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Research Paper

Chemical Constituents of the Roots Extract of *Dryopteris schimperiana* and Evaluation for Antibacterial and Radical Scavenging Activities

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Abstract

Dryopteris schimperiana (Dryopteridaceae) is traditionally used in folk medicine of Ethiopia against bacteria and internal parasites. In view of its traditional use and absence of scientific reports, an attempt was made to explore the chemical constituents, antibacterial and radical scavenging activities of the solvent extracts of the root of *Dryopteris schimperiana.* In this regard, the root was successively extracted with n-hexane, $CHCl_3$ and methanol to afford 5 g (2%), 2 g (0.8%) and 26.6 g (10.6%), respectively. The n-hexane and $CHCl_3$ extracts showing similar TLC profile were mixed and fractionated over silica gel column chromatography which led to the isolation of two compounds identified as heptacosanol (**1**) and isorhmentin (**2**). The structures of the isolated compounds were accomplished using spectroscopic methods including UV-Vis, IR and NMR. To the best of our knowledge, these compounds have not been reported from the genus *Dryopteries.* The extracts and isolated compounds were evaluated for their antibacterial activities using agar well diffusion method against two Gram-positive bacteria (Staphylococcus aureus and Bacillus subtitis) and two Gram-negative bacteria (*E. coli* and Salmonella). The results showed that the n-hexane extract, methanol extracts and isolated compounds were active against all tested bacteria, with compound **2** shown to have an inhibition zone of 17 mm which is comparable with the positive control. Furthermore the extracts and isolated compounds were assessed for their radical scavenging activity using DPPH assay. Isorhmentin (**2**) displayed pronounceable % radical scavenging activity (82.8%) compared with ascorbic acid indicating its strong ability to act as radical scavenger. This study demonstrated that the antibacterial and radical scavenging activity of the root of D. *shimperiana* is accounted to the presence of isorhamnetin. Therefore, the biological activity displayed by the constituents of the roots of *D. shimperiana* corroborates the traditional use of this plant against bacteria.

1. Introduction

Plants have been known from prehistoric times to treat a wide array of diseases affecting human beings and livestocks. Out of a quarter of a million identified plants in the world about one fourth has at one time or other used by some people or cultures for medicinal purpose. The use of traditional medicine for treating human diseases remains widespread in developing countries. The recent reports of World Health Organization (WHO) indicated that 70 to 90% of world population especially from developing countries uses plant remedies for their health care. The principal components responsible for the traditional use of these

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plants are accounted to the presence of secondary metabolites including flavonoids, alkaloids, terpenoids, anthraquinone, lignans, etc (Chanda et al., 2006). Therefore, plants are useful to humans as a source of bioactive pharmaceuticals. Although many have been treated by conventional pharmaceutical approaches, there is a growing interest in the use of natural products by the general public. At the present, many scientists are interested to evaluate the chemical constituents of plants used traditionally by peoples for medicinal purpose.

Some plant species belonging to the genus *Dryopteries* are among medicinal plants that are traditionally used for the treatment of wide array of diseases. Pharmacologically, some species in the genus *Dryopteris* were reported as an effective antibacterial agent against various bacterial pathogens including *Staphylococcus aureus*, *Bacillus subtitis*, *E. coli*, *Salmonella typhus* and *Pseudomonas aeruginosa*. Some species in this genus were shown to have various biological activities including anthelmintic, antiviral, antitumor, antimicrobial, antioxidative and anti-inflammatory (Han et al., 2015). Previous phytochemical investigation of some species in *Dryopteries* has resulted in the isolation of flavonoids, anthraquinones (Kwanzoquinone A, hydroxychryphanol, Kwanzoquinone (B**6**, C**7**, D**8**, E**9**, F**10**)) and alkaloids (Harborne, 1966; Min et al., 2010; Imperato, 2006; Imperato, 2007). *Dryopteris schimperiana* is traditionally used in the folk medicine of Ethiopia against bacterial infections in addition to its use to remove internal parasitic worms. Despite the extensive popular use of this plant as a remedy against bacteria, there is no information describing the chemical constituents, antibacterial and radical scavenging activities of the roots of *D. schimperiana*. Therefore in this paper, we report for the first time the chemical studies, antibacterial and radical scavenging activities of the root extracts of *D. schimperiana.*

