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

Abstract

Oxidative stress (SO) is the imbalance between the generation of reactive oxygen species (REO) and the body's antioxidant defenses in favor of EOR. EORs are chemical species that can be neutralized by natural phytochemicals like the 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 is done by the evaluation of total polyphenols content (TPC), total flavonoids content (TFC) and alkaloids; followed by an in vitro evaluation of theantioxidants activity throughradicals 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 R2) 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 state, induced either by excessive production of reactive oxygen species, or by a decrease in the body's antioxidant defense capacity **(Bonnefont-Rousselot, 2014)**. Reactive oxygen species are unstable chemical species such as free radicals, oxygen ions, peroxides, made chemically very reactive by 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)**.The NO• radical, for example, reacts with the superoxide anion thus forming peroxynitrite (ONOO-); responsible for numerous damages at the level of proteins, lipids and DNA **(Güngör et *al*., 2018)**. It is 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 mainly polyphenols, have aroused growing interest from the scientific community **(Takuissu et al., 2020)**. Indeed, more and more studies show and confirm the many beneficial effects of polyphenols on the prevention of various diseases. This is how research has intensified on food / medicinal plants considered as an enormous source of multiple phytotherapeutic substances endowed with antioxidant activities and which can be the weapon allowing to face oxidative stress and its damage at the level of the organs of living beings **(Mégan et *al*., 2019)**. However, certain plants endowed with biological activities like Alstonia boonei havethe characteristics to produce considerably low quantities of polyphenols; but with very highactivity. 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 thanks 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)**. A 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 is in this order of idea that the objectiveof this study was to evaluate the effect of two extraction solvents on the polyphenols content and antioxidant activity of Alstonia boonei barks.

2. Material and methods

2.1. Chemicals and reagents

Quinin, DPPH, folin-ciocalteu's phenol reagent, gallic acid, quercetin, sodium phosphate dibasic,Acétate de potassium, Trichlorured'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

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

Les composés bioactifs qui ont été déterminés dans les différents extraits étaient : les polyphénols totaux, les flavonoïdes et les alcaloides.

Assay of total phenolic compounds

The polyphenol content of the extracts was evaluated using the protocol described by **Singleton and Rossi (1965)**. Thirty microliters (30 µL) 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 contents were 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 AlCl3 (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 contents were expressed in micrograms of quercetin equivalents per milligram of extract (µg of Quer/mg of extract).

Assay of alkaloids

Thealkaloids 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 [FeCl3 (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 ± 2°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 contents were 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 via 2 mechanisms: scavenging of free radicals and reduction of Iron3+.

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 ($NO^{\cdot}$)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:

% Radical scavenging = [(Control Abs - Sample Abs) / Control Abs] x 100.

The scavenging concentrations 50 (SC_{50}): 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 powerassay

Reducing power of Iron III

The reducing power of the extracts was evaluated via 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:

Reduction of Fe3+ (%) = [(Abs control - Abs sample) / Abs control] x 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. The 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 (R2) 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 the two solvents on bioactive compounds of interest in A. boonei bark

The contents of total phenols, flavonoids and alkaloids 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).

