

Polyphenolic Content, Antioxidant and Antimicrobial Activities of *Vernonia mespilifolia* Less. Used in Folk Medicine in the Eastern Cape Province, South Africa

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Abstract

Vernonia mespilifolia Less. is a shrub of the Asteraceae family used in the South African traditional medicine system for the management of weight loss, hypertension, and heartwater disease. There is a need for scientific evaluation to validate its ethnomedicinal usage. In vitro assays were conducted to evaluate the polyphenolic content, antioxidant and antimicrobial properties of different solvent extracts (acetone, aqueous, and ethanol) of the whole plant of *Vernonia mespilifolia* spectrophotometric and agar dilution techniques, respectively. The result revealed varying amounts of polyphenolics in the different solvent extracts corresponding to the antioxidant activities. Also, only the acetone and ethanol extracts inhibited the growth of the selected bacteria and fungi. These findings reveal that the extracts have strong bioactive compounds and hence support its ethnomedicinal application.

Keywords

agar dilution, antimicrobial activity, *Vernonia mespilifolia*, polyphenolic contents, radical scavenging activity

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Reactive oxygen species are generated as end-products of cellular metabolism or from exogenous sources such as exposure to ionizing radiation, ultraviolet radiation, and carcinogenic agents.¹ These reactive species such as singlet oxygen, superoxide ion, hydroxyl ion, and hydrogen peroxide, are extremely reactive and toxic molecules that cause oxidative damage to important biomolecules such as nucleic acids, proteins, enzymes, and lipids when the antioxidant defense system is overwhelmed, thus resulting in a myriad of chronic and degenerative diseases.² The use of natural sources of antioxidants from plants has attracted much interest as they have the capability of boosting the antioxidant capacity of the plasma and thus reducing the onset of certain disorders such as cancer, diabetes, obesity, cardiovascular diseases, and neurodegenerative diseases. The alarming trend of drug resistance to synthetic antioxidants as well as antimicrobial agents has resulted in the search for medicinal plants with potent antimicrobial and antioxidant capacity.¹ Medicinal plants are sought after because they possess certain bioactive compounds such as flavonoids, alkaloids, tannins, and phenolics with reported antimicrobial as well as other biological activities.³ Herbal medicines are

considered to be relatively inexpensive and safer than synthetic drugs. This has prompted the increased use of medicinal plants in the treatment and management of myriad of ailments. In recent times, the amount of polyphenols in plants and antioxidant activities depends on biological factors such as, genotypes/organ used, and environmental conditions, such as water stress, temperature, and light intensity.^{4,5}

Having a wide distribution in South Africa, *Vernonia mespilifolia* Less. is used for the management of a myriad of ailments. Just like its related species, *V amygdalina*, *V calvaona*, and *V colorata*, which have been shown to exhibit medicinal potentials, such as hypoglycemic and hypolipidemic effects,

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the whole plant of *V mespilifolia* is used in folk and traditional medicine for the management of weight loss and hypertension,⁶ while its stem is used for the management of heartwater disease in goats.⁷

Owing to the alarming rate of microbial resistance to present-day antibiotics, it is of great importance to search for alternative sources of antimicrobial agents, and medicinal plants could be explored for this purpose. However, there is a dearth of information on the antimicrobial and antioxidant properties of *V mespilifolia*; hence, this current study is aimed at evaluating the polyphenolic contents and antimicrobial and antioxidant properties of the whole plant of *V mespilifolia*.

Materials and Methods

The whole plant of *V mespilifolia* was collected in June 2015 from its natural habitat in the wild at Zihlahleni Village in Eastern Cape, South Africa, which lies at latitude 32° 51'41.846"S and longitude 27° 10'59.318"E. The plant was authenticated by Mr Tony Dold of Selmar Schonland Herbarium, Rhodes University, South Africa, and a voucher specimen (Unuofin Med, 2015/1) was prepared and deposited at the Giffen Herbarium, University of Fort Hare.

Preparation of Extracts

The whole plant was rinsed with deionized water and gently blotted with a paper towel to remove the water and subsequently oven-dried (LABOTEC, South Africa) at 40°C for 72 hours until constant weight was achieved. The dried sample was then ground into powder (Polymix PX-MFC 90D, Switzerland) and stored at 4°C until needed for analyses. The ground sample (200 g) was weighed into 3 separate conical flasks containing (2 L) acetone, ethanol, and water, respectively. These were placed on an orbital shaker (Orbital Incubator Shaker, Gallenkamp) for 48 hours. The crude extracts were filtered under pressure using a Buchner funnel and Whatman No. 1 filter paper. The acetone and ethanol extracts were further concentrated to dryness to remove the solvents under reduced pressure using a rotary evaporator (Strike 202 Steroglass, Italy), while the aqueous filtrate obtained was concentrated using a freeze dryer (Vir Tis benchtop K, Vir Tis Co, Gardiner, NY).

