

Research Article

Antioxidant Activities of Methanol Extracts of Thirteen Cameroonian Antibacterial Dietary Plants

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This study falls within the search for alternative solutions to problems related to diseases associated with oxidative stress. It involved the evaluation of antioxidant activities extracts from thirteen antibacterial Cameroonian food plants, namely, *P. nigrum*, *A. cruentus*, *L. sativa*, *S. edule*, *S. nigrum*, *V. amygdalina*, *A. hybridus*, *V. hymenolepis*, *L. capensis*, *M. esculenta*, *C. melo*, *T. occidentalis*, and *T. triangulare*. The thirteen plant extracts with a broad spectrum of antibacterial activity all showed total reducing powers ranging between 2.41 and 27.81 AAE (mg ascorbic acid equivalents per gram of dried extract) and total phenol contents between 2.65 and 35.03 GAE (mg of gallic acid equivalents per gram of dried extract) of dry extract. Except for extracts of *L. capensis*, the other 12 extracts showed flavonoid contents ranging between 0.29 and 5.99 RE (rutin equivalents per gram of dried extract). All 13 plant extracts also showed free radical scavenging activity against DPPH· with IC₅₀ ranging between 1.65 and 310.52 µg/ml, while 12 of these extracts exhibited inhibitory activity against NO· radical (IC₅₀ ranging from 19.77 to 157.72 µg/ml). Statistically insignificant positive correlations ($p > 0.5$) were found between antibacterial activities of these plants and their antioxidant activities. The different results of this study provide scientific evidence for the use of these antibacterial food plants in the control of different conditions associated with oxidative stress.

1. Introduction

Oxidative stress is a condition which arises when reactive oxygen species (ROS) production becomes higher than antioxidant defense of the organism. If antioxidant capacities of the organism decrease or there is an increase in ROS production, the systemic antioxidant/oxidative dysfunction can lead to the accumulation of oxidative damage, which in turn can lead to modification of biomolecules with the occurrence of reactions that lead to protein additions, DNA oxidation, and lipid peroxidation with cellular functional ability reduction and risk of disease increase. Reactive oxygen species (ROS) and reactive nitrogen species (RNS)

levels can be generated through signal transduction pathways and regulation of physiological events such as cell cycle, programmed cell death, and inflammation [1]. It has been established that infectious diseases trigger the production of ROS and RNS which are at the origin of an increase of susceptibility to secondary infections via a mechanism of tissue injury as well as impairment of epithelial barrier functions [2].

The body contains natural cleansing systems against free radicals and other reactive species. However, ROS production sometimes exceeds internal antioxidant capacity leading to oxidative stress. Therefore, external antioxidants have an important function in human health. These

bioactive compounds act preventively and neutralize the formation of new reactive species and free radicals. The exogenous antioxidants are mainly derived from food and medicinal plants including fruits, vegetables, and spices which in recent years gained great interest in their antioxidant activity. These plants contain natural antioxidants mainly made of phenolic compounds. Phenolic compounds include phenolic acids, polyphenols, and flavonoids that protect plants, fruits, and vegetables from oxidative damage [3].

In two previous research papers, we highlighted antibacterial activities of the thirteen Cameroonian antibacterial plants used in this study, namely, *Piper nigrum*, *Amaranthus cruentus*, *Lactuca sativa*, *Sechium edule*, *Solanum nigrum*, *Vernonia amygdalina*, *Amaranthus hybridus*, *Vernonia hymenolepis*, *Lactuca capensis*, *Manihot esculenta*, *Cucurbita melo*, *Telfairia occidentalis*, and the fruits of *Piper nigrum* [4, 5]. These edible plants are used for many purposes in African traditional medicine (Table 1). In our previous studies, we demonstrated that the methanol extracts of these 13 plants inhibit the growth of Gram-negative multidrug-resistant bacteria with MIC values ranging between 32 and 1024 $\mu\text{g/ml}$ and can then be used in the control of bacterial infections including MDR phenotypes [4, 5]. The present study aims to determine the contents in total phenols and total flavonoids and antioxidant activities of methanol extracts of thirteen plants.

2. Material and Methods

2.1. Plant Extract Preparation. In June 2010, thirteen edible Cameroonian edible plants were harvested in Cameroon, West Region, City of Dschang. The collected plant materials were the fruits of *P. nigrum*, *A. cruentus*, *L. sativa*, *S. edule*, *S. nigrum*, *V. amygdalina*, *A. hybridus*, *V. hymenolepis*, *L. capensis*, *M. esculenta*, *C. melo*, *T. occidentalis*, and the fruits of *P. nigrum*. The plants were further identified and authenticated at the National Herbarium of Cameroon under voucher specimen numbers (see Table 1).

Each sample was air-dried at room temperature (RT), and the resulting powder was extracted for 48 h at RT using methanol as solvent. Afterward, the extracts were filtered using Whatman No.1 paper and the crude extracts were obtained after reduced pressure concentration.

