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Phytochemical Screening and Biological Activities of *Diospyros villosa* (L.) De Winter Leaf and Stem-Bark Extracts

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Abstract: This study aimed to evaluate the phytochemical components, antioxidant capacity, and antimicrobial effects of *Diospyros villosa* (L.) De Winter leaves and stem bark. The extracts were obtained using different media (methanol, chloroform, and hexane). The DPPH and FRAP methods were used to assess the antioxidant activity and the Folin–Ciocalteu method was used to determine the total phenolic contents of the crude extracts. The antimicrobial effects of the extracts against five pathogenic bacteria were determined using the MIC, MBC, and agar-well diffusion methods. Flavonoids, alkaloids, and phenols were identified in the *D. villosa* extracts. The mean concentrations of the methanolic leaf and stem-bark extracts against DPPH providing 50% inhibition were $9.53 \pm 0.25 \ \mu g \cdot mL^{-1}$ and $9.52 \pm 0.30 \ \mu g \cdot mL^{-1}$, respectively. In addition, the total phenolic content within the test range of concentrations was found to be 28.45 ± 0.50 mg of gallic acid equivalent per g of sample extract [$mg \cdot g^{-1}$ (GAE)] (methanolic leaf extract) and $4.88 \pm 0.36 \ mg \cdot g^{-1}$ (GAE) (methanolic stem-bark extract). The methanolic leaf extracts further showed promising antimicrobial activity against *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus*, and methicillin-resistant *Staphylococcus aureus* with inhibition zones of 18.0 ± 0.58, 23.5 ± 0.58, 20.0 ± 0.88, and 17.0 ± 2.0 mm, respectively which were comparable to the control (gentamicin and streptomycin). The results suggest that bioactive compounds are abundant in *D. villosa* leaves and stem bark and could serve as a potential source of natural antioxidants as well as an antibacterial agent for the treatment of pathogenic bacterial infections.

Keywords: DPPH; free radicals; minimum inhibitory concentration; phenol content

1. Introduction

The accumulation of free radicals at high levels is attributed to many pathological conditions and chronic diseases in humans [1]. In such situations, humans rely on plant sources of antioxidants for life maintenance and therapeutic measures. Certain research studies have further focused on various plant species that can circumvent the effects of oxidative damage incurred by the generation of free radicals [2–4]. High concentrations of antioxidants in some plant species serve to scavenge free radicals [5], whereas the identified antioxidants, flavonoids, and phenolic compounds have a wide range of structural

features and descriptions as well as characteristics and biological consequences [6]. Bioactive compounds are also recognized for other multiple biological effects including their antimicrobial/bactericidal properties [7,8].

Rasheed et al. [9] indicated that *Diospyros lotus* contains both gallic acid ($C_7H_6O_5$) and quercetin ($C_{15}H_{10}O_7$). *Diospyros montana* has also been reported to contain 8-hydroxydiospyrin ($C_{22}H_{14}O_6$) [10]. These active compounds have diverse defensive responses against different microbial strains through the generation of hydrogen peroxide [11] and alteration of the permeability of the microbial membrane [12,13]. Following these reports, the idea of investigating a novel South African plant for its antimicrobial activity and generation of free radicals comes to mind. Such plants may stand a better chance of alleviating the generation of free radicals and microbes in the disease state.

Diospyros villosa (L.) De Winter is an African plant that occurs naturally in southern parts of the continent. *D. villosa* is a perennial, bushy evergreen plant with a height range of 1–4 m. The leaves are chartaceous, drying dull brown above and much paler beneath. The dimensional length and breadth of the leaf lamina are on average 3 cm and 1.5–6.5 cm, respectively. The shape of the leaves is always obovate but sometimes appears oblong. The leaf apex is usually broadly rounded and slightly emarginated and sometimes obtuse. The leaf base often has a cordate or round shape. The roots of the plant are used locally as toothbrushes and to treat oral infections [14]. This report gives an insight into the concept of this study in such a way as to provide scientific evidence for the medicinal use of the *D. villosa* plant in the pathogenesis of infection in the oral cavity. *Escherichia coli* was reported to be among the top pathogens that manifest in infections, which further result in cases of diarrhoeal illness [15]. Other aerobic Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Klebsiella pneumonia* have been reported as opportunistic bacteria that further contribute to dental caries and sinusitis in orthodontic patients [16,17]. Gram-positive bacteria such as *Staphylococcus aureus* have been reported to co-exist with other microbial strains in the inflamed cavities of immunocompromised individuals and further induce bacterial reactivation in the infected cells [18]. Vellappally et al. [19] established that streptococci were not the only Gram-positive bacteria responsible for bloodstream infections and that *S. aureus* and methicillin-resistant *S. aureus* (MRSA) were often isolated in the hollow cavities of the body. In fact, the scourge MRSA accounted for almost 20–30% of all cases of oral infections [19].

At present, no studies are using *D. villosa* plant extracts against these bacteria strains. Hence, this study was geared toward making a significant contribution to the current search for ways to combat microbial strains involved in microbial infections and perhaps a contribution to existing knowledge of the antioxidant and antimicrobial properties of *D. villosa* extracts. To this end, the study intended to investigate the probable effects of *D. villosa* leaf and stem-bark extracts on identified Gram-negative and Gram-positive bacteria strains in close association with human body infections.

2. Materials and Methods

2.1. Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), gallic acid, ethanoic acid, Folin–Ciocalteu, gentamicin, and streptomycin were procured from Sigma-Aldrich (St. Louis, MO, USA). Mueller Hilton agar media, hexane, methanol, and chloroform were purchased from Merck Chemical Co. (Durban, South Africa).

2.2. Plant Collection

Fresh samples of *D. villosa* (mature leaves and stem bark) were collected in April and August of 2019 in KwaZulu-Natal, Durban, South Africa (29°84′33.6′′ S, 31°4′12′′ E). The plant was identified and a voucher specimen was deposited in the Herbarium (01/18257) at the School of Life Sciences, University of KwaZulu-Natal. The collected plant parts (leaves and stem bark) were air-dried at room temperature for 45 days and crushed into a fine powder. The powdered samples were kept in a cool, dry place for extraction purposes.

2.3. Plant Extraction

Powdered samples of the plant weighing 8 g were heated to a temperature of 40 °C for 15 min with 100 mL of 95% methanol in a round-bottom flask attached to a Soxhlet apparatus. The crude extract was retained and the process was repeated thrice. Successive extractions using chloroform and hexane, respectively, were carried out at 30 min intervals. The condensate was further evaporated to dryness under reduced pressure at 40 °C in a rotary evaporator. The crude extract was stored at 4 °C and used within 48 h for further tests. The extraction yield (%) = $\frac{Weight\ of\ the\ dry\ extract\ (g)}{Weight\ of\ dry\ sample\ used\ for\ the\ extracton\ (g)}$

2.4. Qualitative Phytochemical Tests

The qualitative phytochemical constituent screening of the different extracts of the leaves and stem bark obtained from *D. villosa* was conducted using standard qualitative protocols [20–23].

2.5. Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The extracts were filtered using a Whatman No. 4 and subsequently via a 0.22 μL membrane filter. A GCMS-QP2010 Plus (Shimadzu, Tokyo, Japan) with a capillary chromatography column (30 cm × 0.25 mm ID × 0.25 μm film thickness of 5% phenylmethylsiloxane) was used for the GC–MS analysis of the extracts. At first, the instrument was fixed at a temperature of 50 °C, sustained for 1.5 min, then increased to a temperature of 200 °C using a pace of 4 °C min^{-1}. The temperature was further increased to a temperature of 300 °C using a pace of 10 °C min^{-1} for 7 min. The injector and interface temperatures were 240 and 220 °C, respectively. The helium flow rate was maintained at a rate of 1.2 mL min^{-1}, and 2 μL of each of the crude extracts was dissolved using different solvents (≥99%, GC grade, Sigma-Aldrich) and filtered through a 0.22 μm filter and subsequently injected into the 'splitless' mode system. The range and the total running time for the spectral scan were 40–500 *m/z* and 30 min, respectively. A comparison of each mass spectrum with the data published in Adams [24] and the Mass Spectral Search Program database of the National Institute of Standards and Technology, Washington, DC, USA, aided the identification of each component.

