BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 13, Number 1, April 2024 | Pages: 147-157 | DOI: 10.14421/biomedich.2024.131.147-157 ISSN 2540-9328 (online)

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

Getaneh Worku Moges*, Gizachew Mulugeta Manahelohe, Melese Ababay Asegie
Chemistry Department, College of Natural & Computational Science, University of Gondar, P.O. Box 196, Gondar, Ethiopia.

Corresponding author*
gechchem07@gmail.com

Manuscript received: 01 April, 2024. Revision accepted: 20 May, 2024. Published: 27 May, 2024.

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 IC_{50} indicates greater antioxidant potential. Phenolic compounds and flavonoids are often associated with beneficial health outcomes, including anti-inflammatory and antioxidant effects, anticancer 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. citriodera, Eucalyptus globule, Eucalyptus maculate, E. staigeriana, E. radiate, E. laxophleba, E. cinerea, E. tereticornis,* and *E. leocoxylon* (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, antimalarial, 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).

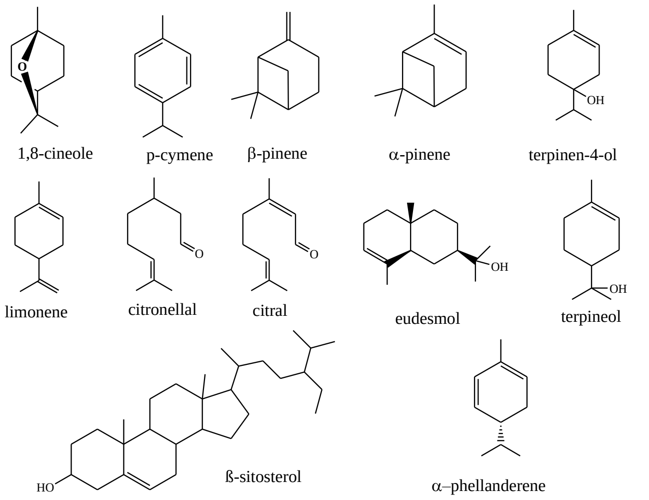

Figure 1. Chemical structures of the main components of *Eucalyptus* fruits, branch tips, and leaf essential oils.

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 *Eucalyptu*s 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, antinociceptive, 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 quercetin, 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).

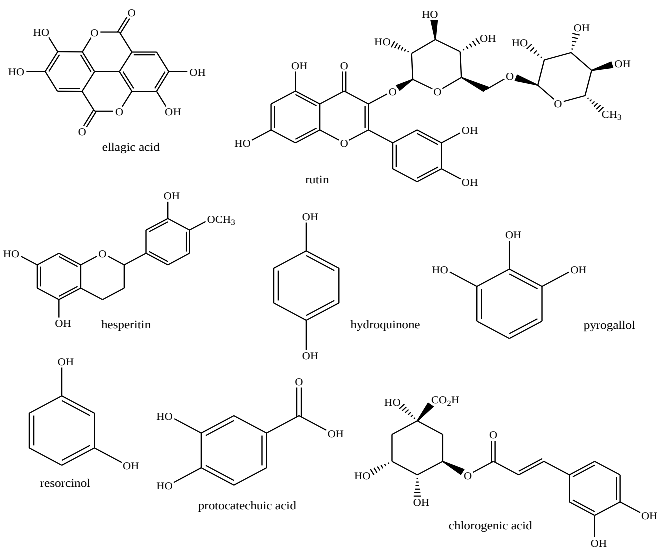

Figure 2. Different phenolic compounds isolated from different *Eucalyptus* extracts.

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, differentiation, the inhibition of DNA binding and cell adhesion, migration, proliferation, and the blockade of signaling pathways (Olawore 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).

Olawore 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.

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

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.

Extract Conditions	**0% ethanol**	**10% ethanol**	**30% ethanol**	**50% ethanol**	**70% ethanol**	**90% ethanol**	**100% ethanol**
TPC (mg GAE/g Extract)	126.7 ± 8.5	422.0 ± 18.4	492.7 ± 13.2	497.7 ± 15.5	448.5 ± 20.1	384.5 ± 10.4	273.2 ± 17.5

FLAVONOID 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, flavanonols, flavanols and catechins (Álvarez et al., 2021; Panche et al., 2016). Quercetin, kaempferol, isorhamnetin, luteolin, phloretin, and catechins are the primary flavonoids found in *Eucalyptus* (Álvarez et al., 2021).

