

RESEARCH

Open Access



# Investigation of antioxidant and antimicrobial activities of *Euclea schimperi* leaf extracts

Alemayehu Mekonnen\*, Minaleshewa Atlabachew and Banchalem Kassie

## Abstract

**Background:** Bioactive components from medicinal plants are said to be responsible for the antioxidant and antimicrobial effects of plant extracts. It was the main purpose of this paper to evaluate the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant and antimicrobial activities of leaf extracts of *Euclea schimperi*.

**Results:** Strong evidence was found when a positive correlation was found between TPC or TFC and antioxidant power. To put it differently, TPC of the *E. schimperi* leaf extracts varied between  $748.78 \pm 3.11$  mgGAE/100 g of dry extract (methanol) and  $40.45 \pm 0.66$  mgGAE/100 g of dry extract (chloroform) while TFC varied from  $3.306 \pm 0.142$  mgQE/100 g of dry extract (methanol) to  $0.334 \pm 0.002$  mgQE/100 g dry extract (chloroform). Similarly, all extracts exhibited significant amount of antioxidant and antimicrobial activities. Ferric reducing power was ranged between  $312.145 \pm 1.542$  mgAAE/100 g extract (methanol) and  $48.177 \pm 0.286$  mgAAE/100 g (chloroform). DPPH radical scavenging activity (%) lied between 83.4% (methanol) and 58.5% (chloroform). The voltammetric analysis of the crude extract gave three different sharp peaks at the oxidation potential side. Finally, the reducing agent capacity of the extracts was further confirmed by synthesizing silver nanoparticles from  $\text{AgNO}_3$  solution and the plant crude extracts. Crude extracts of *E. schimperi* leaves showed various degree of antimicrobial activity towards different standard strains with mean zone of inhibition ranging from 0 to 20.67 mm. Both solvent leaf extracts of *E. schimperi* exhibited MIC ranging from 62.5 to 125 mg/mL towards most bacterial species.

**Conclusions:** *Euclea schimperi* leaf extracts were found to contain high amount of total phenolic and small amount of flavonoid compounds which play a major role in controlling oxidation. In general, therefore, the results prove the effectiveness of the plant for its excellent antioxidant and antimicrobial activities. Accordingly, the positive values of the plant in traditional medicines have been confirmed.

**Keywords:** *Euclea schimperi*, Free radicals, Antioxidants, Total phenolic content, Total flavonoid content, Antibacterial activity, Minimum inhibitory concentration

## Introduction

Traditional medicine is regarded as a combination of knowledge, skill and practice originated from theories, experiences and attitudes native to various cultures that is utilized to treat and diagnose enormous types of physical and mental complications and maintain health [1]. Many people in the world rely on traditional medicine

for their primarily health care needs. It is well understood that several medical techniques and procedures have been discovered from the use and knowledge of traditional medicines. This is because the major part of traditional therapy involves the use of plant extracts or their active principles. As a result, natural products have recently become of immense interest owing to their diverse application [2].

Since in the early stage, plants have been used as a source of medicinal compounds and still continued to play a major role in the human health [3]. They are the

\*Correspondence: negaalex@yahoo.com; alemayehum@bdu.edu.et  
Chemistry Department, Science College, Bahir Dar University, P.O. Box: 79,  
Bahir Dar, Ethiopia

source of drugs in the traditional and modern medicine, nutraceuticals, food supplements, folk medicines and precursors for synthetic drugs [4]. The discoveries of potential new drugs have been led by screening bioactive compounds from plants having efficient activity against various diseases [5].

Phytochemicals are bioactive and naturally occurring compounds originating in plants, which act against pathogens and provide health benefits for humans [6]. Among the prevalent group of phytochemicals, alkaloids, terpenes, flavonoids and phenolic compounds are widely found in most plants and plant parts [7]. Extracts of plant parts (fruits, seeds, leaves, etc.) are used for medicinal effects, among which some of the classes of compounds have antioxidant activity against reactive species in the body [8].

The most recognized antioxidants derived from medicinal plants are phenolic compounds and have high antioxidant activity against free radicals. However, the extent of the activity depends on the structure of the molecules [8, 9]. These antioxidant constituents are closely associated to the prevention of degenerative diseases, such as cardiovascular and neurological illnesses, oxidative stress malfunctions and cancer [9, 10].

*Euclea schimperi*, from the family of *Ebenaceae*, is traditionally used in the treatment of wound, teeth infections, eye disorders, headache, pain and spasm. In Ethiopia, the leaf of this plant is used to treat illness such as gonorrhea, eczema and constipation. In addition, they treat the pots in which milk is kept with the smoke of *E. schimperi* branches to prevent the milk from curdling [11, 12]. The leaves is also used for the treatment of skin disorders, snake biting, scabies, leprosy, Tinea capitis, acne, warts, rheumatic pain and elephantiasis [13].

Despite the wide therapeutic potential presented by *E. schimperi* leaves in Ethiopia, studies on the phytochemical profile, antioxidant and antimicrobial activities of the leaves of this plant are still lacking. Only one other MSc thesis abstract posted on Khartoum University website, to our knowledge, has come up with the antioxidant and antibacterial activity of Sudanese *E. schimperi* leaf extract (<http://khartoumspace.uofk.edu/handle/123456789/16116>). Consequently, in our study, the focus of attention was on the determination of the content of some selected phytochemicals, evaluation of antioxidant and antimicrobial activities of the leaf extracts of Ethiopian *E. schimperi*.

## Experimental section

### Sample collection

The leaves of *E. schimperi* were collected from Bahir Dar University main campus. The plant was identified by its local name “Dedeho” (Amharic) and registered

in Ethiopian folk medicine. A herbarium voucher (Banchalem-001) was identified by Dr. Ali Seid and deposited in the mini-herbarium of a Bahir Dar University (Bahir Dar, Ethiopia). The leaves of the plant were detached from the parent plant and were washed with tap water and placed in shade at room temperature without exposing to sun light and heat for 2 weeks. The air-dried leaves of *E. schimperi* were chopped into small pieces and milled into a uniform powder with a coffee grinder. The powder was packed in polyethylene bags and stored in refrigerator for further use.

### Extraction procedure

The powdered leaf of *E. schimperi* was extracted by two different solvents (methanol and chloroform). In each extraction process, 60 g of dry powder was soaked in 600 mL of each solvent with ratio of 1:10 (W/V) of the plant material to solvents as per standard method for extraction of medicinal plant [14]. The mixtures were extracted up on shaking with mechanical shaker for 48 h. Then, the extracts were filtered and concentrated using a rotary evaporator under reduced pressure at a maximum of 25 °C. Crude extracts obtained using methanol and chloroform solvents were labeled as *ESL<sub>1</sub>*, and *ESL<sub>2</sub>*, respectively. The yields were calculated and preserved using air tight bottles at 4 °C refrigerator. In all cases, the extracts were tested in triplicates and the mean values were tabulated.

### Statistical analysis

Data were presented as mean  $\pm$  SD from triplicate measurements and significance differences between groups were determined by one-way ANOVA followed by Tukey post hoc test and paired *t* test. Differences were considered significant when  $p < 0.05$ .

### Experimental procedures

#### Preliminary phytochemical screening

The leaves of *E. schimperi* were subjected to preliminary phytochemical tests following standard procedures described for the screening of phytochemical constituents (alkaloids, polyphenols, flavonoids, saponins, tannins, glycosides, and terpenoids). Methanol and chloroform extracts were screened for the presence of steroids, terpenoids, flavonoids, phenolics, tannins and saponins following standard method [15]. All tests were performed in triplicates.