2. Material and Methods

2.1. Plant material collection and authentication

The roots of *D. schimperiana* were collected from Kaka mountain Lemu Bilbilo District, Arsi Zone, Oromia Region, Ethiopia. The plant material was identified by a botanist and deposited at the National Herbarium of Addis Ababa University with voucher specimen GM005. The sample was washed with tap water and dried under shade at the laboratory of Department of Chemistry, Adama Science and Technology University.

2.2. Instruments

Melting point was determined in capillary tube with a Thiele tube melting point apparatus. Analytical TLC was run on a 0.25 mm thick layer of silica gel GF254 (Merck) on aluminum plate. Spots were detected by observation under UV light (254 nm) followed by dipping in vanillin/H_2SO_4. Column chromatography was performed using silica gel (230-400 mesh) Merck. Solvent was freed using rotavapor. Infrared (IR) spectra were obtained on Perkin-Elmer 65FT ((IR ν_{max} KBr 4000-400 cm^{-1}) infrared spectrometer using KBr pellets. 1D NMR were obtained on Brucker Avance instrument NMR machine (Bruker Avance 400 NMR spectrometer) at the Chemistry Department of Addis Ababa University.

2.3. Extraction and Isolation

The ground root of *D. schimperiana* (250 g) was successively extracted on maceration with each 1.5 L of *n*-hexane, $CHCl_3$ and MeOH for 72 hrs. The extracts were filtered and concentrated using rotary evaporator to give their corresponding extracts. The profile of each extract was subsequently analyzed by TLC. The hexane and $CHCl_3$ extracts which displayed similar TLC profile were mixed. The combined extract (7 g) was dissolved and fractionated over silica gel (150 g) column chromatography using *n*-hexane:EtOAc:MeOH of increasing polarities as eluent to afford 92 fractions (Table 1), each 50 mL.

Samples showing similar spots on their TLC profiles were mixed. Fractions **14-18** which showed one spot on TLC were combined and dried to afford compound **1**. On the other hand, fraction **25** after rechromatographed over silica gel column chromatography afforded compound **2**.

2.4. Phytochemical screening tests

Qualitative phytochemical screening of the *n*-hexane and methanol extracts of the root of *D. shimpiriana* were done following standard procedures to detect the presence or absence of secondary metabolites including flavonoids, saponins, phenolics, terpenoids, steroids, coumarins, anthraquinones, alkaloids, phenolics and

tannins (Balick, 1996). The detailed experimental procedure used to analyze each phytochemicals is presented as follows:

Flavonoids (Alkaline reagent test): Each 0.25 g crude extracts of *n*-hexane and methanol were treated with ethyl acetate (10 mL) and heated for 3 min using steam bath. The mixture were filtered, and mixed with ammonia (1 mL) solution. Formation of intense yellow color ratifies the presence of flavonoids (Balick, 1996).

Table 1: Column chromatographic fractionation of the combined hexane and chloroform extracts of the root of *D. shimpiriana*

Eluent	Ratio	Fractions
n-hexane	100%	F_1-F_4
n-hexane/EtOAc	98:2	F_5- F_7
"	96:4	F_8-F_{10}
"	90:10	F_{11}-F_{13}
"	80:20	F_{14}-F_{18}
"	75:25	F_{19}-F_{26}
"	70:30	F_{27}-F_{31}
"	60:40	F_{32}-F_{33}
"	50:50	F_{34}-F_{37}

Saponins (Froth test): Each 0.25 g crude extracts of *n*-hexane and methanol were dissolved and diluted with distilled water (10 mL) and shaken for 15 min. Formation of layer of foam indicates the presence of saponnins (Balick, 1996).

