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Phytochemical Profile, Antioxidant Potential, Proximate and Trace Elements Composition of Leaves, Stems and Ashes from 12 *Combretum* spp. Used as Food Additives

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Abstract: In this study, twelve *Combretum* spp. were investigated for their phytochemical content, antioxidant potential, and their proximate and trace elements/minerals composition. The qualitative phytochemical composition of the leaves, stems, and ashes of *Combretum* plants analysed revealed the presence of saponins, tannins, terpenoids, steroids, cardiac glycosides, and flavonoids. The following phytoconstituents were lost in the ashes; tannins apart from *C. mkuzense* and *C. padoides;* cardiac glycosides; and flavonoids. The quantitative phytochemical analyses revealed that both the leaves, stems, and some ashes such as *C. apiculatum* and *C. vendae* contained levels of phenolic compounds, tannins, and flavonoids. DPPH screening method indicated great scavenging activity with the 70% acetone leaf extracts of *C. kraussii*, *C. zeyheriim,* and *C. mkuzense*. There was a significant decrease in the antioxidant activity in the ashes compared to the leaves and the stems. AOAC and ICPE protocols performed the proximate and nutritional analysis of the 70% acetone extracts. The extracts had substantial amounts of ash, moisture, protein, and energy. The leaves and ashes of *C. adenogonium* and *C. apiculatum* could provide a good source of calcium in the diet. This study presents valuable information on the phytochemical composition, nutritional composition, and antioxidant properties of some *Combretum* species.

Keywords: *Combretum*; proximate; antioxidants and phytochemicals

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1. Introduction

Plants are a good source of food, chemicals, and herbal medicines. Plant and fruits are the most important, safest, and most compatible sources of natural bioactives. Natural antioxidants are effectively used to prevent the destructive processes caused by oxidative stress [1]. Substantial evidence has played key roles in reactive oxygen species (ROS), free radicals, and other oxidants in causing numerous disorders and diseases such as cancer, cardiovascular diseases, Alzheimer's, as well as neurodegenerative disorder [2]. Scientists are now focusing on natural antioxidants for the prevention and treatment of diseases and maintenance of human health [3]. Antioxidants often protect and neutralize your cells against free radicals [4]. Recently, interest in naturally occurring antioxidants has considerably increased for use in food, cosmetic and pharmaceutical products, because they possess the ability to neutralize free radicals, they can lower odds of some diseases [4,5]. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been in use for several years as food additives, but now their use is restricted in dietary items due to their implications in chronic diseases such as cancers and cardiovascular disorders [6,7]. So, there is a need to explore natural antioxidants, which have few or no side effects and can be used in medicines in place of synthetic antioxidants

[8]. Many plant materials have been identified and documented as promising sources of natural antioxidants [9,10]. Various studies have highlighted the evidence regarding variation in proximate composition, phenolics, and antioxidant activity with respect to different species of a plant [11,12]. The present study sought to investigate the differences in leaves from twelve *Combretum* plants.

Combretum is the biggest genus of the Combretaceae family with about 370 species [13]. *Combretum* plants are mainly used in traditional medicine against various diseases such mental problems, heart, and worm remedies to fever and microbial infections [14]. All parts of the *Combretum* species, in some cases even the fruits, are used for medicinal purposes [15]; some of the *Combretum* plants have been used in many African countries as food additives. Many species from the Combretaceae family have been reported to contain antioxidant activities. Research studies have shown that many of these antioxidant compounds possess anti-inflammatory, anti-atherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial [16,17]. Studies by Masoko and Eloff [18] and Masoko et al. [14] have also reported antioxidant potential of 24 African combretum species. Combretum plants also possess nutritional components for energy metabolism and vital nutrients to maintain a state of optimal nutrition. The leaves of many Combretum plants are harvested from wild-growing populations and used as popular traditional herbal tea in several tropical West African countries [14,15]. It is very important to investigate plants' nutritional properties, especially those that are easily accessed in all seasons.

2. Materials and Methods

2.1. Collection of Leaves

Fresh leaves of *Combretum caffrum*, *C. vendae*, *C. erythrophyllum*, *C. elaegnoides*, *C. apiculatum*, *C. imberbe*, *C. adenogdium*, *C. padoides*, *C. bractesum*, *C. kraussi*, *C. mkuzense,* and *C. zeyheri* were collected at Nelspruit, National Botanical Gardens, Mpumalanga, South Africa. Voucher specimens and tree labels were used to verify the identity of the plants. The voucher specimens were deposited at the Larry Leach Herbarium (UNIN) for confirmation. The leaves were air-dried for 30 days. When dry, the plant material was ground to a fine powder and stored in paper bags at room temperature.

2.2. Screening of Phytoconstituents

Screening of phytoconstituents included the following: terpenoids flavonoids, cardiac glycosides phlobatannin, steroids, saponins, alkaloids, and tannins [19,20].

Saponins

The persistent frothing test was used to test for saponins by weighing 1 g of plant-powdered leaves and stems and mixed with 30 mL of tap water. The mixture was vigorously shaken and heated at 100 °C. The sample was observed for the formation of persistent froth [21.

Tannins

The presence of tannins was tested by boiling 0.5 g of powdered leaves and stems in 5 mL of purified water in a test tube, then cooled and filtered. A few or three drops of 0.1% ferric chloride was added to 1 mL of the solution in a test tube and observed for brownish green or a blue-black colouration [20].

Phlobotannins

Phlobotannins were tested by weighing 0.2 g of powdered leaves and stems of R. communis into 10 mL of purified water and filtered. The filtrate was boiled with 2% hydrochloric acid solution. The sample was observed for the formation of red colour of precipitate [21].

Terpenes/terpenoids

The Salkowski's test was used, and 5 mg of the leaves and powders were mixed in 2 mL of chloroform and 3 mL concentrated sulphuric acid (H_2SO_4) was carefully added to form a layer. The appearance of a reddish-brown colour indicates the presence of terpenes [21].

Steroids

About 2 mL of acetic anhydride was added to 0.5 g of the powdered leaves and stems, followed by an addition of 2 mL of H2SO4. Blue colour was observed to draw an inference, indicating the presence of steroids [21].

Cardiac glycosides

The Keller–Killani's test was used. About 5 mL of the leaves and stem powders of the plant parts studied were treated with 2 mL of glacial acetic acid, containing one drop of ferric chloride solution. This was followed by an addition of 1 mL concentrated H_2SO_4. The colour changes (brown interface, violet ring below, and greenish ring at the lowest) were observed to draw inference, indicating the presence of cardiac glycosides [22].

Flavonoids

About 5 mL of diluted ammonia solution was added to a portion of the filtrate of each plant extract, followed by the addition of concentrated H_2SO_4. Yellow colour change was observed to draw an inference, indicating the presence of flavonoids [23].

Alkaloids

Drangendorff's reagent was used to test for alkaloids by weighing 0.2 g of ground powdered leaves and stems with 95% ethanol using Soxhlet extractor. The extracting solvent was evaporated to dryness using a vacuum evaporator at 45 °C. The plant residues were dissolved in 5 mL of 1% hydrochloric acid and 5 drops of Drangendorff's reagent was added. Reddish-brown colour change was observed to draw an inference [23,24].

2.2.1. Quantification of Phytochemicals

Total phenolic content

The quantity of phenolics present in leaves was determined by using the Folin–Ciocaltleu reagent method [21] with minor modifications.

Total tannin and total flavonoid content

The Folin–Ciocalteu and aluminium chloride colorimetric assay method described by [22] was used to determine the tannin and flavonoid content in the leaves, stems, and ashes.

2.2.2. Quantitative Antioxidant Activity Assay

Free radical scavenging activity of the plants was quantified and compared using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma, Johannesburg, South Africa) method reported by [25] with modifications.

2.3. *Proximate Analysis*

Proximate analysis of the leaves was performed according to AOAC International [26].