Reagents and Chemicals Used

Solvents and chemicals were purchased from Merck and Sigma-Aldrich, Gauteng, South Africa. All the chemicals used in this study were of analytical grade.

Polyphenolic Acid Determination

Phenol content was estimated spectrophotometrically using the Folin-Ciocalteu's reagent (FCR) method as described by Ghasemzadeh et al⁸ with some modifications. Briefly, 0.5 mL of the plant extracts (1 mg/mL) and standard gallic acid (0.02 to 0.1 mg/mL) were dispensed in test tubes. To this, 2.5 mL of 10% (v/v) FCR reagent was added and the mixture was vortexed. The reaction was allowed to stand at room temperature for about 5 minutes, after which 2 mL of 7.5% (w/v) anhydrous sodium carbonate was added to the solution, vortexed, and incubated at 40°C for 30 minutes. In the control tube, the extract volume was replaced by methanol. After incubation, the

absorbance was measured at 765 nm using a UV-3000 PC spectrophotometer. The phenol content was extrapolated from the gallic acid standard/calibration graph equation— $y = 8.7668x + 0.1977$, $R^2 = 0.9983$ —and calculated using the following formula:

$$C = c \times V/m$$

where C is the total content of phenolic compounds in mg/g GAE or mg GAE/g extract; c is the concentration of gallic acid established from the calibration curve in mg/mL; V is the volume of extract in mL; and m is the weight of extract used in the assay in grams.

Flavonoid Determination

The colorimetric aluminum chloride assay was used to determine the flavonoid content of the plant extract according to the method described by Sen et al⁹ with some modifications. This method is based on the quantification of the yellow-orange color produced by the interaction of flavonoid with $AlCl_3$. Briefly, 0.5 mL (1 mg/mL) aliquots of the different solvent extracts and different concentrations (0.2 to 1 mg/mL) of quercetin standard were dispensed in different test tubes, and 2 mL of distilled water and 0.15 mL of 5% sodium nitrite was added to the test tubes and the mixture was allowed to stand for 6 minutes. Thereafter, 0.15 mL of 10% $AlCl_3$ was added to the solution, allowed to stand for another 5 minutes, and this was followed by the addition of 1 mL of 1 M sodium hydroxide. The solution was made up to 5 mL with distilled water and the absorbance measured using a spectrometer at 420 nm. A control solution containing 0.5 mL of distilled water instead of the extract/standard served as the blank. The flavonoid content was calculated using the calibration curve equation— $y = 1.1734x + 0.1543$, $R^2 = 0.9698$ —and expressed as mg of quercetin equivalent (QE)/g using the formula CV/m , as earlier described for total phenol.

Proanthocyanidin (Condensed Tannin)

Determination of proanthocyanidin was as described by Hanen et al.¹⁰ The reaction mixture contained 0.5 mL of 1 mg/mL of the extract or standard catechin at different concentrations (0.02 to 1 mg/mL) plus 3 mL of vanillin-methanol (4% w/v) and 1.5 mL of hydrochloric acid. The mixture was vortexed and allowed to stand for 15 minutes at room temperature, while a control solution that had neither an extract nor catechin was used as blank. The absorbance was measured at 500 nm using a UV-3000 PC spectrophotometer. Proanthocyanidin content was calculated from the calibration curve equation— $y = 0.9038x + 0.0449$, $R^2 = 0.9951$ —and expressed as mg catechin equivalent (CE)/g sample using the formula CV/m , as earlier described for the determination of total phenol.

Determination of Tannins

Tannin was determined as described previously Mbaebie et al.¹¹ Plant extract (0.2 g) was added to 20 mL of 50% methanol. This was mixed thoroughly and placed in a water bath at 80°C for 60 minutes. The extract was filtered into a 100 mL volumetric flask; 20 mL of distilled water was added, followed by 2.5 mL of FCR and 10 mL of 17% Na_2CO_3 . This was thoroughly mixed and made up to 100 mL using distilled water. The mixture was allowed to stand for 20 minutes until a bluish-green color developed. The tannic acid standard concentrations used ranged from 0 to 10 ppm. The absorbance of the tannic acid standard solutions and plant extracts were measured after color

development at 760 nm using a UV-3000 PC spectrophotometer. The results were expressed as mg/g of tannic acid equivalent using the calibration curve: $Y = 154.45x$; $R^2 = 0.9585$.

