

Determination of total phenolic and flavonoid contents, antioxidant and antibacterial potential of the bark extracts of *Syzygium guineense* (Wild.) DC

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Research Article

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Abstract

Background: *Syzygium guineense* (Wild.) DC. is a wild indigenous tree widely used as a traditional medicine for various human ailments in Ethiopia. The purpose of this study was to quantify total phenolic (TPC) and total flavonoid (TFC) contents and determine antioxidant and antibacterial activities of various solvent extracts of the bark of the plant.

Methods: The TPC and TFC were determined using Folin-Ciocalteu and aluminum chloride methods, respectively. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging, ferric reducing power (FRAP) and total antioxidant capacity (TAC) assays were used to evaluate the antioxidant activities. Antibacterial properties were determined using the disc-diffusion assay based on minimum inhibitory concentration (MIC) against four bacterial strains (*Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhimurium*).

Results: The ethanol extract of the bark was found to have high TPC (37.80 ± 3.70 mgGAE/g) and TFC (19.22 ± 1.44 mgQE/g). Similarly, the ethanol extract showed stronger DPPH scavenging activity ($EC_{50}=5.62$ μ g/mL). The FRAP and TAC were also strong (163.08 ± 11.67 mgAAE/g and 143.72 ± 2.86 mgBHTE/g of dried extract of 1 mg/mL, respectively). The lowest MIC was observed in acetone extract against *S. aureus* and in ethanol extract against *K. pneumoniae*. The chloroform extract was impotent against all microbial strains.

Conclusion: The bark of *S. guineense* possesses high TPC and TFC and strong FRAP and TAC asserting high antioxidant contents. The extracts have shown antibacterial activities against both Gram positive and Gram negative bacterial species. Thus, further in-depth investigations may warrant the isolation of powerful antioxidants and potent antimicrobial agents from the plant.

Introduction

The use of medicinal plants as a fundamental component of the African traditional healthcare system is the oldest with long track record and widely acknowledged of all therapeutic systems. In many parts of rural Africa, traditional healers prescribing medicinal plants are the most easily accessible and affordable health resource available to the local community and at times the only treatment modality that exists [1]. The traditional medicine (TM) in Africa is holistic involving both the body and the mind and traditional healers offer information, counseling, and treatment to patients and their families in a personal manner [1]. Patients' preference, low ratio of medical doctors to the total population and lack of effective modern medical treatment for some ailments in Africa are additional factors for the wider practice of TMs.

Ethiopia is rich in plant biodiversity. It is therefore, not surprising that some of these plants have chemical compounds of therapeutic value that may be used in the treatment of major diseases such as malaria, cancer and pathogenic microorganisms [2]. According to the WHO, 70–80% of Africans today depend either totally or partially on TM [3]. Over 80% of the Ethiopian population also relies TM according to a report as old as 1986 [4] and more than 95% of the preparations are made from plant origin [5]. This

represents the majority of the rural population and sectors of the urban population where there is little or no access to modern health care [6].

Among the highly diverse species of Ethiopian plants used as TMs is *Syzygium guineense* (Wild.) DC. *Syzygium guineense* (Family Myrtaceae) named “Duuwancho” in *Sidamu-Afoo* is a wild tree with edible fruits. Its distribution ranges from tropical Africa, sub-tropical and tropical Asia to Australia [7]. Traditionally the bark of the plant is pounded, macerated, and drunk for the treatment of a health condition locally referred as ‘ARRISHSHO’ and it cures after inducing diarrhea. ‘ARRISHSHO’ is an acute ailment characterized by pain of the hip, irritation during urination, sweating, and loss of appetite as described by the traditional healers [8]. The ripen fruits of the plant are also eaten in small amounts to treat obesity [8]. Other medicinal values of the plant include the treatment of inflammation, various allergic disorders, bronchitis, dysentery and ulcers [7]. Two bioactive 3- β -hydroxylupane-type isoprenoids, betulinic acid methylene diol ester, were isolated from the stem bark of the plant and were reported to have anti-tuberculosis activity [9]. The crude leaf extract of the plant was also reported to have an *in vivo* antimalarial activities [10]. Various studies reported antioxidant [11], anti-diabetic [12], antibacterial [13], antihelmintic activities [14], analgesic and anti-inflammatory [15], antidiarrheal, antifungal, antiviral and anticancer activities among others [16]. Therefore, the main purpose of this study was to document the total phenolic and flavonoid contents and antioxidant and antibacterial activities of various solvent extracts of the bark of this widely used traditional medicinal plant.

Materials and Methods

Sample preparation and extraction

The bark of *S. guineense* was collected from wild habitat of Dalle district, 47 km away from Hawassa, the regional capital city of the Sidama National Regional State, in February 2022. Identification of the plant material was undertaken using the Flora of Ethiopia and Eritrea by comparing with the authenticated specimen and finally confirmed by a plant taxonomic expert at the Addis Ababa University. Finally, the voucher specimen was deposited at the National Herbarium (ETH), Addis Ababa University with the identification number NT061. Chloroform, ethyl acetate, water, acetone, and ethanol extracts of all were prepared by dissolving 10 g of the bark fine powder separately in 100 mL of each solvent. All extractions were conducted in triplicate. After keeping in orbital shaker for 8 h at room temperature, the extract was filtered using Whatman N° 1 filter paper and evaporated to dryness under vacuum at 45 °C under reduced pressure using a rotary evaporator (Buchi, 3000 series, Switzerland). Finally, the dried extracts were stored in a sealed plastic container at 4 °C until assays.