#### Determination of total polyphenols

Total phenolic content of *E. schimperi* leaf extracts was determined according to the Folin–Ciocalteu method with slight modification [16]. Gallic acid was used as reference compound for positive control. Exactly 5 mL

of plant crude extracts (methanol and chloroform) was diluted with 45 mL of distilled water. Folin–Ciocalteu reagent (0.25 mL) was added to 5 mL of the diluted extract solution followed by 0.5 mL of 7% sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) solution. The contents were mixed and the flask made up to volume with distilled water then thoroughly mixed. The mixtures were left for 30 min in the dark and the absorbance was measured at 750 nm.

#### Determination of total flavonoids

The total flavonoid content of *E. shimperi* leaf methanol and chloroform extracts was determined by aluminum chloride assay with slight modifications [17]. Quercetin was used as standard compound. A sample extract of 2.00 mL was mixed with 4.00 mL of distilled water, followed by an immediate addition of 0.30 mL of 5%  $\text{NaNO}_2$ . After 5 min, 0.30 mL of 10%  $\text{AlCl}_3$  solution was added. After 6 min, 2.00 mL of 1.00 M NaOH solution was added and then 10 min later, absorbance of the resulting solution was taken at 510 nm.

#### Measure of antioxidant activities

##### a. Reducing power assay

The reducing power of the extract was determined according to the method reported by Mitic et al. [18] with slight modification. Ascorbic acid was used as reference. Different concentrations (10, 20, 30, 40%, v/v) of 5.00 mg/mL of stock solutions of *E. shimperi* leaf extracts were prepared for each solvent (methanol, and chloroform). From each solution, 2.50 mL was taken and mixed with 2.5 mL of 0.20 M sodium phosphate buffer (pH6.6) and 2.5 mL of potassium ferricyanide solution (1%). The mixtures were incubated in water bath at 50 °C for 20 min then, 2.5 mL of trichloroacetic acid (TCA) solution (10% w/v) was added and the resulting mixture was centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL) was taken, mixed with 2.5 mL of distilled water and 0.5 mL of a ferric chloride solution (0.1% w/v) and the absorbance of the resulting solution was measured at 700 nm.

##### b. DPPH radical scavenging activity determination

The antioxidant activity of *E. shimperi* leaf extracts was also assessed on the basis of the free radical scavenging effect of DPPH (2, 2-diphenyl-1-picrylhydrazyl) using the method described by [19]. Ascorbic acid was used as positive control. The methanol and chloroform extracts from *E. shimperi* leaves with different concentrations [40, 30, 20, 10% (v/v)] of 5.0 mg/mL stock solutions were prepared. 5.0 mL of 0.004% of DPPH solution was mixed with 1 mL of each extract solution. The solutions were kept in the dark for 30 min to complete the reaction

and then the absorbance of the mixture was measured at 517 nm.

##### c. Voltammetric analysis

The 80 mg/mL concentration of leaf extracts of methanol from *E. shimperi* leaves was subjected to voltammetric analysis to measure antioxidant capacity of the sample. It has to be noted that methanol and chloroform extracts were found to contain high and low amount of antioxidants, respectively. Thus, only methanol extract was considered for further comparative analysis using voltammetric method. Exactly 15.00 mL of each sample extract was diluted to 50.00 mL with acetate buffer solution (0.10 M) of pH5. Then, cyclic voltammogram of each sample was obtained by scanning the potential from  $-1000$  to  $1400$  mV at a scan rate of  $100 \text{ mVs}^{-1}$ . Acetate buffer was used as a blank.

All the voltammetric measurements were carried out in a 25-mL cell at room temperature, in a three-electrode configuration. Graphite carbon electrode was used as a working electrode. The reference electrode was an Ag|AgCl salt and the counter electrode was a platinum wire. Cyclic voltammetric measurements were performed in model 700E series electrochemical analyzer connected to a desktop computer. For further relaxation of the peak, the sample was scanned with square wave voltammetry.

##### d. Synthesis of silver nanoparticles

Antioxidant capacity of *E. shimperi* was measured in terms of reduction ability of silver to synthesize silver nanoparticles (AgNPs) based on the method described by Shankar et al. [20] with slight modification. Two different solvent extracts (methanol and chloroform) were prepared with the same concentration. Methanol extract was prepared with different concentrations (10, 20, 30, 40% v/v) of 5 mg/mL stock solution to measure the reduction potential. 10 mL of 5 mM silver nitrate solution was mixed with 1 mL of each sample in the dark by stirring magnetically for 15 min.

The reduction of metallic silver ions was monitored by measuring the absorbance after different time for different concentration of the reaction. The absorbance of the mixture was scanned between 300 and 600 nm using UV–Vis spectrometer after 2, 6, 9 and 24 h of the reaction begins.

#### Antibacterial activity determination

##### Disk diffusion method

The present study was conducted using standard disk diffusion method [21]. Different extract (methanol and chloroform) of *E. shimperi* leaves were used by agar disk diffusion method on Mueller–Hinton Agar (MHA) medium to measure the antimicrobial activity. Standard

**Table 1** Result of phytochemical screening of different solvent extracts of *Euclea schimperii* leaves

Phytochemicals	Types of extracts		Color observed	Reagents/methods used
	Methanol	Chloroform		
Saponins	+++	+	Stable froth	Froth test
Tannins	+++	–	Yellow ppt	Lead acetate test
Phenolics	+++	+	Blue black color	Ferric chloride test
Flavonoids	++	–	Yellow ppt	Lead acetate test
Terpenoids	++	–	Reddish brown ring	CHCl <sub>3</sub> + H <sub>2</sub> SO <sub>4</sub>
Steroids	+++	–	Reddish brown color	Sulfuric acid test

+++ : present in highest concentration, ++ : present in high concentration, + : present in moderate concentration, – : absent [15, 22, 23]

**Table 2** Total phenolic content (TPC) expressed as mg GAE/100 g dry extract and total flavonoids content expressed as mg QE/100 g dry extract of different solvent extracts of *Euclea schimperii* leaves

Type of extract	Total phenolics in mg GAE/100 g dry ext.	Total flavonoids mg QE/100 g dry ext.	Antioxidant activity a FRAP (mg AAE/100 g dry extract)	Antioxidant activity a DPPH (mgAAE/100 g dry ext.)
Methanol extract	748.78 ± 3.11 <sup>a</sup>	3.306 ± 0.142 <sup>a</sup>	312.145 ± 1.542 <sup>a</sup>	19.707 ± 0.186 <sup>a</sup>
Chloroform extract	40.45 ± 0.66 <sup>b</sup>	0.334 ± 0.002 <sup>b</sup>	48.177 ± 0.286 <sup>b</sup>	2.717 ± 0.030 <sup>b</sup>

Sample for three independent measurements (triplicate;  $n = 3$ , mean ± SD). Values in the same column that are followed by a different letters (a, b) are significantly different  $p < 0.05$  by paired  $t$  test

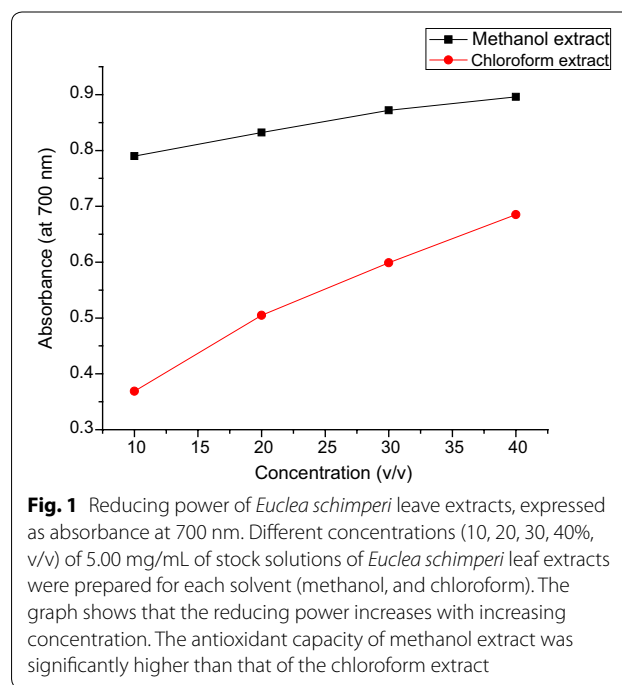
size Whatman No. 1 filter paper disks, 5.0 mm in diameter, sterilized by moist heat at 121 lb in an autoclave for 15 min was used to determine antibacterial activity.