Antioxidant Assay

The antioxidant capacities of the different extracts were measured using DPPH radical scavenging activity, ABTS radical scavenging activity, hydrogen peroxide activity, and nitric oxide scavenging activity. These measurements were made against standard antioxidants, including BHT and Rutin.

ABTS (2,2'-Azino-Bis(3-Ethylbenzothiazoline)-6-Sulfonic Acid) Radical Scavenging Activity. The method described by Khan et al¹² was adopted for the determination of ABTS activity of the plant extract. Briefly, the working solution was prepared by mixing equal volumes of 2 stock solutions of 7 mM ABTS and 2.45 mM potassium persulfate and allowed to react for 12 hours at room temperature in the dark to release ABTS radicals (ABTS⁺). The resultant green-colored solution was further diluted by mixing 1 mL of the ABTS⁺ solution with about 50 mL of methanol to obtain an absorbance of 0.700 ± 0.006 at 734 nm. On obtaining the desired absorbance, 1 mL of the resultant solution was then mixed with 1 mL of the plant extract/or standards at different concentrations (0.005 to 0.08 mg/mL). After 7 minutes, the reduction in absorbance is measured at 734 nm using a spectrophotometer. The percentage inhibition of ABTS⁺ by the extract or standard was calculated from the following equation:

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

DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Radical Scavenging Activity Assay. The method of Kibiti and Afolayan¹³ was used for the determination of DPPH free radical scavenging activity. Briefly, a solution of 0.135 mM DPPH radical in methanol was prepared. One milliliter of this solution was mixed with 1 mL (0.005 to 0.08 mg/mL) each of the plant fractions/standards antioxidants (BHT, Rutin) at different concentrations. The reaction mixture was thereafter vortexed and left in the dark at room temperature for 30 minutes. The absorbance of the mixture was measured spectrophotometrically at 517 nm. The decrease in absorbance was measured against that of the control. The scavenging ability of the plant extract was then calculated using the following equation:

$$\text{DPPH Scavenging activity (\%)} = \frac{[(\text{Abs control} - \text{Abs sample})]}{(\text{Abs control})} \times 100$$

where Abs control is the absorbance of DPPH + methanol and Abs sample is the absorbance of DPPH radical + sample/standard.

Hydrogen Peroxide (H₂O₂) Scavenging Activity. The H₂O₂ inhibition activity of the extracts was assessed by the method of Gülçin et al.¹⁴ Briefly, a solution of 4 mM H₂O₂ was prepared in phosphate buffer (0.1 M; pH 7.4) and incubated for 10 minutes. One milliliter of each plant extract at different concentrations (0.025 to 0.4 mg/mL) was added to 0.6 mL of hydrogen peroxide solution. The absorbance of the hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer solution without hydrogen peroxide. The positive controls used were BHT and

vitamin C. The percentage scavenging of hydrogen peroxide by samples was calculated using the formula:

$$\text{H}_2\text{O}_2 \text{ inhibition capacity (\%)} = \frac{[1 - (\text{H}_2\text{O}_2 \text{ abs of sample})]}{(\text{H}_2\text{O}_2 \text{ abs of blank})} \times 100$$

Nitric Oxide Scavenging Activity. A 2 mL aliquot of 10 mM sodium nitroprusside prepared in 0.5 mM phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of plant fractions, vitamin C, and BHT individually at different concentrations (0.025 to 0.4 mg/mL). The mixture was incubated at 25°C for 150 minutes, and 0.5 mL of the incubated solution was mixed with 0.5 mL of Griess reagent (1 mL sulfanilic acid reagent [0.33% prepared in 20% glacial acetic acid] and 1 mL of naphthalene diamine dichloride [0.1% w/v] at room temperature for 5 minutes). The mixture was incubated at room temperature for 30 minutes, followed by the measurement of the absorbance at 540 nm. A solution containing water instead of the extract/standard was used as a control. Nitric oxide radical scavenging ability was calculated using the following equation:

$$\text{NO radical scavenging activity (\%)} = \frac{[(\text{Abs control} - \text{Abs sample})]}{(\text{Abs control})} \times 100$$

where Abs control is the absorbance of NO radicals + methanol and Abs sample is the absorbance of NO radical + extract or standard.