2.2. Determination of Total Reducing Power. The total reducing power of plant extracts was determined as previously described with some modifications. Briefly, the reaction mixture was prepared in phosphate buffer sodium (PBS) (0.2 M, pH 6.6) with 100 $\mu\text{g/ml}$ of each extract, 440 μL of potassium ferrocyanide ($[\text{K}_3\text{Fe}(\text{CN})_6]$ 1%), and 440 μL of 10% trichloroacetic acid. The samples were then centrifuged for 10 min at 3000 rpm, and 680 μL of supernatant from each tube was collected and introduced into a new tube containing FeCl_3 (140 μL) and demineralized water (680 μL). The absorbance of these new solutions was read at 700 nm and converted into milligrams of ascorbic acid equivalents

(AAE) per gram of dried extract using a calibration curve (prepared using different concentrations of ascorbic acid used in the same manner as extracts).

2.3. Determination of Total Phenol Content. The determination of total phenol content of plant extracts was carried out using the Folin–Ciocalteu technique [98]. Briefly, a stock solution of Folin–Ciocalteu reagent was diluted 10 times. Then 500 μL of this solution was added to 100 μL of extract (10 mg/ml) dissolved in 10% DMSO. After 4 min of incubation at room temperature (RT), 400 μL of 7.5% of Na_2CO_3 solution was added to the mixture and the absorbance of the final solution was read at 765 nm. The absorbance was further converted into milligrams of gallic acid equivalents (GAE) per gram of dried extract using a calibration curve prepared using different concentrations of gallic acid (Figure 1) used in the same manner as extracts.

2.4. Determination of Total Flavonoid Contents. The determination of total flavonoid content of each plant extract was carried out using the spectrophotometric method of aluminum chloride (AlCl_3) using rutin (Figure 1) as a standard flavonoid. Briefly, 100 μL of each plant extract (10 mg/ml) was mixed with 400 μL of MeOH, 20 μL of AlCl_3 10%, 20 μL of acetic acid (CH_3COOH , 1 M), and 560 μL of distilled water. The reaction mixture was then incubated for 4 min at RT. After the incubation period, the absorbance was measured at 415 nm and converted into micrograms of rutin equivalents (RE) per gram of dried extract using a calibration curve (OD vs. rutin concentration).

2.5. Evaluation of the DPPH Radical Scavenging Activity of Plant Extracts. Radical scavenging activity of plant extracts was evaluated as described in a previous study [99]. 100 μL of each plant extract (dissolved in methanol) was added to 900 μL of DPPH (20 mg/l dissolved in methanol), to obtain concentrations of 32, 64, 128, 256, and 512 $\mu\text{g/ml}$. Positive control was made up of L-ascorbic acid (Figure 1) while 1 mL of DPPH (20 mg/L in methanol) served as the negative control. The incubation was made for 30 min at RT in a dark cupboard after mixture. The absorbance was measured at 517 nm and converted into percentage of scavenging activity using the following equation:

$$\% \text{RSA} = \frac{\text{absorbance of DPPH} - \text{absorbance of test sample}}{\text{absorbance of DPPH}} \times 100. \quad (1)$$

Probit table was then used to convert %RSA into probits which were plotted against the logarithmic values of the concentrations, and a linear regression curve was established to calculate the RSA_{50} defined as the quantity of each plant extract which decreases by 50% the free radical DPPH. A comparison was made between percentages of DPPH scavenged by test samples and percentages of DPPH scavenged by L-ascorbic acid (using the results of triplicate experiments).

TABLE 1: Evidence of the activities of the methanol extracts of thirteen antibacterial edible plants.

