Phenolic, Flavonoid Contents, Antioxidant, and Antibacterial Activity of Selected Eucalyptus Species: Review

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Abstract

Many medicinal plants and their essential oils are used to treat or prevent diseases without the risk of mutagenicity, carcinogenicity, or teratogenicity. Eucalyptus essential oils are commonly used as preservatives, flavoring agents, and various consumer goods. Eucalyptus leaves are being widely studied due to their volatile essential oils, including 1,8-cineole, p-cymene, α- and β-pinene, limonene, citronellal, citral, eudesmol, terpinen-4-ol, terpineol, α-phellanderene, and 9β-sitosterol. Among these compounds, 1,8-cineole is the most abundant. Eucalyptus leaves are rich in flavonoids and phenolic compounds and are thus a great source of antioxidants. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays were used to measure antioxidant capacity. The antioxidant activity of Eucalyptus essential oil is stronger than that of standard antibiotics such as erythromycin, cefixime, and gentamicin due to the presence of significant terpenoids in addition to phenolic chemicals and flavonoids. A lower IC50 indicates greater antioxidant potential. Phenolic compounds and flavonoids are often associated with beneficial health outcomes, including anti-inflammatory and antioxidant effects, antiparkinsonian properties, reduced risk of cardiovascular diseases, prevention of age-related neurodegenerative diseases, and prevention of Alzheimer’s disease. Flavonoids also play essential roles in plants, including protecting against reduction, fertility, reproduction, and infection. Due to their anti-inflammatory, antioxidative, and immunomodulatory properties, flavonoids are crucial for pharmacological, medical, and nutraceutical applications.

Keywords: Eucalyptus; medicinal plants; antioxidant activity; reactive oxygen species (ROS); 1,8-cineole.

INTRODUCTION

Plants and their essential oils are often used as medicines to cure or prevent diseases. Medicinal plants are preferred as medication due to their low risk of causing genetic mutagenicity, carcinogenicity, or teratogenicity (Olawore NO & Ololade ZS, 2017). These plants are a significant source of therapeutic drug molecules because they contain secondary metabolites that can serve as potential drugs. Aromatic plants are commonly used in traditional medicine (Sharma et al., 2021). In several countries worldwide, especially in rural areas, aromatic plants are used in primary healthcare (Vecchio et al., 2016).

Eucalyptus is a type of flowering tree belonging to the Myrtaceae family, with more than 900 species and subspecies (Vecchio et al., 2016). These trees are known for their fast growth and ability to tolerate harsh environments such as wildfires, droughts, and acidic soils. The wood industry uses various species of Eucalyptus to produce pulp, timber, and paper. Additionally, the bark, leaves, and branches of these trees are utilized as byproducts to generate energy (Álvarez et al., 2021).

There are several species of Eucalyptus, such as E. camaldulensis, E. saligna, E. citriodora, Eucalyptus globule, Eucalyptus maculate, E. staigeriana, E. radiate, E. leucophleba, E. cinerea, E. tereticornis, and E. lecoxylon (Almas et al., 2021; Surbhi et al., 2023). One of the most popular species is Eucalyptus globulus Labill, or blue gum tree, which is an evergreen tree widely grown in Australia, South Africa, India, Southern Europe, and Ethiopia (Amabye, 2016; Dezsi et al., 2015; Shah et al., 2012). E. globulus is known for its many medical benefits, such as treating wounds, tuberculosis (TB), sore throats, arthritis, asthma, boils, colds, coughs, diabetes, diarrhea, dyspepsia, and bronchitis (Mworia et al., 2019). These plants produce secondary metabolites such as terpenoids, alkaloids, flavonoids, phenols, and saponins as a defense mechanism (Sharma et al., 2021). Eucalyptus trees have been extensively researched due to their essential oils, which have several health benefits, including anti-inflammatory, antimarialar, antibacterial, analgesic, antidiabetic, antiviral, anticancer, antiseptic, antioxidant, and antifungal properties (Panigrahi et al., 2021; Surbhi et al., 2023). Eucalyptus leaves are a great source of antioxidants, particularly...
flavonoids, which offer protection against oxidative stress and free radical damage (Álvarez et al., 2021).

