INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
20–0814/2020/24–6–1781–1787
DOI: 10.17957/IJAB/15.1622
http://www.fspublishers.org

Full Length Article

In vitro Evaluation of the Phytochemical and Antioxidant Properties of *Bulbine abyssinica* Extracts

Emrobowansan M. Idamokoro[1,2,3*] and Anthony J. Afolayan[2]
[1]*Agricultural and Rural Development Research Institute, University of Fort Hare Private Bag X1314, Alice, 5700, South Africa*
[2]*MPED Research Center, Department of Botany, University of Fort Hare, Alice, 5700, South Africa*
[3]*Department of Livestock and Pasture Science, Faculty of Science and Agriculture, University of Fort Hare, Private Bag X1314, Alice 5700, South Africa*
[*]For correspondence: mondayidamokoro@gmail.com; eidamokoro@ufh.ac.za
Received 25 May 2020; Accepted 08 June 2020; Published 10 October 2020

Abstract

This study looked to determine the antioxidant properties and phyto-chemical constituents of *Bulbine abyssinica* leaf using different solvents (aqueous, methanol, ethanol and acetone) against scavenging radicals such as 1,1- diphenyl-2-picrylhydrazyl (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Nitric oxide (NO) and total antioxidant capacity (TAC). The polyphenolic constituent's analysis of the leaf extracts (which include flavonoid, flavonols and phenolic content) were likewise evaluated with the utilization of standard phytochemical reaction procedures. The outcome of the phytochemical analyses showed that the leaf extracts had abundance of phenolic compounds, flavonoids and condensed tannins. From the result, the value of phenolic constituents found in acetone and aqueous samples gave 11.09 and 7.40 mg tannic acid equivalent/g of extract. Likewise, the values for flavonoid composition of acetone, ethanol and methanol, and aqueous leaf extracts gave 376.05, 320.49 153.52, and 85.42 mg quercetin equivalent per gram of extract, accordingly; moreover, the total phenol leaf extracts gave 24.35, 29.38, 16.31, and 16.00 mg of tannic acid per gram of dry extract sample, respectively. The aqueous, acetone, ethanol and methanol leaf extracts of *B. abyssnica* gave significant activity (at 0.08 and 0.4 mg/mL) as an antioxidant for ABTS and total anti-oxidant capacity (TAC) radicals when compared with BHT used as a standard drug. The results from this study indicated the antioxidative capabilities of *B. abyssnica* extracts and the plant may serve as inhibitors (of free radical), which might perform as key antioxidants.

Keywords: Medicinal herb; Phyto-chemicals; Anti-oxidant constituents

Introduction

Damage of biological molecules can be significantly reduced by antioxidants by decreasing oxidative stress (Iqbal *et al*. 2015). Stress generated from oxidation process is widely recognized in the pathogenesis of life style associated diseases such as livestock being confined or in free range pastures. Stress evolving from oxidative process is destructive due to the fact that biological molecules are usually attacked by oxygen free radicals. According to Yoshikawa and Naito (2002), oxidative stress is a condition in which forces of oxidation surpass a particular antioxidant scheme of an organism because of imbalance in the organism. Interestingly, stress resulting from oxidation could encourage harmful actions that could include oxidative DNA damage and lipid peroxidation.

Talking about oxidants, they are compounds formed from oxygen metabolism during oxidative stress. These highly reactive and free molecules produced during oxygen metabolisms such as organic peroxide, hydroxyl radical and superoxide radicals can cause severe destruction to cells and tissues. According to Borneo *et al*. (2009) and Lee *et al*. (2003), synthetic antioxidants (*e.g*., propyl gallate) have been reported to cause external and internal bleeding in animals at high concentration. This has led to the use of antioxidants derived from plant's bioactive phytochemicals such as flavonoids which are proven to be efficient in scavenge of free radicals (Iqbal *et al*. 2015). The bioactive constituents of medicinal plants can be extracted with different methods and then subjected to evaluation. There are reports of significant differences in physiological activities of plant extracts which depends upon the extraction methods and it emphasizes the importance of choosing a fitting extraction method for a specific purpose (Do *et al*. 2014).

Plants are products of primary metabolism which forms secondary metabolites that are primarily produced for protecting/defence against foreign bodies or predators. For instance, some metabolites like alkaloids and flavonoids

stand as the brain that is responsible for causing healing effects in plants (Bhandary *et al*. 2012). Likewise, the polyphenolic constituents present in the extracts of plants produce a countless number of biological activities from which they express their antioxidant ability (Hassan *et al*. 2009). Polyphenolics rarely yield small amount of/or no toxic effect when they are ingested in a cell (Havsteen 2002). According to Hossain *et al*. (2011), antioxidants form a significant part in scavenging oxidative free radicals. Flavonoids which form an example of phenols employ its antioxidant activities by way of mopping up free radicals.