MHA medium for *S. aureus*, *p. aeruginosa*, *K. pneumoniae*, and *S. pneumoniae* was prepared for disk diffusion test. After sterilization, it was poured into sterilized Petri dish plates and allowed to solidify. Then, 1-day-old fresh culture of bacteria was used for inoculum preparation. A suspension that will be just turbid (~0.5 McFarland standard) by visual inspection was prepared by suspending bacteria in 0.9% NaCl solution and the homogeneous suspension was used for inoculation. Using a sterile cotton swab, bacterial cultures were swabbed on the surface of sterile agar plates. Sterile 5-mm disks was impregnated with 50  $\mu$ L of extract and placed on the surface of agar plates inoculated with a microbial culture. After overnight incubation, the plates were observed for the zone of inhibition and the diameter of the inhibition zone was measured in millimeter.

Standard antibiotics, erythromycin, chloramphenicol, gentamycin and ciprofloxacin (10  $\mu$ g/mL) served as positive control and sterile distilled water and chloroform used to dilute plant extract were used as negative control.

#### Determination of minimum inhibitory concentration

The broth dilution technique [22] was employed to determine the MIC of the potent extracts. Standardized inoculums of the test organisms was inoculated into series of



**Fig. 1** Reducing power of *Euclea schimperii* leaf extracts, expressed as absorbance at 700 nm. Different concentrations (10, 20, 30, 40%, v/v) of 5.00 mg/mL of stock solutions of *Euclea schimperii* leaf extracts were prepared for each solvent (methanol, and chloroform). The graph shows that the reducing power increases with increasing concentration. The antioxidant capacity of methanol extract was significantly higher than that of the chloroform extract

sterile tubes of 2 mL nutrient broth containing 500, 250, 125, 62.5 and 31.25 mg/mL of each extracts and incubated at 37 °C for 24 h. The lowest concentration of the extract that produced indication of growth (Turbidity) was recorded as the MIC.

## Results and discussion

### Phytochemical screening tests

The preliminary phytochemical tests are helpful in finding information about chemical constituents present in the plant material [22]. This information could be used to facilitate quantitative estimation and bioassay-guided separation of pharmacologically active compounds from the plant. The preliminary phytochemical screening of the two solvent extracts from *E. schimperi* revealed the presence of several secondary metabolites such as saponins, terpenoids, tannins, steroids, polyphenol and flavonoids in moderate to high yield. As indicated in Table 1 below, significant amount of polyphenols, tannins and saponins were observed in methanol leaf extracts compared to chloroform extract, which was detected from the formation of strongly colored solutions.

### Determination of total phenolic content

The total polyphenol content of the leaf extracts was reported in terms of milligram gallic acid equivalent (mg GAE). As follows from Table 2 shown below, gallic acid standard solutions ranging from 50 to 125 µg/mL were used to construct a calibration curve for the determination of the total phenolic content of each sample. It is apparent from Fig. 1 that a straight line with an equation  $y = 0.00264x + 0.0699$  and a linear regression coefficient ( $R^2$ ) of 0.997 was obtained.

As depicted in Table 2, high total phenolic content was observed from *E. schimperi* leaf extracts; namely, methanol extract exhibited the highest level of phenolic content ( $748.78 \pm 3.11$ ) mgGAE/100 g dry extract while chloroform extracts exhibited the least ( $40.45 \pm 0.66$ ) mgGAE/100 g dry extract. This finding, while preliminary, suggests that extraction of phenolic compounds from this plant is influenced by the polarity of the solvent. Several of the recent literature has reported that aqueous mixture of acetone, methanol and ethanol could be better choice for extraction of low- and high-molecular weight phenolic compounds [6]. In this study, extraction of the phenolic compounds using aqueous mixture of methanol (90% methanol in water) was done but it was noticed that a gummy-type extract resulted and it was not possible to completely redissolve the extract using methanol for further studies. This indicates that in the presence of water as an extracting solvent, the crude extract undergoes in situ polymerization. So this result shows that aqueous solvents are not good solvents particularly for the extraction of *E. schimperi* leaves.

**Table 3 Absorbance of different solvent extract of *Euclea schimperi* leaves at different concentration for ferric reducing power measurement at 700 nm**

Concentration (v/v)	Absorbance at 700 nm of the reduced FRAP product for different extracts	
	Methanol	Chloroform
10%	$0.790 \pm 0.001^a$	$0.369 \pm 0.003^b$
20%	$0.832 \pm 0.002^a$	$0.505 \pm 0.002^a$
30%	$0.872 \pm 0.004^c$	$0.599 \pm 0.003^b$
40%	$0.896 \pm 0.002^b$	$0.685 \pm 0.001^a$

Values in the same column that are followed by a different letters (a–e) are significantly different  $p < 0.05$  by Tukey's HSD multiple comparison test

### Determination of total flavonoid content

Calibration curve was constructed to determine the total flavonoid content of *E. schimperi* leaf extracts. As summarized in Table 2, total flavonoid (TF) contents in the extracts are expressed as mg quercetin equivalent per 100 g of dry extract (QE/100 g of dried extract). Different concentrations of quercetin (2, 4, 6 and 8 µg/mL) were used to construct the calibration curve ( $y = 0.0154x + 0.128$ ,  $R^2 = 0.999$ ). In fact, the total flavonoid content was very small compared to the total phenolics content of leaf extracts of *E. schimperi*. On the other hand, the TFC in methanol extract ( $3.306 \pm 0.142$ ) was relatively higher than that of chloroform extract ( $0.334 \pm 0.002$ ).

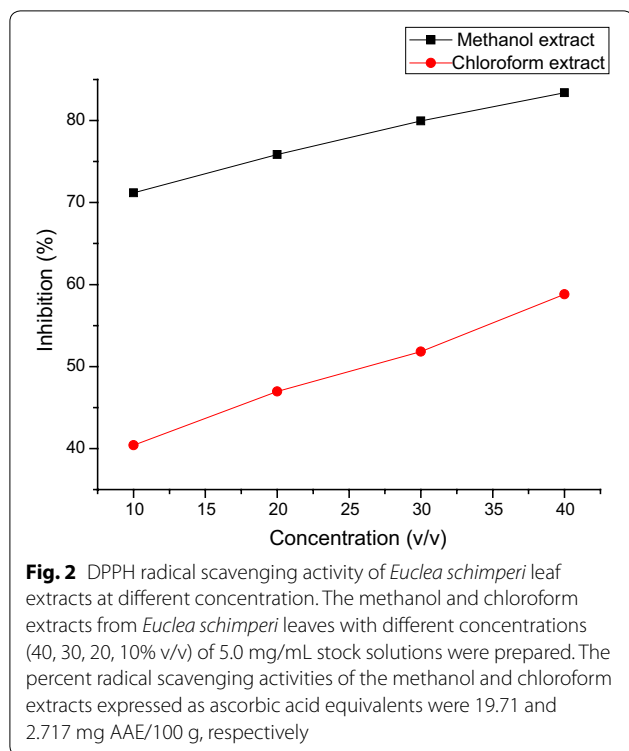
### Antioxidant potential determination of extracts of *E. schimperi* leaves

#### Ferric reducing power (FRAP) assay

The reducing power of the extracts from *E. schimperi* leaves, which may serve as a significant reflection of the antioxidant activity was evaluated using potassium ferricyanide reduction method. The antioxidant capacity of the *E. schimperi* leaf extracts with two solvents was evaluated in terms of ascorbic acid equivalent (mg AAE per g dry weight of sample) and this is tabulated in Table 2. For the antioxidant activity determination, aqueous solutions of ascorbic acid standard at different concentrations (0.2, 0.4, 0.6, 0.8 mg/mL) were used to construct the calibration curve and a straight line with an equation  $y = 0.3525x + 0.491$  and a linear regression coefficient ( $R^2$ ) of 0.997 was obtained.

