

Research Article

Quantitative Phytochemical Analysis and Bioactivities of Hydroethanolic Extracts of *Amorphophallus consimilis*

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Abstract

This study is devoted to the determination of polyphenol, flavonoid and mineral contents and then to the evaluation of antioxidant and antibacterial activities of *Amorphophallus consimilis* extracts. The spectrophotometric method is used for the determination of polyphenol and flavonoid contents with standard solutions of gallic acid and quercetin respectively. The polyphenol content varies from 2.33 (stems) to 4.68 (tubers) $\mu\text{g EAG/g}$ and that of flavonoids from 2.425 (tubers) to 9.373 (stems) $\mu\text{g EQ/g}$. The antioxidant activity is evaluated by the DPPH[•] and ABTS^{•+} method. The 50% Inhibitory Concentration (IC₅₀) values obtained with both methods range from 0.17 ± 0.011 (leaves) to 0.254 ± 0.001 (tubers) mg/mL . Atomic absorption spectrophotometry (AAS) is used for the determination of mineral contents. *Amorphophallus consimilis* contains minerals with average contents of calcium (43 $\mu\text{g/g}$), iron (11 $\mu\text{g/g}$), zinc (0.28 $\mu\text{g/g}$) and copper (0.14 $\mu\text{g/g}$). The disk diffusion method is used for the evaluation of antibacterial activity. A total of five bacterial strains are used, these are: *Escherichia coli* ATCC25922, *Enterococcus faecalis* ATCC29213, *Staphylococcus aureus* ATCC29212, *Pseudomonas* (community strain), *Candida albican* ATCC24433 were used. The minimum inhibitory concentrations (MIC) range from 1.875 to 30 mg/mL , showing overall interesting bacterial activity of the extracts on the strains tested.

Keywords

Amorphophallus Consimilis, Polyphenols, Flavonoids, Minerals, Antioxidants, Antibacterial

1. Introduction

Humans have always lived in an environment full of plant potential. This proximity between humans and nature has led

to a multitude of uses of flora by humans according to their needs. The diversity of flora and ancestral knowledge allow

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humans to feed themselves and cope with various possible ailments. The constant development of chemoresistance of modern drugs against pathogens has allowed the development of pharmacopoeia based on the knowledge of traditional medicine. *Amorphophallus* is a genus that is part of the Araceae family. Plants of this genus are distributed throughout the world and have been used in traditional medicine for a long time, particularly in Ayurvedic and Yunani medical rituals. They are also used in traditional Chinese medicine [1]. They have pharmacological properties, such as analgesic [2], neuroprotective, hepatoprotective, anti-inflammatory [3], anti-convulsant, antibacterial, antioxidant, anticancer, antiobesity and immunomodulatory [4, 5]. They are used for acute rheumatism, tumors, lung swelling, asthma, sinusitis, dysentery, jaundice, lack of appetite, vomiting and abdominal pain [6, 7]. *Amorphophallus consimilis* is a wild, semi-shade plant. It often grows under large trees. The objective of this work is to determine some biological properties such as antioxidant and antibacterial activities and to evaluate the polyphenol,

flavonoid and mineral content of the different parts of the plant.

2. Materials and Methods

2.1. Plant Materials

The choice of *Amorphophallus consimilis* is justified by an ethnobotanical survey coupled with a bibliographic study of plants listed among some traditional practitioners in Senegal. The harvest of the different organs (tubers, fruits, leaves and stems) of this plant was carried out in the commune of Dioulacolon, located in the south of Senegal, with the geographical coordinates 13°05' North and 14°49' West. The different parts of the plant were cut into small pieces then dried in the shade at 27 °C and then crushed using an electric grinder.



Figure 1. Photograph of the parts of *Amorphophallus consimilis* studied.

2.2. Polyphenol and Flavonoid Dosages

The content of total phenolic compounds is determined with the Folin-Ciocalteu reagent [10]. Indeed, 40 µL of each extract are taken and made up to 200 µL with distilled water. A volume of 150 µL of Folin-Ciocalteu reagent, 600 µL of a 20% Na₂CO₃ solution and 2.32 mL of distilled water are added in addition. After 30 minutes of incubation in the dark, the absorbance is read at 760 nm from a Perkin-Elmer Lambda 365 UV/Visible spectrometer. The measurement is compared to a standard curve of gallic acid prepared from a 0.1 mg/mL stock solution. The concentrations are expressed in microgram gallic acid equivalent per gram (µg EAG/g).

