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ORIGINAL ARTICLE / ÖZGÜN MAKALE

FRUITFUL REMEDIES: ANALYZING THERAPEUTIC POTENTIALS IN ESSENTIAL AND FATTY OILS, AND AQUEOUS EXTRACTS FROM *PRUNUS CERASIFERA*, *MALUS SYLVESTRIS*, AND *CORNUS MAS* USING LC-MS AND GC-MS

MEYVELERDEN ŞİFAYA: PRUNUS CERASIFERA, MALUS SYLVESTRIS VE CORNUS MAS TÜRLERİNDEN ELDE EDİLEN UÇUCU YAĞLAR, YAĞ ASİTLERİ VE SU EKSTRELERİNİN LC-MS VE GC-MS YÖNTEMLERİYLE TERAPÖTİK POTANSİYELLERİNİN ANALİZİ

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ABSTRACT

Objective: *The chemical composition and bioactive properties of essential oils, fatty oils and aqueous extracts of Prunus cerasifera, Malus sylvestris and Cornus mas were investigated.*
Material and Method: *Antidiabetic, antimicrobial, anticholinesterase and antioxidant activities of P. cerasifera, M. sylvestris and C. mas were reported. The quantitative determination of some secondary metabolites was also analysed by LC-MS/MS. The chemical composition of the essential oils was also investigated by GC-MS.*
Result and Discussion: *Fatty oils' major compounds were oleic acid (77.1%) in P. cerasifera, palmitic acid (32.5%) in M. sylvestris fruits, and linoleic acid (43.2%) in C. mas seeds. Benzaldehyde (70.1%), nonacosane (30.4%), (E,E)-2,4-Decadienal (43.3%) were found as major compounds of P. cerasifera, M. sylvestris fruits, and C. mas seed essential oils, respectively. Quinic acid was predominant compound in all extracts, ranging from 11262.2996 to 18179.6260 ng/ml. C. mas fatty oil was showed antimicrobial activity against Candida albicans and C. parapsilosis with MIC =625-1250 µg/ml. P. cerasifera, C. mas, and M. sylvestris hold potential as α-glucosidase inhibitors, with varying degrees of potency. IC_{50} values further underscore effectiveness of aqueous extracts, especially in cases of C. mas and M. sylvestris with <10 and 399 µg/ml.*

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ÖZ

Amaç: *Prunus cerasifera, Malus sylvestris ve Cornus mas'ın uçucu yağları, yağ asitleri ve sulu ekstrelerinin kimyasal bileşimi ve biyoaktif özellikleri araştırılmıştır.*

Gereç ve Yöntem: *P. cerasifera, M. sylvestris ve C. mas'ın antidiyabetik, antimikrobiyal, antikolinesteraz ve antioksidan aktiviteleri rapor edilmiştir. Ayrıca bazı sekonder metabolitlerin kantitatif tayini LC-MS/MS ile analiz edilmiştir. Uçucu yağların kimyasal bileşimi de GC-MS ile araştırılmıştır.*

Sonuç ve Tartışma: *Yağların ana bileşikleri P. cerasifera'da oleik asit (%77.1), M. sylvestris meyvelerinde palmitik asit (%32.5) ve C. mas tohumlarında linoleik asit (%43.2) olarak bulunmuştur. Benzaldehit (%70.1), nonakozan (%30.4), (E,E)-2,4-dekadienal (%43.3) sırasıyla P. cerasifera, M. sylvestris meyveleri ve C. mas tohum uçucu yağlarının ana bileşikleri olarak bulunmuştur. Kinik asit, 11262.2996 ila 18179.6260 ng/ml arasında değişen tüm ekstrelerde en çok bulunan bileşik olmuştur. C. mas yağı Candida albicans ve C. parapsilosis türlerine karşı MIC =625-1250 µg/ml ile antimikrobiyal aktivite göstermiştir. P. cerasifera, C. mas ve M. sylvestris, değişen derecelerde etki gücüne sahip α-glukozidaz inhibitörleri olarak potansiyel taşımaktadır. Özellikle <10 ve 399 µg/ml olan C. mas ve M. sylvestris örneklerinde IC_{50} değerleri sulu ekstrelerinin etkinliğini daha da vurgulamaktadır.*

Anahtar Kelimeler: *Antidiyabetik, antikolinesteraz, Cornus mas, LC-MS/MS, Malus sylvestris, Prunus cerasifera*

INTRODUCTION

Diabetes mellitus (DM) presents a significant and growing challenge in global healthcare, with treatment and management complexities. Recent data indicate a troubling rise in DM prevalence among adults aged 20 to 79 worldwide, projected to reach 439 million by 2030, up from 285 million in 2010. Type 2 diabetes ranks as the fourth or fifth leading cause of mortality in many developed nations, with indications of epidemic proportions in several developing regions. Dietary patterns characterized by high glycemic index and low fiber content have been associated with increased DM risk. Furthermore, specific dietary fatty acids may impact insulin resistance and DM risk differently. Certain food categories and dietary constituents, including fatty acids, fruits, vegetables, whole grain cereals, dietary fiber, fish, magnesium, and nuts, have demonstrated potential in improving insulin sensitivity [1]. Diet plays a crucial role in managing diabetes mellitus (DM), and traditional medicinal practices continue to hold relevance, particularly in developing countries, where approximately 80% of the population relies on them. These traditional remedies often incorporate plant-based elements that may not be prevalent in typical diets. Herbs, spices, and vegetables are not only integrated into daily meals but are also consumed in various medicinal forms [2]. Choosing a diet abundant in fruits, vegetables, and whole grains has been associated with a decreased likelihood of developing DM [3].

Alzheimer's disease (AD) is characterized by the presence of amyloid beta (Aβ) plaques, neurofibrillary tangles, diffuse cortical neuronal loss, and cognitive decline. Notably, studies have revealed reduced brain insulin receptor sensitivity and expression in postmortem AD brains. Diabetes mellitus (DM), associated with insulin resistance, is a recognized risk factor for AD. Recent meta-analyses of long-term population studies have shown a 50% increased risk of AD in individuals with diabetes. Intranasal insulin has been shown to reach physiological levels in the brain in humans, mirroring findings from rodent studies where brain insulin influences appetite and energy metabolism. In humans, intranasal insulin has been linked to reduced body fat, changes in food preferences, decreased food intake, and improved peripheral insulin signaling. These findings deepen our understanding of the complex interplay between insulin, cognitive function, and metabolism in AD [4]. Globally, more than 26 million individuals are affected by AD, the most prevalent form of dementia. A hypothesis proposes that even a modest delay of approximately 2 years in the onset of AD could lead to a considerable reduction in the projected worldwide prevalence by 2050. This delay could potentially prevent around 22 million cases and result in significant cost savings. Certain dietary factors have shown protective

effects against cardiovascular diseases, obesity, hypertension, DM, and hypercholesterolemia—all of which are closely associated with the risk of developing AD [5]. Four cholinesterase inhibitors (AChEIs) treat AD. Tacrine, approved in 1993, is rarely used due to hepatotoxicity. Donepezil, rivastigmine, and galantamine are now standard. Donepezil is approved for all AD stages; the others for mild to moderate cases. AChEIs improve neurotransmission by inhibiting acetylcholine breakdown. Clinical trials show modest, temporary benefits, lasting up to 24 months. Despite limitations, AChEIs remain the primary AD treatment [6]. The crucial involvement of insulin in the central nervous system is widely recognized and firmly established [7]. A constellation of risk factors linked to Type 2 diabetes and vascular disease, including elevated blood glucose levels, obesity, hypertension, elevated blood triacylglycerols, and insulin resistance, are interrelated with an increased susceptibility to developing AD and vascular dementia. These common features shared between DM and different types of dementia underscore the complex interplay between metabolic disorders and cognitive well-being [8]. Oxidative stress (OS) arises from an imbalance between the production of reactive oxygen species (ROS) within cells and the body's ability to detoxify them. This phenomenon acts as a catalyst for the initiation and advancement of various diseases, including cardiovascular disease, atherosclerosis, DM, pulmonary disorders, and cancer. The detrimental effects of ROS on cellular and tissue integrity disrupt normal cellular function, providing a fertile ground for the pathogenesis of these diseases [9]. Systemic inflammation, marked by IL-6 and acute-phase reactants, has been linked to type 2 diabetes since 1997. Recent findings also reveal local inflammation in pancreatic islets, with immune cell infiltration, amyloid-associated macrophages, and complement activation. These processes, resembling AD, highlight the role of localized immune responses in diabetes pathogenesis [10].

