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Review

Bioactive Compounds from the Plants of the *Elaeodendron* Genus and Their Biological Activities—A Review

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Abstract: *Elaeodendron* is a genus of tiny trees, shrubs, vines, and herbs consisting of about 23 species. It is used in traditional medicine and has a wide range of pharmacological activities. From the plants in this genus, flavonoids, terpenoids, cardiac glycosides, and cardenolides have been isolated. *Elaeodendron* species have been the subject of numerous *in vitro* investigations; however, not many vivo studies are available. Preclinical investigations have also revealed antiviral, anti-HIV, anticancer, antiproliferative, antioxidant, antifungal, anti-inflammation, cytotoxic, anti-plasmodial, anti-arthritic, antibacterial, and anti-diabetic activities. Bioactive substances found in *Elaedendron* that function in a variety of ways are related to these biological processes. Several databases, including PubMed, Scopus, etc., were searched using keywords such as "*Elaeodendron*", "chemical constituents", "anti-cancer", "anti-viral", "pharmacology", etc., to perform a comprehensive review of the current literature. In this sense, this review intends to provide the most recent developments in the ethnomedical use of *Elaeodendron* species, and their therapeutic benefits and bioactive compounds. Many species of this genus are reported to be toxic. To provide stronger scientific support for their conventional usage, more *in vivo* and clinical research for mechanism-based pharmacological evaluation as well as toxicological studies should be carried out in the future.

Keywords: *Elaeodendron*; Celastraceae; cardenolides; anti-HIV; anticancer; antioxidant

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1. Introduction

There has long been a tradition of using plants as medicines [1,2]. In the latest generations, extensive investigation has been performed on the plants utilized historically or described in ancient literature. Plants grown for ornamental purposes include those used as houseplants, cut flowers, specimen displays, and gardens and landscape design projects. Crops planted for ornamental and floral purposes can offer nutritional and therapeutic benefits. To improve the healing process that takes place in healthcare settings, there has been a resurgence of interest in the use of therapeutic garden surroundings with medicinal value. Several populations, particularly in underdeveloped nations, still depend on conventional therapeutic plant-based therapy in modern times. This is mostly owing to

the scarcity and high cost of traditional medications in underdeveloped nations. Approximately 80% of people from developed nations use conventional medicine, which includes chemicals obtained from medical plants [3]. As a result, the use of bioactive components for pharmaceutical applications has gradually expanded worldwide. Flowering trees are now used in herbal medicine, and it is well known that they have healing properties.

Elaeodendron is a genus in the Celastraceae family [4]. The Celastraceae belongs to the order Celastrales and consists of approximately 96 genera and 1350 species of herbs, vines, shrubs, and small trees [5]. The Celastrales is a flowering plant order that may be found in the tropical and subtropical regions, with just a few genera expanding into temperate areas. *Elaeodendron* is a genus of approximately thirty to forty species native to Africa, Bermuda, the Mexican coast, Madagascar (particularly Mascarene), Australia, Melanesia, and India [5,6]. This genus includes evergreen and, on rare occasions, deciduous shrubs and trees. The lenticels are frequently evident in the yellow pigment layers found in the bark. The leaves are either subopposite or opposite or rotate on occasion. The petals are cream to greenish, and the stamens are upright. The fruits are smooth-surfaced, drupaceous, spherical, white to yellow, and drupaceous. Reddish-brown seeds with succulent cotyledons are squashed [4,5].

Flavonoids [4], terpenoids [4,7] cardiac glycosides [8], and cardenolides [9] have been isolated from these species, which are mainly shrubs and deciduous trees. Plants of this genus have been shown to have antiviral, anti-HIV, anticancer, antiproliferative, antioxidant, anti-inflammation, anti-plasmodial, cytotoxic, antifungal, anti-arthritic, and antibacterial properties in earlier research [10,11].

The purpose of this article is to compile and analyze the scientific data that is currently available regarding pharmacological properties, bioactive chemical components, and traditional usage of *Elaeodendron* species based on online scholarly databases such as Scopus, SciFinder, PubMed, Google Scholar, and published books and proceedings. The existing gaps in the research on the *Elaeodendron* species are also reviewed, along with suggestions for the future.

2. Traditional Uses

Elaeodendron buchananii (Loes.) Loes. is an evergreen shrub or tree with a branching, rounded crown found in eastern Africa, particularly Uganda and Kenya [12]. Despite its toxic nature, *E. buchananii* is occasionally utilized in conventional practice of medicine. Leaf extracts are used to treat fever, as an abortifacient, oxytocic, tonic, and vermifuge [13–15]. Chewing the leaves is considered beneficial for the treatment of diarrhea. Gastrointestinal problems, bloody coughing, excessive uterine bleeding, and infertility are treated using root decoctions. Syphilis is treated using root powder [13,16,17]. On wounds, the root powder is administered topically. The bark decoction is also used to cure leukemia [12].

Elaeodendron croceum (Thunb.) DC., also known as saffron, saffron wood, and forest saffron, is an evergreen tree with a tidy, vertical frame found in various parts of South Africa (Ladismith, KwaZulu-Natal, Limpopo, Southern Cape forests) and in Zimbabwe (Mount Cherinda) [7]. The bark of this plant is used as a febrifuge and emetic in therapeutic approaches to treat opportunistic infections caused by the human immunodeficiency virus (HIV) [7,18]. Tuberculosis and other associated disorders, such as blood in sputum, chest congestion, cough, and sore throat have historically been treated and managed using the bark [18]. The roots, bark, and leaves of the plant are used as herbal treatments to clear the gastrointestinal system and control fever [19].

Preparations of *Elaeodendron glacum* (Rottb.) Pers. have been employed by conventional healers as a remedy for a number of diseases such as diabetes. As sternutatories, the dried and powdered leaves are employed [20]. The dried leaves are also burned, and the resulting smoke is utilized as a disinfectant to treat some nerve illnesses, especially to rouse women from hysterics [21]. Headache is relieved by snuffing the powdered leaves. Fresh root bark is ground into a paste with water and applied to swellings as a poultice. The root

is reported to have anti-snake-venom properties. As an emetic, cold-water infusion of the pulverized roots is employed [4,22].

Elaeodendron orientale Jacq., sometimes known as the fake olive, is an indigenous plant of the Mascarene Islands and Madagascar [9]. The bark has traditionally been used to cure chest infections, venereal illness, and scorpion fish poisoning. The leaves are emetic and astringent. The combination of leaves with those of *Kalanchoe pinnata* (Crassulaceae) generate bufadienolides, used to alleviate hypertension and treat seafood allergies [9].

Elaeodendron roxburghii Wight and Arn. Is found in India, Japan, China, Thailand, and Nepal and its roots are used to treat snake bite [23].

Elaeodendron schweinfurthianum (Loes.) Loes. is a species of evergreen deciduous trees and shrub. The herb exhibits antibacterial, anti-HIV, and anti-plasmodial properties, among many others [4].

In Tanzania, traditional medicinal claims have been made for treating a variety of non-infectious disorders using *Elaeodendron schlechteranum* (Loes.) Loes. An oral decoction of the root is used to treat male dysfunction, female fertility problems, menstrual irregularities, inflammatory diseases, anemia, and heart issues such as high blood pressure and basic body discomfort. On foul-smelling septic wounds, root and stem bark powder is used [24]. Carbuncles and boils are treated using a slurry made from the leaves. Root bark extracts were shown to have a powerful antimicrobial effect. Anti-HIV activity was also reported against HIV-2 (ROD strain) and HIV-1 (IIIB strain) [24].

