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ORIGINAL RESEARCH

Influence of the Solvent and the Harvesting Site on the Content of Phenolic Compounds and the in Vitro Antidiabetic Potential of Leafy Stems and Roots of *Phyllanthus amarus Schum. and Thonn.*

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Purpose: The objective was to verify the impact of the solvent and the harvesting site on the content of phenolic compounds as well as the in vitro antidiabetic activity of leafy stems and roots of *Phyllanthus amarus*.

Methods: The polyphenols and total flavonoids were measured on the crude extracts, obtained after maceration for 48 hours with acetone-water 50:50 (v/v), and ethanol-water 70:30 (v/v) . These extracts were evaluated for their antioxidant properties by DPPH, ABTS, and iron reduction (FRAP) tests. Finally, the α-amylase inhibitory activity of the crude extracts was determined by the method using DNS.

Results: The results show that acetone-water favors polyphenol extraction, with a maximum content of 32.62 ± 0.85 mg EAG/100 mg DE in leafy stems from Banfora (LSBaAw). In contrast, ethanol-water extracted more flavonoids, with 4.59 ± 0.02 mg EQ/100 mg DE in roots from Bobo (RBoEw). For antioxidant activity, the ethanol-water extract of Bobo leafy stems (LSBoEw) showed the highest ABTS free radical scavenging activity (81.34 ± 1.07 µg/mL). In comparison, the ethanol-water extract of Banfora roots (RBaEw) showed the best DPPH free radical scavenging activity (55.71 ± 2.48 µg/mL). On the other hand, the acetone-water extract of Banfora leafy stems (LSBaAw) showed the highest iron reduction activity (15,445.81 ± 835.75 µmol EAA/100 mg DE). Finally, the highest α-amylase inhibitory activity was observed with ethanol-water extracts from roots (RBaEw: 98.45 ± 0.38%; RBoEw: 96.56±0.31%).

Conclusion: These results underline the importance of the choice of solvent, organ, and harvesting site in optimizing the use of *Phyllanthus amarus*. Further studies involving other solvents and environmental conditions will enable us to refine these observations and optimize the pharmacological potential of this plant.

Keywords: *Phyllanthus amarus*, root, leafy stem, solvent, site

Introduction

Worldwide, health problems are increasing by the day, particularly cardiovascular disease [1] due to our high-carbohydrate, high-fat diet.[2] Among these diseases is diabetes, characterized by chronic hyperglycemia resulting from a deficit in insulin production and misuse of this hormone by the body.[3] Oxidative stress is one of the causes of this pathology.[4] In 2019, the worldwide prevalence of diabetes was estimated at 9.3%, and if no action is taken, it could rise to 10.9% by 2045.[3] In response to this growing issue, modern medicine has adopted a therapeutic approach focused on inhibiting carbohydrate digestive enzymes to reduce the hydrolysis of polysaccharide chains.[5] However, while this approach is beneficial, it has limitations, including the side effects of the prescribed medications.[6] Given these challenges, the use of medicinal plants for primary care becomes a viable alternative, considering the abundance of plant resources available.[7]

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Furthermore, *Phyllanthus amarus* Schum. and Thonn. (*Euphorbiaceae*)[8,9] is a plant used in traditional medicine to treat various diseases such as diabetes.[10] In addition, various pharmacological activities of *Phyllanthus amarus*, including antiviral, antibacterial, antiplasmodial, anti-inflammatory, antimalarial, antimicrobial, anticancer, antidiabetic, hypolipidemic, antioxidant, hepatoprotective, nephroprotective and diuretic properties have been reported.[8,11,12] Indeed, the wealth of bioactive compounds present in plants is a potential source of active ingredients used in the manufacture of drugs.[13] These components come from many parts of the plant, such as leaves,[14] roots,[15] and fruits,[16] with varying contents.[17] Among the active principles of plant origin are polyphenols,[18] such as flavonoids, which have antioxidant properties.[19] These compounds are also involved in the inhibition of sugar digestive enzymes.[20] However, existing research has not systematically taken into account the influence of extraction solvents and harvesting sites on the biological activities of plant organs. For this reason, this preliminary study was launched to verify the effect of extraction solvent and harvesting site on the biological activities of *Phyllanthus amarus* leafy stems and roots.

Materials and Methods

Reagents and Solutions

First, the extraction and solubilization required the use of acetone (CL00.0114.2500, Chemlab, Belgium), methanol (CL00.1363.2500, Chemlab, Belgium) and ethanol (CL00.0505.500, Chemlab, Belgium). Then, Folin-Ciocalteu (PCS02220263, Pallav, India), Gallic acid (Sigma-Aldrich, China), sodium carbonate (V7I654128G, Carlo Erba, France), aluminum trichloride (Lab-honeywell, Germany), quercetin (Sigma–Aldrich, China) were used for the determination of phenolic compounds. Then, the antioxidant activities were carried out through the use of 2.2′-azinobis -3-ethylbenzothiazoline-6-sulfonate acid (Meridian Rd, Rockford, USA), L- (+) - acid. ascorbic acid (Sigma-Aldrich, China), 2-2-diphenyl-1-picrylhydrazyl (LOT P19F002, Alfa Aesar, Japan), trichloacetic acid (V9C099200A, Carbo Erba, France), ferric chloride (V7D589039A, Carbo Erba, France) and potassium hexacyanoferrate (V4L501144L, Fisher Chemical, UK). Finally, the use of starch (V9L032160A, Carlo Erba, France), megamylase (LOT 084622) and 3.5-dinitrosalicylic acid (Sigma–Aldrich, India) made it possible to achieve the inhibitory activity α-amylase.

