

## Article

# Antioxidant and Antibacterial Activities of the Leaf and Stem Extracts of *Combretum molle* (R. Br. ex G. Don.) Engl. & Diels

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**Abstract:** Medicinal plants offer reasonable and accessible alternatives to synthetic drugs and are often devoid of the adverse side effects, toxicity, and pathogenic resistance associated with synthetic medicine. *Combretum molle* has been utilized in African traditional medicinal practices and purportedly contains bioactive compounds with medicinally beneficial effects. This study investigated the hexane, chloroform, and methanol leaf and stem extracts for their antioxidant properties using the 2,2'-diphenyl-1-picrylhydrazyl radical scavenging and ferric-reducing antioxidant power assays. The study additionally analyzed the methanol extracts for their antibacterial activity against Gram-negative *Escherichia coli* (ATCC 25922) and Gram-positive *Staphylococcus aureus* (ATCC 25923) bacteria using agar well diffusion. Relative to the scavenging activity of the ascorbic acid control ( $79.15 \pm 0.63\%$  at  $15 \mu\text{g/mL}$  to  $94.61 \pm 0.12\%$  at  $240 \mu\text{g/mL}$ ), the plant's radical scavenging activities were exceptionally high in the methanolic leaf and stem extracts ( $p < 0.05$ ), ranging from  $94.58 \pm 1.10\%$  at  $15 \mu\text{g/mL}$  to  $99.22 \pm 0.30\%$  at  $240 \mu\text{g/mL}$  and  $91.57 \pm 1.71\%$  at  $15 \mu\text{g/mL}$  to  $99.60 \pm 0.20\%$  at  $240 \mu\text{g/mL}$ , respectively, suggesting a strong capacity to donate hydrogen ions. High scavenging activities were additionally observed in the chloroform stem ( $78.68 \pm 1.18\%$  at  $15 \mu\text{g/mL}$  to  $98.14 \pm 1.22\%$  at  $240 \mu\text{g/mL}$ ) and hexane leaf ( $72.12 \pm 4.38\%$  at  $15 \mu\text{g/mL}$  to  $89.87 \pm 1.50\%$  at  $240 \mu\text{g/mL}$ ) extracts ( $p < 0.05$ ). All extracts exhibited poor ferric-reducing abilities in relation to the gallic acid control ( $100 \pm 0.00\%$ ) at all concentrations ( $p < 0.05$ ). The leaf and stem extracts exhibited broad-spectrum antibiotic capabilities against both tested strains, with significant activity at higher concentrations ( $p < 0.05$ ). Overall, both the leaf and stem extracts of *C. molle* exhibited similar antioxidant and antibacterial activities. These findings warrant further pharmacological research on *C. molle* for potential drug development.

**Keywords:** medicinal plants; radical scavenging activity; ferric-reducing antioxidant power; agar well diffusion



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## 1. Introduction

Early civilizations relied on herbal and traditional medicinal practices to treat their medical conditions [1]. Medicinal plant consumption is currently increasing in both traditional and modern practices [2]. In developing African countries, people from disadvantaged and resource-poor communities benefit from the use of medicinal plants, which are relatively cheaper and more accessible than modern medicines [3,4]. Importantly, some synthetic, modern medicines have been noted to cause adverse side effects [5,6], while others have low potencies against certain pathogens [7,8]. In addition, the indiscriminate use of antibiotics has led to numerous bacterial strains developing resistance to synthetic antimicrobial agents [7–9]. Therefore, alternate sources of pharmacologically active compounds are required to effectively treat diseases, resulting in research intensification on

medicinal plant species [10]. Plants contain a large array of bioactive phytochemicals [11] that have been used in medical treatment since historical times [12,13]. Several plant species within the pantropical, medicinal family, Combretaceae R. Br., particularly the genus *Combretum* Loefl., have been purportedly used in African traditional medicinal practices to alleviate symptoms and treat disease [14–16]. The extracts of one such species, *Combretum molle* (R. Br. ex G. Don; Engl. and Diels [velvet bushwillow]), has been reported to treat illnesses associated with oxidation and bacterial infection [14,17,18]. This species' medicinal properties are conceivably attributed to several bioactive phytochemicals that interact synergistically to produce these medicinal effects [19]. Previously isolated bioactive compounds that provide *C. molle* with its antioxidant activities include gallic acid [15] and punicalagin [20], whereas compounds such as Combretenes A and B [21], mollic acid glucoside [22], combregenin, arjungenin, and combreglucoside [20,23] are known to exert antibacterial effects.

Antioxidants are produced endogenously by mammalian bodies to mitigate free-radical damage and prevent oxidative stress caused by increased reactive oxygen species (ROS) [24,25]. Damage by ROS leads to a loss of cell structure and function, inherently causing disease [10,26]. When ROS production exceeds the body's natural antioxidant capacity, exogenous sources are needed to combat oxidative damage [27]. A number of synthetic antioxidants have been shown to produce toxic and mutagenic effects, leading to plant-based compounds being investigated for their abilities to scavenge free radicals and reduce their damage [10]. Several species of the genus *Combretum* have been reported to possess antioxidant activities [28–31]. Koevi et al. [28] showed that ethanolic leaf extracts of *C. molle* presented antiradical activity when tested using the 2,2'-diphenyl-1-picrylhydrazyl and nitric oxide radical scavenging assays. Methanol and acetone extracts of the leaves of *Combretum indicum* and *Combretum racemosum*, respectively, were also reported to produce radical scavenging activities [29,30]. Furthermore, a study on the ethanol extracts of *Combretum albidum* revealed that the plant's ability to reduce oxidative damage was due to elevated superoxide dismutase activity [31].

During bacterial infection, increased ROS production is beneficial in the prevention of the microbial colonization in tissues [8]. However, this ultimately results in an aggravated condition for an infected patient [8]. The adverse side effects of synthetic antimicrobial agents and the increasing resistance of pathogens to current antibiotics is a global health challenge [8,32]. There have been a number of investigations on the antibacterial efficacies of *Combretum* species. Chukwujekwu and Van Staden [33] reported that dichloromethane leaf fractions of *Combretum edwardsii* were highly and moderately effective against *Staphylococcus aureus* (ATCC 11632) and *Escherichia coli* (ATCC 25218), respectively, while ethyl acetate leaf fractions of *Combretum krausii* were more effective against *S. aureus* than *E. coli*. Burman et al. [7] found that aqueous and ethanolic leaf extracts of *Combretum album* showed significant activity against several bacterial strains, including *Pseudomonas aeruginosa* (MTCC 2453), *Bacillus subtilis* (MTCC 441), and *E. coli* (MTCC 739). Acetone, ethyl acetate, and dichloromethane extracts of *C. molle* leaves were found to inhibit the growth of *Enterococcus faecalis* (ATCC 29212), *S. aureus* (ATCC 29213), *E. coli* (ATCC 25922), and *P. aeruginosa* (ATCC 27853) [34].