Phytochemical analysis

Total phenolic content

The total phenolic content (TPC) was estimated by Folin-Ciocalteu method with slight modifications [17]. A 0.1 mL of the extract (1 mg/mL) was mixed with 1 mL of diluted Folin-Ciocalteu reagent. The mixture

was left for 5 min and then 1 mL of sodium carbonate (7.5g/100mL solution) was added. After incubating at 25 °C for 90 min, the absorbance of the resulting blue color was measured at 765 nm using UV-visible spectrophotometer (Spectronic 20, UK). The TPC was expressed in terms of milligram gallic acid equivalent per gram of dried extract (mgGAE/g) using gallic acid calibration curve ($y = 0.024x - 0.014$, $R^2 = 0.996$) and the results were calculated using the equation:

$$C = \frac{cxV}{m}$$

Where; C = TPC (mgGAE/g), c = the concentration established from the gallic acid calibration curve ($\mu\text{g/mL}$), V = volume of extract solution in milliliter, m = weight of dried extract in gram.

Total flavonoid content

The total flavonoid content (TFC) was determined as described by Ayoola *et al.*, [18] with minor modifications. The quantification was based on the formation of yellow color of flavonoid-aluminum complex. Briefly, a volume of 2 mL of 2% aluminum chloride was mixed with the same volume of the extracts (1 mg/mL). Then Absorbance reading at 415 nm was carried out after 1 hour of incubation at room temperature against a blank sample (2 mL of sample solution and 2 mL of ethanol without aluminum chloride). The total flavonoid content was determined using a standard curve of quercetin and values were calculated as milligram of quercetin equivalent per gram of dried extract (mgQE/g) based on the quercetin calibration curve ($y = 0.011x + 0.132$, $R^2 = 0.97$) and the results were calculated using the equation:

$$C = \frac{cxV}{m}$$

Where; C = TFC (mgQE/g), c = the concentration established from the quercetin calibration curve ($\mu\text{g/mL}$), V = the volume of extract solution in milliliter, m = the weight of the dried extract in gram.

DPPH scavenging activity

The DPPH scavenging activity of the extracts and references were determined as described by Engida *et al.*, [17]. Various concentrations ranging from 50 up to 1000 $\mu\text{g/mL}$ of the extracts and commercial antioxidant (ascorbic acid) were taken in different test tubes. The volume of 2 mL of freshly prepared DPPH solution (0.06% w/v) prepared in ethanol was added in each of the test tubes containing 1 mL of the extract. The extract mixtures and the reference standards (ascorbic acid and BHT) were vortexed and left to stand at room temperature in the dark for 30 min. The absorbance of the resulting solution was then measured at 517 nm. Ethanol was used as blank. The ability to scavenge the DPPH radical was calculated as:

$$\text{DPPH scavenging (\%)} = [(A_c - A_s) / A_c] \times 100$$

Where, A_c is the absorbance of the control and A_s is the absorbance in the presence of sample of the extract.

The IC_{50} value is defined as the effective concentration ($\mu\text{g/mL}$) of extracts that scavenges the DPPH radical by 50%. All the tests were performed in triplicate and the graph was plotted with the average of three observations. Using the dose-response curve the IC_{50} values of the commercial antioxidant and crude extracts were calculated.

Ferric reducing antioxidant power (FRAP)

The presence of antioxidants in the extract causes the reduction of yellow ferric/ferric cyanide complex to the ferrous form which can be monitored by measuring the formation of Perl's Prussian blue at 700 nm and was determined as described in Abebie *et al.*, [19].

Different concentrations (0.5 mg/mL and 1 mg/mL) of the bark extract of *S. guineense* in ethanol was mixed with 2.5 mL potassium phosphate buffer (0.2M, P^H 6.6) and 2.5 mL of 1% potassium ferricyanide. Then, the mixture was incubated at 50 $^{\circ}\text{C}$ for 20 min, and then 2.5 mL of 10% trichloroacetic acid was added to the mixture. Finally, 2.5 mL of the supernatant solution was mixed with 2.5 mL of distilled water and 0.5 mL FeCl_3 (0.1%). Absorbance was measured to determine the amount of ferrous cyanide (Prussian blue) at 700 nm against a black in a UV/vis spectrophotometer. An increase in the absorbance of the reaction indicates the reducing power of the samples. The reducing power was expressed as milligram ascorbic acid equivalents per g of the extract (mg AAE/g) using the following equation ($y = 0.0049x + 0.231$, $R^2 = 0.98$) based on ascorbic acid calibration curve.