**Figure 1.** Chemical structures of the main components of *Eucalyptus* fruits, branch tips, and leaf essential oils.

*Eucalyptus* trees are a rich source of essential oils that can be extracted from their fruits, branch tips, and leaves. These oils contain a variety of beneficial phytochemicals, such as 1,8-cineole, p-cymene, α- and β-pinene, limonene, citronellal, citral, eudesmol, terpinen-4-ol, terpineol, α-phellanderene, and 9β-sitosterol. Different methods, such as hydrodistillation, supercritical fluid extraction, and solvent extraction, can be used to extract these chemicals (Surbhi et al., 2023). Among these compounds, 1,8-cineole is the most abundant in all *Eucalyptus* species (Sebei et al., 2015). To treat illnesses, *Eucalyptus* extracts are used both alone and in conjunction with certain other plants. *Eucalyptus* essential oils are safe and nontoxic. They are used as preservatives, flavoring agents, and in a wide variety of consumer goods (Olawore NO & Ololade ZS, 2017). Researchers have recently shown increased interest in essential oils due to their therapeutic properties, which make them useful for preservation and drug formulations. They are also used as flavoring substances in the food, nutraceutical, and pharmaceutical industries. *Eucalyptus* essential oils have been used in medicine to treat a wide range of human and animal ailments. They contain phenolic and flavonoid compounds, which have antioxidant and antibacterial properties (Ololade et al., 2021). This review aims to provide scientific information on the phenolic and flavonoid contents and the antioxidant and antibacterial activities of selected *Eucalyptus* species.

**Eucalyptus** species

*Eucalyptus* is a nonnative and popular tree species classified in the Myrtaceae family. It is a genus of more than 900 species (Vecchio et al., 2016) and has become the most planted genus of tree species in the world (Birara Dessie et al., 2019).

According to the research conducted by Alemu (2016), Brazil possesses the most extensive expansion of *Eucalyptus* plantations globally, with India and China following suit in terms of coverage. In Africa, South Africa proudly claimed the initial position in establishing such plantations, securing a notable fifth place in the worldwide ranking. On the other hand, Ethiopia stands adjacent to South Africa and holds the seventh position on a global scale.

*Eucalyptus* covers 90% of the total planted forest area in the central highlands of Ethiopia. These species of trees are the first nonnative tree species formally introduced to Ethiopia by Emperor Minilik II from Australia in the 1890s (Tesfaye et al., 2020).

Approximately 70 species of *Eucalyptus* are accessible in Ethiopia, most of which are broadly spread in numerous locales of the nation, mostly within the central highlands, where population density is relatively high. The most common and widespread *Eucalyptus* species include *E. globulus*, *E. camaldulensis*, *E. saligna*, *E. grandis*, *E. citriodora*, *E. regnans*, and *E. tereticornis* (Alemu, 2016; Tsegaye Bekele, 2015).

Many products and services, including fuel wood, charcoal, building materials, home and agricultural tools, pulp, *Eucalyptus* oil, lumber, and poles, are known to be provided by *Eucalyptus* trees. The tree proved to be highly helpful for the production of paper because of its rapid growth tendency. Additionally, the tree has traditional and modern medical uses. For example, in Ethiopia, steam from water-boiled *E. globulus* tree leaves is known to cure the flu. Australia, Brazil, Chile, Portugal, South Africa, Spain, and Swaziland are among the nations recognized for their production of *Eucalyptus* oil (Alemu, 2016).

**Benefits of Eucalyptus Species for Health**

The medicinal properties of *Eucalyptus* species have been reported in various studies. Olawore NO & Ololade ZS (2017) studied the therapeutic properties of essential oils derived from the seeds of *E. camaldulensis var. nancy* and *E. camaldulensis var. petford*, including antioxidant, anti-inflammatory, antinoceptive, and antimicrobial activities. *E. citriodora* fruit essential oil has natural antioxidant and antimicrobial activity (Ololade et al., 2021).