Rationale for the Selection of the Microorganisms

The bacteria and fungi used for this study were selected based on their roles as opportunistic pathogens to humans and animals and their association with obesity and diabetes.¹⁵

Microorganism Strains

The selected bacterial strains for this study were 3 gram-positive strains—*Actinomyces odontolyticus* (ATCC 17929), *Lactobacillus sakei* (ATCC 15521), *Staphylococcus aureus* (ATCC 18824)—and 3 gram-negative strains—*Enterobacter cloacae* (ATCC 13047), *Pseudomonas aeruginosa* (ATCC 19582), and *Bacteriodes thetaiotomicon* (ATCC 29741). The fungal strains used for this investigation were *Candida albicans* (ATCC 10231), *Microsporium gypsum* (ATCC 24102), *Penicillium chrysogenum* (ATCC 10106), and *Trichophyton tonsurans* (ATCC 28942).

Preparation of Inocula

Bacterial Inoculum Preparation. The test bacteria strains that were maintained on nutrient agar slants were recovered in sterile MHB and incubated overnight at 37°C. In order to obtain distinct colonies, the 24-hour-old cultures were diluted 1:100 v/v in fresh sterile MHB and cultured on MHA overnight at 37°C. The colony suspension method of EUCAST¹⁶ was used for the preparation of the inoculum. Identical colonies from the culture were suspended in 0.85% sterile saline, adjusted with saline, and compared with 0.5 McFarland standards to obtain a suspension density equivalent to 10⁶ CFU (colony forming unit)/mL. The suspensions were confirmed by spectrophotometric reading at 600 nm. The cell suspensions were finally diluted 1:100 by transferring 0.1 mL of the bacterial suspension into 9.9 mL of sterile broth to give an approximate inoculum of 10⁴ CFU/spot. The inocula suspensions were used for inoculation within 15 minutes.

Table 1. Polyphenolic Content of Various Solvent Extracts of *Vernonia mespilifolia*.

Solvent	Total phenol (mg GAE/g)	Proanthocyanidin (mg CE/g)	Flavonoids (mg QE/g)	Tannins (mg TAE/g)
Aqueous	39.07 ± 0.01 ^a	34.55 ± 0.00 ^c	171.58 ± 0.02 ^c	0.36 ± 0.01 ^c
Acetone	99.04 ± 0.00 ^b	132.10 ± 0.00 ^a	542.87 ± 0.01 ^a	0.984 ± 0.00 ^b
Ethanol	106.99 ± 0.01 ^c	83.90 ± 0.00 ^b	464.75 ± 0.02 ^b	0.932 ± 0.02 ^a

Abbreviations: mg GAE/g, milligram gallic acid equivalent per gram of extract; mg QE/g, milligram quercetin equivalent per gram of extract; mg TAE/g, milligram tannic acid equivalent per gram of extract; mg CE/g, milligram catechin equivalent per gram of extract.

^a Values are expressed as mean ± standard deviation of 3 replicates.

All the superscripts indicate significant difference ($P < 0.005$) between the means. Values within the same column, with different superscript are significantly different.

For the fungal inoculum preparation, the modified method of Therese et al¹⁷ was used for the analysis. The fungi strains were freshly subcultured on SDA and incubated at 25°C for 72 hours. About 1 cm² of 3-day-old spore producing cultures was dropped in sterile distilled water and vortexed for 30 seconds to release the fungal spores. The spore density of each fungus was adjusted with a spectrophotometer at 580 nm to obtain a final concentration of approximately 10⁵ spores/mL. Cell suspensions were finally diluted to 10⁴ CFU/spot.¹⁸ For the *Candida* spp, the inocula were prepared by adding 1.0 mL of overnight *Candida* cultures to 9.0 mL of SDB to yield 10⁴ CFU/spot of the inoculum.¹⁹

Agar Dilution Assay

The method described by Wiegand et al¹⁹ and the EUCAST,¹⁶ which are modifications from the guidelines of the Clinical and Laboratory Standard Institute, were used for this study.

Muller Hinton and Sabouraud Agar were, respectively, prepared according to the manufacturer's description for antibacterial and antifungal screening. The agar was autoclaved at 121°C for 15 minutes and allowed to cool to 50°C in a water bath. One milliliter from the 2-fold serial dilutions were added to the molten agar (19 mL) in the water bath, swirled, poured into petri dishes, and allowed to cool and solidify. Ten microliters each from both the bacterial and fungal inocula was delivered on individual solidified agar surfaces to give the desired final inoculum of 1 × 10⁴ CFU/spot. The extracts and standard antibiotic concentrations for the antibacterial and antifungal evaluation ranged from 5 mg/mL to 0.1563 mg/mL. The concentration of ciprofloxacin ranged from 64 to 2 µg/mL, while nystatin (antifungal standard) ranged from 16 to 0.5 µg/mL. Bacteria plates were incubated at 37°C for 16 to 24 hours, while fungi plates were incubated at 30°C for 48 hours. The minimum inhibitory concentrations (MICs) were determined as the lowest concentration of extracts inhibiting the visible growth of each organism on the agar plate. The presence of 1 or 2 colonies was disregarded.