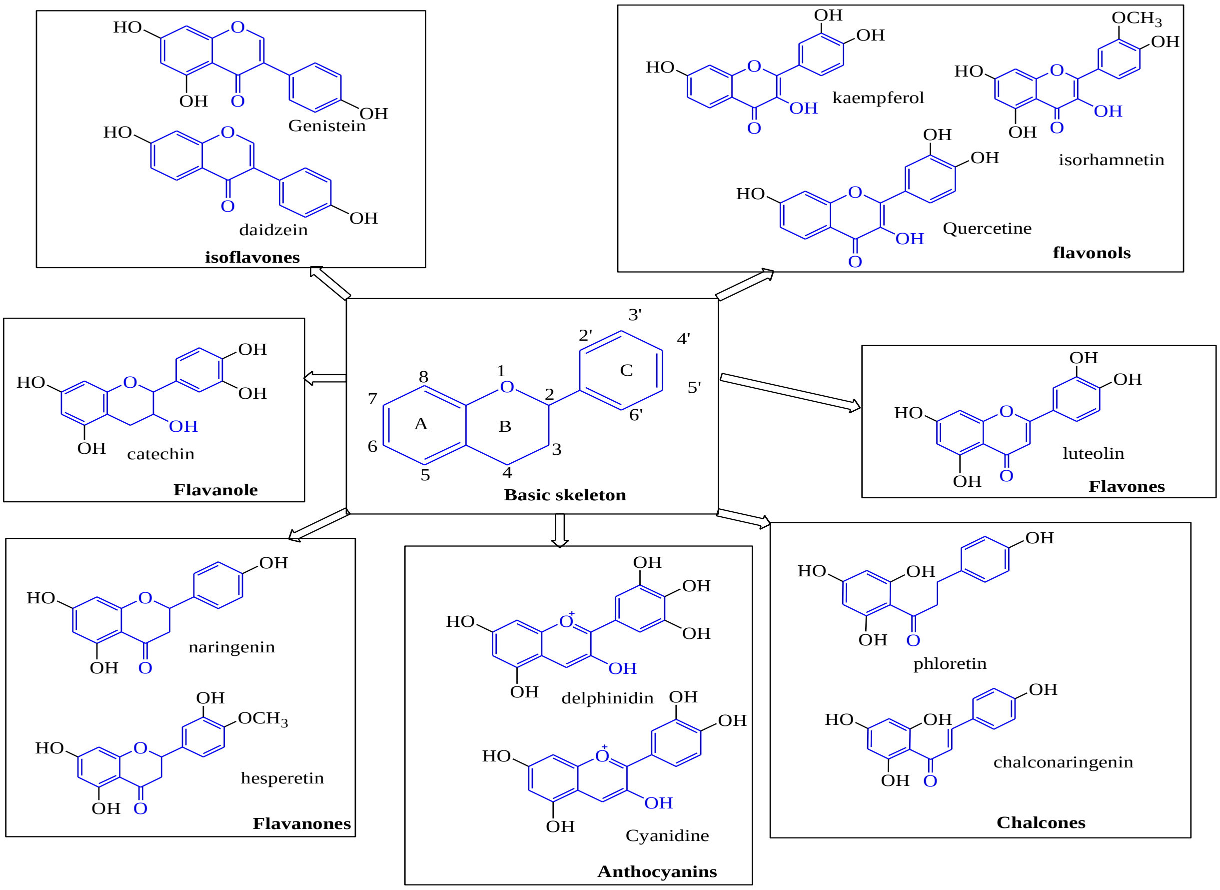

Figure 3. Basic primary structural components of flavonoids and their classes.

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 Rhizophagus 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.

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

***Eucalyptus* species**	**Part of plant**	**Condition**	**Flavonoid content**	**Reference**
E. globulus	leaf	80% methanol	23 ± 0.00 [a]	(Sharma et al., 2021)
E. globulus Labill.	leaves	70% Ethanol extract	35.76 ± 0.95 [a]	(Dezsi et al., 2015)
E. saligna	leaves	pressurized 95% ethanol	160.27 [b]	(Fischer et al., 2020)

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

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

Extract Conditions	**0% ethanol**	**10% ethanol**	**30% ethanol**	**50% ethanol**	**70% ethanol**	**90% ethanol**	**100% ethanol**
TFC (mg QE/g Extract	32.5 ± 5.1	32.7 ± 4.8	40.4 ± 6.7	41.2 ± 7.5	66.5 ± 6.5	156.5 ± 10.4	169.3 ± 12.2

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 vars* (**Table 5**), *Nancy* and *Petersford*, had half maximal inhibitory concentration (IC_{50}) 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 IC_{50} value of 9.00 µg/ml. The essential oils from the seeds of the two *eucalyptuses* 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 IC_{50} value of the fruit essential oil *E. citriodera* is 2.00 µg/ml, and ascorbic acid has an IC_{50} 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 IC_{50} 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.