Elaeodendron transvaalense (Burtt Davy) R. H. Archer is a medium and small deciduous tree frequently known as a bushveld saffron or anthill saffron. In Zambia, Zimbabwe, Mozambique, Swaziland, Botswana, Namibia, Angola, and South Africa, the genus is found as evergreen woods, near rivers, stony highlands, and insect piles [25]. In southern Africa, the root macerate and bark of *E. transvaalense* are used as herbal remedies to treat various ailments. *E. transvaalense* is used to make a natural mixture for diarrhea in Swaziland and South Africa [25], abdominal discomfort in Swaziland and South Africa [10], inflammations, rashes, and skin infections in Swaziland, South Africa, and Namibia, and hemorrhage in Zimbabwe, South Africa, and Botswana [10]. *Peltophorum africanum* Sond. roots are used with *E. transvaalense* roots or root bark to treat women's fertility issues [25]. *E. transvaalense* root bark and roots are also blended with *Ozoroa paniculosa* (Sond.) R.Fern. & A.Fern. roots as a natural remedy for hypertension. *E. transvaalense* roots, *Zanthoxylum capense* (Thunb.) Harv. roots, *Sarcostemma viminale* (L.) R.Br. branches, *Elephantorrhiza elephantina* (Burch.) Skeels, *Sclerocarya birrea* (A. Rich.) Hochst. bark, and *Drimia elata* Jacq. bulb are combined to make a therapeutic plant for treatment of HIV, opportunistic, and sexually transmitted diseases (STIs) [10]. In ethnoveterinary medicine, *E. transvaalense* bark and leaves are used for diarrhea and worm treatment [25].

Elaeodendron trichotomum (Turcz.) Lundell is a plant found in the south and southeast of Mexico, and Central America, where it grows on sand dunes, mangrove edges, and semi-deciduous medium woodland [26]. The species is a big, branching, small evergreen tree that may grow up to 12 feet tall. In terms of ethnobotanical usage, *E. trichotomum* has been employed in Mexico as an anti-infective, antiprotozoal, and anti-tumor agent in febrile-type disorders [26].

Elaeodendron xylocarpum (Vent.) DC. is a tiny evergreen tree or shrub that grows between 2 and 12 m tall [27]. This plant's stem extracts have been proven to have anti-HIV properties in different studies [28–30].

Elaeodendron zeyheri (Sond.) Turcz. is a tiny tree or shrub that grows up to 10 m tall in eastern South Africa and Zimbabwe [5]. Snakebite can be treated using this plant's bark. As an emetic and ordeal poison, a root infusion has been utilized [31].

3. Bioactive Compounds from *Elaeodendron* Species

Bioactive chemicals are extra nutritional components detected in tiny concentrations in plants and foods that provide health advantages in addition to the basic nutritional value [32]. Bioactive substances appear to have significant immunological, behavioural, and physiological

effects. They are being examined extensively to determine their effect on the human body. They are gaining popularity in various fields, including contemporary pharmacology, food business, plant science, nanobioscience, cosmetics, and agrochemicals [32].

Plant bioactive chemicals are categorized using a variety of criteria. Strongly linked species of plants typically generate similar or slightly structurally comparable active compounds. It might be helpful to categorize active molecules based on the genera and families in which they exist. However, there are several situations when genetically unrelated organisms create identical secondary chemicals. The bioactive chemical compounds are the major emphasis. Thus, it is helpful to organize them into biochemical and chemical classes [1].

Elaeodendron species are rich in various biologically active chemicals responsible for a wide range of pharmacological actions. Environmental circumstances, climatic conditions, harvesting season and methods, genetic conditions, species variety, plant part and age, vegetative phase, and soil may all influence the quantitative and qualitative composition of bioactive chemicals [17,33]. Table 1 lists the chemical compounds found in *Elaeodendron* species (Figure 1).

Table 1. Bioactive phytochemicals, traditional uses, part used, and biological activities of *Elaeodendron* species.

Species	Isolated Compounds	Traditional Uses	Part Used	Reported Biological Activity	Reference
E. buchananii	Elabunin; lupeol; 19α, 28-trihydroxyurs-12- en-23-oic acid; 3β, 11α, 3β-acetoxy-19α, 23, 28-trihydroxyurs-12-ene; 3-oxo-19α, buchaninoside; 19α-trihydroxyurs-12-en-23, 28-dioic acid; mutangin; methyl 3β-acetoxy-11α, 28 dihydroxyurs-12-en-24-oic acid	Fever, diarrhea gastrointestinal problems, bloody coughing, excessive uterine bleeding, infertility, syphilis, wounds, and leukemia	Leaves, Roots Bark	Anticancer, gastrointestinal disturbances, antimicrobial	[12,14]
E. croceum	30-Hydroxylup 20(29)-en-3-one; (+)-6R,13R-11,11- dimethyl-1,3,8,10-tetrahydroxy-9-methoxy-peltogynan; galactitol; canophyllol; (−)-4′-0-methoxyepigallocatechin; tingenin B; ouratea-proanthocyanidin A; tingenone; 3-hydroxylupeol; 11α-hydroxy-β-amyrin; naringenin	Tuberculosis, blood in sputum, chest congestion, cough, sore throat, gastrointestinal system, fever	Stem bark	Anti-HIV, antibacterial, anti-arthritic, antimycobacterial, antifungal, antioxidant, anti-inflammatory, cytotoxic	[7,18]
E. glacum	30-Hydroxylup-20(29)-en-3-one; tingenone; canophyllol; tingenin B; 3-hydroxylupeol; elaeodendroside; isocardenolide	Diabetes, sternutatories, nerve illnesses, swellings, headaches, emetic	Leaves, Root bark	Ani-diabetic, anti-snake-bite properties	[20]
E. orientale	Elaeodendroside F; elaeodendroside G; elaeodendroside T; elaeodendroside B; elaeodendroside C; elaeodendroside R; 20(22)-dienolid,6β,8β,11α,14β-tetrahydroxy-12-oxo-2α-O; 11α,14β-dihydroxy-2α-O;3β- O-(30α-methoxy-40-deoxy-50-dehydroxymethyl hexosulose)-card-4; 20(22)-dienolide, 3β-O-(20α,30β-methylendioxy)-40-desoxy-50-deshydroxymethyl-hexosulose]-card-4, 11α,14β-dihydroxy-2α-O; 3β-O-(30 α-methoxy-40-deoxy-50-dehydroxymethyl-hexosulose)-card-4; 20(22)-dienolide	chest infections, venereal illness, scorpion fish poisoning, astringent emetic, hypertension	Leaves, root bark	Anti-arthritic, antiproliferative, anticancer	[6,9]
E. schweinfurthianum	3α-Hydroxyfriedelane; α-amyrin acetate; α-amyrin; 3-oxo-29-hydroxyfriedelane; β-sitosterol; lanosterol; stigmasterol; 3-oxofriedelane; 3-oxofriedelan-28-al	Fever	Roots	Antibacterial, anti-HIV, anti-plasmodial	[4]