Phenolics (Ferric chloride test): Each 0.1 g crude extracts of *n*-hexane and methanol were treated with few drops of 2% of $FeCl_3$ and the formation of bluish green to black color indicates the presence of phenols (Balick, 1996).

Test for tannins (Gelatin test): To the crude extracts 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins (Balick, 1996).

Terpenoids (Salkowski test): The extracts (5 mg) were mixed with 2 mL of chloroform and filtered. The filtrates were treated with few drops of concentrated H_2SO_4, shaken and allowed to stand. Appearance of golden yellow color indicates the presence of terpenoids (Balick, 1996).

Steroids (Salkowski test): One mL of the extracts (1 mg/mL) was dissolved in 10 mL of chloroform and equal volume of concentrated H_2SO_4 was added by sides of the test tube. The upper layer turns red and H_2SO_4 layer showed yellow with green fluorescence. This indicates the presence of steroids (Balick, 1996).

Coumarins: To 2 mL (1 mg/mL) of the extract was added 3 mL of 10% NaOH. Formation of yellow color indicates the presence of coumarins (Balick, 1996).

Anthraquinones: The extracts (1 mg/mL) were boiled with 10% HCl for few min. in water bath and filtered. The filtrate was allowed to cool and equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% ammonia were added to the mixture and heated. The formation of rose-pink color was taken as an indication for the presence of anthraquinones (Balick, 1996).

Alkaloids (Wagner's test): The extracts (1 mg/mL) were dissolved individually in dilute HCl and filtered. Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids (Balick, 1996).

2.5. Antibacterial Activity

The methanol extract, *n*-hexane extract and the two isolated compounds were evaluated for their *in vitro* antibacterial activity using disc diffusion method against two Gram positive bacterial pathogens (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram negative bacteria (*Escherichia coli*, and *Salmonella thyphimurium*). The bacterial cultures were inoculated into the Muller Hinton Agar (MHA). Approximately, 20 mL of sterile MHA were poured into sterile culture plates and allowed to set wells of about 6 mm in diameter which were punched on the plates. The tests were conducted at 5 mg/mL for both crude extracts and isolated compounds. Bacterial concentration was prepared at 1.3×10^8 CFU/mL. The plates were incubated at 37°C. The antibacterial activity of the plant extracts and compounds were evaluated by measuring the zone of inhibition against the test organism after 24 hr (Tiwari, 2011). The results were calculated as averages of triplicate tests. The zone of inhibitions in all cases were includes the diameter of the wells. DMSO was used as negative control during the whole test on bacteria while amoxil was used as positive control.

2.6. DPPH Radical Scavenging Assay

The radical scavenging assay of the extract and constituents of the roots of *Dryopteris shimpiriana* were assessed using DPPH according to the following procedure (Rivero-Perez *et al*., 20017). In view of this, the hexane extract was dissolved in methanol to afford 1 mg/mL. It was serially diluted in methanol to give concentration of 500, 250, 125 and 62.5 µg/mL. To 1 mL of each concentration, 4 mL DPPH (0.04%DPPH in MeOH) was added to make 100, 50, 25 and 12.5 µg/mL solutions. This was repeated for the methanol extracts and isolated compounds. Then all the samples prepared were incubated in an oven at 37°C for 30 min. and then absorbance was recorded at 517 nm using UV-Vis spectrophotometer. The experiments were done triplicates and results were reported as averages of the triplicates. The percentage inhibition was calculated using the formula (Boakye-Yiadom, 1997).

$$(\%)\ \text{inhibition} = \frac{(\text{A control} - \text{A sample})}{\text{A control}} \times 100$$

Where A control was the absorbance of the DPPH solution and A sample was the absorbance in the presence of sample in DPPH solution. Samples were analyzed in triplicate. Ascorbic acid was used as positive control.

