

Comparative study of the effect of two extraction solvents on polyphenols content and antioxidant activity of *Alstonia boonei* bark

Abstract

Oxidative stress (OS) is the imbalance between the generation of reactive oxygen species (ROS) and the body's antioxidant defenses in favor of ROS. ROS are chemical species that can be neutralized by natural phytochemicals like phenolic compounds present in medicinal plants and whose extraction depends on the solvent system used. Thus, the present study aimed to evaluate the effect of two extraction solvents on the polyphenols content and antioxidant activity of *Alstonia boonei* barks. For this, the bark of *A. boonei* were harvested, processed, dried and ground, then the powder was macerated in two solvent systems (water and water-ethanol). Subsequently, the quantitative phytochemical analysis of the different extractions was done by evaluating total polyphenols content (TPC), total flavonoids content (TFC) and alkaloids; followed by an in vitro evaluation of the antioxidants activity through radicals scavenging (DPPH and NO.) and the reducing power of ferric iron to ferrous iron. The relationship between antioxidant potential (DPPH, NO and FRAP) and polyphenols (total polyphenols and total flavonoids) was investigated using simple linear regression. It appears that the aqueous extract allowed better extraction of phenolic compounds from *A. boonei* bark; and exhibited better antioxidant activity. In addition, a strong correlation (r and R^2) was found between polyphenols (TPC and TFC) and antioxidant activity (DPPH, NO and FRAPP). Thus, we preferably recommend the use of water as a solvent for traditional preparations of *A. boonei* bark extract for therapeutic purposes.

Keywords: solvents, polyphenols, antioxidant, *Alstonia boonei* bark

INTRODUCTION

“Oxidative stress is defined as a disturbance of the intracellular oxidative status, induced either by excessive production of reactive oxygen species, or by a decrease in the body's antioxidant defense capacity” (Bonfont-Rousselot, 2014). “Reactive oxygen species are unstable chemical species such as free radicals, oxygen ions, peroxides, due to the presence of unpaired valence electrons in the outermost orbital” (Ziech et al., 2010). “They play an essential role in physiological functions such as gene expression, cell growth, anti-infectious defense and modulating

endothelial function” (Montezano and Touyz, 2012). In a state of stress, they are able to attack biological membranes and cause cell damage (DNA damage, protein glycation, lipid peroxidation) (Petramala et al., 2014). Nitric oxide radical (NO•) for example, reacts with the superoxide anion thus forming peroxynitrite (ONOO-); known to be involved in numerous damages at proteins, lipids and DNA level (Güngör et al., 2018). It's in this context that many studies have shown that limited oxidative stress would have beneficial effects in the management of a wide spectrum of diseases such as diabetes, neurodegenerative diseases and certain cancers (Shariq et al., 2016; Tangvarasittichai, 2015). For a good number of years, natural antioxidants, and more precisely polyphenols, have aroused growing interest from the scientific community (Takuissu et al., 2020). Indeed, more and more studies show and confirm many beneficial effects of polyphenols on various diseases management. This is how research has intensified on food / medicinal plants considered as an enormous source of multiple phytotherapeutic substances with strong antioxidant properties and that can be used to face oxidative stress and cell damage induced in living beings (Mégan et al., 2019). However, certain plants with biological activities such as *Alstonia boonei* have the characteristics to produce considerably low quantities of polyphenols; but with very high activity. Thus, the traditional use of such plants requires preparation from an appropriate solvent to maximize the extraction of phenolic compounds.

boonei, a plant of the Apocynaceae family, is widely used by populations due to the diversity of its geographical location. In traditional medicine, bark decoctions are used for cleaning wounds, fighting insomnia and diarrhea (Gbadamosi et al., 2011). Across the countries of the continent, *A. boonei* is known for its great reputation as an antimalarial in tropical countries (Funmilola et al., 2019). Our recent study revealed that the aqueous extract from the bark of this plant was able to positively modulate hepatic DNA methylation in diabetic rats (Fonkoua et al., 2021). In order to improve and promote the traditional use of this plant, the identification of an appropriate solvent system allowing maximum concentration of total polyphenols is essential. It's in this order of idea that the objective of this study was to evaluate the effect of two extraction solvents (Generally used traditionally for the formulation of *Alstonia boonei* bark for therapeutic purposes) on polyphenols content and antioxidant activity of *Alstonia boonei* bark.

2. Material and methods

2.1. Chemicals and reagents

Quinin, DPPH, folin-ciocalteu's phenol reagent, gallic acid, quercetin, sodium phosphate dibasic, Acetate de potassium, Trichlorure d'aluminium, 1,10-phénanthroline, Nitroprussiate de sodium, Acide sulfonique and Naphtyléthylène diamine (NED) were purchased from Sigma-Aldrich (GmbH, Sternheim, Germany). Iron (III) chloride 6-hydrate and iron (II) sulfate 7-hydrate and ethanol were obtained from Merck (Darmstadt, Germany).