The reducing power of the extracts as a function of different concentrations of extracts is summarized in Table 3 and illustrated in Fig. 1. The graph and the



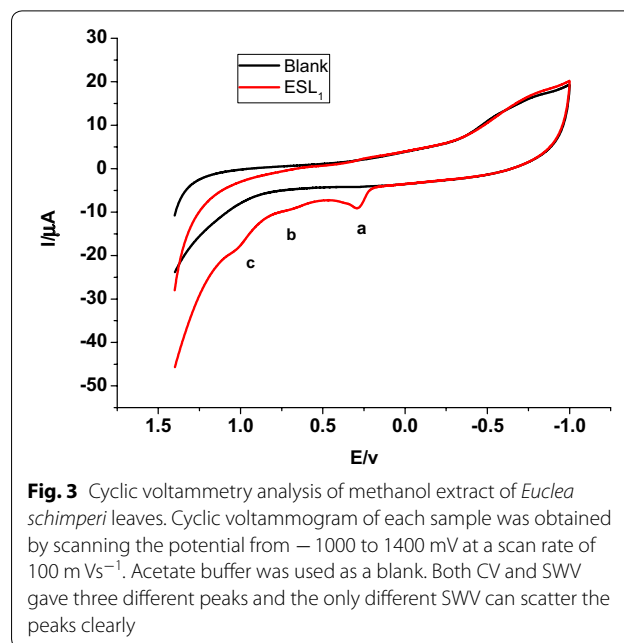


analyzed data confirmed that all the extracts exhibited some degree of reducing power. It is apparent from the table that the reducing power increases with increasing concentration. The antioxidant capacity of methanol extract was significantly higher than that of the chloroform extract. This indicates that polar compounds are more responsible for antioxidant activities and a positive correlation was found between TPC and antioxidant activity.

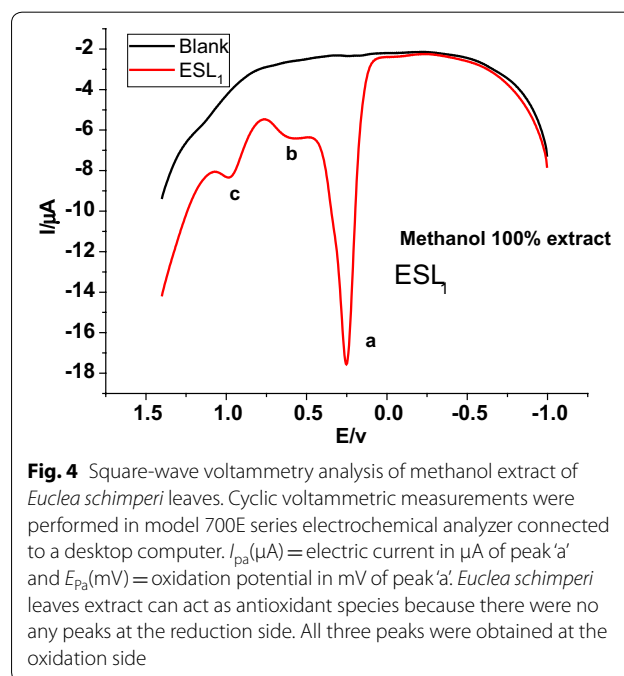
In this assay, the yellow color of the test solution changes to various shades of green when extracts were added which indicates the reducing power of each extracts. The presence of antioxidant causes the conversion of the  $\text{Fe}^{3+}$ /ferricyanide complex to the ferrous/ $\text{Fe}^{2+}$  form. Substances which have reduction potential react with potassium ferricyanide ( $\text{Fe}^{3+}$ ) to form potassium ferrocyanide ( $\text{Fe}^{2+}$ ), then reacts with ferric chloride to form ferrous complex that has an absorption maximum at 700 nm.

#### DPPH radical scavenging activity determination

The DPPH radical scavenging activities of the leaf extracts from *E. schimperi* were estimated by comparing the percentage scavenging activity of the DPPH with a standard, ascorbic acid (Table 2). Figure 2 exemplifies the steady increase in scavenging activity of the methanol and chloroform extracts up to 40% v/v of the 5 mg/mL stock solution of extracts. The methanol and chloroform extracts



from *E. schimperi* were able to scavenge the DPPH radical with percentage scavenging activity of 85.4 and 58.5% at the highest concentration, respectively. In other words, the percent radical scavenging activities of the methanol and chloroform extracts expressed as ascorbic acid equivalents were 19.71 and 2.717 mg AAE/100 g, respectively. A comparison of the results with the standard reveals that the DPPH radical scavenging capacity of the two



**Table 4 Peak parameters of methanol extract of *Euclea schimperi* leaves obtained from voltammetric analysis**

Type of extracts	Oxidation peak characters					
	$I_{pa}$ ( $\mu A$ )	$E_{pa}$ (mV)	$I_{pb}$ ( $\mu A$ )	$E_{pb}$ (mV)	$I_{pc}$ ( $\mu A$ )	$E_{pc}$ (mV)
Methanol	-17.59	248.35	-6.41	622.25	-8.37	989.02

$I_{pa}$  ( $\mu A$ ), electric current in  $\mu A$  of peak 'a';  $I_{pb}$  ( $\mu A$ ), electric current in  $\mu A$  of peak 'b';  $I_{pc}$  ( $\mu A$ ), electric current in  $\mu A$  of peak 'c';  $E_{pa}$  (mV), oxidation potential in mV of peak 'a';  $E_{pb}$  (mV), oxidation potential in mV of peak 'b';  $E_{pc}$  (mV), oxidation potential in mV of peak 'c'

extracts was weaker than ascorbic acid ( $89.95 \pm 0.116\%$  at the concentration of  $125 \mu g/mL$ ). Another point to consider is methanol extract again retained its superiority in the antioxidant activity. This pattern of DPPH inhibition is commonly observed with plant extracts [18].

