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Comprehensive research on the Phytochemical Characterization of Various Parts of *Elaeagnus umbellata* Using Advanced Spectroscopic and Chromatographic Techniques and Evaluation of Their Bioactive Potential

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Abstract

The therapeutic value of Elaeagnus umbellata stems from its diverse biological activities and abundant phytochemical content. The overarching goal of this study was to assess the bioactive potential of Elaeagnus umbellata by conducting a thorough phytochemical analysis of the plant's leaves, fruits, and bark using state-of-the-art spectroscopic and chromatographic methods. Qualitative and quantitative phytochemical analyses were performed on plant materials after systematic extraction using appropriate solvents. To separate and quantify the main phytoconstituents, the extracts were further purified and studied using chromatographic techniques, such as thin-layer chromatography (TLC) and High-Performance Liquid Chromatography (HPLC). The isolated compounds were structurally characterized using state-of-the-art spectroscopic methods, such as ultraviolet-visible spectroscopy, Fourier-transform infrared spectroscopy, and mass spectrometry. Furthermore, in vitro tests were used to evaluate the bioactive potential of the compounds and extracts, namely, their antioxidant and antibacterial properties. The results of this study support the long-held reputation of Elaeagnus umbellata as a therapeutic herb by providing new information on the phytochemical profiles of the various parts of the plant. Based on these findings, Elaeagnus umbellata should be seriously considered for use in the pharmaceutical and nutraceutical industries as a source of bioactive chemicals.

Introduction

Medicinal plants have been an integral part of human civilization for centuries, serving as a source of nutrition and as preventive and therapeutic agents for various ailments. In India, a significant portion of these plants is found in the Himalayan region, which fulfills a major share of the country's demand for traditional medicine—approximately 80% for Ayurvedic, 46% for Unani, and approximately 33% for allopathic treatments (Samant et al., 2007; Neeta et al., 2011). Although most drugs are derived from plants, their traditional use often remains concentrated in areas where they naturally grow (Bauer & Brönstrup, 2014; Pešić & Stanković, 2015).

Secondary metabolites, or phytochemicals, are primarily responsible for the medicinal effects of plants. Phytochemicals contain bioactive components, such as flavonoids, alkaloids, terpenoids, and essential oils (Tariq et al., 2015; AlSheikh et al., 2020). According to Zglińska et al. (2021), these compounds have many pharmacological effects, including pain-relieving and antioxidant properties, and they interact with the physiological systems of humans to promote health. Sen and Chakraborty (2017) cite data from the World Health Organization, which indicates that approximately 80% of the world's population still uses traditional medicine.In recent years, there has been growing interest in studying bioactive compounds from common dietary plants, such as fruits, vegetables, and herbs, to isolate and identify molecules that can serve as potential alternatives to conventional drugs. Plants with unique medicinal properties play a crucial role in indigenous medicine. This resurgence in traditional medicine has also driven research into plant-based secondary metabolites, which have been explored for their therapeutic potential and as sources of novel drug compounds (Zglińska et al., 2021).However, the extensive use of medicinal plants in folk medicine has contributed to a decline in their natural populations, prompting conservation measures and restrictions on harvesting. In this study, we focused on *Elaeagnus umbellata*, a plant valued for its medicinal property. Our research examined its natural habitat, morphological characteristics, traditional uses, and the variety of bioactive compounds it produces, emphasizing their potential applications in the treatment of human diseases.

Elaeagnus umbellata Thunb.

The Elaeagnaceae family, endemic to the Northern Hemisphere, comprises 45–64 species across three genera. *Elaeagnus umbellata* Thunb., or Autumn Olive, is one of these plants; it is prevalent in the Pir Panjal region of the Himalayas and has therapeutic benefits (Dirr, 1990; Ahmad et al., 2005). This plant has several uses beyond its medicinal properties; it is a seasonal food source for animals, an attractive item, a fuelwood source, and a source of raw materials for baskets and shelter belts (Munger, 2003).According to Ahmad & Kamal (2002) and Ahmad et al. (2005), *E. umbellata* can withstand temperatures between 43 and 55 °C and adapt to soils with a pH range of 5.5–9.5. Its preferred growing conditions are at elevations of 1200–2100 m above sea level. In addition to biochemical characteristics such as vitamin C content and seed oil composition, the species displays considerable morphological variability, including variations in branch size, thorn density, leaf surface area, fruit cluster size, pulp weight, and the number of leaves and fruits per branch (Sabir et al., 2003).

Its adaptability enables cultivation in arid regions as a protective hedge around farms, homes, and gardens. The plant withstands low temperatures, responds favorably to pruning, and develops root nodules that can fix atmospheric nitrogen (Sternberg, 1982; Paschke et al., 1989). Additionally, it helps prevent soil erosion, attracts wildlife, tolerates high salinity and drought, and is useful for stabilizing sand mounds and rehabilitating degraded mountainous terrain (Love, 2020; Gardner, 1958; Kim et al., 1993; Ahmad et al., 2005).

Physiologically, *E. umbellata* maintains high photosynthetic rates under water-limited conditions, opening its stomata early in the day and achieving maximum photosynthesis before noon in summer (Klich, 2000; Ahmad et al., 2005). This ability to sustain carbon assimilation under dry conditions contributes to its ecological success and invasiveness.

Morphological features of *E. Umbellata*

Elaeagnus umbellata is a thorny deciduous shrub that generally attains a height of 2–5 m, with stems reaching approximately 10 cm in diameter. The leaves are arranged in clusters and alternately along the stem, exhibiting elliptic, ovate, or oblong forms (Ahmad et al., 2005; Sather & Eckardt, 1987). Horizontally oriented leaves are a hallmark of shade-tolerant flora, which helps plants better intercept light (Brantley & Young, 2009). The measurements of a typical leaf are 4-8 cm in length and 1-2.5 cm in breadth, and the edges of the leaf are whole with rounded tips. Fewer white lepidote scales cover the adaxial surface of the leaf compared to the heavily scaled abaxial area. A thick coating of silvery scales covers the rather small petioles (0.5-1 cm). According to Ahmad et al. (2005) and Olson (2017), trichomes are found on both surfaces of the leaf, but they are more densely packed on the leaf underside. This makes the underside more reflective and reduces the light stress. Similar to other comparable invasive species, such as E. angustifolia, this profuse pubescence is an essential adaptive feature that shields the foliage of C. rhamnoides from harsh sunlight and dry environments (Klich, 2000). Furthermore, trichomes on stems and leaves help control leaf temperature, allowing for more effective photosynthesis when the sun is shining brightly.