3. Results and Discussion

The roots were successively extracted with *n*-hexane, $CHCl_3$ and MeOH to afford 5 g (2%), 2 g (0.8%) and 26.7 g (10.6%) crude extracts, respectively. The latter extract was found to be red in color while the first two were black. As clearly observed, the extract yield obtained using MeOH was higher compared to *n*-hexane and $CHCl_3$. This indicates that the secondary metabolites present in the roots of *D. shimpiriana* are mainly polar.

3.1. Phytochemical Screening

The combined *n*-hexane and chloroform extracts, and methanol extracts of the root of *Dryopteris shimpiriana* was screened for its secondary metabolites including alkaloids, saponins, terpenoids, phenolics, tannins, anthraquinones, flavonoids, steroids and coumarins with the results depicted in Table 2.

As revealed from Table 2, the *n*-hexane/$CHCl_3$ and methanol extract of the root of *Dryopteris shimpiriana* contains secondary metabolites including alkaloids, terpenoids, anthraquinones, and flavonoids. However, neither steroids nor tannins were detected in the *n*-hexane/$CHCl_3$ and methanol extracts. Flavonoids are classes of natural products that are used as an essential component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. This is attributed to their anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function (Aoki *et al*., 2000). Therefore, the presence of flavonoids in this plant is significant as the plant may serves as anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties. Hitherto, secondary metabolites including saponins, flavonoids, alkaloids, anthraquinones, and terpenoids exert antimicrobial activity through different mechanism (Ghamba *et al*., 2014). Therefore, the presence of one or more of these secondary metabolites may account for the traditional use of this plant against bacteria. .

Table 2: Phytochemical screening results of the roots extracts of Dryopteris shimpiriana

Secondary metabolites	Test/reagents	Hexane and $CHCl_3$ extract	Methanol extract
Alkaloids	Wanger test	+	+
Saponins	Froth test	+	+
Terpenoids	Salkowskis test	+	+
Phenolics	Ferric chloride test	+	+
Tannins	Gelatin test	-	-
Anthraquinones	10%HCl & then NH_3	+	+
Coumarins	10%NaOH	+	+
Flavonoids	Alkaline test	+	+
Steroids	Salkowskis test	-	-

(+) indicates presence (-) indicates Absence

3.2. Characterization of isolated compounds

Two compounds were isolated from the combined *n*-hexane and chloroform extracts of the root of *Dryopteris shimpiriana*. The detailed structure elucidations of these compounds are presented below.

Compound **1** (12 mg) was isolated as white solid melting at 82-83ºC (Lit. 81-82ºC) (Koay, C.Y., *et al*., 2013). The UV-Vis spectrum ($CHCl_3$) showed absorption maxima neither in the ultra-violet nor in the visible region indicating the absence of conjugated chromophore. The IR spectrum displayed absorption bands at 3400 and 2950 cm^{-1} due to hydroxy and aliphatic C-H stretching, respectively.

The ^{1}H-NMR spectrum ($CDCl_3$) demonstrated a triplet signal at δ 3.66 (2H, J = 6.8 Hz) assigned to methylene protons on oxygenated carbon. The spectrum also displayed another signal at δ_H 1.61 (4H, m) which were assigned to four protons on two methylene carbons next to carbon bearing oxygen. The presence of many methylene protons integrating for 46 hydrogens is evident at δ_H 1.27 (46H, brs). This clearly indicates the presence of overlapping signals due to many methylenes in the structure of the compound. The upfield signal at δ_H 0.89 (3H, t, J = 6.8 Hz) is diagnostic for the presence of terminal methyl group.

The ^{13}C-NMR spectrum with the aid of DEPT-135 showed the presence of ten well resolved carbon signals of which nine are due to methylenes and one is methyl group. The downfield signal observed at δ_C 63.1 is ascribed to an oxygenated methylene carbon. This agreed very well with the data obtained from the proton NMR spectrum. The spectrum also displayed signals due to methylene carbons in the aliphatic regions at δ_C 32.8, 31.9, 29.7, 29.6, 29.4, 29.3, 25.7 and 22.7. The intense signal observed at δ_C 29.7 is due to the presence of seventeen overlapping methylene carbons which agreed well with the ^{1}H-NMR. Furthermore, the carbon resonance at δ 14.1 is characteristic signal for terminal methyl group. The above NMR spectral data generated indicated that compound **1** is a long chain alcohol named as heptacosanol whose structure is shown in Figure 1.