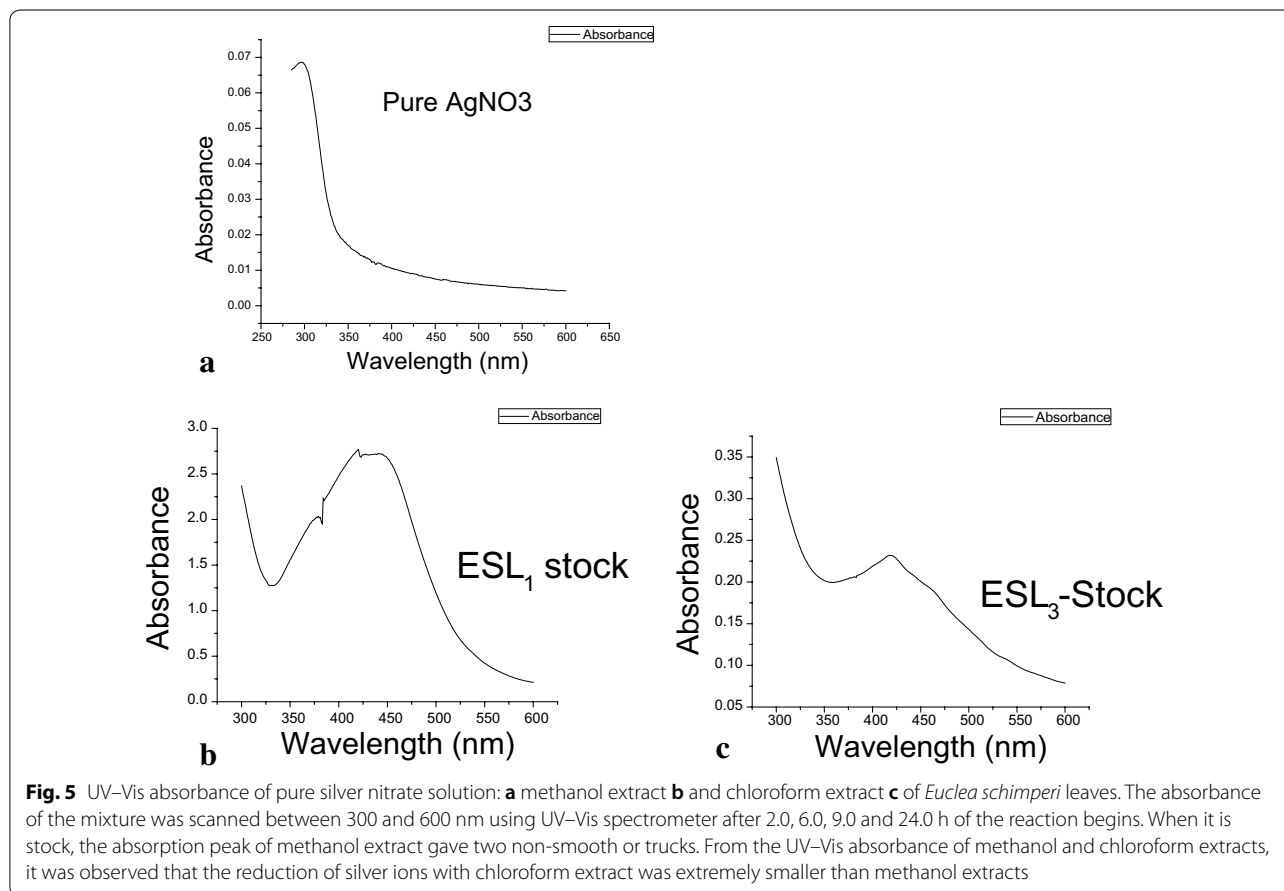
#### Voltammetric analysis, CV

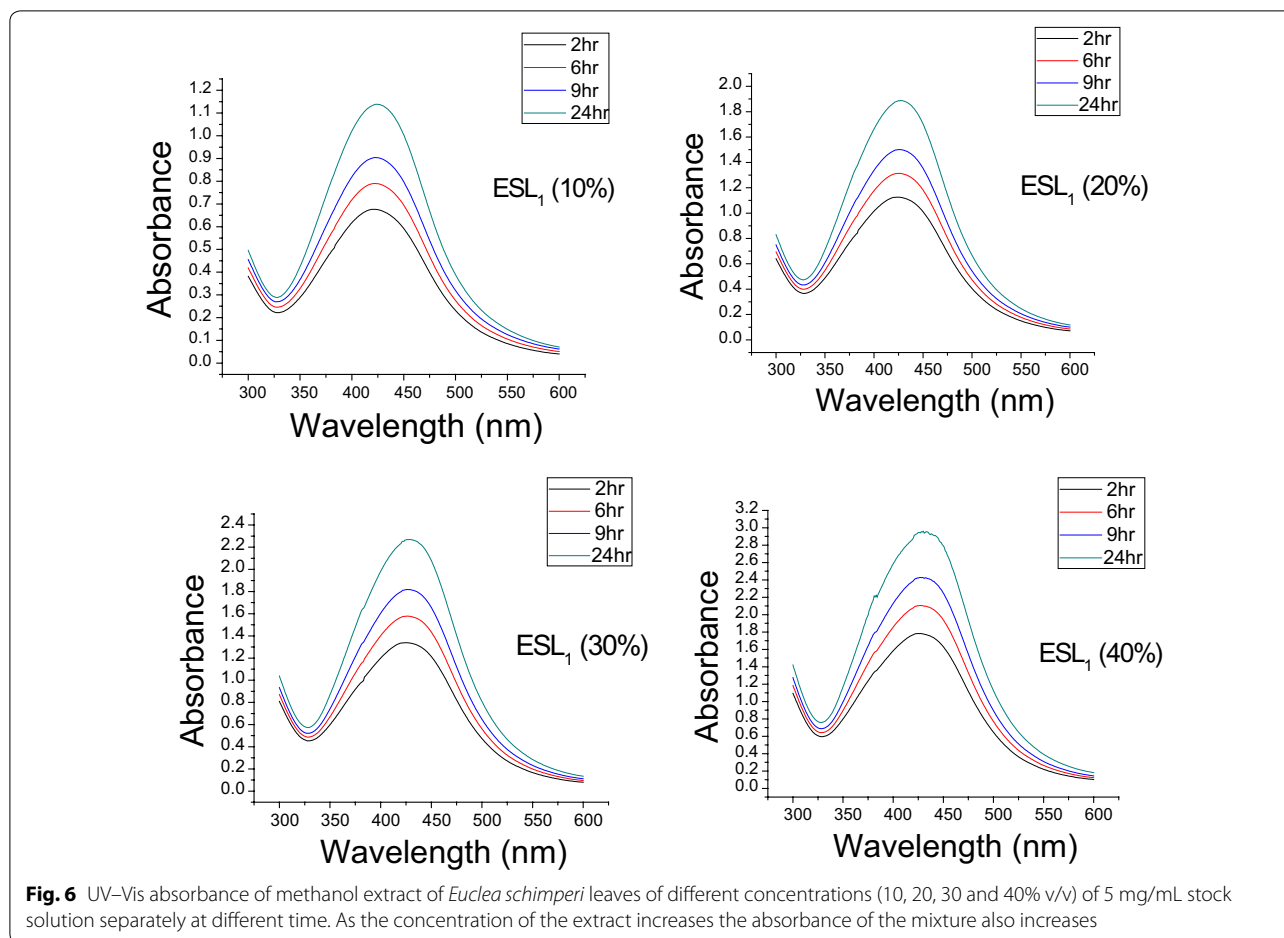
The voltammetric method can be used for the determination of the antioxidant capability in the same manner as the DPPH radical scavenging because of the correlation found between oxidation potentials and antioxidant power. CV method is frequently preferred for various types of physicochemical analysis in redox system due to its high sensitivity, rapidity, simplicity, the possibility of

performing analysis in colored or turbid solutions [24]. It can provide information about the number of redox states as well as qualitative information about the stability of these oxidation states and the electron transfer kinetics [25–27].

Square-wave voltammetry (SWV) is another superior electrochemical technique for both mechanistic and electrokinetic studies of various electrode processes. SWV has several advantages. Among these are its excellent sensitivity, the rejection of background currents and its speed. The current produced is proportional to the combined concentration of the antioxidants [28, 29].

The antioxidant activity of the samples is determined by the ease of phenolic compound to be oxidized. It can





**Fig. 6** UV-Vis absorbance of methanol extract of *Euclea schimperi* leaves of different concentrations (10, 20, 30 and 40% v/v) of 5 mg/mL stock solution separately at different time. As the concentration of the extract increases the absorbance of the mixture also increases

be described as easily oxidizable if its positive oxidation potential is as small as possible (Figs. 3 and 4). Both CV and SWV gave three different peaks; the only different was SWV that can scatter the peaks clearly.

As illustrated in Figs. 3 and 4 above, the voltammetric analysis of *E. schimperi* leaves extract with methanol solvent gives three different sharp peaks at the oxidation side. This indicates that the extract contains three reductant or antioxidant species that can be either three different group of reductant or a reductant that can be reduced to another substance in step-wise process. Out of these three different peaks, one was very long and sharp indicating that this represents the major component of the three antioxidant components.