Plant Material

The plant material consisted of roots and leafy stems of *Phyllanthus amarus*. The samples were collected in Bobo (11°09’22.7“N 4°17’33.6”W) and Banfora (10°38’28.1“N 4°45’50.2”W) in Burkina Faso in July 2024 (Figure 1). Then, this plant was identified by Pr Paulin OUOBA of Nazi BONI University and deposited under the number UNB-930 at the herbarium. After identification, these samples were dried in the dark, pulverized, and stored in appropriate Zip bags before use.

Extraction

Sample moisture was determined using a KERN (MLS 50–3C, Germany). Next, 10 g of each powder was homogenized in 100 mL of ethanol-water (70:30, v/v) and acetone-water (50:50, v/v) respectively. Maceration was carried out at 37°C for 48 hours, following the methodology described by Souhila et al,[21] with certain modifications. After filtration, the solvents were evaporated under vacuum using a rotavapor (Buchi, Switzerland) at 45°C to obtain dry crude extracts. These extracts were weighed and stored in hermetically sealed sterile vials for subsequent biological analysis.

Assay of Polyphenol Content

Folin Ciocalteu method was used to estimate the polyphenol content in crude extract samples.[22] In brief, 50 µL of extracts (10 mg/mL) was mixed with 25 µL of Folin Ciocalteu reagent (1 N), 50 µL of ethanol (95%), and 250 µL of distilled water. After 5 min incubation, 50 µL of 5% sodium carbonate (Na_2CO_3) was added to the previous mix and incubated for 60 min at 37°C. The absorbance of the final mixture was measured at 725 nm using a UV-visible spectrophotometer (BIOBASE, China). The results were expressed in mg gallic acid equivalent per 100 mg dry extract (mg GAE/100 mg DE) using the calibration curve whose equation is (Figure 2).

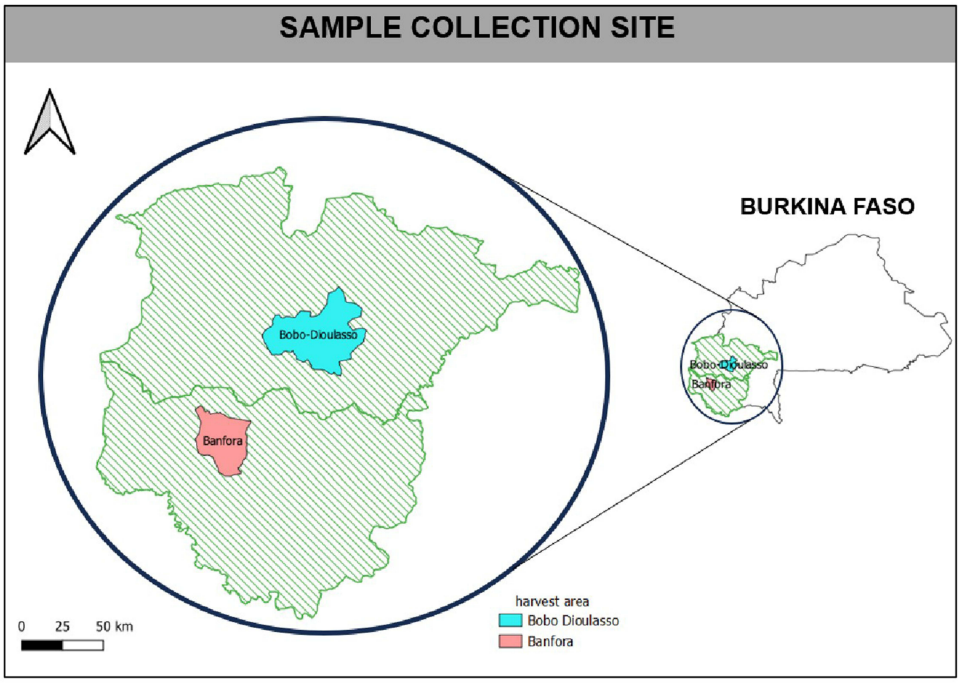

Figure 1 Sample collection site (Banfora in red and Bobo in cyan).