The *E. globulus* Labill species is one of the most well-known. Its leaves have been used as traditional medicine to cure a variety of illnesses, including diabetes, influenza, fungal infections, and pulmonary tuberculosis. Leaf extracts of *E. globulus* have also been used as food additives due to their antioxidant activity (Dezsi et al., 2015).
The *Eucalyptus* plant is traditionally used for its antiseptic and antibacterial effects for treating respiratory tract infections such as colds, flu, sore throats, and chest infections such as bronchitis and pneumonia. This effect on bacteria may be attributed to the dominant presence of eucalyptol (1,8-cineole), which has demonstrated strong antimicrobial activity against many pathogens (Vecchio et al., 2016).

*Eucalyptus* contains phenolic compounds that possess anti-inflammatory, antimicrobial, and antioxidant properties. Moreover, it has neuroprotective effects and could be effective in preventing or delaying the onset of Alzheimer’s disease. Due to their diverse bioactivities, *Eucalyptus* phenolic compounds have potential uses in various industries, such as food, cosmetics, and pharmaceuticals (Park et al., 2023).

TRADITIONAL USE OF EUCALYPTUS IN ETHIOPIA

Ethiopia has a wide range of aromatic and medicinal plant species from which essential oils can be produced for a variety of uses. The most prevalent of these is the *Eucalyptus* plant (Shiferaw et al., 2019). The reforestation of *eucalypt* plants is a popular practice in many countries, particularly in developing countries such as Ethiopia. Ethiopian farmers cultivate many *Eucalyptus* trees on tiny plots of land, and they maintain them well to provide a range of goods, such as small branches and leaves for fuel wood, as well as poles and posts for building houses and other agricultural purposes. *Eucalyptus* trees provide fuel and building materials for a large number of people in Ethiopia (Zerga et al., 2021).

*Eucalyptus* extracts have been used traditionally to cure a wide range of ailments in various nations, including Ethiopia. For example, hot water extracts of both fresh and dry *E. globulus* leaves are used as analgesic, anti-inflammatory, and antipyretic treatments for sinus infections, the common cold, and the flu. This is because *E. globulus* is a unique natural product with antiseptic properties that also helps to clear bronchial tubes and nasal passages, which makes breathing easier. Additionally, they inhale the vapor released when boiling *Eucalyptus* leaves in water to relieve cold, which is particularly common. The essential oils of *Eucalyptus* species have significant biological activities, including diaphoresis, disinfecting, antimalarial, antiseptic, analgesic, anti-inflammatory, antibacterial, and antioxidant properties. *Eucalyptus* oil is one of the main ingredients used to make laundry detergents and toiletries with good deodorizing and antiseptic properties (Shiferaw et al., 2019).

*E. globulus* is used to treat respiratory tract infections; any illness in the respiratory tract that may affect the lungs, bronchi, or nasal passages is referred to as a respiratory tract infection. Because antimicrobial resistance is developing quickly and the effectiveness of currently available drugs is decreasing, these illnesses are becoming major causes of death and morbidity (Teka & Maryo, 2023).

PHENOLIC CONTENTS

A common structural feature of phenolic compounds is an aromatic ring with one or more hydroxyl substituents. These compounds can be classified into many classes, with flavonoids, phenolic acids, tannins, stilbenes, and lignans being the main groups (Álvarez et al., 2021; Zhang et al., 2022). Various phenolic chemicals (Figure 2), including queretin, rutin, ellagic acid, hydroquinone, protocatechuic acid, naringenin, chlorogenic acid, hesperetin, pyrogallol, resorcinol, and catechin, have been extracted from different Eucalyptus extracts (Gullón et al., 2020).

![Figure 2. Different phenolic compounds isolated from different Eucalyptus extracts.](image-url)
Phenolic compounds are a large class of plant secondary metabolites that exist in various plant organs, such as vegetables, fruits, spices, grains, legumes, and nuts, and play important roles in diverse physiological processes, such as plant quality, coloring, flavor, and stress resistance (Zhang et al., 2022). Because of their beneficial effects on human health, polyphenols are very valuable phytochemicals in the food and pharmaceutical industries (Ololade et al., 2021).

Plants with high phenolic content are highly nutritious and beneficial to human health. In addition, they display defense mechanisms against reactive oxygen species (ROS). Natural phenolic compounds play a critical role in both illness prevention and treatment. Phenolic compounds have chemopreventive effects because of a range of therapeutic activities that also regulate carcinogen metabolism and ontogenesis, the inhibition of DNA binding and cell adhesion, migration, proliferation, and the blockade of signaling pathways (Ololade NO & Ololade ZS, 2017).