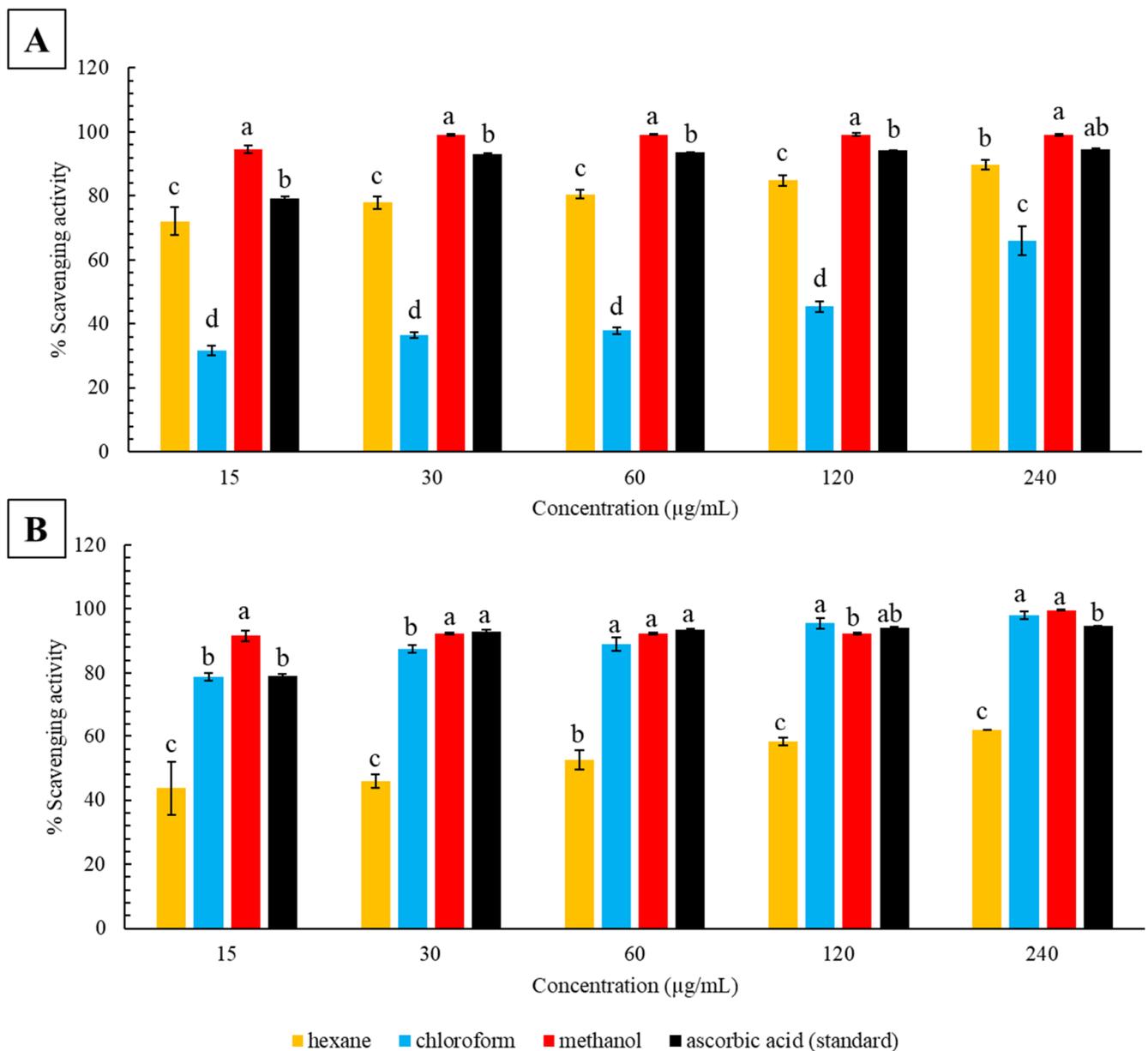
Despite there being several studies on the bioactivities of the plant's leaf extracts, there is a paucity of studies conducted on the stem extracts of *C. molle*. In order to advance the traditional use of plant extracts into modern drug development, there is a need to critically investigate the biological activities of medicinal plant species. Since various species of *Combretum* have been reported to exhibit antioxidant and antibacterial activities, and due to the plethora of bioactive compounds present within *C. molle*, it is imperative to further research and compare this plant's leaf and stem bioactivities when extracted with various solvents. This study aimed to determine the antioxidant potential of hexane, chloroform, and methanol leaf and stem extracts of *C. molle* using the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and the ferric (Fe<sup>3+</sup>)-reducing antioxidant power (FRAP) assays. Additionally, this study aimed to evaluate the antibacterial efficacy of the methanol leaf

and stem extracts against clinical strains of *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) bacteria using the agar well-diffusion technique. To the best of our knowledge, this study is the first report that compares the plant's DPPH scavenging activity with its ferric-reducing abilities.