Plant (family); voucher numbers	Traditional uses	Parts used	Bioactive or potentially bioactive components	Bioactivities of extracts and/or compounds
<i>Amaranthus hybridus</i> Linn (Amaranthaceae); 15630 HNC	Intestinal bleeding, diarrhoea, and excessive menstruation [6, 7]	Leaves, seeds	Flavonoids, steroids, terpenoids, cardiac glycosides [6], alkaloid, saponin, tannins, phenols, hydrocyanic acid, and phytic acid [8, 9]	Antimicrobial [4, 6, 10]
<i>Vernonia hymenolepis</i> (H.F.) Hook (Asteraceae); 42401/HNC	Wounds [11], anticancer [12], fever, stomach ache, diarrhoea, hernia, spleen enlargement [13]	Leaves	Vernolepin [14, 15], vernomenin [15], flavonoids (quercetin, apigenin, and luteolin) [16]	Cytotoxic [14], spasmolytic, antiaggregating and deaggregating activities, antitumor activity, antimicrobial [17], insecticide [18], antifilarial [19]
<i>Lactuca sativa</i> Linn (Asteraceae); 25624/SRF.Cam	Analgesic, conjunctivitis, tired eyes, insomnia, sedative insomnia [20], anxiety, neurosis, dry coughs, rheumatic pain [21], stimulate digestion, enhance appetite, and relieve inflammation [22]	Leaves	Phenolic acids, triterpenoids, saponins, phytol [20], carotenoids [23], flavonoids including kaempferol [16], lettucein-A guaianolide sesquiterpene lactones conjugates, lactucin, deoxylactucin, and lactucopicrin [24]	Antimicrobial [4, 25], antifungal, antibacterial [26], antitumor [27] antioxidating, analgesic, and anti-inflammatory [20], depressant [28] sedative, hypnotic, analgesic, and anticonvulsant [29], hypoglycaemic [30], antioxidant [31, 32], and anxiolytic
<i>Lactuca capensis</i> Thumb (Asteraceae); 27743 HNC	Antispasmodic, digestive, diuretic, hypnotic, narcotic, and sedative properties. Treatment of insomnia, anxiety, neuroses, hyperactivity in children, dry coughs, whooping cough, rheumatic pain, chronic joint pains [33]	Leaves	Lactucarium, sesquiterpene lactone [34]	Antibacterial against Gram-negative multidrug-resistant bacteria [4]
<i>Sechium edule</i> (Jacq) SW (Cucurbitaceae); 42459/HNC	Urine retention, kidney diseases, arteriosclerosis, hypertension [35]	Leaves	C-glycosyl and O-glycosyl, flavones in roots, leaves, stem, and fruits [36], ascorbic acid, gibberellins, flavonoids, and saponins [35]	Diuretic [37], free radical scavenger and antioxidant [38], antibacterial [4, 39], antihypertensive [40], hepatoprotective activity of ethanolic extract, and its different fractions [41]
<i>Manihot esculenta</i> Crantz (Euphorbiaceae); 57650/HNC	Hypertension, headache and pain, irritable bowel syndrome, fever, headache, aches, and pains [42]	Leaves	3-Rutinosides of kaempferol and quercetin; the cyanogenic glycosides, lotaustralin, and linamarin, from the fresh leaves of cassava [43]	Anthelmintic activity of crude extracts antibacterial [4, 44]
<i>Cucurbita pepo</i> Linn (Cucurbitaceae); 15630 HNC	Intestinal infections and kidney problems (seeds), minor injuries (flowers), anthelmintic, hypertension, erysipelas, enteritis, dyspepsia, stomach disorders, liver disorders like jaundice [45]	Leaves	Saponin, tannin, quinone, coumarins, flavonoids, sterol, terpenes, [46] lignin, alkaloids, protein, and sugar curbicin [47] anthocyanin, phenols like syringic acid [47], phytin, lecithin, cucurbitane, and hexanocucurbitane L-2-O- β -glucopyranoside, curbicin [47], flavonoids, vitamins B, C, and E, β -sitosterol	Antihypertensive, antioxidative activities, arthritis, reduce the symptoms of BPH [47, 48]. High cholesterol, antiparasitic activity in vitro [49], alleviates the detrimental effects associated with protein malnutrition [50], antiparasitic [51], nephron, and hepatoprotective, vermifuge, inhibitor of prostaglandin biosynthesis [52], antiparasitic, protects gastric mucosal [45], antibacterial against Gram-negative multidrug-resistant bacteria [4]

TABLE 1: Continued.

<i>Solanum nigrum</i> Linn (Solanaceae); 43000 HNC	Pneumonia aching teeth, stomach ache, tonsillitis, tonic, wing worms [11], pain, inflammation, and fever. Tumor, antioxidant, anti-inflammatory, hepatoprotective, diuretic, antipyretic [53]	Leaves	Kaempferol [16, 54], terpenoids and condensed tannin [55], quercetin, flavonoids [16], polysaccharides, polyphenolic compounds including gallic acid, catechin, caffeic acid, rutin, and naringenin [53]	Anti-inflammatory, antioxidant, anthelmintic activity [55] antinociceptive, antipyretic, antitumor, antiulcerogenic, cancer chemopreventive, hepatoprotective, and immunomodulatory effects [56] mosquito larvicidal [57], antibacterial [4, 58]
<i>Piper nigrum</i> L. (Piperaceae); 25818/SFRcam	Cardiovascular diseases, intoxication, inflammation, bacterial, fungal, and parasitic infections, respiratory diseases, asthma [59]	Seeds	Piperine, pipene [60], piperamides, piperamine [61], pellitorine [62]	Antiapoptotic [63, 64], antibacterial and antibiotic potentiation [5, 59, 65], antidepressant [66], antifungal [67], analgesic, anti-inflammatory [68], antiarrhoeal [69], antimutagenic, antioxidative, increase plasma [70], antipyretic [68], immunomodulatory, antispasmodic [71, 72], asthma, obesity, sinus antispermato-genic, antithyroid, antitumor ciprofloxacin potentiator, transcription inhibitor, insecticidal, hepatoprotective, increase pancreatic enzymes, cytochrome inhibitor [59]
<i>Vernonia amygdalina</i> Del. (Asteraceae); 31149/SRFK	Microbial infections [73], hiccups, kidney problems and stomach discomfort [74], stomach ache and gastrointestinal pain, malarial fever, cough remedy [75], antimalaria, purgative, antiparasitic, eczema blood glucose levels control [76], treatment of eczema [76]	Leaves	Flavonoids, saponins, and alkaloids [75], vernodaline, vernomygdin, vernonioside B1, and vernoniol B1 [77]	Active anticancer [78], antimalarial and antiparasitic agents [79], hypoglycaemic [80], antimicrobial, antibacterial and antibiotic potentiation [5], antihelminthic, antischistosomal, tumor inhibitor [77], hypolipidaemic and antioxidant properties [81]
<i>Telfairia occidentalis</i> (Cucurbitaceae); 33423/HNC	Microbial infections, cholesterolemia, liver problems, and impaired defense immune systems [82]	Leaves	Phenols, alkaloids, and tannins [83]	Antimicrobial and antibiotic potentiation [5], antioxidant and free radical scavenger [83, 84], antiplasmodial, cure lactating properties, hypoglycemic and antidiabetic [82]
<i>Talinum triangulare</i> (Jacq.) Willd. (Portulacaceae); 11537 SRFcam	Against infectious diseases, burns [85], internal heat, cramps, IST [86], cardiovascular diseases [87]	Leaves and entire plants	Essential oil, alpha-tocopherols, beta-tocopherols [88], alkaloids, flavonoids, saponins, tannins [89], β -carotene, minerals [90]	Antioxidant [91] and neuroprotective [92], hemolytic and hyperglycemic [86], prooxidant [93], anti-inflammatory [94], tonic [95]
<i>Amaranthus cruentus</i> Linn (Amaranthaceae); 42335 HNC		Leaves and seeds	Phenols, anthocyanins [96], carbonic anhydrases [97], α -tocopherols, oleic acids, linoleic and folic acids, riboflavin and niacin	Antioxidant against lipid peroxidation, DPPH and ABTS radicals [96], antibacterial against Gram-negative multidrug-resistant bacteria [4]