The utilization of plants with medicinal potentials plays vital part in primary health care of livestock in South Africa. *Bulbine abyssinica* is found in several provinces of South Africa including the Eastern Cape and as far as Ethiopia (Pooley 1998). The plant grows in all weather conditions and dominantly found in rocky grassland and shallow soil overlying rock. *Bulbine abyssinica* is used to treat gastrointestinal parasites in livestock in communal areas of South Africa (Maphosa and Masika 2010). The most parts of the *B. abyssinica* plant that is being utilized by people in this region are the leaves. *Bulbine abyssinica* is also known to have several nutritional contents such as carbohydrate, protein, vitamins, minerals which are beneficial as feed for animals (Kibiti and Afolayan 2018). It is set up through decoctions of the leaves (Maphosa and Masika 2010). Likewise, other parts of *B. abyssinica* such as the stem and root are known to possess vital anthraquinones which include among others knipholone and chrysophanol which are known for their anti-bacterial potentials (Kibiti and Afolayan 2018). This study therefore aim to determine the *in vitro* phytochemical components and anti-oxidant properties of *Bulbine abyssinica* leaf extracts from different solvents; which serve as a plant commonly utilized by local farmers to treat their animals in the Transkei region of South Africa.

Materials and Methods

Preparation of Bulbine abyssinica leaf and extracts

Leaves of *Bulbine abyssinica* were collected in Alice area, Raymond Mhlaba district area. The region of study falls within the latitudes 32° 46' 59.99" S, longitude 26° 49' 59.99" E. The *B. abyssinica* plant was authenticated, and the voucher specimen (KibMed 2014/01) deposited at the Fort Hare University Griffen herbarium. The *B. abyssinica* leaves were cut and kept in a dried condition to a constant weight at room temperature and grounded into a fine powder to pass through a 2 mm sieve. The leaf meal was packaged in airtight polythene plastic bags and kept at room temperature until required for the determination of phytochemical screening, antioxidant activity and anti-nutrient analysis.

Hundred grams (100 g) of *B. abyssinica* powder of the roots were soaked in 500 mL of acetone as was described by Moyo *et al*. (2015). Again, another 100 g plant sample was extracted in aqueous solution. The leaf extract solutions were left to shake at 30°C for a time of 48 h in a shaker (Stuart Scientific Orbital shaker, U.K.). Then, the solutions were sieved separately with the use of a filter paper (Whatman no.1). The acetone extracts were afterward evaporated to dry at 40°C with the use of an evaporator (Laborator 4000-efficient, Heidolph, Germany). Water leaf extracts were dried in a freeze drier (Savant refrigerated vapor Trap with company number: RVT4104, U.S.A.) and it was kept at four degree Celsius. Percentage (%) yields of water and acetone extracts were calculated. The extracts obtained were kept in an air-tight glass bottle for further validation of antioxidant activity, phytochemical screening and anti-nutrient analysis.

Phytochemical analysis

Total phenolics: This was evaluated with the use of Folin–Ciocalteu chemical in a modified technique as explained by Ghasemzadeh *et al*. (2010). The *B. abyssinica* leaf extracts were mixed using five (5 mL) Folin–Ciocalteu chemical and 4 mL of sodium carbonate (at 75 g/l). Mixture of the extracts was shaken in a vortex machine for 15 sec and was left to stay for a period of 30 min for a colour change at 40°C. Absorbance of the extracts was read spectrophotometerically at 765 nm. Furthermore, the extracts were then calculated at 1 mg per mL. The phenolics constituents were calculated as mg/g tannic acid equivalent.

Favonol constituents: This was calculated using the technique as was explained by Kumaran and Karunakaran (2007). Two (2) mL of 2% $AlCl_3$ made in three (3) mL of sodium acetate solutions (50 g/L) and ethanol were poured into 2.0 mL solvent extract. For measurement of absorbance of the solution, it was done at 440 nm for a time of 2.5 h, after incubation normally at 20°C. Furthermore, the extracts were estimated at a concentration of 1 mg/mL. The flavonol constituents were expressed as quercetin (mg/g).

Total proanthocyanidins (condensed) constituent: This was done as was explained by Sun *et al*. (1998) with slight modifications. A solution of 0.5 mL (of 1 mg/mL) of extracts was poured into 3 mL of 4% vanillin–acetone solvent with 1.5 mL of HCl. The absorbance was calculated at 500 nm, before the solution was left to stay for a period of 15 min. The extracts were evaluated at 1 mg per mL. The proanthocyanidin constituent was expressed as catechin equivalents (mg per g).

Total flavonoid constituents: This was done using the technique as was explained by Sen *et al.* (2013). Aliqouts of 0.5 mL of 2% aluminium chloride ($AlCl_{3)}$ prepared in solutions of ethanol were poured into 0.5 mL sample solutions, and this was followed by quantifying the absorbance which was read at room temperature at 420 nm, after 1 h of incubation. A colour change (yellow colour) shows the presence of flavonoids in the solution. The plant extracts are then calculated at a concentration of 1 mg per mL. The flavonoid constituents were expressed as quercetin equivalent (mg per g).