## 2. Results

### 2.1. DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of all extracts (leaves and stems extracted in hexane, chloroform and methanol) of *C. molle* were dose-dependent and increased with increasing concentrations (15 to 240  $\mu\text{g}/\text{mL}$ ) (Figure 1). At each concentration, the scavenging activity of the ascorbic acid standards increased from  $79.15 \pm 0.63\%$  at 15  $\mu\text{g}/\text{mL}$  to  $93.00 \pm 0.41$ ,  $93.62 \pm 0.07$ ,  $94.18 \pm 0.11$ , and  $94.61 \pm 0.12\%$  from 30 to 240  $\mu\text{g}/\text{mL}$ , respectively. Leaves extracted in chloroform produced the lowest radical scavenging activity at all concentrations ( $31.69 \pm 1.39$ ,  $36.51 \pm 0.94$ ,  $37.88 \pm 1.06$ ,  $45.39 \pm 1.67$  and  $65.86 \pm 4.51\%$ ), followed by higher activities in the hexane ( $72.12 \pm 4.38$ ,  $77.86 \pm 2.01$ ,  $80.51 \pm 1.42$ ,  $84.81 \pm 1.75$ , and  $89.87 \pm 1.50\%$ ) and methanol ( $94.58 \pm 1.10$ ,  $98.96 \pm 0.28$ ,  $99.18 \pm 0.24$ ,  $99.20 \pm 0.57$ , and  $99.22 \pm 0.30\%$ ) extracts, respectively (Figure 1A). Relative to the standard, the hexane leaf extracts from 15 to 120  $\mu\text{g}/\text{mL}$  were significantly lower ( $p < 0.05$ ), indicating moderate antioxidant activity, whereas stronger activity was observed at 240  $\mu\text{g}/\text{mL}$  where the extract was comparable to the standard ( $p > 0.05$ ). At all concentrations, the chloroform leaf extracts were significantly lower than the ascorbic acid standards and demonstrated weak-to-moderate scavenging activity with increasing concentration ( $p < 0.05$ ). The methanol leaf extracts were significantly greater than the standard from 15 to 120  $\mu\text{g}/\text{mL}$  ( $p < 0.05$ ) and exhibited similarly elevated scavenging activity at 240  $\mu\text{g}/\text{mL}$  ( $p > 0.05$ ). Within each concentration, each leaf extract produced significantly different radical scavenging activities based on extraction solvent ( $p < 0.05$ ). Stems extracted in hexane produced the lowest activities ( $43.73 \pm 8.22$ ,  $45.96 \pm 2.05$ ,  $52.53 \pm 2.96$ ,  $58.19 \pm 1.26$ , and  $62.03 \pm 0.06\%$ ), followed by those extracted in chloroform ( $78.68 \pm 1.18$ ,  $87.54 \pm 1.28$ ,  $89.03 \pm 2.06$ ,  $95.50 \pm 1.66$ , and  $98.14 \pm 1.22\%$ ) and methanol ( $91.57 \pm 1.71$ ,  $92.34 \pm 0.39$ ,  $92.34 \pm 0.32$ ,  $92.39 \pm 0.26$ , and  $99.60 \pm 0.20\%$ ), respectively (Figure 1B). However, the stems extracted in chloroform at 120  $\mu\text{g}/\text{mL}$  produced significantly greater radical scavenging activity than the methanol extract ( $p < 0.05$ ). When compared to the activity of the control, stems extracted in hexane were significantly lower, suggesting weak scavenging activity ( $p < 0.05$ ). The chloroform extracts were significantly lower than the ascorbic acid control at 30  $\mu\text{g}/\text{mL}$  ( $p < 0.05$ ) and comparable at all other concentrations ( $p > 0.05$ ), representing moderate-to-strong activity with increasing concentrations. Scavenging activity of the stems extracted in methanol was significantly greater than the controls at 15 and 240  $\mu\text{g}/\text{mL}$  ( $p < 0.05$ ) and comparable at all other concentrations ( $p > 0.05$ ), suggesting strong antioxidant activity at all concentrations. Within each concentration, each solvent produced significant differences in radical scavenging activity ( $p < 0.05$ ), except for the chloroform and methanol stem extracts at 60 and 240  $\mu\text{g}/\text{mL}$  ( $p > 0.05$ ). Overall, the leaves extracted in hexane produced significantly higher radical scavenging activities than the stems ( $p < 0.05$ ). In contrast, stems extracted in chloroform demonstrated significantly higher activities ( $p < 0.05$ ). In the methanol extracts, leaves were significantly higher from 30 to 120  $\mu\text{g}/\text{mL}$  ( $p < 0.05$ ), whereas at 15 and 240  $\mu\text{g}/\text{mL}$ , stem extracts presented significantly higher radical scavenging activities ( $p < 0.05$ ).



**Figure 1.** DPPH radical scavenging activity (%) of increasing concentrations (15, 30, 60, 120, and 240 µg/mL) of hexane, chloroform, and methanol extracts of *C. molle* (A) leaves and (B) stems. Values represent the mean ± standard deviation of three replicates. Different letters (a–d) indicate significant differences in scavenging activity between different solvents within each concentration ( $p < 0.05$ ).

For each extract, the concentration of antioxidants needed to scavenge the initial DPPH radicals by 50% are reported in Table 1. Methanol leaf and stem extracts had exceptionally low  $IC_{50}$  values of  $1.52 \times 10^{-7} \pm 2.93 \times 10^{-8}$  and  $2.46 \times 10^{-14} \pm 8.53 \times 10^{-15}$  µg/mL, respectively (Table 1). Low  $IC_{50}$  values were additionally observed in the ascorbic acid standard, followed by the chloroform stem extract, which additionally presented high scavenging activity, and the hexane leaf extract, which exhibited moderate activity. The chloroform leaf and hexane stem extracts produced elevated  $IC_{50}$  values, suggesting poor scavenging capacities. Differences among all extracts were non-significant except for the hexane stem, which displayed significantly higher  $IC_{50}$  values ( $p < 0.05$ ).

**Table 1.** IC<sub>50</sub> values (µg/mL) of the DPPH radical scavenging activities of leaf and stem hexane, chloroform and methanol extracts of *C. molle*.

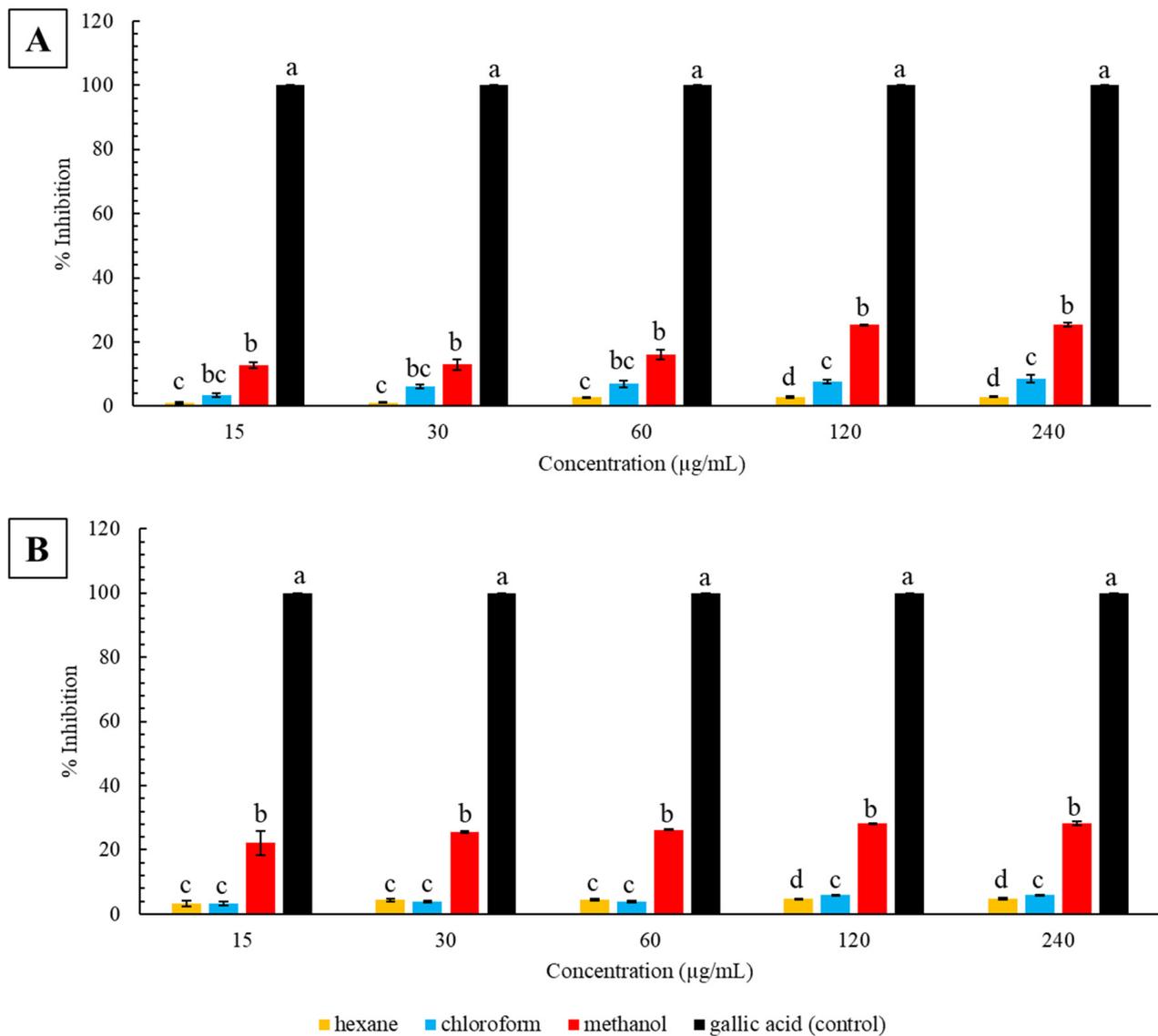
Extract	Solvent	IC <sub>50</sub> (µg/mL)
Leaf	Hexane	42.57 ± 9.88 <sup>b</sup>
	Chloroform	118.12 ± 28.07 <sup>b</sup>
	Methanol	1.52 × 10 <sup>-7</sup> ± 2.93 × 10 <sup>-8b</sup>
Stem	Hexane	1373.30 ± 479.44 <sup>a</sup>
	Chloroform	0.22 ± 0.09 <sup>b</sup>
	Methanol	2.46 × 10 <sup>-14</sup> ± 8.53 × 10 <sup>-15b</sup>
Ascorbic acid (standard)		0.01 ± 0.00 <sup>b</sup>