Plums have a rich historical background dating back to ancient civilizations and have maintained their popularity and commercial significance over the years. In Turkey, a variety of plum species are cultivated, including *Prunus spinosa* L., *P. cerasifera* Ehrh., *P. domestica* L., and *P. insititia* L., The origins of plum cultivation can be traced back to regions around the Caucasus and Caspian Sea, including Turkey, which served as its ancestral homeland before its spread worldwide. Plums are cultivated extensively throughout Turkey, spanning diverse eco-geographical zones from Southeastern Anatolia to the Mediterranean and Aegean regions, and reaching across Central Anatolia. It's worth noting that while plums thrive in many Turkish regions, they are less common in the elevated plateaus of Eastern Anatolia and the arid, warm zones of Southeastern Anatolia [11]. *P. cerasifera,* also referred to as cherry plum or Myrobalan plum, is a versatile species encompassing various subspecies and natural variations. It serves multiple purposes, including being utilized as a rootstock for grafting fruit trees and grown for ornamental purposes. Moreover, it is considered one of the ancestral progenitors of the cultivated garden plum *(P. domestica* L.*)* [12]. *Cornus mas* L., colloquially known as "Cornelian cherry" or "dogwood," is a deciduous plant indigenous to eastern and southern regions of Europe, as well as West Asia. Typically, these trees can attain heights ranging from 7 to 8 meters when cultivated in temperate climates with well-drained soil. The fruits of *C. mas* (Cornaceae) are edible, exhibiting an oval or pear-like shape and showcasing colors that vary from red to purple hues. Renowned for its abundant content of vitamin C and polyphenols, this plant species is esteemed for its nutritional value. The fruits and leaves of *C. mas* harbor substantial quantities of iridoids, anthocyanins, and flavonoids, which contribute to their robust antioxidant and anti-tumor properties. Beyond its nutritional significance, Cornelian cherry finds application as a traditional ingredient in the crafting of liquors, jams, confections, and an array of fruit-based delicacies. The diverse array of bioactive compounds present in *C. mas* renders it not only a flavorful culinary addition but also a potential source of health-promoting benefits [13]. *C. mas* finds its origin in Southern Europe and Southwest Asia. Fruit extracts from this plant are harnessed in Europe for cosmetic purposes, serving as a natural alternative to synthetic astringents, and are reputed to enhance skin complexion. Moreover, the cornelian cherry enjoys popularity as an ornamental plant, celebrated for its striking foliage and profuse, charming blossoms. It is frequently adorned in small gardens and parks, cherished for its aesthetic allure [14]. *Malus sylvestris* (L.) Mill., commonly known as the European crab apple or simply crab-apple, is a member of the Rosaceae family. As the solitary native wild apple species in central Europe, it boasts extensive distribution throughout the European landscape, ranging from southern Scandinavia to the Iberian Peninsula and from the Volga to the British Isles. Typically inhabiting woodlands, scrublands,

and hedgerows, the crab apple thrives in habitats characterized by sparse forests or along the fringes of wooded areas, where ample sunlight is readily available [15]. A small deciduous tree, typically reaching heights between 4 to 10 meters, characterized by its petite spherical fruit, measuring approximately 2.5 x 2.8 cm, and displaying flattened ends. The fruit, glossy and pale green, adorned with sizable white dots, transitions to a flushed or crimson-spotted hue during autumn. Rich in carbohydrates, dietary fats, sugars, proteins, and minerals, this fruit also contains an abundance of polyphenols such as tannins and anthocyanins, alongside saponins, alkaloids, and flavonoids including procyanidin, quercetin, phloretin, myricetin, and epicatechin. Renowned for its diverse pharmacological applications, it serves as a nerve sedative, anxiety reliever, blood pressure regulator, carminative, digestive aid, emollient, hypnotic, laxative, refrigerant, antioxidant, and antibacterial agent. In traditional medicine, crab apples are revered for their effectiveness in treating various ailments, including cancer, malaria, warts, dysentery, fever, scurvy, and spasms. The crushed fruits are applied topically to alleviate inflammation, minor wounds, and sore throats [16].

This study aimed to quantitatively analyze 35 phenolic compounds present in the fruits of three distinct plant species-*Prunus cerasifera* (fruit), *Malus sylvestris* (fruit), and *Cornus mas* (seed)-employing LC-MS/MS methodology. Additionally, the research sought to explore the inhibitory effects of aqueous and hexane extracts derived from these fruits against enzymes such as α-glucosidase, α-amylase, acetylcholinesterase, and butyrylcholinesterase. The antimicrobial activity was assessed by determining the minimum inhibitory concentration (MIC), while antioxidant properties were evaluated using the DPPH and ABTS methods. Moreover, the compositions of fatty acids and essential oils were scrutinized via GC-MS analysis.

MATERIAL AND METHOD

Plant Materials

P. cerasifera, *M. sylvestris* and *C. mas* specimens were collected from natural habitats in Ormanagzi district, Olur, Erzurum-Turkey in 2021. The collection and identification of plants were carried out by Mehmet ÖNAL, who is the Chief Engineer of the Eastern Anatolia Forestry Research Institute. Herbarium specimens identified as M. Önal 114, M. Önal 268, and M. Önal 1019 are meticulously preserved in the Artvin Çoruh University Herbarium (ARTH), respectively. The photos of *P. cerasifera*, *M. sylvestris*, and *C. mas* were presented in Figure 1.

Extraction

To extract the compounds from the *P. cerasifera* (fruit), *M. sylvestris* (fruit), and *C. mas* (seed), the dried plant material was first ground and then subjected to extraction using a Soxhlet apparatus with hexane under a reversing cooler for 3 hours. Subsequently, the obtained extract was subjected to evaporation until complete dryness, after which the resulting residue was carefully weighed [17].

For the aqueous extract, the dried fruit material was macerated in water at room temperature for 3 days (8 hours per day), filtered, and frozen at -80 degrees Celsius. The frozen extract was then subjected to lyophilization and weighed [18].

Essential Oil Extraction and Analysis

The extraction of essential oils from *P. cerasifera* (fruit), *M. sylvestris* (fruit), and *C. mas* (seed) involved the use of a Clevenger apparatus, incorporating water addition throughout the 3-4 hour extraction process. Further details regarding this methodology can be referenced in our previously published work by Karakaya et al. (2023) [19].

Preparation of Fatty Acid Methyl Esters

The process for preparing Fatty Acid Methyl Esters entails a series of sequential steps. Initially, the residual substance (extracted oil via the Soxhlet apparatus) is subjected to reflux with a solution containing 0.5 N sodium hydroxide in methanol for a duration of 10 minutes. Following this, a solution comprising 14-20% BF_3 in methanol is introduced through the condenser, and the mixture is boiled for an additional 2 minutes. Subsequently, 5 ml of n-hexane is added to the mixture, followed by another

minute of boiling. The solution is then allowed to cool, and 5 ml of saturated NaCl solution is incorporated. Gentle agitation of the flask ensures thorough blending of the constituents. Further addition of saturated NaCl solution facilitates the separation of the hexane solution, which floats into the neck of a 1 ml flask. The upper hexane solution is then cautiously transferred into a vial for subsequent application [20].

Figure 1. The photos of *Prunus cerasifera, Malus sylvestris,* and *Cornus mas* by Mehmet Önal

Quantitative Determination of Secondary Metabolites

The quantitative analysis of secondary metabolites in the most effective extracts was conducted using an Agilent 6460 Triple Quadrupole Liquid Chromatography-Tandem Mass Spectrometer (LC-MS/MS) system at the Ataturk University East Anatolia High Technology Application and Research Center (DAYTAM). The separation of analytes was achieved using an Agilent Poroshell 120 EC-C18 column (4.6 × 100 mm, 3.5 µm) coupled with an Agilent 1260 HPLC system. The HPLC system was operated in positive ion mode with electrospray ionization (ESI). For detection and quantification, the protonated product ion $[M + H]^+$ of each compound, prepared at a concentration of 1 mg/mL, was determined using the standard scan mode. The dual mobile phase consisted of phase A (0.5% formic acid in water) and phase B (0.5% formic acid in acetonitrile). The injection volume was 5 µl, and the analysis was performed in Multiple Reaction Monitoring (MRM) mode.

Antimicrobial Activity (MIC, µg/ml)

The antimicrobial efficacy of the extracts was assessed against pathogenic microorganisms including *E. coli* ATCC 8739, *Salmonella enterica* ATCC 14028, *B. subtilis* ATCC 19659, *Pseudomonas aeruginosa* ATCC 9027, *Klebsiella aerogenes* ATCC 13048, *Staphylococcus aureus* ATCC 6538, *Candida albicans* ATCC 10231, and *Candida parapsilosis* ATCC 22019. Further details regarding this methodology can be referenced in our previously published work by Karakaya et al. (2023) [19].

α-Glucosidase Inhibition Assay

The α-glucosidase enzyme inhibition assay was carried out, following the procedure described by Bachhawat et al. (2011) with modifications as proposed by Yuca et al. (2021) [21,22].

α-Amylase Inhibition Assay

The α-amylase enzyme inhibition assay was carried out, following the procedure described by Nampoothiri et al. (2011) with modifications as proposed by Yuca et al. (2021) [22,23].

Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) Inhibition Assay

The assays for inhibiting acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) were performed, utilizing a customized protocol derived from the methodology described by Ingkaninan et al. (2000), with additional enhancements as refined by Karakaya et al. (2023) [19,24].