Total antioxidant using Phosphomolybdenum assay

The total antioxidant capacity (TAC) was evaluated as described earlier [17] with slight modifications. The solution of different concentrations of 0.3 mL (5 and 10 mg/mL) in ethanol was prepared in different test tubes. The reagent solution was prepared by mixing 10 mL of 0.6 M sulphuric acid, 10 mL of 28 mM sodium phosphate and 10 mL of 4 mM ammonium molybdate into a beaker and 3 mL reagent solution was added to all the tubes. A volume of 0.3 mL of ethanol served as a blank. All the tubes were incubated at 95 $^{\circ}\text{C}$ for 90 min. The tubes were measured at 695 nm using UV-visible spectrophotometers. The TAC was expressed as milligram of Butylated hydroxytoluene equivalent per gram of the dried extract (mg BHTE/g) based on the calibration curve ($y = 0.01x + 0.15$, $R^2 = 0.99$).

The test microorganisms

Gram negative bacteria (*Escherichia coli* (ATCC-25922), *Salmonella typhimurium* (ATCC-14028), *Klebsiella pneumoniae* (ATCC-13883), *Pseudomonas aeruginosa* (ATCC-43495)) and Gram positive bacteria *Staphylococcus aureus* (ATCC-25923) were obtained from Southern Nations, Nationalities and Peoples' Region (SNNPR) Health Bureau Public Health Institute, Hawassa, Ethiopia in September 2022.

Each microbial culture was maintained by sub culturing on the appropriate nutrient medium and stored at 4 °C until use [20].

The growth media preparation

The medium was prepared according to manufacturer's instructions. Briefly, 38g Mueller Hinton Agar was added to a flask containing 1000 ml of distilled water and gently heated until the medium was completely dissolved. The medium was sterilized by autoclaving at 121°C for 15 min. After cooling to about 50 °C, approximately 15 ml of the sterilized medium was aseptically poured into 90 mm diameter sterilized Petri dishes and allowed to cool. The sterility of the prepared media was checked by incubation of blindly selected plates at 37°C for 24 h.

The disc diffusion susceptibility test media

The discs of a 6 mm diameter were prepared from Whatman Nº 1 filter paper using a cleaned paper puncher and were sterilized by autoclaving and dried in an oven at 120 °C. Then, the discs were placed in container and stored at 4 °C until further use [20].

The preparation of microbial inocula

Three to five colonies from pure cultures of each of the selected microbe species were transferred with the help of a sterile wire loop into a separately labelled test tube containing 5 mL of nutrient broth and incubated to grow at a temperature of 37 °C for 2 h. The microbial suspension prepared were used to inoculate the sterile MHA plates by streaking with sterile cotton swab after inoculation, sterile discs were soaked in to 10µL of the crude extract at a conc. of 100 mg/mL and then allowed to dry at a room temperature for 15 minutes and placed at the centre of each of the inoculated plates. Plates with gentamycin (10µg) served as positive control and discs with solvents without the plant extracts were used as a negative control. The diameter of inhibition zone was measured using sliding digital micro calliper [20].

Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was determined using the broth dilution test tube method as described earlier [20]. A total of 100 µL of pure nutrient broth was added to each test tube. The total of 10µL stock solution of 100 mg/mL extracts were subjected to two fold serial dilutions ranged between 50 mg/mL to 1.6 mg/mL. Then, 10 µL of the microbial suspension was added to each test tube and were incubated at 37°C for 24 h. After 24 h incubation, the solution were further inoculated in agar plates and incubated. The lowest concentrations of the extracts that inhibited the bacterial growth after a period of 24 h of incubation at 37°C were recorded as MIC.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) using SPSS version 20 (IBM SPSS Inc. Chicago, USA). Linear regression analysis was used to calculate the EC₅₀ value. Duncan's multiple range tests were used to assess mean separation and the statistical significance was considered at a level of $P < 0.05$.

Results

The TPC and TFC

The TPC of the bark of *S. guineense* determined by Folin–Ciocalteu method using gallic acid as the standard showed that the Ethanol fraction had higher TPC of 37.80 ± 3.70 mgGAE/g. Meanwhile, the highest TFC value was obtained for the ethanol followed by acetone extract (19.22 ± 1.44 and 11.70 ± 1.11 , respectively) (Table 1). Generally, the TPC and TFC contents showed similar trends of increasing with the increasing of polarity of the organic solvents.

Table 1
Total phenolic and flavonoid contents of the bark
extract of *S. guineense*

Extract	TPC (mgGAE/g)	TFC (mgQE/g)
Chloroform	10.30 ± 1.23^a	1.88 ± 0.64^a
Ethyl acetate	12.04 ± 1.10^a	4.12 ± 0.80^b
Water	16.61 ± 0.54^b	5.38 ± 0.66^b
Acetone	25.56 ± 2.26^c	11.70 ± 1.11^c
Ethanol	37.80 ± 3.70^d	19.22 ± 1.44^d

Values are expressed as mean $\pm$ SD ($n = 3$) from triplicate experiments. Means with the same letter in a column were not significantly different at the level of $p > 0.05$.