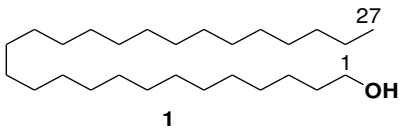

Figure 1: Structure of heptacosanol (**1**)

Comparison of the NMR spectral data of compound **1** with those reported in the literature for 1-heptacosal (Koay *et al*., 2013) was in close agreement (Table 3). This compound was previously reported from the leaves of *Strobilanthes crispus* (Koay *et al*., 2013) but has not been reported from any species of *Dryopteris.*

Compound **2** (17 mg) was isolated as a yellow solid from the combined *n*-hexane and $CHCl_3$ extract of the root of *Dryopteris shimpiriana*. The UV-Vis spectrum (MeOH) showed absorption maxima at 271 and 338 nm suggesting the presence of a flavonoid chromophore. The IR spectrum displayed an absorption band at 1646 cm^{-1} attributable to an α, β-unsaturated carbonyl. The presence of C-O and C-H stretching were evident from the observed absorption bands at 1090 and 2924 cm^{-1}, respectively.

Table 3: ^{1}H- and ^{13}C-NMR spectral data of compound **1** with those reported in the literature for heptacosanol, δ in ppm and J in Hz

NMR spectral data of compound **1**		Data reported for 1-heptacosanol (Koay *et al*., 2013)	
^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
3.66 (2H, *t*, *J* = 6.60, H-1)	63.1 (C-1)	3.64 (2H, *t*, *J* = 6.30, H-1)	63.1 (C-1)
1.61 (4H, *m*, H-2&3)	32.8 (C-2)	1.57 (4H, *m*, H-2&3)	32.8 (C-2)
1.27(46H, *br s*, H-4 to H-26)	31.9 (C-3)	1.25(48H, *br s*, H-3 to H-26)	31.9 (C-3)
	29.7-29.3(C4-24)		29.7-29.3(C4-24)
	25.7 (C-25)		25.7 (C-25)
	22.6 (C-26)		22.6 (C-26)
0.89 (3H, t, *J* = 6.8, H-27)	14.1 (C-27)	0.88 (3H, *t*, *J* = 6.60, H-27)	14.1(C-27)

The ^{1}H-NMR spectrum showed signals in the aromatic region at δ_H 6.45 (1H, br s) and 6.61 (1H, br s) which are apparently assigned to protons on A ring of flavonoid skeleton. The proton signals at δ_H 7.13 (1H, *d*, *J* = 8.4 Hz), 7.76 (^{1}H, unresolved *dd*, *J* = 8.4 Hz*)* and 7.85 (1H, bro s) indicates ABX muntiplicity pattern suggesting a trisubsitituted B-ring of the flavonoid skeleton. The signal due to the presence of one methoxy protons is evident at δ_H 3.98 (3H, s).

The proton decoupled ^{13}C-NMR spectrum with the aid of DEPT-135 displayed the presence of five methine, one methyl and ten quaternary carbons (Table 4). The presence of *α*, *β*-unsaturated ketone is evident from the appearance of the carbonyl carbon signal at δ_C 178.2 (C4). The spectrum showed the presence of seven quaternary carbon signals in the oxygenated aromatic/olefinic regions at δ_C 163.7 (C-7), 161.0 (C-5), 156.5 (C-2), 156.2 (C-9), 144.8 (C-4'), 144.4 (C-3'), 120.9 (C-1') and 137.9 (C-3). Two typical sp^2 quaternary carbon signals were observed at δ_C 137.9 and 104.4 attributed to C-3 and C-5a carbons of flavonoid skeleton. The presence of five methine signals are evident at δ_C 121.3 (C-6'), 114.9 (C-2'), 114.8 (C-5'), 98.4 (C-6) and 93.5 (C-8). The latter two signals are characteristics of C-6 and C8 of the ring A of flavonoid skeleton, respectively, suggesting meta oxygenation substitution pattern of ring A. The up field signal at *δc* 59.4 is typical for the presence of only one methoxyl in the structure of the compound. The above spectral data and comparison with literature suggests that the compound is identical with isorhamnetin (**2**, figure 2, Table 2) (José *et al*., 2012).