Values presented are means ± standard deviation of three replicates. Values followed by different letters are significantly different at  $p < 0.05$ .

## 2.2. Ferric-Reducing Antioxidant Power

The ferric-reducing abilities of all *C. molle* extracts (leaves and stems extracted in hexane, chloroform, and methanol) were dose-dependent and increased with increasing concentrations from 15 to 240 µg/mL (Figure 2). The gallic acid controls had 100 ± 0.00% ferric-reducing abilities at all tested concentrations. The reducing abilities (percent inhibition of oxidation) of the leaf extracts are illustrated in Figure 2A. A low ferric-reducing ability was observed in leaves extracted in hexane (1.03 ± 0.19, 1.11 ± 0.18, 2.70 ± 0.11, 2.74 ± 0.30, and 2.91 ± 0.16%), followed by minor increases in the chloroform (3.51 ± 0.58, 6.13 ± 0.63, 6.99 ± 0.99, 7.65 ± 0.63, and 8.54 ± 1.28%) and methanol (12.77 ± 0.97, 13.01 ± 1.69, 16.07 ± 1.59, 25.33 ± 0.13 and 25.37 ± 0.66%) extracts, respectively. Relative to the gallic acid controls, all leaf extracts were significantly lower, indicating poor reducing abilities ( $p < 0.05$ ). Within each concentration, oxidation inhibition for the leaf extracts was significantly different ( $p < 0.05$ ). However, from 15 to 60 µg/mL, inhibition by the chloroform extracts were comparable to the hexane and methanol extracts ( $p > 0.05$ ). The reducing power of the stem extracts are presented in Figure 2B. The stems extracted in hexane had the lowest oxidation inhibition (3.27 ± 0.98, 4.46 ± 0.47, 4.49 ± 0.24, 4.75 ± 0.14, and 4.86 ± 0.32%), followed by the chloroform (3.39 ± 0.62, 3.88 ± 0.38, 3.93 ± 0.21, 5.87 ± 0.28, and 5.98 ± 0.06%) and methanol (22.15 ± 3.73, 25.57 ± 0.37, 26.31 ± 0.15, 28.17 ± 0.16, and 28.22 ± 0.55%) extracts, respectively. However, at 30 and 60 µg/mL, hexane extracts had a higher inhibition than the chloroform extracts. In relation to the inhibition of the gallic acid controls, all extracts were significantly lower ( $p < 0.05$ ), indicative of poor reducing abilities. Within each concentration, inhibition within the stem extracts was significantly different ( $p < 0.05$ ). However, hexane and chloroform extracts were not significantly different from one another from concentrations of 15 to 60 µg/mL ( $p > 0.05$ ). Overall, hexane and methanol stem extracts exhibited significantly higher inhibition compared to leaf extracts ( $p < 0.05$ ). In contrast, the chloroform leaf extracts exhibited significantly higher inhibition from 30 to 240 µg/mL ( $p < 0.05$ ), and similarly higher inhibition at 15 µg/mL ( $p > 0.05$ ).

The concentration of antioxidants in the extracts required to inhibit oxidation by 50% (IC<sub>50</sub>) are presented in Table 2. All extracts produced elevated IC<sub>50</sub> values ranging from 663.77 ± 74.71 µg/mL in the methanol leaf extract to 8358.53 ± 5514.41 µg/mL in the hexane stem extract. The hexane stem extract was significantly higher than both the methanol leaf extract and the gallic acid control ( $p < 0.05$ ), while the values for all other extracts were similar ( $p > 0.05$ ). The high IC<sub>50</sub> values indicated low reducing abilities relative to the gallic acid control, which produced a low IC<sub>50</sub> of 0.53 ± 0.16 µg/mL, indicating high inhibitory activity.



**Figure 2.** Ferric-reducing antioxidant power (% inhibition) of increasing concentrations (15, 30, 60, 120, and 240 µg/mL) of hexane, chloroform, and methanol extracts of *C. molle* (A) leaves and (B) stems. Values represent the mean  $\pm$  standard deviation of three replicates. Different letters (a–d) indicate significant differences in inhibition between different solvents within each concentration ( $p < 0.05$ ).

**Table 2.** IC<sub>50</sub> values (µg/mL) of the ferric-reducing antioxidant power of the leaf and stem hexane, chloroform, and methanol extracts of *C. molle*.

Extract	Solvent	IC <sub>50</sub> (µg/mL)
Leaf	Hexane	6292.55 $\pm$ 795.31 <sup>cd</sup>
	Chloroform	2948.11 $\pm$ 868.55 <sup>cd</sup>
	Methanol	663.77 $\pm$ 74.71 <sup>bd</sup>
Stem	Hexane	8358.53 $\pm$ 5514.41 <sup>ac</sup>
	Chloroform	2602.06 $\pm$ 1856.93 <sup>cd</sup>
	Methanol	1368.11 $\pm$ 424.54 <sup>cd</sup>
Gallic acid (control)		0.53 $\pm$ 0.16 <sup>bd</sup>

Values presented are means  $\pm$  standard deviation of three replicates. Values followed by different letters are significantly different at  $p < 0.05$ .