***Eucalyptus* species**	**Condition**	**IC_{50} value (µg/ml)**		**Reference**
		DPPH assay	**FRAP**	
E. camaldulensis var. nancy	Seed Essential oil	3.5	5.0	(Olawore NO & Ololade ZS , 2017)
	Ascorbic acid	9.0	11	
E. camaldulensis var. petford	Seed Essential oil	3.5	5.0	
	Ascorbic acid	9.0	11	
E.citriodera	Fruit Essential oil	3.00	2.00	(Ololade et al., 2021)
	Ascorbic acid	9.00	11.00	

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 (SC_{50}), followed by 10%, 50%, and 70% ethanol, with SC_{50} values of 357.9, 505.3, and 509.3 µg/ml, respectively. Among the extraction conditions, 0% ethanol, 90% ethanol, and 100% ethanol had SC_{50} 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 SC_{50} 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 SC_{50} 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 6. DPPH and ABTS radical scavenging activities of different ethanol solutions from *E. globulus* leaf extracts.

Extract Conditions	Radical Scavenging Activity (SC_{50}, µg/mL)	
	DPPH	ABTS
0% ethanol	5841.7 ± 238.4	171.3 ± 10.2
10% ethanol	357.9 ± 28.0	23.1 ± 2.1
30% ethanol	188.2 ± 24.2	14.2 ± 0.9
50% ethanol	505.3 ± 56.2	18.0 ± 1.5
70% ethanol	509.3 ± 57.1	20.8 ± 1.9
90% ethanol	1008.4 ± 121.2	24.8 ± 1.4
100% ethanol	1304.7 ± 156.5	34.9 ± 5.1

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 (H_2O_2), per hydroxyl radical ($HO_2^•$), hydroxyl radical ($OH^•$), superoxide anion radical ($O_2^{•-}$), singlet oxygen ($^1O_2$), 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 $O_2^{•-}$ to form ONOO (Azat Aziz et al., 2019).

Antioxidants are substances or systems that slow autoxidation 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).

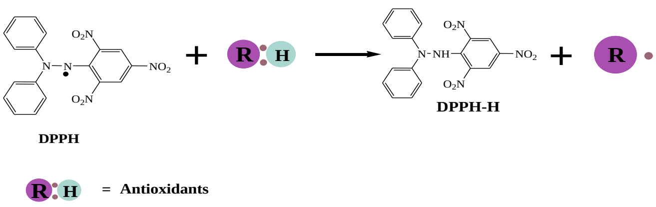

Figure 4. Reaction mechanism of DPPH with an antioxidant.

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 ($(NH_4)_2S_2O_3$) or potassium persulfate ($K_2S_2O_3$) prior to the addition of antioxidants (**Figure 5**) (Sadeer et al., 2020).

Chemical reaction of generating $ABTS^{•+}$ radical

$$(NH_4)_2S_2O_3 + ABTS \longrightarrow ABTS^{•+} + ArOH \longrightarrow ABTS + ArO \quad \text{……….Equ. 1}$$