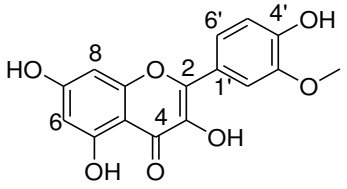

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Figure 2: Structure of isorhamnetin (**2**)

The ^{13}C-NMR spectral data of compound **2** was also compared with the literature reported for isorhamnetin with the data presented in Table 4.

3.3. Antibacterial Activity

The antibacterial activity of the hexane extract, methanol extract and the two isolated compounds from the roots of *Dryopteris shimpiriana* were investigated using agar well diffusion method, against some selected human pathogens such as *E. coli, Bacillus subtilis*, *S. aureus* and *Salmonella typhimurium* (Table 5).

The antibacterial activity of extracts and isolated compounds against bacterial pathogens using agar well diffusion method is expressed using inhibition zones. Antibacterial activity (*x*) is then characterized and classified based on the inhibition growth zone diameters and described as slight (*x*< 4 mm diameter), medium (*x* = 4-8 mm), high (*x* = 8-12 mm), and very high (*x*> 12 mm) (Obdak et al., 2017). In view of this, the extracts and isolated compounds assessed in the present study showed considerable antibacterial activity against three bacterial starins i.e *S. aureus*, *E. coli* and *S. thyphlomurium* compared with the positive and negative controls. The activities displayed by both

Table 4: NMR spectral comparison of isorhamnetin isolated from *Dryopteris shimpiriana* and one that reported in literature (José *et al*., 2012)

S No	^{13}C-NMR data of 2	Literature reported for isorhamnetin	S No	^{13}C-NMR data of 2	Literature reported for isorhamnetin
2	156.5	156.7	5a	104.4	105.5
3	137.9	135.2	1'	120.9	120.9
4	178.2	177.5	2'	114.9	114.6
5	161.0	160.8	3'	144.4	146.3
6	98.4	99.2	4'	144.8	149.5
7	163.7	162.7	5'	114.8	113.5
8	93.5	94.4	6'	121.3	122.1
8a	156.2	155.9	OCH_3	59.4	59.3

Table 5: Zone of bacterial growth inhibition diameter (mm)

Sample (5 mg/mL)	Zone of inhibition diameter (mm)			
	S. aureus	*B. subtitus*	*E. coli*	*S. thyphlomurium*
Hexane extract	13.0 ± 0.42	9.0 ± 0.3	10.3 ± 0.42	9.0 ± 0.51
Methanol extract	10.0 ± 0.5	9.0 ± 0.38	10.0 ± 0.51	9.6 ± 0.2
Compound 1	12.0 ± 0.4	NT	13.6 ± 0.2	NT
Compound 2	17.0 ± 0.2	9.6 ± 0.13	12.6 ± 0.3	10.6 ± 0.24
Amoxil	23.0 ± 0.4	20.0 ± 0.5	20.0 ± 0.34	18.0 ± 0.2
DMSO	-	-	-	-

Results are reported as M± SD; NT: not tested; Amoxil and DMSO were used as positive and negative controls, respectively

compounds are relatively better than the extracts. Compound **1** and **2** showed promising antibacterial activity against *E. coli* and *S. aureus* with zone of inhibition of 13.6 ± 0.2 and 17.0 ± 0.2, respectively, compared to amoxil (20.0 ± 0.34 and 23.0 ± 0.4, respectively) suggesting the activity displayed by the extracts of the root of *D. shimpiriana* is likely accounted to the known flavonoid, isorhamnetin. The activity displayed by the two extracts is likely accounted to the presence of various secondary metabolites including saponins, flavonoids, alkaloids, anthraquinones, and terpenoids. This agrees very well with the previous report which indicated as these secondary metabolites exert antimicrobial activity via different mechanism (Ghamba, *et al*., 2014).

