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Research Article

In-Vitro Assessment of The Nutritional, Anti-Nutritional, And Anti-Diabetic Extracts' Effects of *Xymalos Monospora*

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Abstract

Objective: This study was conducted to evaluate the nutritional, antinutritional, and antidiabetic properties of leaf extracts of *X. monospora*.
Materials and methods: Aqueous (AqSE) and ethanol (EtE) extracts (100, 200 mg/kg) were produced from *X. monospora* leaves. Four experimental samples were prepared from AqSE and EtE. Nutritional, antinutritional, and antidiabetic properties were determined. The α-amylase inhibition assay was performed *in vitro*.
Results: Preliminary phytochemical constituents' investigations revealed that both AqsE(S) and EtE(S) contain antioxidants (1.73±0.10, 14.00±1.31), flavonoids, phenolics (0.68±0.06, 4.45±0.38), saponins (0.37±0.07, 0.39±0.05), oxalate (3.06±0.29, 5.08±0.285), and tannins (0.69±0.01, 0.65±0.04) that may be responsible for the antidiabetic effects of the plant.
Conclusion: Therefore, *X. monospora* leaves have the nutritional, antinutritional, antioxidant, and antidiabetic contents that could help diabetic patients to lower their blood glucose levels, improve blood lipid profiles, and reduce the risk of developing type 2 diabetes.

1. Introduction

Type 2 diabetes is a common metabolic condition that develops when the body fails to produce enough insulin or when insulin fails to work properly [1]. According to the latest report of International Diabetes, by 2045, approximately 783 million people worldwide will suffer from diabetes mellitus (DM), of which more than 90% will have type 2 diabetes mellitus (T2DM) [2, 3]. Type 2 diabetes often develops as a result of overweight, obesity, and lack of physical activity, and the prevalence of type 2 diabetes is on the rise worldwide. Type 2 diabetes is commonly seen in persons older than 45 years and increasingly seen in children, adolescents, and young adults due to rising levels of energy-dense diets. The most common symptoms of type 2 diabetes include increased thirst, fatigue, blurry vision, frequent urination, wounds that do not heal, yeast infections, hunger, and weight loss [4].

According to the International Diabetes Federation (IDF, 2021), the prevalence rates of this health condition are rapidly growing around the world, currently estimated at 10.5%, and the number of diabetic patients is predicted to reach 642 million by 2040. Epidemiologically, type 2 diabetes mellitus represents 90% of all diabetes cases in Africa, mainly attributed to more rural populations with limited resources and low prioritizations of screening [5].

The prevalence of type 2 diabetes is estimated to be 5.7% in urban Cameroon, with more than 1 million people living with the disease, 70% of whom remain undiagnosed [6].

In addition to lowering the risk of developing type 2 diabetes, there is also evidence that plants and diets can be used in the treatment and management of type 2 diabetes, body weight, and cardiovascular risk factors, according to the American Society for Nutrition. Diets such as vegetables, whole grains, fruits, nuts, legumes, and seeds may also be used in the treatment and management of type 2 diabetes. Also, dietary

habits play a significant role in insulin resistance, especially for people who are physically inactive. In view of the side effects associated with the treatment by insulin and synthetic drugs, which are available at present, searching for effective and safer antidiabetic plant drugs is ongoing all over the world. Thus, the development of newer therapeutic approaches remains highly desirable, and herbal medicines play a vital role in preventing the side effects [7].

Also, plant-based diets have many positive effects on secondary factors related to diabetes, including physical/emotional distress, quality of lifestyle, body weight, total cholesterol, and low-density lipoprotein (LDL) cholesterol. Moreover, plant-based diets together with regular exercise not only improved diabetic conditions but also reduced the need for medicine compared to conventional diets, which only reduced caloric and carbohydrate intake [8]. Plants and their extracts have been investigated for their antioxidant activities in recent scientific developments throughout the world. Many of these extracts have been found to exert therapeutic effects against different diseases [9], including their abilities to alleviate toxic effects from free radicals caused by environmental pollutants. Recently, investigations of the stem bark of *X. monospora* isoquinoline alkaloids, benzyl tetrahydro isoquinoline, and benzyl isoquinoline display more potent anticancer activities. Also, in Africa, *X. monospora* is used by tribals for the treatment of parasitic diseases, which include malaria and trypanosomiasis, also known as sleeping sickness [10]. Today, consumers do not only want to depend on pharmaceutical drugs compounded and sold by pharmaceutical industries or pharmacies but also want the drugs (medicines) to be convenient, less expensive, less toxic, sustainable, and healthy.

Also, since the cost price of most pharmaceutical drugs or medicines has been the major challenge to many Cameroonians today, using *Xymalos monospora* leaf extracts will serve as an alternative herbal remedy to individuals and also at the industrial level. In Cameroon, *X. monospora* is used for the treatment of bacteria and microbial infections and was found to display different mechanisms of antibacterial and antibiotic-enhancing activities. However, the traditional use of the plant for its nutritional, antinutritional, and antioxidant properties has attracted the search for new drugs or plant-derived molecules that may enable them to target multiple biochemical pathways [11]. Thus, the present study was aimed to evaluate the nutritional, antinutritional, and antidiabetic properties of extracts of *X. monospora*. Specifically, it was to produce the aqueous and ethanol extracts with the high nutritional, anti-nutritional, and antidiabetic content of *Xymalos monospora*.