Table 1. *Cont.*

Species	Isolated Compounds	Traditional Uses	Part Used	Reported Biological Activity	Reference
E. schlechteranum	4′,4″-Di-O-methyl-prodelphinidin; B_4,3β,29-dihydroxyglutin-5-ene; 4′-*O*-methyl-epigallocatechin; tingenin B; 4′-*O*-methylgallocatechin; cangoronine methyl ester	Menstrual irregularities, anaemia, heart issues, high blood pressure, basic body discomfort, inflammatory disease, carbuncles boils, wounds	Roots, stem bark, root bark, leaf	Anti-HIV, anti-inflammatory	[24]
E. transvaalense	4′-*O*-Methyl-epigallocatechin; canophyllal; (+)-, 11,11-dimethyl-1,3,8,10-trahydroxy-9-methoxypeltogynan; 6β-hydroxy-lup-20(30)-en-3-one; galactitol; hydroxylup-20(29)-ene-3-one; lup-20(29)-ene-30-hydroxy-3-one; Ψ-taraxastanonol; lup-20(30)-ene-3α,29-diol; lup-20(30)-ene-3α,29-diol; β-sitosterol; 3,28-dihydroxylbetuli-20(29)-ene; lup-20(30)-ene-3α,29-diollup-20(29)-ene-30-hydroxy-3-one; 4′-O-methyl-epigallocatechin; 3-oxo-28-hydroxylbetuli-20(29)-ene; 30-hydroxylup-20(29)- ene-3-one.	Diarrhea, stomachache, rashes, skin infections, inflammations, menorrhagia, women's fertility issues, hypertension, HIV, sexually transmitted diseases (STDs).	Root bark	Anti-HIV, anti-inflammatory, antimicrobial, antioxidant, antimalarial, cytotoxic	[10,25,27]
E. xylocarpum	3,25-Epoxy-olean-12-ene; 3β,21a-dihydroxyglut-5-ene; baruol; friedelin; cangoronine; cangoronine methyl ester; glutinol; 3β,29-dihydroxyglut-5-ene; wilforol E; 6β,30-dihydroxylup-20(29)-en-3-one; 6β-hydroxy-3-oxolup-20(29)-en-30-al; 3-oxolup-20(29)-en-30-oic acid; 3β,6β,20-trihydroxylupane; 11α,28-dihydroxylup-20(29)-en-3-one; 3-oxolup-20(29)-en-30-al; ochraceolide A; 12 3-oxo-30 hydroxylupane; 11 3-epiglochidiol; lupenone; botulin; 11 6β,20-dihydroxylupan-3-one; 16 lupan-3β-caffeate; 11 betulin-3β-caffeate; glochidiol; 3-epibetulin; betulone; 11α hydroxyglochidone; lupeol; rigidenol; nepeticin; glochidone; 25-hydroxylupeol; 15 3β,30-dihydroxylupane; 3-epinepeticin; 3b,29-Dihydroxy-olean-18-ene; 29-Hydroxy-3-oxo-olean-18-ene; 6b,29-Dihydroxy-3-oxo-olean-18-ene; 6b-Hydroxy-3-oxo-olean-18-ene; 3b,21a-Dihidroxy-olean-18-ene; 3b,6b-Dihidroxy-olean-18-ene; 21a-Hydroxy-3-oxo-olean-18-ene; 3b,11a,28-Trihydroxy-olean-18-ene; 29-Acetoxy-3-oxo-olean-18-ene; 3b,21a-Diacetoxy-olean-18-ene; 3b-Acetoxy-6b-hydroxy-olean-18-ene; 6β,30-Dihydroxylup-20(29)-en-3-one; 6β-Hydroxy-3-oxolup-20(29)-en-30-al; 3-Oxolup-20(29)-en-30-oic acid; 3β,6β,20-Trihydroxylupane; 1β,3α,28-Trihydroxylup-20(29)-ene; 11α,28-Dihydroxy-3-oxolup-20(29)-ene; 3β,28-Di-O-octanoylbetulin; 28-O-(1-Naphthoyl)botulin; 3β,28-Di-O-(1-naphthoyl)botulin; 28-Oacetyl-3β,20,29-trihydroxylupane; 28-O-acetyl20R,29-epoxy-3β-hydroxylupane; 2 (28-O-acetyl-3β-hydroxylup-20(29)-en30-al; 3β,30-di-O-acetyllup-20(29)-ene; 2-bromo-3-oxolup-20(29)-ene;11α-O-acetyl-3-oxolup-20(29)-ene;11α-O-Acetyl-30-chloro-3-oxolup-1,20(29)-diene	Stimulant	Root bark	Anti-HIV	[29,30]

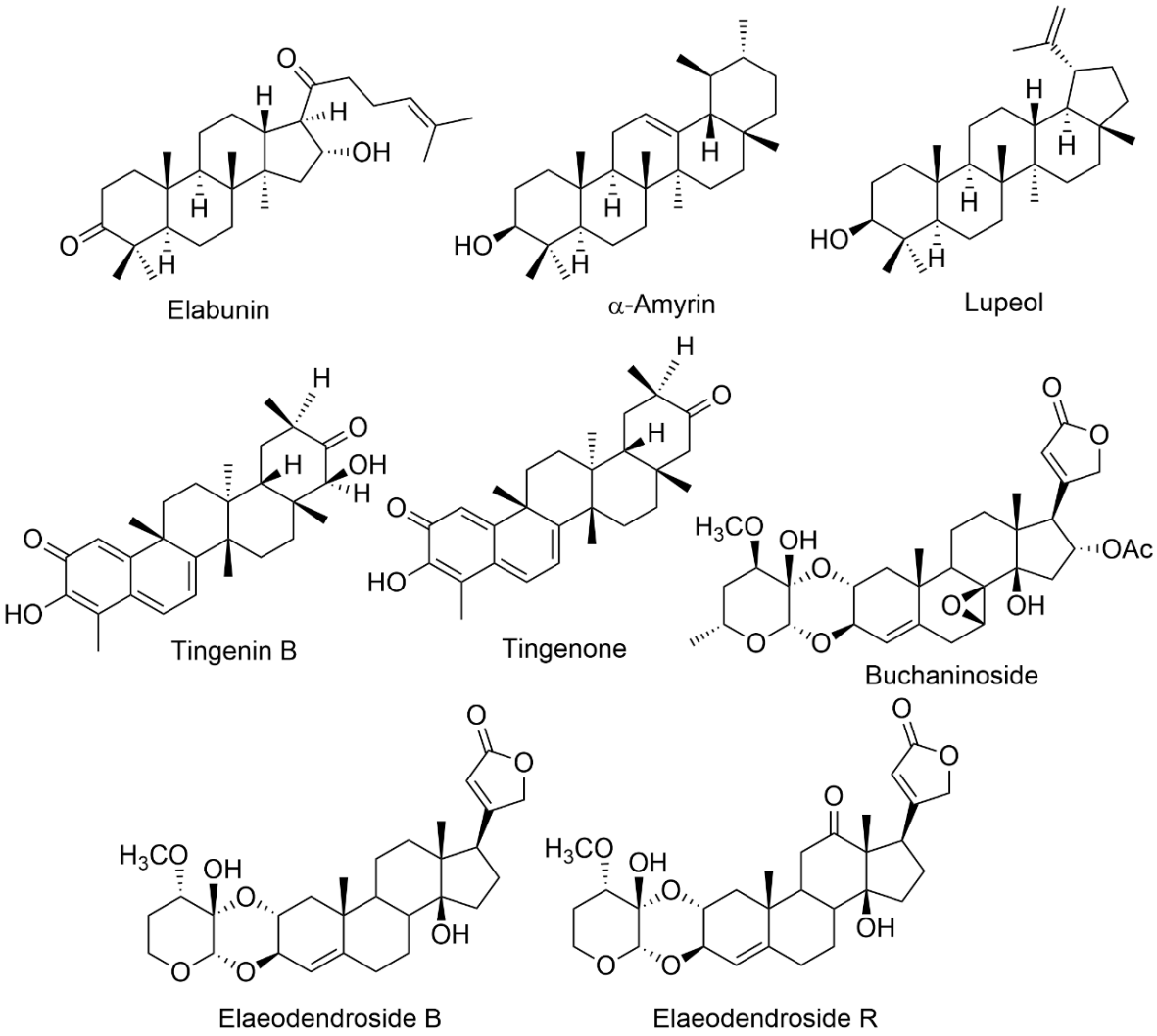

Figure 1. Chemical structures of some selected bioactive compounds isolated from *Elaeodendron* species.

As per published literature, Kubo et al. isolated elabunin, a novel dammarane type triterpene from the methanol crude extract of *E. buchananii* root bark [8]. Dammarane triterpenoids are widely known to be highly significant natural compounds with exceptional biological activity. These substances could be employed as preventative and therapeutic agents for a number of illnesses, including diabetes mellitus, hyperlipidemia, metabolic syndrome, cancer, cardiovascular and cerebrovascular disease, neurodegenerative disease, aging, liver disease, gastrointestinal disease, kidney disease, bone disease, depression-type mental illness, and skin aging [34]. Mutangin, a novel sesquiterpene isolated from the *E. buchananii* fruit, was found to possess moderate antifeedant activity according to Tsanuo et al. [21]. Against *Spodoptera exempta* larvae, Tsujino et al. isolated buchaninoside from the fruits of *E. buchananii* with antifeedant properties [17].