2.6. Fourier Transform Infrared (FT–IR) Analysis

The FT–IR analysis was conducted to characterize the functional groups in the *D. villosa* leaf and stem-bark extracts that are responsible for the biochemical and molecular potential of the plants using a spectrophotometer (Perkin Elmer 100 FT–IR, Waltham, MA, USA). The spectra were examined and imaged at a range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1}. KBr was used as a standard to analyze the samples by dispersing them uniformly in a matrix of dry KBr.

2.7. Sensitivity Test of Leaves and Stem-Bark Extracts of D. villosa on Test Microorganisms

The antimicrobial activities of the methanolic, chloroformic, and hexanolic leaf and stem-bark extracts of *D. villosa* were investigated using the agar-well diffusion method. The discs were prepared using a Whatman No. 1 and obtained by punching and placing them in vials, which were further sterilized in an oven at 150 °C for 15 min. The test microorganisms were reactivated on the nutrient agar broth and further incubated at 37 °C overnight. The test microorganisms were also standardized at an optical density of 0.1 at 625 nm using a UV-vis spectrophotometer (Agilent Cary 60 Spectr., Santa Clara, CA, USA). Following this, 0.2 mL of the standardized test culture was added to 20 mL of molten Muller–Hilton agar and homogenized. This was then poured into sterile plates and allowed to solidify. Thereafter, the wells were aseptically bored into the inoculated Muller–Hilton agar plates using a 6mm sterile cork borer. The test solutions of extracts (100 μL) at graded concentrations of 0.625, 1.25, 2.5, 5, and 10 mg mL^{-1} already dissolved in 10% DMSO were then placed into each designated well on the plate, ensuring that there was no spillage. A standardized amount (10 μg·mL^{-1}) of gentamicin and streptomycin was introduced into the residual wells on

the plate, which was used as a control for the Gram-negative and Gram-positive bacteria strains, respectively. The plates were then allowed to diffuse at room temperature for 1 h in the medium and eventually incubated at 37 °C for 16–18 h in an incubator. The clear zones of inhibition were noted, measured, and recorded in millimeters. Each extract's activity was tested in triplicate.

2.8. Antioxidant Activity

2.8.1. DPPH Scavenging Activity

The free radical scavenging activity of the extracts was determined by DPPH (1, 1-diphenyl-2-picrylhydrazyl) radicals [25]. An aliquot of 3 mL of 0.004% DPPH solution in 95% ethanol and 0.1 mL of each plant extract at concentrations of 15, 30, 60, 120, and 240 µg·mL^{-1} were mixed. The mixture was thoroughly mixed and allowed to sit for 30 min at room temperature. The procedure was repeated for the ascorbic acid (control). The decolorization of DPPH was ascertained by quantifying the absorbance at 517 nm. The control was prepared using 0.1 mL of each constituent and double-distilled water as a replacement for the plant extract or ascorbic acid. The percentage expression of DPPH radical scavenging activity by the plant extracts was calculated as thus:

$$\frac{Absorbance\ of\ Control - Absorbance\ of\ Test\ samples}{Absorbance\ of\ Control} \times 100.$$

2.8.2. Ferric-Reducing Antioxidant Potential (FRAP) Assay

The FRAP assay was conducted as previously described by Juntachote and Berghofer [26]. Multiple graded concentrations of the extract (15, 30, 60, 120, and 240 µg·mL^{-1}) of the extract (1 mL each) were added to 2.5 mL of phosphate buffer (0.2M, pH 6) and 2.5 mL of potassium ferricyanide (1% w/v). The resulting admixture was incubated for 20 min at a temperature of 50 °C. Then, 2.5 mL of 10% trichloroacetic acid was added to the mixture. A quantity of 2.5 mL from each mixture was further diluted twice with deionized water, and 0.5 mL of 0.1% (w/v) FeCl$_3$ was added. The absorbance was later determined at 700 nm after 30 min. The positive control used was ascorbic acid. The half-maximal inhibitory concentration (IC$_{50}$) was calculated from the graph of absorbance against the concentrations of the extracts. The results were generated as thus:

$$\text{Scavenging Effect (\%)} = \left[\frac{Absorbance\ of\ Control - Absorbance\ of\ sample}{Absorbance\ of\ control} \right] \times 100.$$

2.8.3. Total Phenolic Content (TPC)

TPC was investigated using the Folin–Ciocalteu colorimetric method with slight adjustments [27]. A volume of 0.1 mL of each extract was mixed thoroughly with 3 mL of distilled water and 0.5 mL of Folin–Ciocalteu reagent was also added to each sample extract. The mixture was allowed to sit at room temperature for 3 min and 2 mL of 20% sodium carbonate was added. The mixture was further incubated for 30 min at room temperature. The total phenolic content was measured at 725 nm using a spectrophotometer. Gallic acid was used as the positive control. The total phenol values were expressed as mg of gallic acid equivalents (GAE)/g of dry sample extracts.

2.9. Test Micro-Organisms

The bacteria strains used were identified as American-type collection culture strains obtained from the School of Pharmacy and Pharmacological Sciences, University of KwaZulu-Natal. Three Gram-negative bacteria, namely *E. coli* (ATCC 35218), *P. aeruginosa* (ATCC 27853), and *K. pneumoniae* (ATCC 700603), and 2 Gram-positive bacteria, *S. aureus* (ATCC 33591) and MRSA (ATCC 43300), were used in this study.

2.9.1. Determination of Minimum Inhibitory Concentration (MIC) of the *D. villosa* Extracts on Microorganisms

The MIC of the extracts was determined using the method described by Akinpelu and Onakoya [28]. The extract was diluted in two folds and a portion of 2 mL of the extracts at different concentrations was mixed with 18 mL of sterilized molten Mueller–Hilton agar in order to achieve a final concentration regime of 0.313 mg·mL^{-1} to 0.01 mg·mL^{-1}. The resulting medium was transferred into sterile Petri dishes and allowed to set. The dry surface of the medium was achieved before streaking with 18 h-old standardized bacterial cultures. The plates were then incubated at 37 °C for 48 h and later scrutinized for either the absence or presence of growth. The MIC was taken as the lowest concentration that prevents bacterial growth.

2.9.2. Determination of Minimum Bactericidal Concentration (MBC) of the Extracts on Test Microorganisms

The minimum bactericidal concentration of the extract was determined according to Ferrazzano et al. [29]. The inoculum was taken on the line of streaks without visible growth in the MIC assay and subcultured on freshly prepared nutrient agar and incubated at 37 °C for 48 h. The plates were later scrutinized for either the absence or presence of growth. The lowest concentration of the extracts with no indication of any growth on a new set of plates was considered as the MBC of the extracts.

2.10. Statistical Analysis

The results were expressed as means ± SE. Statistical analysis was performed using Graph Pad Prism 5 (Graph Pad Software Inc., San Diego, CA, USA). All outcomes were compared with the control using both one-way and two-way analysis of variance (ANOVA) followed by a Bonferroni post hoc analysis. Effects were considered statistically significant at *p* value ≤ 0.05.

3. Results

3.1. Phytochemical Analysis

The percentage extract yield of *D. villosa* leaves and stem bark are represented in Table 1. It was found that the methanol extract in the leaves produced the highest yield of phytochemicals, at about 10.8%, whereas the chloroform and hexane extracts in the leaves yielded 8.4% and 7.1%, respectively. Similarly, the methanol extract in the stem bark produced a yield of 9.2%, whereas the chloroform and hexane extracts yielded 7.9% and 10.3%, respectively.

Table 1. Yield of extracts of *D. villosa* leaves and stem bark.

	Leaf Extract Yield (%)	Stem-Bark Extract Yield (%)
Methanol	10.8	9.2
Chloroform	8.4	7.9
Hexane	7.1	10.3

The results of the qualitative phytochemical assessment of the *D. villosa* leaves and stem-bark extracts are shown in Table 2. Phytochemical screening showed the presence of alkaloids, terpenoids, and phenols in the methanol extracts of *D. villosa* leaves. In addition, the presence of flavonoids was further observed in the methanol extracts of *D. villosa* stem bark. In addition, terpenoids, flavonoids, and phenols were shown to be present in both the chloroformic and hexanolic stem extracts of *D. villosa*. All the extracts from the plant's leaves and stem cumulatively contained steroids, alkaloids, carbohydrates, and saponins.

