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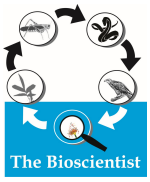

Full Length Research Paper

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Comparative Analysis of Phytochemical Constituents and Antioxidant Properties of Aqueous and Ethanol Extracts of *Alstonia boonei* Stem-Bark

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ABSTRACT

The plant *Alstonia boonei* is extensively used in traditional medicine because of its therapeutic properties. Over the years, researchers have been looking for the active medicinal agents in different plant species and as such, the type of extraction solvent plays a major role. This study aim to investigate the comparative analysis of phytochemical constituents and antioxidant properties of aqueous and ethanol extracts of *A. boonei* stem-bark. The stem-bark of *A. boonei* was collected, dried, pulverized and extracted with ethanol and distilled water using maceration method. The extract was concentrated in a temperature regulated water bath set at 45^{o}C. Qualitative and quantitative phytochemical analysis was carried out in both extracts. Both extracts was also subjected to various antioxidant analytical procedures such as free radical scavenging capacity against 1, 1-diphenyl-2-picrylhydrazyl (DPPH), reducing power, and ferric reducing antioxidant power (FRAP) to determine antioxidant properties. The qualitative phytochemical analysis of both extracts revealed the presence of flavonoids, cardiac glycoside, terpenoids, alkaloids and saponins. However, quantitative studies revealed that proanthocyanidin, flavonoids and total phenol were significantly higher in ethanol extract compared to aqueous extract ($p < 0.05$). Both extracts exhibited antioxidant activities compared to reference antioxidant (Ascorbic acid). However, the reducing power of the aqueous extract was significantly higher than that of the ethanol extract.This result indicates that aqueous extract of *A. boonei*stem-bark had lower phytochemical constituents but higher antioxidant activity compared to ethanol extract. The result of this study revealed that ethanol may be a better solvent for phytochemical extraction of *A. boonei* stem-bark.

Keywords: *Alstonia boonei*, phytochemical analysis, *in vitro*, antioxidant properties

INTRODUCTION

Traditional herbal medicine is the most ancient form of medicine known to man, and plant-based substances account for more than half of all modern clinical drugs (Krishnan, 2018). In the pharmaceutical industry, natural products serve significant functions in drug formulation. Plants have historically served as a great source of drugs, and nearly all of the medications available today originated from plant-based substances either directly or indirectly (Kumar *et al*., 2022). The World Health Organization (WHO), reported that about 80% of the world's population relies on traditional or plant-based medications for primary healthcare. These may served as the foundation of proficient traditional medicine systems and are said to provide great prospects for the development of novel drugs (Ali *et al*., 2019).

Most of the African communities rely on traditional medicines for the treatment of various ailments (Malan and Neuba, 2011). This is necessary because of the medicinal properties that have been exhibited inherbal mixtures and decoctions, reflecting their effectiveness, affordability, and availability. Modern healthcare facilities on the other hand, are out of the reach of majority of the populace (Otu, 2018) or expensive (James *et al*., 2018).

A medicinal plant is defined as any plant that contains substances known as phytochemicals in one or more of its organs that may be used to treat ailments or act as precursors for the synthesis of drugs (Opoku and Akoto, 2015). Phytochemicals are non-nutritive chemical components in plants. They occur during metabolic processes and have been shown possess various therapeutic properties which are helpful in the treatment or alleviation of symptoms of a variety of disease conditions (Opoku and Akoto, 2015). They are secondary metabolites in the leaves, stems, roots,or any part of the plants. Importantly, various researches have shown that they play an important role in human disease prevention and cure (Manash *et al*., 2016). Studies have shown that most plant species possesses phytochemical such as alkaloids, flavonoids, saponins, cardiac glycosides, steroids, tannins, terpenoids, phenolics, triterpenoids, anthraquinones, and so on (Lou *et al*., 2017, Agidew, 2022). These phytochemicals are said to confer pharmacological properties like antimicrobial, antioxidant, antihelmintic, anti-inflammatory, anticancer, anti-diabetic, hypoglycemic, etc, on the plants (Alara *et al*., 2017).