Flavonoid content is measured by the method described by Dirar *et al.* [8, 9]. This method consists of adding 2.5 mL of a 2% AlCl₃ ethanolic solution to 500 µL of each extract. The mixtures are incubated for 1 hour at room temperature and the absorbance is read at 425 nm. The flavonoid content is expressed in micrograms of quercetin equivalent per gram (µg EQ/g) by reference to the calibration curve plotted with a

concentration range obtained from a 0.1 mg/mL quercetin stock solution.

From the equations of the calibration lines of the established standards of the form $Y = ax + b$, the concentration (x) of polyphenols or flavonoids is deduced for a given absorbance at a given wavelength and the content is calculated according to the following formula:

$$\text{Total content (}\mu\text{g/g)} = \frac{x \cdot 50}{1} * Fd$$

x: concentration found from absorbance and calibration line

Fd: dilution factor

50: volume

2.3. Dosage of Some Metals

A mass of 1g of each dried and ground sample (fruits, stems, tubers, seeds) is weighed and subjected to acid mineralization. This consists of a contact time of one hour between the sample and 10 mL of a mixture of 37% HCl (2 volumes) and 68% HNO₃ (1 volume). Mineralization is carried out for 50

minutes in a microwave oven (25 to 170 °C (10 min), 170 °C (10 min), 170 °C to 100 °C (10 min), 100 °C (10 min), 100 °C to 80 °C (5 min), 80 °C (5 min). Thus, mineralization is carried out in triplets for each sample [10]. The mineralized samples are then collected in a volumetric flask and the volume precisely adjusted to 50 mL with demineralized water and stored in the refrigerator at +4 °C. A filtration step using a syringe equipped with a microfilter (0.45 µm diameter) is carried out before analysis with the ICP-OES spectrophotometer. The following elements are specifically targeted using the ICP-OES: Calcium (422.673 nm), Copper (327.395 nm), Iron (238.204 nm), Zinc (213.817 nm). The calibration was carried out using nine points (2 decades) of dilution of a stock solution containing the whole elements at different concentrations. The stock solution was obtained by diluting a certified individual element from a multi-element solution to 100 and 10,000 ppm.

Mineral contents are calculated using the formula below.

$$\text{Mineral content (mg/100g)} = \frac{C \cdot 50 \cdot 100}{P_E \cdot 1000} \cdot F_D$$

C: Concentration of the element to be measured in mg/L read at the SAA

P_E : Test sample in g.

F_D : Dilution factor

2.4. Determination of Antioxidant Activity by the DPPH and ABTS⁺ Method

- 1) The antioxidant test was carried out according to the DPPH[•] method [11-13]. A stock solution of 40 mg/mL is prepared by dissolving 80 mg of dry extract in 2 mL of methanol. Then a range of concentrations was prepared with a dilution factor of 1/2. For each of these different concentrations, 0.2 mL is taken and introduced into test tubes and mixed with 7.8 mL of the previously prepared purple ethanolic solution of DPPH[•]. The tubes are shaken manually for a few seconds and then incubated for 30 min away from light. The absorbance reading is carried out by a spectrophotometer at a wavelength of 517 nm, using ethanol as a blank. Ascorbic acid is used as a reference antioxidant. The DPPH[•] solution is obtained by dissolving 10 mg of DPPH[•] in 250 mL of ethanol with stirring for 30 minutes away from light.
- 2) The method used was that described by Sarr *et al.* [10]. A quantity of 38.40 mg of ABTS⁺ [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)] was previously dissolved in 10 mL of water before adding 6.75 mg of potassium persulfate. The mixture obtained was incubated away from light and at room temperature for 12 h before use. It was subsequently diluted with ethanol to obtain an absorbance of the order of 0.7 for a wavelength of 734 nm. The antioxidant activity was measured by adding 2 mL of an ethanolic solution of the tested extract to 2 mL of the ABTS⁺ solution. The extracts were tested

at the following concentrations: 2.5; 10; 100 and 200 µg/mL. Ascorbic acid and quercetin, used as reference antioxidants, were tested at the same concentrations. Absorbance was measured using a UV-Visible molecular absorption spectrophotometer at 734 nm after 2 minutes of incubation in the dark, using ethanol as a blank. Three absorbance measurements were performed for each concentration tested (n=3).