Phenolic compounds are frequently linked to a number of advantageous health outcomes, including anti-inflammatory and antioxidant effects, anticancer effects, and decreased risk of cardiovascular diseases (Dezsi et al., 2015). Phenolic chemicals exert protective effects against oxidative stress and inflammation caused by airborne particulate matter and play a significant role in protecting plants from ultraviolet radiation and disease attacks (Rahman et al., 2022).

Ololade NO & Ololade ZS (2017) reported that the total phenolic content (TPC) values of E. camaldulensis var. nancy and E. camaldulensis var. petford essential oils were 156.25 ± 0.00 and 167.93 ± 0.00 µg GAE/mg, respectively, which were relatively the same as those of E. citriodera essential oil extracts (175.84±0.00 µg GAE/mg) reported by Ololade et al. (2021).

As shown in Table 1, the total phenolic content of E. saligna leaves extracted with pressurized 95% ethanol was greater than that of the other Eucalyptus species, which was 618.57 mg GAE/g plant material. Fischer et al. (2020) reported that the total phenolic content of an 80% methanol extract of E. globulus was lower than that of other Eucalyptus species (50.00 mg GAE/g plant material) (Table 1).

Table 1. TPC of different Eucalyptus species extracted by different solvents and methods.

<table>
<thead>
<tr>
<th>Eucalyptus species</th>
<th>Part of plant</th>
<th>Condition</th>
<th>Phenolic content (mg GAE/g plant material)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. globulus Labill.</td>
<td>leaves</td>
<td>70% Ethanol</td>
<td>235.87 ± 4.38</td>
<td>(Dezsi et al., 2015)</td>
</tr>
<tr>
<td>E. camaldulensis var. nancy</td>
<td>seed</td>
<td>Essential oil</td>
<td>156.25 ± 0.00</td>
<td>(Ololade NO &amp; Ololade ZS, 2017)</td>
</tr>
<tr>
<td>E. camaldulensis var. petford</td>
<td>seed</td>
<td>Essential oil</td>
<td>167.93 ± 0.00</td>
<td>(Ololade ZS, 2017)</td>
</tr>
<tr>
<td>E. globulus</td>
<td>leaves</td>
<td>80% methanol</td>
<td>50.00 ± 0.00</td>
<td>(Sharma et al., 2021)</td>
</tr>
<tr>
<td>E. citriodera</td>
<td>Fruit</td>
<td>Essential oil</td>
<td>175.84±0.00</td>
<td>(Ololade et al., 2021)</td>
</tr>
<tr>
<td>E. saligna</td>
<td>leaves</td>
<td>pressurized 95% ethanol</td>
<td>618.57±0.00</td>
<td>(Fischer et al., 2020)</td>
</tr>
</tbody>
</table>

As shown in Table 2, Park et al. (2023) reported that the highest TPC values were reported for different concentrations of ethanol in E. globulus leaf extracts, with 422.0, 492.7, 497.7, and 448.5 mg GAE/g extract in 10, 30, 50, and 70% ethanol extracts, respectively. The next highest TPC was 384.5 mg GAE/g extract in the 90% ethanol extract. However, the TPC of the 100% ethanol extract was 273.2 mg GAE/g extract. The lowest TPC value, 126.7 ± 8.5 mg GAE/g extract, was estimated for the 0% ethanol extract. According to the extraction conditions, the E. globulus leaf 50% ethanol extract had the highest TPC relative to the other extracts, as shown in Table 2.

Table 2. TPC of different ethanol concentrations of E. globulus leaf extract.

