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Phytochemical Screening and Evaluation of Antioxidant Power of Hydro-Ethanolic and Aqueous Leaves Extracts of *Annona Muricata* Linn (Soursop)

Criblage phytochimique et évaluation du pouvoir antioxydant d'extraits hydro-éthanoliques et aqueux des feuilles d'Annona muricata Linn (Soursop)

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Mots clés : *Annona muricata*, criblage phytochimique, antioxydant.

RÉSUMÉ

Introduction. *Annona muricata* L. est une plante de la famille des annonacées largement utilisée par les populations de nombreux pays du monde, notamment celles du Cameroun. À la fois cultivée et sauvage, cette plante est présentée comme possédant certaines propriétés médicinales. Le but de cette étude était d'évaluer la composition phytochimique et le pouvoir antioxydant des extraits hydro-éthanoliques et aqueux des feuilles de *Annona muricata* L. **Méthodes**.La composition phytochimique des extraits a été réalisée à l'aide de réactions colorimétriques, tandis que la teneur en phénols totaux a été déterminée par la méthode de Folin-Ciocalteu. Le pouvoir antioxydant des extrais a été évaluée par des méthodes complémentaires (DPPH et RAP). **Résultats**: la composition phytochimique a révélé la présence de composés phénoliques, de glycosides cardiaques et de sucres dans les deux extraits, tandis que les alcaloïdes étaient présents uniquement dans l'extrait hydroéthanolique. La teneur en phénols totaux de l'extrait hydro-éthanolique était significativement supérieure à celle de l'extrait aqueux ($p < 0,05$). Les résultats du test DPPH et ceux du test RAP ont également montré que l'extrait hydro-éthanolique avait un pouvoir antioxydant supérieur à celui de l'extrait aqueux. ($p < 0,05$). **Conclusion**: ces résultats suggèrent que les feuilles de *Annona muricata* L. représentent une source potentielle de composés antioxydants bien que leur nature et leur nombre varient d'un extrait à l'autre.

ABSTRACT

Background. *Annona muricata* L. is a plant of the Annonaceae family widely used by people in many countries of the world, especially those of Cameroon. Both cultivated and wild, this plant is presented as having some medicinal properties. The aim of this study was to evaluate phytochemical composition and antioxydant power of hydro-ethanolic and aqueous leaves extracts of *Annona muricata* L. **Methods**. Phytochemical composition of the extracts was performed using colorimetric reactions while total phenols content was determined by the Folin-Ciocalteu method. Extracts antioxidant power was evaluated by complementary methods (DPPH and RAP). **Results**. phytochemical composition revealed the presence of phenolics compounds, cardiac glycosides and sugars in both extracts, while alkaloids were present only in hydro-ethanolic extract. Total phenols content of hydro-ethanolic extract was significantly higher than that of aqueous extract ($p < 0.05$). The results of DPPH test and those of RAP test also showed that hydro-ethanolic extract had a greater antioxidant power than aqueous extract. ($p < 0.05$). **Conclusion**. These results suggest that *Annona muricata* L. represent a potential source of antioxidant compounds although these compounds vary in kind and number from one extract to another.

INTRODUCTION

In Cameroon and most other parts of the world, plant are still widely used in the treatment of several diseases, especially in areas where access to conventionnal drugs is limited [1]. The plants and plant-based medicines are also used as the basis of many modern pharmaceuticals industries today for the treatment of various ailments [2]. Several phytochemical molecules from these natural products which are capable of exerting physiologic action were studied and characterized. Bioactive compounds such as alkaloids, phenols and cardiac glycosides were considered to be most important. The phytochemical research that has been done based on ethno-pharmacological informations constitutes the effective

approach in the discovery of new bioactive compounds [2, 3].

Annona muricata L. commonly known as graviola, soursop or corrosol, belongs to the Annonaceae family. It's a small, upright tropical and subtropical evergreen tree of 5–6 m in height, with large glossy and dark-green leaves. It produces a large, heart-shaped, edible fruit that is 15–23 cm in diameter, yellow–green in color and has flesh inside. All parts of the *A. muricata* tree have been used in traditional medicine for the treatment of many infectious and non-infectious diseases [1, 2].

MATERIALS AND METHODS

Plant material

Plant material consisted of *A. muricata* leaves harvested in January 2016 at Ahala 1, a suburb located in the district of Yaounde 3rd. The latter was then identified at the National Herbarium of Cameroon in comparison with the sample *Annona muricata* linn. from the specimen of the herbarium collection n° 18681 SFR/YA-CAM.