DPPH scavenging activity

DPPH has a deep purple color in solution and changes to yellow when it encounters a proton-donating substance such as an antioxidant and a radical species which can be quantified by its absorbance reduction at wavelength 520 nm [21]. As shown in Fig. 1, the DPPH scavenging (%) effect of samples increased when the concentration of the extract increased. At 1000 $\mu\text{g/mL}$, DPPH free radical scavenging activities of the extract and ascorbic acid increased in the following order : chloroform ($66.42 \pm 1.44\%$) < ethyl acetate ($71.82 \pm 0.74\%$) water ($80.26 \pm 0.86\%$) < acetone ($94.50 \pm 0.10\%$), ethanol ($97.30 \pm 0.73\%$), ascorbic acid ($98.58 \pm 0.20\%$). This implied that the *S. guineense* extracts contained a compound that can donate electron/hydrogen easily and stabilize free radicals.

In simple terms, the scavenging activities of the bark extracts that corresponds to their concentrations, inhibiting 50% of the DPPH radicals were reported. In the present study it was found that the ethanol extract resulted in the lowest EC_{50} value of $5.62 \mu\text{g/mL}$ which is very close to the positive control, ascorbic acid ($5.27 \mu\text{g/mL}$) (Table 2 and Fig. 1).

Table 2
The EC₅₀ (µg/mL) values of DPPH scavenging activity of the bark extract of *S. guineense* with various solvents of increasing polarity

Extract	EC ₅₀ (µg/mL)
Chloroform	50.39 ± 5.04 ^c
Ethyl acetate	38.90 ± 7.26 ^b
Water	12.90 ± 0.82 ^a
Acetone	8.04 ± 0.47 ^a
Ethanol	5.62 ± 0.08 ^a
Ascorbic acid	5.27 ± 0.10 ^a

Figure 1. DPPH radical scavenging activity (%) of various solvent extracts from dried bark of *S. guineense* and control (L-ascorbic acid) at different concentrations (µg/mL). The values are the average of triplicate measurements (mean ± SD).

Ferric reducing antioxidant power (FRAP)

The ethanol extract of the bark of the *S. guineense* was found to have strong ferric reducing antioxidant power (163.08 ± 11.67 mgAAE/g, treatment dose = 1 mg/mL) increasing in concentration-dependent manner whereas the more non polar solvent, chloroform fraction, was found to have weak ferric reducing antioxidant power (Table 3 and Fig. 2A).

Table 3
The Ferric reducing power (FRAP) and Total antioxidant capacity (TAC) of the bark extract of *S. guineense* with various solvents of increasing polarity

Extract	Ferric reducing power		Total antioxidant capacity	
	mgAAE/g (at 0.5 mg/mL)	mgAAE/g (at 1 mg/mL)	mgBHTE (at 0.5 mg/mL)	mgBHTE/g (at 1 mg/mL)
Chloroform	18.54 ± 0.50	54.22 ± 2.40	37.16 ± 1.26	62.56 ± 2.47
Ethyl acetate	38.78 ± 2.91	63.73 ± 3.26	44.94 ± 1.67	69.97 ± 2.88
Water	30.16 ± 2.80 ^b	55.31 ± 5.13	38.42 ± 1.22	63.80 ± 1.23
Acetone	62.78 ± 1.14	134.06 ± 5.13	51.50 ± 2.07	112.17 ± 5.34
Ethanol	82.83 ± 6.60	163.08 ± 11.67	80.63 ± 1.60	143.72 ± 2.86

Total antioxidant capacity (TAC)

It was determined that the ethanol extract of the bark of the *S. guineense* was found to have strong TAC (143.72 ± 2.86 mgBHTe/g, treatment dose = 1 mg/mL) increasing in concentration-dependent manner whereas the more non polar solvent, chloroform fraction, was found to have less TAC in a similar trend as that of the ferric reducing antioxidant power (Table 3 and Fig. 2B).

Figure 2. Ferric reducing power (mgAAE/g) (A), and total antioxidant capacity (mgBHTe/g) (B) of various solvent extracts from dried bark of *S. guineense*. The values are the average of triplicate measurements (mean $\pm$ SD). The values within the same concentration with the same letter in the histogram bar were not significantly different at $P > 0.05$.

Antibacterial activities

Different solvent extracts of the bark of *S. guineense* were tested against selected bacteria strains (*S. aureus*, *S. typhimurium*, *K. pneumoniae* and *E. coli*) for their antibacterial activities. The inhibition zones were compared with gentamicin 10 μ g which was used as positive control.

The study revealed that all bacteria strains were inhibited by the bark extracts of *S. guineense* with different degrees of inhibition zone (range: 25.39 ± 0.8 mm to 8.49 ± 0.26 mm in diameter). The highest average inhibition zones of 24.39 ± 0.60 mm and 23.35 ± 1.84 mm were recorded from the ethanol extracts against *K. pneumoniae* and *S. aureus*, respectively and the acetone extract (25.39 ± 0.80) against *S. aureus* was the maximum inhibition recorded (Table 4 and Fig. 3).