^aHNC: Cameroon National Herbarium; SRFK: Société des Réserves Forestières du Cameroun.

2.6. Assessment of Nitric Oxide Complexation Radical. At physiological pH, sodium nitroprusside decomposes itself in solution with the production of NO· free radicals. In aerobic conditions, NO· radicals lead to the formation of nitrites. The chromogen formed during diazotization with nitrite ions with Griess reagent has a maximum absorbance at a wavelength of 546 nm [100].

In this study, the nitric oxide was generated from sodium, and nitroprusside was measured using modified Griess reagent. For instance, 1 ml of a solution of sodium nitroprusside dissolved in phosphate buffer (phosphate-buffered saline or PBS) was added to each test tube and mixed with 100 μ L of solutions of extracts to obtain different concentrations (4, 16, 64, 256, and 1024 μ g/ml). The mixture

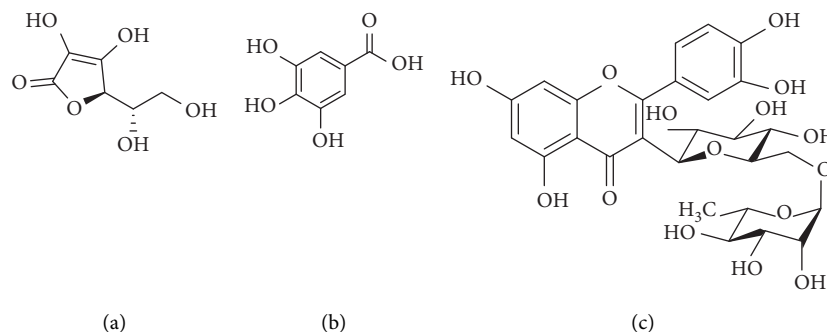


FIGURE 1: Structural formula of some molecules with antioxidant properties used as reference antioxidants. (a) Ascorbic acid. (b) Gallic acid. (c) Rutin.

was then incubated at RT for 180 min. After incubation, 100 μ L of modified Griess reagent was added to each tube. Then, the absorbance was monitored at 540 nm and converted into percentage of radical scavenging activity (%RSA) using the following equation:

$$\%RSA = \frac{\text{absorbance of control} - \text{absorbance of test sample}}{\text{absorbance of control}} \times 100. \quad (2)$$

The probit table was then used to convert %RSA into probits which were plotted against the logarithmic values of the concentrations, and a linear regression curve was established to calculate the RSa_{50} , which are the amounts of sample necessary to decrease by 50% the free radical NO. The experiment was carried out in triplicate and the percentages of NO scavenged by test samples were compared to that of rutin.

2.7. Statistical Analysis. Statistical Package for Social Science (SPSS, version 18.0) was used to carry out statistical analysis. Data from each experience were expressed as mean \pm standard deviation (SD). One-way analysis of variance (ANOVA) followed by the Waller–Duncan post hoc test was used for group comparisons. Differences were considered significant at a p value ≥ 0.05 .

3. Results

The antioxidant properties of extracts were assessed using five different methods. These experiments allowed the obtention of variable results according to the extracts and tests used.