2.2. Plant material

The plant material was consisted of the bark of *Alstonia boonei* harvested in May 2020 in the Center Region, Cameroon (locality of Mbaligui). The plant has been identified in the National Herbarium of Cameroon under the number 43368/HNC by referring to volume 1 of the flora of Cameroon. Once at the Laboratory of Nutrition and Nutritional Biochemistry (LNNB), they were cleaned, dried at room temperature until a constant weight was obtained, then ground in a blender. Two extracts were prepared by maceration of *A. boonei* bark in water and water-ethanol in order to find the best solvent for extracting the bioactive compounds of interest.

2.3. Aqueous and hydroethanolic maceration

A 400 g powder was each macerated in 4000 mL of distilled water (ratio 1/10 w/v) and in 4000 mL of water-ethanol (1:1, v/v) for 24h and 48h respectively at room temperature. The whole was collected and filtered using Whatman paper number 3 (Whatman International Limited, Ken, England). The filtrates obtained were evaporated in an oven (WGLL-65BE) at 50°C for 72 hours. The powder obtained was stored in polyethylene bags to avoid rehydration.

2.4. Quantification of bioactive compounds of interest

The bioactive compounds that were determined in the two extracts were: total polyphenols, flavonoids and alkaloids.

Assay of total phenolic compounds

The polyphenol content of the extracts was evaluated using the protocol described by **Singleton and Rossi (1965)**. Briefly, thirty microliters (30 μ L) of extracts (1 mg/mL) prepared in 95% ethanol were added to 1 mL of Folin's solution (0.2 N), the absorbance was measured at 750 nm after 30 minutes incubation at 25°C using a spectrophotometer. Gallic acid was used as the standard. The total polyphenol content was calculated from the calibration range equation established

with gallic acid ($y = 0,0012x$; $R^2 = 0,894$) and expressed in micrograms of gallic acid equivalents per milligram of extract (μg of EAg/mg of extract).

Assay of total flavonoids (TFC)

The flavonoid content of the extracts was evaluated using the protocol described by **Aiyegoro and Okoh (2010)**. A volume of 1 mL of extracts prepared in 95% ethanol at a concentration of 1 mg/mL was added to 1 mL of AlCl_3 (10%), 1 mL of potassium acetate (1 M) and 5,6 mL of distilled water. After 30 minutes of incubation at 25°C , the absorbance was read at 430 nm using a spectrophotometer. Quercetin was used as the standard. The flavonoid content was calculated from the calibration range equation established with quercetin ($y = 0.0024x$; $R^2 = 0.977$) and expressed in micrograms of quercetin equivalents per milligram of extract (μg of Quer/mg of extract).

Assay of alkaloids

The alkaloids content of the extracts was carried out using the protocol described by **Singh et al, (2004)**. A mass of 100 mg of extracts powder was subjected to extraction in 10 mL of 95% ethanol, then filtered and centrifuged at 5000 g for 10 minutes. In the supernatant obtained, 1mL was taken to which was added 1mL of $[\text{FeCl}_3$ (0.025 M) + HCl (0.5 M)] and 1mL of 1.10 Phenanthroline (0.05 M) prepared in ethanol. The mixture obtained was incubated in a water bath for 30 minutes with the temperature maintained at $70 \pm 2^\circ\text{C}$. The absorbance of the red color of the complex formed was read at 510 nm against white. Quinine was used as the standard. The alkaloid content was calculated from the calibration range equation established with quinine ($y = 0,447x$; $R^2 = 0,9967$) and expressed in micrograms of quinine equivalents per milligram of extract (μg of Qui/mg of extract).

2.5. Antioxidant potential of extracts

The antioxidant potential of the extracts was evaluated through 2 mechanisms: scavenging of free radicals and Iron³⁺ reduction.

2.5.1. Radical scavenging assays

The antiradical activity was evaluated on 2 radicals: one synthetic (DPPH*) and the other biological (NO•).

DPPH scavenging activity

The antiradical activity of extracts prepared in ethanol at different concentrations (2; 2.5; 3; 3.5 and 4 mg/mL) was evaluated using the DPPH method (**Katalinié et al., 2004**). For this, a volume of 50 μL of each extract sample at different concentrations

was added to 1.950 mL of the freshly prepared methanolic solution of DPPH (0.3 mM). The mixture was incubated in the dark for 30 minutes. The control consisting of DPPH without extracts was treated under the same experimental conditions as the tests. Absorbance was read at 515 nm using a spectrophotometer.

Nitric oxide (NO.) scavenging activity

During the experiment, the nitric oxide produced by sodium nitroprusside at physiological pH reacts with oxygen to give nitrite ions which are detected by Greiss' reagent (**Green et al., 1982**). To 0.5mL of extracts prepared in methanol at different concentrations (2; 2.5; 3; 3.5 and 4 mg/mL) were added 2mL of sodium nitroprusside solution (5mM) prepared in phosphate buffer (pH 7.4; 50mM), and the whole was incubated at 25° C. for 5 h. Subsequently, 0.5 mL of each mixture was transferred into tubes corresponding to each sample to which was added 1 mL of NED reagent (1% sulfanilamide, 2% ortho phosphoric acid and 0.1% naphthylene diamine dihydrochloride). Absorbance was read at 540nm by spectrophotometry.

