



# Comparative Study of the Effect of Two Extraction Solvents on Polyphenols Content and Antioxidant Activity of *Alstonia Boonei* Bark

Marielle Anastasie Zali <sup>a</sup>, Martin Fonkoua <sup>a\*</sup>,  
Charles Dakole Daboy <sup>b</sup>, William Arnold Tazon <sup>a</sup>,  
David Goda <sup>a</sup>, Dupon Ambamba Akamba <sup>a</sup>,  
Jules Vidal Nanhah <sup>a</sup> and Judith Laure Ngondi <sup>a</sup>

<sup>a</sup> Laboratory of Nutrition and Nutritional Biochemistry, Department of Biochemistry, The University of Yaoundé 1, Cameroon.

<sup>b</sup> Department of Biochemistry, Faculty of Sciences, The University of Yaoundé 1, Cameroon.

## Authors' contributions

This work was carried out in collaboration among all authors. Author MF designed the work. Authors WAT, MAZ, DG, DAA and JVN conducted the work, collected and analyzed the data. Authors FM, CDD and WAT drafted the manuscript and author JLN revised it critically. All authors agree to be accountable for all aspects of the work.

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## 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

\*Corresponding author: E-mail: fonkoua\_m@yahoo.fr;

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.

## 1. 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” [1]. “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” [2]. “They play an essential role in physiological functions such as gene expression, cell growth, anti-infectious defense and modulating endothelial function” [3]. In a state of stress, they are able to attack biological membranes and cause cell damage (DNA damage, protein glycation, lipid peroxidation) [4]. 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 [5]. 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 [6,7]. For a good number of years, natural antioxidants, and more precisely polyphenols, have aroused growing interest from the scientific community [8]. 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 [9]. 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.

*A.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 [10]. Across the countries of the continent, *A. boonei* is known for its great reputation as an antimalarial in tropical countries [11]. 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 [12]. 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. MATERIALS 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 Naphtyléthylene 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

400g of 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.

### 2.4.1 Assay of total phenolic compounds

The polyphenol content of the extracts was evaluated using the protocol described by Singleton and Rossi [13]. Briefly, thirty microliters (30  $\mu$ 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 ( $\mu$ g of EAg/mg of extract).

### 2.4.2 Assay of total flavonoids (TFC)

The flavonoid content of the extracts was evaluated using the protocol described by Aiyegoro and Okoh [14]. 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 ( $\mu$ g of Quer/mg of extract).

### 2.4.3 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 [ $FeCl_3$  (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  $70 \pm 2^\circ C$ . 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 ( $\mu$ g 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 Iron<sup>3+</sup> 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 [15]. For this, a volume of 50  $\mu$ L 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 [16]. To 0.5 mL of extracts prepared in methanol at different concentrations (2; 2.5; 3; 3.5 and 4 mg/mL) were added 2 mL of sodium nitroprusside solution (5mM) prepared in phosphate buffer (pH 7.4; 50 mM), 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 540 nm 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 (SC<sub>50</sub>): 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<sup>2</sup>). All results with p < 0.05 were considered significant. Results were expressed as the mean ± 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 Fig. 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).