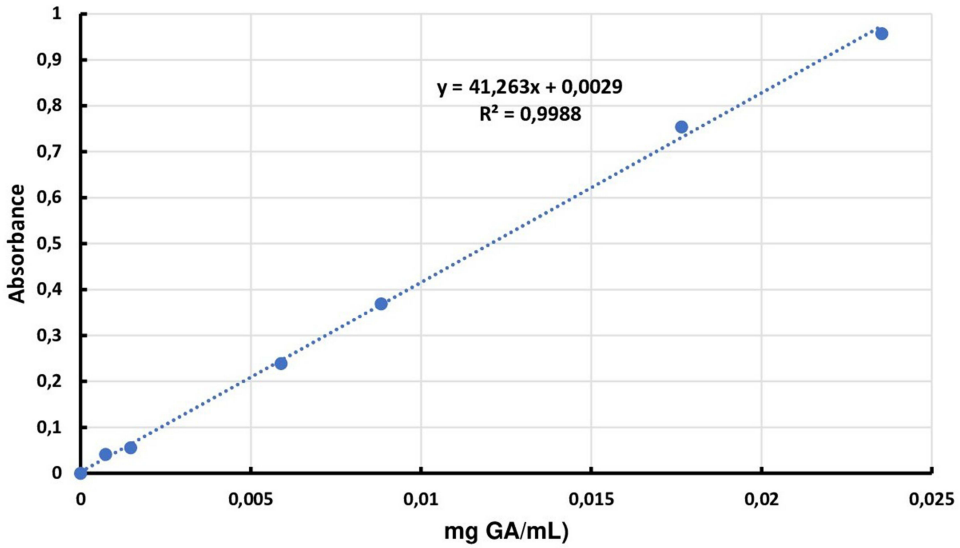

Figure 2 Gallic acid calibration curve for quantifying polyphenols in plant extracts obtained by spectrophotometer at 725 nm. The concentration range used is between 0 and 0.025 mg/mL. The equation of the resulting straight line ($y = 41.263x + 0.0029; R2 = 0.998$) expresses the polyphenol content in gallic acid equivalent.

Assay of Flavonoid Content

The flavonoid content was quantified using the method of Zengin et al[23],100 µL of each extract (10 mg/mL) was added to 100 µL of aluminum trichloride. The absorbances were then read at 430 nm, and the results were expressed as mg quercetin equivalent per 100 mg dry extract (mg QE/100 mg DE) using the quercetin calibration curve (y = 50.315x + 0.0133; R2 = 0.9982) (Figure 3).

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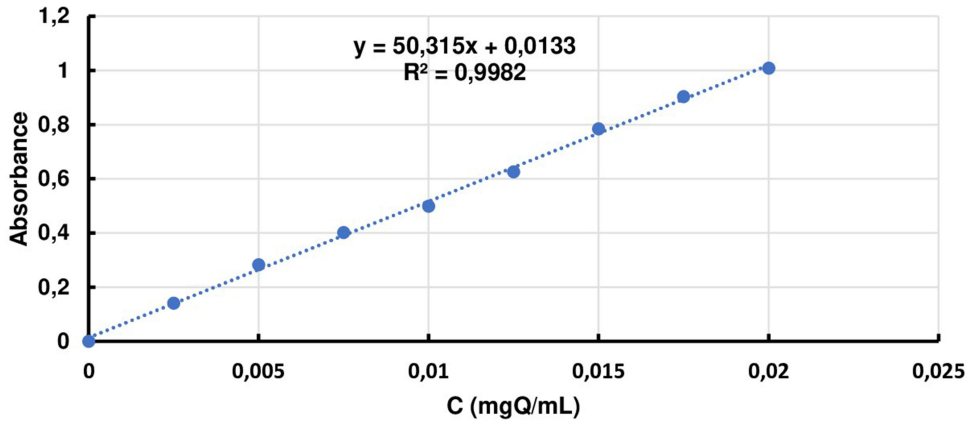

Figure 3 Quercetin (Q) calibration curve for quantifying flavonoids in plant extracts obtained by spectrophotometer at 430 nm. The concentration range used is between 0 and 0.025 mg/mL. The equation of the resulting straight line ($y = 50.315x + 0.0133; R2 = 0.9982$) expresses the flavonoid content in quercetin equivalent.

Assessment of Antioxidant Activity

DPPH (2,2-Diphényl 1-Picrylhydrazyl) Radical Scavenging Test

The DPPH radical scavenging test was evaluated by the method described by Sembiring et al[24] with some modifications. Thus, in a 96-well plate, 40 µL of extract at different concentrations (0–1000 µg/mL) were mixed with 160 µL of DPPH$^{\bullet}$ (0.02 mg/mL). After incubation for 15 min at room temperature, the absorbances were read against a blank consisting only of methanol (200 µL) at 517 nm. The 50% inhibitory concentration (IC_{50}) was determined, and the results were expressed in µg /mL.

ABTS (2.2-Azino-Bis(3éthylbenz-Thiazoline-6-Sulfonic acid)) Radical Inhibition Test

This test was performed using the method described by Khatua et al,[25] with some modifications. Thus, in a 96-well plate, 20 µL of extract at different concentrations (20–500 µg/mL) were mixed with 180 µL of radical ABTS. After incubation for 5 min at room temperature, the absorbances were read against a blank consisting of extract and PBS (pH4.9) at 405 nm. The 50% inhibitory concentration (IC_{50}) was determined, and the results were expressed in µg /mL.

FRAP (Ferric Reducing Antioxidant Power) Test

This test was carried out according to the method used by Hinneburg et al,[26] with some modifications. A 20 min incubation at 50°C was performed by mixing 125 µL extract, 125 µL phosphate buffer (0.2 M, pH 6.6), and 125 µL aqueous potassium hexacyanoferrate solution [K_3 Fe $(CN)_6$] (1%). Next, 125 µL trichloroacetic acid (10%) was added to the previous mixture and allowed to settle for 15 min. Finally, 90 µL of the supernatant of the resulting mixture was added to 90 µL of distilled water and 20 µL of a freshly prepared $FeCl_3$ solution (0.1%). Absorbances were read at 725 nm, and ascorbic acid (0–1000 µg/mL) was used to make the calibration curve ($y = 0.2283x + 0.1046; R2 = 0.9595$) (Figure 4). The iron (III) reducing activity was carried out in triplicate and expressed in mg of ascorbic acid equivalent (EAA)/g dry extract (mg AAE/mL DE).