Table 2. Phytochemical screening of the methanol, chloroform, and hexane extracts of both the leaves and stem bark of *D. villosa*.

Name of Test		Leaves			Stem Bark		
		Methanol	Chloroform	Hexane	Methanol	Chloroform	Hexane
Alkaloids	Mayer's test	+++	-	-	+++	-	-
	Wagner's test	-	+	+	-	+	+
Protein	Dragendorff	-	+	+	-	+	+
Carbohydrates	Benedict's	+	-	-	+	-	-
Steroids	Lieberman-Burchard test	++	+	+	+	+	+
Coumarins	NaOH test	++	-	-	++	-	-
Saponin	Foam test	++	+	+	+++	+	+
Flavonoids	Lead acetate	+++	-	-	+++	-	++
Terpenoids	Salowski's	+++	-	-	++	+	++
Phenols	$FeCl_3$ test	+++	-	-	++	+	-

+ denotes presence; ++ denotes moderate presence; +++ denotes abundant presence; - denotes absence.

3.2. Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The GC–MS analysis of both the leaves and stem of *D. villosa* showed the presence of certain bio-active compounds such as n-hexadecanoic acid, phytol, palmitoleic acid, eicosanoic acid and its derivatives, ascorbic acid and its derivatives, etc. (Tables 3–8). The retention time (RT), compound name, molecular formula, and peak height (%) are presented in Tables 3–8.

Table 3. Compounds identified in the chloroform extracts of *D. villosa* leaves by GC–MS spectral analysis.

S/N	RT	Compound Name	Molecular Formula	Height (%)
1.	3.801	1-Butene, 3-chloro-2-methyl-	C_5H_9cl	1.08
2.	4.254	1, 1-Dimethyl-3-chloropropanol	$C_5H_{11}clO$	0.87
3.	4.296	Pentanoic acid, 2-hydroxy-4-methyl-(S)-	$C_6H_{12}O_3$	7.45
4.	5.252	1-Butene,2,3,3-trimethyl	C_7H_{14}	0.93
5.	10.454	11-Methyldodecanol	$C_{13}H_{28}O$	1.14
6.	10.557	11-Methyldodecanol	$C_{13}H_{28}O$	1.21
7.	10.656	11-Methyldodecanol	$C_{13}H_{28}O$	1.02
8.	11.830	Vanillin	$C_8H_8O_3$	0.28
9.	12.397	10-Methylnonadecane	$C_{20}H_{42}$	0.67
10.	12.545	Eicosane	$C_{20}H_{42}$	0.53
11.	12.680	Heptadecanoic acid, heptadecyl ester	$C_{34}H_{68}O_2$	0.25
12.	12.869	Phenol, 2, 4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	5.54
13.	12.943	1-Dodecanol, 2-hexyl-	$C_{18}H_{38}O$	0.87
14.	13.015	Eicosane	$C_{20}H_{42}$	1.12
15.	13.125	1-Dodecanol, 2-hexyl-	$C_{18}H_{38}O$	0.98
16.	13.222	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4	$C_{11}H_{16}O_2$	2.52
17.	13.585	Fumaric acid, ethyl 2-methyl allyl ester	$C_{10}H_{14}O_4$	1.19
18.	13.697	Decane, 2,3,7-trimethyl-	$C_{13}H_{28}$	0.33
19.	14.193	Trans-3(10)-Caren-2-ol	$C_{10}H_{16}O$	1.04
20.	14.340	Decane, 2,3,7-trimethyl-	$C_{13}H_{28}$	0.33
21.	14.429	2,4a,8,8-Tetramethyldecahydrocyclopropa[d]n	$C_{15}H_{26}$	1.23
22.	14.512	2-Butanone, 4-(2,6,6-trimethyl-1-cyclohexen-1	$C_{13}H_{22}O$	1.02
23.	14.677	Oxalic acid, 6-ethyloct-3-yl heptyl ester	$C_{19}H_{36}O_4$	0.39
24.	14.749	1-{2-[3-(2-Acetyloxiran-2-yl)-1,1-dimethyl propyl]cycloprop-2-enyl} ethanone	$C_{14}H_{20}O_3$	1.27
25.	14.981	11-Methyldodecanol	$C_{13}H_{28}O$	0.68
26.	15.304	11-Methyldodecanol	$C_{13}H_{28}O$	0.67
27.	15.440	Tetradecanoic acid	$C_{14}H_{28}O_2$	3.13
28.	15.550	11-Methyldodecanol	$C_{13}H_{28}O$	0.80

Table 3. *Cont.*

S/N	RT	Compound Name	Molecular Formula	Height (%)
29.	15.685	1-Dodecanol, 2-Octyl	$C_{20}H_{42}O$	0.52
30.	15.879	6-Methyl-cyclodec-5-enol	$C_{11}H_{20}O$	3.88
31.	16.320	Phytol, acetate	$C_{22}H_{42}O_2$	4.14
32.	16.410	2-Pentadecanone,6,10,14-trimethyl-	$C_{18}H_{36}O$	3.77
33.	16.679	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	0.91
34.	16.761	Pentadecanoic acid	$C_{15}H_{30}O_2$	0.48
35.	16.970	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	1.41
36.	17.538	5,9,13-Pentadecatrien-2-one,6,10,14-trimethy	$C_{18}H_{30}O$	0.64
37.	17.741	Hexadecanoic acid,methyl ester	$C_{17}H_{34}O_2$	1.74
38.	18.168	cis-13-Eicosenoic acid	$C_{20}H_{38}O_2$	0.63
39.	18.753	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	21.20
40.	21.024	Eicosanoic acid	$C_{20}H_{40}O_2$	0.40
41.	21.795	Methyl 10-trans,12-cis-octadecadienoate	$C_{19}H_{34}O_2$	0.45
42.	21.991	Methyl 8,11,14-heptadecatrienoate	$C_{18}H_{30}O_2$	1.22
43.	22.537	Phytol	$C_{20}H_{40}O$	5.21
44.	22.935	cis,cis,cis-7,10,13-Hexadecatrienal	$C_{16}H_{26}O$	14.36
45.	24.190	Methyl 5,13-docosadienoate	$C_{23}H_{42}O_2$	0.41

Table 4. Compounds found in the chloroform extracts of *D. villosa* stem by GC–MS analysis.

S/N	RT	Compound Name	Molecular Formula	Height (%)
1.	10.830	Octadecanoic acid	$C_{18}H_{36}O_2$	1.48
2.	10.925	Octadecanoic acid	$C_{18}H_{36}O_2$	3.05
3.	10.991	Undecanoic acid	$C_{11}H_{22}O_2$	3.04
4.	11.050	*n*-Decanoic acid	$C_{10}H_{20}O_2$	2.71
5.	13.890	Disulfide,di-tert-dodecyl	$C_{24}H_{50}S_2$	1.63
6.	14.524	Phytol,acetate	$C_{22}H_{42}O_2$	0.85
7.	16.326	Phytol,acetate	$C_{22}H_{42}O_2$	48.61
8.	16.682	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	10.00
9.	16.976	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	15.54
10.	18.586	1-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	2.79
11.	19.546	Eicosane	$C_{20}H_{42}$	3.17
12.	20.233	6,8a-Epidoxy-4a-methyl-2-oxo-3,4,4a,5,6,7,8	$C_{10}H_{14}O_4$	0.87
13.	22.405	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	$C_{19}H_{34}O_2$	1.92
14.	22.435	2-(2-vinyloxy-ethoxy)-cyclohexanol	$C_{10}H_{18}O_3$	1.60
15.	22.620	Phytol	$C_{20}H_{40}O$	2.76

Table 5. Compounds identified in the methanol extracts of *D. villosa* leaves by GC–MS spectral analysis.