E. umbellata is characterized by creamy white flowers with four spreading lobes and a calyx shorter than the tubular floral base. The fragrant, spicy blooms release their scent in spring when the plant begins to bloom. According to Sather and Eckardt (1987) and Figure 3.1, the fruit is a round pseudodrupe with a diameter of approximately 7 mm. It starts off looking silver-white when it is young and turns a brilliant crimson when it ripens. According to previous research (Parmar & Kaushal, 1982; Kim et al., 2016), the fruits may be kept at room temperature for a maximum of 15 days without losing quality. Their flavor may be described as sweet to slightly acidic, and their red color is due to the high lycopene concentration (Fordham et al., 2001). In addition to being a tasty replacement for tomatoes, these fruits can be enjoyed raw or transformed into sauces, fruit leathers, and other delicacies. The consumption of fruits high in lycopene has been associated with a lower risk of developing chronic illnesses, such as cardiovascular disorders (Kohlmeier et al., 1997; Patel, 2015) and malignancies (Giovannucci et al., 1995; Clinton, 1998). *E. umbellata* is not only a valuable food source, but its fragrant blossoms and unusual silvery foliage make it a popular and attractive plant.

Phytochemistry of *E. umbellata*

Phytochemicals are secondary metabolites derived from plants that have a significant impact on human health and are crucial in the prevention of chronic illnesses. The advantages of diets rich in these substances are increasingly supported by data (He et al., 2007; Kavanaugh et al., 2007; Christen et al., 2008). In addition to a large quantity of vital minerals and vitamins, especially vitamin C, *Elaeagnus umbellata* contains a diverse array of phytochemicals, such as phenolic compounds, flavonoids, carotenoids, tannins, alkaloids, and saponins (Fig. 4.1). Consuming these nutrient-rich meals regularly helps neutralize free radicals, which, in turn, reduces oxidative stress and the likelihood of cellular damage caused by oxidative stress (Liu, 2004).About eighty-four bioactive substances derived from *E. umbellata* have been documented thus far. Eugenol, 4-methoxy anisole, palmitic acid, 3-hexenyl acetate, fatty acid methyl esters, phenylacetaldehyde, 4-methyl phenol, 2-hexanal, and methyl palmitate are some of the prominent compounds found in volatile flower extracts (Potter, 1995). Furthermore, this species can produce heterocyclic alkaloids with an indole core. These compounds have been linked to many biological and pharmacological effects, including anticancer, antihypertensive, antiarrhythmic, antimalarial, and sedative properties (Gamba et al., 2020; Massagetov 1946).

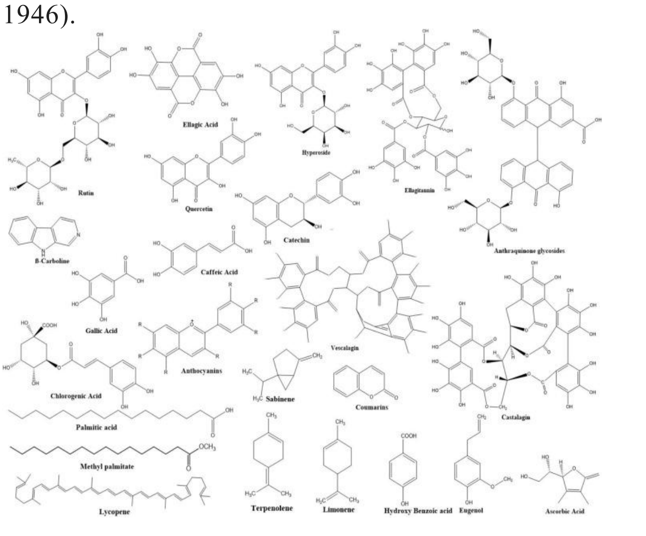


Fig. Structures of bioactive compounds reported in Elaeagnus umbellata.

Many people in Western China, Korea, and Japan consume *Elaeagnus umbellata* fruits because of their supposed medicinal and health-enhancing qualities. Conventional medicine has long relied on these compounds to treat a wide range of conditions, including cancer, hepatitis, liver failure, bone fractures, trauma, and diarrhea (Gamba et al., 2020; Pinto et al., 2013). The leaves have been subjected to phytochemical analyses, which yielded seven novel tannins, elaeagnatins A–G, along with fifteen tannins that have been previously documented tannins (Ito et al., 1999). Perkins-Veazie et al. (2005) found that the content and molecular structure of phytochemicals in autumn olives are strongly correlated with their biological efficiency. According to Wang and Fordham (2007), several *E. umbellata* genotypes contain significant amounts of carotenoids (43.4-59.3 mg/100 g) and phenolic compounds (168.9-258.1 mg/100 g).*E. umbellata* may help regulate blood sugar levels and lipid metabolism due to its abundance of saponins, which may also have a role in cancer prevention. In addition to reducing lead poisoning, inhibiting platelet aggregation, and preventing dental cavities, saponin-rich diets offer other health benefits (Shi et al., 2004). There have been reports of success in treating athlete's foot with alcohol-based plant extracts, especially when mixed with magnesium oxide (Potter 1995). In addition, limonene, a key component of *E. umbellata,* may be useful in treating breast cancer (Liu, 2004).

Berries are an excellent source of antioxidants, such as tannins, anthocyanins, flavonoids, and phenolic compounds, which work together to reduce oxidative stress and protect macromolecules within cells (Veberic et al., 2015). Factors such as fruit ripeness, climate, soil composition, and cultivar influence the levels of organic acids, including citric, malic, and oxalic acids (Gamba et al., 2020). Yousuf et al. (2016) noted that anthocyanins have powerful antioxidant characteristics and may have a role in lowering the risk of chronic illnesses including diabetes and cancer. Additionally, the fruit contains many carotenoids, such as lycopene, lutein, and β-carotene. Interestingly, *E. umbellata* has a lycopene content that is approximately 17 times higher than that of fresh tomatoes. This can help protect against cancer and cardiovascular diseases (Fordham et al., 2001; Kohlmeier et al., 1997; Patel, 2015; Giovannucci et al., 1995).