ArOH = represent phenolic antioxidant

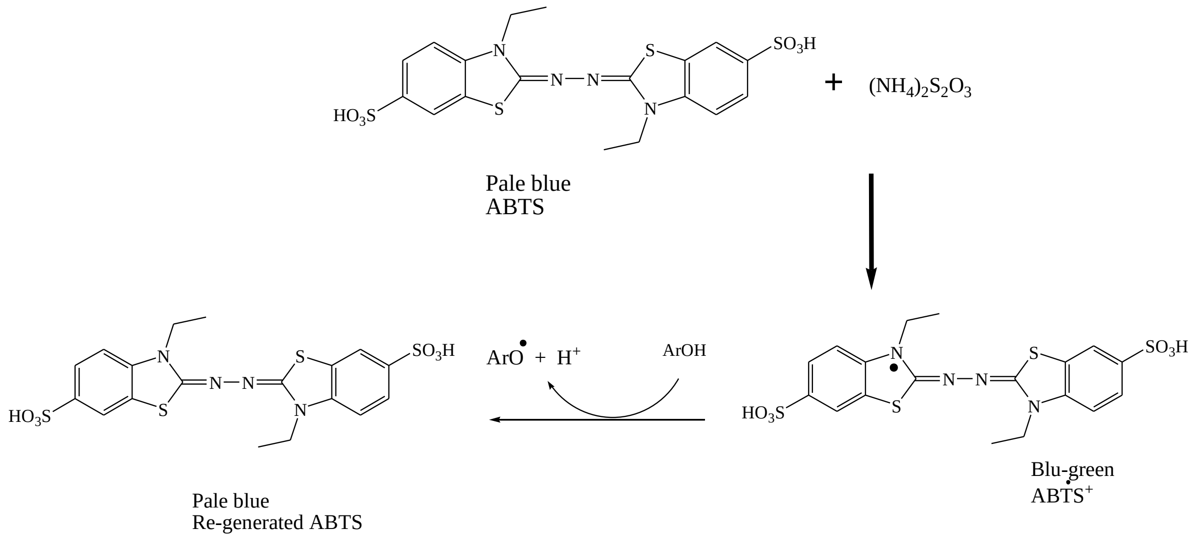

Figure 5. Mechanism of the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) reaction.

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).

$$\text{Antioxidant} + Fe^{3+} \rightleftharpoons Fe^{2+} + \text{oxidized antioxidant}$$
$$Fe^{2+} + Fe(CN)_6^{3-} \rightleftharpoons Fe[Fe(CN)_6]^{-}$$

OR

$$\text{Antioxidant} + Fe(CN)_6^{3-} \rightleftharpoons Fe(CN)_6^{4-} + \text{oxidized antioxidant}$$
$$Fe(CN)_6^{4-} + Fe^{3+} \rightleftharpoons Fe[Fe(CN)_6]^{-}$$

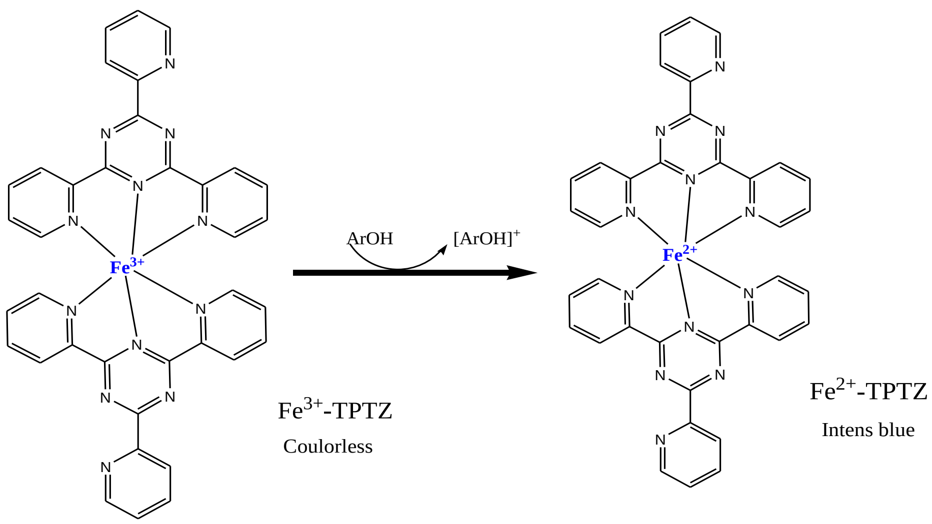

Figure 6. The mechanism of ferric reducing antioxidant power (FRAP) reaction.

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).