Extraction

This procedure derives from that described by Adewole SO and *al*. in 2006 [4]. The leaves were first washed and then dried in an oven for 3 days at 70°C. The dry plant material was pulverized then subjected to successive maceration. Thus, the powder was first macerated in ethanol-water (70: 30 v/v) for 48 hours and then filtered. The residue was dried and macerated again for 48 hours in distilled water. The filtrates obtained were then lyophilized and the final powders were used for further studies.

Phytochemical screening

Determination of secondary metabolites was performed using colorimetric reactions according to the procedures described by Ravalison and *al.* in 2015 [5] and Ayoola and *al.* in 2008 [6].

Total phenols content

Total phenols content of the extracts was determined by the Folin-Ciocalteu method. The protocol used derived from that described by Riwom and *al*. in 2015 [3], with some modifications. In a test tube, introduce successively 2370 µL of distilled water, 30 µL of extract (or standard) at 1 mg/mL and 450 µL of Folin-Ciocalteu reagent. Mix together with a vortex and then add 150 µL of 20% (w/v) sodium carbonate solution. The mixture is finally allowed to incubate for 02 hours in the dark before being read at the UV/visible spectrophotometer at the wavelength of 750 nm. Calculation of the concentration is made from the equation Abs = a x [AC] + b, of the calibration curve established with the reference solution (ascorbic acid).

Antioxidant Activity

DPPH Radical Scavenging

This procedure derives from those described by Riwom and *al.* in 2015 [3] and, Ravalison and *al*. in 2015 [5]. Concentration ranges from 0.05 to 0.8 mg/mL of extracts and 0.025 to 0.4 mg/mL of ascorbic acid have been prepared in methanol. 50µL of each of these solutions were mixed with 1950µL of methanolic solution of DPPH at 0.76mM. After homogenization, the mixture was incubated at room temperature (25°C.), protected from light. After 02 hours of incubation, the absorbance of the samples was read at 517 nm against that of a blank which contained only the methanolic solution of DPPH. The AAR antiradical activity (%) was calculated according to the following equation:

$$\mathbf{AAR} = \frac{Ao - As}{Ao} \mathbf{x\ 100}$$

Ao = Absorbance of blank
As = Absorbance of samples

Antiradical parameters

$$\mathbf{EC50} = \frac{SC50}{[DPPH]} \qquad \mathbf{AP} = \frac{1}{EC50}$$

SC_{50} = Scavenging concentration 50
EC_{50} = Effective concentraton 50
AP = Antiradical power

Reducing Antioxydant Power

The reducing power of the extracts was evaluated according to the method described by Oyaizu [7]. One milliliter (1 mL) of extract at different concentrations (from 0.025 to 0.2 mg/mL) was mixed with 2.5 mL of 0.2 M phosphate buffer solution (pH 6.6) and 2.5 mL of 1% potassium ferricyanide solution $K_3Fe(CN)_6$. The mixture was incubated in a water bath at 50°C for 20 mn then, 2.5 ml of 10% trichloroacetic acid was added and the tubes were centrifuged at 3000 rpm for 10 mn. An aliquot (2.5 mL) of supernatant was combined with 2.5 mL of distilled water and 0.5 mL of 0.1% aqueous $FeCl_3$ solution. The absorbance of the reaction medium was read at 700 nm against a similarly prepared blank by replacing the extract with distilled water. Positive control was represented by an ascorbic acid solution. An increase in absorbance corresponds to an increase in reducing power of the extracts [8, 9].

Statistical analysis

All experiments were done in triplicates and the data was processed with R software 2016 version 3.3.0 and Microsoft Excel 2010. We used ANOVA, Kruskal-Wallis and Jonckheere-Terpstra tests and, the results were considered significant for a p value less than 0.05.

RESULTS

Plant material

Plant material consisted of *A. muricata* leaves harvested in January 2016 at Ahala I. The leaves were oven-dried before being pulverized. We obtained a dark-green powder with an irritating smell.

Extraction

From 807g of powder, we were able to obtain 145.84g of hydro-ethanolic extract and 95.81g of aqueous extract. Table 1 below summarizes the overall results of the extraction process.