Preparation of Extract

A stock solution of 100 mg/mL prepared in little amount of dimethyl sulfoxide and made up with either Muller Hinton or Sabouraud Dextrose Broth for antibacterial and antifungal activities, respectively, was prepared. Two-fold serial dilutions of the (50, 25, 12.5, 6.25, 3.125, 1.5625 mg/mL) were also prepared. Standard antibiotic drugs ciprofloxacin and nystatin for antibacterial and antifungal activities, respectively, were also prepared by 2-fold serial dilutions as described by the Clinical and Laboratory Standard Institute.

Statistical Analysis

All data were expressed as mean ± standard deviation (SD) of 3 replicates. Statistical analysis was performed by ANOVA. Where the data showed significance ($P < .05$), a mean separation was done using Fischer's LSD with the aid of MINITAB 17 statistical package.

Results

The total polyphenol, flavonoid, proanthocyanidin, and tannin concentrations in *V mespilifolia* leaf and stem extracts are shown in Table 1.

The flavonoid, proanthocyanidin, and tannin concentrations were higher in the acetone extracts when compared with the aqueous and ethanol extracts. Also, the amount of phenolic content of plant materials depend on the polarity of the solvent used for extraction.⁵ The acetone extract had the highest concentrations of flavonoid, proanthocyanidin, and tannins, while the aqueous extract had the lowest concentration of these polyphenolics. The total phenol concentration ranged from 39.07 to 106.99 mg GAE/g. Flavonoid concentration ranged from 171.58 to 542.87 mg QE/g. Proanthocyanidin concentration ranged from 34.55 to 89.90 mg CE/g, while the concentration of tannin ranged from 0.36 to 0.984 mg TAE/g.

Antioxidant Activities of the Extracts

The results of this study showed that the antioxidant activities of the plant extracts compared favorably with those of the standard antioxidants (BHT and Rutin) employed in the antioxidant assays (Figures 2–4).

DPPH Free Radical Scavenging Assay

The DPPH radical scavenging activities of the extracts and standards are presented in Figure 1. At the maximum concentration tested (0.08 mg/mL), Rutin exhibited the highest DPPH scavenging activity (94.15%), followed by the ethanol extract (89.60%), BHT (88.63%), acetone (63.50%), and the aqueous extract (11.37%). The IC₅₀ values of the tested extracts/standards were in the following order: Rutin > BHT > ethanol extract > acetone extract > aqueous extract (Table 2).

ABTS Radical Scavenging Assay

The results of this assay, as shown in Figure 2, revealed that all the extracts and standards possessed considerable ABTS

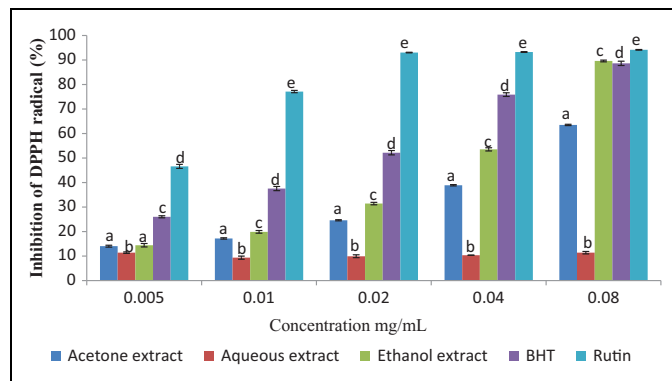


Figure 1. DPPH radical scavenging activity of the different extracts of *Vernonia mespilifolia*. Values are mean \pm SD of 3 replications. Set of bars with the same letter are not significantly different ($P < .05$).

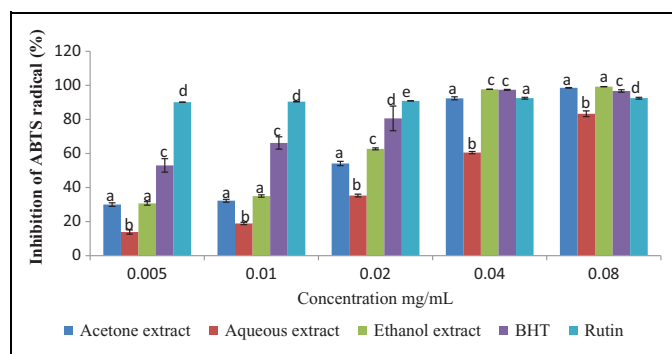


Figure 2. ABTS radical scavenging activity of the different extracts of *Vernonia mespilifolia*. Values are mean \pm SD of 3 replications. Set of bars with the same letter are not significantly different ($P < .05$).