2.3.1. Estimation of Moisture Content

Two grams of the dried sample was put the moisture analyser. The moisture analyser (Fisher Scientific, Johannesburg, South Africa) used the basic "loss-on-drying" technique to simultaneously weigh and heat the sample. Moisture was recorded as percentage moisture.

2.3.2. Estimation of Ash Content

Two grams of the dried sample was weighed into a dry porcelain dish and then heated in the maffle furnace at 600 °C for 6 h. It was cooled in desiccators and weighed. The percentage ash content was calculated by using the following formula:

$$\% \text{ Ash} = \frac{\textit{weight of ash} - \textit{weight of crusible}}{\textit{weight of the original sample x dry coefficient factor}} \times 100$$

2.3.3. Protein Determination

Crude protein was determined following the Dumas AOAC method 992.23 using LECO Corp, Truspec (Johannesburg, South Africa). Two grams of the sample was combusted at high temperature in an oxygen atmosphere. Via subsequent oxidation and reduction tubes, nitrogen is quantitatively converted to N_2. A thermal conductivity detector measured the nitrogen content of the plant samples and was subsequently multiplied by 6.25 to get the protein content. A factor of 6.25 was used because most protein contains approximately 16% nitrogen.

2.3.4. Determination of Energy

The gross energy was determined for individual samples using the Isoperibol bomb calorimeter AC500 (LECO, Johannesburg, South Africa). About 1g of the sample was weighed in a crucible and placed inside a stainless-steel container (the "decomposition vessel") filled with oxygen. Next, the sample was ignited through a cotton thread connected to an ignition wire inside the decomposition vessel and burned (combusted). The measurement result was presented in kilojoules.

2.4. Mineral or Trace Elements Analysis

Digestion of the Dried Leaves and Stems

Four hundred milligrams of the sample were weighed into the digestion vessels. Five millilitres of HNO_3 and 3 mL of H_2O_2 was added and the mixture was shaken. A waiting period of 10 min was observed before closing the vessel. The microwave heated following the program in the Table 1.

Table 1. Microwave digester conditions.

Step	Target Temp °C	Pressure Max [bar]	Ramp Time	Hold Time Min	% Power
1	150	30	10	5	50
2	150	35	5	15	80
3	50	35	1	10	0

Following digestion, the mineral analysis was performed by ICPE 9000 (Shimadzu) (Limpopo, South Africa).

2.5. Statistical Analysis

Statistical analysis of results was performed using Statistix 10 data analysis software; a completely randomised test and the Welch's Test was used to compare any significant differences between the means. Statistical analysis was performed to determine variation between the plants in terms of proximate, nutritional, phytoconstituents, and antioxidant properties. Values were considered significantly different when $p < 0.05$.

3. Results

3.1. Phytochemicals

Phytochemicals are compounds of plants known to exhibit diverse pharmacological and biochemical effects on living organisms [27]. The qualitative phytochemical composition of the leaves *Combretum* plants analysed in this study revealed the presence of saponins, tannins, terpenoids, steroids, cardiac glycosides, and flavonoids (Table 2).

Table 2. Qualitative test for phytochemicals in the leaves.

Plant	Saponins	Tannins	Phlabatannins	Terpenoids	Steroids	Cardiac Glyocosides	Flavonoids	Alkaloids
C. adenogonium	+	+	-	+	+	+	+	-
C. apiculatum	+	+	-	+	+	+	+	-
C. bracteosum	+	+	-	+	+	+	+	-
C. caffrum	+	+	-	+	+	+	+	-
C. elaegnoides	+	+	-	+	+	+	+	-
C. erythrophyllum	+	+	-	+	+	+	+	-
C. imberbe	+	+	-	+	+	+	+	-
C. krausii	-	+	-	+	+	+	+	-
C. mkuzense	+	+	-	+	+	+	+	-
C. padoides	+	+	-	+	+	+	+	-
C. vendae	+	+	-	+	+	+	+	-
C. zeyherii	+	+	-	+	+	+	+	-

Key: + present; - absent.

The ashes of the plants contain only saponins, terpenoids, and steroids. However, tannins were also detected in the ashes of *C. mkuzense* and *C. padoides*—cardiac glycosides and flavonoids (Table 3).

Table 3. Qualitative test for phytochemicals in the ashes of twelve *Combretum* species.

Plant	Saponins	Tannins	Phlabatannins	Terpenoids	Steroids	Cardiac Glycosides	Flavonoids	Alkaloids
C. adenogdnium	+	-	-	+	+	-	-	-
C. apiculatum	+	-	-	+	+	-	-	-
C. bracteosum	+	-	-	+	+	-	-	-
C. caffrum	+	-	-	+	+	-	-	-
C. elaeagnoides	+	-	-	+	+	-	-	-
C. erythrophyllum	+	-	-	+	+	-	-	-
C. imberbe	+	-	-	+	+	-	-	-
C. krausii	-	-	-	+	+	-	-	-
C. mkuzense	+	+	-	+	+	-	-	-
C. padoides	+	+	-	+	+	-	-	-
C. vendae	+	-	-	+	+	-	-	-
C. zeyherii	+	-	-	+	+	-	-	-

Key: + present; - absent.

The ashes of the plants contain only saponins, terpenoids, and steroids. However, tannins were also detected in the ashes of *C. mkuzense* and *C. padoides*—cardiac glycosides and flavonoids (Table 4).

Table 4. Qualitative test for phytochemicals in the ashes of twelve *Combretum* species.

Plant	Saponins	Tannins	Phlabatannins	Terpenoids	Steroids	Cardiac Glycosides	Flavonoids	Alkaloids
C. adenogdnium	+	-	-	+	+	-	-	-
C. apiculatum	+	-	-	+	+	-	-	-
C. bracteosum	+	-	-	+	+	-	-	-
C. caffrum	+	-	-	+	+	-	-	-
C. elaegnoides	+	-	-	+	+	-	-	-
C. erythrophyllum	+	-	-	+	+	-	-	-
C. imberbe	+	-	-	+	+	-	-	-
C. krausii	-	-	-	+	+	-	-	-
C. mkuzense	+	+	-	+	+	-	-	-
C. padoides	+	+	-	+	+	-	-	-
C. vendae	+	-	-	+	+	-	-	-
C. zeyherii	+	-	-	+	+	-	-	-

Key: + present; - absent.

Quantitative Phytochemical Composition of the Combretum Plants

The leaves showed the presence of high amounts of phenolic compounds when compared to the stems with *C. pardoides*, *C apiculatum*, and *C. bracteosum* having the highest concentration of phenols. The phenolic compounds of the leaves, stems, and ashes of the plants ranged between 172–851, 35–350, and 0–43 mg of GAE/g of sample, respectively. (Figure 1).

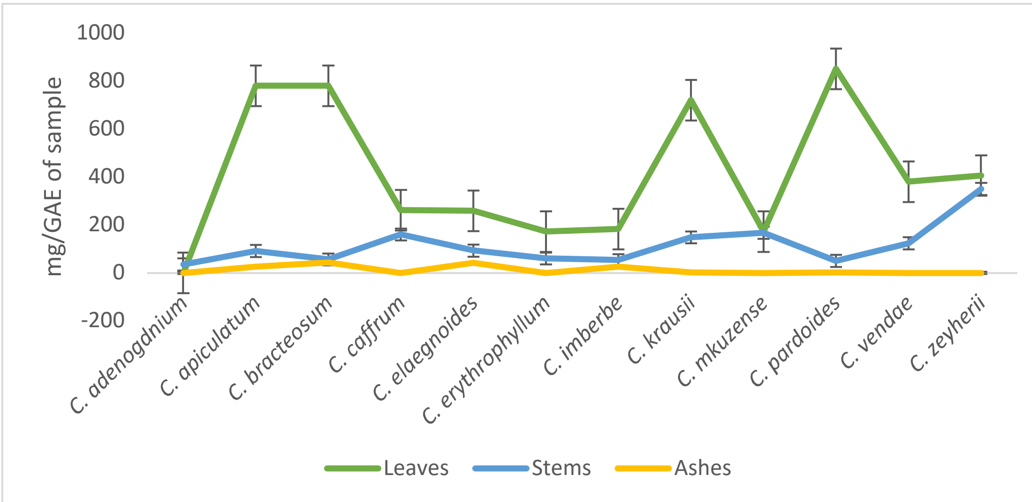

Figure 1. Evaluation of the phenolic compounds in the leaves, stems, and ashes of twelve *Combretum* species.