Determination of anti-oxidant activities

The antioxidant potentials were done by measuring the total antioxidant capacity (TAC), 1,1- diphenyl-2-picrylhydrazyl (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Nitric Oxide (NO) radical scavenging activity. These measurements were compared to butylated hydroxytoluene (BHT) as a standard antioxidant.

2,2-diphenyl-1-picrylhydrazyl (DPPH) Scavenging activity

This was carried out by the use of the technique as explained by Liyana-Pathiana and Shahidi (2005). Briefly, an estimate of 0.135 m*M* of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in acetone was prepared and 1.0 mL of the mixture (from *B. abyssinica*) was poured into 1.0 mL of extract in water and acetone that contains 0.2–1.0 mg/mL of extracts. The solution was kept for a period of 30 min at room temperature (in the dark) to determine the reaction of extracts on the 2,2-diphenyl-1-picrylhydrazyl radical. The absorbance of the mixture of extracts was read at 517 nm on spectrophotometer with ascorbic acid and butylated hydroxytoluene (BHT) as standards for comparisons. Again, activity of the extract to scavenge the 2,2-diphenyl-1-picrylhydrazyl radical were done by the use of the given equation:

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

Where $Abs_{control}$ ≡ absorbance of 2,2-diphenyl-1-picrylhydrazyl radical + ethanol and Abs_{sample} ≡ absorbance of 2,2-diphenyl-1-picrylhydrazyl radical + sample extract/standard

Activities of NO scavenging ability

The adjusted technique as was explained by Odeyemi *et al*. (2010), was utilized to ascertain the NO scavenging ability of the given extracts (namely aqueous, methanol, acetone, and ethanol) of *B. abyssinica* leaf. Two (2 mL) of sodium nitroprusside in 0.5 mL of phosphate buffer solution (pH 7.4) was poured into 5 mL of the extracts of butylated hydroxytoluene and ascorbic acid at concentrations ranging between 0.2–1.0 mg per mL. The mixture solution was afterward incubated for a period of 150 min (at a temperature of 25 degree Celsius). Conversely, 0.5 mL of the solution was poured into a 0.5 mL of sulfanilic acid chemical after which it was then incubated for a period of 5 min at standard temperature. One milliliter (1 mL) of naphthylethylenediamine dihydrochloride (0.1% *w*/*v*) was eventually poured into the solution before it was incubated for 30 min at standard temperature. The absorbance was measured spectrophotometerically at 540 nm. Likewise, calculation was done for the extracts to scavenge NO radicals with the use of the formula as expressed below:

$$\% \text{ inhibition} = [(Abs_{control}) - (Abs_{sample})]/[(Abs_{control})] \times 100.$$

Where; $Abs_{control}$ ≡ Absorbance of NO radical + acetone and Abs_{sample} ≡ Absorbance of NO radical + sample extract/standard.

Determination of total antioxidant activity (TAC)

This was done using the technique as was explained in the study of Olugbami *et al*. (2015). In brief, about 0.3 mL of the various solvent extracts with specified drug (25 μg/mL to 400 μg/mL) were placed inside the test tubes and were dissolved in 3 mL of chemical mixture which initially contained 4 m*M* of ammonium molybdate, 0.6 *M* of sulphuric acid, together with 28 m*M* of sodium phosphate, accordingly. After that, the tubes that were used were sealed and placed in a water bath for a period of 95 min at 95°C. Furthermore, a mixture of the different solvents was cooled to a standard temperature after which absorbance was read spectophotometerically at 695 nm. The control was made from a mixture of distilled water for the samples. The specified drugs used were gallic acid and ascorbic acid for comparison. It should be noted that higher absorbance used for this study indicates higher total antioxidant property. Conversely, the percentage (%) inhibition that was calculated from the formula given below: [(absorbance of sample – absorbance of control)/ (absorbance of sample)] multiplied by 100.

Determination of 1,1- diphenyl-2-picrylhydrazyl scavenging activity

This was done from the technique previously explained by Hsu *et al*. (2011). The solution used was prepared by mixing an equal volume of 2.45 m*M* of potassium persulfate and 7 m*M* of 1,1- diphenyl-2-picrylhydrazyl (ABTS). This mixture was left to react for a period of 12 h in the dark at standard temperature in order to produce 1,1- diphenyl-2-picrylhydrazyl radicals (ABTS+). Furthermore, the ABTS+ stock mixture was then diluted in one (1 mL) of the ABTS+ mixture with 50 mL of liquid methanol so as to get a given absorbance (0.700 ± 0.006 at 734 nm). Subsequently, 1 mL of the ABTS + mixture was added together with 1 mL of extract/or standards at varied chemical strength (0.005–0.080 mg/mL). The reduction of the absorbance was then determined after 7 min at 734 nm. The inhibition % of ABTS+ by the extract/standard was determined using the formula below:

Percentage inhibition = [(Abs control – Abs sample)/ (Abs control)] multiplied by 100

Data analysis

The MINITAB 17 statistical software package was used for all statistical analysis of data. The results of the data were given as standard deviation (SD) of mean ± in three replications. The statistical analysis was done using analysis of variance (ANOVA). In situations where there is significance at $P < 0.05$, a mean ± separation was carried out using the Fischer's least significant difference (LSD).