At the end of these antiradical tests, the scavenging percentages were calculated according to the following formula:

$$\% \text{ Radical scavenging} = [(\text{Control Abs} - \text{Sample Abs}) / \text{Control Abs}] \times 100.$$

The scavenging concentrations 50 (SC₅₀): concentrations which trap 50% of the radicals, were calculated from the graph of the scavenging percentages as a function of the concentrations of extracts.

2.5.2. Reducing power assay

Reducing power of Iron III

The reducing power of the extracts was evaluated through their ability to reduce ferric iron to ferrous iron (**Oyaizu et al., 1986**). To 1,25 mL of phosphate buffer (pH 6,6; 200 mM), were added 1,25 mL of potassium ferrocyanide (1%) and 0,5 mL of each extract prepared at different concentrations (2; 2,5; 3; 3,5 and 4 mg/mL). The whole was incubated at 50°C for 20 min. Subsequently, 2,5 mL of trichloroacetic acid (10%) was added and the reaction mixture was centrifuged at 3000g for 10 min. Then 1,25 mL of the supernatant was mixed with 1,25 mL of distilled water and 0,25 mL of iron chloride (0,1%). The absorbance of the final solution was read at 700 nm against the blank prepared in parallel by replacing the extract with distilled water. The results were expressed as percentage reduction according to the formula:

$$\text{Reduction of Fe}^{3+} (\%) = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100$$

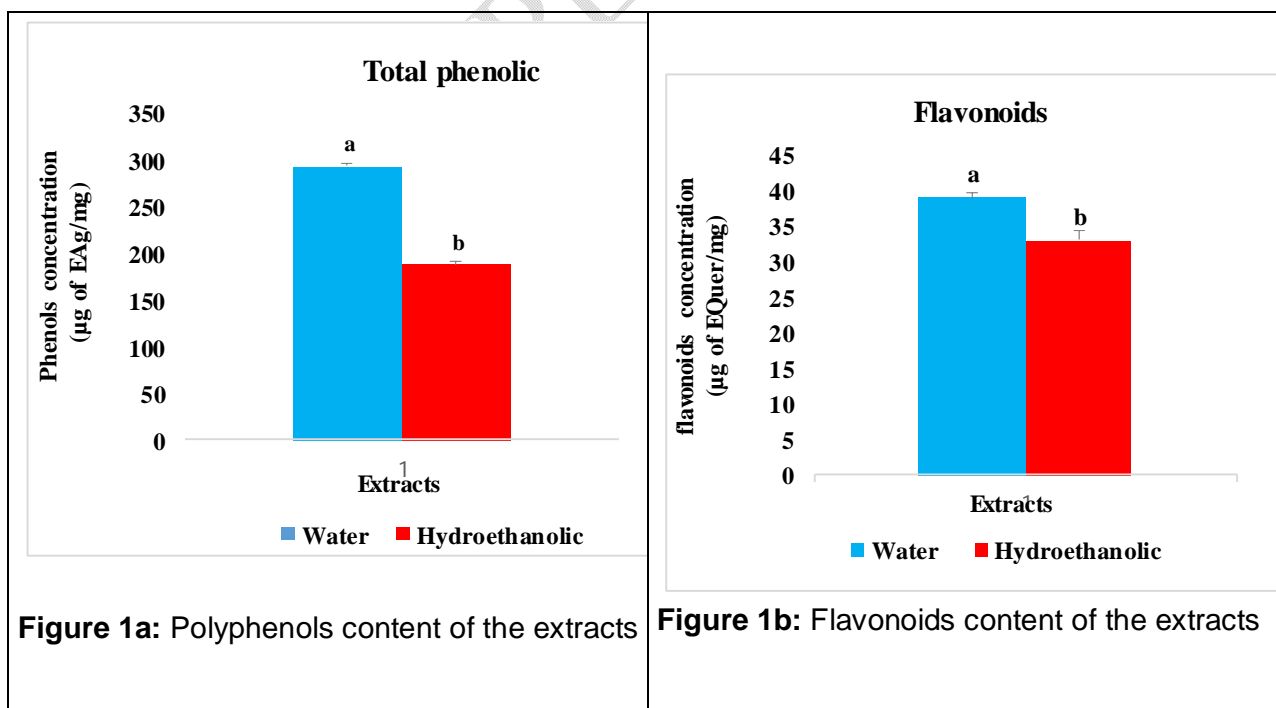
2.6. Data processing and analysis

Microsoft Excel software was used to process the data and draw the graphs. SPSS (Statistical Package for Social Science) software version 20.0 for Windows was used for the statistical analysis of the results. One-factor ANOVA (Analysis Of Variance) test followed by a post-hoc test (LSD) was performed to compare the means. Correlation analyzes among different parameters were also performed using both correlation coefficient (r) and coefficient of determination (R^2). All results with $p < 0.05$ were considered significant. Results were expressed as the mean \pm standard error of the mean.