The inhibitory powers are determined using the formula below:

$$\text{Scavenging activity (\%)} = \frac{A_C - A_S}{A_C} \cdot 100$$

A_C : Control absorbance, A_S : Sample absorbance

2.5. Antibacterial Activities

2.5.1. Sample Sterility Test

This test is intended to check whether the extracts contain germs or not. To this end, 0.1 g of the extract must be added to 10 mL of thioglycolate broth, and the broth incubated at 37 °C for 24 hours. After this time, inoculate the broth onto a Petri dish containing nutrient agar and another containing Sabouraud agar, and incubate the Petri dishes at 37 °C [14]. The substance is declared sterile if no colony is visible on the agar dish after 24, 48, and 72 hours of incubation at 37 °C [15].

2.5.2. Bacterial Susceptibility Testing

The disc diffusion method was used to test the susceptibility of bacterial strains to different extracts [12]. Bacteria were inoculated onto Muller-Hinton (MH) agar and Sabouraud agar for *Candida albican*. From a bacterial culture of 18 to 24 hours, inocula equivalent to the 0.5 Mac-Ferland standard (106 bacteria per mL) were prepared. A drop of the culture dilution (inoculum) prepared with a few micrograms of bacteria in a biological fluid (sodium chloride) was placed on each Petri dish and spread by tight streaks with a sterile swab, over the entire MH agar and the Sabouraud agar for *Candida albican*. Sabouraud cups of 0.7 cm diameter were then placed on the upper layer of the agar medium, seeded with bacteria. The cups were impregnated with 100 µL of solution at a concentration of 30 mg/mL and the dishes were incubated in the incubator at 37 °C for 24 hours. Throughout the incubation period (24 hours at 37 °C), the strain studied competes with the inhibitory effect of the plant extract. A circular inhibition zone forms around the cup when the strain is sensitive to the extracts and an absence of inhibition zones if the strain is resistant [8]. The extract is not effective if the diameter is less than 4.8 mm; effective if the diameter is between 4.8 and 9.6 mm and very effective if the diameter is greater than 9.6 mm.

2.5.3. Determination of Minimum Inhibitory Concentrations (MIC)

The MIC is the lowest concentration of antibiotic capable of causing complete inhibition of the growth of a given bacterium, appreciable by the naked eye, after a given incubation period. The MIC determination is carried out for extracts that have shown zones of inhibition against the microorganism by the disk diffusion method. Rectangular plates containing 96 wells are used, concentration ranges are prepared from a stock solution of concentration 30 mg/mL and a solution of biological fluid (sodium chloride). On each well, 20 μ L of bacterial culture suspension is added. For each bacterial strain, a solution of dimethyl sulfoxide (DMSO) is used as a positive control, and as a negative control ceftriaxone (C-tri 10) [8, 12, 16] is used. Each microplate is covered and incubated for 24 hours at 37 $^{\circ}$ C. A clear color of the well indicates no growth and a cloudiness of the well indicates growth of bacteria. Each

experiment is repeated three times.

3. Results and Discussions

3.1. Total Polyphenols and Flavonoids in *Amorphophallus consimilis*

The contents of polyphenols and flavonoids were determined by the spectrometric method. Standard solutions of gallic acid for polyphenols and quercetin for flavonoids at concentrations ranging from 0.1 to 0.0125 mg/mL were prepared and their absorbances were measured. The curves representing the absorbances as a function of concentrations are shown in Figure 2. From the equation of the regression line and the absorbance of the extract, the evaluation of the concentration and content of polyphenols and flavonoids was carried out.