Analysis of the antibacterial activity revealed as compound **1** and **2** were both active against all tested bacteria. The activities displayed by both compounds are relatively better than the extracts. Especially noteworthy is the very high activity of compound 2 (17 mm) shown against *S. aureus*. The result is superior compared with compound **1** and the two extracts, and comparable with the positive control. Furthermore the antibacterial activity displayed by the extracts and isolated constituents of the root of *D. shimpiriana* substantiates the traditional use of this plant against bacteria.

3.4. Radical scavenging activity

DPPH radical scavenging assay is a simple method for finding antioxidants by measuring absorbance at 517 nm due to the stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical. Those compounds that exhibit antioxidant activity change the purpled colored DPPH reagent to yellow in addition to their ability of lowering the absorbance at 517 nm. This phenomenon results due to the quenching of the DPPH radical and formation of DPPH-H that would lower absorption. In this work, the extracts and isolated compounds from the roots *D. shimpiriana* were evaluated using DPPH assay (Table 6).

Table 6: Results of radical scavenger activity of extracts and isolated compounds

Samples	%DPPH inhibition at			
	100(µg/mL)	50 (µg/mL)	25 (µg/mL)	12.5 (µg/mL)
Hexane/$CHCl_3$ extract	60.3±0.05	54.1±0.01	47.5±0.08	41.2±0.02
MeOH extract	63.9±0.01	51.6±0.01	44.7±0.07	36.5±0.10
Compound 1	44.7±0.03	40.0±0.05	33.3±0.01	25.7±0.02
Compound 2	82.8±0.01	77.1±0.01	71.4±0.02	63.8±0.03
Ascorbic acid	90.0±0.02			

The % DPPH inhibition shown by the methanol extract and the combined *n*-hexane/$CHCl_3$ extract is 63.9 and 60.3%, respectively. The activity displayed by the extract is accounted to the presence of flavonoids which was confirmed by the alkaline test. On the other hand, compound **2** displayed pronounceable free radical scavenging activity with an average percent inhibition of 82.8, 77.1, 71.4, and 63.8% at 100, 50, 25 and 12.5 $\mu g\ mL^{-1}$, respectively. The result is significant compared to ascorbic acid which inhibited the radical by 90% at 100 $\mu g\ mL^{-1}$. Further confirmation of the antioxidant activity of compound **2** was made due to the observed immediate discoloration of the purple DPPH solution to yellow. Therefore, the radical scavenging activity of the roots of *D. schimperiana* is accounted to the presence of the known flavonoids, namely, isorhamnetin (**2**).

4. Conclusions

In conclusion, phytochemical screening analysis conducted on roots extract of *Dryopteris schimperiana* revealed the presence of alkaloids, terpenes, anthraquinone, flavanoids, and tannins. The combined *n*-hexane and $CHCl_3$ extract after silica gel column chromatography furnished two compounds identified as spectroscopic methods as heptacosanol (**1**) and isorhamnetin (**2**). The extracts of the roots of *D. shimeriana* were shown to contain compounds with wide-spectrum antibacterial activity, capable of inhibiting the growth of Gram-positive and negative bacteria. The radical scavenging activity of the extracts and isolated compounds were increasing with increasing dose with isorhamnetin (**2**) shown to have the high % DPPH inhibition at concentration of 100 µg/mL which is comparable with ascorbic acid.

Reference

Aoki, T., Akashi, T., Ayabe, S. (2000). Flavonoids of leguminous plants: structure, biological activity, and biosynthesis. *J Plant Res,* 113: 475–488.