S/N	RT	Compound Name	Molecular Formula	Height (%)
1.	3.971	Toluene	C_7H_8	2.05
2.	4.349	Tetrachloroethylene	C_2cl_4	2.33
3.	13.240	Nonanedioic acid, dimethyl ester	$C_{11}H_{20}O_4$	1.24
4.	13.931	Ethyl-1-thio-beta-d-glucopyranoside	$C_8H_{16}O_5S$	0.66
5.	14.956	Methyl-21-methyldocosanoate	$C_{24}H_{48}O_2$	1.12
6.	16.131	Carda-4,20(22)-dienolide,3-(6-deoxy-3-O-methyl)	$C_{30}H_{44}O_9$	0.68
7.	16.185	Carda-4,20(22)-dienolide,3-(6-deoxy-3-O-methyl)	$C_{30}H_{44}O_9$	0.67
8.	16.325	Phytol acetate	$C_{22}H_{42}O_2$	9.80
9.	16.430	Cyclohexadecanone	$C_{16}H_{30}O$	1.10
10.	16.683	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	4.98
11.	16.785	Pseudosmilagenin bis(3,5-dinitrobenzoate)	$C_{41}H_{48}N_4O_{13}$	0.52
12.	16.972	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	7.28
13.	17.295	Acetamide,N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl	$C_{11}H_{18}N_2O_2$	0.20
14.	17.449	1-oxaspiro(2,5)octan-4-one,2,2,6-trimethyl-trans	$C_{10}H_{16}O_2$	0.65

Table 5. *Cont.*

S/N	RT	Compound Name	Molecular Formula	Height (%)
15.	17.669	Methylhexadec-9-enoate	$C_{17}H_{32}O_2$	2.60
16.	17.756	Pentadecanoic acid,14-methyl-,methyl ester	$C_{17}H_{34}O_2$	29.89
17.	17.925	Methyl 2,3,4-tri-O-acetyl-6,7-di-O-methyl-beta-D-glucoheptopyranoside	$C_{16}H_{26}O_{10}$	0.26
18.	18.635	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	7.65
19.	18.825	1,1,1,4,7,7,7-Heptamethyl-4-vinyltrisilethylene	$C_{13}H_{32}Si_3$	0.56
20.	21.820	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	6.48
21.	22.023	11,14,17-Eicosatrienoic acid, methyl ester	$C_{21}H_{36}O_2$	15.19
22.	22.964	Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	1.86
23.	23.450	Acetamide, N-(4-piperidinylmehyl)-	$C_8H_{16}N_2O$	0.32
24.	23.620	3-[3-[1-Aziridinyl]propoxy]-2,5-dimethylpyraz	$C_{11}H_{17}N_3O$	1.01
25.	23.641	3-Tridecen-1-yne,(Z)-	$C_{13}H_{22}$	1.01

Table 6. Compounds identified in the methanol extracts of *D. villosa* stem by GC–MS spectral analysis.

S/N	RT	Compound Name	Molecular Formula	Height (%)
1.	3.965	Toluene	C_7H_8	2.71
2.	4.343	Tetrachloroethylene	C_2cl_4	3.17
3.	13.425	2,2,6,7-Tetramethyl-10-oxatricyclo [4,3,1,0 (1,6)-decan-5-ol	$C_{13}H_{22}O_2$	0.34
4.	14.339	tau-Muurolol	$C_{15}H_{26}O$	2.41
5.	14.462	A-Cardinol	$C_{15}H_{26}O$	3.28
6.	15.858	2-Methyltetracosane	$C_{25}H_{52}$	1.53
7.	16.325	Phytol, acetate	$C_{22}H_{42}O_2$	0.99
8.	17.766	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	26.89
9.	17.905	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-methyl ester	$C_{18}H_{28}O_3$	3.35
10.	18.030	Borane, diethyl(1-ethyl-2-methyl-1-butenyl)-(Z)	$C_{11}H_{23}B$	0.73
11.	18.608	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	25.93
12.	19.040	2,7-dithiatricyclo [4,3,1,0(3,8)] decane,10-bromo	$C_8H_{11}BrS_2$	0.40
13.	19.146	Ethyl 15-methyl-hexadecanoate	$C_{19}H_{38}O_2$	1.51
14.	21.825	Methyl 10-trans, 12-cis-octadecadienoate	$C_{19}H_{34}O_2$	10.79
15.	22.037	6-Octadecenoic acid, methyl ester, (Z)-	$C_{19}H_{36}O_2$	8.20
16.	22.223	6-Octadecenoic acid, methyl ester, (Z)-	$C_{19}H_{36}O_2$	1.47
17.	22.957	Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	2.01
18.	23.469	1-[3-Aminopropyl]-2[1H]-pyridone	$C_8H_{12}N_2O$	0.91
19.	23.640	Bicyclo [3,3,2] decan-9-one	$C_{10}H_{16}O$	2.59
20.	23.710	1H-Inden-1-one,octahydro-7a-methyl-,trans	$C_{10}H_{16}O$	0.81

Table 7. Compounds found in the hexane extracts of *D. villosa* leaves by GC–MS analysis.

S/N	RT	Compound Name	Molecular Formula	Height (%)
1.	3.836	3-Hexanone	$C_6H_{12}O$	0.86
2.	3.885	2-Hexanone	$C_6H_{12}O$	1.43
3.	3.985	Cyclopentanol,1-methyl-	$C_6H_{12}O$	1.03
4.	4.639	Cyclopentanone,3-methyl-	$C_6H_{12}O$	1.42
5.	5.425	2-Pentanethiol,2-methyl-	$C_5H_{14}S$	6.21
6.	5.678	Valeric acid,2-ethoxyethyl ester	$C_9H_{18}O_3$	10.80
7.	5.924	Pentanoic acid,2-propenyl ester	$C_8H_{14}O_2$	4.33
8.	6.061	2-Furanmethanol, tetrahydro-5-methyl-	$C_6H_{12}O_2$	3.55
9.	6.308	Oxalic acid, cyclohexyl ethyl ester	$C_{10}H_{16}O_2$	22.48
10.	6.611	2-Pentene,4,4-dimethyl-,(E)-	C_7H_{14}	2.55
11.	6.650	2-Pentene,4,4-dimethyl-,(E)-	C_7H_{14}	2.36
12.	6.740	Phosphorus dibromide, cyclohexyl-	$C_6H_{11}Br_2P$	0.86

Table 7. *Cont.*

S/N	RT	Compound Name	Molecular Formula	Height (%)
13.	10.458	2-Isopropyl-5-methyl-1-heptanol	$C_{11}H_{24}O$	0.41
14.	10.659	2-Isopropyl-5-methyl-1-heptanol	$C_{11}H_{24}O$	0.26
15.	11.765	Octadecanoic acid	$C_{18}H_{36}O_2$	0.29
16.	11.845	Tetradecanoic acid	$C_{14}H_{28}O_2$	0.41
17.	11.948	Octadecanoic acid	$C_{18}H_{36}O_2$	1.00
18.	12.159	5,9-Undecadien-2-one,6,10-dimethyl-,(E)	$C_{13}H_{22}O_2$	0.45
19.	12.874	Phenol,2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	1.39
20.	13.022	Hexadecanoic acid, butyl ester	$C_{20}H_{40}O_2$	1.87
21.	13.155	Dodecane,4-methyl-	$C_{13}H_{28}$	0.40
22.	13.222	2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4	$C_{11}H_{16}O_2$	0.99
23.	15.473	Tetradecanoic acid	$C_{14}H_{28}O_2$	1.85
24.	16.336	Phytol,acetate	$C_{22}H_{42}O$	1.57
25.	16.432	2-Pentadecanone,6,10,14-trimethyl-	$C_{18}H_{36}O$	4.43
26.	16.690	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	0.27
27.	16.798	Pentadecanoic acid	$C_{15}H_{30}O_2$	0.23
28.	16.987	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	0.42
29.	17.554	5,9,13-Pentadecatrien-2-one,6,10,14-trimethyl	$C_{18}H_{30}O$	0.41
30.	17.675	Oxirane,hexadecyl-	$C_{18}H_{36}O$	0.14
31.	17.764	Pentadecanoic acid,14-methyl-,methyl ester	$C_{17}H_{34}O_2$	1.99
32.	18.204	Palmitoleic acid	$C_{16}H_{30}O_2$	0.45
33.	18.871	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	11.77
34.	19.235	2-Hexadecene,3,7,11,15-tetramethyl-,[R-[R	$C_{20}H_{40}$	0.17
35.	21.093	Eicosanoic acid	$C_{20}H_{40}O_2$	0.26
36.	21.846	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	0.70
37.	22.052	Methyl 8,11,14-heptadecatrienoate	$C_{18}H_{30}O_2$	1.40
38.	22.579	Phytol	$C_{20}H_{40}O$	2.52
39.	23.187	Butyl 9,12-Octadecadienoate	$C_{22}H_{40}O_2$	0.41
40.	24.158	cis,cis,cis-7,10,13-Hexadecatrienal	$C_{16}H_{26}O$	5.65

Table 8. Compounds found in the hexane extracts of *D. villosa* stem by GC–MS spectral analysis.