3. Results

3.1. Effect of solvents on bioactive compounds contents in *A. boonei* bark

Total phenols, flavonoids and alkaloids contents of the aqueous and hydroethanolic extract are represented in figure 1 below. The aqueous extract shows significantly high levels of phenols and flavonoids (293.33 μg of EAg/mg and 38.88 μg of EQer/mg of extract) compared to the hydroethanolic extract (187.5 μg of EAg/mg and 32.91 μg of EQuer/mg of extract). However, the alkaloid contents were significantly higher in the hydroethanolic extract (209.26 μg QuiE/mg extract) compared to the aqueous extract (208.13 μg QuiE/mg extract).



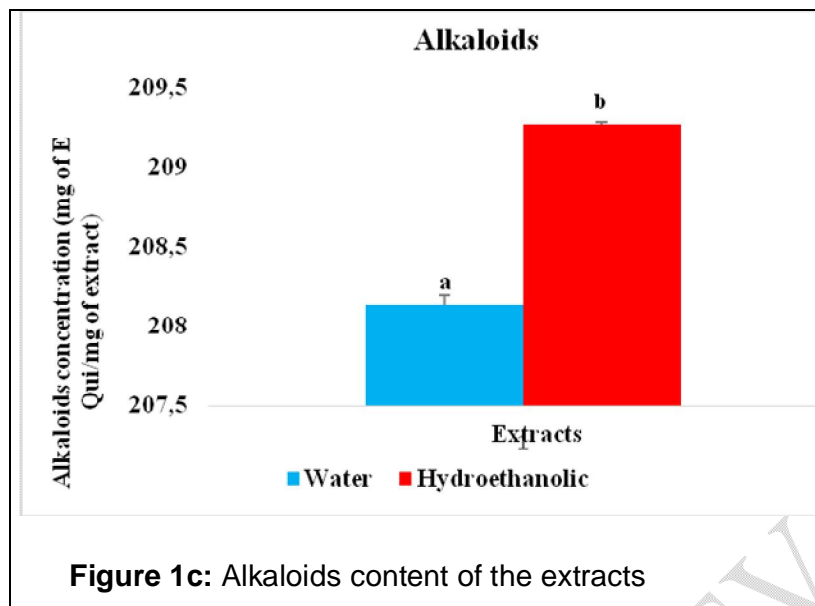
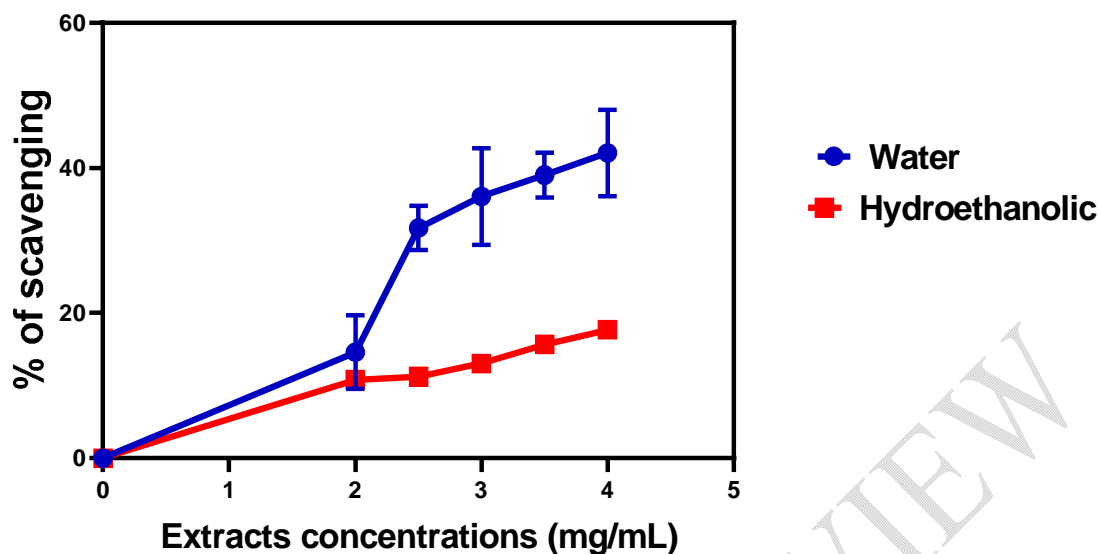


Figure 1: Effect of different solvents on total phenolic, flavonoid and alkaloids content of *A. boonei* barks

EAg: Gallic acid equivalent; EQuer: Quercetin equivalent; EQui: Quinine equivalent. Values are expressed as mean \pm standard error. The values assigned different letters (a, b) are significantly different ($P < 0.05$).

3.2. Effect of different solvents on DPPH scavenging activity of *A. boonei* barks

Study of the capacity of the extracts to scavenge the DPPH radical according to the concentration (figure 2) showed that the DPPH antiradical activity was dose dependent with scavenging percentages between 14.61% and 42.08% for the aqueous extract, against 9.90% and 17.69% for the hydroethanolic extract. The aqueous extract presented scavenging percentages higher than that of the hydroethanolic extract.