### 2.3. Antibacterial Activity

Based on the antioxidant activities, stems and leaves of *C. molle* extracted in methanol displayed higher scavenging activity ( $p < 0.05$ ) than the other extracts and were, hence, chosen for the antibacterial assay. The results of the antibacterial assay revealed that the inhibition of both *E. coli* and *S. aureus* growth notably increased as the leaf and stem methanol extract concentrations increased from 0.625 to 10 mg/mL (Table 3). No inhibition was observed for the negative controls. Relative to the antibiotic positive controls (10 µg/mL of gentamicin and streptomycin), the inhibition of bacterial growth by the leaf extracts was significantly lower against *E. coli* and *S. aureus* from 0.625 to 2.5 mg/mL ( $p < 0.05$ ). However, at 5 and 10 mg/mL, the inhibition was similar to the controls, suggesting an effective inhibitory activity against both strains at these concentrations ( $p > 0.05$ ). At 10 mg/mL, *S. aureus* exhibited higher susceptibility to the leaf extract than to streptomycin, producing a greater inhibition zone ( $14.50 \pm 1.08$  mm) than the antibiotic ( $12.33 \pm 1.25$  mm). When compared to the antibiotics, the antibacterial activity of the stem extracts was significantly lower from 0.625 to 5 mg/mL ( $p < 0.05$ ). However, at 10 mg/mL, bacterial inhibition was comparable to the controls, indicating effective inhibitory activity against both *E. coli* and *S. aureus* ( $p > 0.05$ ). For both strains, the leaf and stem extracts produced similar inhibition zones at all concentrations ( $p > 0.05$ ). However, at 2.5 mg/mL, the leaf extract against *E. coli* produced significantly higher antibacterial activity than the stem extract ( $p < 0.05$ ). Furthermore, the leaf and stem extracts exhibited similar activity against both strains ( $p > 0.05$ ), although *S. aureus* was significantly more susceptible to the leaf extract at 10 mg/mL than *E. coli* ( $p < 0.05$ ).

**Table 3.** Zones of inhibition (mm) of the methanolic leaf and stem extracts of *C. molle* against Gram-negative *E. coli* and Gram-positive *S. aureus* bacteria.

Bacterial Strain	Extract Concentration (mg/mL)					Positive Control (10 µg/mL)
	0.625	1.25	2.5	5	10	
	Leaf					
<i>E. coli</i>	6.67 ± 0.24 *	7.33 ± 1.18 *	7.50 ± 1.08 *†	10.00 ± 1.22	12.00 ± 0.00 §	12.33 ± 0.47
<i>S. aureus</i>	7.00 ± 0.00 *	7.00 ± 0.00 *	8.67 ± 0.24 *	11.00 ± 0.00	14.50 ± 1.08 §	12.33 ± 1.25
	Stem					
<i>E. coli</i>	6.33 ± 0.24 *	7.00 ± 0.00 *	7.00 ± 0.41 *†	9.67 ± 0.47*	11.83 ± 0.62	12.67 ± 0.47
<i>S. aureus</i>	7.17 ± 0.62 *	7.17 ± 0.62 *	7.50 ± 1.08 *	10.33 ± 0.47*	12.33 ± 0.47	13.33 ± 0.94

Values presented are means ± standard deviation of three replicates. *E. coli* = *Escherichia coli* (ATCC 25922); *S. aureus* = *Staphylococcus aureus* (ATCC 25923); positive controls: 10 µg/mL of gentamicin (Gram-negative antibiotic) and 10 µg/mL of streptomycin (Gram-positive antibiotic); \* indicates significant differences in inhibition relative to the respective control; † indicates significant differences in inhibition between different extracts against the same strain; § indicates significant differences in inhibition between the same extract against different strains; ( $p < 0.05$ ).

## 3. Discussion

### 3.1. Antioxidant Activity

The antioxidant abilities of the leaf and stem extracts of *C. molle* were assessed using the spectrophotometric DPPH radical scavenging and FRAP inhibition assays. These assays provided insight on the extracts' abilities to scavenge ROS, reduce oxidative substances, and, thus, inhibit oxidation [35,36]. In this study, the DPPH assay was based on the ability of the *C. molle* leaf and stem extracts to donate a hydrogen ion to the DPPH radical, essentially reducing it from 2,2'-diphenyl-1-picrylhydrazyl to 2,2'-diphenyl-1-picrylhydrazine (DPPH-H) [37–39]. The data from the assay revealed that a number of the analyzed extracts of *C. molle* had significant antioxidant activities, and that higher extract concentrations produced higher activities (Figure 1). The dose-dependent nature of the plant's antioxidant activity is possibly due to higher amounts of phytochemicals with radical scavenging abilities occurring at higher extract concentrations [40]. At the tested concentrations of 15 to 240 µg/mL, scavenging activity of the methanol leaf and stem extracts were signifi-

cantly higher or comparable to the ascorbic acid standards, representing strong antioxidant abilities. Similar scavenging activity within the leaves and stems suggests that similar phytochemical constituents responsible for the antioxidant effects were extracted from these organs. Low  $IC_{50}$  values are indicative of high antioxidant activity. The  $IC_{50}$  values of the methanol extracts supplemented the radical scavenging data with remarkably low values (Table 1). The *C. molle* stem extracted in chloroform was additionally found to be effective in scavenging the DPPH free radicals, with activity ranging from moderate to strong as the concentration increased. At higher concentrations (120 and 240  $\mu\text{g}/\text{mL}$ ), these extracts were more effective than the ascorbic acid controls. Conversely, leaves extracted in chloroform produced low-to-moderate activities, as evident from their correspondingly high  $IC_{50}$  values. In addition, the hexane leaf extracts exhibited moderate-to-high scavenging activities, with similar activity to the ascorbic acid standard at 240  $\mu\text{g}/\text{mL}$ , while stems extracted in hexane produced low activities. Notably, extracts of differing concentrations produced similar activities. This may be due to the extracts containing a favorable number of phytochemicals responsible for the plant's antioxidant properties even at lower concentrations and increasing only slightly with increasing concentrations.