The concentration of tannins in leaves, stems, and ashes of the plants ranged from 172–851, 93.3–508.3, and 0–50 mg of GAE/g of sample, respectively. The ashes of *C. apiculatum* had the highest concentration of tannins when compared to the stems (Figure 2).

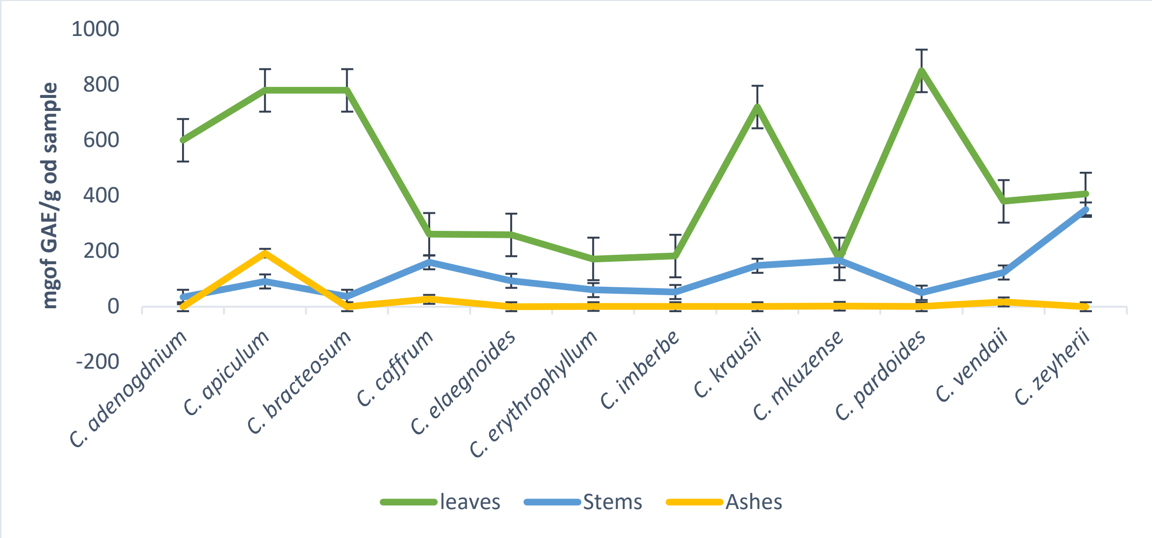

Figure 2. Total tannins content concentrations of the 70% aqueous acetone extracts of twelve *Combretum* species.

Most of the leaves had the highest flavonoids concentration when compared to the stems apart from *C. kraussii*, *C. apiculatum*, *C. pardoides*, and *C. adenogdnium*. Interestingly, ashes of *C. apiculatum*, *C. elaegnoides*, and *C. vendae* had the highest concentrations of the flavonoids when compared with other species of *Combretum* (Figure 3).

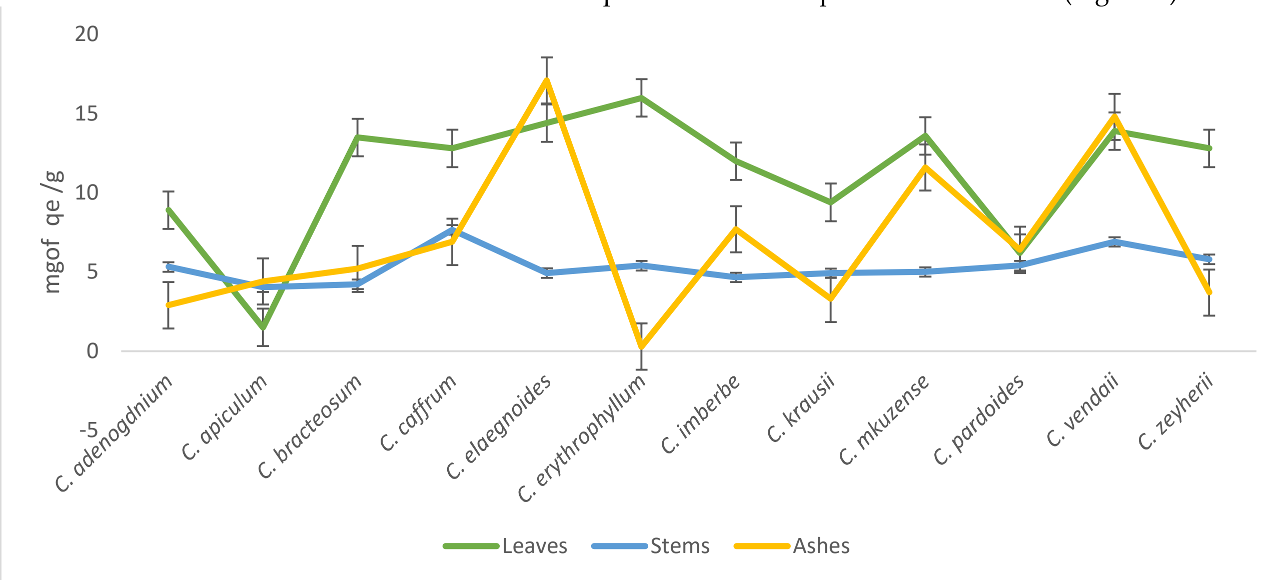

Figure 3. Total flavonoids content concentrations of the 70% aqueous acetone extracts of twelve *Combretum* species.

3.2. Antioxidants

Free radical scavenging properties of 70% of leaf extracts are presented in Figure 4. Most of the leaves tested exhibited a dose-dependent manner of antioxidant activity. The following leaf extracts showed highest scavenging activity: C *mkuzense, C. zeyherii, C. kraussii,* and *C. padoides.* Statistically, there was no significant difference (p = 001) in the scavenging activity of the following plants: *C. mkuzense* (0.0625; 0.125; 0.25; and 0.5 mg/mL); *C. zeyherii* (0.25 and 0.5 mg/mL); *C. kraussii* (25 and 0.5 mg/mL), *and C. padoides* (1 mg/mL). *C. elaegnoides, C. erythrophyllum,* and *C. bracteosum* had the lowest radical scavenging potential compared to other leaves in the study, as depicted in Figure 4.