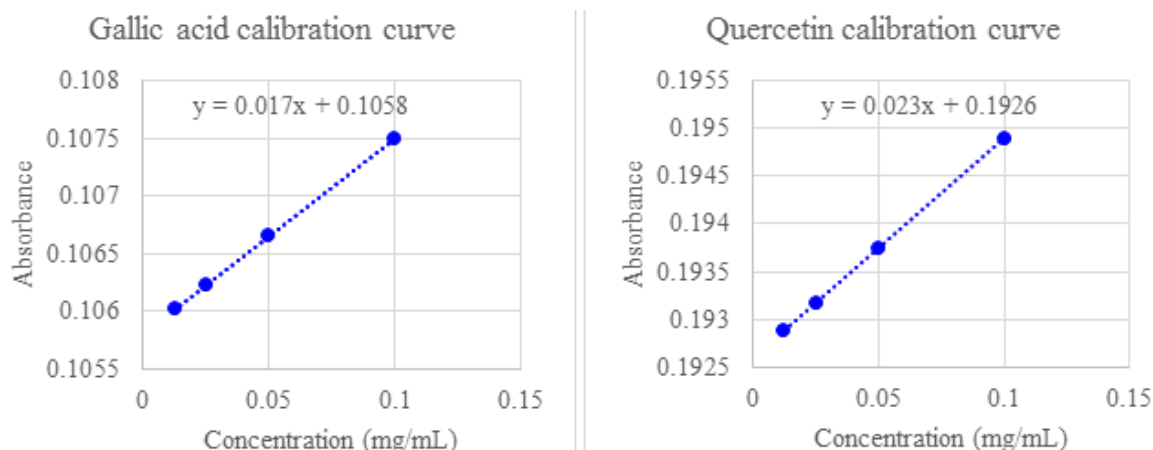


Figure 2. Calibration curves of gallic acid and quercitrin.

The total polyphenol and flavonoid contents of the determined samples are reported in Table 1. The polyphenol contents of the samples vary between 2 and 5 μ g EAG/g. The lowest polyphenol content is obtained with the stem extract (2.33 ± 0.011 μ g EAG/g). The tuber, fruit and leaf extracts give practically identical values respectively (4.68; 4.565; 4.625

μ g EAG/g). The extracts of tubers, fruits and leaves are richer in polyphenols. The flavonoid content of the stem extract is higher (9.373 ± 0.112 μ g EQ/g) than that of tubers, fruits and leaves. It can also be noted that the flavonoid content in fruits is twice that in tubers.

Table 1. Polyphenol and flavonoid composition of extracts from parts of *Amorphophallus consimilis*.

Dosed compounds	Tubers	Stems	Fruits	Leaves
Polyphenol (μ g EAG/g)	4.68 ± 0.002	2.33 ± 0.011	4.565 ± 0.102	4.625 ± 0.011
Flavonoid (μ g EQ/g)	2.425 ± 0.001	9.373 ± 0.112	4.96 ± 0.013	3.75 ± 0.020

3.2. Distribution of Minerals in Different Parts of *Amorphophallus Consimilis*

The results obtained are recorded in Table 2 below. Minerals such as calcium, iron, zinc and copper are unevenly distributed in the different parts of *Amorphophallus consimilis*. The leaf extract contains the highest levels of calcium (54.67 ± 0.001 $\mu\text{g/g}$) and zinc (0.44 ± 0.001 $\mu\text{g/g}$). However, it

has the lowest levels of iron (9.95 ± 0.001 $\mu\text{g/g}$) and copper (0.08 ± 0.0001 $\mu\text{g/g}$). The distribution of calcium and iron between the tubers, fruits and stems is almost identical with average values of 39 and 11 $\mu\text{g/g}$ respectively. The zinc content of the leaves is double, or 0.44 $\mu\text{g/g}$, the zinc content of the other parts of the plant alone. Zinc is an essential trace mineral that acts as an antioxidant by neutralizing free radicals while iron is a pro-oxidant.

Table 2. Mineral content expressed in micrograms per gram of sample.

Samples	Element content ($\mu\text{g/g}$)			
	Ca	Fe	Zn	Cu
Leaves	54.67 ± 0.001	9.95 ± 0.001	0.44 ± 0.001	0.08 ± 0.0001
Tubers	39.39 ± 0.012	12.35 ± 0.011	0.26 ± 0.041	0.21 ± 0.012
Fruits	39.71 ± 0.001	11.76 ± 0.001	0.24 ± 0.002	0.15 ± 0.0001
Stems	38.63 ± 0.013	10.98 ± 0.002	0.18 ± 0.001	0.11 ± 0.0002

The copper content of the tuber extract is three times that of the leaves and double that of the fruits and stems. Therefore, the tubers are richer in copper (0.21 ± 0.012 $\mu\text{g/g}$). The mineral composition is established as follows: there is more calcium followed by iron, zinc and lastly comes copper. This trend is observed in all parts of the plant. The iron content of the different parts of the plant is higher than that of oat flakes (5.9 $\mu\text{g/g}$), cooked green beans (9.4 $\mu\text{g/g}$) and almost similar to that of tuna (12.1 $\mu\text{g/g}$) [17]. The zinc contents are relatively low compared to that of cooked fish (5.5 $\mu\text{g/g}$) and cooked lentils (10.3 $\mu\text{g/g}$).