S/N	RT	Compound Name	Molecular Formula	Height (%)
1.	3.881	2-Hexanone	$C_6H_{12}O$	1.08
2.	3.979	Cyclopentanol,1-methyl-	$C_6H_{12}O$	0.69
3.	4.636	Cyclopentanone,3-methyl-	$C_6H_{10}O$	1.07
4.	5.418	2-Pentanethiol,2-methyl-	$C_6H_{14}S$	5.96
5.	5.669	Valeric acid, 2-ethoxy ethyl ester	$C_9H_{18}O_3$	10.71
6.	5.916	Pentanoic acid, 2-propenyl ester	$C_8H_{14}O_2$	3.88
7.	6.054	2-Furanmethanol, tetrahydro-5-methyl-	$C_6H_{12}O_2$	3.59
8.	6.299	Oxalic acid, cyclohexyl propyl ester	$C_{11}H_{18}O_4$	21.93
9.	6.603	2-Pentene,4,4-dimethyl-,(Z)-	C_7H_{14}	2.26
10.	6.644	2-Pentene,4,4-dimethyl-,(Z)-	C_7H_{14}	2.04
11.	6.733	Cyclohexane,nitro	$C_6H_{11}NO_2$	0.77
12.	12.679	Heptadecane,2,6,10,15-tetramethyl-	$C_{21}H_{44}$	0.54
13.	12.868	Phenol,2,4-bis(1,1-dimethyl ethyl)-	$C_{14}H_{22}O$	1.30
14.	13.365	1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl,(E)	$C_{15}H_{26}O$	0.68
15.	13.737	Carophyllene oxide	$C_{15}H_{24}O$	1.96
16.	14.014	12-Oxabicyclo [9,1,0]dodeca-3-7-diene,1,5, 5,8-tetramethyl-[1R,3E,7E,11R]	$C_{15}H_{24}O$	0.69
17.	14.122	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8, 8a-octahydronaphthalene-2-ol	$C_{15}H_{24}O$	0.50
18.	14.156	Cubenol	$C_{15}H_{26}O$	0.39
19.	14.313	*α*-cadinol	$C_{15}H_{26}O$	2.44
20.	14.429	*α*-cadinol	$C_{15}H_{26}O$	3.86

Table 8. *Cont.*

S/N	RT	Compound Name	Molecular Formula	Height (%)
21.	14.684	Octadecane,1-chloro	$C_{18}H_{37}cl$	0.62
22.	14.871	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8, 8a-octahydronaphthalene-2-ol	$C_{15}H_{24}O$	0.38
23.	15.450	Tetradecanoic acid	$C_{14}H_{28}O_2$	1.32
24.	16.327	Phytol, acetate	$C_{22}H_{42}O_2$	1.73
25.	16.419	2-Pentadecanone,6,1014-trimethyl-	$C_{18}H_{36}O$	2.36
26.	16.545	Cyclopentadecanone,2-hydroxy	$C_{15}H_{28}O_2$	0.31
27.	16.683	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	0.36
28.	16.780	Pentadecanoic acid	$C_{15}H_{30}O_2$	0.53
29.	16.979	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	0.55
30.	17.753	Pentadecanoic acid, 14-methyl-,methyl ester	$C_{17}H_{34}O_2$	2.41
31.	18.921	*n*-Hexadecanoic acid, ethyl ester	$C_{16}H_{32}O_2$	13.98
32.	19.155	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	0.32
33.	19.315	2-Bromotetradecane	$C_{14}H_{29}Br$	0.20
34.	21.079	Heptadecanoic acid	$C_{17}H_{34}O_2$	0.44
35.	21.828	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	0.65
36.	22.056	8-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	0.72
37.	22.600	Phytol	$C_{20}H_{40}O$	0.91
38.	22.971	Methylstearate	$C_{19}H_{38}O_2$	0.17
39.	23.840	1,E-11,Z-13-Octadecatriene	$C_{18}H_{32}$	2.69
40.	24.125	6-Octadecenoic acid,(Z)-	$C_{18}H_{34}O_2$	3.04

3.3. Antimicrobial Effects

The zone of inhibition of *D. villosa* leaf and stem-bark extracts at 10 mg·mL^{-1} was significantly higher compared to the control ($p < 0.05$), except for the activity of the methanol stem-bark extract against *E. coli*, where the zone of inhibition at 10 mg·mL^{-1} was lower compared to the control (Table 9). At a concentration of 5 mg·mL^{-1}, the zones of inhibition of the methanol leaf extract as observed against *S. aureus*, methicillin-resistant *S. aureus*, and *K. pneumoniae* were found to be higher compared to the control (streptomycin and gentamicin) (Table 9). The zone of inhibition of the methanol stem-bark extract at 5 mg·mL^{-1} as observed against *S. aureus* was significantly higher compared to the control ($p < 0.05$).

There was an effect of the chloroform extract of *D. villosa* leaves on *S. aureus*. The zone of inhibition of the chloroform leaf extract against *K. pneumoniae* was found to be higher compared to the control (gentamicin) at a concentration of 10 mg·mL^{-1} (Table 9). At all concentrations, the zone of inhibition of the hexane leaf extract against *K. pneumoniae* was lower compared to the control (gentamicin). Meanwhile, the zone of inhibition against *K. pneumoniae* at a concentration of 10 mg·mL^{-1} was higher compared to the control (Table 9). The zones of inhibition at all other doses were lower compared to the control.

Following the serial dilution of 0.625 mg·mL^{-1} in the lowest concentration of the extract, all the bacterial strains were observed to be resistant to the activities of the methanolic leaf extract of *D. villosa* at a concentration of 0.01 mg·mL^{-1} (Table 10). In addition, the same trend was observed with the methanol stem-bark extract of the plant at 0.01 mg·mL^{-1}. It was also observed that the bacterial strains were sparred as there was no reactivity at a concentration of 0.01 mg·mL^{-1}.

Table 9. Zone of inhibition (mm) of the graded doses of *D. villosa* leaf and stem-bark extracts against bacteria strains.