Numerous therapeutic plants contain cardenolides, which are prominent cardioactive secondary metabolites. Cardenolides' cytotoxicity and cardiac activity have both been investigated [6]. Cardenolides are abundant in the Celastraceae family, primarily those that are found in Elaeodendron. Some cardenolides of this family have peculiar sugar linkage dioxane-type six-membered rings and methylendioxy moiety, which make them structurally interesting [9]. *Elaeodendron* sp. from the Madagascar rain forest produced two novel antiproliferative cardenolides, elaeodendrosides T and U, which were extracted from the ethanol extract [6]. Osorio discovered novel cytotoxic cardenolides in the fruits and leaves of *Elaeodendron orientale*, including elaeodendroside F, elaeodendroside G, elaeodendroside T, elaeodendroside B, elaeodendroside C, and elaeodendroside R [9].

4. Pharmacological Properties of *Elaeodendron* Species

Elaeodendron species have been reported to have antiviral, anti-HIV, anticancer, antiproliferative, antioxidant, anti-inflammation, anti-plasmodial, cytotoxic, antifungal, anti-arthritic, anti-diabetic, and antibacterial properties.

4.1. Antioxidant Activity

Odeyemi and Afolayan used FRAP (reducing power), ABTS (2,2′-azinobis-3-ethylbenzothiazoline-6-sulphonate), and DPPH (2,2-diphenyl-l-picrylhydrazyl) free radical scavenging tests to assess the antioxidant properties of *E. croceum* stem bark and leaf acetone extracts [35]. Rutin, butylated hydroxytoluene (BHT), and ascorbic acid were used as reference antioxidant compounds. The leaf acetone extract IC_{50} (Inhibitory Concentration) values were 0.1 mg/mL for the DPPH test, 2.5 mg/mL for FRAP, and 0.09 mg/mL for ABTS, whereas the IC_{50} values of bark extract were 0.07 mg/mL for DPPH, 9.2 mg/mL for FRAP, and 0.2 mg/mL for ABTS. The reference medicines, ascorbic acid, BHT, and rutin, have IC_{50} values ranging between 0.02 mg/mL to 3.5 mg/mL. This antioxidant activity demonstrated that the extracts have excellent ability with diverse free radical species, equivalent to ascorbic acid [35]. Elisha et al. used the TEAC, FRAP, ABTS, DPPH free radical scavenging tests with Trolox and ascorbic acid as standard medicines to assess the antioxidant properties of *E. croceum* acetone extract. The extracts had strong DPPH and ABTS activity, with IC_{50} values of 7.7 µg/mL and 3.1 µg/mL, respectively, which were also similar to Trolox and ascorbic acid, the two positive standards with IC_{50} values in the range between 2.9 µg/mL to 5.6 µg/mL. The IC_{50} for TEAC and FRAP were 1.3 µg/mL and 1.0 µg/mL respectively, whereas the values for Trolox and ascorbic acid ranged from 1.0 to 3.7 [36]. The antioxidant properties of *E. croceum* extracts are most likely due to flavonoids and phenolics extracted from the plant.

Motlhanka et al. examined the antioxidant properties of *E. transvaalensis* ethanol and water root extracts, as well as 4′-O-methyl-epigallocatechin, a compound obtained from the plant, using a DPPH free radical scavenging experiment with ascorbic acid, rutin, and quercetin as standard drug. The ethanol extract had an 80% radical scavenging activity above 100 µg/mL concentration, which was equivalent to the standard antioxidant substances ascorbic acid, rutin, and quercetin, while the water extract had 80% scavenging activity above 200 µg/mL concentration. Between 25.0 µg/mL and 50 µg/mL concentration, the molecule 4′-O-methyl-epigallocatechin had a 65% radical scavenging activity, which was higher than the activity of both ethanol and water extracts. However, at levels more than 50 µg/mL, the ethanol extract outperformed the chemical 4′-O-methyl-epigallocatechin [37].

Nethengwe et al., used the ABTS, iron chelating property assay, DPPH, sulphur hydryl content, hydroxyl radical scavenging, total antioxidant capacity, superoxide, and nitric oxide (NO) radical scavenging tests to examine the antioxidant effects of *E. transvaalense* methanolic bark extracts. The IC_{50} values for the superoxide was 1.6 µg/mL, hydroxyl was 3.6 µg/mL, ABTS was 4.1 µg/mL, iron chelating was 3.9 µg/mL, DPPH assay was 0.7 µg/mL, and NO was 3.6 µg/mL [38]. Makhafola et al. used the DPPH radical scavenging activity test with ascorbic acid as the control sample to assess the antioxidant properties of *E. transvaalense* methanolic leaf extracts. The extract had an EC_{50} value of 2.8 µg/mL, which was equivalent to the positive control, ascorbic acid (EC_{50} = 2.3 µg/mL) [39]. The antioxidant properties of *E. transvaalense* crude extracts are most likely attributable to phenolics and flavonoids extracted from the species [39].

More et al., used lipopolysaccharides (LPS)-induced intracellular reactive oxygen/nitrogen species measurement, $ABTS^{+}$ radical scavenging assay, and nitrite concentrations as a measure of reactive nitrogen species to assess the antioxidant activity of south African medicinal plant extracts including *E. croceum* and *E. transvaalense* [40]. Non-cell-based and cell-based tests were used to assess the extracts' reactive oxygen/nitrogen species (ROS/RNS) and antioxidant inhibiting activities. The test was carried out using a modified version of Volsteedt's technique [41]. According to the approach published by More et al., [40], the vero cells were used to measure ROS by producing external oxidative stress using lipopolysac-

charides (LPS) and a Gram-negative *Eccherichia coli* bacteria's endotoxin. The decrease in ABTS radicals was demonstrated in eight extracts, and the EC_{50} values were recorded. *E. croceum* exhibited considerably stronger $ABTS^+$ reducing capacity with EC_{50} values 10 µg/mL, whereas *E. transvaalense* had EC_{50} values > 10 µg/mL. The strong ABTS scavenging activity of the extracts has EC_{50} values equivalent to ascorbic acid, the control sample, which had excellent ABTS reducing power and an EC_{50} value of 2.30 µg/mL. The cell-permeant probe 2′,7′-dichlorodihydrofluorescein diacetate (H2DCFDA), which is non-fluorescent but is transformed to the highly fluorescent 2′,7′-dichlorofluorescein (DCF) when acetate groups are cleaved by intracellular esterases, was used to detect ROS generation [42]. The data show that following exposure to LPS alone, ROS levels rose by roughly 95% as compared to the control group (untreated cells). The LPS-induced ROS levels were significantly lowered after pre-treatment with extracts (100 µg/mL). *E. natalensis, E. croceum, E. elephantina*, and *E. transvaalense* decreased ROS levels by 60% When cells were activated with LPS (1 µg/mL), they generated more NO than cells that were not stimulated. LPS-induced NO levels were considerably lowered after treatment with extracts (100 µg/mL). Extracts of *E. croceum*, *E. transvaalense*, and *E. natalensis* showed significant NO inhibitory activity of 80, 78, and 75%, respectively [40]. These findings imply that the inhibitory effect of plant extracts on reactive species is based on a variety of metabolites found within the extracts, each of which has a different mechanism of action [40].

The DPPH technique works by reducing a stable electron/hydrogen acceptor to DPPH [40,43], while a cation $ABTS^+$ interacts with antioxidants through an electronic transport mechanism [40]. The DPPH and $ABTS^+$ scavenging activities were evaluated in this work and found to be almost identical, indicating that the two techniques are in a positive way associated. Lipopolysaccharides (LPS) are a prominent component of microbial cell membrane and are involved in tissue and organ harm in both humans and animals [44]. Furthermore, the increased generation of nitric oxide, ROS/RNS such as superoxide anion radical ($O_2^{\bullet -}$) and lipid peroxides, which generate oxidative stress, causes LPS to promote the release of inflammatory cytokines and mediators. This investigation decreased of LPS-induced ROS and RNS revealed nearly equivalent outcomes. The strong correlation between anti-inflammatory and antioxidant approaches suggests that a particular metabolite is engaged in liquefying radicals by activating pathways which target various radicals but could utilize multiple techniques, which is consistent with previous research, despite the fact that various free radical—scavenging techniques were utilised [44].