An imbalance between oxidant production and the ability of a biological system to detoxify reactive intermediates or repair the consequent damage lead to oxidative stress. Oxidative stress has been implicated in several degenerative diseases such as atherosclerosis, diabetes, hypercholesterolemia, aging, hypertension (Keshari *et al*., 2015). Oxidants are endogenously produced by cells during normal aerobic respiration. However, exogenous sources may include environmental stressors (Olubodun and Eriyamremu, 2013), chemical toxicants, antibiotic resistance in pathogenic organisms, etc. The human body has the ability to protect itself against these oxidants (reactive oxygen species) due to antioxidant systems (Kone *et al*., 2015). Antioxidants are also largely present in medicinal plants *(Aliyuet al., 2020).* Most antioxidants have been shown to possess phenolic hydroxyl groups in their structures, and the antioxidant properties are attributed to their ability to scavenge free radicals (Kone *et al*., 2015).

Alstonia booneiis found majorly in West Africa. Itbelongs to the family *Apocynaceae*. It is found in the tropics and rain forests of West and Central Africa (Opoku and Akoto, 2015). The plant is known in English as

Alstonia, cheese wood, pattern wood; Egbu, Egbo-ora (Igbo); Ahun (Yoruba); and Ukhu (Edo) (Okoye *et al*., 2022). Pharmacological studies have shown that the plant extracts possesses anti-malarial, analgesic, antipyretic, anti-inflammatory, diuretic, and hypotensive properties, and so on (Otuu *et al*., 2020; Opoku and Akoto, 2015). The plant's parts have been used for the treatment of malaria, jaundice, rheumatism, malaria, fever, painful micturition, chronic diarrhea, insomnia, intestinal helminthes, and hypertension, as well as anti-venom against snake bite and other forms of disease in Nigeria and other West African countries (Opoku and Akoto 2015). In order to evaluate the therapeutic properties of this plant, the study aims to investigate the comparative analysis of phytochemical constituents and antioxidant property of the aqueous and ethanol extracts of *A. boonei* stem-bark.

MATERIALS AND METHODS

Collection and Identification

Stem-bark of *Alstonei boonei* was obtained with the help of an herbalist in Benin City, Nigeria. The plant was identified by Dr. H. A. Akinnibosun of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria. The stem-bark was allowed to dry at room temperature for a period. It was grind into fine powder with sterile mortar and pestle and stored until needed for use.

Preparation of Ethanol and Aqueous Extracts

The stem-bark of *A. boonei* was dried at room temperature for a period. It was reduced to fine powder after showing brittle properties when crushed with hand then with sterile mortar and pestle. The powdered sample (1000g) was subjected to Soxhlet extraction with 2000ml ethanol. The extract was concentrated with a rotary evaporator at 40^{o}C. The concentrated extract was stored in a refrigerator at -4^{o}C until required for further studies. Another quantity of the stem-bark of *Alstonei boonei*was macerated with distilled water (250ml) for 48hours. The extract was filtered and concentrated to dryness with a rotary evaporator in vacuo. The dried powdery sample was stored at -4ºC until required for use.

Qualitative and Quantitative Phytochemical Analysis

The crude ethanol and aqueous extracts were subjected to simple qualitative and quantitative phytochemical analysis using standard methods [Trease and Evans, (1989); Sofowora, (1993)] to determine the presence of alkaloids, tannins, saponins, anthraquinones, flavonoids and other phenolic compounds. Total phenolic content was estimated by Folin-Ciocalteau method (Gulcin *et al*., 2003). Total flavonoid content was estimated by the method described by Ebrahimzadeh *et al.* (2008). Proanthocyanidin content was estimated by the method described by Sun*et al.* (1998).

Determination of*in vitro* Antioxidant Activity

The antioxidant activity of the stem-bark of *Alstonei boonei* extracts was evaluated using 1,1-Diphenyl-2-picryl-hydrazyl (DPPH)-free radical scavenging method described by Manzocco *et al*., (1998). The extract was serially diluted to concentrations of 10, 20, 40, 60, 80 and 100 µg/ml. One ml of each dilution was mixed with 1ml of DPPH solution (0.004% in ethanol) and incubated at 37°C for 30min. The absorbance of mixture was then measured at 517nm. Absolute ethanol was used as a negative control. The DPPH scavenging activity was calculated as DPPH scavenging activity (%) = A0"A/A0 × 100 Where A is the absorbance of sample containing extract and A0 is the absorbance of the negative control

(0.004% DPPH solution). Ascorbic acid was used as a positive control. The concentration required for a 50% inhibition of DPPH (IC50) was then calculated by plotting the percentage of residual DPPH against the sample concentration. Nitric oxide radical scavenging assay was conducted in accordance to Griess reaction method of Marcocci *et al*., (1994); Determination of Ferric Reducing Antioxidant Potential (FRAP) was according to the method of Benzie and Strain, (1996).