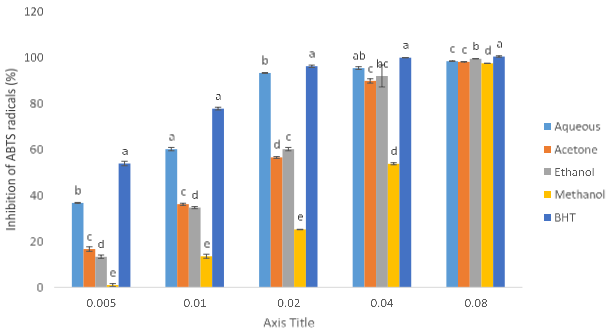

Fig. 1: ABTS radical scavenging activity of the different extracts of *B. abyssinica*. Values are mean ± SD of 3 replications. Set of bars with the same letter are not significantly different ($P < 0.05$)

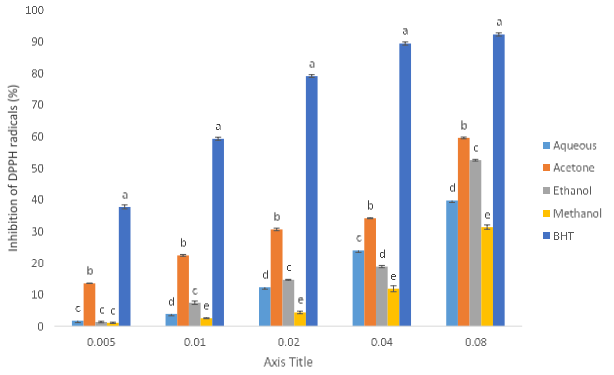

Fig. 2: DPPH radical scavenging activity of the different extracts of *B. abyssinica*. Values are mean ± SD of 3 replications. Set of bars with the same letter are not significantly different ($P < 0.05$)

Results

Polyphenolic Component of plant extracts

The polyphenolic compound result of the *B. abyssinica* plant sample showed that there are occurrences of phenols, flavonoids and condensed tannins as depicted in Table 1. The results for the chemical strength of phenols in the different solvents showed that extracts were substantially high for flavonoids and condensed tannins compared to total phenols. The condensed tannins and flavonoid constituents of the extracts were higher in terms of concentration in acetone as compared to aqueous, ethanol and the methanol solvents (Table 1). Conversely, the phenol constituents of leaf extract gave higher mean for ethanol compared to aqueous, acetone and methanol solvents. The IC_{50} value for all the solvents (including acetone, aqueous, ethanol and methanol extracts), with the standards *i.e.*, ascorbic acid and BHT are given in Table 2.

Radical scavenging activities of ABTS

Fig. 1 showed the results of ABTS assay. It was shown that every of the extracts and standard had substantial 1,1-diphenyl-2-picrylhydrazyl scavenging abilities (Fig. 1). The inhibition percentage of the ABTS plant extract and the standard were dependent on the concentration. Judging from the highest concentration that was tried (at 0.08 mg/mL), the values obtained for aqueous, acetone, ethanol, and methanol extracts were 98.50, 98.22, 99.55 and 97.54%, respectively, while that of the standard (BHT) gave inhibition as high as 100.52%. Likewise, the IC_{50} values that were given for the tried extracts/ standard were as follows: BHT < aqueous extract < acetone extract < ethanol extract < methanol extract (Table 2).

2,2-diphenyl-1-picrylhydrazyl scavenging activities

The DPPH radical scavenging activity of aqueous extracts of *B. abyssinica* extract compared fairly well with BHT (Fig. 2). Likewise, from the result of the current study, acetone leaf extract of *B. abyssinica* had higher activity compared to aqueous, ethanol and methanol extracts, but lesser activity than the standard drug (BHT). Likewise, there was increase in the DPPH activities as the concentration of the solvents increases (*i.e.*, from 0.005 – 0.08 mg/mL). The IC_{50} value for all the solvents (such as aqueous, ethanol, acetone, and methanol) leaf extracts and BHT are presented in Table 2.

Nitric Oxide scavenging activity

The extracts from acetone and aqueous solvents had effective scavenging activity on the nitric oxide (NO) radicals (Fig. 3). The scavenging activity of the extracts including methanol, aqueous, ethanol and acetone were lesser in comparison to BHT. The inhibition values for the different solvents were highest at 0.4 mg/mL. Furthermore, the result showed significant difference in the nitric oxide (NO) activities at different concentrations with the BHT having a higher inhibition values (%) than the different solvents used. The ethanol solvent had a significantly higher concentration at 0.025, 0.05, 0.1 and 0.4 mg/mL, accordingly as compared with aqueous, methanol and acetone leaf extracts. The values of the IC_{50} of the extracts/standard drug are as follows: ethanol extract < acetone extract < BHT < aqueous extract < methanol extract (Table 2) with ethanol leaf extract possessing the lowest IC_{50}, demonstrating strong NO scavenging ability.