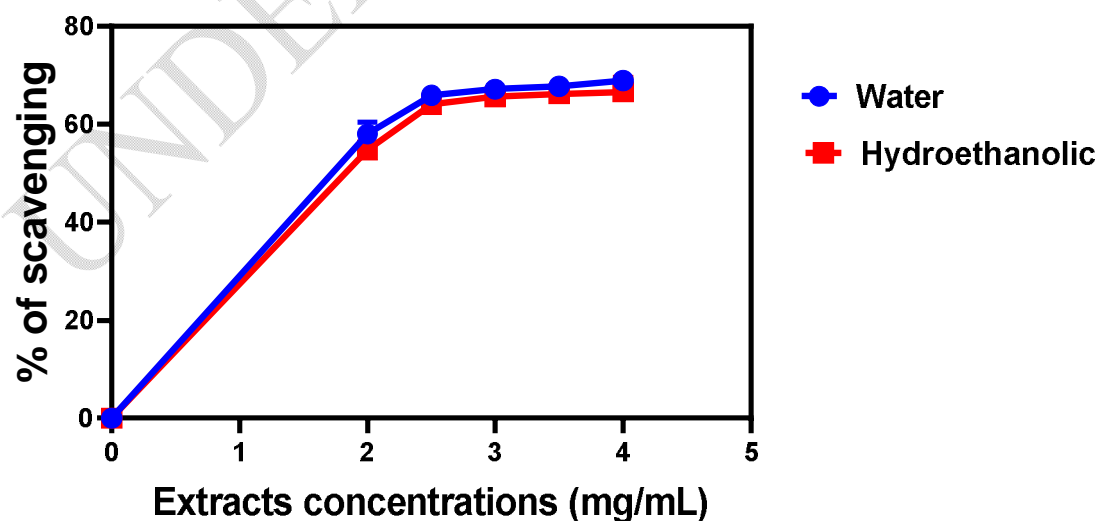


Results were expressed as the mean \pm standard error of the mean.

Figure 2: Effect of different solvents on DPPH radical scavenging activity of *A. boonei* barks

3.3. Effect of different solvents on nitric oxide (NO \cdot) radical scavenging activity of *A. boonei* barks

The ability of extracts to scavenge the NO \cdot radical. at different concentrations was explored and recorded in Figure 3. It appears that the extracts scavenged the NO \cdot radical in proportion to the concentrations. The scavenging percentages varied from 65.03% to 68.90% for the aqueous extract, against 60.45% to 66.58% for the hydroethanolic extract. However, the aqueous extract resulted in better activity.



Results were expressed as the mean \pm standard error of the mean

Figure 3 : Effect of different solvents on nitric oxide (NO·) radical scavenging activity of *A. boonei* barks.

The scavenging concentrations 50 (SC₅₀) on the DPPH and NO radicals have been summarized in Table 1 below.

Table 1: SC₅₀ of the two solvents on the DPPH radicals. and No

SC ₅₀ DPPH (mg/mL)		SC ₅₀ NO· (mg/mL)	
Water	Hydroethanolic	Water	Hydroethanolic
7,58 ^a	201,2 ^b	2,53 ^a	2,67 ^b

SC₅₀: Scavenging concentration 50. values assigned different letters (a and b) are significantly different

3.4. Effect of the two solvents on the ferric ion reducing power (FRAP) of *A. boonei* bark

The ferric iron reducing power of the extracts at different concentrations is shown in Table 2 below. It appears that the reduction percentage is proportional to the concentrations of extracts, and varying from 51.98% to 68.35% for the aqueous extract, against 48.42% to 62.12% for the hydroethanolic extract. The aqueous extract showed a better reducing activity with a reduction concentration 50 of 2.91mg/mL against 3.46 mg/mL for the hydroethanolic extract.

Table 2: Effect of different solvents on FRAP reducing power activity

Concentration	2 mg/mL	2.5 mg/mL	3 mg/mL	3.5 mg/mL	4 mg/MI
Water	51.98± 0.11	57.60±0.04	61.38±0.09	65.54±0.04	68.35±0.05
Hydroethanolic	48.42 ± 0.14	51.02 ±0.13	53.05±0.51	57.99 ± 0.13	62.12±0.33

Values are expressed as mean ± standard error

3.5. Correlation between polyphenols contents and DPPH scavenging activity

To assess the relationship between antioxidant potential (DPPH scavenging activity) and polyphenols (TPC and TFC); the correlation coefficient (r) and the coefficient of determination (R²) between DPPH scavenging activity of the extracts and polyphenols (TPC and TFC) were studied using simple linear regression. By comparing these two solvents; a strong correlation (r and R²) was noted between

polyphenols (TPC and TFC) and DPPH scavenging activity in aqueous extract (Figure 4).