2. Materials and Methods

Materials

2.1. Raw materials

The raw material for the preparation of aqueous and ethanol extracts was *Xymalos monospora* leaves.

2.2. Plant identification and sample collection

The plant, together with the leaves, flowers, roots, bark, seeds, and fruits, were harvested from Oshie in the Njikwa Subdivision of the Northwest Region of Cameroon. The plant species was identified at the natural herbarium center in Yaoundé, found in the Centre region of Cameroon, as *Xymalos monospora* (Harv.) Baill ex Warb under the reference number No 50557/HNC.

2.3. Study area

Preparation of *Xymalos Monospora* leaves, production of extracts of *Xymalos Monospora*, analysis of nutrients, antinutrients, antioxidants properties.

2.4. Chemicals/Reagents

All chemicals and reagents used were of analytical grade. 70% ethanol, 70% distilled water, metformin tablets (60 mg/kg), dexamethasone (11 mg/kg), ascorbic acid ($C_6H_8O_6$) with 99% purity, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and β-carotene, acetate buffer, and diazepam (5 mg/mL).

2.5. Other materials

Equipment: Centrifuge (Hettich), UV-VIS spectrophotometer (MODEL: 752), water bath, refrigerator, deep freezer, pH meter, stopwatch, beakers, volumetric cylinder, aluminum foil paper, glassware, electronic scale, domestic mixer (Philip), 800 µm-mesh sieve, knife, analytical balance, micropipettes, aluminum trays (45 cm × 33 cm), conical flask, beakers, mortar and pestle, stopwatch, thermostat oven DHG-ISA PEC, test tubes, storage containers, graduated syringes, labeling tapes, bold markers, and Whatman filtered papers.

Methods

2.6. Preparation of aqueous and ethanol extracts

Preparation of aqueous and ethanol extracts were done according to the method reported by [12].

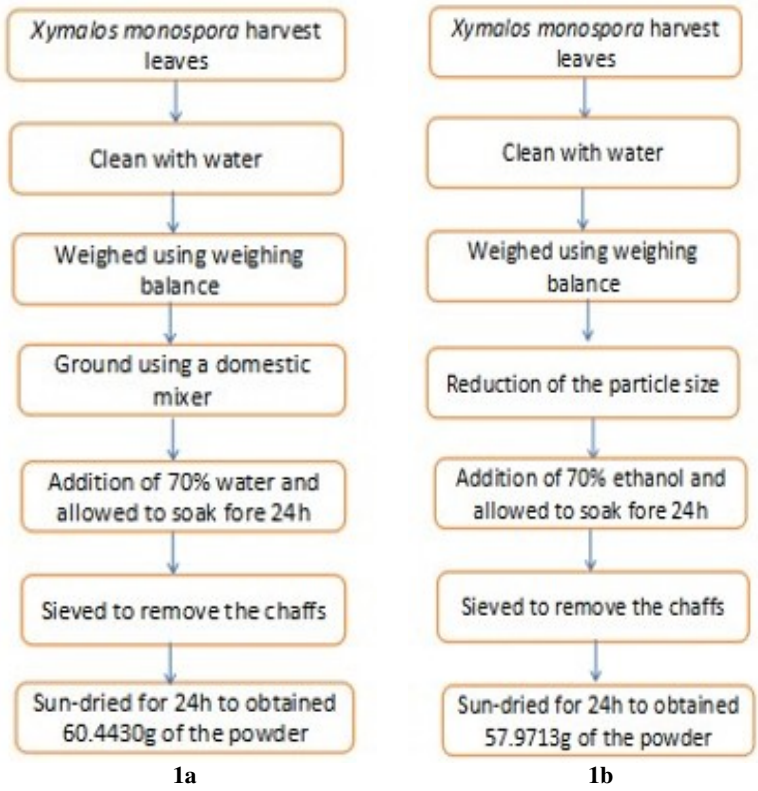

Figure 1: Preparation of aqueous (AqsE:1a) and ethanol extracts (EtE: 1b) of *X. monospora*

2.7. Calculation of yield

Preparation of aqueous extracts

Three kilograms (3 kg) of leaves were weighed using a weighing balance, washed, and ground using the domestic mixer (Philip), and 70% water was added. It was allowed to soak for 24 h. After 24 h, it was sieved using an 800 µm-mesh sieve to remove the chaff. The extract was dried for 24 h, followed by oven drying for 24 h at the temperature of 50°C to obtain the powder Figure 1.

Preparation of ethanol extracts

Also, 3 kg of leaves were weighed using a weighing balance, washed with clean water, and the particle size reduced using a knife. The leaves were allowed to dry in the sun for 24 h. After drying, the leaves were ground using the domestic mixer, and 70% ethanol was added. It was allowed for 24 h under room temperature. After 24 h, the leaves were sieved, concentrated, and dried for 24 h to obtain the powder. Immediately after drying, the leaves were weighed using the analytical balance. The mass was recorded, and the extract was stored in an airtight plastic container Figure 1.