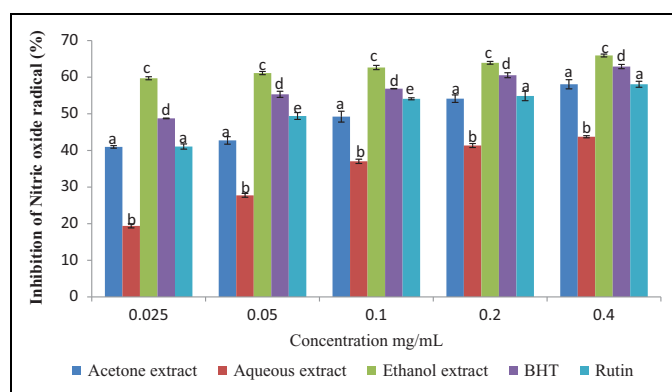


Figure 3. Nitric oxide radical scavenging activity of the different extracts of *Vernonia mespilifolia*. Values are mean \pm SD of 3 replications. Set of bars with the same letter are not significantly different ($P < .05$).

radical scavenging activities. The percentage inhibition of ABTS by the plant and the standards were concentration-dependent. At the highest concentration tested (0.08 mg/mL), the values for acetone, aqueous, and ethanol extracts were 98.55%, 83.29%, and 99.23%, respectively, while the standards (BHT and Rutin) produced inhibition as high as 96.68% and

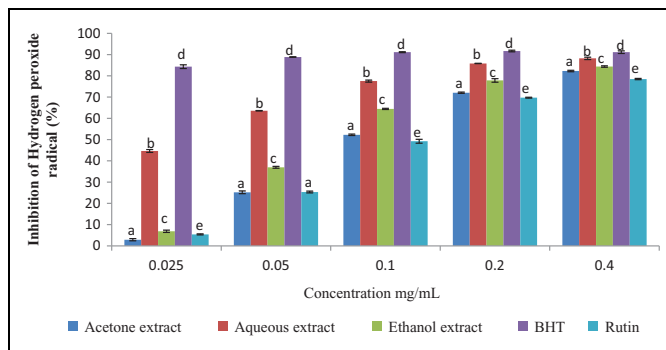


Figure 4. Hydrogen peroxide scavenging activity of the different extracts of *Vernonia mespilifolia*. Values are mean \pm SD of 3 replications. Set of bars with the same letter are not significantly different ($P < .05$).

92.49%, respectively. The IC_{50} values of the tested extracts/standards were in the following order: BHT > ethanol extract > acetone extract > aqueous extract > Rutin. The results are presented in Table 2.

Nitric Oxide Scavenging Assay

The percentage nitric oxide inhibitory activities of the extracts and standards (Figure 3) were in the following order: ethanol extract > BHT > Rutin > acetone extract > aqueous extract, at the highest concentration (0.40 mg/mL). The IC_{50} values of the tested extracts/standards were in the following order: ethanol extract > BHT > Rutin > acetone extract > aqueous extract (Table 2) with ethanol extract possessing the lowest IC_{50} , indicating its strong nitric oxide scavenging ability.

Hydrogen Peroxide Scavenging Assay

The scavenging ability of the plant extracts against hydrogen peroxide is shown in Figure 4. The scavenging activity of the plant in different solvents were in the following order: BHT > aqueous extract > ethanol extract > acetone extract > Rutin (Table 2). The percentage inhibition of hydrogen peroxide by the different solvents were concentration-dependent and significantly different ($P < .05$). The aqueous extract had highest radical scavenging potential among the extracts used but did not scavenge as much as BHT.

The results of the antibacterial MICs using agar dilution is shown in Table 3. The result revealed that both the gram-positive and gram-negative bacteria tested were susceptible to the various extracts of *V mespilifolia*. The MIC values ranged from 2.5 to 5 mg/mL for both gram-positive and gram-negative bacteria except for *P aeruginosa* and *S aureus* (acetone extract), which had MIC values greater than 5 mg/mL. The aqueous extract had the lowest activity against both gram-positive and gram-negative bacteria with MIC values greater than 5 mg/mL. The standard drug (ciprofloxacin) showed higher antibacterial activity with MIC value ranging from 2 to 32 μ g/mL.

Table 2. IC₅₀ Values of the Various Extracts of *Vernonia mespilifolia* Whole Plant and Standard Drugs^a.