$ABTS^{\bullet+}$ Scavenging Activity

The evaluation of $ABTS^{\bullet+}$ scavenging activity was conducted in accordance with the methodology established by Re et al. (1999). For more comprehensive information on this methodology, readers are encouraged to consult our prior publication by Karakaya et al. (2023) [19,25].

$DPPH^{\bullet}$ Scavenging Activity

The evaluation of $DPPH^{\bullet}$ scavenging activity adhered to the methodology devised by Blois (1958). For additional insights into this methodology, readers are directed to our earlier publication by Karakaya et al. (2023)[19,26].

Total Phenolic Content

The quantification of total phenolic content in the extracts was conducted using a modified approach based on the method initially established by Folin and Denis (1912) and later improved by Slinkard and Singleton (1977), with minor adjustments [27,28]. For a deeper understanding of this methodology, readers are encouraged to consult our earlier research by Karakaya et al. (2023) [19]. The gallic acid standard graph was given in Figure 2.

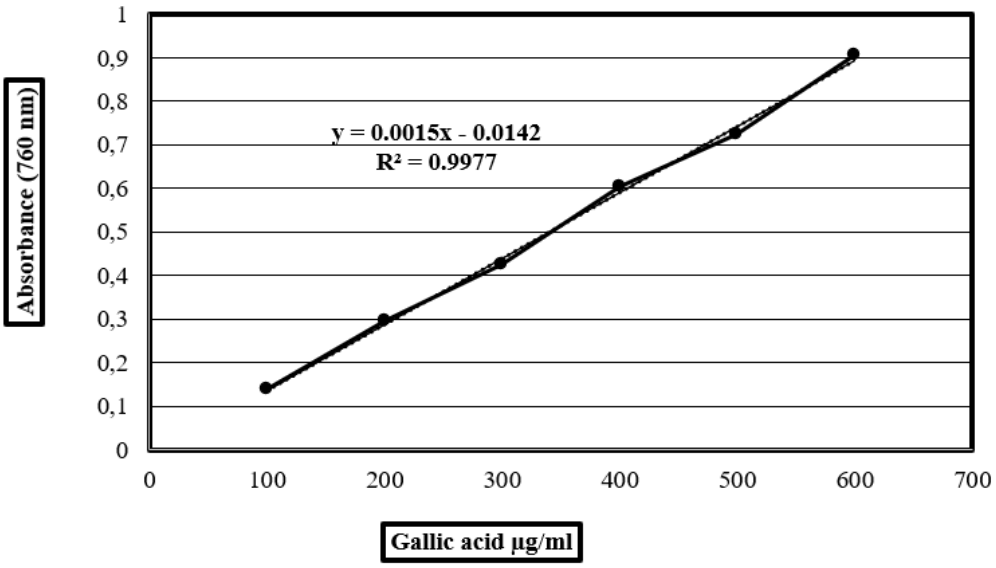

Figure 2. The gallic acid standard graph. [(760 nm) Absorbance = 0,0015 × Gallic acid – 0.0142]

Total Tannin Content

The assessment of total tannin content in aqueous extracts and fatty oils from the species involved a customized method derived from the Folin-Ciocalteu method, as outlined by Makkar (2003) [29]. For additional insights into this methodology, readers are directed to our prior publication by Karakaya et al. (2023) [19]. The tannic acid standard graph was given in Figure 3.

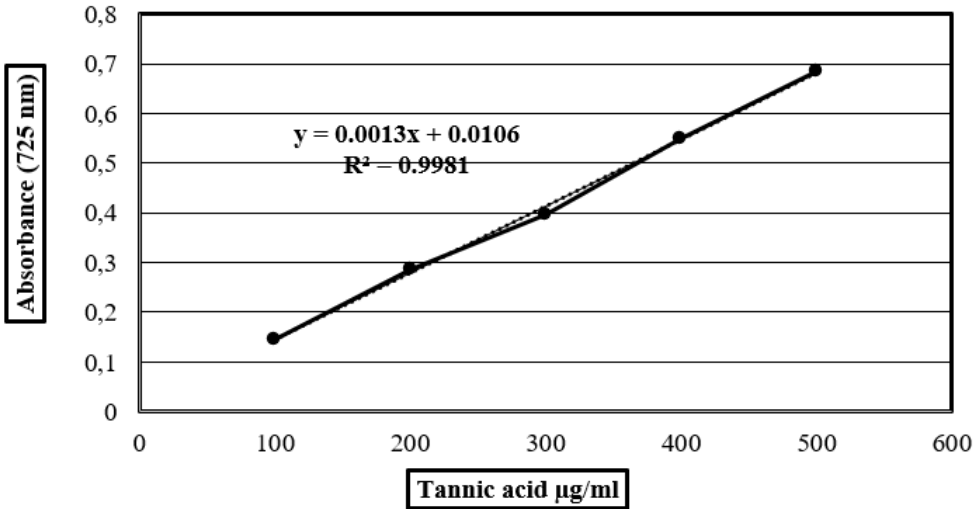

Figure 3. The tannic acid standard graph. [(725 nm) Absorbance = 0,0013 × Tannic acid + 0.0106]

RESULT AND DISCUSSION

Extraction

The aqueous extract of *P. cerasifera* had the highest yield among the all extracts (66%), while *M. sylvestris* hexane extract had the least yield (5.99%). The dried powdered of samples and yields of extracts were given in Table 1.

Table 1. The dried powdered of samples and yields of extracts

Samples		Dried powdered (g)	Yield (%)
P. cerasifera	Hexane	16	7.25
	Aqueous	50	66.00
M. sylvestris	Hexane	12	5.99
	Aqueous	50	29.47
C. mas	Hexane	15	6.27
	Aqueous	50	12.00

Common solvents for polyphenol extraction include methanol, water, ethanol, acetone, and others, each with varying polarity affecting their efficiency. Organic solvents, especially in aqueous mixtures, often enhance extraction yields. For instance, acetone outperformed methanol, water, and ethanol in extracting polyphenols from lychee flowers, while water was more effective for walnut husks. Generally, solvents with higher polarity are more effective due to the greater solubility of polyphenols [30]. Methanol has shown marginally greater efficiency than ethanol in extracting polyphenols and anthocyanins [31].

Fatty and Essential Oils Analysis

A total of nine compounds, constituting 100% of the fatty oil in *P. cerasifera* fruit, were identified. The predominant compound was oleic acid, representing 77.1% of the composition. In the fatty oil of *M. sylvestris* fruit, twelve compounds, totaling 99.9%, were identified, with palmitic acid as the major compound at 32.5%. *C. mas* seed fatty oil, composed of nine compounds and representing 97.1% of the oil, was primarily composed of linoleic acid at 43.2%. The fatty acid compositions of *P. cerasifera*, *M. sylvestris*, and *C. mas* are detailed in Table 2.

Earlier investigations aimed at assessing the oil content and fatty acid composition in kernels from 15 varieties of *Prunus* species in Turkey. The oil yields obtained from these kernels ranged from 46.3% to 55.5%. Oleic acid was identified as the predominant fatty acid in *Prunus* kernel oils, varying from 43.9% to 78.5%, followed by linoleic acid in the range of 9.7% to 37%, and palmitic acid ranging from 4.9% to 7.3% [32]. Oils extracted from by-products of *Malus* spp., particularly cv. "Ola," exhibit rich fatty acid content, including linolenic acid (57.8%), α-linolenic acid (54.3%), and oleic acid (25.5%) (Radenkovs et al. 2018). For *C. mas*, the supercritical CO2 (SC-CO2) extraction technique yielded a seed oil with concentrations ranging from 2.35% to 5.18%. The fatty acid profile of the seed oil included predominantly linoleic acid (65.73%), followed by oleic acid (23.69%), palmitic acid (8.05%), stearic acid (1.92%), erucic acid (0.48%), and arachidic acid (0.13%) [33]. Omega-3 (N-3) polyunsaturated fatty acids (PUFAs) are indispensable for proper neuronal and brain function. These fatty acids serve as vital components of cell membranes and play critical roles in various physiological processes, including inflammation and oxidative stress modulation. The main N-3 PUFAs encompass docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3). While both can be synthesized internally from alpha-linolenic acid (18:3n-3), the conversion rate in humans is notably limited. Hence, dietary intake remains the primary source of DHA and EPA [5].