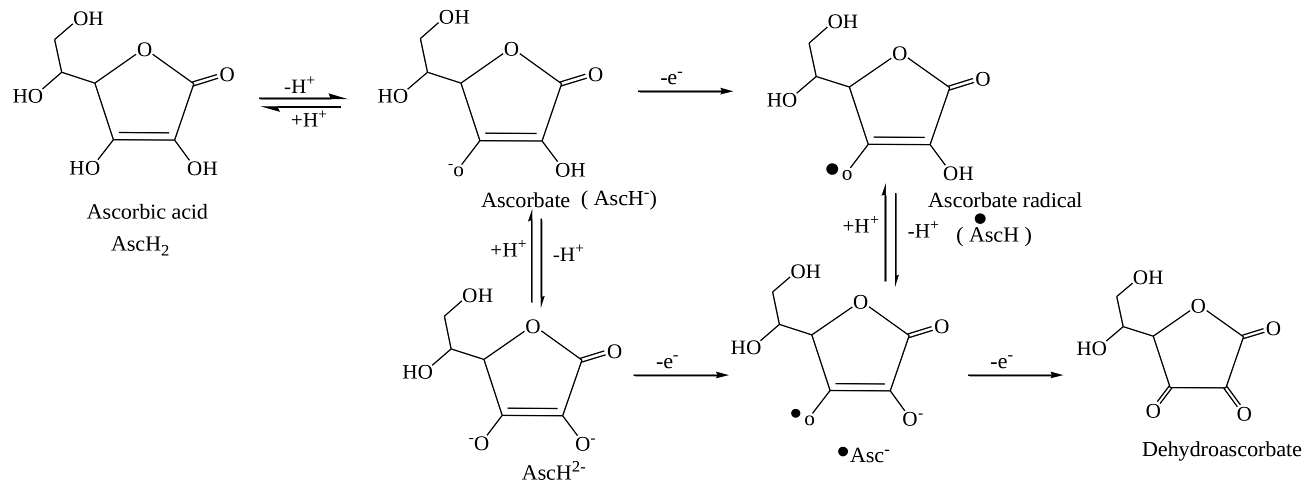

Figure 7. Mechanism of the radical scavenging activity of vitamin C.

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).

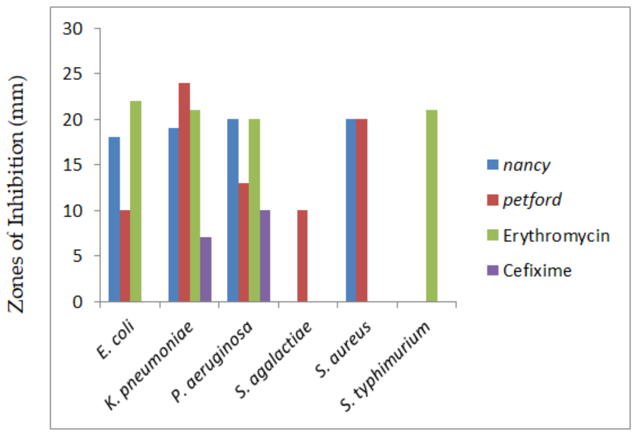

Figure 8. Zones of inhibition (mm) showing the antimicrobial properties of the seed essential oils of the two varieties of *E. camaldulensis.*

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).

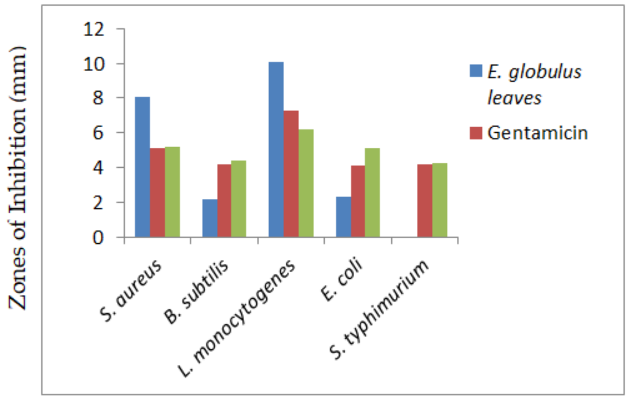

Figure 9. Effects of zones of inhibition (mm) on the antibacterial activity of *E. globulus* extracts and antibiotics against bacterial species, as determined by a disc diffusion assay.

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 cefuroxine antibiotics, as shown in **Figure 10** (Ololade et al., 2021).

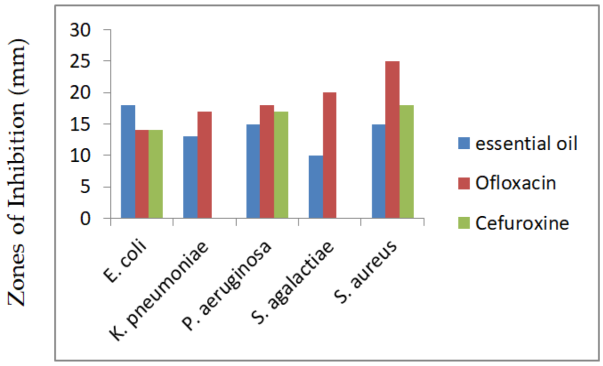

Figure 10. Zones of Inhibition (mm) on Antibacterial Activity of the Essential Oil of *E. Citriodora* and Standard Antibiotic Discs against Bacterial Isolates.

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-inflammatory, 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.