4.2. Anti-inflammatory Activity

Odeyemi and Afolayan used the protein denaturation test using diclofenac as a control sample to assess the anti-inflammatory effects of *E. croceum* stem bark and leaf acetone extracts. The extracts indicated activity, with IC_{50} values of 0.9 mg/mL and 1.9 mg/mL for leaf and stem bark extracts, respectively, whereas diclofenac (positive control) had an IC_{50} value of 0.3 mg/mL. The extracts had a considerable inhibitory effect on protein denaturation, indicating that they may have anti-inflammatory properties [35]. Olaokun et al. examined the inhibitory effects of *E. croceum* bark acetone extracts on the pro-inflammatory enzyme 5-lipoxygenase (5-LOX) using HeLa cervix carcinoma cells and human pancreatic cancer cell lines Panc-1 and Capan-2 as the cell lines and using quercetin as the control sample to investigate if the extract reduce inflammation. The extract reduced 5-lipoxygenase activity with an IC_{50} of 75.5 g/mL [45]. Elisha et al. examined the anti-inflammatory properties of *E. croceum* leaves acetone extract by measuring nitric oxide (NO) generation suppression and 15-lipoxygenase enzyme inhibition in lipopolysaccharide (LPS) activated RAW 264.7 macrophages. The preparations decreased NO generation in LPS-stimulated RAW 264.7 macrophage in a dose dependent manner. The extract *E. croceum* (IC_{50} = 26.2 µg/mL) demonstrated significant activity against 15-lipoxygenase activity than that of the control sample quercetin IC_{50} of 53.7 µg/mL [36]. Mamba et al. investigated the anti-inflammatory properties of *E. croceum* and *E. transvaalense* bark ethanol (70%) extract by measuring inhibitory activity on the pro-inflammatory enzyme 15-LOX using quercetin

as a control sample. The *E. croceum* and *E. transvaalense* extract had an IC_{50} value of 82.5 and 80.2 µg/mL, respectively, whereas the positive control, quercetin, had an IC_{50} value of 48.9 µg/mL [19]. *E. croceum* possesses a flavonoid called naringenin, which is known for anti-inflammatory properties. Flavonoids can control the inflammation mediated by lymphocytes and macrophages. These results indicate that *E. croceum* and *E. transvaalense* might be used as herbal medications to treat inflammatory wounds, ailments, and discomfort [19].

Motlhanka and Habtemariam examined the anti-inflammatory activities of *E. transvaalense* root bark aqueous crude extract using the COX inhibition test and indomethacin as a control sample. In LPS stimulated RAW 264.7 macrophages, the extract (125 mg/mL) inhibited PGE2 by 90%, which is equivalent to indomethacin, the control medication, which inhibited PGE2 by 100% at 0.4 mg/mL [46].

More et al. evaluated the anti-inflammatory effect of the extract from the medicinal plants of South Africa including *E. croceum*, and *E. transvaalense* [40]. The extracts' inhibitory action against RNS/ROS was tested using cell-based and non-cell-based assays. In both ROS and RNS assays, *E. croceum*, and *E. transvaalense* demonstrated a decrease in LPS-induced ROS and RNS with elevated activity >60%. The study's findings are in line with the positive effects of radical scavenging [40]. Because cell viability assessments and cellular oxidative stress can be markers of high ROS manufacturing, which can result in apoptosis [47], suppressing ROS could explain why the extracts were considerably less harmful to Vero cells. The DPPH and $ABTS^{+}$ radicals were substantially suppressed by *E. croceum*, and *E. transvaalense* in this investigation, and the formation of LPS-induced reactive oxygen species was decreased [40].

4.3. Antibacterial Activity

Odak et al. evaluated canophyllol, a new triterpene isolated from the *E. buchananii* stem bark ethyl acetate crude extract using the agar diffusion method against the pathogens; *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Neisseria meningitides*. The compound showed promising antibacterial activity against *Neisseria meningitides* with minimum inhibitory concentrations (MIC) value of 31.25 µg/mL [14].

Eloff et al. used the agar diffusion technique to test the antibacterial activity of an acetone extract of *E. croceum* bark against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Escherichia coli*. The extract exhibited antimicrobial activity against the microorganisms examined, with maximum activity levels ranging from 8.0 µg/mL to >275.0 µg/mL [48]. Using a two-fold serial microdilution approach, Kaikabo et al. analyzed the antimicrobial properties of *E. croceum* bark acetone extract against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Enterococcus faecalis*. Screening for MIC (minimum inhibitory concentrations) was carried at values ranging from 0.02 mg/mL to 2.5 mg/mL. The extracts were effective against at minimum a few of the test species for 1, 2, or 24 h, with typical MIC values ranging from 0.2 mg/mL to 1.8 mg/mL against the microorganism evaluated [49]. Elisha et al. used the microplate serial dilution technique with gentamicin as a positive control to investigate the antibacterial activity of *E. croceum* leaves acetone extract against *Bacillus anthracis*. The extract demonstrated antibacterial activity with a MIC of 0.3 mg/mL and total antimicrobial property of 290.0 mg/mL [50].

Elisha et al. investigated the antimicrobial activities of *E. croceum* leaves acetone extract against *Escherichia coli*, *Salmonella typhimurium*, *Stenotrophomonas maltophilia*, *Proteus mirabilis*, *Enterobacter cloacae*, and *Klebsiella pneumoniae* using a serial microdilution method and gentamicin as a reference drug. The extract had overall antimicrobial activity values ranging from 290.2 mL/g to 1124.6 mL/g and MIC values ranging from 0.08 mg/mL to 0.31 mg/mL [51]. Odeyemi and Afolayan used the agar well diffusion and broth microdilution methods to test the antibacterial activity of *E. croceum* stem bark and leaves aqueous extract against *Staphylococcus aureus*, *Enterococcus faecalis*, *Shigella flexneri*, *Klebsiella pneumoniae*, *Salmonella typhimurium* and *Proteus vulgaris* [35]. The extracts were active against all the pathogens tested, with zones of inhibition that range from 13.0 to 15.7 mm. Amoxicillin, the positive control, was effective against *S. aureus*, *E. faecalis*, *S. flexneri*, and

K. pneumoniae with zones of inhibition that ranges from 12.5 to 26.5 mm. The extracts and the control had a minimum inhibitory concentration ranging from 0.6 mg/mL to >5.0 mg/mL against all pathogens [35].

Odeyemi et al. investigated silver nanoparticles' biosynthesis from the leaves and stem bark of *E. croceum* as well as their antimicrobial and anticancer properties [52]. *E. croceum* leaf and stem bark extracts (ECL-AgNPs and ECB-AgNPs, respectively) were used to make the silver nanoparticles, which were characterized using transmission electron microscopy, X-ray diffraction, UV-visible spectroscopy, and Fourier transform infrared spectroscopy. The experiments included Gram-negative and Gram-positive bacteria, as well as MCF-7 and MDA-MB-231 cells. For ECBAgNPs and ECL-AgNPs, the nanoparticles were stable aqueous dispersive, crystalline, and spherical in form, with average particle sizes of 22.26 nm and 10.01 nm, respectively. For ECBAgNPs and ECL-AgNPs, the nanoparticles were stable aqueous dispersive, crystalline, and spherical in form, with average particle sizes of 22.26 nm and 10.01 nm, respectively. The X-ray Diffraction (XRD) patterns exhibited four diffraction peaks indexed to (111), (200), (220), and (311) planes fcc at 32.24°, 48.11°, 58.64°, and 77.47°, respectively. The lowest inhibitory concentrations and IC_{50} values, on the other hand, were greater than the norms. In conclusion, a single-step technique for producing silver nanoparticles from *E. croceum* has been developed [52]. The antibacterial activity of *E. croceum* extracts demonstrates the species' potential as an herbal medication against bacterial infections and verifies the species' traditional usage as a herbal remedy for sore throat in South Africa [53].