Bacteria Strain	Meth. Lv. Extr. Conc.(mg·mL^{-1})						Meth. Stem Extr. Conc (mg·mL^{-1})					
	10	**5**	**2.5**	**1.25**	**0.625**	**Control**	**10**	**5**	**2.5**	**1.25**	**0.625**	**Control**
E. coli	18.3 ± 0.67	19 ± 0	19.5 ± 0.5	19.67 ± 1.28	0.90 ± 0.10	21 ± 3.08	16.3 ± 0.88	15 ± 0.88	13.3 ± 1.05	12.3 ± 1.05	0.8 ± 0	23.3 ± 0.77
P. aeruginosa	18.0 ± 0.58	13.0 ± 0.58	12.0 ± 1.53	13.33 ± 0.88	0.90 ± 0	11.67 ± 0.77	14.3 ± 0.67	11.0 ± 0.58	0.9 ± 0	10.67 ± 0.33	0.9 ± 0.1	10.33 ± 0.29
S. aureus	20.3 ± 0.88	15.3 ± 0.33	10 ± 1.15	12.33 ± 0.67	0.83 ± 0.10	15 ± 1.15	14.0 ± 0.58	11.3 ± 0.88	12.50 ± 0.50	10.33 ± 0.33	0.90 ± 0	5.45 ± 4.55
MRSA	17 ± 2	15.5 ± 1.50	12.67 ± 0.67	11.67 ± 0.88	0.2 ± 0	17.67 ± 1.45	16 ± 0	15.33 ± 0.88	13.33 ± 0.33	11.33 ± 0.67	0.63 ± 0.09	19 ± 1.15
K. pneumonia	23.50 ± 0.50	21.5 ± 0.50	16.67 ± 3.33	13.67 ± 1.20	10.67 ± 5.46	19.3 ± 2.91	20.33 ± 1.33	17.33 ± 1.86	17.0 ± 2.08	11.67 ± 0.88	0.73 ± 0.12	21.67 ± 1.20
	Chl. Lv. Extr. Conc (mg·mL^{-1})						Chl. Lv. Extr. Conc (mg·mL^{-1})					
	10	**5**	**2.5**	**1.25**	**0.625**	**Control**	**10**	**5**	**2.5**	**1.25**	**0.625**	**Control**
K. pneumonia	24.50 ± 0.5	22 ± 1.0	21.50 ± 1.50	20 ± 1.0	15.0 ± 2.0	20 ± 0	20.33 ± 1.33	20 ± 0	18.0 ± 0.58	16.33 ± 1.33	14.33 ± 2.33	19 ± 0.88
	Hex. Lv. Extr. Conc (mg·mL^{-1})						Hex. Stem-bark Extr. Conc (mg·mL^{-1})					
	10	**5**	**2.5**	**1.25**	**0.625**	**Control**	**10**	**5**	**2.5**	**1.25**	**0.625**	**Control**
K. pneumonia	17.50 ± 0.50	15 ± 2.0	14 ± 0	13 ± 1.0	0.85 ± 0.05	19.5 ± 0.50	24.0 ± 1.0	21.0 ± 2.0	20.5 ± 3.50	15.50 ± 3.50	13.50 ± 3.50	21.50 ± 1.50

E. coli = *Escherichia coli*; *P. aeruginosa* = *Pseudomonas aeruginosa*; *S. aureus* = *Staphylococcus aureus*; *MRSA* = methicillin-resistant *Staphylococcus aureus*; *K. pneumonia* = *Klebsiella pneumonia*; Chl.= Chloroform; Hex = Hexane, Meth = Methanol; Lv = leaves; Extr. = Extract; Conc. = Concentration.

Table 10. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *D. villosa* leaf and stem-bark extracts against different bacterial strains.

| | Meth. Lv. Extr. | | Meth. Stem Extr. | | Chl. Lv. Extr. | | Chl. Stem Extr. | |
| | Conc. (mg·mL^{-1}) | | Conc. (mg·mL^{-1}) | | Conc. (mg·mL^{-1}) | | Conc. (mg·mL^{-1}) | |
Bacteria strain	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
K. pneumonia	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
P. aeruginosa	0.01	0.01	0.01	0.01				
S. aureus	0.01	0.01	0.01	0.01				
MRSA	0.01	0.01	0.01	0.01				
E. coli	0.01	0.01	0.01	0.01				

P. aeruginosa = *Pseudomonas aeruginosa*; *S. aureus* = *Staphylococcus aureus*; MRSA = methicillin-resistant *Staphylococcus aureus*; *K. pneumonia* = *Klebsiella pneumonia*; Chl. = Chloroform; Hex = Hexane, Meth. = Methanol; Lv. = leaves; Extr. = Extract; Conc. = Concentration.

3.4. DPPH Radical Scavenging Activity

The free radical scavenging activity and IC$_{50}$ values of the leaf and stem-bark extracts are summarized in Figures 1 and 2, respectively. The simultaneous occurrence of a greater percentage of radical scavenging activity and lower IC$_{50}$ values indicated excellent antioxidant activity. Out of all the different leaf extracts, the methanol leaf extract showed higher scavenging activity (85.29%) compared to the other extracts, whereas the ascorbic acid at the same concentration showed 92.82%, which are somewhat close to each other (Figure 1a). In addition, the methanol leaf extract showed excellent DPPH radical scavenging activity with an IC$_{50}$ value of 9.53 µg·mL^{-1} compared to that of the ascorbic acid (10.3 µg·mL^{-1}) (Figure 1b).

In addition, both the chloroform and hexane leaf extracts showed weak antioxidant behavior with higher IC$_{50}$ values of 10.7 µg·mL^{-1} and 11.8 µg·mL^{-1}, respectively, compared to that of the ascorbic acid. The methanol and hexane stem-bark extracts also showed high scavenging activity at 85.76% and 87.22%, respectively, but these were not as high as the ascorbic acid (92.82%) (Figure 2a). The IC$_{50}$ of both the methanol (9.53 µg·mL^{-1}) and hexane (9.53 µg·mL^{-1}) stem-bark extracts were lower compared to the ascorbic acid (10.0 µg·mL^{-1}) (Figure 2b). Meanwhile, the chloroform stem-bark extract indicated a higher IC$_{50}$ compared to the ascorbic acid.

Figure 1. Comparison of DPPH radical scavenging (**a**) IC$_{50}$ values of *D. villosa* (%) of *D. villosa* leaf extracts in DPPH scavenging assay (**b**). Chl = Chloroform; Hex = Hexane, Meth = Methanol; Lv. = leaves; Extr. = Extract.
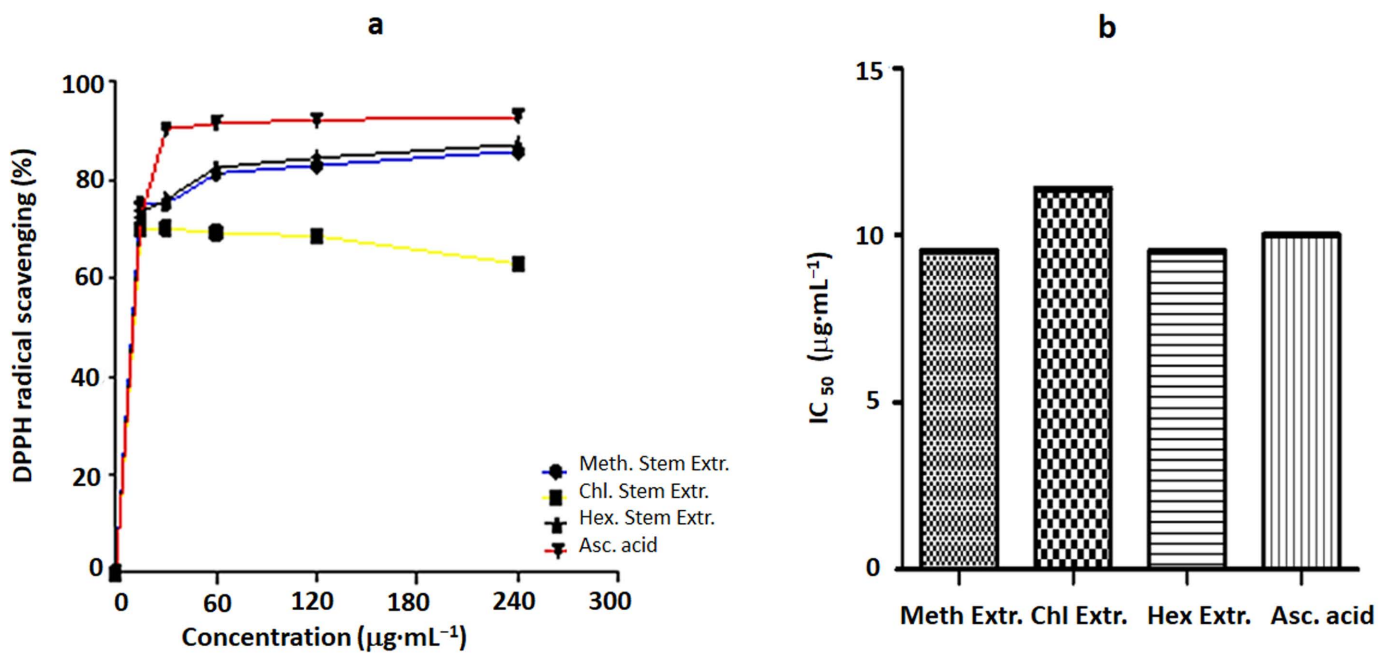
Figure 2. Comparison of DPPH radical (**a**) IC$_{50}$ values of *D. villosa* stem scavenging (%) of *D. villosa* stem-bark extracts in DPPH radical scavenging assay (**b**). Chl. = Chloroform; Hex = Hexane, Meth. = Methanol; Lv. = leaves; Extr. = Extract; Asc. Acid = Ascorbic acid.