<table>
<thead>
<tr>
<th>Extract Conditions</th>
<th>0% ethanol (mg GAE/g Extract)</th>
<th>10% ethanol</th>
<th>30% ethanol</th>
<th>50% ethanol</th>
<th>70% ethanol</th>
<th>90% ethanol</th>
<th>100% ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPC</td>
<td>126.7 ± 8.5</td>
<td>422.0 ± 18.4</td>
<td>492.7 ± 13.2</td>
<td>497.7 ± 15.5</td>
<td>448.5 ± 20.1</td>
<td>384.5 ± 10.4</td>
<td>273.2 ± 17.5</td>
</tr>
</tbody>
</table>

**FLAVONOIDS CONTENTS**

Flavonoids are a class of secondary metabolites. Their main structural element is a benzopyrone ring that has variously positioned phenolic or polyphenolic groups. Based on the degree of unsaturation and oxidation of the C ring as well as the carbon of the C ring to which the B ring is attached, flavonoids are divided into subgroups, as shown in Figure 3. Flavonoids in which the B ring is linked to position 3 of the C ring are called isoflavones. Those in which the B ring is linked at position 4 are called neoflavonoids, whereas those in which the B ring is linked at position 2 can be further separated into multiple subgroups according to the structural characteristics of the C ring. These subgroups include anthocyanins, chalcones, flavones, flavonols, flavanones,
Flavonoids are found in many plants, fruits, vegetables, and leaves. These compounds have potential biological and medicinal uses. It offers several health benefits, including the potential to prevent age-related neurodegenerative diseases and Alzheimer’s disease. They also possess antioxidant, antiviral, and anticancer properties (Ullah et al., 2020; Zulkefli et al., 2023).

Flavonoids play many important roles in plants, including protecting against reduction, fertility and reproduction and protecting against infection and the rhizosphere. Flavonoids function as signaling molecules, detoxifying agents, and phytoalexins and promote seed germination, temperature acclimatization, and drought resistance. Flavonoids are also used to reduce reactive oxygen species in plant tissue, and they play other roles in the fragrance, color, and taste of fruits, flowers, or seeds. This fragrance and color attract pollinators that aid in the pollination and dispersal of seeds (Roy et al., 2022). Flavonoids in humans and plants are relatively important as signaling molecules and reducing agents (Brunetti et al., 2013). Flavonoids facilitate the colonization of tomato roots and the germination of Rhizobatus irregularis spores (Lidoy et al., 2023).

Flavonoids have shown the ability to control or prevent inflammation. Due to their anti-inflammatory, antioxidative, and immunomodulatory qualities, flavonoids are essential for pharmacological, medical, and nutraceutical applications (Rakha et al., 2022). The number and position of free OH groups on the flavonoid skeleton appear to determine the antioxidant potential of naturally occurring polyphenolic compounds. The B-ring substitution pattern plays a key role in the ability of flavonols to scavenge free radicals. Flavonoids with multiple hydroxyl groups are more potent antioxidants than those with only one hydroxyl group (Brewer, 2011).

As shown in Table 3, the flavonoid content of the E. saligna leaves extracted with pressurized 95% ethanol was 160.27 mg QE/g of extract. Sharma et al. (2021) reported that the flavonoid content of an 80% methanol extract of E. globulus leaves was lower, at 23 mg RE/g plant material.
According to Park et al. (2023) (Table 4), the total flavonoid content (TFC) of *E. globulus* leaf extracts increased as the concentration of ethanol increased from 0% to 100%. Under 0 and 50% ethanol extraction conditions, the TFC values ranged from 32.5 to 41.2 mg QE/g extract, with similar values. The 70% ethanol extract had a value of 66.5 mg QE/g extract, which is approximately 1.5-fold greater than that of the 0, 10, 30, and 50% ethanol extracts. The highest TFC values were 156.5 and 169.3 mg QE/g extract in 90% and 100% ethanol, respectively. The best extraction conditions for TFC were 100% ethanol.

### Table 3. Flavonoid content of different *Eucalyptus* species extracted by different solvents and methods.

<table>
<thead>
<tr>
<th><em>Eucalyptus</em> species</th>
<th>Part of plant</th>
<th>Condition</th>
<th>Flavonoid content</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. globulus</em></td>
<td>leaf</td>
<td>80% methanol</td>
<td>23 ± 0.00 a</td>
<td>(Sharma et al., 2021)</td>
</tr>
<tr>
<td><em>E. globulus</em> Labill.</td>
<td>leaves</td>
<td>70% Ethanol extract</td>
<td>35.76 ± 0.95 a</td>
<td>(Dezsi et al., 2015)</td>
</tr>
<tr>
<td><em>E. saligna</em></td>
<td>leaves</td>
<td>pressurized 95% ethanol</td>
<td>160.27 b</td>
<td>(Fischer et al., 2020)</td>
</tr>
</tbody>
</table>

mg RE/g plant material a, mg QE/g extract b, RE= Rutin equivalent, QE= Quercetin equivalent.