Table 4
Inhibition zones (mm) of various solvent extracts of the bark of *S. guineense*

Solvents	<i>Staphylococcus aureus</i>	<i>Salmonella typhimurium</i>	<i>Klebsiella pneumoniae</i>	<i>Escherichia coli</i>
Chloroform	-	-	-	-
Ethyl acetate	14.24 ± 0.60^{aC}	12.72 ± 2.36^{aB}	15.60 ± 1.38^{aC}	8.49 ± 0.38^{aA}
Water	22.24 ± 1.30^{bC}	23.87 ± 0.21^{bC}	16.64 ± 0.92^{abB}	8.98 ± 0.95^{aA}
Acetone	25.39 ± 0.80^{cC}	23.32 ± 0.52^{bC}	18.26 ± 0.90^{bB}	11.54 ± 0.98^{bA}
Ethanol	23.35 ± 1.84^{bCB}	21.76 ± 0.23^{bB}	24.39 ± 0.60^{cB}	8.49 ± 0.26^{aA}
Gentamicin 10 μ g	24.08 ± 1.42^{cA}	26.88 ± 1.08^{cB}	29.06 ± 1.41^{dC}	25.63 ± 0.84^{cA}

The values are expressed as mean $\pm$ SD from triplicate experiments. Means with the same letter in a column (lower case) and in a row (upper case) were not significantly different at $P > 0.05$.

Figure 3. Zone of inhibitions of the bark extracts of *S. guineense* in comparison with gentamicin (positive control) against *S. aurous*. Inhibition zone of the bark extracts of *S. guineense* with acetone – A; ethyl acetate – EA; ethanol – E; water – W; and chloroform – C extracts.

In addition to the inhibition zone determination, the MIC was determined for five extracts of the bark of the plant against four different bacterial strains. *Staphylococcus aureus* was relatively the most sensitive strain against acetone and ethanol extracts of the bark of *S. guineense* with the MIC values of 1.56mg/mL and 3.12 mg/mL, respectively. *Klebsiella pneumonia* was also found to be highly sensitive against the ethanol extracts of the bark of *S. guineense* (MIC = 1.56 mg/mL). The bacterial strain *Escherichia coli* was found to be less sensitive even at higher doses of 50 mg/mL of solvent extracts (Table 5). Standard antibiotics were used as positive control and completely inhibited the growth at 10 μ g while solvents without extracts were used as negative control and thus normal growth was recorded.

Table 5
The MIC of various solvent extracts (mg/mL) of the bark of *S. guineense*

Solvents	<i>S. aureus</i>	<i>S. typhi</i>	<i>K.pneumoniae</i>	<i>E. coli</i>
Acetone	1.56	3.12	6.25	50
Ethyl acetate	12.5	12.5	12.5	50
Chloroform	-	-	-	-
Ethanol	3.12	6.25	1.56	50
water	6.25	3.12	6.25	50
(-) = No inhibition at 100 mg/mL				

Discussion

In this study, we have quantitatively evaluated the dominant biologically useful phytochemicals and antibacterial activities of the bark of *S. guineense*, a traditionally useful plant in most rural parts of Ethiopia. It is well established that the information derived from various TM systems all over the world is utilized in the process of discovering lifesaving drugs [22]. The TM practiced today are maintained on the basis of perceived safety profiles by the indigenous people which is based on its own version of clinical trials, where the TMs have been used only when they have shown to be effective and safe [23]. The authors are ascertained that the trials may take place over very long periods of time from generation to generation and this might have led to very detailed knowledge and understanding of the medicinal properties of many natural products. The TM in use, however, needs rigorous validation studies for safe practice and wider application.

The fact that *S. guineense* is widely used in the TM all over the world [24, 25] is a remarkable indication that the plant has biologically active secondary metabolites as also revealed by the present study. Different parts of the plant reported in TM use include leaves [10, 26], roots [7], fruits [27] and the bark [11]. Various forms of human ailments such as diabetes [28], chronic diarrhea [29], malaria [10, 30], chest pain, stomachache and ringworm [31], hookworms [32], wounds [24], opportunistic infections [33], infertility, paresthesia and cancer [34] are among diseases traditionally treated from preparations of different parts of *S. guineense*.

The extract of the bark of the plant has high TPC and TFC especially the extract with more polar organic solvents. This is in agreement with previous studies in that different parts of the plant extracted with more polar solvents showed high TPC and TFC [24]. The TPC and TFC reported in the present study were found to be high when compared to the reports elsewhere [11, 35]. The antioxidant properties are correlated with the phenolic content of any plants which act as hydrogen donors, reducing agents, and are capable of scavenging free radicals [36, 37]. The TAC, increasing in concentration-dependent manner, high DPPH activities and FRAP are high in a more polar organic solvent extracts in the present study. This finding is corroborated by previous studies [38–40].

Free radicals are generated in our body by various endogenous systems and a balance between free radicals and antioxidants is necessary for proper physiological state. Recently, interests towards flavonoids and other polyphenolic compounds has increased due to their potent antioxidant and free-radical scavenging activities [41, 42]. The high contents of TPC and TFC, and the TAC reported here makes the *S. guineense* the potential source of antioxidants.

In the present study, the remarkable DPPH scavenging activity with the lowest EC₅₀ recorded value of 5.62 µg/mL was very close to the positive control, ascorbic acid (5.27 µg/mL). This result further signifies that the plant can be a good source of free radical scavenging compounds and is supported by earlier reports [11]. The organo-protective effect of the bark extract reported elsewhere also attributed to the high antioxidant activities of the plant and thus agrees with the present finding [43].