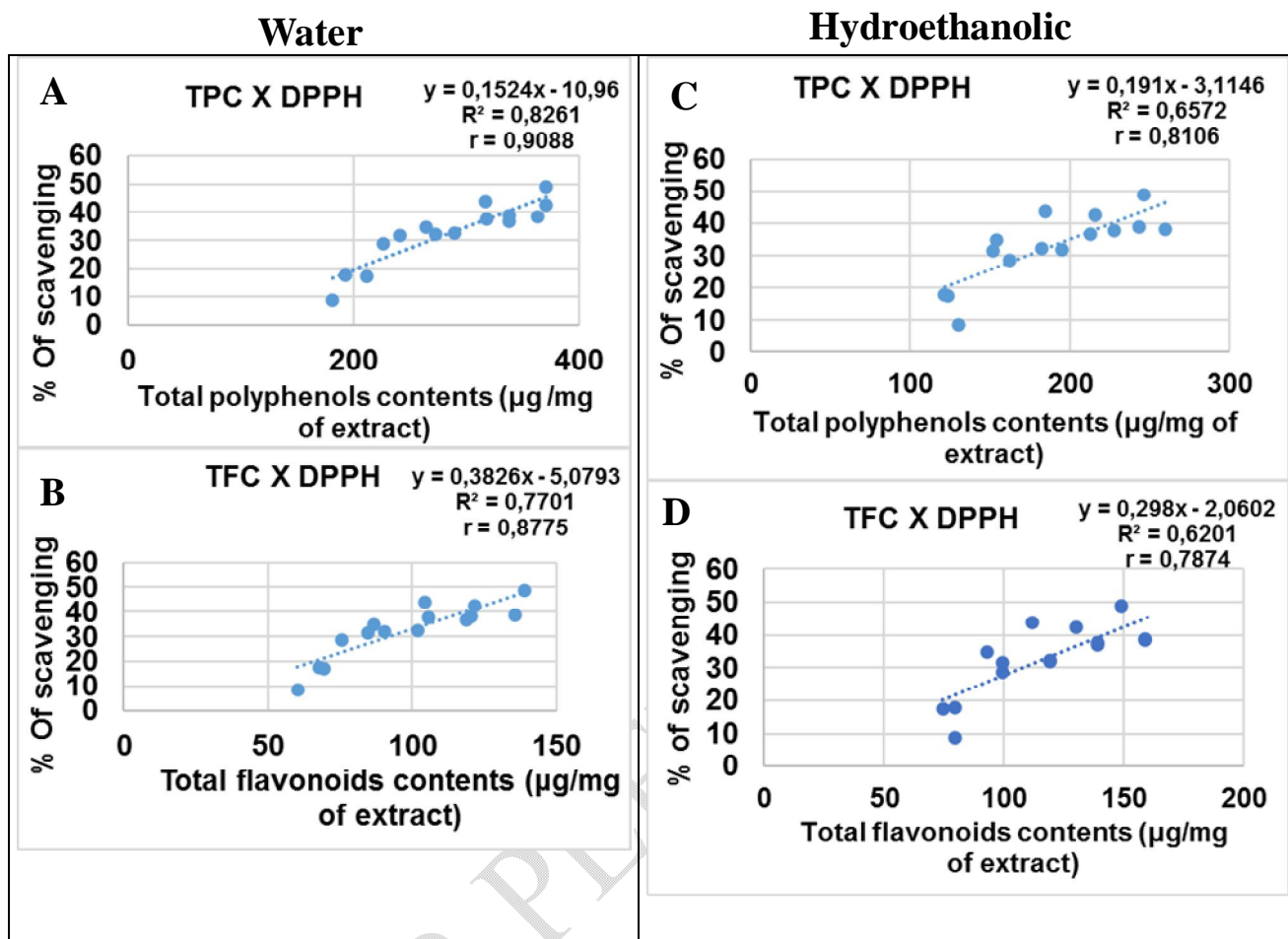


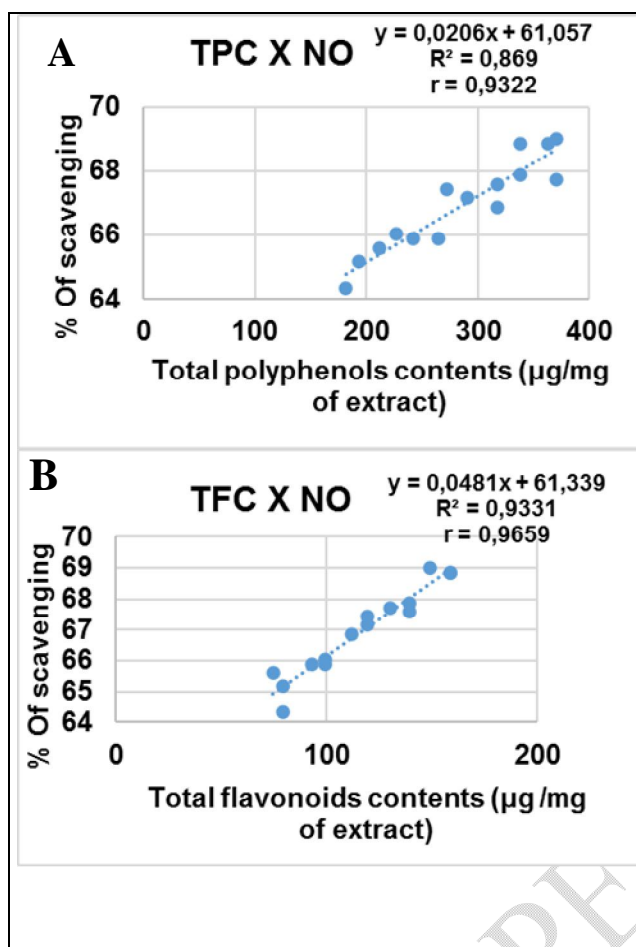
Figure 4: Correlation between total phenolic, total flavonoids contents and DPPH radical scavenging using different solvent extracts of *A. boonei*.