The findings from this study are congruent with other analyses on the scavenging ability of *C. molle* extracts. Rademan et al. [41] found high DPPH radical scavenging activities in *C. molle* leaves and fruit extracted in ethanol, with correspondingly low  $IC_{50}$  values of  $1.9 \pm 0.01$  and  $5.1 \pm 0.05$   $\mu\text{g}/\text{mL}$ , respectively. Furthermore, Koevi et al. [28] investigated the radical scavenging activity of the plant's ethanol leaf extracts but obtained a moderate  $IC_{50}$  value of  $42 \pm 0.07$   $\mu\text{g}/\text{mL}$ . The  $IC_{50}$  value of the leaves in the present study was comparatively low, indicating that methanol extracts may be more effective in scavenging free radicals. The high scavenging abilities present in the methanol extracts may be attributed to the solvent's ability to extract phytochemical constituents such as phenolic compounds and flavonoids, which are associated with producing antioxidant effects [26,40]. Structurally, these compounds comprise an aromatic ring, which enables free radical scavenging by the donation of hydrogen atoms [40,42]. There are various reports of extracts of *C. molle* containing phenolic compounds and flavonoids [15,19,28,43], corroborating these findings. In contrast, Ntshanka et al. [19] found that when compared with the acetone, chloroform, and ethanol fractions of the plant's leaf, methanol fractions presented lower scavenging activities with elevated  $IC_{50}$  values. These deviations may be due to variations in location, climate, harvesting season, and extraction methods [44,45]. Nevertheless, methanol extracts of other species in the genus have displayed high radical scavenging activities. *Combretum apiculatum* subsp. *apiculatum* leaves extracted in methanol were reported to produce significant activities, with a half maximal effective concentration of  $14.5 \pm 0.12$   $\mu\text{g}/\text{mL}$  [46]. Manga [47] revealed that methanol leaf and root bark extracts of *C. racemosum* additionally produced considerably high activities with  $IC_{50}$  values of  $3.00 \pm 0.30$  and  $2.90 \pm 0.40$   $\mu\text{g}/\text{mL}$ , respectively. The investigation further analyzed the scavenging activities of the methanolic leaf extracts of *Combretum celastroides* subsp. *Laxiflorum*, which demonstrated high activities with an  $IC_{50}$  value of  $5.00 \pm 0.10$   $\mu\text{g}/\text{mL}$ . Methanol extracts of the leaves of *C. indicum* produced an  $IC_{50}$  value of 48.87  $\mu\text{g}/\text{mL}$ , indicative of moderate scavenging activity [30]. Moreover, *Combretum micranthum* was analyzed for its DPPH scavenging activity. In addition, a hydroalcoholic leaf extract comprising ethanol and water produced an  $IC_{50}$  of  $2.49 \pm 0.53$   $\mu\text{g}/\text{mL}$  [48]. Effective antioxidant activities from both methanol and ethanol extracts may be attributed to the high polarity of these alcoholic solvents, resulting in the extraction of similar phytochemicals [49,50].

The FRAP assay was used to determine the antioxidant capabilities of the *C. molle* leaf and stem extracts by its ability to donate an electron, causing the reduction of potassium ferricyanide ( $\text{Fe}^{3+}$ ) to potassium ferrocyanide ( $\text{Fe}^{2+}$ ) [51,52]. The results showed that all extracts had low reducing powers, indicative of a low inhibition of oxidation (Figure 2). The extracts were highly ineffective in comparison to the gallic acid control, as substantiated by the elevated  $IC_{50}$  values (Table 2). Regardless of the poor inhibition, *C. molle* leaves and stems extracted in methanol had a significantly higher reducing power than the chloroform

and hexane extracts, corresponding with the findings from the DPPH assay. A recent analysis on ethyl acetate and aqueous fractions of methanol extracts of *C. micranthum* leaves revealed high antioxidant capabilities when analyzed using both DPPH and FRAP assays [53]. These findings are in contrast to the data of the present study, which comprises a large variation in antioxidant activities, involving high scavenging activity but low ferric-reducing abilities. This implies that the *C. molle* leaf and stem extracts were effective in donating hydrogen ions as illustrated by the reduction of DPPH but may not be effective in donating electrons as shown by the poor ferric-reducing abilities. A study on the antioxidant activities of *C. racemosum* revealed comparable findings, with higher DPPH scavenging activity than ferric-reducing ability in methanol, ethyl-acetate, and *n*-butanol extracts of the plant's leaves [54]. Reduction of the ferric ion is dependent on the presence of specific reducing agents within the plant extracts [55,56]. Alternatively, the difference in scavenging activity with ferric-reducing abilities could be due to varied experimental conditions.

### 3.2. Antibacterial Activity

The antibacterial effectiveness of the methanol extracts of *C. molle* were determined using the agar well-diffusion technique. This was based on the ability of the extracts to inhibit bacterial growth upon diffusion into the agarose medium [57]. The inhibition of growth was characterized by a clear zone surrounding the well containing the extract [58,59]. Results from the antibacterial assay of this study revealed that the methanol leaf and stem extracts of *C. molle* exhibited activity against both Gram-negative *E. coli* and Gram-positive *S. aureus* bacteria, with significant activity at higher concentrations (5 and 10 mg/mL) where the activity is similar to the antibiotics; 10 µg/mL of gentamicin and streptomycin were demonstrated (Table 3). The extracts' bacterial inhibition was dose-dependent, possibly attributed to higher quantities of antibacterial-associated phytochemical constituents (e.g., phenolic compounds, flavonoids, and alkaloids) being present at higher extract concentrations [60–62]. However, the dose-dependent antibacterial activity was notably unexceptional, insinuating that the responsible phytochemicals did not increase excessively with increasing extract concentration. Plants with antioxidant activities also possess antibacterial properties [19,63,64] due to specific phytochemicals that augment the plant's antioxidant capabilities; additionally, they are responsible for activity against bacterial pathogens [10,60,65]. Due to their high polarity, there is evidence that methanolic extracts increase the presence of antioxidant- and antibacterial-associated phytochemicals due to possessing a high extraction capacity for these compounds [49,50]. In conjunction with phenolic compounds, flavonoids and alkaloids, tannins, terpenoids, and essential oils are likewise responsible for a plant's antibacterial effects [7,8,66]. Several studies have reported the presence of these compounds in the leaves and stems of *C. molle* [15,19,28,67], substantiating the plant's antibacterial efficacy. There are several probable modes of action in which these phytochemicals produce their effects. However, a likely mechanism may be the alteration of the bacterial membrane permeability, causing cell destruction and a subsequent decrease in pathogenicity [10].