Extracts/Standard	DPPH		ABTS		Nitric Oxide		H ₂ O ₂	
	IC ₅₀	R ²	IC ₅₀	R ²	IC ₅₀	R ²	IC ₅₀	R ²
Aqueous	1.87	0.9291	0.039	0.9527	0.6033	0.9646	0.0249	0.9176
Acetone	0.06	0.9985	0.013	0.9210	0.1158	0.9792	0.1108	0.9786
Ethanol	0.039	0.9378	0.012	0.9233	0.0003	0.9747	0.0865	0.9332
BHT	0.015	0.9857	0.003	0.9418	0.0294	0.9897	<<0.025 ^b	0.7246
Rutin	0.003	0.7427	0.996	0.9059	0.0768	0.8933	0.1172	0.9805

Abbreviations: IC₅₀, the concentration (mg/mL) required to scavenge/inhibit 50% of the radical; R², coefficient of determination.

^a Values obtained from regression lines with 95% confidence level.

^b Far less than 0.025.

Table 3. Minimum Inhibitory Concentrations (MICs) of the Different Solvent Extracts of *Vernonia mespilifolia* on Selected Gram Negative and Gram Positive Bacteria Using the Agar Dilution Assay.

Bacteria	Gram	Extract (mg/mL)			Ciprofloxacin (μg/mL)
		Acetone	Aqueous	Ethanol	
<i>Bacteriodes thetaiotomicon</i>	–	2.5	>5	5	2
<i>Actinomyces odontolyticus</i>	+	2.5	>5	2.5	2
<i>Lactobacillus sakei</i>	+	2.5	>5	5	2
<i>Enterobacter cloacae</i>	–	5	>5	5	2
<i>Pseudomonas aeruginosa</i>	–	>5	>5	2.5	2
<i>Staphylococcus aureus</i>	+	>5	>5	5	32

Table 4. Minimum Inhibitory Concentrations (MICs) of the Different Solvent Extracts of *Vernonia mespilifolia* on Selected Human Pathogenic Fungi.

Fungi	Extract (mg/mL)			Nystatin (μg/mL)
	Acetone	Aqueous	Ethanol	
<i>Microsporium gypsum</i>	2.5	>5	2.5	8
<i>Penicillium chrysogenum</i>	0.3125	>5	1.25	4
<i>Trichophyton tonsurans</i>	>5	>5	>5	4
<i>Candida albicans</i>	0.3125	>5	5	>16

The antifungal activities of the extracts of *V mespilifolia* on some selected fungi associated with obesity are shown in Table 4. The MICs were taken after 3 days of incubation. The acetone extract was more active against the 4 fungi tested. Its MICs ranged from 0.3125 to 2.5 mg/mL, except for *T tonsurans*, with MIC greater than 5 mg/mL. The ethanol extract was also active against the various fungi tested but not as much as the acetone extract. Its MIC ranged from 1.25 to 5 mg/mL. The aqueous extract had the least activity of all the extracts tested, with an MIC value greater than 5 mg/mL for all the fungi used for the study.

Discussion

In this study, flavonoid, total phenol, proanthocyanidins, tannin concentrations, antioxidant activity, and antimicrobial activity were determined for different extracts of *V mespilifolia*. These parameters usually depend on the type and polarity of solvent.^{20,21} In the present study, acetone extract of *V mespilifolia* had the highest concentration of flavonoids, proanthocyanidins, and tannins. The ethanol extract had the highest concentration of polyphenol. This is similar to results of Sharifi-Rad et al²¹ obtained using *Chrozaphora tinctoria*, where the acetone extract gave the highest yield of total polyphenols, flavonoids, and tannins. In recent times, various scientists have been appraising the effects of extraction solvents on natural products. The polarity of solvent used for extraction has a great impact on the yield of different phytochemical classes present in the plant.^{21,22} According to Eloff,²³ acetone extract contains more bioactive components with better antimicrobial potency.

According to Farasat et al,²⁴ the highest antioxidant activity in plants is attributed to polyphenols among other secondary metabolites. Phenolics have the ability of oxidizing a broad spectrum of free radicals to their stable radical intermediates.²⁵ They also serve as electron donors, metal chelators, and singlet and triplet oxygen quenchers.²⁶ Tannins (a polyphenol) bind and precipitate microbial proteins, thus starving bacteria of major nutrients.²⁷