Data Analysis

The Instat-Graphpad software, San Diego, California, USA, was used to carry out all analyses in triplicate and the results expressed as mean ± standard error of mean (SEM). The data were subjected to one way analysis of variance (ANOVA) where applicable and a P values < 0.05 was regarded as statistically significant.

RESULTS

Phytochemical Analysis: The qualitative phytochemical analysisof the aqueous and ethanol stem-bark extracts is presented in Table 1. The phytochemical analysisreveals that both extracts possesses flavonoids, terpenoids, cardiac glycosides, alkaloids, and phenol. However, tannins and steroids were not present in both extracts. The quantitative phytochemical analysis of the aqueous and ethanol extracts of *A. boonei* stem-bark is presented in Figures 1–3. Quantitatively, the ethanol extract showed significantly higher prothocyanadine, flavonoid, and phenol contents compared to the aqueous extract.

Table 1:Qualitative Analysis of Aqueous and Ethanol Extracts of *A.boonei*

Phytochemicals	Ethanol Extracts	Aqueous Extracts
Flavonoids	+	+
Tannins	-	-
Cardiac glycosides	+	+
Steroids	-	-
Terpenoids	++	++
Alkaloids	+	+
Saponins	+	+

Legend: ++ = moderately high, +=low, -= not detected

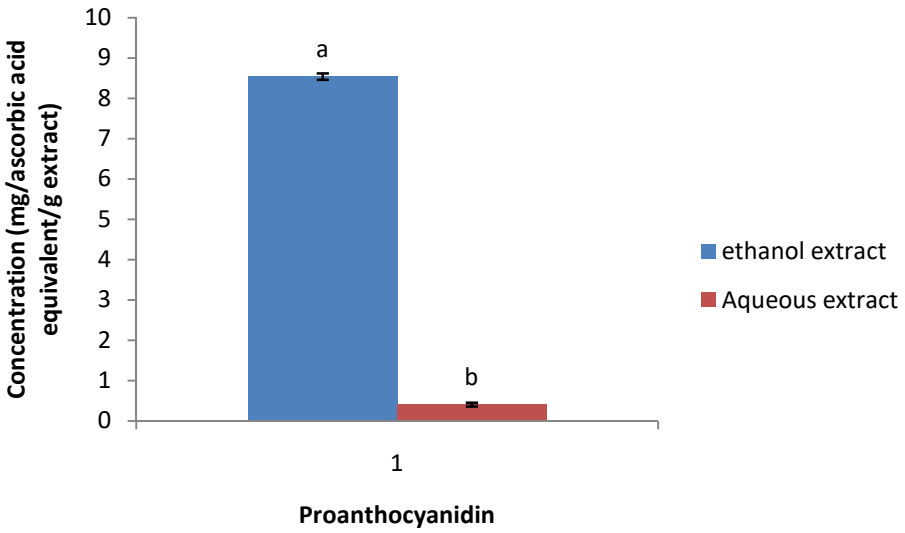

Fig. 1: Proanthocyanidin Content of Aqueous and Ethanol Extracts of *A. boonei*. Values are mean ± SEM (n = 3). Different alphabets on the bars signifies significant difference at (p < 0 .0 5).

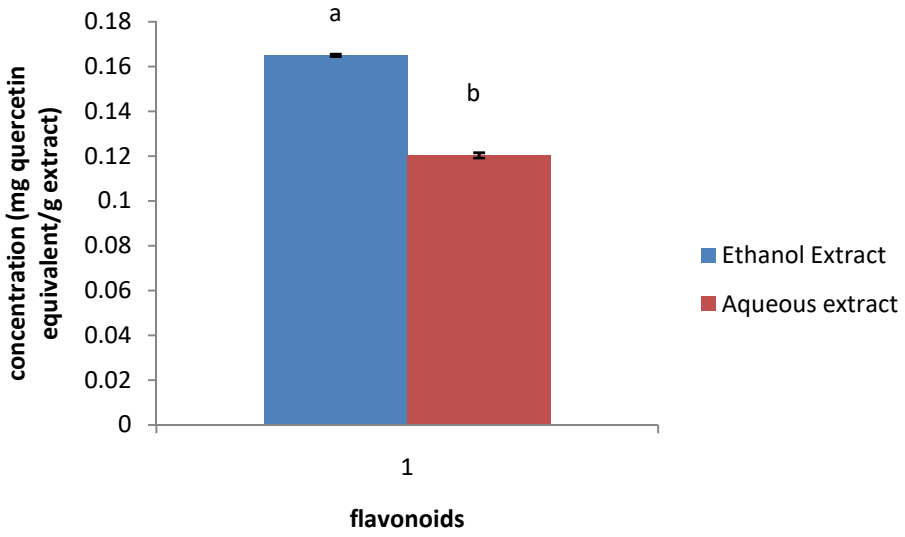

Fig. 2: Flavonoid Content of Aqueous and Ethanol Extracts of *A. boonei.* Values are mean ± SEM (n = 3). Different alphabets on the bars signifies significant difference at (p < 0 .0 5).