Khumalo et al. examined the antimicrobial property of *E. transvaalense* stem bark extracts and components in methanol and dichloromethane. 6β-hydroxy-lup-20(29)-ene-3-one,4′O-methylepigallocatechin, lup-20(30)-ene-3α,29-diol, and 30-hydroxylup-20(29)-ene-3-one were tested against *Salmonella typhimurium*, *Staphylococcus epidermidis* [54] *Staphylococcus aureus, Escherichia coli*, *Shigella sonnei*, and *Pseudomonas aeruginosa* using a micro-titer plate broth two-fold serial dilution experiment with ciprofloxacin as the control sample. The extract and compounds had moderate antibacterial activity, with minimum inhibitory concentration values 0.1 mg/mL to 1.7 mg/mL. Using the serial broth microdilution assay and ciprofloxacin as a positive control. Mamba et al. investigated the antimicrobial activities of *E. transvaalense* bark ethanol extracts and the molecules 4′-O-methyl-epigallocatechin, lup-20(30)-ene-3,29-diol and lup-20(29)-ene-30-hydroxy-3-one isolated from the plant against *Neisseria gonorrhoeae*, *Oligella ureolytica*, and *Gardnerella vaginalis.* MIC values for the extracts and compounds varied from 1.6 mg/mL to 12.5 mg/mL, whereas the positive control had a MIC of 0.01 mg/mL [19]. McGaw et al. used disc-diffusion and micro-dilution assays to test the antimicrobial activity of *E. transvaalense* bark aqueous and hexane ethanol extracts against *Staphylococcus aureus, Klebsiella pneumoniae, Bacillus subtilis*, and *Escherichia coli* with neomycin as a positive control. Water and ethanolic extracts were potent against *Bacillus subtilis and Staphylococcus aureus*, with MICs ranging from 0.1 mg/mL to 0.8 mg/mL [55]. Using the agar dilution method, Tshikalanga et al. investigated the antimicrobial activities of *E. transvaalense* chloroform and aqueous bark extracts against *Enterobacter cloacae*, *Enterobacter aerogenes*, *Bacillus pumilus*, *Bacillus cereus*, *Klebsiella pneumoniae, Bacillus subtilis,* and *Escherichia coli*. The extracts had MIC values between 20 mg/mL to 50 mg/mL against *Bacillus cereus*, *Bacillus pumilus*, *Bacillus subtilis*, and *Staphylococcus aureus* [10]. Steenkamp et al. investigated the antibacterial activities of *E. transvaalense* bark methanolic and water extracts against *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* utilizing broth microdilution and plate-hole diffusion methods with ampicillin as the control sample. The extracts had minimum inhibitory concentration values from 1.3 mg/mL to 17.2 mg/mL against *Staphylococcus aureus* and *Staphylococcus epidermidis*. The control sample had a minimum inhibitory concentration of 0.2 mg/mL [56]. These findings supported the species traditional usage as an herbal remedy for venereal illnesses, sexually transmitted infections, stomach pains, diarrhoea, sore throat, skin infections, and wounds [10,25].

4.4. Cytotoxic Activity and Antiproliferative Activity

Prinsloo et al. used 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay on Vero cells to assess the cytotoxicity of semi-purified *E. croceum* stem bark extracts. On cell lines with a treatment response of 250 (therapeutic index), the extract demonstrated only 20% toxicity at a concentration of 25 µg/mL [57]. Using the MTT assay on Vero cells, Elisha et al. assessed the cytotoxic effects of an acetone extract of *E. croceum* leaves. The extract revealed activity with an LC_{50} of 5.2 µg/mL and SI (selectivity index) values between 0.01 to 0.07, whereas the standard drug, doxorubicin, had an LC_{50} of 8.3 µg/mL [50,58]. The extract was highly cytotoxic, matching the findings of Yelani et al., who claimed that the poisonous triterpenoids naringenin, tingenone, 20-hydroxy-20-epi-tingenone, tingenine B, and 11-hydroxy-amyrin obtained from the species' leaves cause cytotoxicity in Vero cells [7].

Odeyemi et al. investigated the cytotoxicity of biologically produced silver nanoparticles from *E. croceum in vitro* and *in vivo* [59]. The stem of *E. croceum* was used to make silver nanoparticles, which were then described. The oral acute toxicity experiments were conducted on Wister rats in groups of 500, 1000, and 2000 mg/kg body weight. The WST-1 Cell Growth test was used to assess an *in vitro* anticancer assay in MDA-MB-231 breast cancer cells. The percent of viable cells after therapy with *E. croceum* silver nanoparticles (ECAgNPs) and aqueous extracts of *E. croceum* (ECE) was compared to the percentage of cell viability following treatment with paclitaxel. In vivo investigations demonstrated that the LD_{50} was greater than 2000 mg/kg, and that there was no significant change ($p > 0.05$) in mean organ-to-body weight ratio and all haematological parameters except WBC and haematocrit between both the experimental and comparison groups. For serum electrolytes (Cl, Na^+, K^+ Mg^{2+}, and Ca^{2+}), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, alkaline phosphatase (ALP), urea, albumin, glutamyl transferase (GGT), and total and conjugated bilirubin, there was no significant difference ($p > 0.05$) between the treatment and control groups. Cholesterol levels, creatinine, and urea, on the other hand, changed. *In vitro* studies revealed that ECE and ECAgNPs had IC_{50} values of 70.87 µg/mL and 138.8 µg/mL against MDA-MB-231 cells, respectively, compared to paclitaxel, which had an IC_{50} value of 80 ng/mL. The LD_{50} of ECE and ECAgNPs in Wister rats was shown to be larger than 2000 mg/kg body weight, according to the findings. The hazardous chemicals in the aqueous extract were likewise found to be more cytotoxic than the ECAgNPs, suggesting that the toxic compounds in the aqueous extract were involved in the capping of the AgNPs [59]. Although *E. croceum* has traditionally been used as a medicinal herb in South Africa and Swaziland to cure various human ailments, traditional healers have been accused of using the species roots to induce death [7,18]. According to Maroyi 2019, *E. croceum* is classified as harmful to cattle in the KwaZulu Natal region of South Africa and causes mortality. In rabbit experiments, 2.5 g/kg body mass and 10 g/kg body mass of fresh leaves caused mortality 15 min after treatment [18]. These toxicological findings suggest that *E. croceum* should be used with precaution when used as an herbal medication.

Deutschländer et al., investigated the cytotoxic properties of stem bark extract of *E. transvaalense* by testing their effects on hepatocyte and preadipocyte cell lines. At 12.5 g/mL, the extract was cytotoxic to Chang liver cells and 3T3-L1 preadipocytes [60]. Tshikalange and Hussein used the sodium 3′-[1-(phenyl amino-carbonyl)-3,4-tetrazolium]-bis-[4-methoxy-6-nitro] benzene sulfonic acid hydrate (XTT) colorimetric technique to test the cytotoxicity of the crude ethanol extract and the compounds Ψ-taraxastanonol, β-sitosterol, 4′-O-methyl-epigallocatechin, lup-20(30)-ene-3α,29-diol, and lup-20(29)-ene-30-hydroxy-3-one isolated from the bark extract of *E. transvaalense* against MCF-7 breast and Vero cancer cell lines, using zelaralenone and doxorubicin as a positive control. All substances inhibited the cell lines at the maximum tested materials (200 µg/mL), excluding Ψ-taraxastanonol, and crude extract. 4′-O-methyl-epigallocatechin, Ψ-taraxastanonol, and the crude extract had IC_{50} values larger than 100 µg/mL in Vero cells, and Ψ -taraxastanonol and the crude extract had IC_{50} values greater than 100 µg/mL in the MCF-7 cell line. In the MCF-7 cell and Vero cells line, the IC_{50} values for other substances ranged from 19.4 µg/mL

to 96.0 µg/mL [61]. Morobe et al. assessed the antitumor effect of *E. transvaalense* aqueous and methanolic extracts against HeLa MAGI (membrane-associated guanylate Kinase with inverted orientation) CCR5 (CC chemokine receptor 5) cells using the MTT test (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide). The extracts had a half maximum CC_{50} (cytotoxic concentration) of 3.7 mg/mL indicating that they were active [62]. Using the MTT cell viability test against HEK293 (human embryonic kidney) and HepG2 (human hepatocellular carcinoma) cells, Nethengwe et al. examined the antitumor effects of *E. transvaalense* bark of methanolic, dichloromethane, and aqueous extracts [39]. The remaining extracts, apart from dichloromethane, which had an LC_{50} of 512.0 µg/mL against HEK293 and 394.0 µg/mL against HepG2, were inactive [38]. Mthethwa et al. used the MTT test with berberine as a standard drug to assess the cytotoxic effects of *Cassine transvaalensis* (*E. transvaalense*) bark extracts. The extract had a CC_{50} value of 200 µg/mL, which was more significant than the 27 µg/mL displayed by the standard drug berberine and the International System of Units (SI) value of 57.1 [63]. Using the MTT cell proliferation test, Sigidi et al. assessed the antitumor effects of *E. transvaalense* bark aqueous extract against Vero cellU937 and MeWo, lines. The extract was effective against all three specific tumor carcinoma cells [64].