Babaa and Malik²⁸ stated that flavonoids such as flavones, flavanols, and condensed tannins possess antioxidant potentials as a result of their free OH groups and thus they can suppress the formation of reactive oxygen species, mast cell histamine release, and antimicrobial activities. Antioxidants scavenge free radicals by different modes of action, which include using transition metal chelation, singlet oxygen quenchers, free radical scavenger (donate H), and peroxide stabilizers.²⁹ Because of these numerous modes of action of scavenging free radicals, we decided to assay for 4 different antioxidant assays. DPPH radical shows a maximum absorption at 517 nm. On encountering proton radical scavengers, its purple color fades rapidly. Antioxidants with the capacity of donating a hydrogen atom or electrons can quench DPPH free radicals, thus converting them to colorless bleached products, which brings about the reduction in absorbance.³⁰ The antioxidant activity was confirmed

by a decrease in absorbance band on increasing concentrations of *V mespilifolia*. The ethanol extract had the highest DPPH scavenging potential when compared with other extracts used, and this may be due to its high phenolic content, which contribute to their electron transfer/hydrogen donating ability.

The ABTS radical assay measures the relative antioxidant ability to scavenge the radical. It is an excellent tool for determining the antioxidant capacity of hydrogen-donating antioxidants.³¹ According to Heim et al,³² flavonoids and phenolic acids exhibit antiradical and antioxidant activities. The ethanol extract thus may have scavenged best due to its high phenolic content because of its high phenolic acid content.

Nitric oxide is classified as a free radical because of its unpaired electron and displays important reactivity with certain types of proteins and other free radicals. Its toxic effect becomes adverse when it reacts with superoxide radical, forming a highly reactive peroxynitrite anion (ONOO⁻).³³ Our finding suggests that the polyphenolics present in the *V mespilifolia* could be responsible for nitric oxide scavenging effect as ethanolic extract of *V mespilifolia* had the highest inhibitory effect on nitric oxide production.

Hydrogen peroxide itself is not very reactive, but when it becomes hydroxyl radical in the cells its toxic nature is revealed. Thus, the removal of H₂O₂ as well as O₂⁻· is very important for antioxidant defense in cell or food systems.³⁴ The various extracts of *V mespilifolia* scavenged H₂O₂ and this may be attributed to the phenolics, which can donate electrons to H₂O₂, thereby neutralizing it into water. The aqueous extract had the highest inhibitory effect on hydrogen peroxide radical.

Antimicrobial activity is one of the important properties of polyphenolic compounds.²¹ The antimicrobial activity of phenolics is due to their partially hydrophobic nature. They inhibit the hydrolytic enzymes (proteases), microbial adhesion, as well as cell envelope transport proteins, and so on.³⁵ Additionally, flavonols can bind with extracellular and soluble proteins and with bacterial cell walls. Most antibacterial medicinal plants are effective on gram-positive bacteria, but few against gram-negative bacteria; this is due to the presence of lipopolysaccharide barrier on the outer membrane of gram-negative bacteria, which is absent on gram-positive bacteria.³⁶

The antibacterial and antifungal activities of the acetone, aqueous, and ethanol extracts of *V mespilifolia* were investigated in this study. In this study, acetone and ethanol extracts of *V mespilifolia* showed the greatest antibacterial and antifungal activities, while the aqueous extract had the lowest (Tables 3 and 4).

The disparity in the activities of the crude extracts and standard antimicrobial drugs could be attributed to the combination of bioactive components inherent in the crude extracts as compared to standard drugs, which are pure compounds.^{18,37} However, the active components in the crude extracts could act in synergy to bring about the antimicrobial effects.²³ The weak activity of the aqueous extract in this current study could be due to a very low concentration of the compounds present in the crude extracts that are active against the various organisms. The weak activity of the aqueous extract against all bacterial

strains investigated in this study is in agreement with previous reports.^{18,38} According to Saglam and Arar,³⁸ medicinal plant extracts and pure compounds have displayed both antibacterial and fungicidal potentials both in vitro and in vivo. Therefore, plant extracts with renowned antimicrobial properties could be employed for therapeutic purposes³⁹ and as such these would boost the use of herbal remedies for microbial infections.¹⁸ The results from this study are in line with several reports on antimicrobial activities of many medicinal plants.^{40,41}

Conclusion

This study reveals that *Vernonia mespilifolia* has high amount of polyphenolic compounds. The ethanol extract had the highest polyphenolic content, which could have contributed to the high antioxidant activities observed. Also, the acetone extract was the most promising against gram-positive and gram-negative bacteria and also exhibited higher antifungal ability.

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Author Contributions

JOU, GAO, and AJA conceived and designed the study. JOU performed the experiments and wrote the draft manuscript. GAO and AJA coordinated and helped revise the manuscript. JOU, GAO, and AJA read and approved the final manuscript.


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The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Approval

Ethical approval is not required for this article as it does not contain any studies involving human or animal participants.

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