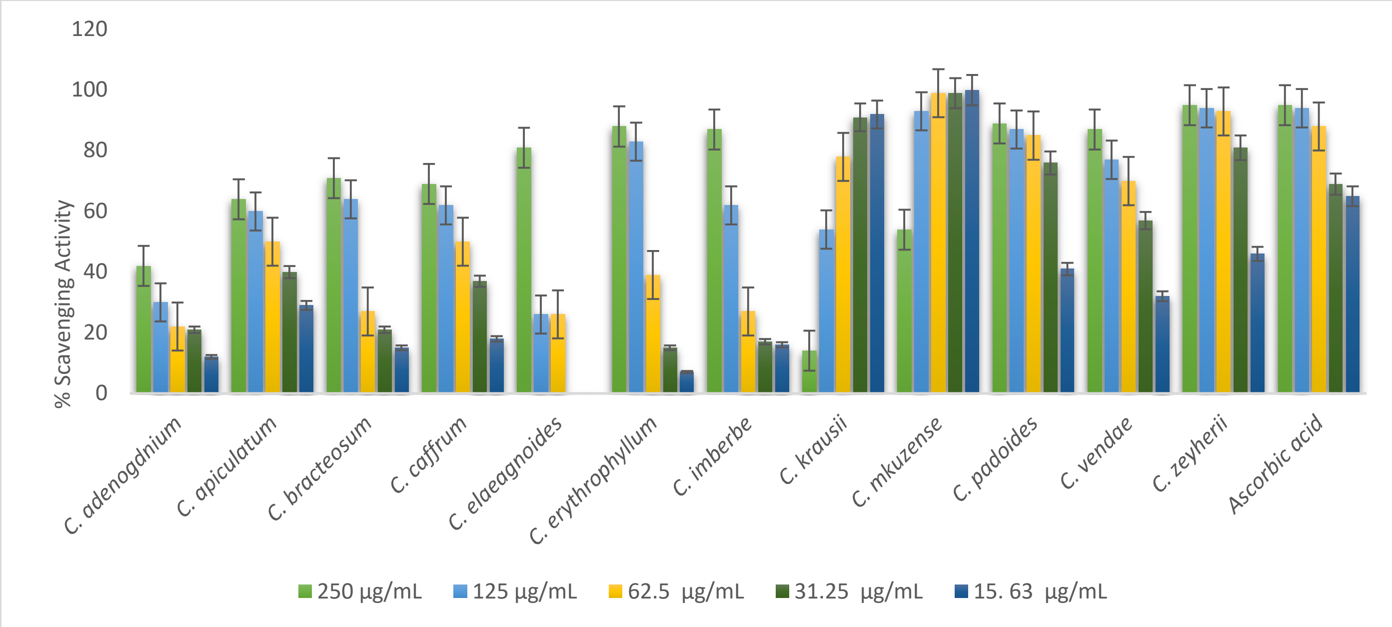

Figure 4. Evaluation of the antioxidant activity of the 70% acetone leaf extracts of the twelve *Combretum* species.

Figure 5 shows the concentration-dependent activity of the leaf extracts of the plants on DPPH radical scavenging by stems of *Combretum spp.* except for *C. kraussii*. As sample concentration increased, the percentage inhibition of DPPH radical also increased. In the plants that acted in a concentration-dependent manner, the following stems: *C. zeyherii, C. bracteosum, C. imberbe,* and *C. adenogonium*, showed the greatest percentage scavenging activity (Figure 4).

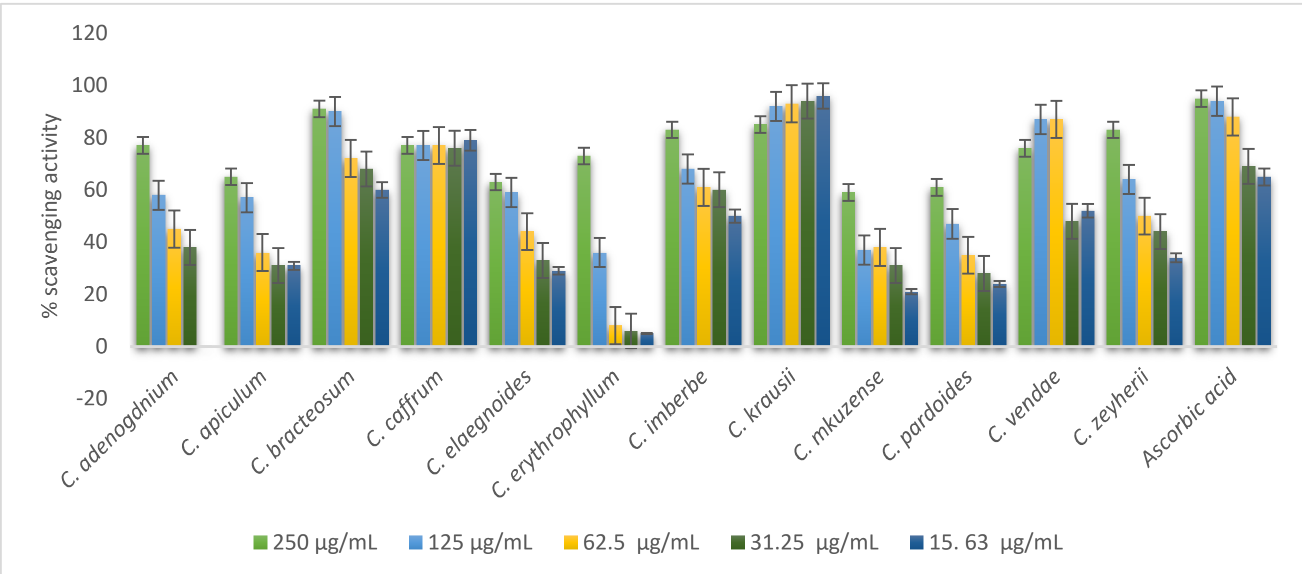

Figure 5. Evaluation of the antioxidant activity of the stems of the twelve *Combretum* species.

The majority of the *Combretum* ash extracts such as *C. elaegnoides* and *C. zeyherii* did not possess any antioxidant activity. Ashes from *C. apiculatum, C. caffrum, C padoides,* and *C. vendae* showed some antioxidant activity in a concentration-dependent manner while the activity of *C. imberbe* was observed to be opposite, as depicted in Figure 6.

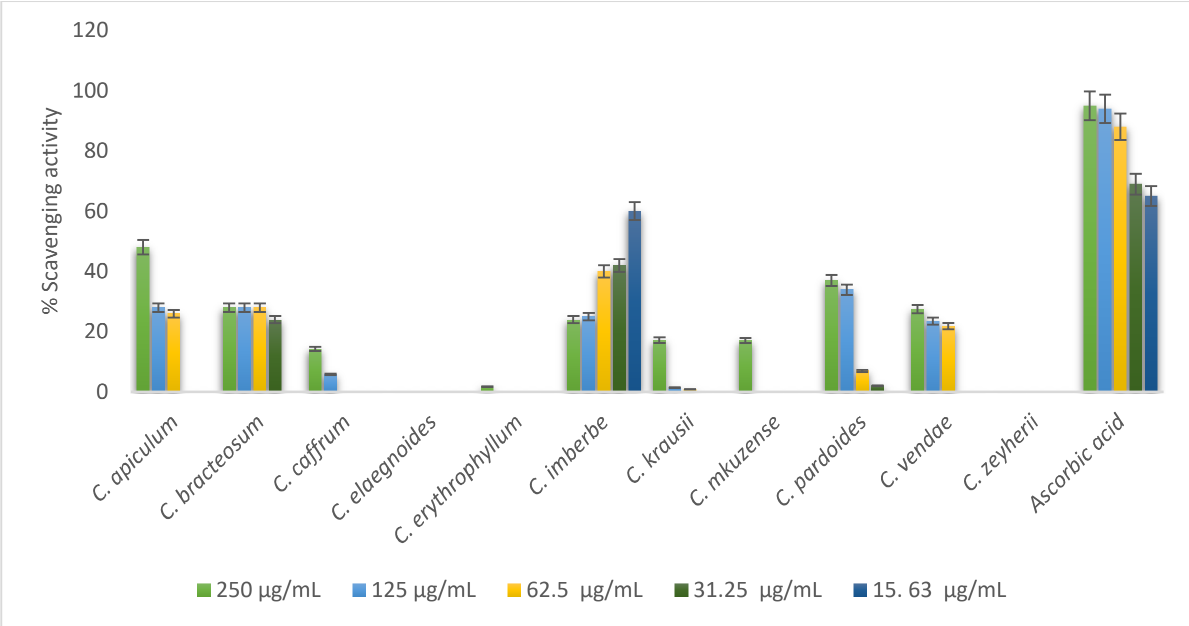

Figure 6. DPPH radical scavenging activity of 70% acetone ash extracts of twelve *Combretum* species.