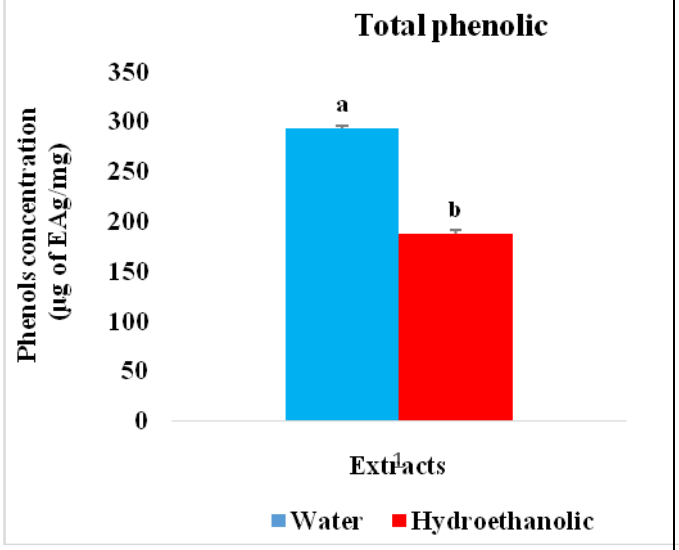


Figure 1a: Polyphenols content of the extracts

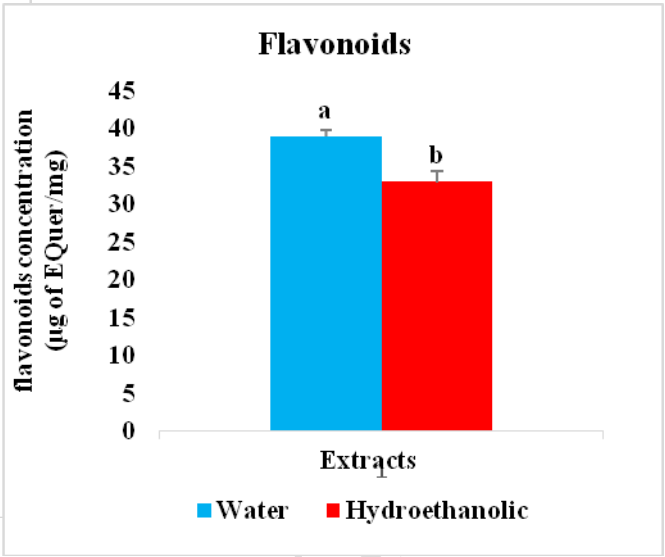


Figure 1b: Flavonoids content of the extracts

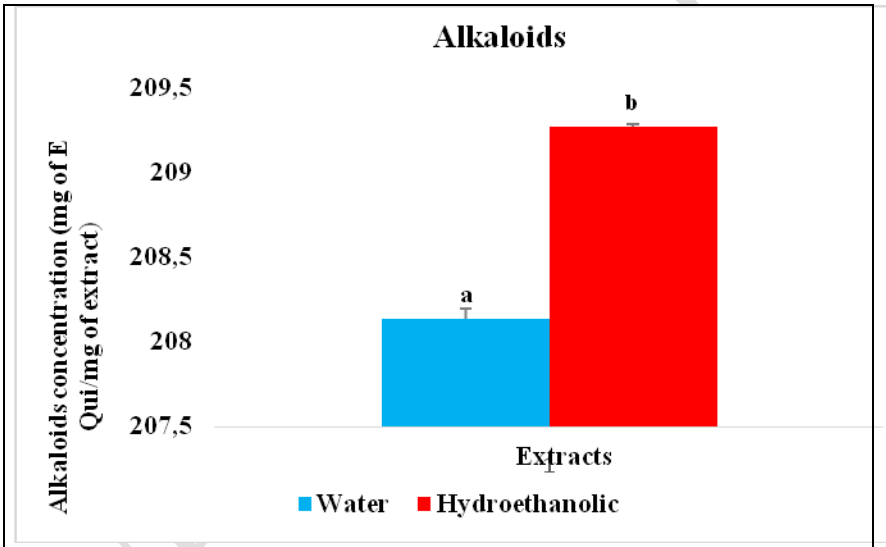


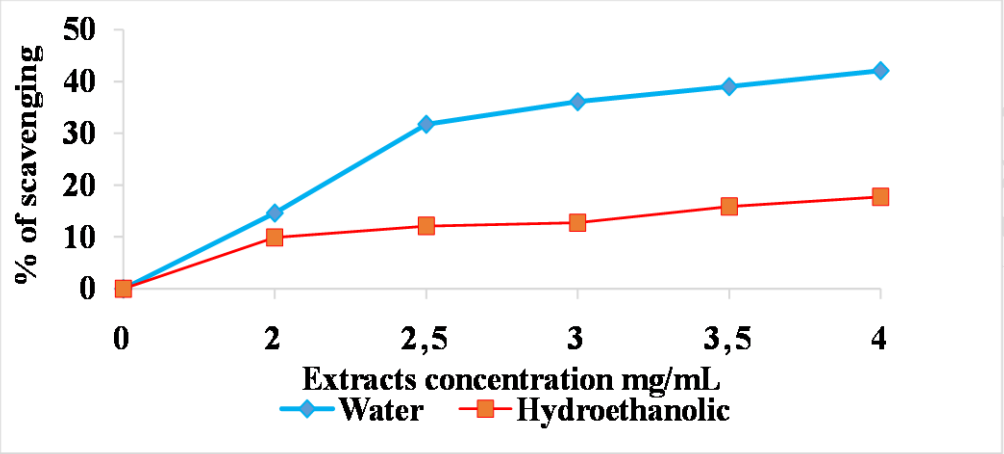
Figure 1c: Alkaloids content of the extracts

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

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

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

The 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.