3.3. DPPH and ABTS Antioxidant Activities of Hydroethanolic Extracts of *Amorphophallus consimilis*

The absorbances measured as a function of the concentrations of the extracts made it possible to calculate the corresponding inhibitory percentages. The results obtained are represented in the diagrams in order to plot the regression lines and determine the minimum inhibitory concentrations at 50% of our different extracts. Ascorbic acid was used as a standard. If the correlation coefficient (R^2) of the regression line is less than 0.9; the extract is discarded and considered inactive because it cannot inhibit 50% of the DPPH $^{\bullet}$ radical or ABTS $^{\bullet+}$; in other words, the highest value of the inhibitory power is less than 50%.

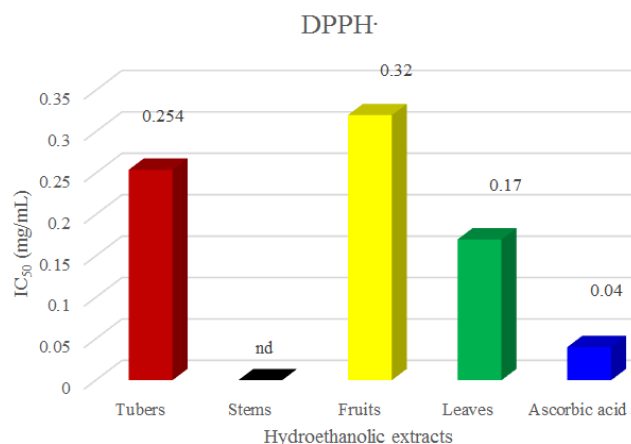


Figure 3. Diagram of 50% inhibitory concentrations (IC_{50}) of extracts from different parts of the plant.

The minimum inhibitory concentration of an extract provides information on its effectiveness; the lower it is, the more active the extract is considered. The antioxidant or anti-radical property of a sample is its capacity or ability to reduce a radical such as DPPH $^{\bullet}$ once in contact with it. The inhibitory concentrations obtained with DPPH $^{\bullet}$ for the different extracts vary between 0.17 and 0.32 mg/mL. The hydro-ethanolic extract of the stems does not present remarkable antioxidant activity. Among the extracts tested, the leaf extract has the lowest IC_{50} (0.17 ± 0.011 mg/mL) followed by the tuber and fruit extract respectively (0.254 ± 0.001 and 0.32 ± 0.001 mg/mL).

Table 3. Inhibitory concentration at 50% (DPPH and ABTS⁺ method).

Inhibitory concentration at 50% (mg/mL)					
Method	Hydro-ethanolic extract				Standard
	Tubers	Stems	Fruits	Leaves	Ascorbic acid
DPPH [•]	0,254 ± 0,001	nd	0,32 ± 0,001	0,17 ± 0,011	0,04
ABTS ⁺	0,243 ± 0,002	nd	0,274 ± 0,013	0,194 ± 0,012	0,042

nd: not determined

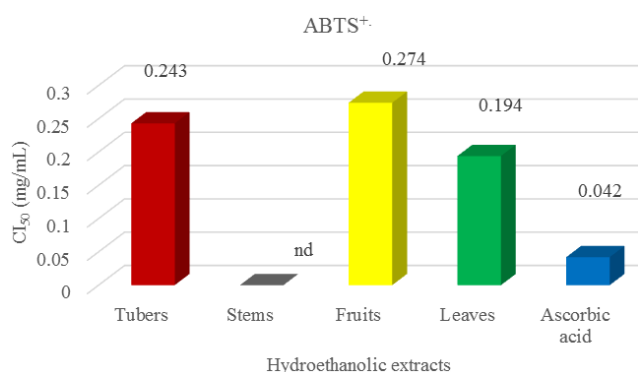
The values of the minimum inhibitory concentrations obtained for each sample are in the same order of magnitude. This fact gives reliability to our values.

The lower IC₅₀ of the leaf extract may be due to its higher zinc content, which plays an antioxidant role and therefore enhances the extract's anti-free radical capacity.