In the present study, at 10 mg/mL, *S. aureus* was more susceptible to the leaf extract than *E. coli*. The extract was additionally more effective than the control antibiotic (streptomycin). Gram-positive bacteria generally have a higher susceptibility to antibiotics than Gram-negative bacteria due to the outer peptidoglycan layer of the cell wall being an ineffective antibiotic barrier [10,68]. In contrast, the outer membrane of Gram-negative bacteria contains a periplasmic space harbouring lipopolysaccharides that more effectively prevent the passage of antibiotics into the cell [10,68]. Nevertheless, the leaf extract's activity against *E. coli* was significant, with similar effectiveness as the antibiotic control (gentamicin). The exhibition of significant antibacterial activity against both Gram-negative and Gram-positive strains suggests the latent use of *C. molle* extracts as a broad-spectrum antibiotic. An antibacterial evaluation by Cock and Van Vuuren [69] on aqueous and methanolic leaf extracts of *C. molle* further revealed the plant's broad-spectrum nature by exhibiting comparable activity against Gram-negative and Gram-positive strains. The

study further reported broad-spectrum capabilities for the leaves of *Combretum cilium*, *Combretum erythrophloeum*, *Combretum erythrophyllum*, *Combretum hereroense*, and *Combretum microphyllum*. Broad-spectrum antibacterial activities were additionally observed in the leaf extracts of *C. album* [7] in flavonoids isolated from *C. erythrophyllum* [63] and in gold nanoparticles synthesized from leaf extracts of *C. erythrophyllum* [70]. Conclusively, *C. molle* extracts in the present study were found to be as effective as the standard, synthetic antibiotics. Regardless of the extracts being at significantly higher concentrations than the control antibiotics, it is important to consider that plant extracts are a safer alternative with potentially no toxicity or adverse side effects [1,71,72]. Furthermore, plants are cheaper and more accessible, resulting in better availability and consumption for all communities [71,73]. Moreover, the production and consumption of synthetic antibiotics have been reported to exert detrimental effects on the environment [74,75].

With regards to distinguishing between the plant's leaf and stem antibacterial effectiveness, it was found that both organs exhibited a similar degree of inhibition of bacterial growth (Table 3). As reported for the plant's antioxidant activity, comparable antibacterial activities within the extracts of the leaves and stems suggest the presence of similar phytochemicals in both organs. An exception to this was found at extract concentrations of 2.5 mg/mL, where *E. coli* was more susceptible to the leaf extract than the stem. Contrasting results were revealed in a study on the ethanol extracts of *C. molle*, where leaf and bark extracts produced considerable antibacterial activity against *S. aureus*, while seed and stem extracts produced insignificant activities even at high concentrations (100 mg/mL) [76]. These differences may be attributed to variations in locality, climate, season, and extraction methods [44,45]. Furthermore, plant extracts comprise several bioactive constituents, which may be present in minute quantities or subdue the activities of one another [17]. Referring to the findings of this study, it is suggested that both leaf and stem extracts can be used as antibacterial agents due to similar effectiveness. The synergistic usage of both leaves and stems may prevent the exploitation of a single organ, ultimately reducing the risk of destructive harvesting [3,77]. Data from several studies correspond to the findings of *C. molle*'s positive antibacterial activity. Asres et al. [18] investigated the plant's stem bark via the disc diffusion technique and found that effective activity was produced against Gram-negative strains; *E. coli* (K99, K88, 306, LT37, 872, ROW 7/12, 3:37C, and CD/99/1) and several *Shigella* species were comparable to the control antibiotic ciprofloxacin. Mogashoa et al. [34] investigated the antimicrobial activity of *C. molle* leaves via microdilution. The study revealed effective activity from the acetone, ethyl acetate, and dichloromethane extracts against *E. faecalis* (ATCC 29212), *S. aureus* (ATCC 29213), *E. coli* (ATCC 25922), and *P. aeruginosa* (ATCC 27853). However, the acetone extracts produced the highest antibacterial effect on all strains, with an average minimum inhibitory concentration of 0.20 mg/mL. Another study revealed that leaves of *C. molle* produced moderate activity in comparison to the standard antibiotic chloramphenicol via a microdilution technique, where minimum inhibitory concentrations ranged from 128 µg/mL against *Enterobacter aerogenes* (EA294) to 512 µg/mL against various *E. coli* strains (ATCC10536, AG100, AG100A, and W3110) [66]. Ntshanka et al. [19] used the disc-diffusion technique to examine the plant's antibacterial activities and found significant activity against *E. coli* from the plant's acetone leaf extracts. In addition, numerous studies on the antibacterial activities of other *Combretum* species revealed results that suggest high antibacterial activities present within the genus. Fyhrquist et al. [17] showed that methanol extracts of the *Combretum fragrans* root, as well as the *Combretum padoides* root and stem bark, were highly effective against *S. aureus* (FOMK). Moreover, *C. microphyllum* extracts produced significant activity against *S. aureus* (ATCC 29213) and *E. coli* (ATCC 25922). A study by Chukwujekwu and Van Staden [33] revealed that a dichloromethane fraction of the leaves of *C. edwardsii* was highly effective against *S. aureus* (ATCC 11632) when compared to *E. coli* (ATCC 25218), with a minimum inhibitory concentration of 0.195 µg/mL. Against *E. coli*, the most effective activity was obtained by the ethyl acetate leaf fraction, with a minimum inhibitory concentration of 390 µg/mL. The study further revealed that *C. krausii* displayed moderate and weak antibacterial activity

against *S. aureus* and *E. coli*, respectively. Furthermore, cold aqueous, hot aqueous, and ethanol extracts of the leaves of *C. album* were effective against *E. coli* (MTCC 739) [7]. From all the extracts, leaves extracted in ethanol produced the lowest minimum inhibitory concentration (15 µg/mL).

#### 4. Materials and Methods

##### 4.1. Plant Material Collection and Extract Preparation

Leaves and stems of *C. molle* were collected from a single tree located in a roadside garden on Pitlochry Road (29°49.0985' S, 30°56.1057' E), Westville North, Durban, KwaZulu-Natal, South Africa. A voucher specimen was deposited at the Bews Herbarium in the School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg Campus, with accession No. NU0092543, collected by M. Parusnath (collector no. 1). The leaves and stems were dried in an oven (EcoTherm, Labotec, South Africa) for three weeks at 30 °C. The dried material was ground separately into fine powders using a blender (Russell Hobbs, RHB048, Failsworth, Manchester, UK). Subsequently, 10 g each of the leaf and stem-powdered material was placed into separate 250-mL round-bottom flasks, to which 100 mL of solvent was added. Using a reflux apparatus, the solutions were boiled thrice for three hours, each with an intervening filtration step using a Whatman® No. 1 filter paper. Three different solvents were used; therefore, the reflux process was repeated for each solvent in order of increasing polarity, i.e., hexane, chloroform, and then methanol (Merck, Darmstadt, Germany). The extracted filtrates were stored in hermetic glass bottles at room temperature in the dark.

##### 4.2. Antioxidant Assays

For the antioxidant assays, each extract was made up of concentrations of 15, 30, 60, 120, and 240 µg/mL by diluting the sample with its respective solvent (hexane, chloroform, or methanol).