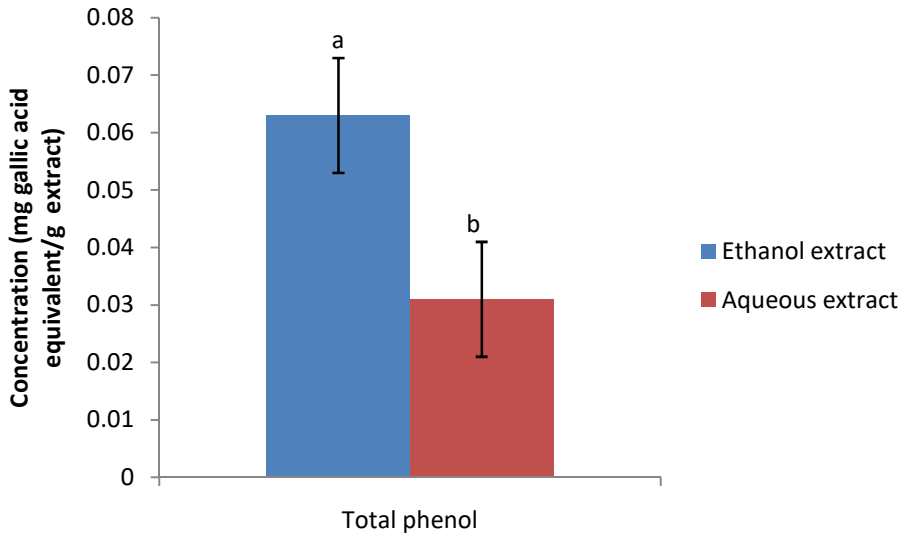

Fig. 3: Total Phenol Content of Aqueous and Ethanol Extracts of *A. boonei.* Values are mean ± SEM (n = 3). Different alphabets on the bars signifies significant difference at (p < 0 .0 5).

***In-vitro* Antioxidant Activity**

Ferric-reducing Antioxidant Potential (FRAP): The ferric-reducing antioxidant potential of aqueous andethanol extracts of *A. boonei* stem-bark and ascorbic acid is presented in Figure 4. Ferric antioxidant reducing power was significantly higher in aqueous extract compared to ethanol extract and ascorbic acid (standard).

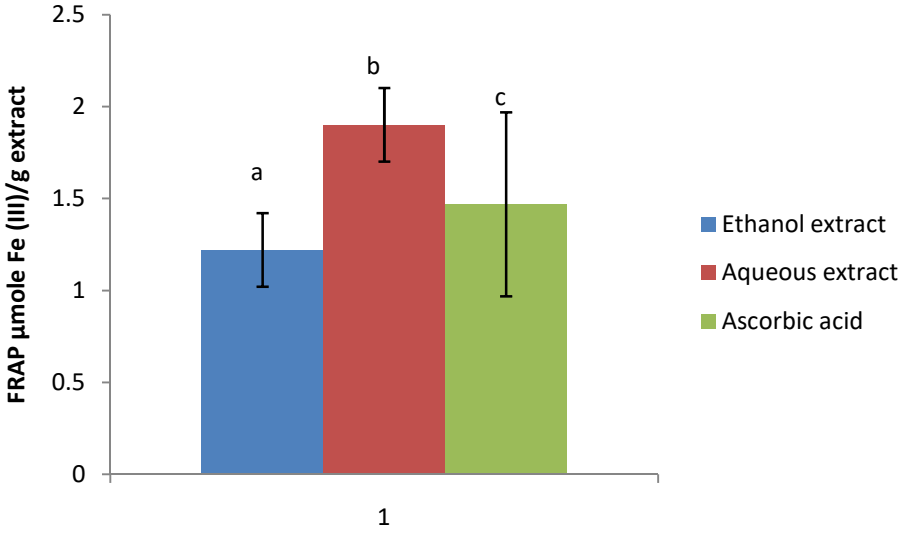

Fig. 4: Ferric-reducing Antioxidant Potential of Aqueous and Ethanol Extracts of *A. boonei.* Values are mean ± SEM (n = 3). Different alphabets on the bars signifies significant difference at (p < 0 .0 5).