Total antioxidant capacity radical scavenging activity

The result is presented in Fig. 4. The antioxidant capacity of all the solvent fractions increased with an increase in concentration which indicated that they are concentration dependent. The solvent (aqueous, acetone, ethanol and methanol) extracts compared favorably with the BHT at 0.1, 0.2 and 0.4 mg/mL. The IC50 of the different plant extracts and the standard drug BHT < acetone < aqueous < ethanol < methanol, respectively (Table 2).

Table 1: Polyphenolic constituent of different solvent extracts of *B. abyssinica*

Phenolics	Aqueous	Acetone	Ethanol	Methanol
Total phenol (mg GAE/g)	16.00 ± 0.12	24.35 ± 0.30	29.38 ± 0.94	16.31 ± 1.01
Condensed tannin (mg TAE/g)	18.31 ± 1.92	295.83 ± 1.94	194.59 ± 1.11	124.28 ± 1.69
Flavonoids (mg QE/g)	85.42 ± 0.52	376.05 ± 0.52	320.49 ± 0.83	153.52 ± 1.37

STD = standard deviation. Values are means of triplicate determinations ± STD. mg GAE/g = Expressed as mg tannic acid/g of dry plant material; mg TAE/g = milligram tannic acid equivalent per gram of extract; mg QE/g = milligram quercetin equivalent per gram of extract

Table 2: Inhibitory concentrations at 50% (IC_{50}) of the antioxidant activities of *B. abyssinica* leaf extracts compared to known standard

Solvents	ABTS		DPPH		Nitric Oxide		TAC	
	IC_{50}	R^2	IC_{50}	R^2	IC_{50}	R^2	IC_{50}	R^2
Aqueous	0.0062	0.8481	0.0962	0.9802	2.8276	0.9626	0.0224	0.9441
Acetone	0.0148	0.9773	0.0631	0.9575	0.5747	0.9850	0.0172	0.9432
Ethanol	0.0418	0.9761	0.0800	0.9663	0.3797	0.9754	0.0253	0.9861
Methanol	1.0412	0.9919	0.1380	0.9634	3.0449	0.9020	0.0773	0.9795
BHT	0.0023	0.8723	0.0068	0.9230	0.7524	0.9456	0.0023	0.9795

IC_{50}: Expressed as the concentration (mg/mL) adequate to obtain 50% of a maximum scavenging activity of leaf extracts. R^2: regression co-efficient

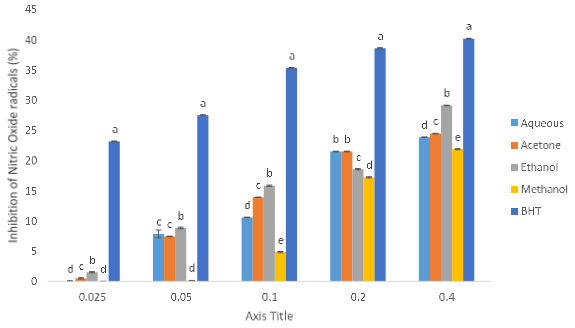

Fig. 3: Nitric oxide radical scavenging activity of the different extracts of *B. abyssinica*. Values are mean ± SD of 3 replications. Set of bars with the same letter are not significantly different ($P < 0.05$)

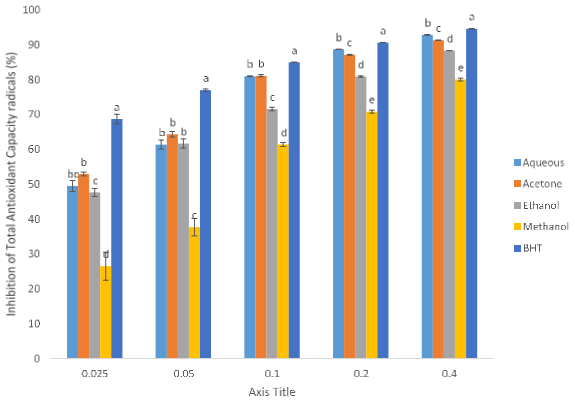

Fig. 4: Total antioxidant capacity radical scavenging activity of the different extracts of *B. abyssinica*. Values are mean ± SD of 3 replications. Set of bars with the same letter are not significantly different ($P < 0.05$)

Discussion

The major compounds available in *B. abyssinica* (plant) leaf extracts are polyphenols and they displayed some potentials for antioxidant properties. Polyphenols are known to display antioxidant activities as a result of their redox properties (Zheng and Wang 2001). The redox reaction of polyphenols is very important as a result of their effectiveness to destroy and defuse loss radicals, putrefy peroxides, in addition, extinguish ROS activities (Aiyegoro and Okoh 2010). The present study showed that the solvents utilized to extract *B. abyssinica* leaf powder gave a substantial yield of phyto-chemicals including phenols and flavonoids, however, it was observed that the yield from acetone solvent was higher in comparison with ethanol, water, and methanol solvents. A similar observation was made by Idamokoro *et al*. (2017) and Omoruyi *et al*. (2012) in their studies as they also reported that acetone extracted more phenols from *V. karroo* and *Carpobrotus edulis* leaves in comparison to water solvent, respectively. Solvents used in the extraction of compounds from leaf samples usually depend on the amount of yields it will give. An explanation for the obvious variations in the extraction capability of the solvents used in the current study may have led to the cause of the difference in polarity of acetone and other solvents such as water, methanol and ethanol.