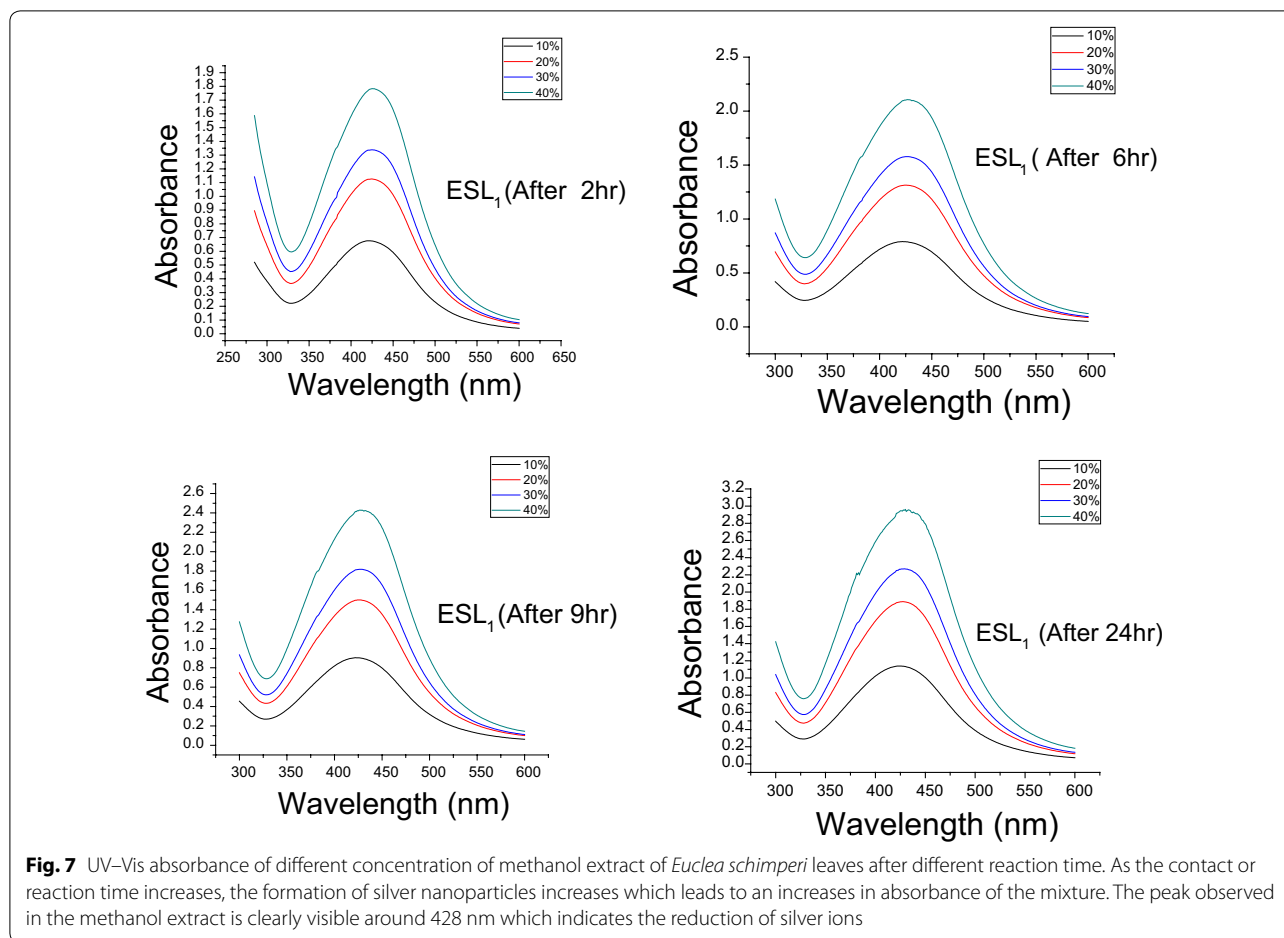
As a result, *E. schimperi* leaves extract can act as antioxidant species because there were no any peaks at the reduction side (all three peaks were obtained at the oxidation side). The peak parameters obtained from the cyclic voltammograms of each sample are tabulated in Table 4.

#### Synthesis of silver nanoparticles

On account of noble metal nanoparticles are widely applied to areas of human health, there is a growing interest to develop environmentally friendly processes for nanoparticles synthesis [30]. Biological methods of synthesis have paved way for the “greener synthesis” of nanoparticles and these have proven to be better methods due to slower kinetics, offer better manipulation and control over crystal growth and their stabilization [31, 32].

Phytochemicals are involved directly in the reduction of the ions and formation of silver nanoparticles using biological methods [30, 33]. *E. schimperi* leaf extracts contain a variety of phytochemicals such as polyphenols, flavonoids and terpenoids which help in the reduction of metal ion to form nanoparticles. Preliminary identification of nanoparticle formation was carried out by observing the color change of the reaction mixture. It was observed that when  $\text{AgNO}_3$  solution was mixed with the plant extracts, brown coloration was observed due to the formation of silver nanoparticles. This is a clear indication of the formation of silver nanoparticles [34].





**Table 5 Comparison of inhibition zone in mm among different crude extracts of *Euclea schimperi* leaves and positive controls against standard drug resistance Gram-positive and Gram-negative bacteria**

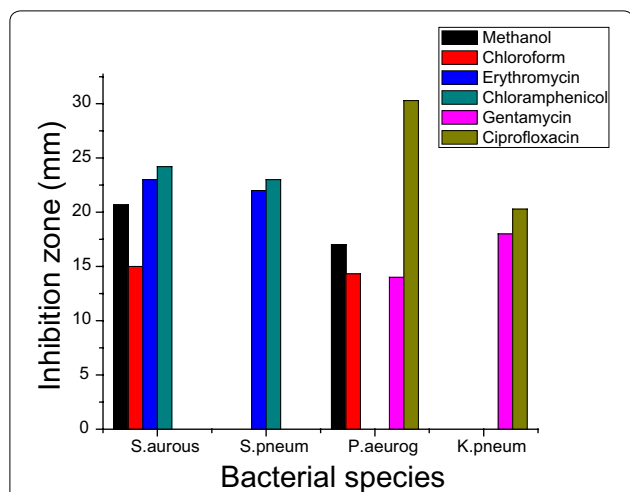
Types of extracts or positive control	Types of bacteria strain			
	Gram positive		Gram negative	
	<i>S. aureus</i>	<i>S. pneumoniae</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>
Methanol extract	20.67 ± 1.53 <sup>a</sup>	0.00 <sup>a</sup>	17.00 ± 1.00 <sup>a</sup>	0.00 <sup>a</sup>
Chloroform	15.00 ± 1.00 <sup>b</sup>	0.00 <sup>a</sup>	14.33 ± 0.58 <sup>b</sup>	0.00 <sup>a</sup>
Erythromycin	23.00 ± 0.05	22.00 ± 0.20	–	–
Chloramphenicol	24.20 ± 0.30	23.00 ± 0.07	–	–
Gentamycin	–	–	14.00 ± 0.05	18.00 ± 0.35
Ciprofloxacin	–	–	30.30 ± 1.23	20.30 ± 0.89

*S. pneumoniae*, *Streptococcus pneumoniae*; *S. aureus*, *Staphylococcus aureus*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *K. pneumoniae*, *Klebsiella pneumoniae*

Values in the same column that are followed by a different letters (a, b) are significantly different  $p < 0.05$  by paired *t* test

Therefore, the present investigation demonstrates the formation of silver nanoparticles by the reduction of aqueous silver ions from *E. schimperi* leaf extracts using different solvents. The properties of the nanoparticle and its reduction time depend on various characteristics

of plant extract, namely concentration of the metal salt and the extracts, contact time and reaction temperature. Nevertheless, this study considered two major factors, contact time and concentration of the plant extracts. As a result, the formation of silver nanoparticles by reduction



**Fig. 8** Comparison of inhibition zone among crude extracts of *Eucllea schimperi* leaves using different solvent (methanol and chloroform) against standard Gram-positive and Gram-negative bacteria. Standardized inoculums of the test organisms were inoculated into series of sterile tubes of 2 mL nutrient broth containing 500, 250, 125, 62.5 and 31.25 mg/mL of each extracts and incubated at 37 °C for 24 h. The methanol extract showed maximum antibacterial activity against *S. aureus* and *P. aeruginosa* with diameter of 20.67 ± 1.53 and 17 ± 1 mm at concentrations of 500 mg/mL, respectively. Chloroform extract also exhibited antibacterial activity against *S. aureus* and *P. aeruginosa* with diameter of 15 ± 1 and 14.33 ± 0.58 mm at concentrations of 500 mg/mL, respectively

of silver ions was measured with different concentrations (10, 20, 30, 40% v/v) of *E. schimperi* leaf extracts at different reaction time (2, 6, 9 and 24 h).