TPC: Total polyphenols contents; TFC: Total flavonoids contents

3.6. Correlation between polyphenols contents and nitric oxide scavenging activity

Figure 5 represents the relationship between antioxidant potential (nitric oxide scavenging activity) and polyphenols (TPC and TFC). By comparing these two solvents; a strong correlation (r and R^2) was noted between polyphenols (TPC and TFC) and nitric oxide scavenging activity in aqueous extract of *A. boonei* bark (Figure 5).

Water



Hydroethanolic

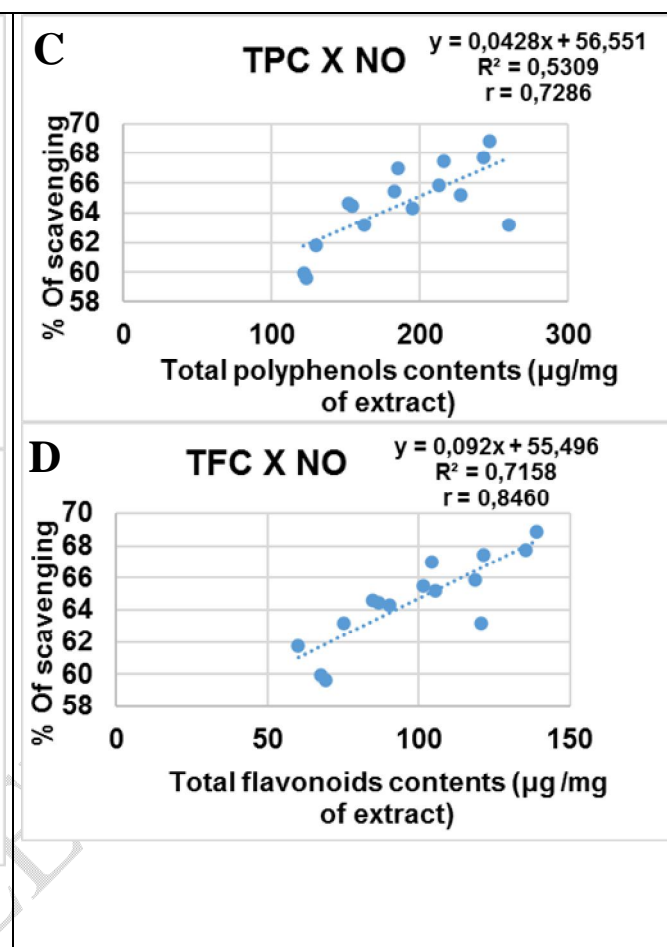


Figure 5: Correlation between total phenolic content, total flavonoids contents and nitric oxide radical scavenging using different solvent extracts of *A. boonei*.

TPC: Total polyphenols contents; TFC: Total flavonoids contents

3.7. Correlation between polyphenols contents and ferric ion reducing power

Figure 6 represents the relationship between antioxidant potential (nitric oxide scavenging activity) and polyphenols (TPC and TFC) using simple linear regression. By comparing these two solvents; a strong correlation (r and R^2) was noted between polyphenols (TPC and TFC) and ferric ion reducing power in aqueous extract of *A. boonei* bark (Figure 5). Similarly, in the hydroethanolic extract showed strong correlation with the ferric ion reducing power.