### Table 4. TFC of different ethanol concentrations of the *E. globulus* leaf extract.

<table>
<thead>
<tr>
<th>Extract Conditions</th>
<th>0% ethanol</th>
<th>10% ethanol</th>
<th>30% ethanol</th>
<th>50% ethanol</th>
<th>70% ethanol</th>
<th>90% ethanol</th>
<th>100% ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>TFC (mg QE/g Extract)</td>
<td>32.5 ± 5.1</td>
<td>32.7 ± 4.8</td>
<td>40.4 ± 6.7</td>
<td>41.2 ± 7.5</td>
<td>66.5 ± 6.5</td>
<td>156.5 ± 10.4</td>
<td>169.3 ± 12.2</td>
</tr>
</tbody>
</table>

### ANTIOXIDANT ACTIVITIES

Antioxidants are molecules that can scavenge ROS or free radicals, protecting cells from damage and death. In biological systems, free radicals are crucial for the synthesis of some biomolecules, energy production, phagocytosis, and cell development. An imbalance between free radical generation and unfavorable antioxidant defenses leads to oxidative stress, resulting in DNA or tissue damage (Vecchio et al., 2016).

The 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays are commonly used to measure antioxidant capacity. The main differences between these reactions are their reaction mechanisms and types of radicals (Park et al., 2023).

Olawore NO & Ololade ZS (2017) reported that the two seed essential oils of *E. camaldulensis* var. *Nancy* and *Petersford* had half maximal inhibitory concentration (IC₅₀) values of 3.50 μg/ml, indicating that these compounds are more effective as antioxidants and free radical scavengers than ascorbic acid, which had an IC₅₀ value of 9.00 μg/ml. The essential oils from the seeds of the two *eucalyptus* species under investigation showed a twofold greater reduction in antioxidant activity (5.0 μg/ml) than ascorbic acid (11.0 μg/ml). This is because, unlike ascorbic acid, which contains only one molecule, seed essential oils include several significant terpinoids in addition to phenolic compounds. According to Ololade et al. (2021), the antioxidant IC₅₀ value of the fruit essential oil *E. citriodera* is 2.00 μg/ml, and ascorbic acid has an IC₅₀ value of 11.00 μg/ml. This shows that the antioxidant activity of the essential oil is more potent than that of ascorbic acid.

The lower the IC₅₀ value is, the greater the antioxidant potential (Ololade et al., 2021). The presence of terpenoids in the essential oil of *Eucalyptus* species plays an active role in the antioxidant potential and its greater reduction antioxidant effect (Olawore NO & Ololade ZS, 2017; Ololade et al., 2021). The presence of terpenoids in the oil contributed to its greater antioxidant effect since these compounds are known to chelate metal ions (Ololade et al., 2021).