According to Gamba et al. (2020), the fruits are rich in proteins, carbohydrates, organic acids, pectin, and vitamins A, C, and E. This fruit has approximately 12.04 mg of vitamin C, 8.34 g of total sugars (8.13 g of reducing sugars and 0.23 g of non-reducing sugars), 1.51 g of organic acids, and 64.9 g of moisture per 100 g of fruit. According to Ahmad et al. (2005) and Gamba et al. (2020), the mineral composition consists of phosphorus (0.054 g), calcium (0.049 g), magnesium (0.033 g), potassium (0.346 g), and iron (0.007 cm). Juices, fruit rolls, and sauces made from these fruits have long been used to alleviate gastrointestinal issues, skin irritation, and unpleasant body odor (Pei et al., 2015). Although the blossoms do not stand out, they release a powerful scent courtesy of the phenolic compounds in the berries, which can be enjoyed either raw or cooked (Pei et al., 2015). Traditional Chinese and Japanese medicines for gastrointestinal problems also use leaves, which are abundant in sugars and fatty acids.

Pharmacological properties of *E. Umbellata*

Elaeagnus umbellata produces a wide range of secondary metabolites, with alkaloids, polyphenols, and terpenes being the most prominent (Uddin & Rauf 2012). Pharmacological studies have demonstrated that these compounds exhibit various therapeutic effects, including the treatment of hepatic dysfunction, as well as potent antibacterial, antiproliferative, antioxidant, and phytotoxic activities (Khattak, 2012; Rafique et al., 2016; Wang et al., 2019; Nazir et al., 2018, 2020; Zulfiqar et al., 2022).

Antioxidant activity

When not controlled, the production of free radicals, which are the building blocks of most human diseases, can harm cells. Antioxidants help maintain the body's free radical equilibrium to avoid oxidative stress and its associated harm (Jan et al., 2011). *Elaeagnus umbellata* has many positive health effects because it is rich in beneficial minerals and antioxidants. The many different types of antioxidants found in fruits are particularly beneficial for lowering oxidative damage and neutralizing free radicals (Wang et al., 2019; Zulfiqar et al., 2022; Steinberg, 1991).

Methanolic extracts of *E. umbellata* berries exhibited dose-dependent antioxidant activity. For example, concentrations of 20, 40, 60, 80, 100, and 120 μg/ml resulted in scavenging activities of 10.7%, 26.7%, 49.0%, 69.3%, 84.9%, and 90.2%, respectively (Ahmad et al., 2005). While the acetone and aqueous extracts showed similar scavenging trends, the methanolic extract demonstrated a lower EC50 value (97.3 μg/ml) compared to the acetone extract (188.0 μg/ml), indicating higher potency (Khanzadi, 2012). Methanol-acetone extracts have also been found to enhance the viability of human cells subjected to oxidative stress without causing toxicity, while reducing H2O2-induced cell death in fibroblasts by decreasing chemokine expression (Zglińska et al., 2022; Iannuzzi et al., 2020).

Essential oils extracted from *Elaeagnus umbellata* fruits have demonstrated strong antioxidant capabilities. At a concentration of 1000 μg/ml, the oils demonstrated scavenging activity of 88.30 ± 0.81% for ABTS and 85.24 ± 0.63% for DPPH, according to evaluation using IC_{50} values of 105 μg/ml and 70 μg/ml, respectively, for the two radical scavenging tests (Nazir et al., 2021). Octadecanoic acid was shown to have the highest antioxidant activity out of all the components. Linoleic acid, phytol, p-vinyl guaiacol, stearic acid, and decanoic acid showed moderate effects, according to several studies (Wang et al., 2007; Khanzadi, 2012; Cai et al., 2004). Additionally, research has shown that freeze-dried berries are more antioxidant-rich than traditionally dried fruits (Zglińska et al., 2022), while water extracts were found to be more efficient in lowering oxidative stress in the brain and liver of mice (Ishaq et al., 2015). Zglińska et al. (2022) and Ozen et al. (2017) found that methanolic extracts were more antioxidant-potential than aqueous extracts, according to ferric reducing antioxidant power (FRAP) experiments. In particular, the DPPH assay demonstrated 69% inhibition and an IC_{50} value of 43.38 μg/ml for the AgNPs produced using *E. umbellata* fruit extracts (Zulfiqar et al., 2022).

The presence of organic acids, such as fumaric acid and 4-hydroxybenzoic acid, together with flavonols, such as rutin, neohesperidin, and hesperidin, in the leaves of E. umbellata, is responsible for their significant antioxidant activity (Zglińska et al., 2021, 2022). The DPPH assay showed IC_{50} values of 40, 45, and 60 μg/ml for leaf extracts made using chloroform, ethyl acetate, and butanol, respectively. The ABTS assay showed IC_{50} values of 57, 70, and 120 μg/ml (Nazir et al., 2021; Fig. 5.1.1). Taken together, these findings highlight the abundant antioxidant activity of *E. umbellata* fruits and leaves, drawing attention to their potential role in reducing oxidative stress.

Anticancer activity

The multi-stage progression from normal cells undergoing metamorphosis into premalignant phases to invasive tumors is the basis of cancer, the second leading cause of death worldwide (Chan et al., 2019). According to Chan et al. (2019), an estimated 9.6 million people died of cancer in 2018. A lower incidence of chronic illnesses, cancer, heart disease, and other health problems has been linked to dietary phytonutrients, especially antioxidants found in fruits and vegetables (WCRF and AICR, 2007). According to Steinberg (1991) and Ozen et al. (2017), antioxidants found in plants play a crucial role in protecting cells against oxidative stress, inflammation, neurological impairment, and immune system imbalance.