3.3. Proximate Analysis

3.3.1. Ash

Ash percentage ranged from 1–15 in the leaves and 4–35 in the stems. The leaves of *C. vendae* and *C. adenogdnium* had the highest ash levels. Generally, the stems (83%) had the highest ash percentage levels when compared to the leaves (17%), as shown in Figure 7.

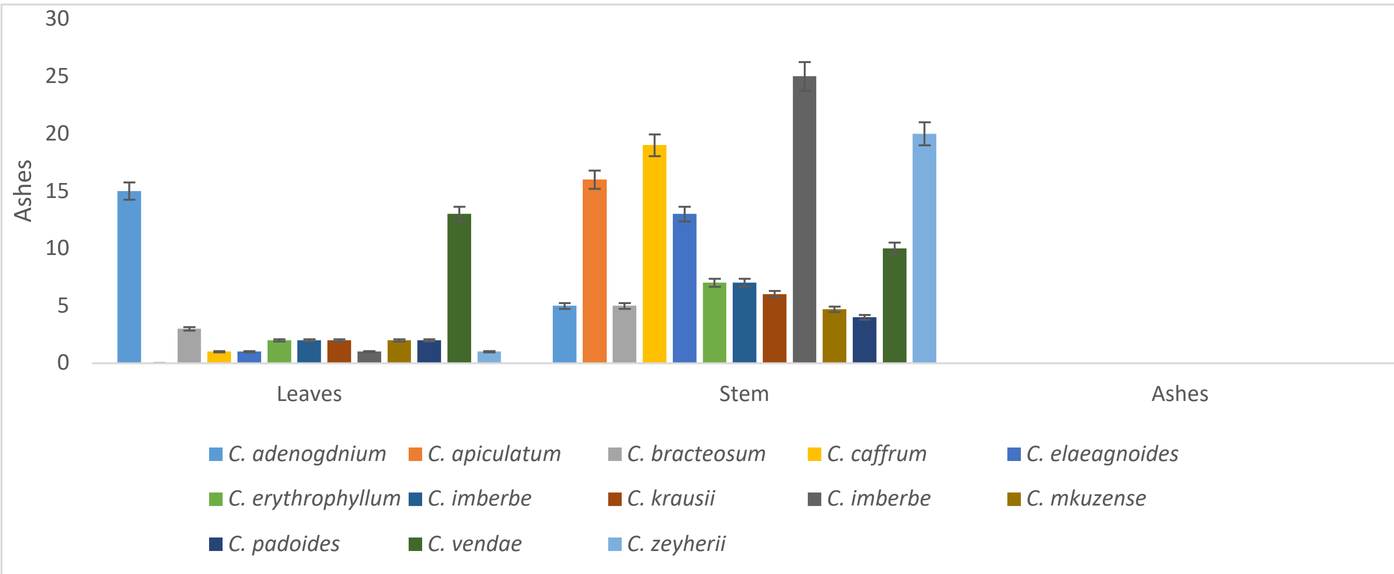

Figure 7. Ash percentage of the leaves and stems of the *Combretum* species.

3.3.2. Moisture

Moisture content of the plants ranged from 0–9% in both the ash, leaves, and stems. The leaves of *C. vendae* and *C. adenogonium* had no moisture; it was only detected in the stems. The leaves of *C. caffrum* had the highest moisture content, however; the stems had 0% of moisture content, as depicted in Figure 8.

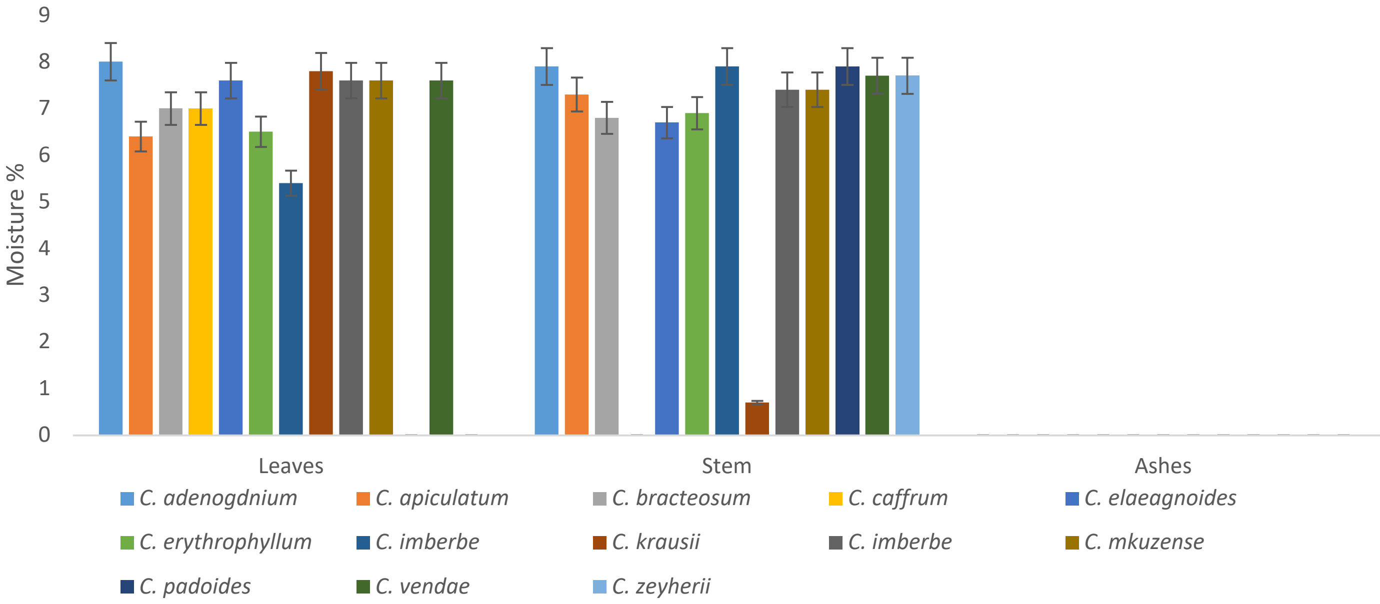

Figure 8. Moisture content (%) of the leaves and stems of the *Combretum* plants.

3.3.3. Protein percentage.

The protein content in the leaves ranged between, 2 and 14.4%, stems 4.04 and 7.66%, while the ashes contain protein in the range of 0.29–17.1%, as depicted in Figure 9. Generally, the leaves had higher protein content compared to the stems and ashes of other species. However, the protein contents of the *C. elaegnoides* and *C. padoides* were interestingly high.