The results of the present research should be interpreted cautiously in that high doses of *S. guineense* extracts of different parts were reported with adverse indications elsewhere. For instance, administration of high doses of the hydroethanolic extract of its leaves to the pregnant dams showed teratogenic effects and toxicity to the reproductive system in female rat [44, 45]. However, effective doses in our finding are much less than the suggested high dose resulted in adverse effects.

The present finding revealed that both Gram positive (e.g., *S. aureus*) as well as Gram negative bacteria strains (e.g., *K. pneumonia*) were found to be sensitive against the bark extracts of *S. guineense* with more polar solvents such as water, ethanol and acetone. Both groups of bacteria were found to be non-sensitive to less polar solvent, chloroform, extract which might indicate the biologically active principle is soluble in more polar solvents [46]. From the very nature, Gram negative bacteria have structural organizations that make them more resistant over Gram positive bacteria making them the most

important public health threat resulting in high morbidity and mortality worldwide. Therefore, it can be argued that the plant can be a potential source of lead materials for new drug in quest to fight drug resistance, an ever-present enormous global health crisis[47].

Conclusion

The present results clearly indicate that the extracts of the bark of a traditional medicinal plant *S. guineense* possess high TPC and TFC endowing it high antioxidant activities exhibited through DPPH and FRAP assays. The total antioxidant content was also high. The polar solvent extracts of the bark have shown antibacterial activities against both Gram positive and Gram negative bacterial species. Therefore, carefully designed further investigations, targeting a lead compound isolation and antioxidant compound characterization, are recommended.

Abbreviations

AAE	Ascorbic acid equivalents
<i>BHT</i>	Butylatedhydroxytoluene
BHTE	Butylated hydroxytoluene equivalents
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	Ferric reducing antioxidant power
MHA	Mueller Hinton Agar
MIC	Minimum inhibitory concentration
TAC	Total antioxidant capacity
TAC	Total antioxidant capacity
TFC	Total flavonoid content
TM	Traditional medicine

Declarations

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Authors contribution Conceptual framework: ED, MM, HG, NT. Plant material collection, identification, and extraction: MM, NT. Methodology: ED, MM, HG, NT. Phytochemistry experiments, spectrophotometry, and formal analysis: ED, MM, HG. Data curation – NT. Writing – original draft: NT. Writing – review & editing: ED, MM, HG, NT. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Data Availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was approved by the research ethics committee of the Hawassa College of Teacher Education (Ref. Nº: HCTE/RIC/021/21). All the reagents used in this study were prepared, used, and disposed of according to the set laboratory guidelines and the material safety and data sheets (MSDS).

Consent for publication

Not applicable.