Compared to the total reducing power and content of total phenols, *P. nigrum* stood out clearly with the largest respective values of 30.12 AAE (Table 2) and 14.01 GAE (Table 3), while the other 12 extracts showed reducing power between 1.62 and 8.41 AAE (Table 2), and total phenols comprised between 2.34 and 7.71 GAE (Table 3) and a strong correlation was also observed between the results of these two methods with a coefficient of correlation of 0.868 (Table 4), reflecting the strong involvement of phenols in the reducing power of the extracts of this study. However, higher levels of flavonoids were not obtained with extracts showing the greatest levels of total phenols. Actually, among the 4 extracts

TABLE 2: Total reducing power of the methanol extracts of thirteen antibacterial edible plants.

Plant extracts	Total reducing power (AAE)*
<i>Amaranthus hybridus</i>	1.62 \pm 0.12 ^g
<i>Amaranthus cruentus</i>	3.21 \pm 0.49 ^f
<i>Cucumis melo</i>	8.41 \pm 0.60^b
<i>Lactuca capensis</i>	2.83 \pm 0.04 ^f
<i>Lactuca sativa</i>	6.37 \pm 0.54 ^d
<i>Manihot esculenta</i>	8.22 \pm 0.40^{bc}
<i>Piper nigrum</i>	30.13 \pm 0.92^a
<i>Sechium edule</i>	4.87 \pm 0.15 ^e
<i>Solanum nigrum</i>	3.37 \pm 0.71 ^f
<i>Talinum triangulare</i>	3.31 \pm 0.43 ^f
<i>Telfairia occidentalis</i>	4.54 \pm 0.23 ^e
<i>Vernonia hymenolepis</i>	5.98 \pm 0.11 ^d
<i>Vernonia amygdalina</i>	7.44 \pm 0.36^c

*AAE: μ g of ascorbic acid equivalents per gram of dried extract.

TABLE 3: Total phenol content of the methanol extracts of thirteen antibacterial edible plants.

Plant extracts	Total phenols content (GAE)*
<i>Amaranthus hybridus</i>	7.31 \pm 0.22^{bc}
<i>Amaranthus cruentus</i>	1.23 \pm 0.25 ^g
<i>Cucumis melo</i>	7.71 \pm 0.03^b
<i>Lactuca capensis</i>	5.62 \pm 0.22 ^d
<i>Lactuca sativa</i>	6.82 \pm 0.27^c
<i>Manihot esculenta</i>	4.93 \pm 0.13 ^d
<i>Piper nigrum</i>	14.01 \pm 0.10^a
<i>Sechium edule</i>	2.58 \pm 0.32 ^f
<i>Solanum nigrum</i>	2.98 \pm 0.08 ^{ef}
<i>Talinum triangulare</i>	3.51 \pm 0.44 ^e
<i>Telfairia occidentalis</i>	2.65 \pm 0.03 ^{ef}
<i>Vernonia hymenolepis</i>	3.12 \pm 0.21 ^{ef}
<i>Vernonia amygdalina</i>	2.34 \pm 0.31 ^f

*GAE: mg of gallic acid equivalents per gram of dried extract.

showing the highest flavonoid content (Table 5), we noticed the presence of *M. esculenta* (the only one of the 4 extracts with one of the highest phenolic contents) and especially that of *A. cruentus* which showed the lowest phenolic content. This lack of correlation between flavonoid contents and phenolic contents and between flavonoid content and total reducing power of the extracts on the other hand was further confirmed through correlation factors (Table 4).

TABLE 4: Total flavonoid content of the methanol extracts of thirteen antibacterial edible plants.

Plant extracts	Total flavonoids content (RE*)
<i>Amaranthus hybridus</i>	1.91 ± 0.32 ^e
<i>Amaranthus cruentus</i>	5.59 ± 0.27 ^a
<i>Cucumis melo</i>	4.50 ± 0.27 ^{ab}
<i>Lactuca capensis</i>	0.00 ± 0.00 ^f
<i>Lactuca sativa</i>	5.59 ± 0.50 ^a
<i>Manihot esculenta</i>	5.99 ± 0.53 ^a
<i>Piper nigrum</i>	3.88 ± 0.10 ^b
<i>Sechium edule</i>	3.78 ± 0.41 ^{bc}
<i>Solanum nigrum</i>	1.80 ± 0.23 ^{de}
<i>Talinum triangulare</i>	5.50 ± 0.58 ^a
<i>Telfairia occidentalis</i>	0.29 ± 0.03 ^{cf}
<i>Vernonia hymenolepis</i>	1.02 ± 0.22 ^{de}
<i>Vernonia amygdalina</i>	2.27 ± 0.02 ^{cd}

*RE: μg of rutin equivalents per gram of dried extract.

Correlation analysis was also made between the antibacterial activities of extracts (Supplementary data available here) and the antioxidant activities obtained with each of the five methods used in this work. The results presented in the last row of Table 4 showed that there are statistically insignificant positive correlations ($p > 0.5$) between the antibacterial activities of these plants and the antioxidant activities obtained with each of the five methods. The strongest correlation was observed between antibacterial activities and total phenols content, followed by free radical scavenging activity against nitric oxide. The weakest correlation was observed between antibacterial activities and total flavonoid content, followed by free radical scavenging activity against DPPH and total reducing power. This correlation analysis could suggest that although the correlation between the antibacterial and the antioxidant activities of the extracts in this study is not very high, the phenols they contain may exert both antibacterial and antioxidant activities.