Kubo et al. researched the cytotoxic activity of elabunin, a novel triterpene obtained from the methanol crude extract of *E. buchananii* root bark, against L-1210 leukemic cells. Elabunin has modest cytotoxic action against L-1210 leukemic cells, with a median effective dosage (ED_{50}) of 1 µg/mL [8].

Cao et al. evaluated the antiproliferative activity of two novel cardenolides, elaeodendrosides T and U, isolated from the ethanol extract of *E. orientale* root bark against human ovarian cancer cells (A2780). Having IC_{50} values of 0.085 and 0.019M, respectively, compounds 1 and 2 demonstrated substantial antitumor activity against human ovarian cancer cells (A2780) [6].

4.5. Anti-fungal Activity

Mamba et al. used the broth microdilution technique to test the antifungal activity of *E. croceum* ethanol bark extract using ciprofloxacin as the control sample against *Candida albicans*. The extract showed activity with a minimum inhibitory concentration (MIC) of 1.6 mg/mL, whereas ciprofloxacin had an MIC of <0.01 mg/mL [19]. The antifungal properties of *E. croceum* extracts demonstrate the species' potential as an herbal remedy against fungal and microbial diseases.

Steenkamp et al. used broth microdilution and plate-hole diffusion procedures to test the antifungal activity of *E. transvaalense* bark water and methanol extracts against an ATCC 10231 (*Candida albicans*) standard strain and five clinical isolates with amphotericin B as the control sample. Only the methanol extract had an MIC of 20.2 mg/mL against the reference strain (ATCC 10231), but the control sample amphotericin B suppressed the development of all tested organisms with an MIC of <10 mg/mL [65]. Using the agar diffusion and microdilution procedures, Samie et al. examined the antifungal properties of *E. transvaalense* bark of *hexane* and acetone extracts against *Cryptococcus neoformans*, *Candida albicans*, and *Candida krusei* with flucytosine and nystatin as positive controls. Only hexane extract showed activity with inhibition zones ranging from 8 to 16 mm, compared to 22 mm for both nystatin and flucytosine, the two positive controls. The minimum inhibitory concentration values against the organisms examined spanned from 0.5 mg/mL to 1.9 mg/mL, with MIC values of 0.2 µg/mL and 1.9 µg/mL for the positive controls, nystatin, and flucytosine, respectively. Minimum fungicidal concentrations (MFC) varied from 1.9 mg/mL to 7.5 mg/mL in this study. The duration research showed that the hexane extract had a strong time-dependent fungicidal activity against *Candida albicans*, killing >90% of all organisms at a dosage of 1.9 mg/mL following a ten-hour gestation [66]. Using the serial broth microdilution assay, Mamba et al. assessed the antifungal activities of *E. transvaalense* bark ethanol extracts and the compounds 4′-O-methyl-epigallocatechin, lup-20(29)-ene-30-hydroxy-3-one, and lup-20(30)-ene-3,29-diol isolated from the plant against

Candida albicans. The compounds and extracts had minimum inhibitory concentration values that range from 3.1 mg/mL to 12.5 mg/mL [19], indicating that they were active. These antifungal properties back up the species' usage as a medicinal herb in South Africa, where it was used to treat rashes, candidiasis, and skin infections [10,25].

4.6. Anti-HIV Activity

Prinsloo et al. tested the anti-HIV effects of *E. croceum* stem bark by measuring signaling pathway inhibition in the MT-2 VSV-pseudotyped and HeLa-TAT-Luc recombinant virus tests. At 100 ng/mL, the extracts inhibited signaling pathways effectively [67]. Mamba et al. used an RT (non-radioactive HIV-reverse transcriptase) colorimetric test with doxorubicin as a standard drug to assess the anti-HIV activity of *E. croceum* ethanol bark extract against recombinant HIV-1 enzyme. The extract had a lower inhibitory activity of 30.2%, whereas doxorubicin, a positive control, had a 96.5% inhibitory activity [19]. The anti-HIV effects of *E. croceum* extracts and the substance digitoxigenin-glucoside identified from the plant support the plants' traditional use in South Africa to treat HIV opportunistic infections [19].

E. schlechteranum 80% MeOH extract and 4-O-Methylgallocatechin-(48)-4-O-methylepigallocatechin isolated from the extract were evaluated against HIV-1 (strain IIIB) and HIV-2 (strain ROD) [68,69]. The anti-HIV testing and cytotoxicity evaluation of the fractions in MT-4 cells (expressing HTLV-1 Tax and permissive for replication of an HIV-1 gp41 mutant lacking the cytoplasmic tail) revealed that polyphenolic chemicals are responsible for at least some of the anti-HIV-1 action. HIV-1 reverse transcriptase and HIV-1 integrase were suppressed by *E. schlechteranum* [70]. The anti-HIV drug was discovered to be digitoxigenin-3-O-glucoside, a cardiac glycoside. Regardless of the fact cardiac glycosides are recognized for their toxicity, which may be connected to their anti-HIV effect, this chemical showed just a slight anticancer activity (20% suppression on Vero cells at 25 µg/mL) [57,67]. At 100 ng/mL, approximately 90% of the recombinant virus was inhibited.

Using the anti-HIV-1_{iiiB} test, Morobe et al. assessed the anti-HIV activity of *E. transvaalense* bark methanolic extracts. At half maximum dose levels (EC_{50}) of 0.1 µg/mL and 0.2 µg/mL, the extract suppressed HIV-1_{iiB} [62]. Tshikalange et al. tested the anti-HIV activity of *E. transvaalense* stem bark of 70% acetone, ethyl acetate, and chloroform extracts by measuring suppression of viral proteins, α-glycohydrolase, reverse transcriptase Tat (Transactivator of transcription), and NF-kB (Nuclear Factor Kappa B), using mesuol as a standard drug. The extracts had no inhibitory activity against α -glycohydrolase in an *in vitro* experiment, while the ethyl acetate and chloroform extracts had high inhibitory effects of 76% and 64%. The extracts demonstrated no inhibitory action against α -glycohydrolase in an *in vitro* experiment. Still, the chloroform and ethyl acetate extracts had significant inhibitory effects of 64% and 76%, correspondingly, in the NF-kB assay at the lowest dosage tested (1 µg/mL). At 15 µg/mL, chloroform and ethyl acetate extracts had strong activity of 73 and 75%, respectively, while 70% acetone extract had a lower activity (54%). In the case of cell death percentage, all the three extracts (70% acetone, chloroform, and ethyl acetate) showed less cell fatality percentages against the MT2 cell line at the highest dose (15 µg/mL), ranging from 17.1% to 27.6% [71]. Using the anti-HIV-1_{iiiB} test, Mthethwa et al. assessed the anti-HIV properties of *E. transvaalense* bark extracts. With a half-maximum effective concentration (EC_{50}) of 3.5 µg/mL, the extract was shown to suppress HIV-1iiB [63]. To assess the anti-HIV activities of *E. transvaalense* bark ethanol extracts and the 4′-O-methyl-epigallocatechin, lup-20(30)-ene-3,29-diol, and lup-20(29)-ene-30-hydroxy-3-one, Mamba et al. used the non-radioactive HIV-RT colorimetric assay with doxorubicin as a standard drug. The standard drug had a high inhibitory activity of 96.5%, 4′-O-methyl-epigallocatechin had a moderate inhibitory activity of 63.7%, and the ethanolic extract had a low inhibitory activity of 20% [19]. Using the reverse transcriptase (RT) test, Sigidi et al. assessed the anti-HIV effects of the *E. transvaalense* bark aqueous extract. The extract demonstrated inhibition

levels ranging from 25% to 40% [68]. These anti-HIV actions support the usage of the plant as a traditional medication in South Africa to treat HIV opportunistic infections [72,73].