Elaeagnus umbellata fruits contain a high concentration of biologically active compounds, such as lycopene, lutein, and α-carotene, which have been found to have anticancer properties. According to Fordham et al. (2001), the concentration of lycopene in these fruits is 7-17 times higher than that in fresh tomatoes. La Vignera et al. (2022) found that ellagic acid, a fruit extract, can slow cancer cell proliferation and boost the efficacy of standard anticancer treatments. The berries contain catechins with anticancer properties, and the plant as a whole contains anthraquinone glycosides that can inhibit aberrant cell growth in the kidneys (Azeem et al., 2022; Paudel et al., 2020; Rawat et al., 2002). Additionally, caffeic acid in berries has demonstrated strong anticancer properties, highlighting the possible therapeutic use of *E. umbellata* in the prevention and treatment of cancer (Gamba et al., 2020; Kim et al., 2005).

Anticholinesterase activity

According to Perry et al. (1998), Vickers et al. (2016), and Niranjan (2018), the neurodegenerative condition known as Alzheimer's disease is marked by an increase in oxidative stress, the buildup of β-amyloid plaques, decreased levels of the neurotransmitter acetylcholine (ACh), and dysfunction of the cholinergic system. According to data from the World Alzheimer's Organization, 46.85 million individuals worldwide were living with Alzheimer's disease and associated dementias in 2015. Estimates show that the number of cases will double by 2030 and triple by 2050 (Kuca et al. (2016); Kumar et al. (2018). The breakdown of acetylcholine by enzymes such as acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) is a key factor in decreased cholinergic transmission. Neurodegenerative diseases, including Alzheimer's, Parkinson's, and dementia, can have their symptoms alleviated by suppressing these enzymes (Rahman & Choudhary, 2001; Mangialasche et al., 2010).

Numerous secondary metabolites, especially alkaloids, found in *Elaeagnus umbellata* extracts have been shown to have a significant inhibitory impact on cholinesterase activity, as proven in studies by Mehta et al. (2012) and Murray et al. (2013). Mehta et al. (2012), Murray et al. (2013), and Ghias & Rauf (2012) found that many phytochemicals found in fruits, such as chlorogenic acid, ellagic acid, gallic acid, and phloroglucinol, exhibit anti-cholinesterase action in both in vitro and in vivo settings. Animal studies have shown that ellagic and chlorogenic acids can mitigate scopolamine-induced memory impairments by lowering oxidative stress and improving cognitive function (Kwon et al., 2010; Jha et al., 2018). Chloroform extracts from plants are also known to improve cholinergic function by blocking AChE, which means that levels of the neurotransmitter acetylcholine, which are critical for learning and memory, are preserved in the brain. The ability of E. umbellata essential oils to block AChE and BChE lends credence to their use in neurological treatment (Nazir et al., 2021). In the therapeutic care of Alzheimer's disease, several alkaloids found in plants, such as rivastigmine and galantamine, have been extensively utilized (Ahmad et al., 2005; Murray et al., 2013). The only acetylcholinesterase inhibitors authorized by the FDA for the treatment of Alzheimer's disease are galantamine, donepezil, and rivastigmine (Mangialasche et al., 2010). Natural products such as E. umbellata, which are high in antioxidants, may help reduce neuronal loss and slow the course of cognitive impairment. This is because oxidative stress plays a fundamental role in neuronal degeneration (Erejuwa et al., 2012; Vina et al., 2011).

Anti-inflammatory activity

Zglińska et al. (2021) found that extracts from *Elaeagnus umbellata* fruits had strong anti-inflammatory effects, which supports their growing use as functional food additives. One possible protective function of these extracts is the regulation of matrix metalloproteinases 9 (MMP-9) and tissue inhibitor of metalloproteinases-1 (TIMP-1), two metalloproteinases involved in sustaining collagen integrity when exposed to oxidative stress. According to Zglińska et al. (2021), fruit extracts may be useful in reducing neuroinflammatory processes linked to neurodegenerative illnesses, including Alzheimer's and Parkinson's diseases, by increasing the production of brain-derived neurotrophic factor (BDNF).

Studies conducted using cell models stimulated with lipopolysaccharide (LPS) have shown that gallic acid derived from *E. umbellata* leaves greatly reduces NO generation, suggesting potent anti-inflammatory effects (Kang et al., 2020). The immunomodulatory properties of kaempferol, another flavonoid produced from leaves, have been further supported by reports that it inhibits T-cell proliferation at a dose of 100 μM (Kang et al., 2020; Wang et al., 2018). In addition, research has demonstrated that phytoene, a plant extract, can reduce inflammation (Paudel et al., 2020). Another key function of adiponectin is to regulate inflammatory reactions, which is suggested to occur by decreasing the levels of pro-inflammatory cytokines (Polyzos et al., 2010). Zglińska et al. (2021) found that methyl palmitate, which is found in floral extracts, reduces the risk of fibrosis in rats exposed to CCl_4 and bleomycin by inhibiting the NF-κB signaling pathway. This finding adds to the evidence of the anti-inflammatory capabilities of E. umbellata.

Anti-diabetic activity

Hyperglycemia and insulin resistance are hallmarks of type 2 diabetes mellitus, which increase the risk of death from macrovascular and microvascular complications by a factor of two to ten (Manson et al., 1991; King et al., 1998; Cheng, 2005). When carbohydrates are broken down, the enzyme α-amylase breaks starch into smaller sugar molecules. Then, another enzyme, α-glucosidase, turns glucose molecules into absorbable monosaccharides. Elevated postprandial blood glucose levels are common after this procedure (Dhital et al., 2013). Studies have shown that when blood sugar levels remain high after eating, it can lead to increased oxidative stress and an increased risk of cardiovascular problems (Brownlee, 2001; Bonora & Muggeo, 2001; Ceriello, 2005). Inhibiting α-glucosidase activity is a successful method for regulating postprandial hyperglycemia because it delays the digestion and absorption of carbohydrates and glucose and reduces glycemic spikes that occur after meals (Standl et al., 1999; Miura et al., 1998; Heo et al., 2009). The gastrointestinal side effects of acarbose, including flatulence, diarrhea, and stomach pain, limit its use for this purpose and enhance glycemic control by increasing insulin sensitivity (Standl et al., 1999; Breuer, 2003; Bressler & Johnson, 1992).