The radical scavenging activities of extracts expressed here as RSa_{50} varied according to radicals and extracts. Thus, with the NO^\bullet radical, the greatest activity was observed with *A. hybridus* ($\text{RSa}_{50} = 19.77 \mu\text{g}/\text{ml}$), greater than that of rutin used as reference antioxidant ($\text{RSa}_{50} = 157.72 \text{ g}/\text{ml}$). Four other extracts (*T. occidentalis*, *V. amygdalina*, *L. capensis*, and *M. esculenta*) also showed activities higher than that of rutin. Extracts of *V. hymenolepis* and *S. nigrum* exhibited free radical scavenging activity statistically similar to that of rutin. The remaining six samples exhibited activities, which, although significantly lower than that of rutin, is still important in most cases (Table 6).

Against the DPPH \cdot free radical, all the 13 extracts exerted scavenging activities with RSa_{50} ranging from 1.65 (*P. nigrum*) to 109.22 mg/ml (*C. melo*). Apart from the extract of *P. nigrum* which showed best radical scavenging activities against DPPH \cdot , seven other extracts showed RSa_{50} values which were lower than 50 mg/ml. These extracts include *S. edule*, *M. esculenta*, *L. capensis*, *V. hymenolepis*, *A. cruentus*, and *V. amygdalina* (Table 7).

Negative correlation was observed between free radical scavenging activity of extracts against NO and phenol contents, while there were insignificant correlations between

scavenging activity, reducing power, and total flavonoid content, suggesting that these elements do not play a significant role in the trapping of NO. A significant correlation was observed between the scavenging activity vis-à-vis the DPPH and total reducing power, showing that these two activities are linked to the same compounds, while non-significant correlations were observed between DPPH and the contents of flavonoids and total phenols.

Antioxidant activities undertaken in this study corroborate the results of other studies that reported the benefits of fruits and vegetables and give information about the subject. Fruits and vegetables are known for their ability to protect organisms through antioxidant properties. They are very rich in antioxidants, and several publications were already made on different plants of this study (Table 1). Some highlights concern the wealth of some of these fruits and vegetables antioxidant reference such as vitamin C, vitamin E, and quercetin in *A. hybridus*, *M. esculenta*, *P. vulgaris*, and *S. nigrum* (Table 1).

Previous studies showed the plants of the genus *Amaranthus* used in this study, namely, *A. hybridus* and *A. cruentus* presented phenols in varying degrees depending on the extraction solvent [101]. Without determining the RSa_{50} , the same study showed that *A. cruentus* and *A. hybridus* exerted antiradical activity against different radicals including NO^\bullet . Moreover, it has been proven that the extract of *A. cruentus* helps to fight against oxidative stress in rats by increasing the activity of different antioxidant enzymes of plasma [102].

A study has shown that the ethanolic, aqueous, and acetone extracts pomace of roots of *B. vulgaris* contains phenols (316.30 to 564.50 GAE) and flavonoids (200.50 to 253.50 RE) [103]. Much higher levels than those of the methanolic extract of the whole root were found in this study (9.28 GAE and 0.25 RE, respectively, for phenols and flavonoids). However, with an RSa_{50} of 175.49 $\mu\text{g}/\text{ml}$, the extract presented an antiradical activity against DPPH $^\bullet$ comparable to the one found in a previous study (RSa_{50} between 133 and 275 mg/ml) [103]. It was also demonstrated that the methanol extract inhibits lipid peroxidation, preserves the activity of plasma antioxidant enzymes, and prevents excessive fluidity of plasma membranes in rats that received CCl_4 [104].

It was previously shown that *C. melo* may be used as a source of natural antioxidant due to its contents in phenols of 26.4 ± 0.3 GAE and flavonoids of 69.7 ± 3.37 RE [105]. It is also capable of reducing the production of superoxide, nitrite, and peroxynitrite. These antioxidant activities would be partly explained by its richness in antioxidants such as glutathione, carotenoids, or vitamin E [106]. *C. pepo* also contains phenols including flavonoids and a scavenging activity against DPPH $^\bullet$ variables with content specifically fertilizer and soil composition in general [107].

It was also reported that ethanol extracts of leaves and seeds of aqueous extracts of *S. edule* possess antioxidant activities performed through the inhibition of lipid peroxidation, the activity of free radical scavenging against the β -carotene and DPPH $^\bullet$, with an RSa_{50} of 2 mg/ml. These previous studies showed that the aqueous and ethanolic

TABLE 5: DPPH radical scavenging activities of the methanol extracts of thirteen antibacterial edible plants.