Table 1 : Extraction yield and appearance of extracts obtained

	Hydro-ethanolic extract	Aqueous extract
Mass (g)	145,84	95,81
Yield (%)	18.07	11.87
Aspect	Irregularly shaped dark-green crystals	Irregularly shaped brown crystals
Final yield (%)	29.94	

Phytochemical screening

Colorimetric tests performed on our two extracts revealed the presence of numerous groups of secondary metabolites. The results are shown in Table 2 below.

Tableau 2 : Some groups of secondary metabolites of *A. muricata* leaves

Secondary métabolites	Hydro-éthanolic extract	Aqueous extract
Alcaloïds	+	-
Anthocyans	+	+
Flavonoïds	+	+
Tanins	+	+
Saponins	+	+
Sterols and triterpenes	+	+
Coumarins	+	+
Cardiac glycosides	+	+
Sugars	+	+

Presence + ; Absence –

Total phenols content

Total phenols content of the extracts was determined from the equation of the calibration curve established using the reference solution (ascorbic acid).

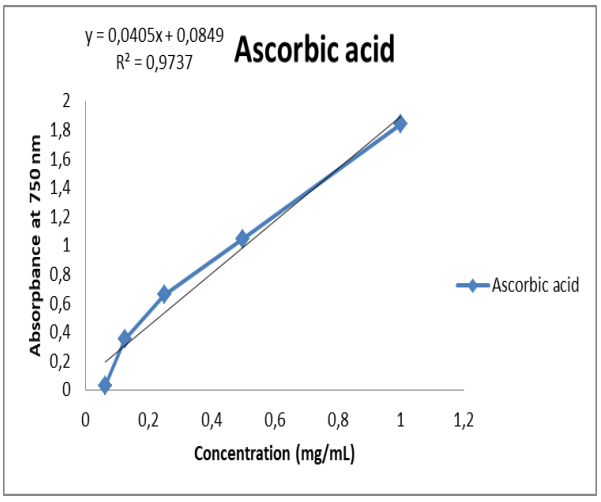

Figure 1 : Ascorbic acid calibration curve

The results obtained (Table 3) revealed that there was a significant difference (p value <0.05) between the levels of phenols content in our two extracts. In fact, the hydro-ethanolic extract had higher phenols content than aqueous extract.

Table 3 : Extracs total phenols content

Extracts	Hydro-ethanolic	Aqueous
Phenols content (µg EAA/mgw)	22,52 ± 0.0011	16,94 ± 0.0015

Antioxidant Activity

DPPH Radical Scavenging

The ability to scavenge DPPH radical by our extracts was quantitatively evaluated using a UV-Visible spectrophotometer at the wavelength of 517 nm. The Figure 2 represents the curves of inhibition percentages of DPPH radical as a function of concentration of the extracts tested and ascorbic acid (reference antioxidant). The analysis of this figure shows that inhibition percentage increases significantly with the concentration of the samples; the different correlation coefficients being 0.927 for ascorbic acid, 0.982 for the hydro-ethanolic extract and 0.981 for the aqueous extract. As other information, it's also noted that inhibition percentages depend on type of sample tested (p value <0.05).The equations of the straight lines in Figure 2 allowed us to calculate the antiradical parameters namely SC_{50}, EC_{50}, AP (Table 4).

Table 4 : DPPH test results

Antiradical parameters	Aqueous extract	Hydro-ethanolic extarct	Ascorbic acid
SC 50	0,281	0,219	0,11
EC 50	0,369	0,288	0,14
AP	2,71	3,47	7,14

Reducing Antioxydant Power

Reducing power (or, antioxidant capacity) of the extracts was determined and the results are shown in Figure 3. Analysis of this figure revealed that reducing power increases significantly with the concentration of the samples; the different correlation coefficients being 0.966 for the hydro-ethanolic extract and 0.978 for the aqueous extract. In addition, it appears that this reducing power also depends on the type of sample tested (p value <0.05). Thus, the hydro-ethanolic extract showed a better profile compared to aqueous extract.