3.4. Antibacterial Activities

3.4.1. Extract Sterility

Since the environment is full of microbial strains, our extracts can be contaminated during handling processes. Sterility tests for our various extracts are carried out in petri dishes. This involves checking whether the extracts are infested with bacteria or not during processing.

**Figure 4.** Inhibitory concentration at 50% (IC₅₀) diagram.**Table 4.** Results of sterility tests of extracts.

Hydro-ethanolic Extract	<i>Amorphophallus Consimilis</i>			
	Tubers	Stems	Fruits	Leaves
Results	-	-	-	-

-: absence of bacterial colony development

+: presence of bacterial colony development

These results show that no infection was noted after 24 hours of incubation of the dishes containing the culture of the extracts at 27 °C.

3.4.2. Bacterial Sensitivity

Sensitivity is the effect of the extract on the growth of the corresponding bacteria. If the extract prevents or inhibits the

growth of the bacteria, the bacteria is said to be sensitive to the extract; otherwise, the bacteria is not sensitive to the corresponding extract. The concentration of the extract used in this sensitivity test is 30 mg/mL.

Table 5. Diameters of sensitivity of bacteria to hydro-ethanolic extracts in millimeters (mm).

Bacteria	Hydro-ethanolic Extracts			
	Tubers	Stems	Fruits	Leaves
Escherichia Coli ATCC25922	16	na	11	13
Enterococcus faecalis ATCC 29213	12	na	9	10
Staphylococcus aureus ATCC 29212	11	na	8	11
Pseudomonas (communitystrain)	9	na	8	8
Candida Albican ATCC24433	11	na	na	na

na: not active

Only extracts with an inhibition diameter greater than 5 mm for an initial concentration of 30 mg/mL exhibit activity against the tested strain. The results show that the inhibition diameters vary between 8 and 16 mm. It is noted that all bacteria are sensitive to the hydro-ethanolic extract of the tubers with inhibition diameters varying between 9 and 16 mm. However, no sensitivity of the different strains to the stem extract is noted. The *Candida albican ATCC24433* strain is only sensitive to the tuber extract with a diameter of 11 mm.

3.4.3. Minimum Inhibitory Concentration (MIC)

The MIC is the lowest concentration of antibiotic capable of causing complete inhibition of the growth of a given bacterium, appreciable by the naked eye, after a given incubation period. The MIC is determined in well microplates with extracts that have shown zones of inhibition against the strains tested by the disk diffusion method.

Table 6. Minimum inhibitory concentration MIC (mg/mL).

Bacteria	MIC (mg/mL)		
	Tubers	Fruits	Leaves
Escherichia Coli ATCC25922	1,875	15	3,75
Enterococcus faecalis ATCC 29213	3,75	30	7,5
Staphylococcus aureus ATCC 29212	7,5	30	15
Pseudomonas (communitystrain)	15	30	30
Candida Albican ATCC24433	7,5	nd	nd

nd: not determined

The minimum inhibitory concentrations of the extracts vary between 1.875 and 30 mg/mL. The MICs obtained with the E.Coli strain are lower, showing the efficacy of the extracts against the latter. Overall, the MICs obtained with the hydro-ethanolic extract of the fruits are higher than those obtained with the other organs of the plant. These results reflect the low antibacterial activity of the hydro-ethanolic extract of the leaves on the strains tested. The highest MICs are obtained with the community strain (pseudomonas) on the one hand and with the fruit extract on the other hand. This result is in correlation with the IC_{50} found previously and which are higher with the fruit extract. It should be noted that the higher

the IC_{50} , the less active the extract.

4. Conclusion

Amorphophallus consimilis exhibits very interesting antioxidant and antibacterial activities with relatively low inhibitory concentrations. It is relatively rich in minerals, particularly calcium and iron. Furthermore, there is an almost equal distribution of polyphenols, unlike flavonoids, within the plant. The highest MICs are obtained with the fruit extract. The results show that *Amorphophallus consimilis* could be a potential source of natural antioxidants that could be benefi-

cial in the prevention of degenerative diseases linked to oxidative stress. It can also contribute to the nutritional management of populations with dietary imbalances. To our knowledge, this study is the first conducted on *Amorphophallus* consimilis.

Abbreviations

MIC	Minimum Inhibitory Concentration
IC ₅₀	Inhibitory Concentration at 50%
DPPH	2,2-Diphenyl-1-Picrylhydrazyl Radical

Conflicts of Interest

The authors declare no conflicts of interest.

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