The fatty acid composition of the fruits from *P. cerasifera*, *M. sylvestris*, and *C. mas* reveals significant variations that underscore the unique biochemical profiles of these species. Each fruit displays a distinct fatty acid profile, highlighting the diversity in bioactive lipids that could contribute to their therapeutic potential. In *P. cerasifera*, oleic acid was the predominant fatty acid, constituting 77.1% of the total fatty acid composition. Oleic acid, a monounsaturated omega-9 fatty acid, is well-known for its beneficial effects on human health, particularly its role in cardiovascular health by improving lipid profiles and reducing inflammation. Its high concentration in the fatty oil of *P. cerasifera* suggests that this fruit could be a valuable source of bioactive lipids, potentially contributing to the prevention of cardiovascular diseases, as well as possessing antioxidant and anti-inflammatory properties. This result is consistent with previous studies that have highlighted the health-promoting effects of oleic acid, especially in the context of olive oils and other plant-based oils. In contrast, *M. sylvestris* exhibited a fatty acid profile with palmitic acid as the major component (32.5%). Palmitic

acid, a saturated fatty acid, has been widely studied due to its potential implications for human health. While saturated fatty acids are generally considered to contribute to atherosclerosis and other metabolic disorders when consumed in excess, recent studies have suggested that their effects may be context-dependent, influenced by factors such as the overall fatty acid composition of the diet. In this case, the relatively moderate concentration of palmitic acid in *M. sylvestris* fruit oil may not have the same negative impact as higher concentrations found in other sources. Additionally, the presence of other unsaturated fatty acids in the oil may balance the potential adverse effects of palmitic acid. *C. mas* seed oil was dominated by linoleic acid, an essential polyunsaturated omega-6 fatty acid, which comprised 43.2% of the oil. Linoleic acid is recognized for its beneficial effects on skin health, inflammation modulation, and potential anti-cancer properties. Its abundance in *C. mas* seed oil suggests that this species could serve as a promising source of essential fatty acids, contributing to various therapeutic applications, including dermatological and anti-inflammatory treatments. Furthermore, linoleic acid is known to support the integrity of cellular membranes, enhance skin barrier functions, and improve the overall lipid profile, adding to the potential health benefits of *C. mas* oil.

The diversity of fatty acids observed in these fruit oils-monounsaturated oleic acid, saturated palmitic acid, and polyunsaturated linoleic acid-illustrates the potential of these species as sources of nutraceuticals. Each species offers distinct advantages depending on the specific health outcomes sought. The predominance of oleic acid in *P. cerasifera* highlights its suitability for cardiovascular health, while the linoleic acid content in *C. mas* seeds positions it as a valuable oil for inflammatory and dermatological applications. The presence of palmitic acid in *M. sylvestris* may require more careful consideration, especially in the context of overall fat consumption, but its moderate concentration may still provide nutritional benefits when consumed as part of a balanced diet.

In conclusion, the fatty acid compositions of these three species highlight their potential as sources of bioactive lipids with diverse health benefits. Further studies on the antioxidant, anti-inflammatory, and other biological properties of these oils, particularly in vivo models, are warranted to fully understand their therapeutic potentials and to guide their use in functional foods and medicinal applications.

Table 2. The fatty acid compositions of *P. cerasifera*, *M. sylvestris*, and *C. mas*

Compound	*P. cerasifera* %	*M. sylvestris* %	*C. mas* %
Caprylic acid (C8:0)	-	1.2	-
Pelargonic acid (C9:0)	-	0.6	-
Capric acid (C10:0)	-	-	-
Myristic acid (14:0)	tr	1.1	0.1
Palmitic acid (16:0)	3.0	32.5	10.4
Margaric acid (17:0)	-	1.0	-
Stearic acid (18:0)	3.0	19.8	3.1
Oleic acid (18:1)	77.1	18.5	36.3
Elaidic acid (18:1)	1.8	-	1.6
Linoleic acid (18:2)	11.1	1.2	43.2
Linolenic acid (18:3)	1.1	-	1.2
Nonadecanoic acid (20:0)	-	1.4	-
Arachidic acid (20:0)	1.5	13.0	tr
Heneicosanoic acid ** (21:0)	-	1.8	-
Behenic acid (22:0)	1.4	7.8	1.2
Total	100	99.9	97.1

% calculated from FID data
tr Trace (< 0.1 %)
** Tentative identification

The essential oil extraction from *P. cerasifera* fruit yielded 0.11%, and a total of 39 compounds, constituting 86.3% of the essential oil, were identified. The predominant component was benzaldehyde, representing 70.1%. *M. sylvestris* fruit essential oil had a yield of 0.09%, with 32 compounds identified, making up 79.6% of the essential oil. Nonacosane (30.4%) and hexadecanoic acid (19.1%) were the

major constituents. *C. mas* seed essential oil had a yield of 0.14%, with 17 compounds identified, constituting 83.4% of the essential oil. The primary components were (E,E)-2,4-Decadienal (43.3%) and (E,Z)-2,4-Decadienal (12.1%).

Prior research has indicated that the principal constituents found in *P. armeniaca* leaf essential oil consist predominantly of (Z)-phytol (27.18%), pentacosane (15.11%), nonacosane (8.76%), and benzaldehyde (7.25%) [34]. Benzaldehyde plays a crucial role in the flavor industry, with its demand primarily satisfied through synthetic manufacturing processes. However, in the leaf essential oil of *P. persica*, extracted across various seasons and subjected to analysis via GC-FID and GC-MS, notably elevated levels of benzaldehyde (ranging from 63.1% to 98.3%) were observed [35]. In the oil extracted from *M. domestica* leaves, the primary constituents were identified as eucalyptol (43.7%), phytol (11.5%), α-farnesene (9.6%), and pentacosane (7.6%) [36]. Meanwhile, in *M. sylvestris* fruits, the predominant sesquiterpenes detected included E-β-Farnesene (35.03%), E-caryophyllene (7.17%), and germacrene D (5.76%). Noteworthy sesquiterpene oxides comprised bisabolol oxide B (4.82%), spathulenol (4.78%), α-eudesmol (4.52%), caryophyllene oxide (4.46%), 2Z, 6 E-Farnesol (4.34%), and Z-dihydro-apofarnesol (2.22%) [37]. In the dehydrated fruit of *C. officinalis*, prominent constituents included palmitic acid (11.1%), benzyl cinnamate (10.2%), isobutyl alcohol (9.6%), isoamyl alcohol (9.2%), furfural (9.2%), methyl eugenol (7.4%), isoasarone (7.1%), β-phenylethyl alcohol (4.1%), trans-linalool oxide (3.3%), and elemicine (3.2%) [38]. The essential oil extracted from *C. mas* flowers was distinguished by the abundance of monoterpenoids, including camphor, verbenone, borneol, α-terpineol, β-thujone, carvone, and 1,8-cineole [39]. Based on our literature review, this is the first study to comprehensively compare both fatty and essential oil contents of *P. cerasifera, M. sylvestris,* and *C. mas* species.

The essential oil profiles of *P. cerasifera*, *M. sylvestris*, and *C. mas* provide valuable insights into the chemical composition and potential applications of these oils, showcasing distinct variations in their primary constituents and their respective yields. These differences highlight the unique therapeutic potentials and industrial applications of the essential oils from these species. In *P. cerasifera*, the essential oil yield was 0.11%, with 39 compounds identified, contributing to 86.3% of the oil. Benzaldehyde was the dominant compound, comprising 70.1% of the essential oil. Benzaldehyde is a volatile aromatic compound commonly associated with a pleasant, almond-like aroma, and has demonstrated various bioactive properties, including antimicrobial and antioxidant activities. Its high concentration in *P. cerasifera* essential oil suggests that it may offer significant therapeutic potential, particularly in applications related to fragrance, antimicrobial agents, and food preservation. The dominance of a single compound like benzaldehyde also indicates that *P. cerasifera* could provide a relatively straightforward and potent source of bioactive volatiles for pharmaceutical and cosmetic industries. In contrast, *M. sylvestris* fruit essential oil showed a yield of 0.09% with 32 identified compounds, accounting for 79.6% of the oil. The major components, nonacosane (30.4%) and hexadecanoic acid (19.1%), are both long-chain hydrocarbons. Nonacosane, a saturated hydrocarbon, is often found in plant waxes and has been reported to possess antimicrobial and insecticidal properties. Hexadecanoic acid, a saturated fatty acid, is known for its anti-inflammatory and potential antimicrobial effects. The significant presence of these compounds in *M. sylvestris* essential oil suggests its potential use in applications requiring both antimicrobial properties and stable long-chain molecules, such as in the development of functional food additives or natural preservatives. *C. mas* seed essential oil yielded 0.14%, and the oil was composed of 17 identified compounds, which made up 83.4% of the oil. The two predominant components were (E,E)-2,4-Decadienal (43.3%) and (E,Z)-2,4-Decadienal (12.1%), both of which are unsaturated aldehydes with distinct aromatic profiles. These compounds have been shown to possess antimicrobial, antifungal, and antioxidant properties, which makes *C. mas* essential oil promising for therapeutic applications, particularly in natural antimicrobial formulations. The high concentration of (E,E)-2,4-Decadienal, in particular, could also offer strong potential for use in perfumery and flavoring due to its characteristic aroma. The unsaturated nature of these aldehydes may also contribute to the oil's stability and potency as a bioactive agent. The yield differences observed between the three species-*P. cerasifera* (0.11%), *M. sylvestris* (0.09%), and *C. mas* (0.14%)-highlight the variability in essential oil production even within the same plant family, suggesting that extraction methods, plant variety, and environmental conditions may play significant roles in determining oil

yields. The variation in chemical composition further emphasizes the importance of species selection for specific applications, as each oil shows a distinct profile of bioactive compounds (Table 3).