The fact that *B. abyssinica* leaf possesses abundant amount of phenols as was observed from the present study, might be a strong reason for its traditional therapeutic utilization in livestock farming. For an example, flavonoids, are very effective aqueous-soluble antioxidants. According to Loots *et al*. (2007), flavonoids inhibit damage in cell tissues that may result from stress during oxidation, due to the fact that they act as potent antioxidant agents fighting against loss radicals. It was noticed from the current study that, flavonoids extracted from *B. abyssinica* leaf was relatively substantial in all the solvents that was used; however, it was more for acetone and ethanol solvents. The flavonoid concentrations generated for acetone (376.05 mg per g) and aqueous (85.42 mg per g) leaf extracts from *B. abyssinica* plant were higher in comparison to the ones reported by Omoruyi *et al*. (2012), for acetone (0.65 mg per g) and aqueous (0.2 mg per g) extracts for leaf of *Carpobrotus edulis* (L.) *bolus*.

Condensed tannins usually referred to as proanthocyanidins are a group of polyphenolic bioflavonoids. The compounds are ubiquitous and they are available as the second greatest available naturally occurring phenolic next to lignin. Conversely, condensed tannins are of significant importance in animal diets and as alternative medicine, as a result of its effective antioxidant properties and its probable protective effects to secure human health (Gu *et al*. 2004). Conversely, it shows that *B. abyssinica* is rich in proanthocyanidin content in reference to catechin as observed from the present study. Furthermore, the acetone extracts had a higher significant difference amount than the other solvent extracts ($P < 0.05$) and hence it could be said that solvent of extraction of phenolic compounds determines the amount of condensed tannins extracted from the leaf extract.

Additionally, the result gotten for the 2, 2-diphenyl-1-picrylhydrazyl scavenging activity assay showed that *B. abyssinica* extracts were active against loss radicals. The ability of *B. abyssinica* leaf extracts to scavenge 2,2-diphenyl-1-picrylhydrazyl radicals was lesser in comparison to butylated hydroxytoluene (BHT). Nonetheless, aqueous, acetone and ethanol leaf extracts (IC_{50}: 0.0062 mg per mL, IC_{50}: 0.0148 mg per mL and 0.0418 mg per mL) show comparable activities to that of the butylated hydroxytoluene (IC_{50}: 0.0023 mg per mL). This attribute points to the fact that *B. abyssinica* extracts are able to release hydrogen protons to loss radical, with that, they might be utilized as main antioxidant agent. The antioxidant activities of plant extracts on 2-diphenyl-1-picrylhydrazyl is made possible because their attributes to release protons to loss radicals (Krishna-Kumar *et al*. 2012).

Generally, it is known that NO are very reactive loss radicals that are formed in the body cells for it to provide a lot of reactive species including peroxynitrite, which may decomposed to produce hydroxyl (OH) radicals. From the present study, it was shown that at a 0.2 and 0.4 mg per mL concentrations, the NO scavenging characteristics of acetone, water and ethanol extracts compared favourably with butylated hydroxytoluene used in the study. In line with the present study, it was observed by (Aiyegoro and Okoh 2010), that water extracts gotten from the plant *Helichrysum longifolium* DC leaf had a significant effect on the level of nitric oxide radicals. It has been found out that, some browse plants sometimes secrete some form of substances (as mechanical defence) together with bioactive components (which include phenolics, glucosinolates, alkaloids, terpenes) used as protection from predators; conversely, they may result into a beneficial compound to livestock to give medicinal benefits to animals when leaf parts are utilized by local farmers to treat/feed their animals (Woll *et al*. 2013).

Worthy of noting is the fact that isofuranonaphthoquinones, anthraquinones, and phenylanthraquinones have previously been isolated from other parts of *B. abyssinica* (Kibiti and Afolayan 2018). These compounds are known to be effective as anti-helminths. Furthermore, other compounds like bulbine-knipholone, phenylanthraquinone has been extracted from the root part of *B. abyssinica*. According to Bringmann *et al*. (2002), these compounds showed a significant *in-vitro* antiplasmodial activity without any cytotoxic effects on the cells of mammal. The presence of bioactive compounds extracted from *B. abyssinica* leaves may explain why it is been used by farmers to treat their animals even though they are not aware of the scientific relevance of the bioactive compounds in the plant.