***Acknowledgments*:** I would like to express my gratitude to the University of Gondar, College of Natural and Computational Science, Department of Chemistry, for giving me the opportunity to pursue my postgraduate study. I would also like to thank my advisor, Gizachew Mulugeta (Ph.D.), for his valuable suggestions, constructive advice, and guidance in preparing my review. Furthermore, I would like to express my appreciation to Tilahun Belayineh (Ph.D.) for his feedback on my review paper.

***Author Contributions*:** G. W. M conceived the idea and prepared the original draft of the manuscript. G.W.M., G.M.M., and M.A.A. proofread, reviewed and edited the manuscript.

***Conflict of Interest*:** The authors declare that they have no conflicts of interest.

REFERENCES

Alemu, M. M. (2016). Eucalyptus Tree Production in Wolayita Sodo, Southern Ethiopia. *OALib*, *03*(12), 1–10. https://doi.org/10.4236/oalib.1103280

Almas, I., Innocent, E., Machumi, F., & Kisinza, W. (2021). Chemical composition of essential oils from Eucalyptus globulus and Eucalyptus maculata grown in Tanzania. *Scientific African*, *12*, 1–7. https://doi.org/10.1016/j.sciaf.2021.e00758

Álvarez, X., Cancela, Á., Merchán, Y., & Sánchez, Á. (2021). Anthocyanins, phenolic compounds, and antioxidants from extractions of six eucalyptus species. *Applied Sciences (Switzerland)*, *11*(21), 1–12. https://doi.org/10.3390/app11219818

Amabye, T. G. (2016). Phytochemical and Antimicrobial Potentials Leaves Extract of Eucalyptus Globulus Oil from Maichew Tigray Ethiopia. *International Journal of Complementary & Alternative Medicine*, *3*(1), 1–4. https://doi.org/10.15406/ijcam.2016.03.00056

Azat Aziz, M., Shehab Diab, A., & Abdulrazak Mohammed, A. (2019). Antioxidant Categories and Mode of Action. In *Antioxidants* (pp. 1–20). https://doi.org/10.5772/intechopen.83544

Birara Dessie, A., Mire Abate, T., & Melese Mekie, T. (2019). Eucalyptus: The Popular Exotic Tree Crop in Ethiopia. *Acta Scientific Agriculture*, *3*(9), 50–56. https://doi.org/10.31080/asag.2019.03.0607

Brewer, M. S. (2011). Natural Antioxidants: Sources, Compounds, Mechanisms of Action, and Potential Applications. *Comprehensive Reviews in Food Science and Food Safety*, *10*(4), 221–247. https://doi.org/10.1111/j.1541-4337.2011.00156.x

Brunetti, C., Di Ferdinando, M., Fini, A., Pollastri, S., & Tattini, M. (2013). Flavonoids as antioxidants and developmental regulators: Relative significance in plants and humans. In *International Journal of Molecular Sciences* (Vol. 14, Issue 2, pp. 3540–3555). https://doi.org/10.3390/ijms14023540

Dezsi, Ș., Bədərəu, A. S., Bischin, C., Vodnar, D. C., Silaghi-Dumitrescu, R., Gheldiu, A. M., Mocan, A., & Vlase, L. (2015). Antimicrobial and antioxidant activities and phenolic profile of Eucalyptus globulus Labill. and Corymbia ficifolia (F. Muell.) K.D. Hill & L.A.S. Johnson leaves. *Molecules*, *20*(3), 4720–4734. https://doi.org/10.3390/molecules20034720

Fischer, B., Gowzdz, E. P., Feltes, G., Griep, P., Junges, A., Paroul, N., & Cansian, R. L. (2020). Antioxidant and Antimicrobial Properties of Eucalyptus Leaf Extract Obtained Using Pressurized Ethanol. *Brazilian Journal of Development*, *6*(10), 74499–74512. https://doi.org/10.34117/bjdv6n10-036

Francenia Santos-Sánchez, N., Salas-Coronado, R., Villanueva-Cañongo, C., & Hernández-Carlos, B. (2019). Antioxidant Compounds and Their Antioxidant Mechanism. In *Antioxidants* (pp. 1–28). https://doi.org/10.5772/intechopen.85270

Gullón, P., Gullón, B., Astray, G., Munekata, P. E. S., Pateiro, M., & Lorenzo, J. M. (2020). Value-added compound recovery from invasive forest for biofunctional applications: Eucalyptus species as a case study. *Molecules*, *25*(18), 1–19. https://doi.org/10.3390/molecules25184227