In conclusion, the essential oils of *P. cerasifera*, *M. sylvestris*, and *C. mas* each present unique chemical signatures with substantial potential for various applications. *P. cerasifera* is particularly notable for its high concentration of benzaldehyde, making it a promising candidate for use in antimicrobial and fragrance-based industries. *M. sylvestris* oil, with its predominance of nonacosane and hexadecanoic acid, offers opportunities for applications in food preservation and antimicrobial formulations. Finally, *C. mas* essential oil, rich in unsaturated aldehydes, holds promise for both medicinal and industrial uses, especially in antimicrobial and flavoring applications. These findings suggest that these oils may offer diverse and valuable bioactive properties, deserving further investigation for their potential therapeutic and industrial uses.

Table 3. The composition of the essential oil *P. cerasifera*, *M. sylvestris*, and *C. mas*

RRI	Compound	*P. cerasifera*	*M. sylvestris*	*C. mas*
1244	2-Pentyl furan	0.1	-	-
1360	1-Hexanol	0.4	0.1	-
1374	4-Hydroxy-4-methyl-2-pentanone	-	0.1	-
1400	Nonanal	0.4	0.1	-
1400	Tetradecane	-	0.4	2.9
1452	1-Octen-3-ol	-	-	0.4
1479	Furfural	0.3	0.7	0.3
1541	Benzaldehyde	70.1	5.9	-
1542	Vitispirane	-	2.3	-
1543	Ethyl vitispirane	-	0.6	-
1548	(*E*)-2-Nonenal	-	-	tr
1553	Linalool	0.1	-	-
1600	Hexadecane	tr	0.5	3.2
1655	(*E*)-2-Decenal	-	0.2	0.6
1661	Safranal	0.2	-	-
1694	*p*-Vinylanisole	0.1	-	-
1706	*α*-Terpineol	0.2	-	-
1751	Carvone	tr	-	-
1765	(*E*)-2-Undecanal	-	-	0.6
1773	*δ*-Cadinene	tr	-	-
1779	(*E,Z*)-2,4-Decadienal	-	0.1	12.1
1800	Octadecane	0.1	0.4	2.9
1827	(*E,E*)-2,4-Decadienal	0.2	1.0	43.3
1838	(*E*)-*β*-Damascenone	tr	-	-
1853	Ethyl dodecanoate	0.4	-	-
1868	(*E*)-Geranyl acetone	0.2	-	-
1900	Nonadecane	-	0.7	-
1958	(*E*)-*β*-Ionone	0.3	-	-
1973	1-Dodecanol	tr	-	-
2000	Eicosane	0.1	1.1	1.9
2050	(*E*)-Nerolidol	-	tr	-
2056	Ethyl tetradecanoate (=*E. myristate*)	0.8	0.6	-
2100	Heneicosane	0.8	0.4	0.7
2131	Hexahydrofarnesyl acetone	0.2	1.2	1.1
2226	Methyl palmitate	0.8	0.3	-

RRI Relative retention indices calculated against *n*-alkanes
% calculated from FID data
tr Trace (< 0.1 %)
Unknown I: EIMS, 70 eV, m/z (rel. .int.): 117[M]+ (0.5), 115(10.2), 81 (52.8), 54 (38.9), 41 (100), 39 (47.4)

Table 3 *(continue).* The composition of the essential oil *P. cerasifera, M. sylvestris,* and *C. mas*

RRI	Compound	*P. cerasifera*	*M. sylvestris*	*C. mas*
2262	Ethyl palmitate	1.5	0.7	-
2300	Tricosane	0.4	1.8	-
2369	(*2E,6E*)-Farnesol	-	4.4	
2380	*epi*-Manoyl oxide	-	-	1.3
2384	Farnesyl acetone	0.1	tr	-
2400	Tetracosane	-	-	1.3
2438	Kaur-16-ene	-	-	4.7
2456	Methyl oleate	0.3	1.1	-
2467	Ethyl stearate	0.2	-	-
2492	Ethyl oleate	0.7	-	-
2500	Pentacosane	tr	-	-
2503	Dodecanoic acid	1.4	tr	-
2509	Methyl linoleate	0.2	tr	-
2538	Ethyl linoleate	0.8	tr	-
2583	Methyl linolenate	0.3	-	-
2613	Ethyl linolenate	0.5	-	-
2670	Tetradecanoic acid	1.1	2.5	-
2700	Heptacosane	-	2.9	-
2900	Nonacosane	0.4	30.4	6.1
2931	Hexadecanoic acid	2.6	19.1	-
	TOTAL	**86.3**	**79.6**	**83.4**

RRI Relative retention indices calculated against *n*-alkanes
% calculated from FID data
tr Trace (< 0.1 %)
Unknown I: EIMS, 70 eV, m/z (rel. .int.): 117[M]+ (0.5), 115(10.2), 81 (52.8), 54 (38.9), 41 (100), 39 (47.4)

Quantitative Determination of Secondary Metabolites

A comprehensive examination was conducted on three aqueous extracts derived from *P. cerasifera*, *M. sylvestris*, and *C. mas*, revealing the presence of 35 unique phenolic compounds using LC-MS/MS. These compounds encompass a diverse array, including cyanidin-3-O-glucoside, quinic acid, caffeic acid, fumaric acid, p-coumaric acid, gallic acid, pyrogallol, chlorogenic acid, catechin, peonidin-3-O-glucoside, 4-OH-benzoic acid, luteolin, syringic acid, epicatechin, rosmarinic acid, epigallocatechin gallate, taxifolin, vanillic acid, vanillin, vitexin, naringin, ellagic acid, naringenin, hesperidin, ferulic acid, resveratrol, keracyanin chloride, quercetin, myricetin, apigenin, isorhamnetin, chrysin, sinapic acid, curcumin, and galangin.

Among these compounds, quinic acid, fumaric acid, gallic acid, cyanidin-3-O-glucoside, chlorogenic acid, catechin, epicatechin, ellagic acid, and quercetin were detected in all three extracts. Notably, quinic acid exhibited the highest concentration across all three extracts, with values of 11262.2996 ng/ml, 14802.2687 ng/ml, and 18179.6260 ng/ml for *P. cerasifera*, *M. sylvestris*, and *C. mas* extracts, respectively. Detailed results of the quantitative analysis of these phenolic compounds are outlined in Table 4.

The research investigated the combined effects of quercetin and quinic acid on a streptozotocin (STZ)-induced diabetic rat model. Diabetic rats were administered single and combined doses of quercetin and quinic acid over a period of 45 days, followed by analysis of their impact on liver, kidney, and pancreatic tissues. The findings revealed that treatment with quercetin and quinic acid led to increased levels of insulin and C-peptide, while also reducing hyperglycemia and oxidative stress. These results indicate a potential therapeutic role of quercetin and quinic acid in managing diabetes and its associated complications [40]. The study explored the neuroprotective properties of four derivatives of quinic acid sourced from Aster scaber in mitigating toxicity induced by amyloid Aβ in PC12 cells. The results demonstrated a significant reduction in cell toxicity caused by Aβ when cells were pre-treated with these quinic acid derivatives. Notably, among the derivatives, (−)4,5-dicaffeoyl quinic acid

emerged as the most effective in protecting against Aβ-induced cell toxicity. This underscores the potential of (−)4,5-dicaffeoyl quinic acid as a promising candidate for neuroprotection against Aβ-induced toxicity in PC12 cells [41]. Peaches, rich in polyphenols, protect against various health issues like obesity and diabetes. It was analyzed methanol, ethanol, and hexane extracts from fresh red peaches in Mersin, Turkey. Thirteen compounds, including quinic acid and chlorogenic acid, were identified in the ethanol extract by LC-MS/MS, which showed the highest antioxidant, antibacterial, and enzyme inhibition activities. These results highlight ethanol extract as a potential ingredient for antidiabetic and antibacterial formulations [42]. It was analyzed 14 *Malus* spp. genotypes for polyphenol content and composition, with concentrations ranging from 560 to 4860 mg/L. Using RP-HPLC and LC-MS/MS, 19 polyphenols were quantified, with hydroxycinnamates and flavan-3-ols being the most abundant. The 'Kola' hybrid showed the highest total polyphenol concentration (1429 mg/l), mainly composed of chlorogenic acid. 'Kaz 95 18–06' had a high level of flavan-3-ols, while 'Zapta' contained the highest phlorizin. LC-MS identified 120 polyphenols, including some with potential health benefits [43]. The Cornus fructus was treated with varying osmotic pressures, pH, heat, and ethanol concentrations. The highest gallic acid extraction (1.57 mg/g) was achieved using 100% ethanol for 1 hour. Extracts with 70% ethanol for 24 and 48 hours resulted in 1.35 and 1.50 mg/g of gallic acid, respectively. The analysis was performed using HPLC and LC-MS/MS [44].