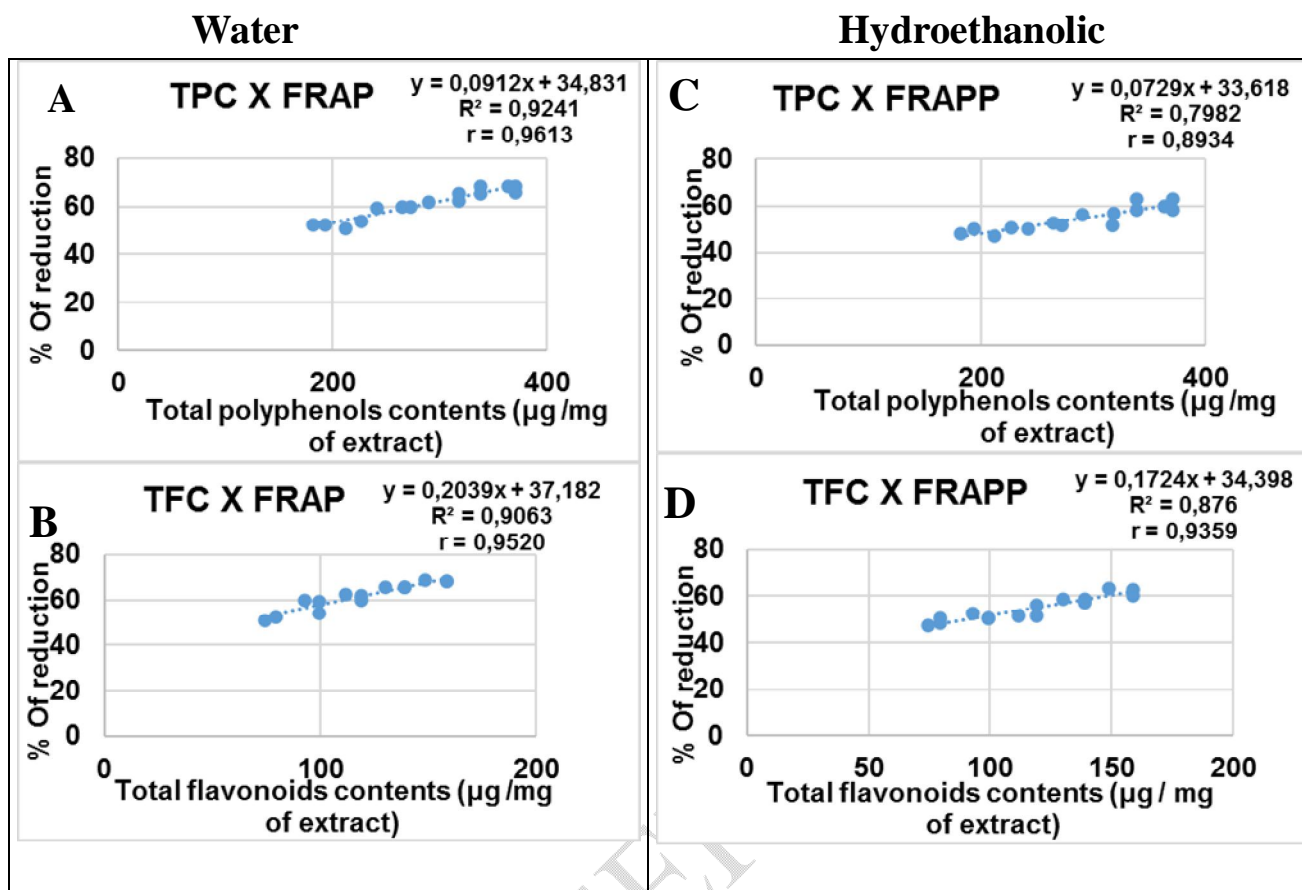


Figure 6: Correlation between total phenolic, total flavonoids contents and ferric ion reducing power using different solvent extracts of *A. boonei*.

TPC: Total polyphenols contents; TFC: Total flavonoids contents

4. Discussion

The antioxidant activity of a plant is most often attributed to its polyphenol contents, in particular flavonoids (Ighodaro and Akinloye, 2017). However, a better extraction of these metabolites requires an appropriate solvent. The present study was to evaluate the effect of two extraction solvents on the polyphenol contents and antioxidant activity of *Alstonia boonei* barks. After aqueous and hydroethanolic maceration of *A. boonei* bark; the analysis of total phenols, flavonoids and alkaloids of the extracts revealed significantly high contents of total polyphenols and flavonoids and low alkaloids in the aqueous extract compared to the hydroethanolic extract (figure 1). This would be explained by the fact that polyphenols by their structure are more soluble in polar solvents (Sara et al., 2020). Indeed, due to the presence of heteroatoms, they can easily form hydrogen bonds with water molecules, thereby promoting their solubility (Bourgou et al., 2016). Although studies like Muhammad et al. (2016) demonstrated that the hydroalcoholic solvent is very suitable for the

extraction of phenolic compounds from a large number of plants such as halophyte plants; our results show that compared to the hydroethanolic extract, water is the most appropriate solvent for the extraction of phenolic compounds from *A. boonei* barks.

The nature of the solvent can influence the polyphenol content in a plant extract; and consequently, have repercussions on its antioxidant potential (**Roby et al., 2013**). Thus, the evaluation of the effect of the two solvents on antioxidant activity of the barks of *A. boonei* reveal that polyphenols and flavonoids of the aqueous extract showed a very strong correlation with the antiradical activity DPPH and NO as well as the reducing power of Iron III (figure 4, 5 et 6). This result is similar to that of **Obame-Engonga et al. (2019)** which showed that the aqueous extract of *A. boonei* barks showed better antioxidant activity compared to the methanolic extract. In addition, several scientific research works have shown that the antioxidant activity of a plant is correlated with its flavonoid content (**Sara et al., 2020; Fredes et al., 2014; Katalinic et al., 2013**). "Indeed, due to the redox potential associated to the OH groups of flavonoids, they can easily supply a proton and/or an electron, thus scavenging the DPPH• and NO• radicals and giving rise to stable compounds" (**Procházková et al., 2011**). Thus, in general, the study reveals that the aqueous extract allows better extraction of phenolic compounds from *A. boonei* bark; and exhibits better antioxidant activity.

Conclusion

The results of this study reveal that water is a good solvent for extracting compounds with antioxidant attributes from *A. boonei* barks. Thus, due to the multiple beneficial effects of *A. boonei* in traditional management of a broad spectrum of diseases; we preferably recommend the use of water as a solvent when preparing traditional *A. boonei* bark maceras. Additive work is needed to extend the study using multiple solvent systems.

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