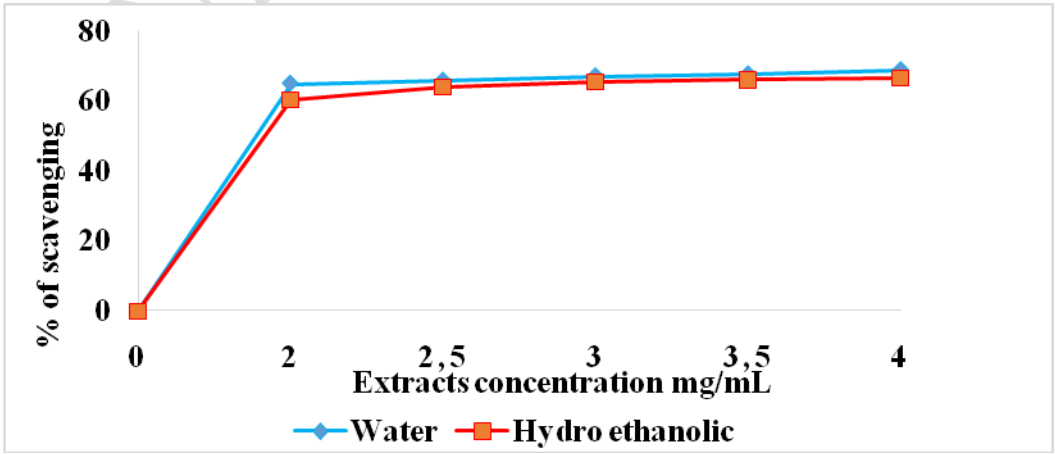


Results were expressed as the mean ± standard error of the mean.

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.) radical scavenging activity of A. boonei barks

The ability of extracts to scavenge the NO· radical. at different concentrations was explored and recorded in Figure 3. It appears that the extracts scavenged the NO· 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 ± standard error of the mean

Figure 3 : Effect of different solvents onnitric oxide(NO·) radical scavenging activityof A. boonei barks.

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[a]	201,2[b]	2,53[a]	2,67[b]

*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 study of the ferric ironreducing 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, varying from 51.98% to 68.35% for the aqueous extract, against 48.42% to 62.12% for the hydroethanolic extract. The aqueous extract resulted in a better activity with a fifty-reduction concentration 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/mL
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 polyphenolic content and antioxidant activity

To assess the relationship between antioxidant potential (DPPH, NO and FRAP) and polyphenols (TPC and TFC); the correlation coefficient (r) and the coefficient of determination (R^2) between the antioxidant potential (DPPH, NO and FRAP) and the polyphenols (TPC and TFC) of different extracts of A. boonei were studied using

simple linear regression. The study shows that by comparing these two solvents; a strong correlation (r and R^2) was noted between polyphenols (TPC and TFC) and antioxidant activity (DPPH, NO and FRAPP) in the aqueous extract of A. boonei bark (Table 3 and 4).

Table 3: Correlation between total phenolic content (TPC) and each DPPH, NO radical scavenging and FRAP reducing power activities using different solvent extracts of A. boonei

Solvents	TPC x DPPH			TPC x NO			TPC x FRAP		
	r	R^2	Equation	r	R^2	Equation	r	R^2	Equation
Water	0.98*	0.96	y = 0.02x + 2.03	0.99**	0.99	y = 0.006x + 61.22	0.99**	0.98	y = 0.02x +36.56
Hydro ethanolic	0.91*	0.82	y = 0.04x - 4.62	0.91*	0.82	y = 0.01x + 55.97	0.98**	0.97	y = 0.03x + 33.91

TPC: Total polyphenols content; values of r carrying ** mean P < 0.02; the values of r carrying *means P < 0.05

Table 4: Correlation between total flavonoids content (TFC) and each DPPH, NO radical scavenging and FRAP reducing power activities using different solvent extracts of A. boonei

Solvents	TFC x DPPH			TFC x NO			TFC x FRAP		
	r	R^2	Equation	r	R^2	Equation	r	R^2	Equation
Water	0.98**	0.82	y = 0.32x - 4.62	0.99**	0.99	y = 0.04x + 61.22	0.99**	0.98	y = 0.20x + 36.56
Hydro ethanolic	0.91*	0.96	y = 0.11x + 2.03	0.91*	0.82	y = 0.08x + 55.97	0.98**	0.97	y = 0.20x + 33.91

TFC: Total flavonoid content; values of r carrying ** mean P < 0.02; the values of r carrying *means P < 0.05

4. Discussion

The antioxidant activity of a plant is most often attributed to its content of polyphenolic compounds, in particular flavonoids **(Ighodaro and Akinloye, 2017)**. However, a better extraction of these secondary metabolites requires an appropriate solvent.The present study was to evaluate the effect of two extraction solvents on the polyphenols content 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)**study 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 the 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 (table 3 and 4). 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 thanks to the OH groups of the 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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