### 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 (Fig. 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.

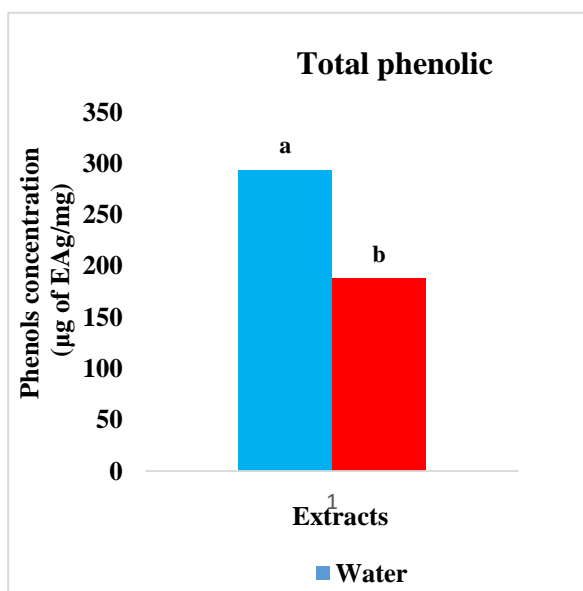


Fig. 1a. Polyphenols content of the extracts

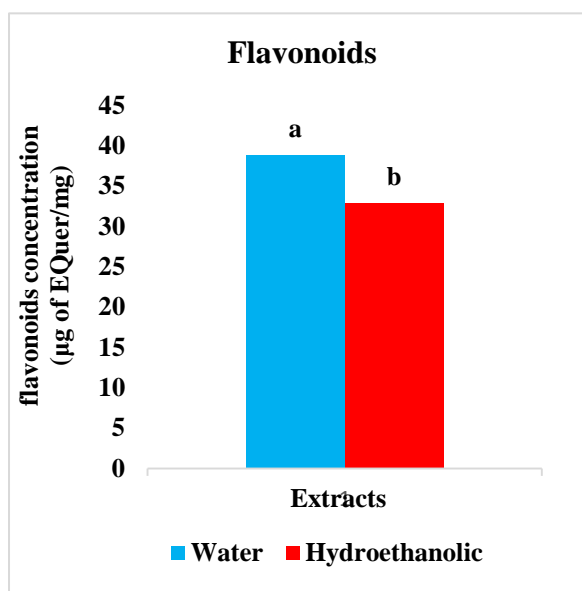


Fig. 1b. Flavonoids content of the extracts

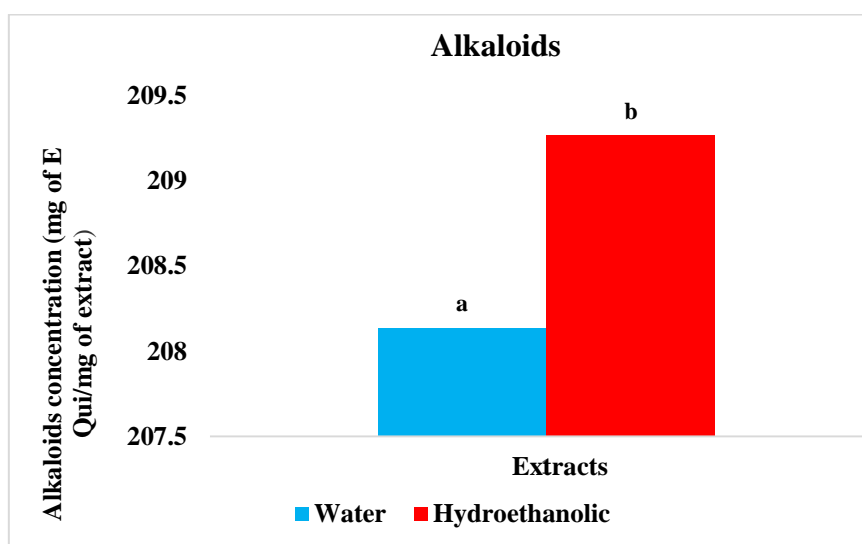


Fig. 1c. Alkaloids content of the extracts

Fig. 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 ( $P < 0.05$ )

### 3.3 Effect of Different Solvents on Nitric Oxide (NO.) 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 Fig. 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.