Evaluation of Anti-α-Amylase Activity

Extraction of α-Amylase From Megamylase

The extraction method Kabré et al[27] involved removing the surface layer of the one megamylase tablet and crushing it to obtain a powder. This powder was then dissolved in 10 mL distilled water containing 1 mL 0.1% calcium chloride. The final solution after the mixture filtration contains 300 U/mL megamylase. For the experiment a 3 U/mL stock solution has been prepared using the last one 300 U/mL megamylase.

A-Amylase Inhibition Test

The inhibitory activity of α-amylase was evaluated according to the method of Gazali et al,[28] with some modifications. Briefly, a mixture of 100 µL pH 6.9; 0.02 M phosphate buffer, 1 mL plant extract (1mg/mL) or Acarbose (1mg/mL), and

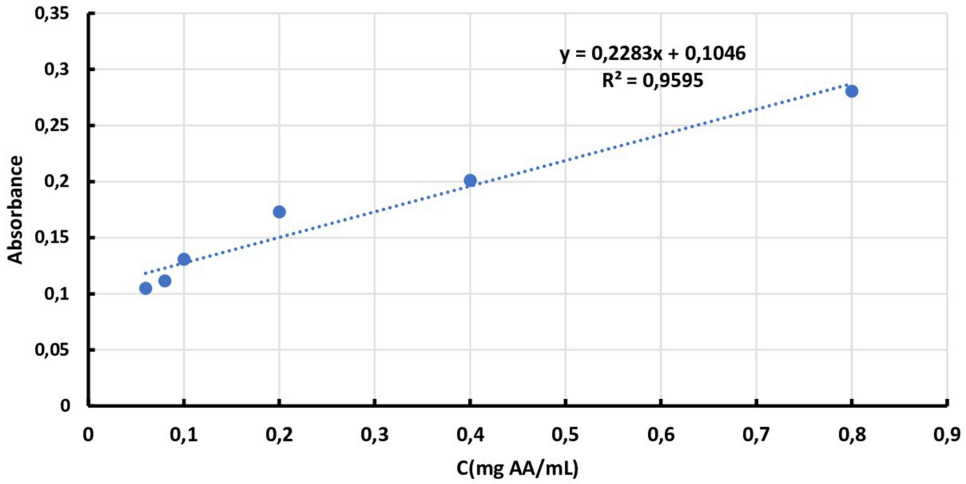

Figure 4 Ascorbic acid (AA) calibration curve used to assess the iron-reducing activity of plant extracts, determined spectrophotometrically at 725 nm. The concentration range used is 0 to 0.8 mg/mL. The equation of the straight line obtained ($y = 0.2283x + 0.1046; R2 = 0.9595$) expresses the iron-reducing capacity of each extract in ascorbic acid equivalent.

100 µL enzyme solution were incubated at 37 °C for 20 min. Then, 100 µL 1% starch were added to the mixture, and incubated at 37°C for 20 min. Finally, 100 µL 1% DNS stop solution were had. After 10 min incubation at 100 °C following by an ice bath cooling the absorbance measurement was performed at 540 nm. The results were expressed according the following formula: $I(\%) = [(A\ Control - A\ test)/A\ Control]$ with I: inhibition.

Data Analysis

Results are presented as means ± SD. Data visualization was performed using GraphPad Prism 8.0.2 and Excel 2016. One-way ANOVA followed by Tukey's post hoc test (R Commander) was used to determine statistical significance. Values of $p < 0.05$ were considered statistically significant. Principal Component Analysis (PCA) was performed to assess the relationships between phenolic compounds and biological activities, allowing the characterization of each extract.

Results

Polyphenol Content

The highest polyphenol content was obtained with the LSBaAw extract (32.62 ± 0.85 mg GAE/100 mg DE) (Figure 5). The results show a highly significant difference between this extract and the ethanol-water extract (LSBaEw: 25.89 ± 0.33 mg GAE/100 mg DE), indicating that the solvent influences polyphenol content. Furthermore, for the same solvent, a significant difference was observed between LSBaAw and LSBoAw (24.97 ± 0.34 mg GAE/100 mg DE) ($p < 0.001$), also highlighting the impact of the harvesting site.

Flavonoid Content

The results show that the ethanol-water extract (RBoEw: 4.59 ± 0.02 mg EQ/100 mg DE) has the highest flavonoid content (Figure 6). A highly significant difference was observed between RBoEw and RBoAw (4.13 ± 0.07 mg EQ/100 mg DE), as well as between RBoEw and RBaEw (3.65 ± 0.02 mg EQ/100 mg DE) ($p < 0.001$), highlighting the influence of solvent and collection zone on this flavonoid content.

Antioxidant Activity

ABTS Radical Scavenging Activity

The results reveal that the ethanol-water extract (LSBoEw = 81.34 ± 1.07 µg/mL) exhibits the highest ABTS free radical scavenging activity (Table 1). A significant difference was observed compared to LSBoAw (93.04 ± 3.84 µg/mL) and