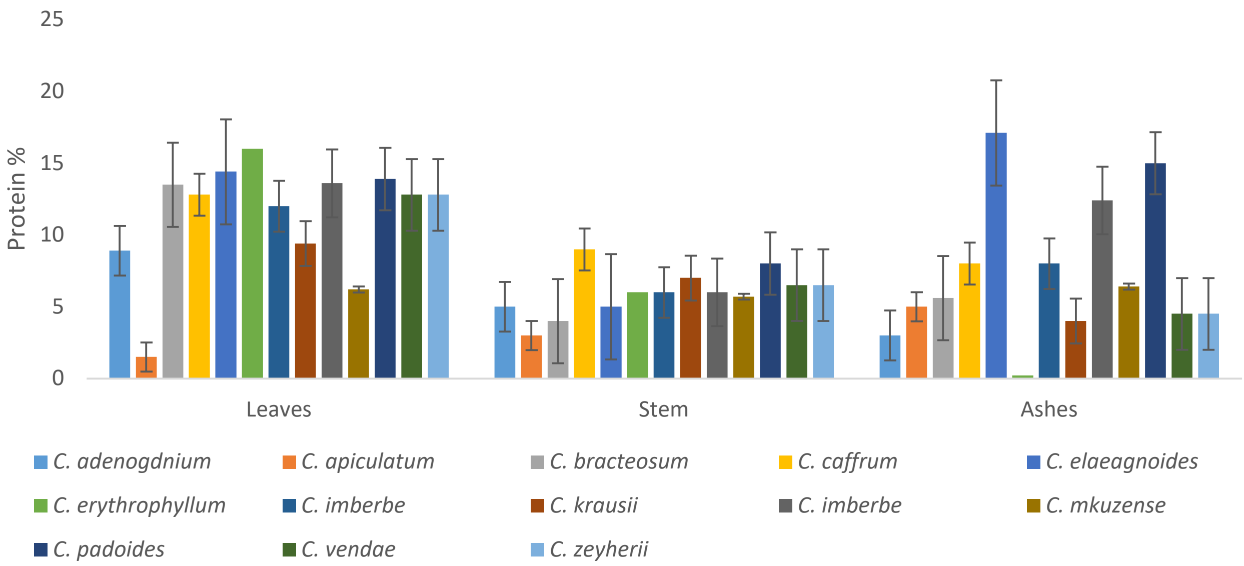

Figure 9. Protein percentage of the leaves, stems, and ashes of the *Combretum* species.

All the plants had appreciable amounts of energy, with *C. adenogdnium* exhibiting the highest concentrations for both leaves and stems, as illustrated in Figure 10. The ashes *C. erythrophyllum* had the lowest energy overall.

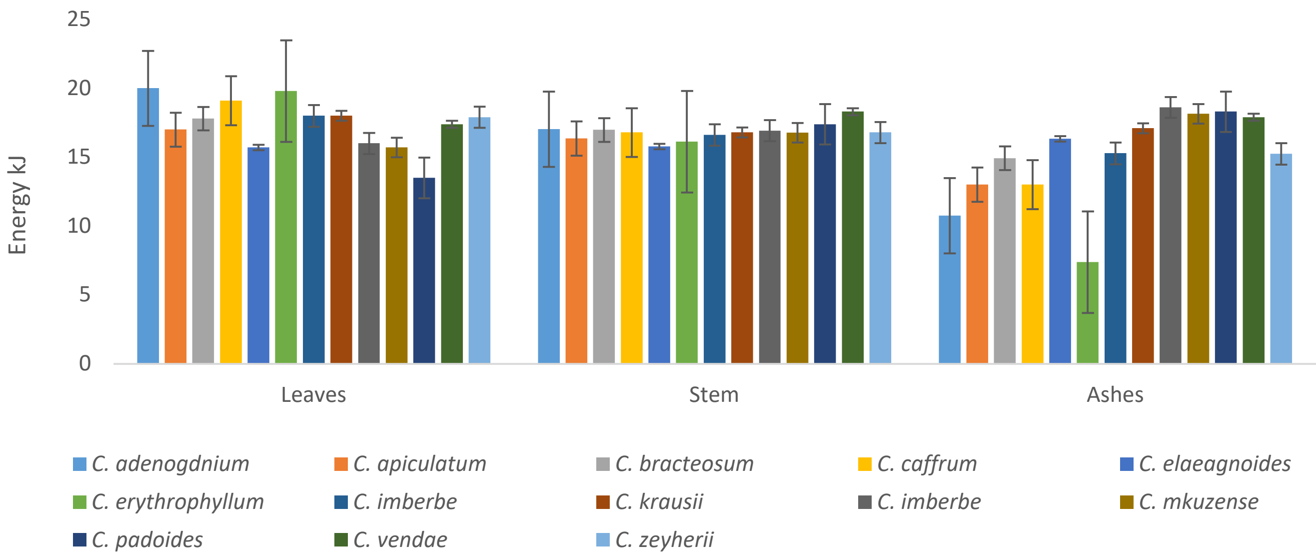

Figure 10. Energy content of the leaves, stems, and ashes of the *Combretum* plants tested in the study.

3.3.4. Mineral and Trace Metals Composition of the Leaves

The minerals detected in the leaves ranged from 4.86–283 (Ca); 0.114–166 (Co); 0.15–0.661 (Cu); 0.209–3.19 (Fe); 2.98–65.5 (K); 2.03–51.2 (Mg) 0.0319–3.98 (Mn); 1.8–14 (Na); 0.0139–3.5 (Ni); and 0.006–1.04 (Zn). *C. elaegnoides* had the lowest concentrations of minerals apart from Mg and Zn. *C. adenogonium* had the highest Ca, Cu, and Fe concentration while *C. apiculatum* had the highest concentration of Co, K, Mg, and Mn (Table 5).

Table 5. Concentration (mg/mL) of trace minerals in the leaves of some *Combretum* species.

Plant	Ca	Co	Cu	Fe	K	Mg	Mn	Na	Ni	Zn
C. adenogonium	241	1.03	0.661	3.14	59.9	23.1	1.67	0 [f]	3.5	0.941
C. apiculatum	198	1.66	0.508	2.07	65.5	51.2	3.98	12	1.45	0.801
C. bracteosum	283	1.54	0.584	3.19	63.4	28.7	1.43	14	1.61	1.03
C. caffrum	119	1.14	0.444	2.63	63.3	37.7	2.96	9.5	1.01	0.688
C. elaegnoides	4.86	0.114	0.15	0.209	2.98	2.03	0.0319	1.8	0.0139	0.177
C. erythrophyllum	131	1.02	0.432	1.71	35.1	26.3	1.32	9.1	0.983	1.04
C. imberbe	46.6	0.816	0.328	0.586	16.7	18.8	0.23	6.6	0.751	0.279
C. kraussii	117	1.11	0.423	1.49	39	27.4	1.69	9.7	1.07	0.599
C. mkuzense	176	1.36	0.509	2.74	63.2	24.3	2.26	11	1.25	0.792
C. padoides	132	1.26	0.467	1.98	37.1	34.7	2.57	10	1.17	0.601
C. vendae	3.9	0.701	0.367	2.51	51.7	22.3	1.84	6.7	0.693	0.351
C. zeyherii	36.1	0.456	0.258	0.955	23.2	8.53	0.929	4.3	0.36	0.006

C. krausii had the lowest concentration of the following minerals: Ca, Co, Cu, K, and Mn while *C. erythrophyllum* had the highest concentration of Cu and Zn. The mineral that was detected in high concentrations was Ca whereas Zn is lowest as shown in Table 6.

Table 6. Concentration (mg/mL) of trace minerals in the stem of some *Combretum* species.

Plant	Trace Elements/Minerals									
	Ca	Co	Cu	Fe	K	Mg	Mn	Na	Ni	Zn
C. apiculatum	253	1.15	0.784	1.2	30	25	2	10.5	3.7	0.968
C. bracteosum	80	0.526	0.45	1.52	28	11	0.798	12.5	2.88	0.472
C. caffrum	95	0.782	0.527	2	88	18	3.4	6.8	3.1	0.63

C. elaegnoides	176	0.81	0.524	1.41	26	14	0.995	5	3.34	0.782
C. erythrophyllum	284	0.978	0.679	2.1	31	19	1.19	9.1	3.43	1.09
C. imberbe	235	1.08	0.678	2.1	33	22	1.45	5.8	3.56	0.877
C. kraussii	2.67	0.13	0.0547	0.145	2.89	2.61	0.307	8.7	1.99	0.53
C. mkuzense	85.1	0.546	0.386	2.37	16.1	7.6	0.997	8	2.87	0.387
C. padoides	104	0.7	0.44	1.18	26.1	13.5	1.23	5.1	2.98	0.397
C. vendae	8.08	0.156	0.0707	0.117	11.6	2.19	0.428	3.5	1.89	0.567
C. zeyherii	94.5	0.623	0.523	1.92	31	16.1	2.17	7.8	2.9	0.342

The mineral compositions in percentage in the ashes ranged from 38.9–980 (Ca); 0–1.73 (Co), 0–1.07 (Cu) 0–25.1(Fe); 0–196 (K); 0–57.9 (Mg); 0–2.71 (Mn); 0–24.4 (Na); 0–2.22 (Ni); 0–18。7 (Zn). Overall *C. zeyherii* had the highest concentration of all the minerals, as highlighted in Table 7.