Plant extracts	Radical scavenging activities expressed as RSa ₅₀ (µg/ml)
<i>Amaranthus hybridus</i>	310.52 ± 41.19 ^g
<i>Amaranthus cruentus</i>	38.48 ± 3.03 ^{bcd}
<i>Cucumis melo</i>	109.22 ± 10.04 ^e
<i>Lactuca capensis</i>	19.44 ± 0.99^{abc}
<i>Lactuca sativa</i>	163.21 ± 10.09 ^f
<i>Manihot esculenta</i>	16.22 ± 0.51^{ab}
<i>Piper nigrum</i>	1.65 ± 0.16^a
<i>Sechium edule</i>	11.39 ± 0.26^{ab}
<i>Solanum nigrum</i>	48.89 ± 5.66 ^{cd}
<i>Talinum triangulare</i>	64.34 ± 4.39 ^d
<i>Telfairia occidentalis</i>	48.23 ± 2.93 ^{cd}
<i>Vernonia hymenolepis</i>	38.48 ± 7.28^{bcd}
<i>Vernonia amygdalina</i>	24.91 ± 2.23^{abc}
L-ascorbic acid	1.86 ± 0.10^a

RSa₅₀: concentration of tested sample necessary to decrease by 50% the free radical DPPH. Values bearing the different superscript letters are significantly different ($p < 0.05$).

TABLE 6: NO radical scavenging activities of the methanol extracts of thirteen antibacterial edible plants.

Plant extracts	Radical scavenging activities expressed as RSa ₅₀ (µg/ml)
<i>Amaranthus hybridus</i>	760.25 ± 65.02 ^f
<i>Amaranthus cruentus</i>	524.13 ± 37.30 ^e
<i>Cucumis melo</i>	58.38 ± 3.27 ^a
<i>Lactuca capensis</i>	74.58 ± 3.82 ^{ab}
<i>Lactuca sativa</i>	19.77 ± 1.80 ^a
<i>Manihot esculenta</i>	118.60 ± 6.60 ^{abc}
<i>Piper nigrum</i>	477.09 ± 19.32 ^e
<i>Sechium edule</i>	738.77 ± 35.49 ^f
<i>Solanum nigrum</i>	233.27 ± 7.26 ^d
<i>Talinum triangulare</i>	—
<i>Telfairia occidentalis</i>	53.05 ± 2.06 ^a
<i>Vernonia hymenolepis</i>	191.97 ± 20.85 ^{cd}
<i>Vernonia amygdalina</i>	66.86 ± 4.05 ^a
<i>Rutin</i>	157.72 ± 7.38 ^b

Note: “—”: >1024 µg/ml; RSa₅₀: concentration of tested sample necessary to decrease by 50% the free radical NO. Values bearing the different superscript letters are significantly different ($p < 0.05$).

TABLE 7: Correlation between different antioxidant activity and antibacterial parameters of the methanol extracts of thirteen antibacterial edible plants.

	Total reducing power	Total flavonoids content	Total phenols content	NO· radical scavenging activity	DPPH· radical scavenging activity
Total reducing power					
Total flavonoid content	0.449** (0.0003)				
Total phenol content	0.263* (0.015)	0.114 (0.292)			
NO· radical scavenging activity	0.254* (0.018)	0.273* (0.011)	-0.138 (0.2)		
DPPH· radical scavenging activity	0.362* (0.01)	0.125 (0.246)	0.095 (0.379)	-0.114 (0.288)	
Antibacterial activity	0.116 (0.483)	0.077 (0.640)	0.236 (0.148)	0.225 (0.169)	0.113 (0.495)

Note: values in parentheses are p values. ** Significant correlation with probability threshold 0.01. *Significant correlation with probability threshold 0.05.

extracts possess higher activity than that obtained (11.39 mg/ml) with the methanol extract by sheets which *S. edule* still presented one of the most reducing powers against DPPH° [38].

In addition to phenols and other phytochemicals (alkaloids, steroids, and saponins) highlighted in this work, the antioxidant activities of *M. esculenta* could also be explained by its high anthocyanins and vitamin C [43]. Indeed, it has also highlighted antiradical activities (against DPPH° and

superoxide radical) of methanol extracts and acetone from the said plant and their metal chelator and antioxidant against deoxyribose degradation effect. All these activities only expressed in percentages were variable depending on the test sample and in particular with a reduction percentage of DPPH° of 15.2% with 0.1 mM methanolic extract [43].

The methanol extract of *S. nigrum* has already made radical scavenging activity against DPPH° up to 92% (a much lower activity with the aqueous extract) with a correlation with its phenolic content. Isolated from this plant glycoproteins could also justify highlighted in this study activity because they exert concentration-dependent antiradical activity against DPPH° and superoxide anion, as well as activity against the oxidation of 2-deoxyribose [108].

It has been shown that three flavones (luteolin, luteolin 7-O- β -glucuronide, and luteolin 7-O- β -glucoside) isolated from *V. amygdalina* have protective effects against the oxidation of β -carotene and oleic acid, lutein being more active than BHT [109]. It was also shown that three daily administrations of the aqueous extract of the plant at doses of 50 and 100 mg/kg can prevent the induction (acetaminophen) in oxidative stress and lipid peroxidation in rats [110].

Several parameters must be considered to maximize the biological effects of different vegetables, fruits, and spices of this study.

The oxidation is an essential biological process for the production of energy in many living organisms. However, certain pathological conditions such as microbial attacks cause an excessive production of reactive oxygen species (ROS) [111]. This excess of ROS in turn can cause many physiological disorders [112].