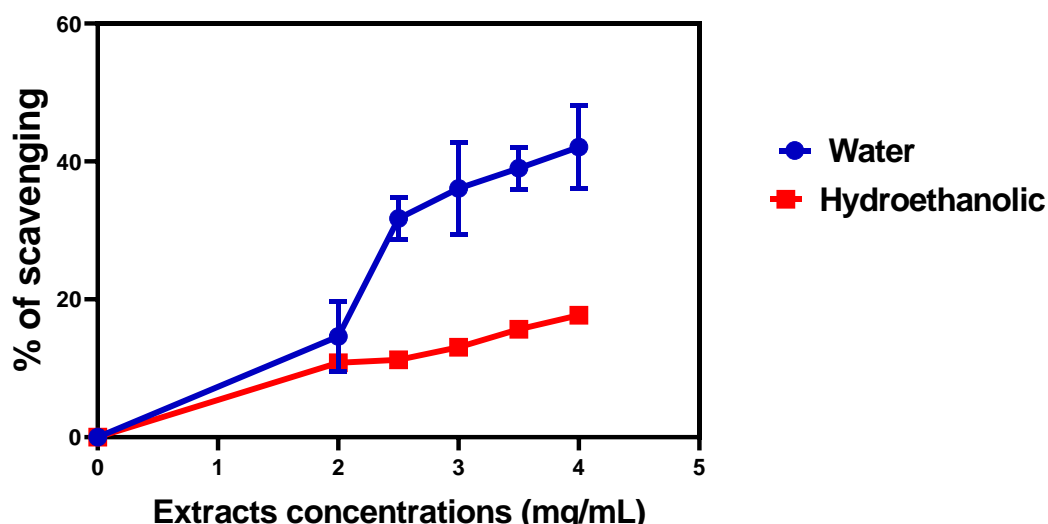


Fig. 2. Effect of different solvents on DPPH radical scavenging activity of *A. boonei* barks  
Results were expressed as the mean  $\pm$  standard error of the mean

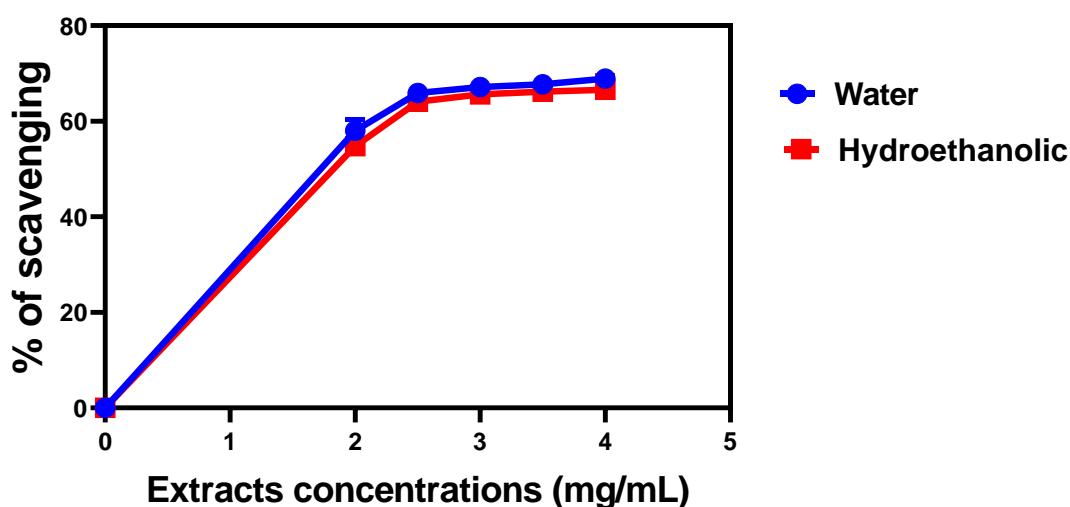


Fig. 3. Effect of different solvents on nitric oxide (NO.) radical scavenging activity of *A. boonei* barks  
Results were expressed as the mean  $\pm$  standard error of the mean

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

Table 1.  $SC_{50}$  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 <sup>a</sup>	201,2 <sup>b</sup>	2,53 <sup>a</sup>	2,67 <sup>b</sup>

$SC_{50}$ : 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.