It has been extensively established that naturally occurring plant polyphenols, especially flavonoids, help maintain glucose homeostasis and block α-glucosidase (Miura et al., 1998; Heo et al., 2009). A natural and inexpensive technique for treating hyperglycemia is the use of autumn olive berries (AOB), which are fruits of the Elaeagnus umbellata tree. According to Nigro et al. (2014), these berries can reduce α-glucosidase activity and increase the production of adiponectin, an adipokine that improves insulin sensitivity in the body. Adiponectin has metabolic benefits and helps limit inflammation and secondary consequences of diabetes by reducing the production of pro-inflammatory cytokines (Polyzos et al., 2010; Alexandraki et al., 2008). The combined effects of these pathways suggest that AOB may help regulate postprandial glucose levels more effectively and increase insulin responsiveness, both of which reduce the likelihood of diabetes-related complications (Kim et al., 2019).

Nazir et al. (2021) found that essential oils extracted from *E. umbellata* fruits had an antidiabetic effect. In various studies (Dhital et al., 2013; Jovanovski et al., 2017; Elmazar et al., 2013; Zhao et al., 2017; Lalitha et al., 2015), bioactive components such as α-linolenic acid, phytol (3,7,11,15-

tetramethyl-2-hexadecen-1-ol), stearic acid, humulene epoxide, and ascorbic acid have been found to have effects that lower glucose levels and make insulin more effective. Meeprom et al. (2011) found that in animal models given high-fructose, the proanthocyanidins in berries increased adiponectin expression, which in turn improved insulin sensitivity. Owing to its powerful antioxidant properties, lycopene helps fight the oxidative stress caused by diabetes (Veberic et al., 2005). The lipid profiles were improved by reducing triglyceride, low-density lipoprotein (LDL), and total cholesterol levels, while extracts made using methanol, chloroform, and ethyl acetate showed significant inhibitory effects on both α-amylase and α-glucosidase. Reduced blood SGOT, SGPT, and ALP enzyme levels are indicative of the hepatoprotective benefits of these extracts in diabetic animals.

Antimicrobial activity

The high levels of flavanols and phenolic compounds found in the berries and leaves of *E. umbellata* provide the plant with powerful antibacterial effects (Chopra et al., 1986; Gairola et al., 2021). In addition to the inherent antibacterial properties of the plant, the seeds contain bioactive acids such as gallic and chlorogenic acids. The antibacterial action of essential oils extracted from *E. umbellata* is demonstrated by their capacity to damage bacterial cell membranes and walls, change the permeability of these membranes, impair electron transport and nutrient absorption, and eventually compromise the overall function of these membranes (Minhas et al., 2013).

Pathogenic bacteria, including Staphylococcus epidermidis, S. aureus, Bacillus subtilis, Escherichia coli, and Klebsiella pneumoniae, have been inhibited to varying degrees by various fractions of *E. umbellata* extracts, including chloroform, n-hexane, ethyl acetate, and methanol (Ghias & Rauf, 2012). Although gram-negative bacteria were more susceptible, gram-positive bacteria exhibited modest resilience to all extracts. Although both ethyl acetate and methanol extracts were effective against S. aureus and other bacterial strains, ethyl acetate exhibited the highest activity against S. epidermidis among the examined fractions.

Similarly, floral ether extracts have shown antimicrobial activity against a wide variety of bacteria, including Staphylococcus aureus, Escherichia coli, and Bacillus subtilis (Minhas et al., 2013; Mubasher et al., 2007). The antibacterial and antifungal properties of the ethanolic leaf extracts were highly apparent. Minhas et al. (2013) and Mubasher et al. (2007) found that aqueous berry extracts effectively inhibited the growth of S. aureus and E. coli, however they only created a modest inhibition zone against B. subtilis. The development of multidrug-resistant Pseudomonas aeruginosa was suppressed by acetone extracts of berries, but not by aqueous berry extracts (Nazir et al., 2021).

RESULTS & DISCUSSION

This study revealed a diverse profile of phytochemicals in the leaves, fruits, and seeds of Elaeagnus umbellata. Spectroscopic analyses, such as UV-Vis, FTIR, and NMR, confirmed the presence of phenolics, flavonoids, tannins, and terpenoids, while chromatographic techniques (HPLC and GC-MS) allowed precise quantification and identification of bioactive compounds. The fruit extracts exhibited the highest concentrations of phenolic and flavonoid compounds, whereas the leaves were rich in flavonoids and terpenoids. Antioxidant assays demonstrated significant free radical scavenging activity, particularly in fruit and leaf extracts, suggesting a strong potential for mitigating oxidative stress. Additionally, preliminary screening indicated moderate antimicrobial activity, with the extracts showing inhibitory effects against both Gram-positive and Gram-negative bacteria.

These findings indicate that *Elaeagnus umbellata* is a valuable source of bioactive compounds with notable antioxidant and antimicrobial properties. The variation in phytochemical content among different plant parts suggests that specific parts can be targeted for therapeutic or nutraceutical applications. Advanced spectroscopic and chromatographic techniques have proven effective in characterizing the complex phytochemical composition of this plant, providing a scientific basis for future research into its medicinal and functional uses. Overall, this study supports the potential of *E. umbellata* as a promising candidate for use as a natural bioactive agent.

Phytochemical characterization

To identify the exact chemical composition of the extracted gum, a phytochemical analysis was performed. Various experiments were performed.

Molisch'sTest

A solution of α-naphthol in ethanol was then combined with the mucilage. Subsequently, a coating of concentrated sulfuric acid (H_2SO_4) was intentionally formed at the bottom of the test tube by carefully adding it along the edges without mixing. Carbohydrates were present if an interface ring of violet or purple color was formed.

Ferric Chloride Test

A small amount of ferric chloride ($FeCl_3$) solution was added to the sample after it was dissolved in water. The presence of phenolic compounds was determined by observing any changes in the color of the solution.

Ninhydrin Test

One milliliter of a 0.1% ninhydrin solution (1,2,3-indanetrione monohydrate) was added to the mucilage solution in a test tube. While stirring, the ingredients were slowly brought to a boil in a water bath. When proteins or amino acids were no longer bound to the sample, a blue or purple color developed.