Suffice to state that, livestock raised in the area of study would be able to benefit more from the medicinal usefulness of *B. abyssinica*. Reason being that, apart from the plant's nutritional constituents (Kibiti and Afolayan 2018), they could obtain bioactive constituents from the leaves of *B. abyssinica*, and this could be very beneficial for their body metabolism. For instance, the presence of flavonoids which is an important constituent that was isolated from the leaf extract of *B. abyssinica*, are vital hydroxylated phenolic compounds occurring abundantly in plants. According to Hodek *et al*. (2002), flavonoids has an extensive range of health benefiting biological activities which includes among others; anti-inflammatory, analgesic, antiallergic, antimicrobial, anticancer, antioxidant and antidiabetic potentials. Conversely, gastrointestinal parasites in animals show symptoms as result of pathological variations in different animal organs. As a reference, a study by Meeusen (1999), showed that the response to intestinal helminth problem caused inflammation of parasite products in the gut of the host. According to Maphosa and Masika (2010), several medicinal plants including *B. abyssinica* utilized by rural people are said to possess activities which ranged from anti-inflammatory, anti-helminth, antiedema, antimicrobial potentials. Rural farmers utilize *B. abyssinica* leaves as decoctions to treat gastrointestinal parasites in livestock and most especially in small ruminants (Maphosa and Masika 2010). The use of *B. abyssinica* may however, be imperative to treat helminths, and to enhance the body immunity of livestock due the presence of several bioactive compounds.

From direct conversations with local livestock farmers from the study area, we obtained information that goat farmers use this plant (*B. abyssinica*) to treat their animal, reason being that they perceived it to be a potent medicinal natural plant resource that improves the well-being of their livestock, even though there are no scientific evidence for their claim. According to the study by Masika and Mafu (2004), it was gathered that goats raised under an extensive system of farming are often confronted with circumstances of metabolic stress resulting from their daily search for pastures under an extensive system of farming. Goats fed with *B. abyssinica* leaf parts may also profit from several other medicinal benefits such as antioxidant potentials of phenols (*e.g*., flavonoids) that are available in the plant. The consumption of naturally resourceful plants could protect animals from loss radicals often produced in animal body cells during stress caused by oxidation (Loots *et al*. 2007).

Conclusion

The availability of phenols, flavonoids and condensed tannins in the leaves of *B. abyssinica* provides some imperial proof for its traditional use by indigenous people for mitigating intestinal parasites farm animals. The acetone and aqueous extracts of *B. abyssinica* gave appreciable activities (at 0.08 and 0.4 mg per mL) as an antioxidant substances; nonetheless, more investigation and research is imperative to be done, so as to establish the active components available in *B. abyssinica* leaf which could be responsible for its use as an anti-helminth in the control of internal parasites of livestock as used by communal farmers in South Africa.

Author Contributions

Emrobowansan Monday Idamokoro and Anthony, J. Afolayan conceptualized and design the work; Emrobowansan Monday Idamokoro collected and analyzed the data; Emrobowansan Monday Idamokoro visualized the results; Emrobowansan Monday Idamokoro wrote the paper; Anthony, J. Afolayan provided laboratory facilities for analyzing the plant samples and financial support.

References

Aiyegoro AO, AI Okoh (2010). Prelimary phytochemical screening and *in vitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium*. *BMC Complem Altern Med* 10:2–8

Bhandary SK, N Kumari, VS Bhat, K Sharmila, MP Bekal (2012). Preliminary phytochemical screening of various extracts of *Punica granatum* peel, whole fruit and seeds. *J Health Allied Sci* 2:34–38

Borneo R, AE Le´on, A Aguirre, P Ribotta, JJ Cantero (2009). Antioxidant capacity of medicinal plants from the Province of C´ordoba (Argentina) and their *in vitro* testing in a model food system. *Food Chem* 112:664–670

Bringmann G, D Menche, R Brun, T Msuta, B Abegaz (2002). Bulbine-knipholone, a new, axially chiral phenylanthraquinone from *Bulbine abyssinica* (Asphodelaceae): Isolation, structural elucidation, synthesis, and antiplasmodial activity. *Eur J Org Chem* 6:1107–1111

Do QD, AE Angkawijaya, PL Tran-Nguyen, LH Huynh, FE Soetaredjo, S Ismadji (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *J Food Drug Anal* 22:296–302

Ghasemzadeh A, HA Jaafar, A Rahmat (2010). Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (*Zingiber officinale* Roscoe). *Molecules* 15:4324–4333

Gu L, MA Kelm, JF Hammerstone, G Beecher, J Holden, D Haytowitz, S Gebhardt, RL Prior (2004). Concentrations of proanthocyanidins in common foods and estimations of normal consumption. *J Nutr* 134:613–702

Hassan MDK, AKL Kabir, S Mistry (2009). Chemical and biological investigation of leaves of *Polygonum Plebejum*. *J Pharm Sci* 2:66–71

Havsteen BH (2002). The biochemistry and medical significance of the Flavonoids. *Pharmacol Therapeut* 96:67–202