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

### 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 ( $R^2$ ) 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  $R^2$ ) was noted between

### 3.6 Correlation between Polyphenols Contents and Nitric Oxide Scavenging Activity

Fig. 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 (Fig. 5).

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

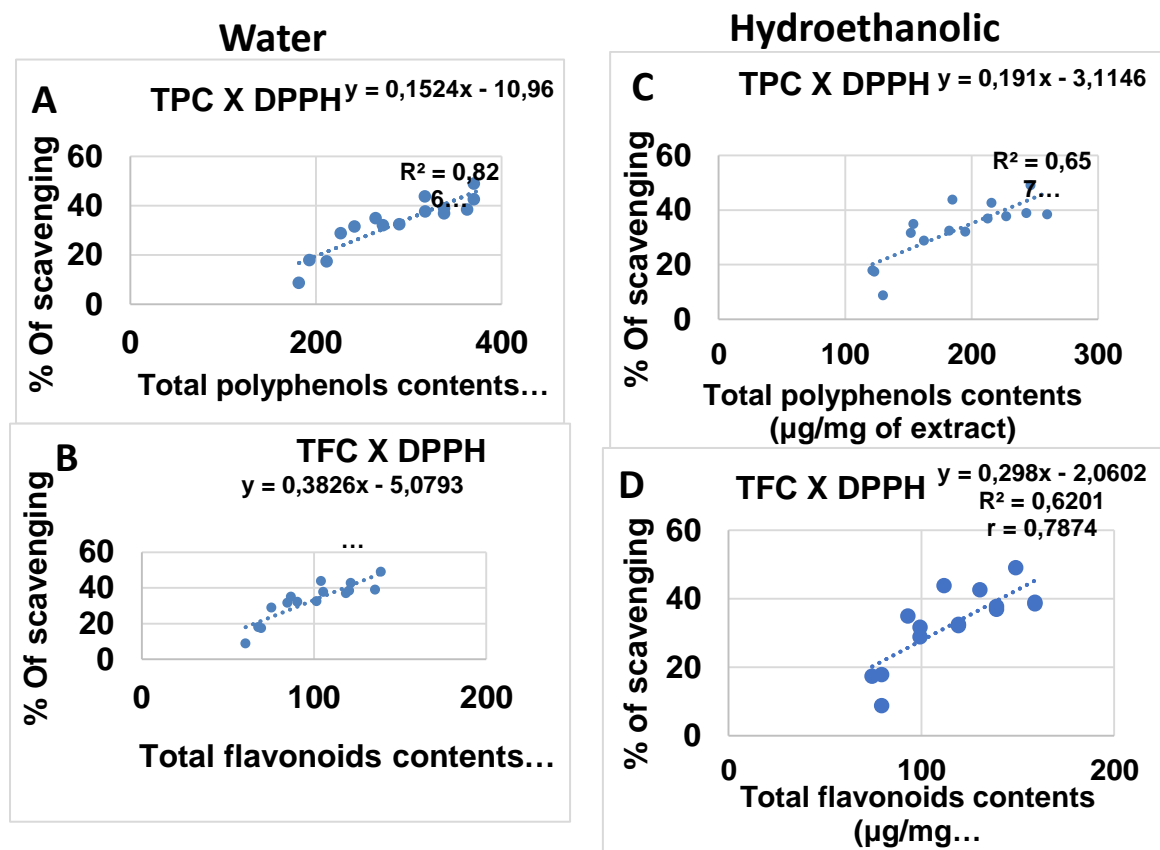


Fig. 4. Correlation between total phenolic, total flavonoids contents and DPPH radical scavenging using different solvent extracts of *A. boonei*  
 TPC: Total polyphenols contents; TFC: Total flavonoids contents