Table 4. Quantitative assessment of 35 distinct phenolic compounds in aqueous extracts utilizing LC-MS/MS

NO	Compound	Samples	Final Conc (ng/ml)
1.	Quinic Acid	*P. cerasifera*	11262.2996
		M. sylvestris	14802.2687
		C. mas	18179.6260
2.	Fumaric Acid	*P. cerasifera*	144.3326
		M. sylvestris	32.8088
		C. mas	165.3011
3.	Gallic Acid	*P. cerasifera*	0.0000
		M. sylvestris	0.0000
		C. mas	4987.8455
4.	Cyanidin-3-*O*-glucoside	*P. cerasifera*	80.3648
		M. sylvestris	192.1741
		C. mas	40.7629
5.	Chlorogenic Acid	*P. cerasifera*	3861.2059
		M. sylvestris	3234.2241
		C. mas	15.4365
6.	Catechin	*P. cerasifera*	316.6816
		M. sylvestris	260.3998
		C. mas	0.0000
7.	Epicatechin	*P. cerasifera*	230.0535
		M. sylvestris	162.3451
		C. mas	0.0000
8.	Ellagic Acid	*P. cerasifera*	0.0000
		M. sylvestris	0.0000
		C. mas	1590.5162
9.	Quercetin	*P. cerasifera*	2.0335
		M. sylvestris	2.3522
		C. mas	0.1642

This study identified 35 phenolic compounds in the aqueous extracts of *Prunus cerasifera*, *Malus sylvestris*, and *Cornus mas* using LC-MS/MS, highlighting their potential therapeutic value. Compounds like quinic acid, fumaric acid, gallic acid, cyanidin-3-O-glucoside, chlorogenic acid, catechin, epicatechin, ellagic acid, and quercetin were found in all three extracts, suggesting they may play a key

role in the plants' biological activities. Quinic acid was present in the highest concentrations across all extracts, indicating its potential importance in the therapeutic effects of these plants. It is known for its antioxidant, anti-inflammatory, and hepatoprotective properties. Other identified compounds, such as catechins, caffeic acid, and gallic acid, are also recognized for their antioxidant and anti-inflammatory effects, which could contribute to the health benefits of these plants. The variations in concentrations of these compounds across the different species suggest that each plant may offer unique health benefits. For example, *C. mas* had the highest concentration of quinic acid, which may point to its stronger antioxidant effects compared to the others. Overall, the diverse phenolic profiles of these plants indicate their potential as sources of bioactive compounds with therapeutic properties, warranting further research into their biological effects and potential health applications.

Antimicrobial Activity

The fatty oils of fruit and aqueous extracts *of P. cerasifera, M. sylvestris,* and *C. mas* were evaluated against some of Gram (+), Gram (-), and yeasts. MIC values were presented in Table 5. Generally, the MIC value was observed between 1250-5000 µg/ml.

Table 5. Minimum inhibitory concentrations (µg/ml)

Samples	*E. coli*	*S. enterica*	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*K. aerogenes*	*C. albicans*	*C. parapsilosis*
Fatty oil of fruit from *M. slyvestris*	2500	1250	>2500	2500	1250	1250	5000	5000
Fatty oil of fruit from *P. cerasifera*	2500	2500	>2500	2500	2500	1250	5000	2500
Fatty oil of fruit from *C. mas*	2500	>2500	>2500	>2500	>2500	>2500	625	1250
Aqueous extract of *P. cerasifera*	>2500	>2500	>2500	>2500	>2500	ND	2500	2500
Aqueous extract of *M. sylvestris*	>2500	>2500	>2500	>2500	>2500	ND	2500	2500
Aqueous extract of *C. mas*	625	>2500	312.5	312.5	>2500	ND	625	5000
Moxifloxacin	0125>	0125>	0125>	0125>	0125>	ND	-	-
Ampicilline	0.125>	1.0	0.125>	0.125>	>64	32	-	-
Terbinafine	-	-	-	-	-	-	4.0	32
Fluconazole	-	-	-	-	-	-	1	1

ND: Not detected

Fatty oil of fruit from *M. slyvestris* was found effective against *Salmonella enterica* ATCC 14028, *Pseudomonas aeruginosa* ATCC 9027 and *Klebsiella aerogenes* ATCC 13048 with MIC= 1250 µg/ml. Fatty oil of fruit from *P. cerasifera* was more effective against *Klebsiella aerogenes* ATCC 13048. Fatty oil of seed from *C. mas* was showed antimicrobial activity against *Candida* species with MIC =625-1250 µg/ml. Generally, fatty oil of fruit from *C. mas* was found effective MIC=> 2500 µg/ml against Gram (+) and Gram (-).

Generally, the MIC value was observed between >2500 µg/ml for the aqueous extracts of *P. cerasifera, M. sylvestris,* and *C. mas.* The aqueous extract of *C. mas* was found more effective againts *S. aureus* and *B. Subtilis* with MIC=312.5 µg/ml. Also the exract of *C. mas* was more effective against *C. albicans* at MIC = 625 µg/ml than *C. parapsilosis.*

In scientific literature, the antimicrobial efficacy of leaf extracts from *P. divaricata* subsp. *divaricata* was assessed using petroleum ether, dichloromethane, methanol, and distilled water against various bacterial strains including *Bacillus subtilis* NRS-744, *Staphylococcus aureus* NRRL B-767, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 7644, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* NRRL B-4420, and *Klebsiella pneumonia* ATCC 700603. The results revealed that the petroleum ether extract exhibited antimicrobial activity against *E. faecalis*, with an inhibition zone of 11 mm [45]. The antimicrobial potential of methanol extracts from the fruit of *P.*

divaricata subsp. *divaricata* was assessed against eight bacterial strains and two yeast strains. Among the clinical isolates, the most pronounced antimicrobial effects against Gram-negative organisms were observed for *E. coli* and *K. pneumonia*, whereas *C. albicans* and *C. parapsilosis* exhibited the least susceptibility to the extracts [46].

The antibacterial efficacy of peel extract derived from *M. sylvestris* was examined against *Salmonella typhi*. The findings of this investigation revealed a MIC of 12.5%, accompanied by an average diameter of inhibition zone measuring 13.67 mm [47]. The peel extract of *M. sylvestris* in various solvent formulations containing ethanol concentration demonstrated inhibitory effects on the growth of *Streptococcus agalactiae* and *E. coli* [48].

Ethanol or methanol extracts derived from various parts of C. mas, including the bark, fruits, leaves, and seeds, were evaluated for their efficacy against a spectrum of pathogens including *S. aureus, S. pyogenes, E. coli, P. aeruginosa, C. albicans, Aspergillus fumigatus,* and *Trichophyton mentagrophytes*. Utilizing the disc-diffusion method, leaf and seed extracts exhibiting inhibition zone diameters ranging between 10-15 mm demonstrated the most potent antibacterial activity against *S. aureus* and *C. albicans* [49]. To evaluate the antimicrobial efficacy, water and methanol extracts of fruit from *C. mas* were tested against 93 clinical isolates of human pathogenic strains, including C. *albicans, E. coli, Proteus mirabilis, P. aeruginosa, S. aureus, C. glabrata, C. krusei, C. parapisilosis,* and *C. tropicalis*. The methanol and water extracts of the fruit exhibited potent antibacterial activity against *S. aureus*, yielding a notable 25 mm inhibition zone and a MIC value of 0.156 mg/ml [50]. In our investigation, the fatty oil extracted from *C. mas* fruits demonstrated superior efficacy against *C. albicans*. The observed activity can be attributed to the bioactive compounds in the extracts, such as phenolics and fatty acids, known for their antimicrobial properties. These extracts showed broad-spectrum activity, effective against both Gram (+) and Gram (-) bacteria, as well as yeasts. These results suggest that the plant extracts have potential as natural antimicrobial agents. Further studies are needed to explore their mechanisms of action and optimize extraction methods for enhanced efficacy.

α-Glucosidase and α-Amylase Inhibition Assay

Based on the assays, the results indicate noteworthy *α*-glucosidase inhibition activity in various samples, including *P. cerasifera, C. mas*, and *M. sylvestris.* The inhibitory effects on α-glucosidase were evaluated by measuring both the percentage inhibition at a concentration of 5000 µg/ml and the determination of IC_{50} values.

At a concentration of 5000 µg/ml, the aqueous extract from *P. cerasifera* exhibited significant inhibition of α-glucosidase, showing a notable inhibitory activity of 67.73%, albeit slightly lower compared to acarbose (77.61%). Similarly, the aqueous extract of *C. mas* and *M. sylvestris* exhibited notable *α*-glucosidase inhibition activities, with values of 81.58% and 70.11%, respectively. In comparison, the fatty oil of *P. cerasifera, C. mas,* and *M. sylvestris* demonstrated relatively lower *α*-glucosidase inhibition activities, with values of 0.22%, 18.73%, and 51.17%, respectively.