Table 7. Concentration (mg/mL) of trace minerals in the ashes of some *Combretum* species.

Plant	Trace Elements/Minerals									
	Ca	Co	Cu	Fe	K	Mg	Mn	Na	Ni	Zn
C. adenogonium	869	1.13	0.53	0.8	29	22	0.867	18.8	1.57	2.93
C. apiculatum	804	1.15	0.5	2.32	93	35.1	2.19	18.01	1.66	3.4
C. bracteosum	0	0	0	0	0	0	0	0	0	0
C. caffrum	38.9	0.312	0.208	0.211	36.2	2.58	0.172	3.75	0.386	0.36
C. elaegnoides	579	0.92	0.375	0.766	69.9	30.9	1.15	12.4	1.22	1.58
C. erythrophyllum	516	0.944	0.614	3.17	78.8	19.1	0.735	16.2	1.25	3.14
C. imberbe	0	0	0	0	0	0	0	0	0	0
C. kraussii	231	0.669	0.338	3.17	70.3	20	1.03	8.48	0.874	5.88
C. padoides	0	0	0	0	0	0	0	0	0	0
C. mkuzense	61	0.245	0.19	0.188	8.67	3.2	0.143	2.35	0.268	0.586
C. vendae	26.3	0.248	0.185	0.315	8.67	2.08	0.119	2.14	0.284	0.411
C. zeyherii	980	1.73	1.07	25.1	196	57.9	2.71	24.4	2.22	18.7

4. Discussion

All the plants investigated are traditionally used for medicinal purposes in South Africa and other African countries. Most of the plants are indigenous to South Africa.

Phytochemicals: Phytochemicals are substances produced by plants known to possess diverse pharmacological and biochemical effects on living organisms [28]. The qualitative phytochemical composition of both the leaves and stems of some *Combretum* species analysed in this study revealed the presence of saponins, tannins, terpenoids, steroids, cardiac glycosides, and flavonoids. These phytoconstituents were not detected in the ashes of the plants. However, tannins were present in *C. mkuzense*, while cardiac glycosides and flavonoids were contained in *C. padoides.* These phytoconstituents, which were not detected in the ashes, may have been destroyed by heat. The quantitative phytochemical analyses revealed that both the leaves, stems, and some ashes such as *C. apiculatum* and *C. vendae* contained appreciable levels of phenolic compounds, tannins, and flavonoids. As detected in this study, these phytoconstituents have been associated with antimicrobial activities and numerous physiological activities in mammalian cells in various studies [23–26]. Generally, the leaves showed to have higher concentrations of the phytoconstituents when compared to the stems. The phenolic compounds contained in the plants' leaves were found to vary in the following order: *C. padoides* > *C. apiculatum* ≥ *C. bracteosum* > *C. krausii* > *C. zeyheri* > *C. vendae* > *C. caffrum* > *C. adenogdnium* >*C. elaeagnoides* > *C. imberbe* > *C. mkuzense* > *C. erythrophyllum.* These indicate that the leaves of *C. padoides, C. bracteosum, and C. apiculatum* are a good source of phenolic compounds when compared to other plant leaves in the study. These results support results presented by Masoko and Eloff [18], who investigated the qualitative antioxidant activity and phytochemical properties of 30 members of the Combretaceae. The phenolic compounds of the stems were found to be in the following order *C. mkuzense* > *C. caffrum* > *C. krausii* > *C. vendae* > *C. apiculum* = *C. elaeagnoides* > *C. bracteosum* > *C. imberbe* > *C. padoides* > *C. zeyheri* > *C. adenogdnium* > *C. erythrophyllum.* Although there was a significant decrease (p = 0.01) in the concentration of phenolic compounds in the stems when compared to the leaves, they still possessed substantial amounts and could still be used during seasons when leaves are scarce. Phenolic compounds exhibit various physiological properties, such as anti-allergenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardio-protective, and vasodilatory effects [29–31]. Phenolic compounds have been associated with the health benefits of consuming high fruits and vegetables [32,33]. The antioxidant activities found in phenols causes them to have great health benefits [34]. In the ashes, notable amounts of phenolic compounds and tannins were only observed in *C. apiculatum, C. bracteosum,* and *C. caffrum.* Flavonoids play various biological activities in living organisms [35]. The study revealed that the all the *Combretum* plants tested possess substantial concentrations of flavonoids, with their leaves having higher concentrations than the stem. It was interesting to observe that the ashes of *C. apiculatum, C. mkuzense,* and *C. vendae* were significantly higher than those of the leaves and stems (p = 001). Currently, few or no studies report the presence of phytoconstituents in the ashes of these plants. The flavonoids in the leaves were found to be in the following order: *C. elaeagnoides* > *C. vendae* > *C. padoides* > *C. imberbe* > *C. apiculatum* > *C. bracteosum* > *C. erythrophyllum* > *C. caffrum* > *C. zeyheri* > *C. krausii* > *C. mkuzense.* Several studies [36–38] have reported the presence of flavonoids in *C. apiculatum, C. caffrum, C. kraussii,* and *C. erythrophyllum,* supporting the findings of this study. Several reports have revealed that other *Combretum* plants that were not included such as *C. hereroense* [39], *C. nigricans, C. leprosum* [40], and *C. micranthum* [41–43] contain flavonoids. These reports, together with these study findings, indicate that *Combretum* plants are a good source of flavonoids. Flavonoids have a capacity to act as antioxidants because of polyphenolic compounds. The functional groups mediate antioxidants by scavenging free radicals or by chelating metals ions [44–46]. As a dietary component, flavonoids are thought to have health-promoting properties due to their high antioxidant capacity in both in vivo and in vitro systems [47,48]. Saponins are used to treat the following medical conditions: hypercholesterolemia, hyperglycaemia, antioxidant, anticancer, anti-inflammatory, and weight loss. Reports have shown that they also possess antifungal properties [49]. Saponins have great cytotoxic effect and growth inhibition against different cell lines, making them potential anticancer agents [50]. Terpenes are a crucial group of organic compounds that have been reported as potent drugs used to treat a wide range of ailments. Terpenes are the most rapidly acting anti-malarial Artemisinin and its derivate [51]. Phenols, also found in plant sources, are a major group of compounds acting as primary antioxidants or free radical scavengers [52].

Antioxidants: The antioxidant activities of many plants are of great interest in the food, cosmetics, and pharmaceutical industries since their possible use as natural additives emerged from a growing tendency to replace synthetic preservatives with natural ones. DPPH assay is widely used to evaluate antioxidant activity of biological samples. DPPH is a stable free radical with characteristic absorption at 520 nm, and antioxidants react with DPPH radical and convert it to diamagnetic 2,2-diphenyl-1-picrylhydrazine molecule. The discolouration degree indicates the antioxidant extract's scavenging potential, which is due to the hydrogen-donating ability [53,54]. In this study, as the sample concentration increased, the percentage inhibition of DPPH radical also increased. However, in the case of *C. kraussii* and *C. mkuzense*, the opposite was observed, i.e., at the lowest concentration, the scavenging activity was the highest. This means that, out of all the leaves tested, *C. kraussii* and *C. mkuzense* are good sources of antioxidants. Concentration-dependently, C. *zeyherii* leaves showed the overall highest scavenging activity when compared to the other leaves. It was observed to have scavenging activity, which was tested at concentrations of 250 μg/mL and 125 μg/mL. The methanol and acetone extracts of the leaves of *C. zeyherii* were found to possess antioxidant activity [55]. The stems of the plants had a relatively good antioxidant activity, which was found to be concentration-dependent, except for *C. kraussii* (Figure 6). There was a significant decrease in the antioxidant activity in the ashes ($p = 0.01$), compared to both the leaves and the stems. This may be due to the loss of some phytoconstituents, (tannins, cardiac glycosides, and flavonoids). Flavonoids and tannins have been shown to act as a secondary antioxidant defence system in plant tissues exposed to different abiotic and biotic stresses [56]. Ashes from *C. imberbe, C. apiculatum,* and *C. padoides* still possessed antioxidant activity after the burning process. *Combretum* plants are a good source of phytochemicals that possess an antioxidant capacity.