###### 4.2.1. Assay of 2,2'-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging

The antioxidant activity of hexane, chloroform, and methanol leaf and stem extracts of *C. molle* was determined using DPPH radical scavenging assay [37–39], with modifications as implemented by Akwu et al. [78]. For each extract, 1 mL of its respective concentration and 500 µL of 0.135 mM DPPH (Sigma–Aldrich, Burlington, MA, USA) were added. The solutions were mixed and incubated in the dark at room temperature for 30 min. Following incubation, absorbance was measured at 517 nm using a UV–2600 UV–VIS Spectrophotometer (Shimadzu, Kyoto, Japan), with methanol as a blank. Ascorbic acid (Sigma–Aldrich, Burlington, MA, USA) was used as the standard. All analyses were conducted in triplicate. To determine the DPPH scavenging activity of the extracts, Formula (1) was utilized:

$$\text{DPPH scavenging activity (\%)} = \left( \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right) \times 100; \quad (1)$$

where  $\text{Abs}_{\text{control}}$  was the absorbance of the control (a solution of DPPH with methanol), and  $\text{Abs}_{\text{sample}}$  was the absorbance of the solution of DPPH with the sample (or standard). To evaluate the antioxidant activities of the extracts, the half-maximal inhibitory concentrations or  $\text{IC}_{50}$  values (the concentration of antioxidants needed to scavenge 50% of the initial DPPH radicals) of each extract were derived from inhibition curves by plotting percentage activity against concentration.

###### 4.2.2. Ferric ( $\text{Fe}^{3+}$ )-Reducing Antioxidant Power (FRAP) Assay

The antioxidant power of hexane, chloroform and methanol leaf, and stem extracts of *C. molle* was determined using the FRAP assay [51], with modifications as per an analysis by Akwu et al. [78]. Briefly, 50 µL of 0.2 M sodium phosphate buffer (pH 6.6) (Sigma–Aldrich,

USA) and 100  $\mu\text{L}$  of 1% potassium ferricyanide were added to 50  $\mu\text{L}$  of each sample of each concentration. The solutions were mixed and incubated for 30 min at 50  $^{\circ}\text{C}$  in a water bath (Labcon Laboratory Equipment, Krugersdorp, South Africa). Thereafter, 50  $\mu\text{L}$  of 10% trichloroacetic acid was added to each sample, followed by the addition of 50  $\mu\text{L}$  of distilled water and 10  $\mu\text{L}$  of 0.1% iron (III) chloride ( $\text{FeCl}_3$ ) (Merck, Darmstadt, Germany). The solutions were transferred to a 96-well microtiter plate and absorbance was measured at 700 nm using a Synergy HTX Multi-mode Reader (BioTek Instruments Inc., Winooski, VT, USA). Gallic acid was used as the positive control. All analyses were conducted in triplicate. The results were expressed as a percentage of the absorbance of the extracts relative to gallic acid using Formula (2).

$$\% \text{ Inhibition} = \left( \frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{gallic acid}}} \right) \times 100 \quad (2)$$

where  $\text{Abs}_{\text{sample}}$  was the absorbance of the sample and  $\text{Abs}_{\text{gallic acid}}$  was the absorbance of gallic acid (positive control). To evaluate the antioxidant activities of the extracts,  $\text{IC}_{50}$  values (the concentration of antioxidants required to inhibit oxidation by 50%) of each extract were derived from inhibition curves by plotting percentage inhibition against concentration.

#### 4.3. Antibacterial Assay

The antibacterial activity of the methanol leaf and stem extracts of *C. molle* was determined using the agar well-diffusion technique as described by [58], with modifications. The assay was performed against clinical strains of Gram-negative *E. coli* (ATCC 25922) and Gram-positive *S. aureus* (ATCC 25923) bacteria (procured from Prof. J. Lin, Discipline of Microbiology, School of Life Sciences, UKZN, Westville). The previously prepared methanol extracts were dried and maintained at room temperature. Each extract was dissolved in 10% dimethyl sulfoxide (DMSO) to produce concentrations of 0.625, 1.25, 2.5, 5, and 10 mg/mL. The bacterial strains were cultured in Nutrient Broth (Biolab, Pretoria, South Africa) for 18 h at 37  $^{\circ}\text{C}$ . Thereafter, the strains were diluted with Nutrient Broth to an optical density of 0.08–0.1, determined using a UV–2600 UV–VIS Spectrophotometer (Shimadzu, Kyoto, Japan), yielding a final concentration of approximately  $1 \times 10^8$ – $1 \times 10^9$  bacterial cells/mL. The medium was prepared by mixing 38 g of Mueller–Hinton Agar (MHA) (Biolab, Pretoria, South Africa) in 1 L of distilled water, followed by boiling for 1 min and autoclaving at 121  $^{\circ}\text{C}$  for 20 min. After cooling to room temperature, the medium was poured into sterile 90 mm Petri dishes where it was allowed to set under laminar flow conditions. Each bacterial strain was uniformly swabbed onto the MHA plates using sterile cotton swabs. Sterile micropipette tips were used to punch wells with a diameter of 6 mm into the plates. Subsequently, 100  $\mu\text{L}$  of the methanolic extracts at each concentration was added to the wells. The plates were incubated in an oven (EcoTherm, Labotec, uMhlanga, Durban, South Africa) at 37  $^{\circ}\text{C}$ , and the antibacterial activity was assessed after 18 h by measuring the zones of inhibition (mm). The positive controls used in the assay were antibiotics, viz. 10  $\mu\text{g}/\text{mL}$  of gentamicin and 10  $\mu\text{g}/\text{mL}$  of streptomycin for the Gram-negative and Gram-positive strains, respectively, whereas 10% DMSO was used as the negative control. The assay was conducted in triplicate.

#### 4.4. Statistical Analyses

The data were analyzed using RStudio 2022.02.1+461 for Windows (Boston, MA, USA). A one-way analysis of variance (ANOVA) was used to test for inter-treatment differences in antioxidant and antibacterial activity. Means for all analyses of variance were separated using a Tukey post-hoc test. All differences were considered significant at the 0.05 level.

## 5. Conclusions

Assays of DPPH radical scavenging and agar well-diffusion revealed that the methanol leaf and stem extracts of *C. molle* were highly effective as antioxidant and antibacterial agents, respectively. The observed activity was significantly higher than the controls, suggesting a strong hydrogen donating capacity and a strong ability to inhibit bacterial growth. Both leaf and stem extracts exhibited similar antioxidant and antibacterial activities, proposing their synergistic use. However, the extracts' poor ability to reduce ferric ions, as shown by the data obtained from the FRAP assay, limits knowledge on the plant's oxidant reducing capabilities. Therefore, it is recommended that the assay be performed on the plant's isolated phytochemicals. We propose that these extracts and their bioactive compounds may produce optimum alleviation of symptoms and disease related to oxidative stress and bacterial infection, and may, hence, be used in drug development. These findings additionally validate the use of this species in traditional medicine and potentially provide disadvantaged communities with an alternative source of medicinal treatment. However, assays about the toxicity of *C. molle* extracts should be performed to assure its validation and safe use. Additionally, it is recommended that further research be conducted on the identification and isolation of the specific phytochemical constituents responsible for the plant's antioxidant and antibacterial capabilities.

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