The extract of *P. nigrum* has both the highest antimicrobial activity and the highest antioxidant activity. Although the correlation between antioxidant and antibacterial activity is difficult to establish with other plant extracts of this study, the combination of these two activities observed in all these could be of great importance in the treatment of infectious diseases, as in addition to the elimination of the causative agent or the inhibition of growth, they may inhibit the oxidative processes that often aggravate the symptoms of these diseases.

Different plant extracts studied in this work showed antiradical activity against DPPH radicals and NO°. The antiradical force varies greatly between antioxidants and radical to another. According to the proposed scale [113], antioxidants activities are considered significant or high if the IC₅₀ < 50 mg/ml, moderate if 50 mg/ml < IC₅₀ < 100 mcg/ml, and low if IC₅₀ > 100 mcg/ml. According to this scale, extracts of *P. nigrum*, *S. edule*, *M. esculenta*, *L. capensis*, *V. amygdalina*, *V. hymenolepis*, *A. cruentus*, *T. occidentalis*, and *S. nigrum* can be classified as extracts with high activity against DPPH° free radical scavenging activity and extract of *T. triangulare* can be classified as extract with moderate scavenging activity against this radical. According to the same scale, although significant differences were not observed between the activity of many extracts (*M. esculenta*, *L. capensis*, *V. hymenolepis*, *C. melo*, *S. nigrum*, *A. hybridus*, *T. occidentalis*, and *V. amygdalina*) and that of rutin (reference), only extracts of *M. esculenta* presented high activity against the NO° free radical, while

moderate activities were observed with extracts of *S. edule* and *V. hymenolepis* using the same scale [113].

The radical scavenging activity of chemical compounds against DPPH radical is related to their ability to provide electrons and/or protons. Sodium nitroprusside, at physiological pH, decomposes in solution by producing NO° radical. In the presence of oxygen, radical leads to the formation of nitrite from nitrate. The chromogen formed during diazotization with nitrite ions with Griess reagent has a maximum absorbance at a wavelength of 546 nm [100]. By complexing with the NO° radical, compounds contained in extracts of plants thus inhibited the formation of the chromogen.

The elevated reducing powers of plant extracts of this study could be explained by high levels of total phenolics and flavonoids [114]. Reducing power is very often linked to the effect of the compounds that are electron donors [115]. The extract of *P. nigrum* would be the richest in reductones extracted followed by extracts *T. triangulare*, *A. hybridus*, *M. esculenta*, and *V. amygdalina*.

As demonstrated with the strong correlation between the phenol content and total reducing power, the action of phenols partly explains the reducing power and antiradical activity against DPPH plant extracts. The presence of phenols in all extracts is not a surprising fact, since they are present in all vascular plants [116]. Phenolic compounds from plants are powerful antioxidants capable of preventing oxidative damage to biomolecules such as DNA, lipids, and proteins that play a role in chronic diseases such as cancer and cardiovascular diseases [117, 118]. It has also been shown that there is a linear relationship between the antioxidant power plant and the concentration of phenolic compounds [56] acting through their hydroxyl groups [119].

The subset of the most abundant polyphenols in our diet, flavonoids are known primarily for their antioxidant activity [119, 120]. They are a group of phenols particularly clever and quick to transfer an electron. This electron stabilizing free radicals explains the scavenging activity of various flavonoids. However, no significant correlation between total phenolic content and flavonoid content could explain the lack of a significant correlation between reducing power and total flavonoid content, which also presented a negative correlation with NO.

4. Conclusion

This study was aimed at evaluating the antioxidant activity of thirteen Cameroonian plants which previously displayed antibacterial activities. The thirteen plant extracts exhibited high antioxidant activities correlated to their reducing power. They possess total reducing powers (from 2.41 to 27.81 μ g ascorbic acid equivalents/g of dried extract), total phenol contents (2.65 and 35.03 mg of gallic acid equivalents/g of dried extract), total flavonoid contents (0.15 and 5.99 μ g of rutin equivalents/g of dried extract), and free radical scavenging power against DPPH (IC₅₀ varying between 1.65 and 375.43 μ g/ml) and NO (IC₅₀ varying from 19.77 to 157.72 μ g/ml). Statistically insignificant positive correlations ($p > 0.5$) exist between the antibacterial

activities of these plants and the antioxidant activities with the strongest correlations observed between antibacterial activities and total phenols content, followed by free radical scavenging activity against nitric oxide. These important antioxidant activities demonstrated in the present study provide enough evidence to support the fact that the thirteen Cameroonian antibacterial plants can be used as a source of natural antioxidants and help combat oxidative stress which is sometimes associated with infectious conditions.

Data Availability

The experimental data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

DED VK, JAKN, JRK, and MM designed the experiments, carried out the study, and wrote the manuscript; all authors read and approved the final manuscript.

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Supplementary Materials

Table S1: bacterial strains and features. Table S2: antibacterial activities/minimal inhibitory concentrations ($\mu\text{g/ml}$) of methanol extracts from the thirteen studied plants and chloramphenicol. (*Supplementary Materials*)

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