Lidoy, J., Berrio, E., García, M., España-Luque, L., Pozo, M. J., & López-Ráez, J. A. (2023). Flavonoids promote Rhizophagus irregularis spore germination and tomato root colonization: A target for sustainable agriculture. *Frontiers in Plant Science*, *13*, 1–9. https://doi.org/10.3389/fpls.2022.1094194

Munteanu, I. G., & Apetrei, C. (2021). Analytical methods used in determining antioxidant activity: A review. In *International Journal of Molecular Sciences* (Vol. 22, Issue 7, pp. 1–30). https://doi.org/10.3390/ijms22073380

Mworia, J. K., Kibiti, C. M., Ngugi, M. P., & Ngeranwa, J. N. (2019). Antipyretic potential of dichloromethane leaf extract of Eucalyptus globulus (Labill) and Senna didymobotrya (Fresenius) in rats models. *Heliyon*, *5*(12), 1–8. https://doi.org/10.1016/j.heliyon.2019.e02924

Nimse, S. B., & Pal, D. (2015). Free radicals, natural antioxidants, and their reaction mechanisms. *RSC Advances*, *5*(35), 27986–28006. https://doi.org/10.1039/c4ra13315c

Olawore NO. & Ololade ZS. (2017). Eucalyptus camaldulensis var. nancy and Eucalyptus camaldulensis var. petford Seed Essential Oils: Phytochemicals and Therapeutic Potentials. *Chemical Sciences Journal*, *08*(01), 1–6. https://doi.org/10.4172/2150-3494.1000148

Ololade, S, Z., Olawore, & O, N. (2021). Health Benefits of the Fruit Essential Oil of Eucalyptus Citriodora: Secondary Metabolites, Radical Scavenging, Antioxidant, Anti-inflammatory, Analgesic, Antimicrobial Potential. *Int J Drug Discov*, *13*(4), 1–7. https://www.ijddr.in/%0Ahttps://medical-case-reports.imedpub.com/

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: An overview. In *Journal of Nutritional Science* (Vol. 5, pp. 1–15). https://doi.org/10.1017/jns.2016.41

Panigrahi, A., Jena, N. C., Tripathi, S., Tiwari, V., & Sharma, V. (2021). Eucalyptus : A Review on Agronomic and Medicinal Properties. *Biological Forum*, *13*(1), 342–349.

Park, J. Y., Kim, J. Y., Son, Y. G., Kang, S. D., Lee, S. W., Kim, K. D., & Kim, J. Y. (2023). Characterization of Chemical Composition and Antioxidant Activity of Eucalyptus globulus Leaves under Different Extraction Conditions. *Applied Sciences (Switzerland)*, *13*(17), 1–11. https://doi.org/10.3390/app13179984

Rahman, M. M., Rahaman, M. S., Islam, M. R., Rahman, F., Mithi, F. M., Alqahtani, T., Almikhlafi, M. A., Alghamdi, S. Q., Alruwaili, A. S., Hossain, M. S., Ahmed, M., Das, R., Emran, T. Bin, & Uddin, M. S. (2022). Role of phenolic compounds in human disease: Current knowledge and future prospects. In *Molecules* (Vol. 27, Issue 1, pp. 1–36). https://doi.org/10.3390/molecules27010233

Rakha, A., Umar, N., Rabail, R., Butt, M. S., Kieliszek, M., Hassoun, A., & Aadil, R. M. (2022). Anti-inflammatory and anti-allergic potential of dietary flavonoids: A review. In *Biomedicine and Pharmacotherapy* (Vol. 156, pp. 1–10). Elsevier Masson s.r.l. https://doi.org/10.1016/j.biopha.2022.113945

Roy, A., Khan, A., Ahmad, I., Alghamdi, S., Rajab, B. S., Babalghith, A. O., Alshahrani, M. Y., Islam, S., & Islam, M. R. (2022). Flavonoids a Bioactive Compound from Medicinal Plants and Its Therapeutic Applications. In *BioMed Research International* (Vol. 2022, pp. 1–9). https://doi.org/10.1155/2022/5445291

Sadeer, N. B., Montesano, D., Albrizio, S., Zengin, G., & Mahomoodally, M. F. (2020). The versatility of antioxidant assays in food science and safety—chemistry, applications, strengths, and limitations. In *Antioxidants* (Vol. 9, Issue 8, pp. 1–39). https://doi.org/10.3390/antiox9080709