### Table 5. Antioxidant activity of different *Eucalyptus* species.

<table>
<thead>
<tr>
<th><em>Eucalyptus</em> species</th>
<th>Condition</th>
<th>IC₅₀ value (μg/ml)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DPPH assay</td>
<td>FRAP</td>
</tr>
<tr>
<td><em>E. camaldulensis</em> var. <em>nancy</em></td>
<td>Seed Essential oil</td>
<td>3.5</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>Ascorbic acid</td>
<td>9.0</td>
<td>11</td>
</tr>
<tr>
<td><em>E. camaldulensis</em> var. <em>petford</em></td>
<td>Seed Essential oil</td>
<td>3.5</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>Ascorbic acid</td>
<td>9.0</td>
<td>11</td>
</tr>
<tr>
<td><em>E. citriodera</em></td>
<td>Fruit Essential oil</td>
<td>3.00</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>Ascorbic acid</td>
<td>9.00</td>
<td>11.00</td>
</tr>
</tbody>
</table>
Park et al. (2023) reported the antioxidant ability of E. globulus leaves using DPPH and ABTS radical assays. As shown in Table 6, the highest radical scavenging activity against DPPH radicals was observed for 30% ethanol, with a 188.2 µg/ml scavenging capacity (SC₅₀), followed by 10%, 50%, and 70% ethanol, with SC₅₀ values of 357.9, 505.3, and 509.3 µg/ml, respectively. Among the extraction conditions, 0% ethanol, 90% ethanol, and 100% ethanol had SC₅₀ values of 5841.7, 1008.4, and 1304.7 µg/ml, respectively, indicating little antioxidant ability. However, compared to the DPPH assay, the ABTS radical scavenging activity assay often produced better results. The results indicated that the E. globulus leaf extracts with 30% and 50% ethanol had the greatest antioxidant effects, with SC₅₀ values of 14.2 and 18.0 µg/ml, respectively. The results showed that the 100% and 0% ethanol extracts had the lowest antioxidant effectiveness, with corresponding SC₅₀ values of 34.9 and 171.3 µg/ml, respectively. These findings verified that extracting E. globulus leaves with the right ratio of water to ethanol produced a more potent antioxidant effect than extracting the leaves with either water or ethanol alone. The findings showed that to optimize the antioxidant potential, a mixture of extraction solvents was necessary.

<table>
<thead>
<tr>
<th>Table 6. DPPH and ABTS radical scavenging activities of different ethanol solutions from E. globulus leaf extracts.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Extract Conditions</strong></td>
</tr>
<tr>
<td>0% ethanol</td>
</tr>
<tr>
<td>10% ethanol</td>
</tr>
<tr>
<td>30% ethanol</td>
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<tr>
<td>50% ethanol</td>
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<tr>
<td>70% ethanol</td>
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<tr>
<td>90% ethanol</td>
</tr>
<tr>
<td>100% ethanol</td>
</tr>
</tbody>
</table>

**ANTIOXIDANT MODE AND MECHANISM OF ACTION**

The term "oxidative stress" describes the imbalance between antioxidants and oxidants in the body as a result of excess reactive sulfur species (RSSs), reactive nitrogen species (RNAs), or reactive oxygen species (ROS) that can damage cells (Azat Aziz et al., 2019). Under various pathophysiological circumstances, oxidative stress is caused by ROS and RNS. Oxidative stress conditions modify the cellular components of the body, leading to different disease states. Antioxidants are useful tools for strengthening cellular defenses against oxidative stress. Certain compounds act as in vivo antioxidants by increasing the levels of endogenous antioxidant defenses. The expression of genes encoding enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSHPx) increases the level of endogenous antioxidants (Nimse & Pal, 2015).

All highly reactive forms of oxygen, including free radicals, are collectively referred to as ROS. The ROS categories included hypochlorous acid (HOCl), hypochlorite radical (OCl⁻), peroxynitrite (ONOO⁻), hydrogen peroxide (H₂O₂), per hydroxyl radical (HO₂⁺), hydroxyl radical (OH⁻), superoxide anion radical (O₂⁻), singlet oxygen (¹O₂), nitric oxide radical (NO⁻), and different lipid peroxides. RSS are easily produced from thiols through a reaction with ROS, while RNS are derived from nitric oxide by a reaction with O₂⁻ to form ONOO⁻ (Azat Aziz et al., 2019).

Antioxidants are substances or systems that slow autodestruction by preventing the production of free radicals or by stopping the spread of free radicals through one or more different mechanisms (Brewer, 2011). Antioxidative chemicals have multiple chemical modes of action, including single electron transfer, hydrogen atom transfer, and transition metal chelation (Francenia Santos-Sánchez et al., 2019).

**Figure 4. Reaction mechanism of DPPH with an antioxidant.**

DPPH is a stable radical by nature compared with the ABTS⁺ radical; it is a radical that should be generated by chemical reactions (Equation 1). Generally, the improved method generates the ABTS⁺ radical in only one reaction by reacting ABTS with ammonium persulfate ((NH₄)₂S₂O₅) or potassium persulfate (K₂S₂O₅) prior to the addition of antioxidants (Figure 5) (Sadeer et al., 2020).