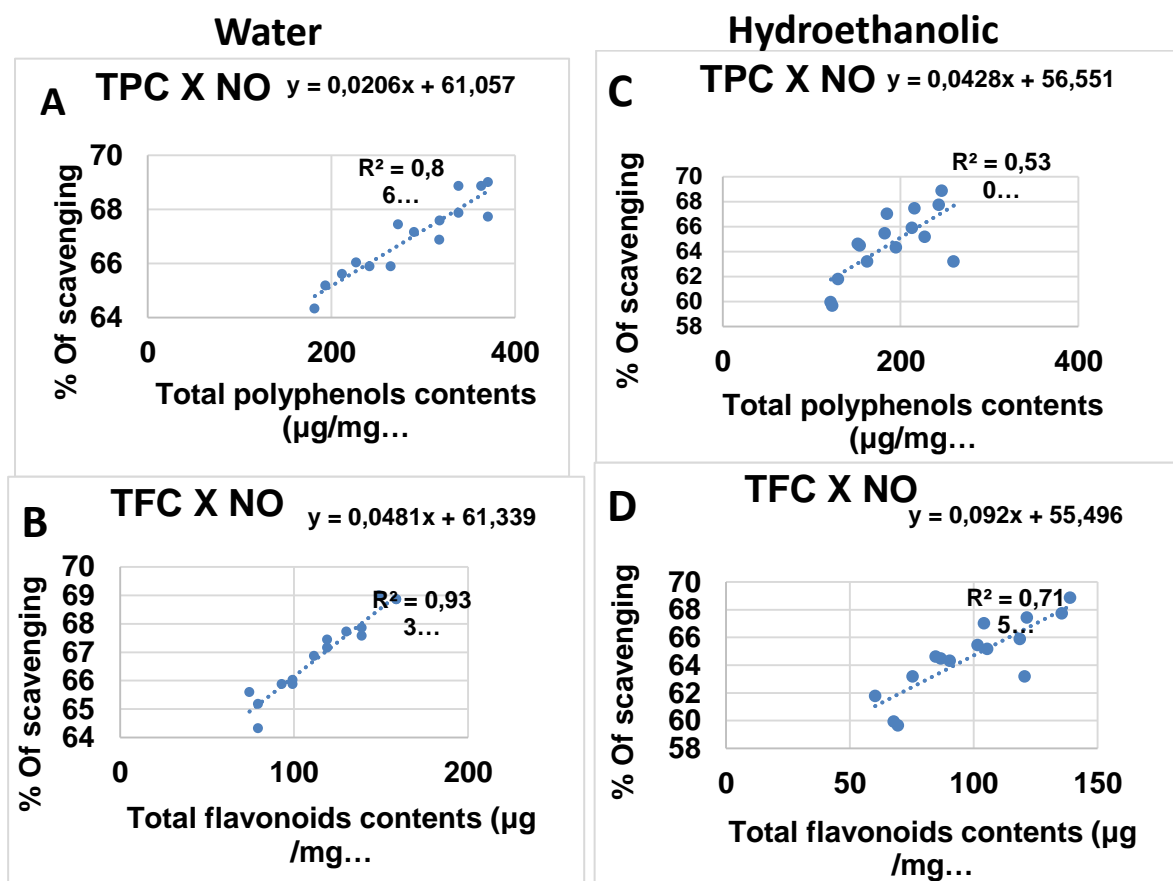


Fig. 5. Correlation between total phenolic content, total flavonoids contents and nitric oxide radical scavenging using different solvent extracts of *A. boonei*  
 TPC: Total polyphenols contents; TFC: Total flavonoids contents