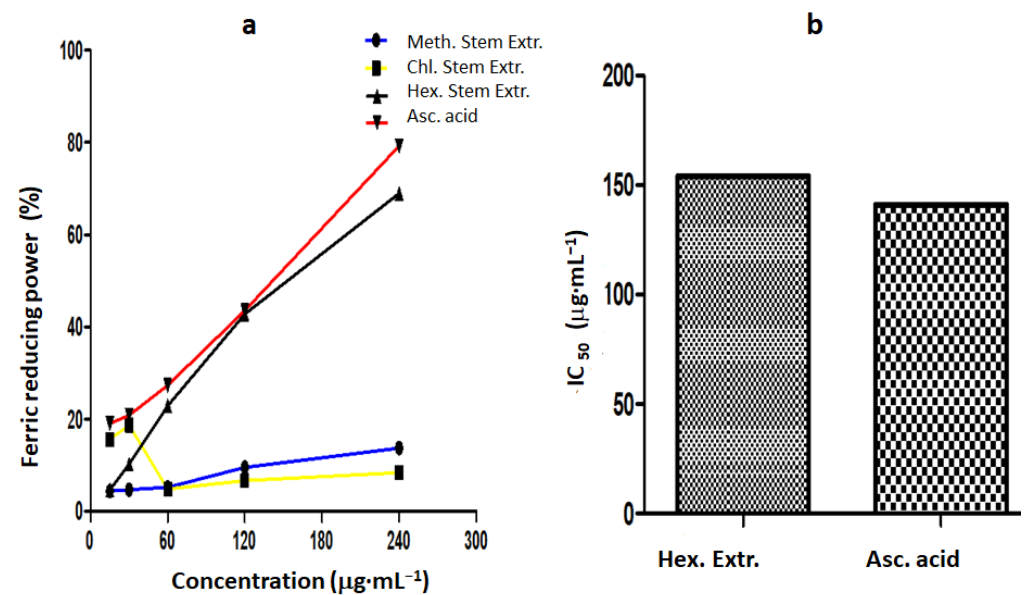*3.5. Ferric-Reducing Antioxidant Potential*

The ferric-reducing potential and IC$_{50}$ values of the leaf and stem-bark extracts of *D. villosa* are summarized in Figures 3 and 4, respectively. Among the different leaf extracts, the hexanic leaf extract showed high scavenging activity (85.2%), whereas the ascorbic acid indicated 79.3% at the same concentration (Figure 3a). The chloroform and methanol leaf extracts showed a higher reducing power compared to the ascorbic acid. Similarly, the methanol leaf extract showed ferric-reducing power with an IC$_{50}$ value of 112 µg·mL^{-1} compared to that of the ascorbic acid (143 µg·mL^{-1}) (Figure 3b). On the other hand, both the chloroform and hexane leaf extracts showed excellent antioxidant behavior with IC$_{50}$ values of 11.0 µg·mL^{-1} and 13.3 µg·mL^{-1}, respectively, compared to that of the ascorbic acid (Figure 3b). The methanol and hexane stem-bark extracts also showed high scavenging activity at 85.8% and 87.22%, respectively, but these were not as high as the ascorbic acid (92.8%) (Figure 4a). The IC$_{50}$ of both methanol and chloroform stem extracts was lower compared to that of the ascorbic acid (141.0 µg·mL^{-1}) (Figure 4b). Similarly, the hexane stem-bark extract further indicated a higher IC$_{50}$ compared to the ascorbic acid.

Figure 3. Comparison of ferric-reducing power (**a**) IC_{50} values for *D. villosa* extracts of *D. villosa* leaf extract in ferric-reducing antioxidant potential (**b**). Chl. = Chloroform; Hex = Hexane, Meth. = Methanol; Lv. = leaves; Extr. = Extract; Asc. Acid= Ascorbic acid.

Figure 4. Comparison of ferric-reducing power in (%) of *D. villosa* stem-bark extract (**a**), IC_{50} values for *D. villosa* stem ferric-reducing antioxidant potential (**b**). Chl. = Chloroform; Hex = Hexane, Meth. = Methanol; Extr. = Extract; Asc. Acid = Ascorbic acid.

3.6. Total Phenolic Content

The TPC in the leaves and stem bark of *D. villosa* was estimated and analyzed. One-way analysis of variance showed that there was a significant difference in the total phenol contents in *D. villosa* leaves and stem bark $F_{(2, 6)} = 225.8$, $p \leq 0.001$. The highest TPC in *D. villosa* leaves was found in the methanol extract (28.45 ± 0.50) mg gallic acid equivalent per gram of dry weight. Meanwhile, the highest TPC in the stem bark was found in the hexane extract (14.40 ± 0.58) mg gallic acid equivalent per gram of dry weight (Figure 5).

Figure 5. Total phenol content of the solvent extracts of both *D. villosa* leaves and stem bark. $F_{(2, 6)} = 225.8$, $p \leq 0.0001$. * (methanol vs. chloroform), $p < 0.05$. * (hexane vs. methanol), $p < 0.05$. Values are expressed as means ± SEM; n = 3/group. Chl. = Chloroform; Hex = Hexane, Meth. = Methanol; Lv. = leaves; Extr. = Extract.

3.7. Fourier Transform Infrared (FT–IR) Spectral Analysis

The FT–IR spectra of the *D. villosa* leaf and stem-bark extracts using methanol, chloroform, and hexane as the media for extraction are presented in Figure 6. The spectra analysis revealed the vibrational frequencies of the various functional groups observed to be present in the crude extracts. Absorption peaks with a broad range of 2500–3300 cm^{-1} were characteristic of the hydroxyl (O-H) group, particularly from carboxylic acid (Figure 6a,b). The peaks around 1650–1750 cm^{-1} were assigned to the carbonyl (C=O) group. The stretching peaks at 1599 and 1605 cm^{-1} occurred due to the presence of (C≡N) nitrile. The C-H (hydrocarbon) stretches appeared at 2927 cm^{-1} (Figure 6c,d). The peak at 2849 cm^{-1} was meant to further characterize the (C-H) stretching vibration. The strong peaks absorbed at 1735 and 1617 cm^{-1} (Figure 6c,d) were the (C=O) stretches in the aldehydes and ketones. The peaks at 3369 and 3309 cm^{-1} (Figure 6c,d) occurred due to the presence of the (O-H) functional group. In addition, the strong peaks at 2916 and 2848 cm^{-1}, as well as at 2917 and 2849 cm^{-1} (Figure 6e,f), were due to the presence of the (C-H) functional group.

Figure 6. FT–IR spectra of crude plant extracts of *D. villosa* (**a**) crude leaf methanol extract; (**b**) crude stem-bark methanol extract; (**c**) crude leaf chloroform extract; (**d**) crude stem-bark chloroform extract; (**e**) crude leaf hexane extract; (**f**) crude stem-bark hexane extract at room temperature (24 °C).

4. Discussion

Several studies have reported that herbal antioxidants act against free radicals [30–32]. The presence of secondary metabolites such as terpenoids, alkaloids, flavonoids, and phenolic compounds have been implicated as antioxidant factors in different plant materials [33]. It is interesting to note that the methanol extract of *D. villosa* (both leaves and stem bark) showed the presence of flavonoids and alkaloids. Following the qualitative phytochemical analysis, the methanol leaf extract presented with a color intensity to indicate the presence of terpenoids, flavonoids, and even phenolic compounds. It is without a doubt that the

confirmed compounds (terpenoids, flavonoids, and phenols) could provide the justifiable underlying factors for the antioxidant activity of *D. villosa*. This confirms the findings of Echeverría et al. [34] that showed that the antioxidant activity of natural flavonoids of *Chilean flora* (Flora of Chile) is a result of the embedded hydroxyl group for the oxygenation-substitution pattern. The presence of a hydroxyl group in the *D. villosa* leaves and stem bark supports hydroxylation as the plant's mechanism for exhibiting its antioxidant function and thereby conferring stability to free radicals. In addition, this study revealed the presence of alkaloids in the methanol leaf and stem-bark extracts only. Many alkaloids are potent antioxidants and are used for the treatment and/or management of skin cancer [35]. The detection of alkaloids in the plant extracts is consistent with Dangoggo et al. [36], who revealed that the aqueous extract of *Diospyros mespiliformis* (ebony diospiros) leaves was quite rich in alkaloids and may further be responsible for the effective inhibitory effect on DPPH, thereby helping in the detoxification of the generated radical oxygen species.