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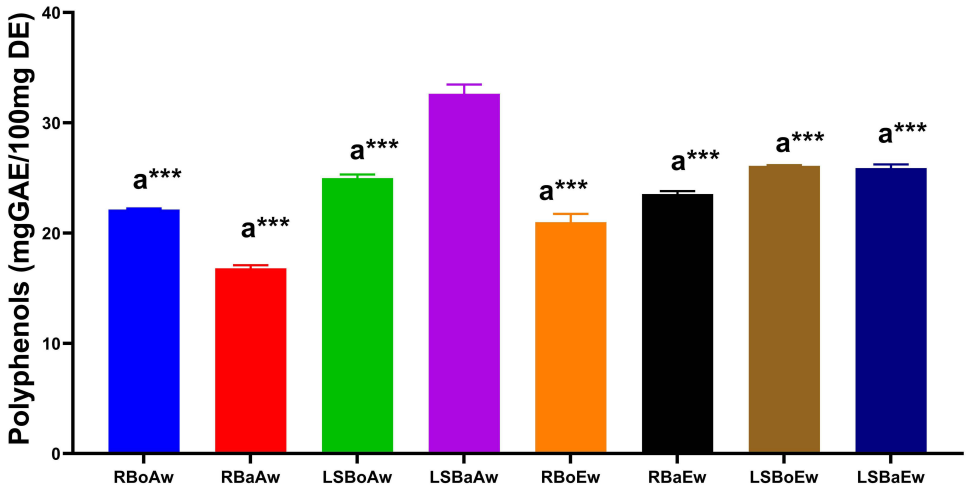

Figure 5 Impact of the solvent and harvesting site on the polyphenol content in the organs (roots and leafy stems) of *Phyllanthus amarus*.
Notes: Each value represents means ± SD (n = 3). Analysis was performed using one-way ANOVA (Analysis of Variance) followed by a Tukey post hoc test. [a]Against LSBaAw; ***p<0.001.
Abbreviations: RBoAw, Acetone-water extract of the roots of Bobo; RBoEw, Ethanol-water extract of the roots of Bobo; LSBoEw, Ethanol-water extract of the Leafy Stem of Bobo; LSBoAw, Acetone-water extract of the Leafy Stem of Bobo; RBaAw, Acetone-water extract of the roots of Banfora; RBaEw, Ethanol-water extract of the roots of Banfora; LSBaEw, Ethanol-water extract of the Leafy Stem of Banfora; LSBaAw, Acetone-water extract of the Leafy Stem of Banfora.

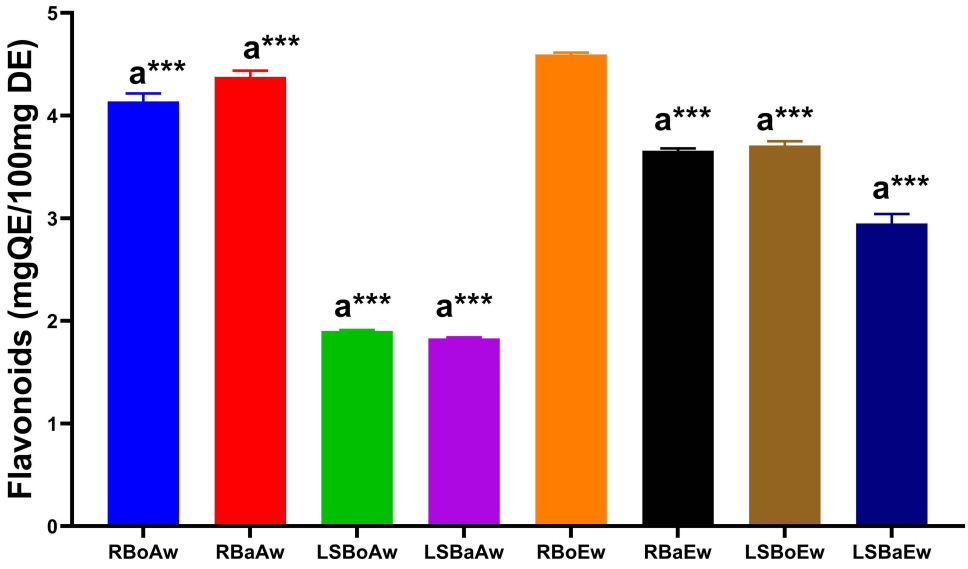

Figure 6 Impact of the solvent and harvesting site on the flavonoid content in the organs (roots and leafy stems) of *Phyllanthus amarus*.
Notes: Each value represents means ± SD (n = 3). Analysis was performed using one-way ANOVA (Analysis of Variance) followed by a Tukey post hoc test. [a]Against RBoEw; ***p<0.001.
Abbreviations: RBoAw, Acetone-water extract of the roots of Bobo; RBoEw, Ethanol-water extract of the roots of Bobo; LSBoEw, Ethanol-water extract of the Leafy Stem of Bobo; LSBoAw, Acetone-water extract of the Leafy Stem of Bobo; RBaAw, Acetone-water extract of the roots of Banfora; RBaEw, Ethanol-water extract of the roots of Banfora; LSBaEw, Ethanol-water extract of the Leafy Stem of Banfora; LSBaAw, Acetone-water extract of the Leafy Stem of Banfora.

LSBaEw (184.16 ± 3.25 µg/mL) extracts ($p < 0.001$). These results highlight the influence of solvent and collection site on this activity.

DPPH Radical Scavenging Activity

The influence of solvent and collection zone on DPPH free radical scavenging activity was demonstrated. A highly significant difference was observed between the RBaEw extract (55.71 ± 2.48 µg/mL) and the RBaAw (115.82 ± 1.21 µg/mL) and RBoEw (67.24 ± 0.34 µg/mL) extracts ($p < 0.001$). The ethanol-water extract of Banfora roots (RBaEw) thus showed the highest DPPH free radical scavenging activity (Table 2).