### 3.7 Correlation between Polyphenols Contents and Ferric Ion Reducing Power

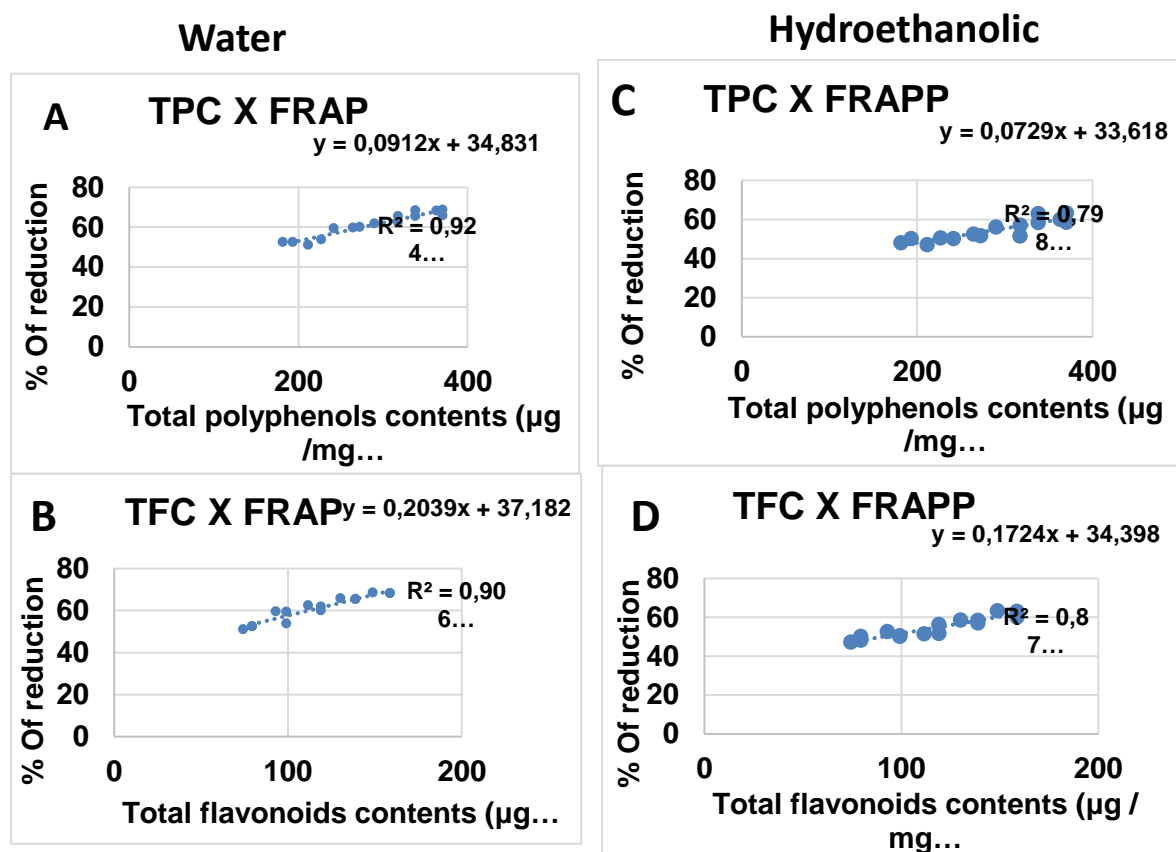
Fig. 6 represents the relationship between antioxidant potential (nitric oxide scavenging activity) and polyphenols (TPC and TFC) using simple linear regression. By comparing these two solvents; a strong correlation ( $r$  and  $R^2$ ) was noted between polyphenols (TPC and TFC) and ferric ion reducing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

## 4. DISCUSSION

The antioxidant activity of a plant is most often attributed to its polyphenol contents, in particular flavonoids [17]. 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 (Fig. 1). This would be explained by the fact that polyphenols by their structure are more soluble in polar solvents [18]. Indeed, due to the presence of heteroatoms, they can easily form hydrogen bonds with water molecules, thereby promoting their solubility [19]. Although studies like the one of Muhammad *et al.* [20] 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.





**Fig. 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

The nature of the solvent can influence the polyphenol content in a plant extract; and consequently, have repercussions on its antioxidant potential [21]. 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 (Figs. 4, 5 et 6). This result is similar to that of Obame-Engonga et al. [22] 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 [18,23,24]. "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" [25]. 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.

## 5. 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 macerates. Additive work is needed to extend the study using multiple solvent systems.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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