4.7. Anti-plasmodial Activity

Using the parasite lactate dehydrogenase test, Nethengwe et al. examined the anti-plasmodial effects of *E. transvaalense* bark of dichloromethane, methanolic, and aqueous extracts against *Plasmodium falciparum* the chloroquine-sensitive strain of (D10). Excluding dichloromethane, which had an IC_{50} of 5.1 µg/mL, the other extracts were inactive [38]. These findings supported the idea that *E. transvaalense* might be a source of antimalarials and, to a certain extent, back up the species' historical usage as an herbal treatment for fever and malaria [38].

4.8. Larvicidal Activities

Using the mosquito larvicidal assay of *Culex quinquefascitus* larvae, Nethengwe et al. examined the larvicidal properties of *E. transvaalense* bark of dichloromethane, methanolic, and aqueous extracts. The percentage mortality of *Culex quinquefascitus* fourth instar larvae revealed that aqueous extracts (35%) had the least larvicidal action, followed by methanol (47%) and dichloromethane (60%). Methanol and dichloromethane extracts had IC_{50} values of 9.8 µg/mL and 18.2 µg/mL, respectively [38]. These data confirmed the utilization of *E. transvaalense* as an anti-malarial herbal medication [38].

4.9. Anti-pyretic Activities

Nethengwe et al. investigated the anti-pyretic effects of *E. transvaalense* bark of methanolic and dichloromethane extracts in male and female Sprague-Dawley rats, using paracetamol as a control medication. The extracts reduced pyrexia in the provoked rats. Their effects were concentration and time course-dependent, with the extracts exhibiting action as soon as thirty minutes, even at the least dose of 100 mg/kg. The activity of the methanol extract was equivalent to that of paracetamol, the reference medication [38]. These data reinforce the use of *E. transvaalense* as a fever-fighting herbal medication.

4.10. Hypoglycaemic Activity

The inhibitory effects of *E. transvaalense* stem bark acetone extract on carbohydrate-hydrolysing enzymes α-glucosidase and α-amylase on hypoglycaemic activity were researched by Deutschländer et al. By assessing glucose absorption, the acetone extracts were tested against Chang liver, C2C12 myocyte, and 3T3-L1 preadipocyte cells. At 50 µg/mL concentration, the extracts demonstrated a 138.6% potential to reduce blood glucose levels in 3T3-L1 preadipocytes in an *in vitro* experiment. The extracts' 50% IC_{50} for α-glucosidase and α-amylase were reported to be 50.6 µg/mL and 1.1 µg/mL, correspondingly [60]. These results demonstrate the use of *E. transvaalense* as an antidiabetic herbal medication [60].

4.11. Anti-arthritic Activity

Using an anti-protein denaturation experiment, Elisha et al. examined the anti-arthritic effects of *E. croceum* acetone leaves extract. In an *in vitro* anti-arthritic test, the extract displayed an amount of the drug response, with an IC_{50} value of 80.0 µg/mL, greater than the positive control diclofenac sodium's IC_{50} value of 32.4 µg/mL [36]. The extracts' promising properties back up the species' longstanding use for inflammatory diseases [36].

4.12. Anti-diabetic Activity

In an alloxanized rat model, Lanjhiyana et al. investigated the anti-diabetic effect of stem bark methanolic extract of *E. glaucum* [20]. The goal of the investigation was to quantify the total phenolic content of ED methanolic extract (MED) and assess its antidiabetic potential in normal and alloxan-induced diabetic rats. The trial employed inbred adult male Charles-Foster (CF) albino rats for antidiabetic activity in OGTT and nondiabetic rats, as well as antidiabetic activity in alloxan-induced rats. MED responded positively for

carbohydrates, flavonoids, alkaloids, tannins saponins, triterpenes, and sterols, according to phytochemical analysis. The MED also revealed a total phenolic content of 285.2 mg/g. In diabetic control experimental rats, the increasing level of glycosylated hemoglobin (HbA1c) is exactly proportionate to the reduced level of total hemoglobin. For assessing the degree of protein glycation during diabetes mellitus, glycosylated hemoglobin (HbA1c) is utilized as the most accurate marker and standard diagnostic technique. Protein glycation is a non-enzymatic process that occurs when excess glucose in the blood reacts with free amino groups on hemoglobin's globin component. The HbA1c level is used to determine long-term glycemic status and to connect with different problems associated with diabetes. In experimental rats, oral treatment with MED dramatically reduced HbA1c levels, probably due to normoglycemic control mechanisms, which also reflected lower protein glycation condensation reactions, and the results were consistent with prior findings [20]. The continuing post-treatment with MED for 21 days demonstrated potential hypoglycemic action in OGTT and normoglycemic rats, as well as antidiabetic activity in alloxan-induced rat models, according to the findings. This suggests that plants may have an insulin-like function, which might assist to lower the risk of lipid-related problems. Significant lipid management may help to prevent the coexistence of hypercholesterolemia and hypertriglyceridemia, as well as lower cardiovascular risk factors [20]. In an alloxan-induced diabetes model, MED was discovered to be a promising antidiabetic extract by lowering oxidative damage and regulating antioxidant enzymes in a dose-dependent way. In the future, isolation and establishment of the exact mechanism of action of particular chemicals derived from MED will be carried out [20].

5. Future Prospects

The pharmacokinetic properties of *Elaeodendron* species, including their antioxidant, hepatoprotective, neuroprotective, cytotoxic, anti-diabetic, and anti-inflammatory potential, have received extensive research. However, most of these studies have concentrated on the *in vitro* evaluation of biological activities, and relatively few studies have used *in vivo* models that concentrated on the specific mechanism of action. Similar to research conducted on the screening of biological activities, studies that focus on the bioactivity-guided separation and identification of the active components are likewise scarce. These research gaps should be filled by further investigations. Designing research to demonstrate their applicability to conventional therapeutic usage is also expected as these are some of the most commonly utilized crude drugs in traditional therapeutic uses. Similar to this, future research should also concentrate on the pharmacokinetics of active substances and thoroughly examine any potential interactions between them and other natural products or manufactured medications.

6. Conclusions

Elaeodendron species are commonly utilized in herbal medicine worldwide for a variety of well-known medicinal purposes. Antiviral, anti-HIV, anticancer, antiproliferative, antioxidant, anti-inflammation, anti-plasmodial, cytotoxic, antifungal, anti-arthritic, and antibacterial activities have been discovered in them. Different phytochemicals, whose primary components include flavonoids, terpenoids, cardiac glycosides, and cardenolides, exert these diverse biological activities. *Elaeodendron* has a lot of promise for human health, and its medicinal benefits should be studied further thoroughly. *Elaeodendron* species have been the topic of phytochemical and pharmacological study over the past 30 years. However, there is still insufficient evidence to link the species' therapeutic benefits to their phytochemical and pharmacological features. Detailed pharmacokinetic, *in vivo*, and clinical trials investigations utilizing chemicals identified from *Elaeodendron* and extracts from the plants are needed. The discovery of this bioactive compounds and their pharmacological activity should be a subject of research involving physiological pathways with identification of various cell signalling targets, molecular mechanisms of action, and pharmacokinetics. Randomized clinical trials, experimental animal research, and target-organ

toxicity studies should be included in these investigations. Because the bark of *Elaeodendron* species is known to be toxic, extensive toxicological analyses are required to tread a fine line between the therapeutic promise and the species' unfavorable and harmful consequences. There is insufficient knowledge on the toxicological qualities of *Elaeodendron* species, such as if they produce minor pain or major toxicity when used as traditional remedies. In the absence of such comprehensive toxicological studies, patients should utilize *Elaeodendron* species as herbal preparations with concern since they have the possibility to trigger long-term harm.

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