amylase inhibition (IC_{50} = 28.31 µg/mL), moderate anti-inflammatory effect (IC_{50} = 366.64 µg/mL), and good biocompatibility, supporting its safe application in various industries. Myrtle powder was incorporated into mayonnaise at concentrations of 0.3%, 0.6%, and 1%. The highest concentration (1%) significantly increased phenolic content and antioxidant stability during 28 days of storage, without altering key physicochemical properties such as pH and viscosity. Overall, the study highlights *Myrtus communis* as a promising natural resource with diverse bioactive properties, demonstrating its potential as a functional ingredient in food products, nutraceuticals, and pharmaceuticals. The findings provide strong scientific evidence for the incorporation of myrtle in value-added applications, contributing to human health and sustainable product development.
Keywords: *Myrtus communis* L.; phenolic compounds; drying methods; optimization; antioxidant and biological activities; functional foods.

Résumé
La demande croissante d'ingrédients naturels bioactifs a ravivé l'intérêt pour les plantes médicinales telles que *Myrtus communis* L., une espèce méditerranéenne traditionnelle reconnue pour sa richesse en composés phénoliques. Cette étude visait à valoriser les fruits de myrte par la caractérisation de leur profil phénolique, l'évaluation de leurs activités biologiques et l'exploration de leur potentiel dans les applications alimentaires fonctionnelles. L'impact de différentes méthodes de séchage sur la préservation des composés bioactifs a été évalué, le lyophilisat s'étant révélé le plus efficace pour conserver les composés phénoliques, notamment les flavonoïdes et les anthocyanes, ainsi que l'activité antioxydante. Les conditions optimisées d'extraction (50 % d'acétone, 40 °C, 180 minutes) ont permis d'obtenir un extrait riche en polyphénols présentant de fortes propriétés antioxydantes, comme en témoignent plusieurs essais, dont DPPH-RSA, ABTS-RSA et le pouvoir réducteur ferrique. L'extrait a montré une inhibition marquée de l'α-amylase (IC_{50} = 28,31 µg/mL), un effet anti-inflammatoire modéré (IC_{50} = 366,64 µg/mL) et une bonne biocompatibilité, confirmant son innocuité et son potentiel d'application dans divers domaines industriels. La poudre de myrte a été incorporée dans une mayonnaise à des concentrations de 0,3 %, 0,6 % et 1 %. La plus forte concentration (1 %) a significativement augmenté la teneur en composés phénoliques et la stabilité antioxydante au cours de 28 jours de stockage, sans altérer les principales propriétés physico-chimiques telles que le pH et la viscosité.
Dans l'ensemble, l'étude met en évidence *Myrtus communis* comme une ressource naturelle prometteuse, riche en composés bioactifs variés, et souligne son potentiel en tant qu'ingrédient fonctionnel pour les produits alimentaires, les nutraceutiques et les produits pharmaceutiques. Ces résultats apportent une base scientifique solide pour l'intégration du myrte dans des applications à haute valeur ajoutée, contribuant à la santé humaine et au développement durable.
Mots-clés : *Myrtus communis* L. ; composés phénoliques ; méthodes de séchage ; optimisation ; activités antioxydantes et biologiques ; aliments fonctionnels.

الملخص

الريحان (*Myrtus communis* L.) ، وهي نوع متوسطي تقليدي معروف بغناه بالمركّبات الفينولية. هدفت هذه الدراسة إلى تثمين ثمار الريحان (الآس) من خلال توصيف تركيبها الفينولي، وتقييم أنشطتها البيولوجية، واستكشاف إمكانياتها في التطبيقات الغذائية الوظيفية.

تم تقييم تأثير طرق التجفيف المختلفة على حفظ المركّبات النشطة حيوياً، حيث أثبتت طريقة التجفيف بالتجميد فعاليتها العالية في الحفاظ على المركّبات الفينولية، وخاصة الفلافونويدات والأنثوسيانينات، إضافةً إلى النشاط المضاد للأكسدة. وقد مكّنت شروط الاستخلاص المثلى (50% أسيتون، 40 درجة مئوية، 180 دقيقة) من الحصول على مستخلص غني بالبوليفينولات يتميز بخصائص قوية مضادة للأكسدة، كما أظهرتها عدة اختبارات مثل DPPH-RSA و ABTS-RSAوقدرة الاختزال الحديدي. أظهر المستخلص تثبيطاً واضحاً لإنزيم-α أميلاز (IC_{50} = 28.31 ميكروغرام/مل)، ونشاطاً مضاداً للالتهابات بدرجة معتدلة = IC_{50} (366.64ميكروغرام/مل)، إضافةً إلى توافقٍ حيوي جيد يؤكد سلامته وإمكانية استخدامه في مجالات صناعية مختلفة.

تم إدخال مسحوق فاكهة الريحان في تركيبة المايونيز بتركيزات 0.3%، 0.6%، و1%. وقد أدّى أعلى تركيز (1%) إلى زيادة ملحوظة في محتوى المركّبات الفينولية والاستقرار المضاد للأكسدة خلال 28 يوماً من التخزين، دون أن يؤثر على الخواص الفيزيوكيميائية الأساسية مثل الرقم الهيدروجيني (pH) واللزوجة.

بشكل عام، تبرز الدراسة نبات الريحان كمصدر طبيعي واعد غني بالمركّبات النشطة بيولوجياً، وتؤكد على إمكانيته كعنصر وظيفي في المنتجات الغذائية والمغذيات الدوائية والمستحضرات الصيدلانية. وتوفر هذه النتائج أساساً علمياً متيناً لإدماج الآس في تطبيقات عالية القيمة المضافة تُسهم في تحسين صحة الإنسان وتعزيز التنمية المستدامة.

الكلمات المفتاحية: الريحان (*Myrtus communis* L.) ؛ المركّبات الفينولية؛ طرق التجفيف؛ الاستخلاص الأمثل؛ الأنشطة المضادة للأكسدة والبيولوجية؛ الأغذية الوظيفية.