Reducing Power Estimation: Results of the reducing power of aqueous and ethanol extracts of *A. boonei* and ascorbic acid is presented in Figure 5. The aqueous extract had significantly higher reducing power than ethanol extract. However, both extracts were significantly lower than the standard (ascorbic acid).

DPPH Radical Scavenging: The DPPH radical scavenging activities of aqueous and ethanol extracts of *A. boonei* stem-back and ascorbic acidis presented in Figure 6. Results showed that the ethanol extracts are the best inhibitors of the DPPH radical at higher concentrations (50-100 g/mL), and they were observed to have a dose-dependent increase in percentage inhibition as concentration increased.

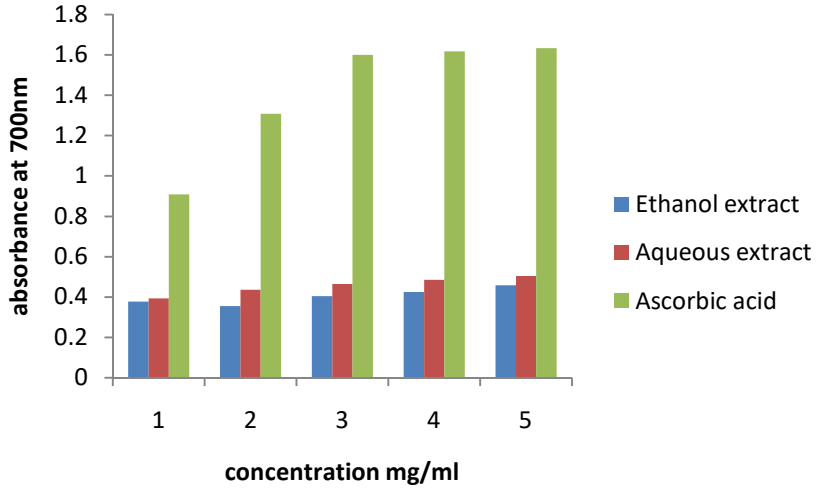

Fig. 5: Reducing Power Assay of the Aqueous and Ethanol Extracts of *A. boonei*. Values are mean ± SEM (n = 3). Different alphabets on the bars signifies significant difference at (p < 0 .0 5)

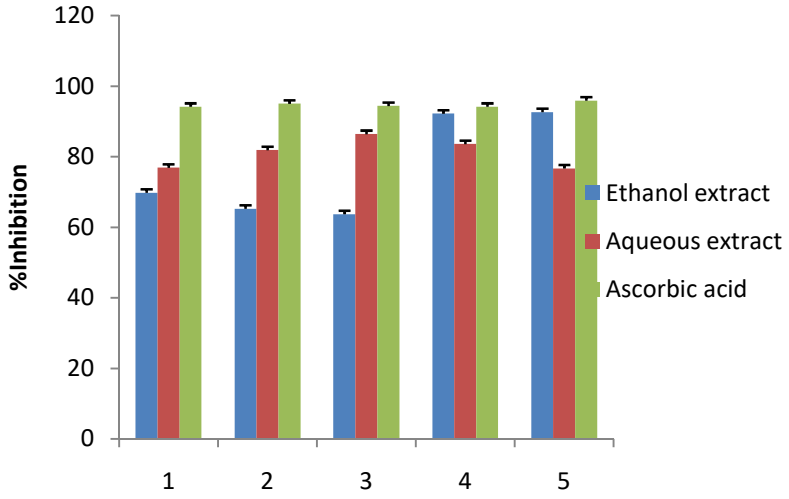

Fig.6: DPPH Radical Scavenging Activity of the Aqueous and Ethanol Extracts of *A. boonei.* Values are mean ± SEM (n = 3). Different alphabets on the bars signifies significant difference at (p < 0 .0 5).

DISCUSSION

The phytochemical analysis of aqueous and ethanol extracts of *A. boonei* stem-bark showed presence of alkaloids, terpenoids, cardiac glycosides, flavonoids, and phenol. Other researchs also reported presences of phytochemicals in previous studies(Ali *et al*., (2019); Otuu *et al*., 2020). Phytochemicals exhibit varied biochemical and pharmacological properties and have been shown to ameliorate diverse health issues because of its antioxidant activity (Ali *et al*., 2019).