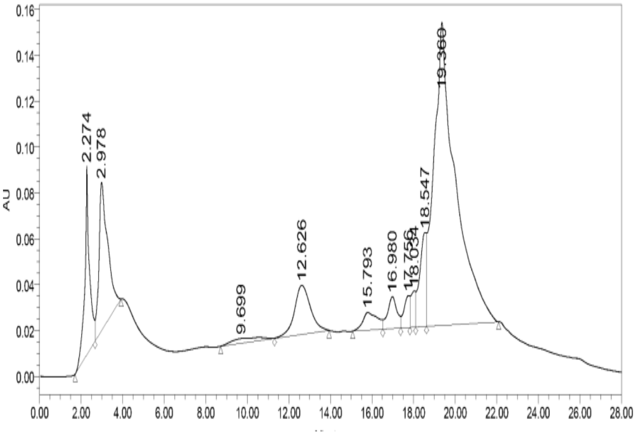
Wagner's Test

To treat the mucilage solution, Wagner's reagent was mixed with 2 g of iodine and 6 g of potassium iodide in 100 mL of water. We noted any shift in hue as a promising sign.

Keller-Killiani Test

In a test tube, the mucilage solution was combined with approximately 3 mL of glacial acetic acid and a little quantity of a 0.1% ferric chloride ($FeCl_3$) solution. Subsequently, 1 mL of sulfuric acid solution was added dropwise. A favorable outcome was recorded when there were changes in the color.

Ruthenium Red Test



Ammoniated ruthenium oxychloride, a ruthenium red dye, was added to the mucilage solution to stain the mucilage. No change in hue was observed as a result.

Salkowski Test

Chloroform and concentrated sulfuric acid were added to the mucilage solution. A reddish-blue hue was used to identify the chloroform layer, and green fluorescence was used to identify the acid layer.

Shinoda Test

The mucilage solution was supplemented with four small pieces of magnesium ribbon, and a few drops of strong hydrochloric acid (HCl) were added. Flavonoids were identified by their pink/red coloration.

Fehling's Test

To prepare Fehling's solution, we divided Fehling's A (a solution of copper(II) sulfate) and Fehling's B (a solution of sodium potassium tartrate) in half. All reagents were added to the boiling mucilage solution. The formation of a brick-red precipitate indicated the presence of reducing sugars or aldehydes.

Iodine Test

The mucilage solution was then treated with potassium iodide solution. Any color change indicated the presence of starch.

Silver Nitrate Test

A small amount of silver nitrate solution was added to the sample after it was dissolved in water. The presence of chloride ions was confirmed by the formation of a white precipitate.

Barium Chloride Test

A barium chloride solution was added to the mucilage. The formation of a white precipitate indicated the presence of sulfate ions.

The leaves and stems of *E. umbellata* were analyzed for their total phenolic, catechin, and flavonoid contents. The leaves contained 2.72% phenolics, while the stems contained a similar amount (2.54 %). Both plant parts also exhibited comparable levels of total catechins and flavonoids, with catechins ranging from 0.49% to 0.53% and flavonoids between 0.35% and 0.48%, as summarized in Table 6.1 and illustrated in Figure 6.1.

Table. Contents of polyphenols, catechins, and flavonoids in *Elaegnus umbellate*

Chemical (% DW)	Plant part	
	Leaf	Stem
Polyphenols	2.71	2.51
Catechines	0.51	0.50
Flavonols	0.49	0.38

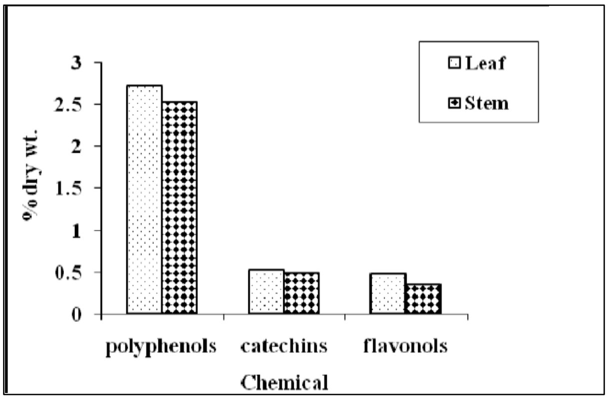


Fig. The flavonoids, catechines, and polyphenols found in Elaegnus umbellata

The use of high-performance liquid chromatography (HPLC) for the identification and quantification of catechins

We used reverse-phase high-performance liquid chromatography (RP-HPLC) to examine catechins, including EGC, CAT, EGCG, EC, GCG, CG, and ECG. The phenolic components of E. *umbellata* extracts (roots, leaves, and stems) were quantified using a gradient elution-based RP-HPLC technique. Catechins were identified by comparing their retention times with those of reference standards run under the same conditions. To quantify these compounds, we used standard calibration curves created for each catechin.

We also used high-performance liquid chromatography to examine the catechin profile of *E. umbellata* stems and leaves. Catechin was most abundant in the leaves (0.12% concentration), and all catechin concentrations were higher in the leaves than in the stems. Alternatively, the stems only contained gallic acid, catechin, and epigallocatechin, and the remaining catechins were either not present or were too small for the device to identify.

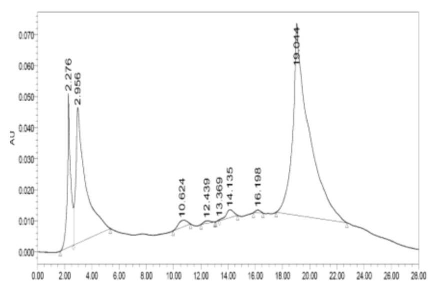
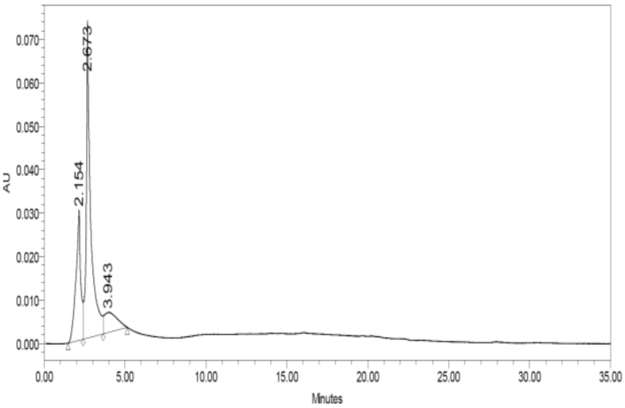


Fig. Catechins in *E. umbellata* were profiled using high-



performance liquid chromatography. A stem and B leaves. In order from 1 to 7, we have gallic acid, ECG, CAT, EGCG, GCG, ECG, and CG.