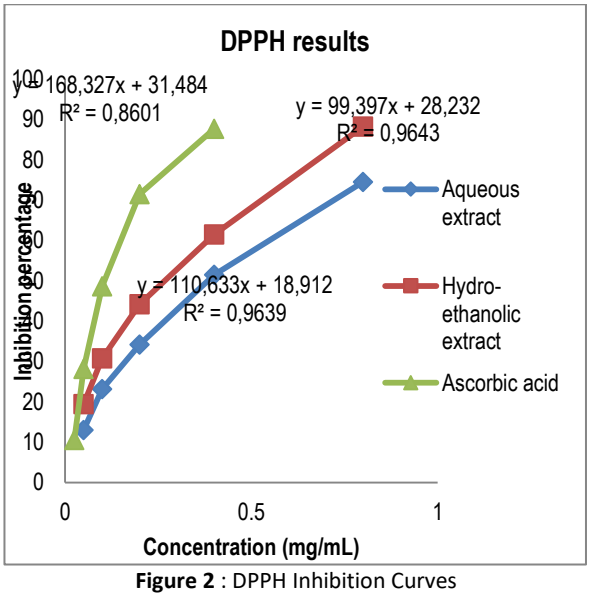

Figure 2 : DPPH Inhibition Curves

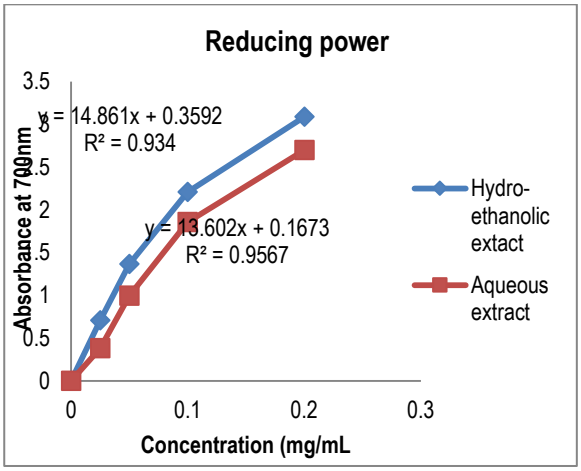

Figure 3 Reducing Antioxydant Power

DISCUSSION

The successive maceration of *A. muricata* leaves with two differents solvents allowed us to obtain two extracts of variable quantities. The best yield was obtained with hydro-ethanolic extract; which suggests that substances present in the leaves of *A. muricata* are mostly lipophilic compounds [10, 11]. Phytochemical characterization showed that *A. muricata* leaves contained secondary metabolites such as alkaloids, anthocyanins, flavonoids, tannins, coumarins, saponins, sterols, terpenoïds, cardiac glycosides and sugars. These secondary metabolites have demonstrated numerous pharmacological activities both *in vitro* and *in vivo* [10, 11]. The use of *A. muricata* in traditional medicine is therefore validated by the presence of these phytochemicals of known health benefits. Total phenols content revealed that hydro-ethanolic extract contained higher phenols compounds than aqueous extract. This is explained by the fact that ethanol correctly solubilizes medium polar phenol compounds and, the addition of water to the extraction system improves the yield of glycosylated phenolic compounds and phenolic compounds with a very high degree of polymerization [12, 13, 14]. DPPH assay revealed that hydro-ethanolic extract had a greater scavenging capacity than aqueous extract. The DPPH radical is capable of accepting an electron as well as a hydrogen but data supports the latter mechanism as a predominant, if not exclusive pathway [15, 16, 17]. Therefore, this higher radical scavenging activity could be due to the high content in phenolic compounds compared to aqueous extract. Indeed, several studies have shown the relationship between antioxidant activity and total phenols content [18, 19]. For example, Phenolic compounds like flavonoids, due to their chemical structure, are ideal donors of hydrogen to the DPPH radical by facilitating HAT (Hydrogen Atom Transfer) to take place [9, 20]. Concerning Reducing Antioxidant Power, hydro-ethanolic extract also showed higher potency than aqueous extract. This is in agreement with the findings of such research groups that such positive correlation between total phenols content and antioxidant activity [9, 18, 21], which highlights the electrons donating capacity of polyphenols due to the number, configuration and glycosylation of their hydroxyl groups [22]. This result could also be due to the presence of alkaloids, which are compounds known for their antioxidant power. Many studies have demonstrated that these compounds are antioxidants because they are donors of hydrogen atoms and possess a single electron transfer mechanism [3, 23, 24].

CONCLUSION

Given the results, it appears that *A. muricata* leaves contain several secondary metabolites that have demonstrated numerous pharmacological activities including antioxidant activity. Although these compounds vary in kind and in number from one extract to another, it doesn't seem exaggerated to say that leaves of *A. muricata* represent a potential source of antioxidant compounds. It is therefore easy to understand why this plant is used in traditional medicine in managing oxidative stress related diseases [10, 11].

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