Proximate analysis: The study of the proximate content—for instance, the moisture content presented in the different parts of the plants—could reflect the plant's ability to resist harsh environmental conditions such as drought. Reports have shown that most Combretum spp, such as *C. erythrophyllum* and *C. zeyherii*, are drought-resistant [57]. Moisture content is measuring the amount of water contained in a material. Water is an essential compound of many food products. About 20% of the total water is consumed through food [58].

The percentage ash content of the plants could be important reflection of the nutritional mineral contents [59]. Ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter in a foodstuff. Ash contains inorganic material of the plant, which includes oxides and salts containing anions [60]. The ash content is a measure of the total amount of minerals present within a food product. Generally, the leaves had the lowest ash percentage when compared to the stems, apart from *C. adenogdium* and *C. vendae. C. kraussii* had the highest ash percentage, followed by *C. zeyherii*. This means that these plants should possess the highest concentration of mineral content. High ash content indicates the presence of heavy amounts of inorganic nutrients in plant material [61]. Proteins are chains of amino acids which are involved in nearly every process in the body. These macromolecules are involved in metabolic reactions such DNA replication, transcription, and act as enzymes as well as signalling proteins [62,63]. The protein contents in the plants were observed in the following order: leaves > stem > ashes for the following plants (*C. adenogdium, C. caffrum, C. erythrophyllu*m, *krausii,* and *C. zeyherii*); ashes > leaves > stem (*C. elaegnoides, C. apiculatum,* and *C. vendae*); leaves > ashes > stem (*C. bracteosum, C. mkuzense,* and *C. padoides*). Overall, the leaves had the highest protein content apart from *C. elaegnoides* at 17.1%. Foods that provide more than 12% of their calorific value from proteins are considered a good source of proteins [64]. Since many of the plants tested in the study had appreciable levels of protein content, they can be considered for use in the food industry. These plants showed appreciable levels of energy content within them. Plant-based proteins are regarded as functional ingredients in food formulations, including thickening and gelling agents, stabilisers of emulsions and foams, and binding agents for fat and water. Moreover, some proteins have biological activities such as antioxidant or antimicrobial properties [65].

Minerals: Trace elements are dietary minerals essential for proper growth, development, and maintaining and recovering the organism's health [66]. Some trace elements control important biological processes through such actions as catalysts in enzymes systems and oxidation-reduction in metabolism. In this study, the leaves contain the highest calcium concentration, while zinc has the lowest concentration. *C. bracteosum* had the lowest levels of minerals such as calcium, copper, nickel, and zinc when compared to leaves of other Combretum species used in this study. *C. adenogonium* had the highest levels of copper and iron, while *C. caffrum* had the highest levels of magnesium and manganese. *C. elaegnoides* had the lowest concentrations of the following minerals: Ca, Co, Cu, Fe, K, Mg, Mn, and Ni.

The variation of elemental content from plant to plant was mainly attributed to the differences between the botanical structure and the mineral composition of the soil in which plants are cultivated. Other factors responsible for the variation include absorbability of the plants, the use of fertilizers, irrigation water, and climatic conditions [64]. Compared to other foods, they are good sources of minerals. The analysed leaves were found to have higher concentrations of minerals when compared to consumable vegetables such as *Allium sativum* and *Allium tuberosum* [67]. In addition, they were found to have higher concentrations than some wild plants that are used as spices such as *F. xanthoxyloïdes, H. gabonii (bark and fruit), M. myristica, M. whitei, P. brazzeana, P. guineense, P. umbellatum, S. melongena, S. striatinux, S. zenkeri (fruit), S zenkeri (bark), T. tetraptera,* and *X. aethiopica* [65]. Regarding stems, the mineral content was found to have decreased significantly compared to the leaves. Apart from calcium, there was a further decrease in mineral content regarding the ashes. It was interesting to observe that the calcium concentration in all the ashes increased significantly, approximately four times more than the leaves and stems. Based on these findings, it can be concluded that the leaves and ashes of *C. adenogonium* and *C. apiculatum* could provide a good source of calcium. Calcium is also important for blood coagulation and the normal functioning of the cardiac muscles [68]. It is also noteworthy to highlight that *C. zeyherii* has high mineral content. These minerals are necessary for the maintenance of good health in both animals and humans. Furthermore, the mineral elements affect biochemical processes and play crucial roles in living organisms, specifically the biological, metabolic, and enzymatic reactions leading to the development of active organic components [69]. Sodium and potassium maintain the human body's ionic balance and tissue excitability. Sodium plays an important role in the transport of metabolites [70]. The ratio of potassium/sodium in any food is an important factor associated with hypertension and arteriosclerosis. Sodium enhances and potassium depresses blood pressure [71]. In this study, the leaves and ashes of *C. adenogonium, C. bracteosum,* and *C. apiculatum* proved to be a good source of sodium. Iron is essential in oxygen binding to haemoglobin and acts as a catalyst for many enzymes such as cytochrome oxidase [72]. In the current study, the leaves of *C. adenogonium, C. bracteosum,* and ashes of *C. zeyherii* possessed high levels of concentrations of iron. Thus, these plants can be recommended against anaemia. Magnesium helps to support muscle and nerve function and energy production and prevent impaired spermatogenesis and bleeding disorders [73]. The leaves of *C. bracteosum,* stems of *C. apiculatum,* and ashes of *C. caffrum* and *C. bracteosum* possessed appreciable amounts compared to other plants in the study. The recommended dietary allowance (RDA) for calcium (1000 mg/day), magnesium (400 mg/day), and iron (8 mg/day) suggest that these plants contribute substantially to improving the diet in terms of mineral requirement. The manganese concentration ranged between 0.00 and 3.9 mg/mg, which is higher than the values obtained from wild edible plants such as *Aegle marmelos (L.) Corrêa, Argyreia speciosa (L. f.) Sweet, Butea monosperma (Lam* Taub) reported by [74] Zn concentration ranged between 0.006 and 1, 04mg/mL, which is like the levels reported in some wild and leafy vegetables in India [74–76]. According to Shirwaikar et al. [38], minerals such as copper, manganese, and zinc are well-known antioxidants. The presence of these minerals might be attributed to the increase in the antioxidant activity of certain plants.

5. Conclusions

The pharmacological effect of the phytochemical constituents such as alkaloids, glycoside, tannins, and flavonoids as well as the antioxidant activity of the plants and ashes in the study explains the rationale for using these plants in traditional medicine and their use as food additives. The outcome of this study suggests that the selected plants and ashes could probably be a cheaper alternative to conventional drugs and preservatives since the plants are easily obtainable and can be cultivated on a sustainable basis. The plants and ashes contained appreciable amounts of nutrients such as protein, energy, and mineral elements that could enhance the nutrition of humans and livestock. Furthermore, the results suggest that these plants and their ashes could serve as a feed supplement to improve health and growth performance in humans and livestock. It can be ascertained that the *Combretum* extracts, together with the ashes, can be exploited as a source of natural nutrients and minerals, especially *C. adenogonium, C. bracteosum,* and *C. apiculatum.* Therefore, *Combretum* species could be potential nutraceuticals as nutritional supplements. This indicates that the *Combretum* plants will offer their users both nutritional and medicinal benefits.

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