Table 1 Effect of the Solvent and Harvesting Site on the ABTS Radical Scavenging Activity of the Roots and Leafy Stems of *Phyllanthus amarus*

Extracts/Reference	IC_{50} ABTS (µg /mL)
RBoAw	155.31 ± 2.57a***b***
RBoEw	154.73 ± 2.63a***b***
LSBoEw	81.34 ± 1.07a***
LSBoAw	93.04 ± 3.84a***b*
RBaAw	143.99 ± 4.63a***b***
RBaEw	171.04 ± 4.32a***b***
LSBaAw	114.42 ± 5.96a***b***
LSBaEw	184.16 ± 3.25a***b***
Ascorbic acid	42.24 ± 0.13

Note: Each value represents means ± SD (n = 3). Analysis was performed using one-way ANOVA (Analysis of Variance) followed by a Tukey post hoc test. [a]Against Ascorbic acid; [b]Against LSBoEw; ***p<0.001; *p<0.05.

Abbreviations: RBoAw, Acetone-water extract of the roots of Bobo; RBoEw, Ethanol-water extract of the roots of Bobo; LSBoEw, Ethanol-water extract of the Leafy Stem of Bobo; LSBoAw, Acetone-water extract of the Leafy Stem of Bobo; RBaAw, Acetone-water extract of the roots of Banfora; RBaEw, Ethanol-water extract of the roots of Banfora; LSBaEw, Ethanol-water extract of the Leafy Stem of Banfora; LSBaAw, Acetone-water extract of the Leafy Stem of Banfora. ABTS, 2.2-azino-bis(3éthylbenz-thiazoline-6-sulfonique acid).

Table 2 Effect of the Solvent and Harvesting Site on the DPPH Radical Scavenging Activity of the Roots and Leafy Stems of *Phyllanthus amarus*

Extracts/Reference	IC_{50} DPPH (µg /mL)
RBoAw	90.34 ± 3.33a***b***
RBoEw	67.24 ± 0.34a***b***
LSBoEw	89.48 ± 1.09a***b***
LSBoAw	68.27 ± 0.63a***b***
RBaAw	115.82 ± 1.21a***b***
RBaEw	55.71 ± 2.48a***
LSBaAw	83.46 ± 1.61a***b***
LSBaEw	93.97 ± 3.86a***b***
Ascorbic acid	28.58 ± 0.11

Note: Each value represents means ± SD (n = 3). Analysis was performed using one-way ANOVA (Analysis of Variance) followed by a Tukey post hoc test. [a]Against Ascorbic acid; [b]Against RBaEw; ***p<0.001.

Abbreviations: RBoAw, Acetone-water extract of the roots of Bobo; RBoEw, Ethanol-water extract of the roots of Bobo; LSBoEw, Ethanol-water extract of the Leafy Stem of Bobo; LSBoAw, Acetone-water extract of the Leafy Stem of Bobo; RBaAw, Acetone-water extract of the roots of Banfora; RBaEw, Ethanol-water extract of the roots of Banfora; LSBaEw, Ethanol-water extract of the Leafy Stem of Banfora; LSBaAw, Acetone-water extract of the Leafy Stem of Banfora. DPPH, 2.2-diphényl 1-picrylhydrazyl.

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Iron Reducing Activity

The results show that the acetone-water extract (LSBaAw: 15,445.81 ± 835.75 µmol EAA/100 mg DE) has the highest iron reduction activity (Table 3). A highly significant difference was observed between the LSBaAw extract and those of LSBaEw (9,936.08 ± 580.37 µmol EAA/100 mg DE) and LSBoAw (9,721.98 ± 966.19 µmol EAA/100 mg DE) ($p < 0.001$). This indicates an influence of solvent and collection site on this activity.

Anti-α-Amylase Activity

The results show that the ethanol-water extract (RBaEw: 98.45 ± 0.38%) has the highest α-amylase inhibitory activity (Figure 7). However, this activity was not significantly different from that observed for the Bobo ethanol-water extract (RBoEw: 96.56 ± 0.31%) and the positive control (acarbose: 98.23 ± 0.21%) ($p > 0.05$). On the other hand, a significant difference was observed with Banfora acetone-water extract (RBaAw: 88.93 ± 1.27%), suggesting that inhibitory activity is mainly influenced by solvent and not by collection site. While taking into account the influence of solvent or harvesting site, a principal component analysis (PCA) was carried out to verify which of the organs appeared to be most impacted by the biological activities (Figure 8). Two principal axes explain 68.4% of the total variance (Dim1, associated with α-amylase inhibitory activity, and Dim2, characterized by antioxidant activities). The results show that root extracts, in particular, exhibit strong α-amylase inhibitory activity, irrespective of solvent or harvesting site, suggesting that the organ (roots) plays a key role, in possessing specific bioactive metabolites. On the other hand, antioxidant activity varies according to solvent and harvesting site. However, the organ has a greater influence on inhibitory activity, which is more pronounced in roots than in leafy stems.

Table 3 Impact of the Solvent and the Harvesting Site on the Iron-Reducing Activity of the Organs (Roots and Leafy Stems) of *Phyllanthus amarus*

Extracts/ Reference	FRAP (µmol EAA/100mg DE)
RBoAw	10214.66 ± 379.18a***
RBoEw	6790.43 ± 81.25a***
LSBoEw	5249.20 ± 254.46a***
LSBoAw	9721.98 ± 966.19a***
RBaAw	10381.04 ± 243.75a***
RBaEw	9056.48 ± 31.27a***
LSBaAw	15445.81 ± 835.75
LSBaEw	9936.08 ± 580.37a***
Ascorbic acid	-

Note: Each value represents means ± SD (n = 3). Analysis was performed using one-way ANOVA (Analysis of Variance) followed by a Tukey post hoc test. [a]Against LSBaAw; ***p<0.001.