Balick, M.J. (1996). Transforming ethnobotany for the new millennium. *Ann Mo Bot Gard.* 83: 58-66

Boakye-Yiadom, K., Fiagbe, N.I.Y., Ayim, J. S. K. (1977). Antimicrobial properties of some West African Medicinal Plants. Antimicrobial activity of xylopic and other diterpenes from the fruits of *Xylopia aethiopica* (Annonaceae) Lloydia, 40: 543–545.

Chanda, S.V, Parekh, J., Karathia, N. (2006). Evaluation of antibacterial activity and phytochemical analysis of *Bauhinia variegate*. L bark *Afr J Biomed Res.* 9:53–56.

Ghamba, P.E., Balla, H., Goje, L.J., Halidu, A., Dauda, M.D. (2014). *In vitro* antimicrobial activities of *Vernonia amygdalina* on selected clinical isolates, *Int. J. Curr. Microbiol. App. Sci,* 3(4): 1103-1113.

Harborne, J.B. (1966). Comparative biochemistry of flavonoids-II: 3-desoxyanthocyanins and their systematic distribution in ferns and gesnerads. *Phytochemistry, 5*: 589–600.

Harborne, J.B. (1988). *The Flavonoids, Advances in Research since 1980*. London: Chapman and Hall; 427–468.

Han, X., Lia, Z., Li, C., Jia, W., Wang, H., and Wang, C. (2015). Phytochemical Constituents and Biological Activities of Plants from the Genus *Dryopteris*, *Chemistry & Biodiversity*, 12: 1131

Imperato, F. (2006). Kempferol 3-O-(acetylrutinoside), a new flavonoid and two new fern constituents, quercetin 3-O-(acetylglucoside) and 3-O-(acetylrutinoside), *Am. Fern J*. 96: 93–95.

Imperato, F. (2007). A new flavonoid, quercetin-3-O-(X″-acetyl-X″-cinnomoyl -glucoside) and a new fern constituent quescetin-3-O-(glucosylrhamnoside) from *Dryopteris villarii*. *Am. Fern J.,* 97:124–126.

Imperato, F. (2007). Three new flavonoid glycosides, kaempferol 3-O-(caffeoylrhamnoside), apigenin 4›-O-(caffeoylglucoside) and 4›-O-(feruloylglucoside) from *Dryopteris villarii*. *Am. Fern J*, 97:233–236.

José, G., Napolitano, David, C., Lankin, Shao-Nong Chen and Guido, F., Pauli. (2012) Complete ^1H NMR spectral analysis of ten chemical markers of *Ginkgo biloba. Magn Reson Chem.,* 50(8: 569–575.

Koay, C.Y., Wong, C.K., Osman, H., Eldeen, I.; Asmawi, Z.M. (2013). Chemical Constituents and Biological Activities of *Strobilanthes crispus* L., *Rec. Nat. Prod*. 7(1): 59–64.

Min, B.S., Tomiyama, M., Ma, C.M., Nakamura, N., Hattori, M. (2001). Kaempferol acetylrhamnosides from the rhizome of Dryopteris crassirhizoma and their inhibitory effects on three different activities of human immunodeficiency virus-1 reverse transcriptase. *Chem. Pharm. Bull*. 49:546–550.

Obdak, A., Zielinska, D., Rzepkowska, A., KoBohyn-Krajewska,D. (2017). Comparison of antibacterial activity of *Lactobacillus plantarum*strains isolated from two different kinds of regional cheeses from Poland: Oscypek and Korycinski Cheese, *BioMed Research International*, 9:1-10.

Rivero-Perez, M.D., Muniz, P., Sanjase, M.L. (2007). Antioxidant profile of red coines evaluated by total antioxidant capacity, Scavenger activity, and biomarkers of oxidative stress methodologies. *Journal of Agricultural and Food chemistry*, 55(14): 5476-5483.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., Kaur, H**.** (2011). Phytochemical screening and extraction: A review. *Internationale Pharmaceutica Sci-encia*, 1: 98-106