(NH₄)₂S₂O₅ + ABTS⁺ + ArOH → ABTS⁺ + ArOH → ABTS⁺ + ArO⁻

ArOH = represent phenolic antioxidant
The FRAP test is a typical SET-based method for measuring the reduction of a complex of ferric ions (Fe\(^{3+}\))-ligand to an intensely blue ferrous complex (Fe\(^{2+}\)) by means of antioxidants in acidic environments (Figure 6). The original FRAP test employed tripyridyltriazine (TPTZ) as the connecting ligand to the iron ion. Alternative ligands, such as ferrozine, were also used to bind iron ions to evaluate the reducing power of ascorbic acid. Potassium ferricyanide has recently emerged as the most commonly utilized ferric reagent in FRAP experiments (Munteanu & Apetrei, 2021).

Vitamin C (ascorbic acid) is a water-soluble free radical scavenger. Vitamin C changes to the ascorbate radical by donating an electron to the lipid radical to terminate the lipid peroxidation chain reaction (Figure 7). One ascorbate molecule and one dehydroascorbate molecule are produced by the quick reaction of the pairs of ascorbate radicals. There is no antioxidant capacity for dehydroascorbate. Thus, the addition of two electrons transforms dehydroascorbate back into ascorbate. It has been suggested that oxidoreductase completes the final step of adding two electrons to dehydroascorbate (Nimse & Pal, 2015).
ANTIBACTERIAL ACTIVITIES

Medicinal plants are important sources of potentially useful structures for the development of novel chemotherapy drugs. Evaluating their antibacterial activity in vitro is the initial step toward achieving this goal (Dezsi et al., 2015).

The antibacterial activities of the two seed essential oils of the E. camaldulensis varieties against the tested bacteria were variable (Figure 8). E. camaldulensis var. nancy had the greatest inhibitory effect on P. aeruginosa (20 mm), S. aureus (20 mm), K. pneumoniae (19 mm), and E. coli (18 mm) but was resistant to S. agalactiae and S. typhimurium, while E. camaldulensis var. petford had inhibitory effects on K. pneumoniae (24 mm), S. aureus (20 mm), S. agalactiae (15 mm), P. aeruginosa (13 mm) and E. coli (10 mm) but was resistant to S. typhimurium. The tested bacteria S. agalactiae and S. aureus were found to be resistant to erythromycin, and E. coli, S. agalactiae, S. aureus, and S. typhimurium were found to be resistant to cefixime antibiotics (Olawore NO & Ololade ZS, 2017).

As shown in Figure 9, the antibacterial activity of the E. globulus leaf extracts against L. monocytogenes (10.1 ± 0.4 mm) and S. aureus (8.1 ± 0.1 mm) was greater than that of the standard antibiotics gentamicin and ciprofloxacin against the bacterial strains of L. monocytogenes and S. aureus (Dezsi et al., 2015).

The antibacterial activities of the essential oil of E. citriodora were determined against five bacteria, namely, E. coli, K. pneumoniae, P. aeruginosa, S. agalactiae, and S. aureus, which were found to have the greatest inhibitory effects on E. coli (18 mm), P. aeruginosa (15.00 mm), S. aureus (15.00 mm), S. agalactiae (10.00 mm) and K. pneumoniae (13.00 mm). The bacteria were found to be sensitive to ofloxacin, and some were resistant to conventional cefuroxime antibiotics, as shown in Figure 10 (Ololade et al., 2021).
CONCLUSION

Medicinal plants are used to cure a variety of diseases and ailments in both humans and animals. These plants are an important source of therapeutic drug molecules that contain secondary metabolites, which are potential sources of drugs. The major bioactive components in medicinal plants are secondary metabolites such as alkaloids, flavonoids, phenols, and saponins, which have several broad biological activities. These include antibacterial, antiseptic, antioxidant, and antifungal properties. Eucalyptus plants have several medical benefits, such as antioxidant, anti-inflamatory, antinociceptive, and antimicrobial activities. They also have the potential to prevent age-related neurodegenerative and Alzheimer’s disease. Additionally, these plants possess antiviral and anticancer properties due to the presence of polyphenols and flavonoids. Research on aromatic and medicinal plants has a bright future due to the application of advanced technologies, interdisciplinary methods, and sustainable practices. This may result in the creation of novel and more effective plant-based medications, as well as new therapies.

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