The quantitative phytochemical analysis revealed significantly more proanthocyanidin, flavonoids and total phenol in ethanol extract compared to aqueous extract (Figures 1,2 and 3). The level of proanthocyanidins in plant extracts has been associated with several *in vitro*antioxidant activities. This result agrees with those recorded by other researcherswho reported the presence of some phytochemicals in different solvent extracts of the leaves, fruit or other plant parts of *A. boonei* and other plant species [Omoregie *et al*., (2014); Olubodun and Osagie, (2016); Olubodun *et al*., (2021)]. The observed differences may be a result of the differences in the plant materials, the variety of chemical constituents in the plant materials and their differing solubility properties in different solvents, the compounds to be isolated and/or methods of extraction (Olubodun *et al*., 2021).The study revealed that ethanol is better for extraction of phytochemicals in *A. boonei* stem-bark when compared with aqueous extraction

The potential antioxidant activities of phytochemicals were suggested to be the reason for their medical properties since oxidative stress is implicated in most disease conditions (Nkono *et al*., 2014). The antioxidant potential of aqueous and ethanol extracts of *A. boonei* estimated with the use of FRAP, reducing power estimation and DPPH radical scavenging activities showed that even though DPPH of both extracts were high, they were significantly lower ($p < 0.05$) than the ascorbic acid standard. The results of both extracts also revealed that they exhibited radical scavenging activities against DPPH with the ethanol extracts havinga significantly higher DPPH radical scavenging potential at increasing concentration compared to aqueous extract. At increasing extract concentration, there is a corresponding increase in the percentage DPPH scavenging activity of the ethanol extract when compared with the aqueous extracts. This increase in concentration brought about a decrease in the absorbance of the DPPH. This decrease is a result of free radicals mopping by the extracts. The results indicates that the extracts contains phytochemicals which may have the ability to remove free radicals and/or prevent them from occurring by donating hydrogen to stabilize the free radicals (Baliyan *et al.,* 2022), hence, reducing DPPH to DPPH.H complex that gives the characteristics yellow colour (Marrassini *et al.,* 2018; Olubodun *et al*., 2021). This observation in both extracts may be due to the variety of phytochemicals contained in the extracts and their differing solubility properties in the different solvents used for extraction (Truong *et al*., 2019; Olubodun *et al*., 2021). Ascorbic acid (the standard)on the other hand,had the highest DPPH radical scavenging activity when compared with both extracts. This may be due to the purity of the standard when compared with both crude extracts. This result showed that the extracts contain phytochemicals which may possess the ability to remove free radicals by reducing DPPH (Olubodun *et al*., 2021).

Ferric Reducing Antioxidant Potential (FRAP) is directly proportional to the molar concentration of antioxidants present in biological samples. The results revealed significant differences between both extracts

and ascorbic acid. While the aqueous extract significantly possess higher FRAP potential than the ethanol extract and standard, the ethanol extract was significantly lower($p < 0.05$) than the ascorbic acid FRAP value. Antioxidant activity may be regarded as a significant indicator of the reducing capacity of a compound. This is because the presence of reductants such as antioxidant substances in the sample, may cause the reduction of Fe^{3+} ferricyanidine to the ferric form (Rahman *et al.,* 2015). In the result of this study, there was a slight increase in the reductive potential as concentration increased, especially in the ethanol extract, while the aqueous extract maintained a relatively steady range. This study agrees with the study of other researchers who recorded that *A. boonei* leaves (Omoregie *et al*., 2014), root-bark possesses antioxidant effects (Obiagwu *et al*., 2014). This study therefore suggests that the antioxidant activity of the extracts of *A. boonei* may be responsible for its medicinal properties, hence its use for the management of different ailments.

This study revealed that *A. boonei* stem-bark extracts have significant amount of proanthocyanidin which is higher in the ethanol extract than the aqueous extract.The result indicates that aqueous extract of *A. boonei*stem-bark had lower phytochemical constituents but higher antioxidant activity when compared with the ethanol extract.The result of the comparative analysis in this study indicates that ethanol may be a better solvent for extraction of *A. boonei* stem-bark.

CONCLUSION

The study revealed that the aqueous extract of *A. boonei* stem-bark had lower phytochemical constituents but higher antioxidant activity compared to ethanol extract.The resultsalso revealed that ethanol may be a better solvent for phytochemical extraction of *A. boonei* stem-bark.

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