Abbreviations: RBoAw, Acetone-water extract of the roots of Bobo; RBoEw, Ethanol-water extract of the roots of Bobo; LSBoEw, Ethanol-water extract of the Leafy Stem of Bobo; LSBoAw, Acetone-water extract of the Leafy Stem of Bobo; RBaAw, Acetone-water extract of the roots of Banfora; RBaEw, Ethanol-water extract of the roots of Banfora; LSBaEw, Ethanol-water extract of the Leafy Stem of Banfora; LSBaAw, Acetone-water extract of the Leafy Stem of Banfora. FRAP, Ferric reducing antioxidant power.

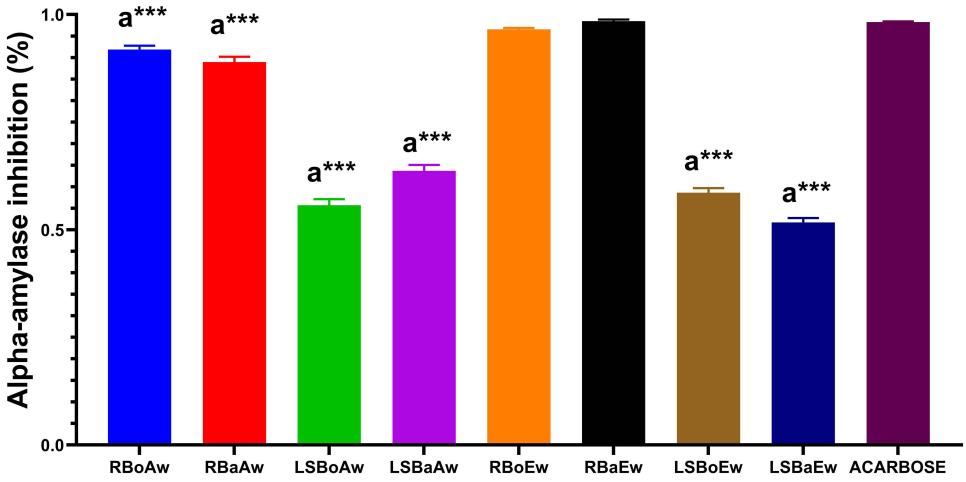

Figure 7 Impact of the solvent and collection site on the α-amylase inhibitory activity of the organs (roots and leafy stems) of *Phyllanthus amarus*.
Notes: Each value represents means ± SD (n = 3). Analysis was performed using one-way ANOVA (Analysis of Variance) followed by a Tukey post hoc test. [a]Against ACARBOSE; ***p<0.001.
Abbreviations: RBoAw, Acetone-water extract of the roots of Bobo; RBoEw, Ethanol-water extract of the roots of Bobo; LSBoEw, Ethanol-water extract of the Leafy Stem of Bobo; LSBoAw, Acetone-water extract of the Leafy Stem of Bobo; RBaAw, Acetone-water extract of the roots of Banfora; RBaEw, Ethanol-water extract of the roots of Banfora; LSBaEw, Ethanol-water extract of the Leafy Stem of Banfora; LSBaAw, Acetone-water extract of the Leafy Stem of Banfora.