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Figures

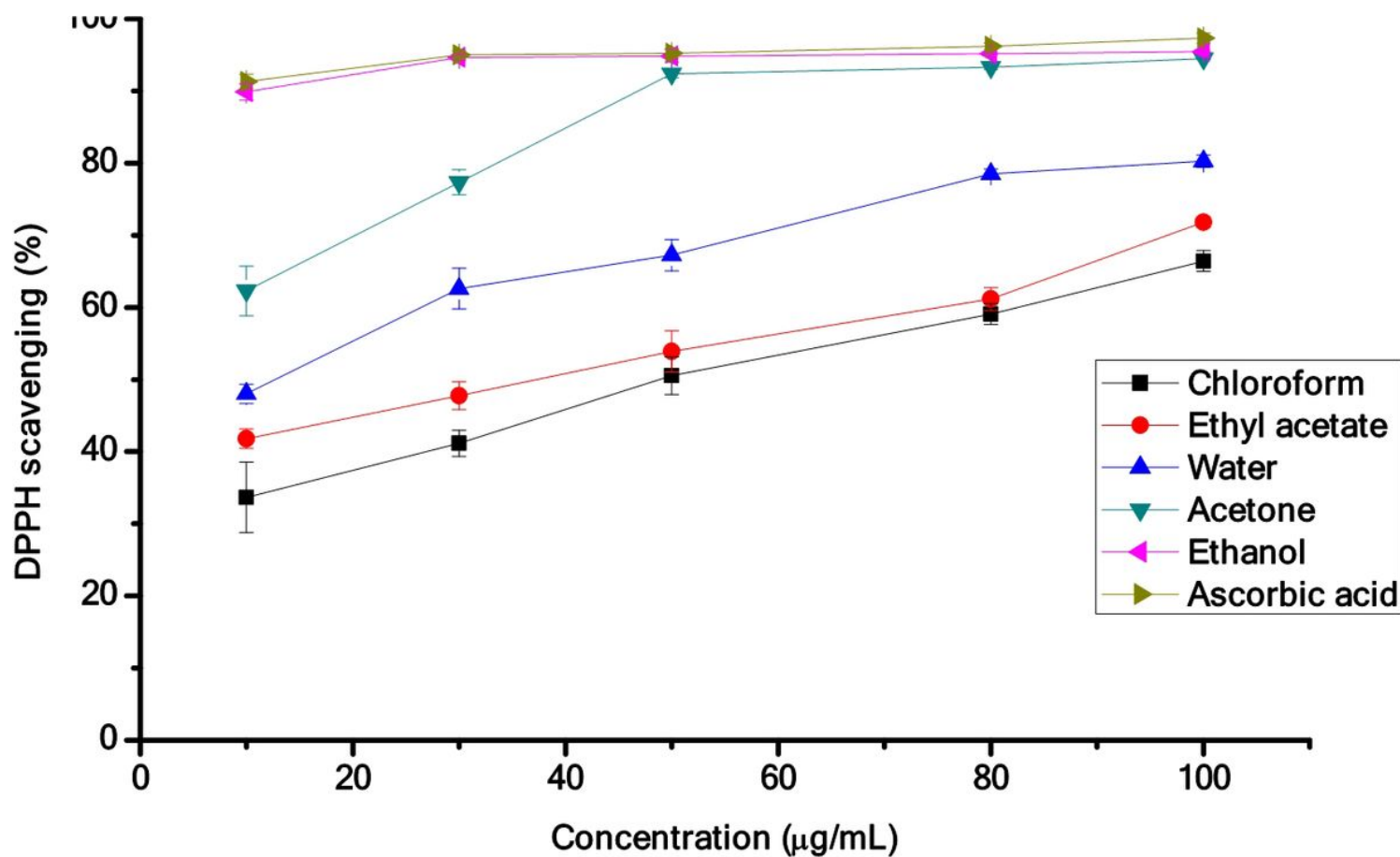


Figure 1

DPPH radical scavenging activity (%) of various solvent extracts from dried bark of *S. guineense* and control (L-ascorbic acid) at different concentrations (µg/mL). The values are the average of triplicate measurements (mean $\pm$ SD).

