Antioxidant power of hydro-ethanolic and aqueous leaves extracts of Annona Muricata Linn

Logmo Mahi et al

Article Original

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

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Introduction. 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 antioxidant 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 conventional 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

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References:


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

*Annona muricata* L. commonly known as graviola, soursop or corosol, 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:

\[
\text{AAR} = \frac{A_0 - A_s}{A_0} \times 100
\]

Ao = Absorbance of blank  
As = Absorbance of samples

**Antiradical parameters**

\[
\frac{\text{SC}_{50}}{\text{EC}_{50}} = \frac{\text{AP}}{1 - \text{AP}}
\]

EC50 = Effective concentration 50  
SC50 = Scavenging concentration 50

**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 K₃Fe(CN)₆. The mixture was incubated in a water bath at 50°C for 20 mins then, 2.5 mL of 10% trichloroacetic acid was added and the tubes were centrifuged at 3000 rpm for 10 min. An aliquot (2.5 mL) of supernatant was combined with 2.5 mL of distilled water and 0.5 mL of 0.1% aqueous FeCl₃ 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.
Antioxidant power of hydro-ethanolic and aqueous leaves extracts of *Annona Muricata* Linn

**Table 1 : Extraction yield and appearance of extracts obtained**

<table>
<thead>
<tr>
<th>Type of Extract</th>
<th>Mass (g)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydro-ethanolic</td>
<td>145.84</td>
<td>18.07</td>
</tr>
<tr>
<td>Aqueous</td>
<td>95.81</td>
<td>11.87</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Secondary metabolites</th>
<th>Hydro-ethanolic extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sterols and triterpenes</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Coumarins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sugars</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Table 3 : Extracts total phenols content**

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Hydro-ethanolic</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenols content (µg EAA/mgw)</td>
<td>22.52 ± 0.0011</td>
<td>16.94 ± 0.0015</td>
</tr>
</tbody>
</table>

**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.

**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).

![Ascorbic acid calibration curve](https://www.hsd-fmsb.org)

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.

**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 SC50, EC50, AP (Table 4).

**Reducing Antioxidant 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.

![DPPH Inhibition Curves](https://www.hsd-fmsb.org)

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.
DISCUSSION

The successive maceration of A. muricata leaves with two different 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, terpenoids, 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 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].

ACKNOWLEDGEMENTS

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Logmo Mahi et al