Hodek P, P Trefil, M Stiborova (2002). Flavonoids-potent and versatile biologically active compounds interacting with cytochrome P450. *Chem-Biol Interact* 139:1–21

Hossain MA, MD Shah, C Gnanaraj, M Iqbal (2011). *In vitro* total phenolics, flavonoids contents and antioxidant activity of essential oil, various organic extracts from the leaves of tropical medicinal plant *Tetrastigma* from Sabah. *Asian Pac J Trop Biomed* 4:717–721

Hsu CF, H Peng, C Basle, JT Sejdic, PA Kilmartin (2011). ABTS scavenging activity of polypyrrole, polyaniline and poly (3, 4-ethylenedioxythiopene). *Poly Intern* 60:69–77

Idamokoro EM, PJ Masika, V Muchenje (2017). A Report on the In Vitro Antioxidant Properties of *Vachellia karroo* Leaf Extract: A plant widely grazed by goats in the Central Eastern Cape of South Africa. *Sustainability* 9:164–171

Iqbal E, KA Salim, LB Lim (2015). Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutinus* (Airy Shaw) from Brunei Darussalam. *J King Saud Univ Sci* 27:224–232

Kibiti CM, AJ Afolayan (2018). Mineral composition and nutritive analysis of *Bulbine abyssinica* A. Rich. Used in the treatment of infections and complications associated with diabetes mellitus in the Eastern Cape Province of South Africa. *Afr J Trad Complem Altern Med* 15:38–46

Kumaran A, RJ Karunakaran (2007). *In vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. Lebensmittel-Wissenschaft and Technologie. *Food Sci Technol* 40:344–352

Krishna-Kumar HN, SN Navyashree, HR Rakshitha, JB Chauhan (2012). Studies on the free radical scavenging activity of *Syagrus romanzoffiana*. *Intern J Pharm Biomed Res* 3:81–84

Lee SE, HJ Hwang, JS Ha, HS Jeong, JH Kim (2003). Screening of medicinal plant extracts for antioxidant activity. *Life Sci* 73:167–179

Liyana-Pathiana CM, F Shahidi (2005). Antioxidant activity of commercial soft and hard wheat (*Triticum aestivium* L.) as affected by gastric pH conditions. *J Agric Food Chem* 53:2433–2440

Loots DT, FHVD Weststhuizen, L Botes (2007). Aloe ferox leaf gel phytochemical content, antioxidant capacity, and possible health benefits. *J Agric Food Chem* 55:6891–6896

Maphosa V, PJ Masika (2010). Ethnoveterinary uses of medicinal plants: A survey of plants used in the ethnoveterinary control of gastro-intestinal parasites of goats in the Eastern Cape Province, South Africa. *Pharm Biol* 48:697–702

Masika PJ, JV Mafu (2004). Aspects of goat farming in the communal farming systems of the central Eastern Cape, South Africa. *Small Rumin Res* 52:161–164

Meeusen ENT (1999**)**. Immunology of helminth infections, with special reference to immunopathology. *Vet Parasitol* 84:259–273

Moyo M, AO Aremu, JV Staden (2015**)**. Medicinal plants: An invaluable, dwindling resource in sub-Saharan Africa. *J Ethnopharmacol* 174:595–606

Odeyemi SO, G Bradley, AJ Afolayan (2010**)**. *In vitro* and *in vivo* antioxidant activities of aqueous extract of *Strychonos henningsii* Gilg. *Afr J Pharm Pharmacol* 4:70–78

Olugbami JO, MA Gbadegesin, OA Odunola (2015). *In vitro* free radical scavenging and antioxidant properties of ethanol extract of *Terminalia glaucescens*. *Pharmacogn Res* 7:49–56

Omoruyi BE, G Bradley, AJ Afolayan (2012). Antioxidant and phytochemical properties of *Carpobrotus edulis* (L.) bolus leaf used for the management of common infections in HIV/AIDS patients in Eastern Cape Province. *BMC Complement Altern Med* 12:215–224

Pooley E (1998). *A field guide to wild flowers of KwaZulu-Natal and the Eastern Region*. Natal Flora Trust Publications, Durban

Sen S, B De, N Devanna, R Chakraborty (2013). Total phenolic, total flavonoid content, and antioxidant capacity of the leaves of *Meyna spinosa* Roxb., an Indian medicinal plant. *Chin J Nat Med* 11:149–157

Sun B, JM Richardo-da-Silva, I Spranger (1998). Critical factors of vanillin assay for catechins and proanthocyanidins. *J Agric Food Chem* 46:4267–4274

Woll S, SH Kim, HJ Greten, T Efferth (2013). Animal plant warfare and secondary metabolite evolution. *Nat Prod Bioprospect* 3:1–7

Yoshikawa T, Y Naito (2002). What Is Oxidative Stress? *Jap Med Assoc J* 45:271–276

Zheng W, SY Wang (2001). Antioxidant activity and phenolic compounds in selected herbs. *J Agric Food Chem* 49:5165–5170