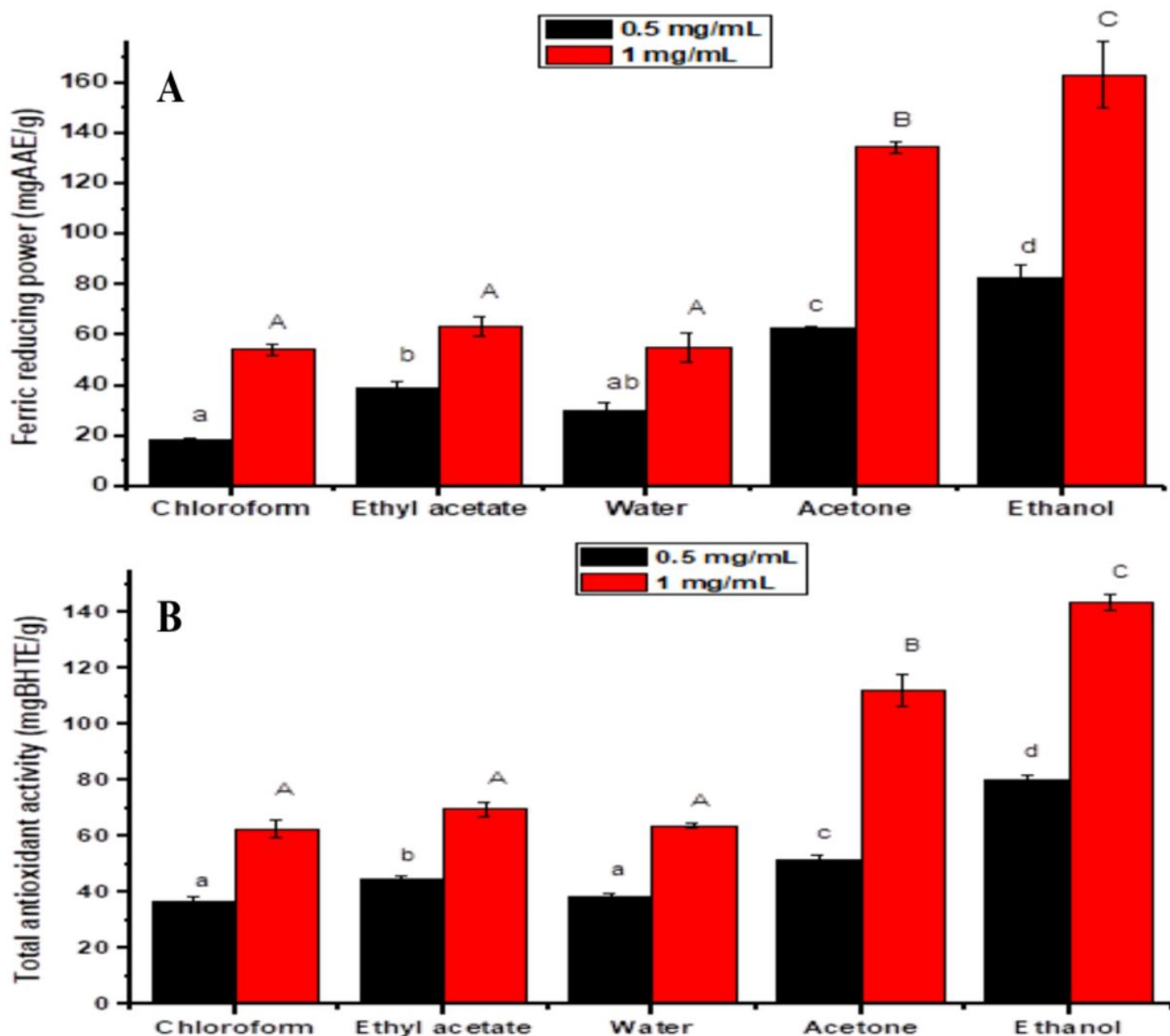


Figure 2

Ferric reducing power (mgAAE/g) **(A)**, and total antioxidant capacity (mgBHTE/g) **(B)** of various solvent extracts from dried bark of *S. guineense*. The values are the average of triplicate measurements (mean $\pm$ SD). The values within the same concentration with the same letter in the histogram bar were not significantly different at $P > 0.05$.

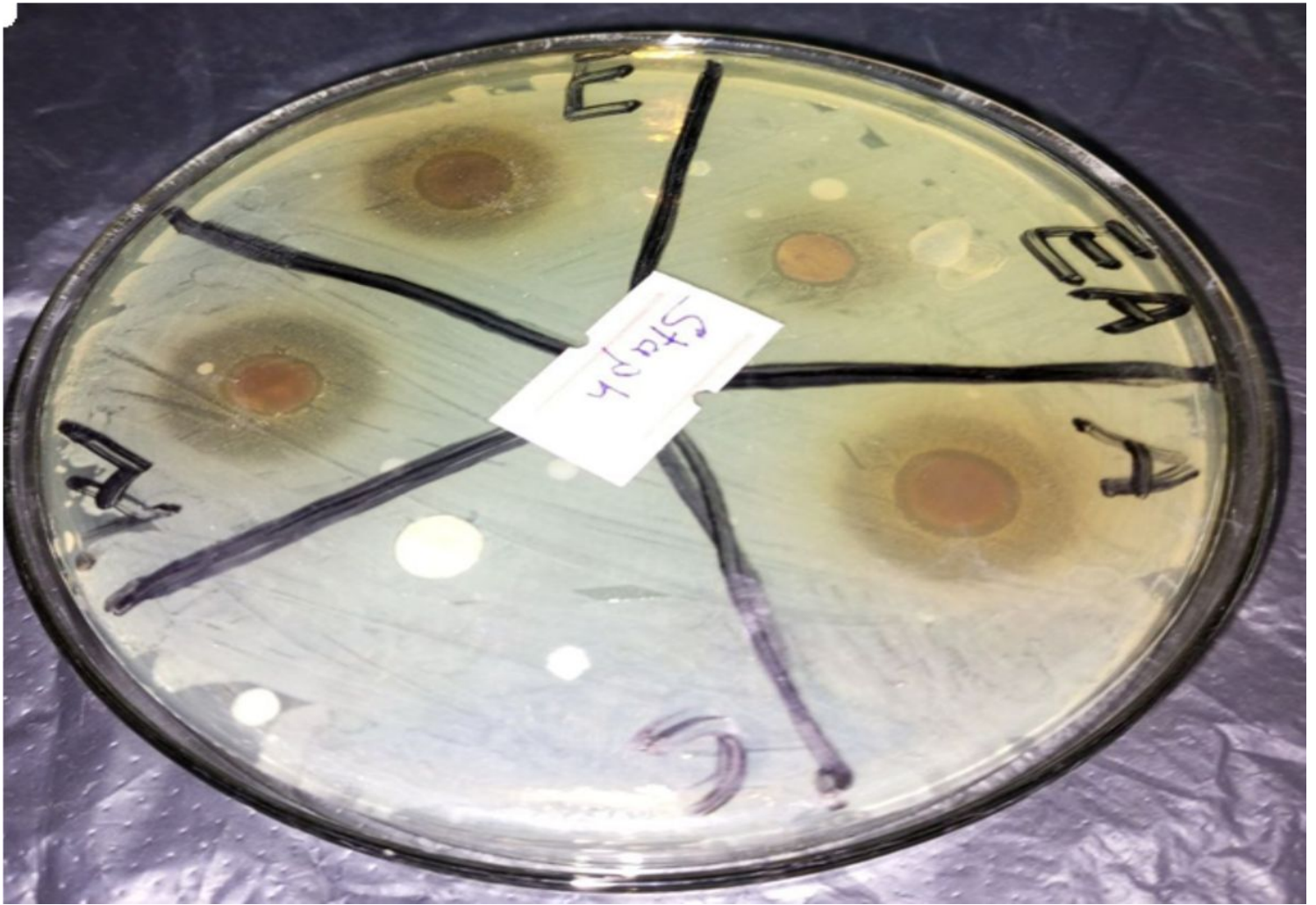


Figure 3

Zone of inhibitions of the bark extracts of *S. guineense* in comparison with gentamicin (positive control) against *S. aureus*. Inhibition zone of the bark extracts of *S. guineense* with acetone – A; ethyl acetate – EA; ethanol – E; water – W; and chloroform – C extracts.