The GC–MS analysis indicted the existence of a number of phytocompounds such as n-hexadecanoic acid; phytol; palmitoleic acid; oxalic acid; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; acetate; alpha-cadinol; tau-Muurolol; eisosane; and vitamin E. The major compounds found in the methanol leaf extracts were 11, 14, 17-eicosatrienoic acid, methyl ester (15.19%), pentadecanoic acid, and methyl ester (29.89%), whereas in the methanol stem extracts, n-hexadecanoic acid (25.93%), hexadecanoic methyl ester (25.93%), and methyl 10-trans, 12-cis octadecadienoate (10.79%) were present. In the chloroform leaf extract, the major compounds were n-hexadecanoic acid (20.12%) and cis, cis, cis-7, 10, 13-Hexadecatrienal (14.36%), whereas phytol acetate (48.61%) and 3,7,11,15-tetramethyl-2-hexadecen-1-ol (15.54%) were found in the chloroform stem extract of *D. villosa*. Similarly, oxalic acid, cyclohexyl ethylester (22.48%), and n-hexadecanoic acid (11.77%) were found in the hexane leaf extract, whereas in the hexane stem extract, oxalic acid, cyclohexylpropyl ester (21.93%), n-hexadecanoic acid, and ethyl ester (13.98%) were found in high proportions. The high concentrations of valeric acid in the hexane extracts of the leaves and stem bark may be associated with the antioxidant activity of the extracts. These results support the findings of Vishwakarma et al. [37], where valeric acid isolated from *Valeriana wallichii* was scientifically proven to have anti-inflammatory properties by reducing lipid peroxidation and restoring glutathione levels in intracerebrovascular streptozotocin-induced neurodegeneration, and further suggests that it could be used in the management of inflammatory diseases.

Phytol is not just a diterpene compound but can also act as an anti-inflammatory, anticancer, antimicrobial, and diuretic. Phytol acetate as found in the *D. villosa* extracts was revealed to be in high concentrations and could be used as a novel class of pharmaceuticals as a therapeutic measure for rheumatoid arthritis and especially for chronic inflammatory diseases. This is further corroborated by Ogunlesi et al. [38], where phytol increased oxidative burst in vivo and further corrected the effect of the genetic polymorphism in the translational model of arthritis. Phytol may further be considered a novel class of drugs for treating chronic inflammatory diseases. In fact, phytol as an acyclic diterpene alcohol could be considered a precursor for the industrial synthesis of vitamin E [38]. Among the found compounds, *n*-hexadecanoic acid, hexadecanoic acid, and palmitoleic acid have antioxidant, hypocholesterolemic, nematicide, pesticide, and lubricating properties [39]. In addition, *n*-hexadecanoic acid and ethyl ester have antitumor, antifungal, and antibacterial properties. Hexadecanoic acid as found in *D. villosa* possesses antioxidant and haemolytic properties and is also an effective pesticide.

Similarly, Rathee et al. [40] reported that the methanolic extract of *Mentha longifolia* showed remarkable antioxidant activity via the reactive oxygen species scavenging efficacy and lipid peroxidation inhibition. In this study, the methanol extracts of *D. villosa* (both leaves and stem bark) showed notable antioxidant activity in comparison with the reference drug (ascorbic acid). This activity could further be associated with the presence of alkaloids and flavonoids as well as the phenolic content. The observed lower IC_{50} values of these extracts support the relevance of *D. villosa* leaves and stem bark as a potential organic source of antioxidants and hence they can be used for the prevention of free-radical-mediated

diseases. Furthermore, the results of the DPPH radical scavenging ability showed that the methanol leaf and stem-bark extracts can prevent radical-induced oxidative damage. This is reflected in the phenol content in the methanol leaf and stem-bark extracts. Accordingly, Saeed et al. [41] established a correlation between the health benefits of polyphenolic-rich plants and their antioxidant properties, and the possible mechanism responsible for the phenolic activity could be the redox properties of its hydroxyl group.

The functional groups in any bio-organic compound influence the biological activities of the compound. The influence is a result of the contribution of the embedded functional groups to the inherent properties of the compounds such as solubility, stereochemistry, partition coefficient, acid–base properties, etc. All these proficiencies are believed to induce the metabolic extraction, absorption, distribution, and toxicity of bioactive molecules [42]. Therefore, the analysis of the functional group showed that it performs a dynamic function in identifying the physicochemical properties of the extracts. The detection of the functional groups, therefore, helped to assess the structure–function relationship of the bio-organic compound. In this study, the FT–IR spectral analysis of the leaf and stem-bark extracts of *D. villosa* showed the presence of phytochemicals carrying a hydrogen-bonded OH functional group. The functionality of most phenolic compounds such as tannins and flavonoids owes to the presence of a hydroxyl functional group [43].

Although the mechanism of antibacterial activity could not be ascertained in this study, it was however noted that higher zones of inhibition were produced by the graded doses of the methanol leaf extract compared to those of the stem bark. It is not a mere coincidence that the chloroform and hexane extracts showed antibacterial activity against *K. pneumonia* only. The presence of alkaloids in the methanol extracts may be identified as an additional key factor for the antibacterial activity of the *D. villosa* plant. This is supported by Bai et al. [39]'s evidence and confirms the antibacterial activity of the alkaloids as well as the mechanism of action through intercalation with bacterial DNA. There has been motivation and justification for the production of new antimicrobial agents to treat infections [44]. The newest trend shows that plant-based antimicrobial agents have high medicinal efficacy since they pose no hazardous threats to human life [45]. The fact that plant extracts produce zones of inhibition against different bacteria strains indicates their antimicrobial activity and further confirms their use as anti-infection agents. In addition, the production of zones of inhibition against both Gram-negative and Gram-positive bacteria shows their applicability for a wide spectrum of activity. The methanol extract of *D. villosa* leaves further indicates a higher zone of inhibition against *S. aureus*, *P. aeruginosa,* and *K. pneumoniae* compared to conventional antibiotics of high concentrations. Although the mechanisms of action of *D. villosa* are yet to be ascertained, there is no doubt that the chemical contents of the plants, such as phenols, flavonoids, and alkaloids, are much more likely to be responsible for antimicrobial activities. This is similar to Linuma et al. [46], where the flavonoid contents of the extract were revealed to be a typical phytochemical responsible for microbial inhibition. In addition, the methanol stem-bark extract of *D. villosa* further showed a reaction against these strains but it was not as high as the control drug. The observed reduction in the degree of inhibitory activity could be ascribed to the lower concentration of phenolic content in the stem-bark extract of *D. villosa*. Vaquero et al. [47] indicated that phenolic compounds possess high antibacterial effects. Similarly, Majhenič et al. [48] found that methanol extracts of *Paullinia cupana* (guarana) seed showed high antibacterial activity due to their high phenolic contents [48]. Hence, it can be said that *D. villosa* possesses exceptional phytochemicals that account for bacterial metabolism inhibition.

5. Conclusions

The present study indicated that the leaf and stem-bark extracts of *D. villosa* displayed the occurrence of flavonoids, alkaloids, phenols, and even terpenoids. The leaves of the plant further revealed strong antioxidant activity owing to the high concentration of total phenolic content as well as the absorption peaks with a wide range for the hydroxyl group. In addition, the plant showed strong antimicrobial activities against Gram-negative and

Gram-positive bacteria strains with a minimum inhibitory concentration of 0.01 mg·mL^{-1}. Therefore, this study suggests that the leaf and stem-bark extracts of *D. villosa* are a notable source of natural antioxidant and antimicrobial agents. It is expected that this study could lead to the design of bio-compounds that could be used to investigate novel and efficacious antioxidants as well as the antimicrobial agents of plant origin. Further research is needed to isolate the active molecules from the crude extract and also to evaluate in detail the in vivo biological activities of these isolated compounds.

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