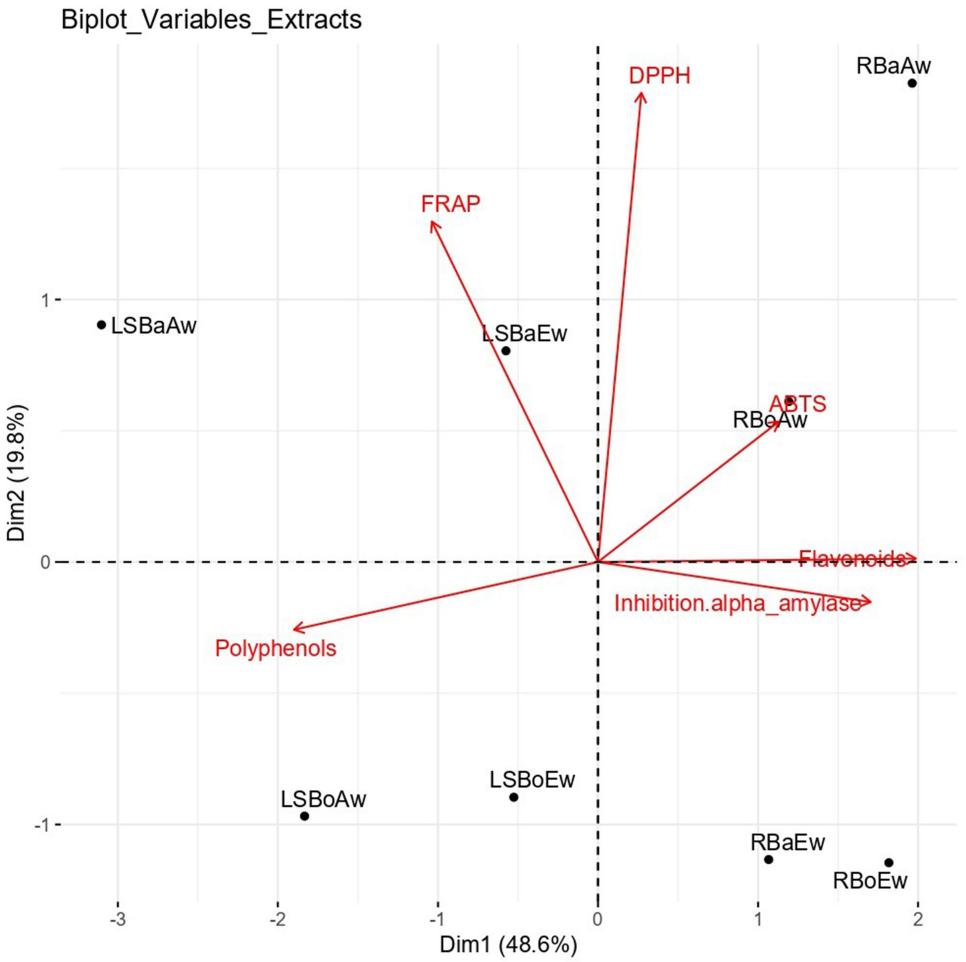

Figure 8 Biplot of variables (red) and extracts (black) on Dim1 and Dim2. Dim 1 is characterized by alpha-amylase inhibitory activity, while Dim 2 is defined by free radical scavenging activity (DPPH: IC_{50}) and iron-reducing activity (FRAP). FRAP, Ferric reducing antioxidant power; DPPH, 2.2-diphényl 1-picrylhydrazyl.

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Discussion

In this study, the efficacy of acetone and ethanol for the extraction of polyphenols and flavonoids from *Phyllanthus amarus* extracts was compared. The results show that these solvents influence the chemical composition of the extracts differently due to their differences in polarity[29] and their ability to solubilize various secondary metabolites.[30] Acetone and water have proved particularly effective for polyphenol extraction, in line with the work of Zhou et al.[31] The addition of water to organic solvents improves their solubility, as reported by Sripad et al,[32] this solubility depends mainly on the presence of hydroxyl groups, the molecular weight, and the structure of the compounds.[33] In contrast, the ethanol-water mixture favors flavonoid extraction, probably due to a proportion of water (~30%) sufficient to improve solubility without compromising extraction. This observation is consistent with the results of Do et al,[34] who indicate that increasing the water content in a solvent-water mixture can reduce the concentration of flavonoids in the extract. Despite this solvent selectivity, extracted polyphenols and flavonoids play a key role in antioxidant activity. Several studies have shown that these compounds share common properties, including the ability to scavenge free radicals via their hydroxyl groups and to chelate transition metal ions.[35,36] Moreover, flavonoids are not limited to their antioxidant effects but possess α-amylase inhibitory activity.[20] The results show that ethanol-water extracts exert a stronger inhibitory effect on α-amylase than acetone-water extracts. A positive correlation (r=0.74) between flavonoid content and this activity (Figure 8) suggests that ethanol-water favors the extraction of the most active flavonoids. These results are in agreement with previous studies,[37–39] notably that of Lo Piparo et al,[37] who showed that the inhibition of α-amylase by flavonoids correlates with the number of hydroxyl groups on the B-ring, involving hydrogen bonds with the enzyme's catalytic residues and a π-conjugate system stabilizing affinity for the active site, a mechanism similar to that of acarbose.[20] On the other hand, Perera et al[40] observed that *Phyllanthus amarus* roots contain a higher content of flavonoids, which play a major role in α-amylase inhibition. In addition to the influence of the solvent, the results highlight the impact of the harvesting site on the phenolic compound content and biological activities of the extracts. This variability could be attributed to abiotic factors[41,42] such as rainfall and soil conditions, which influence the biosynthesis of secondary metabolites. Finally, the distribution of polyphenols and flavonoids in the various plant organs directly influences their biological activities. Polyphenols, mainly present in leafy stems, are well known for their antioxidant properties, notably their ability to neutralize free radicals and chelate metal ions,[35,36] which could explain the high antioxidant activity of leafy stem extracts. Conversely, flavonoids, mostly concentrated in roots, seem to be involved in α-amylase inhibition, as confirmed by the positive correlation between their content and this activity (Figure 8). This distinction between organs underlines the importance of choosing the right part of the plant for targeted exploitation of *Phyllanthus amarus* in pharmacology. This study highlights the influence of solvent and harvesting sites on the chemical composition and biological activities of *Phyllanthus amarus* extracts. To better understand these interactions, further investigations on other geographical sites and solvents would be necessary. This approach would optimize the extraction of bioactive compounds and enhance the pharmacological potential of the species.

Conclusion

This prospective study highlighted the influence of extraction solvent and harvesting site on the polyphenol and flavonoid content and the antioxidant and α-amylase inhibitory activities of *Phyllanthus amarus* extracts. Acetone water favors polyphenol extraction, while ethanol water is more suitable for flavonoid extraction. These differences influence the biological properties of the extracts, with greater antioxidant activity in polyphenol-rich extracts and stronger α-amylase inhibition in flavonoid-rich extracts. The study also highlights the impact of harvesting sites, which influences phenolic compound content and biological activities, probably due to abiotic factors affecting their biosynthesis. In addition, the distribution of secondary metabolites varies according to the organ: polyphenols, mainly present in leafy stems, explain the high antioxidant activity, while flavonoids, concentrated in roots, play a key role in α-amylase inhibition. Finally, these results highlight the importance of the choice of solvent, organ, and harvesting site for optimal valorization of *Phyllanthus amarus*. Further studies, including other solvents and geographical sites, would enable us to extend these observations and optimize the pharmacological use of this plant.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these sites; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare that there is no conflict of interest.

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