Sebei, K., Sakouhi, F., Herchi, W., Khouja, M. L., & Boukhchina, S. (2015). Chemical composition and antibacterial activities of seven Eucalyptus species essential oils leaves. *Biological Research*, *48*, 1–5. https://doi.org/10.1186/0717-6287-48-7

Shah, G., Kaur, M., Singh, P. S., Rahar, S., Dhabliya, F., Arya, Y., & Shri, R. (2012). Pharmacognostic Parameters of Eucalyptus globulus Leaves. *Pharmacognosy Journal*, *4*(34), 38–43. https://doi.org/10.5530/pj.2012.34.7

Sharma, A., Chahal, M., & Kaur, I. (2021). Antioxidant and bioactivities of leaf extracts from eucalyptus globulus. *Arabian Journal of Medicinal and Aromatic Plants*, *7*(3), 385–406. https://doi.org/10.48347/IMIST.PRSM/ajmap-v7i3.28443

Shiferaw, Y., Kassahun, A., Tedla, A., Feleke, G., & Abebe, A. A. (2019). Investigation of Essential Oil Composition Variation with Age of Eucalyptus globulus Growing in Ethiopia. *Natural Products Chemistry & Research*, *07*(02), 1–5. https://doi.org/10.35248/2329-6836.19.7.360

Surbhi, Kumar, A., Singh, S., Kumari, P., & Rasane, P. (2023). Eucalyptus: phytochemical composition, extraction methods and food and medicinal applications. In *Advances in Traditional Medicine* (Vol. 23, Issue 2, pp. 369–380). https://doi.org/10.1007/s13596-021-00582-7

Teka, A., & Maryo, M. (2023). Ethiopian Medicinal Plants Used for Respiratory Tract Disorders: Ethnomedicinal Review. *Evidence-Based Complementary and Alternative Medicine*, *2023*, 1–9. https://doi.org/10.1155/2023/7612804

Tesfaye, M. A., Gardi, O., Anbessa, T. B., & Blaser, J. (2020). Aboveground biomass, growth and yield for some selected introduced tree species, namely, Cupressus lusitanica, Eucalyptus saligna, and Pinus patula in Central Highlands of Ethiopia. *Journal of Ecology and Environment*, *44*(1), 1–18. https://doi.org/10.1186/s41610-019-0146-z

Tsegaye Bekele. (2015). Integrated Utilization of Eucalyptus globulus grown on the Ethiopian Highlands and its Contribution to Rural Livelihood: A Case Study of Oromia, Amhara and Southern Nations Nationalities and People's Regional State Ethiopia. *Journals International Journal of Basic and Applied Sciences*, *4*(2), 80–87. www.crdeep.com

Ullah, A., Munir, S., Badshah, S. L., Khan, N., Ghani, L., Poulson, B. G., Emwas, A. H., & Jaremko, M. (2020). Important flavonoids and their role as a therapeutic agent. In *Molecules* (Vol. 25, Issue 22, pp. 1–39). https://doi.org/10.3390/molecules25225243

Vecchio, M. G., Loganes, C., & Minto, C. (2016). Beneficial and Healthy Properties of Eucalyptus Plants: A Great Potential Use. *The Open Agriculture Journal*, *10*(1), 52–57. https://doi.org/10.2174/1874331501610010052

Zerga, B., Warkineh, B., Teketay, D., & Woldetsadik, M. (2021). The sustainability of reforesting landscapes with exotic species: a case study of eucalypts in Ethiopia. *Sustainable Earth*, *4*(1), 1–11. https://doi.org/10.1186/s42055-021-00044-7

Zhang, Y., Cai, P., Cheng, G., & Zhang, Y. (2022). A Brief Review of Phenolic Compounds Identified from Plants: Their Extraction, Analysis, and Biological Activity. In *Natural Product Communications* (Vol. 17, Issue 1, pp. 1–14). https://doi.org/10.1177/1934578X211069721

Zulkefli, N., Che Zahari, C. N. M., Sayuti, N. H., Kamarudin, A. A., Saad, N., Hamezah, H. S., Bunawan, H., Baharum, S. N., Mediani, A., Ahmed, Q. U., Ismail, A. F. H., & Sarian, M. N. (2023). Flavonoids as Potential Wound-Healing Molecules: Emphasis on Pathways Perspective. In *International Journal of Molecular Sciences* (Vol. 24, Issue 5, pp. 1–29). https://doi.org/10.3390/ijms24054607

THIS PAGE INTENTIONALLY LEFT BLANK