Furthermore, the IC_{50} values for *α*-glucosidase inhibition were calculated, with acarbose serving as a reference. The IC_{50} value of acarbose was 2434 µg/ml. Among the samples, the aqueous extract of *P. cerasifera* exhibited an IC_{50} value of 650 µg/ml, indicating significant *α*-glucosidase inhibitory potential. *C. mas*'s aqueous extract displayed an even more potent inhibition, with an IC_{50} value of less than 10 µg/ml. Additionally, *M. sylvestris*'s aqueous extract demonstrated an IC_{50} value of 399 µg/ml. However, the fatty oil samples did not yield IC_{50} values, suggesting that their inhibitory effects were not as pronounced.

In terms of *α*-amylase inhibition activity, acarbose exhibited an inhibition of 65.79% at a concentration of 5000 µg/ml. Among the samples, *P. cerasifera*'s fatty oil displayed an *α*-amylase inhibition of 36.23%, while *C. mas* and *M. sylvestris* exhibited lower inhibitions of 11.36% and 20.72%, respectively. The aqueous extracts of all samples did not exhibit significant *α*-amylase inhibition (Table 6).

In a prior investigation, we assessed the antidiabetic properties of a 70% methanolic extract obtained from *P. cerasifera* fruit. Intriguingly, the extract exhibited no inhibitory effects against both α-glucosidase and α-amylase enzymes [51]. In another study, health benefits of various traditional *Prunus* fruits cultivated in Serbia were investigated. The study focused on assessing their inhibitory activities

against *α*-glucosidase and *α*-amylase. For the extracts used in the study, a 50% ethanol preparation method was employed. Noteworthy results showed that the white cherry plum (*P. cerasifera*) extract had a substantial inhibitory effect on *α*-glucosidase (6.18 mg/ml), while it exhibited no detectable inhibitory effect on *α*-amylase. In comparison, the reference compound acarbose showed an inhibitory effect of 0.11 mg/ml on *α*-amylase and 3.73 mg/mL on *α*-glucosidase [52].

Table 6. The results of *α*-glucosidase and *α*-amylase inhibition assays

α-Glucosidase Inhibition Activity (% Inhibition of 5000 µg/ml ± standard deviation)			
Acarbose	77.61 ± 4.70		
Samples	***Prunus cerasifera***	***Cornus mas***	***Malus sylvestris***
Fatty oil	0.22 ± 2.31	18.73 ± 5.75	51.17 ± 3.89
Aqueous extract	67.73 ± 2.72	81.58 ± 1.29	70.11 ± 2.79
α-Glucosidase Inhibition Activity (IC_{50} values, µg/ml)			
Acarbose	2434		
Samples	***Prunus cerasifera***	***Cornus mas***	***Malus sylvestris***
Fatty oil	-	-	3887
Aqueous extract	650	<10	399
α-Amylase Inhibition Activity (% Inhibition of 5000 µg/ml ± standard deviation)			
Acarbose	65.79 ± 3.03		
Samples	***Prunus cerasifera***	***Cornus mas***	***Malus sylvestris***
Fatty oil	36.23 ± 5.13	11.36 ± 9.10	20.72 ± 3.41
Aqueous extract	ND	ND	ND

ND: Not Determined

A study aimed to investigate the potential of hydroalcoholic (80% ethanol) extract derived from cornelian cherry fruit as inhibitors of key carbohydrate digestive enzymes, pancreatic α-amylase, and intestinal α-glucosidase. The findings revealed that the cornelian cherry extract exhibited inhibition of α-glucosidase and α-amylase, with IC_{50} values of 6.87 and 6.05 mg/ml, respectively. In comparison, the reference compound acarbose demonstrated IC_{50} values of 0.023 mg/ml against α-amylase and 0.043 mg/mL against α-glucosidase [53]. A study aimed to evaluate the bioactivity of extracts obtained from red and yellow Cornelian cherry fruits. These extracts were investigated for their inhibitory effects against α-glucosidase. The results indicated that at concentrations of 25.7 and 30.0 µg/ml, the extract derived from red fruits displayed a more pronounced inhibitory effect on α-glucosidase activity compared to the extract from yellow fruits at equivalent concentrations. Specifically, the red fruit extract exhibited an IC50 value of 25.68 µg/ml, whereas the yellow fruit extract showed a slightly higher IC_{50} value of 28.46 µg/ml. In contrast, the reference compound acarbose demonstrated a significantly higher IC_{50} value of 5.68 × 103 µg/ml [54]. A research study was undertaken to explore the inhibitory effects on α-amylase and α-glucosidase activities using aqueous extracts from three apple varieties: *M. sylvestris* (green apple), *M. pumila* (red apple), and *Syzygium samarangense* (wax apple). The findings revealed that all apple varieties exhibited dose-dependent inhibition of α-amylase (with IC_{50} values ranging from 12.66 to 16.98 µg/ml) and α-glucosidase (ranging from 13.55 to 16.23 µg/ml) activities. Notably, green apple demonstrated the most potent inhibitory activity, while wax apple exhibited the least inhibitory effect [55]. These results highlight the potential of aqueous extracts, particularly from *C. mas*, as effective α-glucosidase inhibitors. However, the fatty oils' limited inhibitory activity suggests that they may not be as effective for inhibiting carbohydrate-digesting enzymes. Further studies are required to explore the specific compounds responsible for these effects and their potential use in diabetes management.

Anticholinesterase Assays

In terms of acetylcholinesterase inhibition activity at 100 µg/ml concentration, the reference compound donepezil demonstrated a strong inhibitory effect of 99.20%. Among the samples tested, the *Prunus cerasifera* extract displayed an inhibition of 11.56%, *Cornus mas* had an inhibition of 10.70%,

and *Malus sylvestris* exhibited an inhibition of 11.71% for the fatty oil. For the aqueous extract, *P. cerasifera* showed an inhibition of 10.02%, *C. mas* had an inhibition of 9.31%, and *M. sylvestris* displayed an inhibition of 8.13%.

In terms of butyrylcholinesterase inhibition activity at 1000 µg/ml, donepezil exhibited complete inhibition with a value of 100%. Among the samples, *P. cerasifera* extract showed an inhibition of 10.39%, *C. mas* exhibited an inhibition of 8.96%, and *M. sylvestris* displayed an inhibition of 6.39% for the fatty oil. For the aqueous extract, *P. cerasifera* had an inhibition of 1.13%, *C. mas* showed an inhibition of 11.28%, and *M. sylvestris* exhibited an inhibition of 8.93% (Table 7).

Table 7. The results of acetylcholinesterase and butyrylcholinesterase inhibition assays

Acetylcholinesterase Inhibition Activity (% Inhibition of 100 µg/ml ± standard deviation)			
Donepezil	99.20 ± 0.22		
Samples	***Prunus cerasifera***	***Cornus mas***	***Malus sylvestris***
Fatty oil	11.56 ± 7.70	10.70 ± 1.82	11.71 ± 4.26
Aqueous extract	10.02 ± 0.87	9.31 ± 4.58	8.13 ± 2.32
Butyrylcholinesterase Inhibition Activity (% Inhibition of 1000 µg/ml ± standard deviation)			
Donepezil	100 ± 0.49		
Samples	***Prunus cerasifera***	***Cornus mas***	***Malus sylvestris***
Fatty oil	10.39 ± 9.64	8.96 ± 3.19	6.39 ± 1.75
Aqueous extract	1.13 ± 2.69	11.28 ± 1.37	8.93 ± 3.85

Our study stands as the inaugural exploration into the anticholinesterase potential of *Prunus cerasifera*, in line with our existing knowledge. When examining research conducted on the *Prunus* genus, a study aimed to assess the anticholinesterase property of *P. mahaleb*. Results indicated that the methanolic extract's lower IC_{50} values ($p<0.05$) for acetylthiocholinesterase (AChE), and butyrylcholinesterase (BChE) (52.1 and 86.2 µg/ml, respectively) compared to the hexane extract (85.2 and 110.7 µg/ml, respectively). In comparison to the standard drug galantamine (with IC_{50} values of 12.8 and 10.7 µg/ml, respectively), both extracts exhibited very limited activity [56]. Another study investigated the inhibitory effects of sweet and bitter apricot (*P. armeniaca*) kernel extracts on cholinesterase enzymes utilizing Ellman's method. The aqueous extract of the bitter variety exhibited the most prominent AChE inhibitory activity (IC_{50}= 134.93 µg/ml). Notably, none of the extracts displayed inhibitory activity against BChE. Comparatively, the reference compound rivastigmine showed AChE inhibitory activity with an IC_{50} value of 2.77 µg/ml and BChE inhibitory activity with an IC_{50} value of 1.93 µg/ml [57].