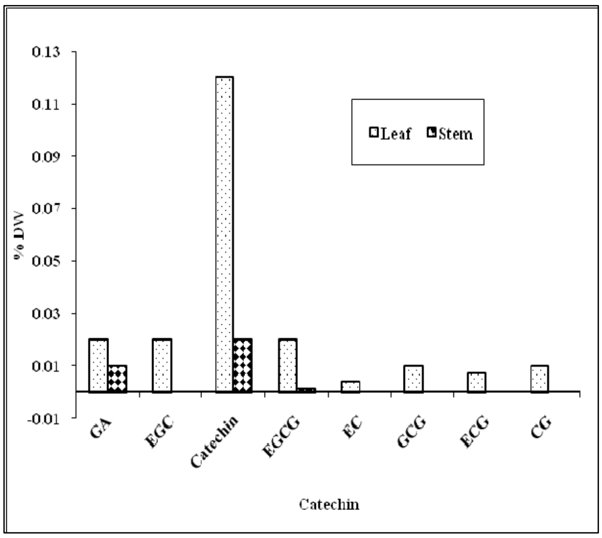


Fig. analyses of *E. umbellata* for catechin concentrations using high-performance liquid chromatography. Gallic acid (GA), epigallocatechin (EGC), gallocatechin gallate (GCG), epicatechin (EC), epicatechin gallate (ECG), and catechin gallate (CG) are the acronyms used for gallic acid-related compounds.

Table: Distinct E. umballeta components' Catechine profiles, as determined by high-performance liquid chromatography

Catechine (% DW)	Plant part		Total content
	Leaf	Stem	
GA	0.021	0.010	**0.031**
EGC	0.022	0	**0.022**
Catechine	0.120	0.020	**0.140**
EGCG	0.022	0.001	**0.023**
EC	0.003	0	**0.003**
GCG	0.011	0	**0.011**
ECG	0.008	0	**0.008**
CG	0.010	0	**0.010**

High Performance Liquid Chromatography for the detection and quantification of flavonoids

Fig. Concentrations of catechins in different parts of *E. umbellata*, as determined by HPLC. Abbreviations: GA, gallic acid; EGC, epigallocatechin; EGCG, epigallocatechin gallate; GCG, gallocatechin gallate; EC, epicatechin; ECG, epicatechin gallate; CG, catechin gallate.

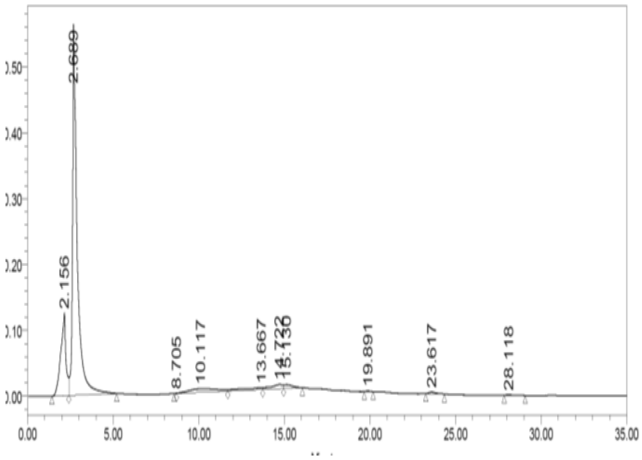


Fig. HPLC profile of flavonoids in different parts of *E. umbellata*. a: leaf, b: stem1: rutin

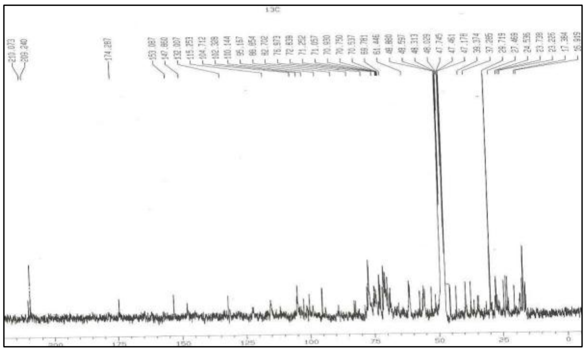
Table. Flavonoids of various parts of *E. umbellata* by HPLC

S.No	*Elaegnus umbellata* (%dry wt.)	Leaf	Stem
1	Chlorogenic acid	Nd	Nd
2	Rutin	0.041	Nd
3	Quercitn	Nd	Nd
4	Myrecetin	Nd	Nd
5	Kaempferol	0.026	Nd
6	Quercitn-3-O- galactoside	Nd	Nd
7	Iso-rhamnetin	Nd	Nd

Isolation and purification of major phytochemicals from *E. umbellata*:

The crude butanolic fraction of the *E. umbellata* extract was further purified using a Diaion HP-20 column, as described in the Materials and Methods section. This fraction was produced using solvent extraction based on the polarity. Column chromatography on silica gel (60-120 mesh) was used to further separate the 70% acetone fraction recovered from this method. Two fractions, eluted with solvent systems of 70:30 (MeOH:$CHCl_t$) and 90:10 (MeOH:$CHCl_t$), respectively, were identified on thin-layer chromatography (TLC) as single spots, indicating their relative purity. The yields of the two isolated compounds were 52 mg and 47 mg.