When it is stock, the absorption peak of methanol extract gave two non-smooth or trucks. This may be due to higher deposition of silver nanoparticles. From the UV–Vis absorbance of methanol and chloroform extracts of *E. schimperi* leaves, it was observed that the reduction of silver ions with chloroform extract was extremely smaller than methanol extracts (Fig. 5).

Initially, the reacting mixture was a slightly yellowish liquid; as the reaction proceeded, the solutions became dark brown. Different concentrations (10, 20, 30 and

40%) of methanol extract of *E. schimperi* leaves result in the formation of different absorption peaks. As the concentration of the extract increases the absorbance of the mixture also increases (Fig. 6). The visual inspection of the color change of the mixture from yellow to intense brown was demonstrated with increase in concentration of the leaf extracts. This also ascertains the dependence of the formation of silver nanoparticles on the concentration of the extracts. The absorbance of silver nanoparticles in the presence of different concentrations of the extract at different time can be found in Fig. 6.

Another point to consider is as the contact or reaction time increases, the formation of silver nanoparticles increases which leads to an increases in absorbance of the mixture. This result is shown in Fig. 7.

Additionally, as both the concentration of the plant extract and the reaction time increase, non-smooth part of the peak around the same wave length region was increased. This is due to the increase in the concentration of silver nanoparticles formed in the reaction.

As can be seen from the Figs. 6 and 7 above, the peak observed in the methanol extract of *E. schimperi* leaves is clearly visible around 428 nm which indicate the reduction of silver ions.

**Determination of antibacterial activity of *E. schimperi* leaf extracts**

**Agar disk diffusion method**

Methanol extract of *E. schimperi* leaves exhibited prominent antibacterial activity against both one Gram-positive and one Gram-negative bacterial species. The chloroform extract of the plant sample also exhibited inhibition zone on some bacterial species but it was not as strong as methanol extract. The inhibition zones obtained by each solvent extract of leaves of *E. schimperi* and the control against each tested microorganism are summarized in Table 5. The results were expressed as mean ± standard deviation of triplicate measurements.

As shown in Fig. 8 and Table 5, zone of inhibition of the tested extract against different bacterial species of the methanol extract showed maximum antibacterial activity against *S. aureus* and *P. aeruginosa* with diameter of

**Table 6** MIC determination of *Eucllea schimperi* leave extracts against Gram-positive and Gram-negative bacteria

Bacterial species	Types of extract	Concentration (mg/mL)					MIC
		500.0	250.0	125.0	62.5	31.25	
<i>S. aureus</i>	Methanol	–	–	–	–	+	62.5
	Chloroform	–	–	–	+	+	125
<i>P. aeruginosa</i>	Methanol	–	–	–	–	+	62.5
	Chloroform	–	–	–	+	+	125

–, inhibition of bacterial growth; +, turbidity (slight bacterial growth)

20.67 ± 1.53 and 17 ± 1 mm at concentrations of 500 mg/mL, respectively. Chloroform extract also exhibited antibacterial activity against *S. aureus* and *P. aeruginosa* with diameter of 15 ± 1 and 14.33 ± 0.58 mm at concentrations of 500 mg/mL, respectively. The antimicrobial activity of methanol leaf extracts of *E. schimperi* was stronger than chloroform extracts towards the tested pathogens. This substantiates that more polar bioactive compounds are extracted more by methanol than chloroform. In other words, alcoholic extracts of tested plants provided better antibacterial effect than other solvents [35]. Both methanol and chloroform extracts showed no activity against *S. pneumoniae* and *K. pneumoniae* (multidrug resistance).

The presence of zone of inhibition around the well was considered positive. The extracts were found to cause marked inhibition of bacteria in a dose-dependent manner. Among bacteria, Gram-positive bacteria have shown higher sensitivity to the extracts compared to Gram-negative bacteria. The inhibition caused by methanol extract was lesser than that of standard antibiotic since the standard antibiotic are found in pure form [36].

Alkaloids, tannins, saponins, glycosides and steroid derived from plants have been shown to have antimicrobial effect and pharmacological activities [37, 38]. Flavonoids, phenols, saponins, steroids, tannins, and terpenes detected from the extracts are responsible to observed antibacterial action of *E. schimperi* leaf extracts. In summary, this antibacterial action may serve as justification to the traditional use of the *E. schimperi* plant against various human disorders.

#### Determination of minimum inhibitory concentration (MIC)

The MIC was taken as the lowest concentration that prevented bacterial growth and it was determined using a broth dilution test.

As shown in Table 6, the MIC values were evaluated relatively at higher concentrations of the methanol (62.5 mg/mL) and chloroform (125 mg/mL) extracts. Correspondingly, methanol extract afforded notable MIC value at 62.5 mg/mL against both *S. aureus* and *P. aeruginosa*. An implication of these findings is that *E. schimperi* leaf extracts can inhibit some of the bacterial species at relatively high concentration.

#### Conclusion

The outcome of our investigation clearly indicated that *E. schimperi* leaf extracts were found to contain high amount of total phenolic and small amount of flavonoids content. Likewise, the extracts revealed significant antioxidant activities against various tests; FRAP, DPPH, voltammetric analysis and silver nanoparticle reduction. Based on the results, the methanol extract showed higher reducing capacity, DPPH radical scavenging

activity, anodic peak current and smaller positive oxidation potential than the chloroform extract. The methanol extract can also reduce silver ion and able to form significant amount of silver nanoparticles. Last, the result can also prove the effectiveness of *E. schimperi* leaf extracts for antimicrobial activity.

In general, the methanol extracts demonstrated superior antioxidant and antimicrobial effects than chloroform extracts, which is related to the quantity of phytochemicals present in the crude extracts. To this end, the findings are quite convincing to scientifically confirm the traditional use of this plant for medicinal purpose by the population.

#### Authors' contributions

AM and MA conceived and designed the experiments; AM analyzed data and wrote the paper; BK performed the experiments. All authors participated in the revisions of the manuscript. All authors read and approved the final manuscript.

#### Acknowledgements

The authors are thankful to Bahir Dar University for the provision of lab facility and Dr. Maereg Amare for obtaining voltammetric data.

#### Competing interests

The authors declare that they have no competing interests.

#### Availability of data and materials

Aherbarium voucher (Banchalem-001) was identified by Dr. Ali Seid and deposited in the mini-herbarium of a Bahir Dar University, (Bahir Dar, Ethiopia). All data generated or analyzed during this study are included in this published article.

#### Consent for publication

Not applicable.

#### Ethics approval and consent to participate

Not applicable.

#### Funding

Not applicable.

#### Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 5 June 2018 Accepted: 11 July 2018

Published online: 08 August 2018

#### References

1. Ignacimuthu S, Ayyanar M, Sankara Sivaraman K. Ethnobotanical investigations among tribes in Madurai district of Tamil Nadu (India). *J Ethnobiol Ethnomed*. 2006;11(25):1–7.
2. Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. *J Appl Microbiol*. 1999;86:985–90.
3. Giday M, Asfaw Z, Elmqvist T, Woldu Z. An ethnobotanical study of medicinal plants used by the Zay people in Ethiopia. *J Ethnopharmacol*. 2003;85:43–52.
4. Sukhdev S, Suman PSK, Gennaro L, Dev DR. Extraction technologies for medicinal and aromatic plants. Trieste: International Centre for Science and High Technology; 2008.
5. Hasler CM, Blumberg JB. Phytochemicals: biochemistry and physiology. Introduction. *J Nutr*. 1999;129(3):756–75.

6. Evans WC. General methods associated with the phytochemical investigation of herbal products. Trease and Evans pharmacognosy. 15th ed. Philadelphia: Saunders/Elsevier; 2008. p. 137–48.
7. Hamilton AC. Medicinal plants, conservation and livelihoods. Biodivers Conserv. 2004;13:1477–517.
8. Wang H, Liu YM, Qi ZM, Wang SY, Liu SX, Li X, Wang HJ, Xia XC. An overview on natural polysaccharides with antioxidant properties. Curr Med Chem. 2013;20:2899–913.
9. Liu Y, Sun Y, Huang G. Preparation and antioxidant activities of important traditional plant polysaccharides. Int J Biol Macromol. 2018;111:780–6.
10. Huang G, Mei X, Hu J. The antioxidant activities of natural polysaccharides. Curr Drug Targets. 2017;18(11):1296–300.
11. Nwachukwu CU, Umeh CN, Kalu IG, Okere S, Nwoko M. Identification and traditional uses of some common medicinal plants in Ezinihitte Mbaise LGA, of Imo State, Nigeria. Rep Opin. 2010;2:1–10.
12. De Moura KC, Emery FS, Neves-Pinto C, Pinto MD, Dantas AP, Salomão K, Castro SL, Pinto AV. Trypanocidal activity of isolated naphthoquinones from *Tabebuia* and some heterocyclic derivatives: a review from an interdisciplinary study. J Br Chem Soc. 2001;12:325–38.
13. Gebre-Mariam T, Neubert R, Schmidt PC, Wutzler P, Schmidtke M. Antiviral activities of some Ethiopian medicinal plants used for the treatment of dermatological disorders. J Ethnopharmacol. 2006;104:182–7.
14. Mikayel G, Margarit P, Armen T. Antimicrobial activity of some plant materials used in Armenian traditional medicine. BMC Complement Altern Med. 2017;17:50–8.
15. Gohari AR, Hajimehdipour H, Saeidnia S, Ajani Y, Hadjiakhoondi A. Antioxidant activity of some medicinal species using FRAP assay. J Med Plants. 2011;10(37):54–60.
16. Amin I, Norazaidah Y, Hainida KE. Antioxidant activity and phenolic content of raw and blanched *Amaranthus* species. Food Chem. 2006;94:47–52.
17. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002;10:178–82.
18. Milan C, Hana C, Petko D, Maria K, Anton S, Antonin L. Different methods for control and comparison of the antioxidant properties of vegetables. Food Control. 2010;21:518–23.
19. Louli V, Ragoussis N, Magoulas K. Recovery of phenolic antioxidants from wine industry by-products. Bioresour Technol. 2004;92:201–8.
20. Shankar SS, Rai A, Ahmad A, Sastry M. Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth. J Colloid Interface Sci. 2004;275:496–502.
21. Enzo AP. Traditional medicinal plant extracts and natural products with activity against oral bacteria: Potential application in the prevention and treatment of oral diseases. Evid Based Complement Altern Med. 2011;2011:1–15.
22. Akinyemi KO, Oladapo O, Okwara CE, Ibe CC, Fasure KA. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity. BMC Complement Altern Med. 2005;5(6):1–7.
23. Tahiya HAA, Amira HSAM, Mohammad AH, Afaf MW, Qasim AR. Comparative study of phytochemical screening, antioxidant and antimicrobial capacities of fresh and dry leaves crude plant extracts of *Datura metel* L. J King Saud Univ. 2014;26(3):237–43.
24. David IG, Bizgan AM, Popa DE, Buleandra M, Moldovan Z, Badea IA, Tekiner TA, Basaga H, Ciucu AA. Rapid determination of total polyphenolic content in tea samples based on caffeic acid voltammetric behaviour on a disposable graphite electrode. Food Chem. 2015;173:1059–65.
25. Jelen F, Kourilova A, Hason S, Kizek R, Trnkova L. Voltammetric study of adenine complex with copper on mercury electrode. Electroanalysis. 2009;21(3–5):439–44.
26. Adam V, Fabrik I, Kizek R, Eckschlager T, Stiborova M, Trnkova L. Vertebrate metallothioneins as target molecules for analytical techniques. TrAC Trends Anal Chem. 2010;29(5):409–18.
27. Hynek D, Krejcová L, Sochor J, Cernei N, Kynicky J, Adam V, Trnkova L, Hubalek J, Vrba R, Kizek R. Study of interactions between cysteine and cadmium (II) ions using automatic pipetting system off-line coupled with electrochemical analyser. Int J Electrochem Sci. 2012;7(3):1802–19.
28. Kilmartin PA, Zou H, Waterhouse AL. Correlation of wine phenolic composition versus cyclic voltammetry response. Am J Enol Vitic. 2002;53(4):294–302.
29. Huang T, Gao P, Hageman MJ. Rapid screening of antioxidants in pharmaceutical formulation development using cyclic voltammetry-potential and limitations. Curr Drug Discov Technol. 2004;1(2):173–9.
30. Song JY, Kim BS. Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess Biosyst Eng. 2009;32(1):79–84.
31. Sonvico F, Dubernet C, Colombo P, Couvreur P. Metallic colloid nanotechnology, applications in diagnosis and therapeutics. Curr Pharm Des. 2005;11(16):2091–105.
32. Shipway AN, Katz E, Willner I. Nanoparticle arrays on surfaces for electronic, optical, and sensor applications. Chemphyschem. 2000;1(1):18–52.
33. Bar H, Bhui DK, Sahoo GP, Sarkar P, De SP, Misra A. Green synthesis of silver nanoparticles using latex of *Jatropha curcas*. Colloids Surf A Physicochem Eng Asp. 2009;339:134–9.
34. Jain D, Daima HK, Kachhwaha S, Kothari SL. Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their antimicrobial activities. Dig J Nanomat Biopstruct. 2009;4:557–63.
35. Bakht N, Humaira F, Madiha A, Ihsan-ul-Haq. Recent trends and methods in antimicrobial drug discovery from plant sources. Austin J Microbiol. 2015;1(1):1–12.
36. Prescott LM, Harley JP, Klein OA. Microbial nutrition, types of media. New York: McGraw Hill Publishers; 2005. p. 95–105.
37. Ieven M, Vanden BD, Mertens F, Vlietinck A, Lammens E. Screening of higher plants for biological activities I. Antimicrobial activity. Planta Medica. 1979;36:311–21.
38. Frei B, Heinrich M, Bork PM, Herrmann D, Jaki B, Kato T, Kuhn M, Schmitt J, Schühly W, Volken C, Sticher O. Multiple screening of medicinal plants from Oaxaca, Mexico: ethnobotany and bioassays as a basis for phytochemical investigation. Phytomedicine. 1998;5:177–86.

Submit your manuscript to a SpringerOpen® journal and benefit from:

- Convenient online submission
- Rigorous peer review
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article

---

Submit your next manuscript at ► [springeropen.com](http://springeropen.com)

---