Our research represents the first investigation into the potential anticholinesterase properties of *Cornus mas*, aligning with our current understanding. Upon reviewing studies carried out within the *Cornus* genus, the objective of a study was to explore the anticholinesterase activity of methanol extracts from *C. sanguinea* leaves and fruits. Specifically, leaves extract exhibited dose-dependent AChE inhibitory effects, ranging from 16.84% to 50.89% at concentrations of 12.5 to 100 µg/ml. In contrast, fruits extract displayed no AChE inhibition at 12.5 and 25 µg/ml, but demonstrated inhibitory effects of 11.59% and 24.58% at 50 and 100 µg/ml, respectively. The IC_{50} values for both extracts were determined as 93.64 and > 100 µg/ml, respectively. Notably, both extracts exhibited lower inhibitory effects compared to the positive control, galantamine [58].

A study was conducted to evaluate the potential of fresh fruit juice derived from *M. domestica* x *M. sylvestris* in mitigating AD symptoms in mice. Cognitive enhancement was assessed through behavioral tests including the Morris water maze (MWM) and Passive shock avoidance paradigm (PSAP), alongside the estimation of AChE activity. Two doses (1 ml/kg and 1.5 ml/kg, b.w, p.o) of juice were administered against AD induced by scopolamine (0.4 mg/kg, i.p), with piracetam (400 mg/kg, i.p) used as the standard. Prolonged administration of both low and high doses of juice notably reduced transfer latency (TL) and escape latency time (ELT) in PSAP and MWM ($P < 0.01$ and $P < 0.05$, respectively). Enhanced memory retention was evidenced by higher time spent in the target quadrant (TSTQ) values in the MWM model. Furthermore, the higher dose of fruit juice significantly ($p < 0.01$)

lowered AChE activity in the brain, indicating improved learning and memory retention [59]. These findings suggest that while these extracts have some cholinesterase inhibitory effects, they are far less potent than donepezil. However, they may still hold potential for future research, particularly in combination with other treatments for neurodegenerative diseases. Further studies are needed to identify the specific compounds responsible for these effects.

ABTS•+ and DPPH• Scavenging Activity

Table 8 summarizes the antioxidant activity of aqueous extracts and fatty oils from the investigated species. In ABTS cation radical scavenging tests, the analysis of % inhibition values at a concentration of 100 µg/ml indicated that the *C. mas* aqueous extract showed significantly higher % inhibition on $ABTS^{\cdot+}$ compared to the standards (α-tocopherol (TC) and trolox (TR)). Other samples exhibited antioxidant capacities that were generally similar to the standards.

Table 8. Antioxidant activity test results

$ABTS^{\cdot+}$ Scavenging Activity of Standards and Aqueous Extracts (% Inhibition of 100 µg/ml ± standard deviation)			
***α*- Tocopherol**	94.099 ± 0.0017		
Trolox	99.614 ± 0.0012		
Samples	***Prunus cerasifera***	***Cornus mas***	***Malus sylvestris***
Fatty oil	0.949 ± 0.0106	6.546 ± 0.0141	5.775 ± 0.0229
Aqueous extract	8.491 ± 0.0037	**98.693 ± 0.0026**	12.442 ± 0.0093
$DPPH^{\cdot}$ Scavenging Activity of Standards and Aqueous Extracts (% Inhibition of 100 µg/ml ± standard deviation)			
***α*- Tocopherol**	88.886 ± 0.001		
Trolox	91.285 ± 0.0004		
Samples	***Prunus cerasifera***	***Cornus mas***	***Malus sylvestris***
Fatty oil	ND	5.338 ± 0.0566	0.823 ± 0.0345
Aqueous extract	2.726 ± 0.0797	**89.515 ± 0.0002**	9.492 ± 0.0142

ND: Not detected

Similarly, in DPPH radical scavenging assays at a concentration of 100 µg/ml, the *C. mas* aqueous extract demonstrated a superior % inhibition value on $DPPH^{\cdot}$, closely approaching that of the standards. This underscores the substantial antioxidant potential of the *C. mas* aqueous extract. The results from both antioxidant activity tests were consistent with each other.

In a separate investigation evaluating the total antioxidant capacity, phenolic composition, organic acid, and vitamin C content of three plum species, it was observed that *P. spinosa*, characterized by the highest phenolic content, organic acids, and vitamin C composition, exhibited stronger antioxidant capacity compared to *P. domestica* and *P. cerasifera*. Remarkably, a positive correlation was identified between the vitamin C content and the overall antioxidant capacity [10].

Through ferric reducing ability of plasma and ABTS radical scavenging tests conducted to evaluate the *in vitro* antioxidant capacity of *C. mas*, it was evident that the plant exhibits significant antioxidant properties. As a result, it was deduced that *C. mas* possesses the capability to mitigate acute inflammation [60].

In a literature-registered study, 38 extracts derived from fruits and leaves of various species were investigated for their antioxidant properties. Comparing the DPPH radical scavenging activities of 50% methanolic extracts from the fruits, it was observed that C. mas exhibited more potent antioxidant effects compared to P. divaricata (with EC_{50} values on DPPH radical of 1.078 and 1.692, respectively) [61]. This suggests that *C. mas* is a strong natural antioxidant, potentially more effective than some common standards. Other samples showed antioxidant activity similar to TC and TR, indicating their moderate effectiveness. These results highlight *C. mas* as a promising source of antioxidants for potential applications in food and pharmaceuticals to combat oxidative stress-related diseases. Further studies on the active compounds are needed to confirm their benefits.

Total Phenolic and Tannin Content

The test results for total phenolic and tannin content of the samples were provided in Table 9. Upon evaluating the studies conducted on these samples, it was determined that the aqueous extract of *Cornus mas*, in particular, exhibited high levels of total phenol and total tannin content. Additionally, this extract demonstrated the highest antioxidant activity. Consequently, the findings of our study are consistent and mutually supportive.

Table 9. Total phenolic and tannin content test results

Total phenolic content (µg GAE/mg extract ± standard deviation)			
Samples	***Prunus cerasifera***	***Cornus mas***	***Malus sylvestris***
Fatty oil	14.511 ± 0.0017	24.777 ± 0.0008	18.866 ± 0.0019
Aqueous extract	34.666 ± 0.0007	**198.511 ± 0.0041**	41.755 ± 0.001
Total tannin content (µg TAE/mg extract± standard deviation)			
Samples	***Prunus cerasifera***	***Cornus mas***	***Malus sylvestris***
Fatty oil	ND	9.512 ± 0.0008	2.692 ± 0.0019
Aqueous extract	20.923 ± 0.0007	**209.974 ± 0.0041**	29.102 ± 0.001

GAE: Gallic acid equivalent, TAE: Tannic acid equivalent

Previous research has reported the total phenolic contents of *Cornus* genotypes to be within the range of 25.90–74.83 mg gallic acid equivalent per gram of fresh weight [62,63]. However, in the investigation conducted by Hamid et al., the total phenol content was determined to be within the range of 1097.19–2695.75 mg gallic acid equivalent per 100 grams of fresh weight. The discrepancy in these values compared to previous studies was attributed to variations in total phenolic content influenced by environmental factors and post-harvest processes [64]. In our research, we observed that the total phenolic content of the aqueous extract from *C. mas* was notably higher. Another study investigating the phenolic content of 11 wild fruit species, including *P. spinosa*, *C. mas*, and *M. sylvestris*, using the HPLC-UV method, revealed that catechin hydrate was most abundant in *C. mas*, with a concentration of 268.16 mg per 100 grams of dry weight [65]. It was also demonstrated the strongest antioxidant activity, which correlates well with its bioactive content. These findings suggest that the extraction method plays a significant role in the quantity of bioactive compounds extracted, and this, in turn, affects the antioxidant potential of the extract.

Conclusions

In conclusion, this study highlighted the relationship between the chemical content and bioactive activities of the essential oils, fatty oils, and aqueous extracts of *P. cerasifera*, *M. sylvestris*, and *C. mas*. The fatty oils from these species were primarily composed of oleic acid (*P. cerasifera*), palmitic acid (*M. sylvestris*), and linoleic acid (*C. mas*), which are known for their potential health benefits, including anti-inflammatory and antioxidant properties. The essential oils revealed key compounds such as benzaldehyde in *P. cerasifera*, nonacosane in *M. sylvestris*, and (E,E)-2,4-Decadienal in *C. mas*, which may contribute to their therapeutic effects. Quinic acid, the most predominant compound in all extracts, was associated with antioxidant and antidiabetic activities, supporting its role as a bioactive agent in these plants. Notably, *C. mas* showed strong antimicrobial activity against *Candida* species, with MIC values indicating its potential as an antifungal agent. Furthermore, the aqueous extracts demonstrated promising α-glucosidase inhibition, with particularly potent activity observed in *C. mas* and *M. sylvestris*, as reflected in their low IC_{50} values. These findings demonstrate that the chemical composition of these extracts directly correlates with their bioactivity, underlining the potential of these plants for future therapeutic applications, especially in the management of diabetes, infections, and oxidative stress.

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CONFLICTS OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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