The purified compounds were structurally characterized



using LC-MS/MS and NMR spectroscopy (^{1}H and ^{13}C NMR). The compound from the 70:30 (MeOH:$CHCl_3$) fraction appeared as a light-brown amorphous powder. LC-MS analysis suggested a molecular formula of $C_{52}H_{38}O_{35}$ based on the observed $[M+H]^+$ peak at m/z 1241. The ^{1}H-NMR and ^{13}C-NMR spectra were complex, and the detailed data are presented in Figures 6.5–6.7 and in Table 6.4. Analysis of the combined spectral information indicated that the compound consists of a triterpenoidal moiety conjugated with two glucuronic acid units (Figure 6.8). This proposed framework provides a basic structural outline, while further detailed characterization is currently ongoing.

Evaluation of the structure using LC-MS and NMR tech

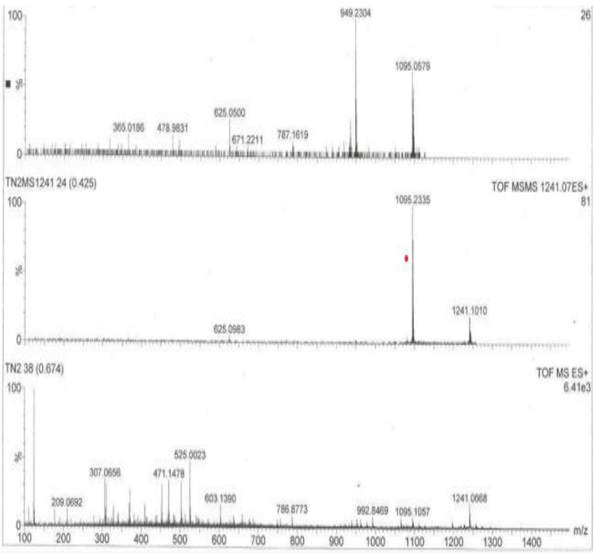


Fig. LC-MS of purified compound isolated from *E. umbellate*

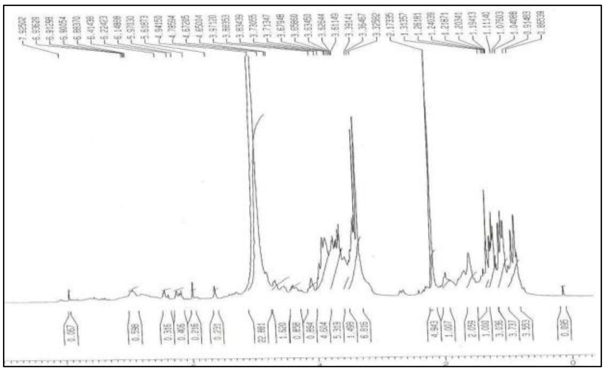


Fig. ^{1}H-NMR spectra of purified compound from *E. umbellata*

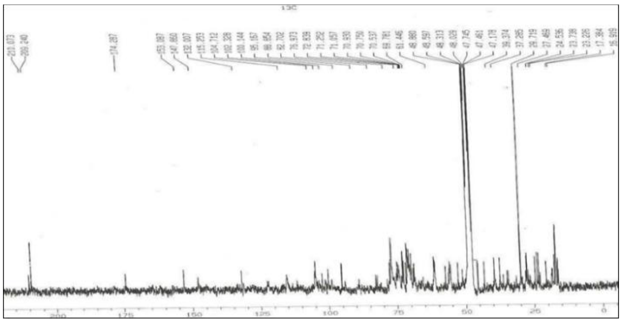


Fig. ^{13}C-NMR spectra of purified compound from *Elaegnus umbellate*

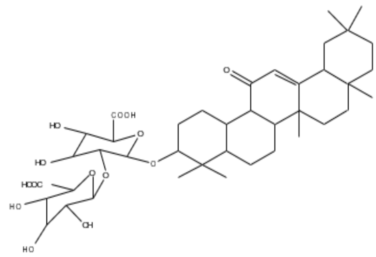


Fig. Proposed structure of the purified compound isolated from *Elaeagnus umbellata*.

Another compound isolated from the 90:10 (MeOH:$CHCl_3$) fraction of *E. umbellata* exhibited similar spectral characteristics, indicating a comparable skeleton. The detailed interpretation and structural characterization of this compound are currently in progress.

Conclusion

This comprehensive investigation systematically elucidated the phytochemical composition of different parts of *Elaeagnus umbellata* using advanced spectroscopic and chromatographic techniques, alongside an assessment of their bioactive properties. The combined analytical strategies facilitated the detection and characterization of a diverse array of secondary metabolites, such as phenolic compounds, flavonoids, terpenoids, fatty acids, and related phytoconstituents, with clear variations observed across the individual plant organs.

Detailed chromatographic profiling demonstrated distinct organ-specific phytochemical patterns, and spectroscopic analyses provided critical structural information for the identified bioactive molecules. These findings underscore the significant chemical heterogeneity of *E. umbellata* and validate the use of integrated multi-analytical methodologies for comprehensive phytochemical evaluation.

Biological activity assessments revealed that extracts derived from various plant parts possess noteworthy bioactive properties closely associated with their phytochemical richness. The demonstrated antioxidant and related biological activities indicate that *E. umbellata* is a valuable natural reservoir of bioactive compounds with promising prospects in pharmaceutical, nutraceutical, and functional food applications.

In conclusion, the findings of this study provide robust scientific support for the medicinal and functional significance of *Elaeagnus umbellata*. Moreover, this study expands the existing knowledge of phytochemical diversity and establishes a solid platform for future research aimed at isolating specific active constituents, elucidating their mechanisms of action, and exploring their potential therapeutic utility.

Submission Declaration:
This manuscript has not been published previously and is not under consideration for publication elsewhere. The authors confirm that the work is original and have read and approved the final manuscript for submission.

Conflict Of Interest:
The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study.

Declaration Of Competing Interest:
The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Ethics Statement:
This review paper, involves no experimental research, human subjects, or animal studies that need ethical approval; instead, it is based entirely on publicly available literature. For academic openness and integrity, all acknowledged sources were appropriately referenced. I have done all in my power to provide an objective, accurate, and thorough literature review free from any conflicts of interest that could affect how the data are interpreted. The development of this study did not involve any instances of scientific misconduct, data manipulation, or plagiarism. Let me know if you need refinement

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