The percentage yield of different extractive values of the fresh sample of the AqsE and EtE was calculated as

$$\text{Yield} = \frac{\text{Weight of extract}}{\text{Weight of fresh sample}} \times 100$$

2.8. Nutritional content

Determination of mineral contents Zinc (Zn), Iron (Fe), Magnesium (Mg), and Potassium (K))

Principle: The principle of the method is based on nebulizing a sample solution into an air-acetylene flame where it is vaporized. Elemental ions would be atomized, and the atoms would then absorb radiation of a characteristic wavelength from a hollow cathode lamp. The absorbance measured is proportional to the number of analysts in the sample solution. The amount of each element in the sample solution is determined by reference to a calibration curve.

Procedure: The procedure described by [13] was employed. The minerals zinc, magnesium, iron, and potassium were determined using atomic absorption spectrometry (Perkin Elmer atomic absorption spectrophotometer analyst 700). All equipment used, except for the

spectrometer, were first immersed in 10% nitric acid for at least 2 hours, after which it was rinsed with deionized water and dried in an oven at 40°C. A mass of 2 g of sample was weighed into Teflon capsules and digested in 8 ml of concentrated nitric acid. The digest was left to cool for 6 hrs and then quantitatively transferred to a 50 ml volumetric flask and made up to the volume mark with deionized water. A blank digest was carried out in the same way using deionized water. Readings were performed at different wavelengths for Zn, Fe, K, and Mg, respectively.

Expression of results
The amount of each mineral (mg/100) was obtained using the formula

$$\text{Mineral content (mg/100)} = \frac{V_t(c_s - c_b) \times 100 \times 100}{M \times \text{dry matter}}$$

Where,

- v_t = volume of sample
- C_s = concentration of mineral in the sample analyzed
- C_b = concentration of blank
- M = concentration of the sample used

Determination of vitamin A

Principle: The principle for determining vitamin A (β-carotene content) involves the use of spectrophotometric methods, specifically UV-visible spectrophotometry. In these methods, a specific wavelength of light was used to detect the presence and quantity of vitamin A (β-carotene content). The absorption of light at the specified wavelength is used to quantify the concentration of vitamin A in the sample.

Procedure: Beta-carotene extraction from the sample was done according to the method reported by [14]. The 0.5 g sample was weighed and soaked in 25 mL of methanol at room temperature under dark conditions for 24 h. The extracts were centrifuged to separate the supernatant, and these operations were repeated until the sample was completely colorless. The volume was made up to 50 mL with methanol. The absorbance of the extract were read at 543 nm. β-carotene in the extract (μg/mL) was calculated using the β-carotene standard curve. A 15 μg/mL standard stock solution of β-carotene was prepared by dissolving 750 μg of standard β-carotene in 50 mL of methanol. From the stock solution, serial dilutions were made to obtain 0.1, 0.5, 1.5, 3.6, 9, and 12 μg/mL β-carotene. These working standard solutions were scanned in the range of 350-600 nm. The absorption spectra data were collected from typical absorption peaks, with the maximum obtained from 543 nm for plotting the calibration curve.

Expression of results
β-carotene content in μg/mL was obtained using the formula From

$$\text{C} = \frac{\text{CV}}{M}$$

Where,

- C=β-carotene content in micrograms per milliliter (μg/mL)
- C = concentration of the standard stock solution (blank) in g/mL
- V = volume of extract in μg/mL
- M = mass of the extract in grams (g)

Determination of vitamin C by iodometric titration

Principle: The principle for determining vitamin C is generally based on titration methods, which involve the reaction between vitamin C (ascorbic acid) and an oxidizing agent such as iodine.

Procedure: Ascorbic acid determination was carried out by iodine titration. When iodine is added to a starch solution, it reacts to produce a dark blue color. However, if there is any vitamin C, it neutralizes the iodine, preventing the formation of a blue color. As iodine is added during the titration, the ascorbic acid is oxidized to dehydro ascorbic acid while the iodine is reduced to iodide anions. The endpoint of the titration is determined by the first excess of iodine in the reaction medium that reacts with starch, forming a starch-iodine complex with an intensive dark blue-violet color. 1 g of the sample was weighed, and 10 mL of distilled water was added. The solution was shaken vigorously and filtered, followed by rinsing to 10 mL volume. 1 mL of starch solution (1%) was added and titrated with Lugol's iodine solution. The titer values were recorded, and ascorbic acid content was calculated from the standard calibration curve.

Expression of results
Ascorbic acid content in parts per million (ppm) was obtained using the formula
From

$$\text{C} = \frac{\text{CV}}{M}$$

Where

- C=ascorbic acid content in ppm
- C = concentration of the standard stock solution (blank) in mg/mL
- V = volume of extract in mL
- M = mass of the extract in grams (g)

2.9. Antinutrient contents

Determination of saponin content

Principle: The principle for determining saponin content is based on the ability to form stable foam (foam index), commonly referred to as the "hemolysis test." In this test, a solution containing saponin is shaken vigorously to generate foam, which is then measured and compared to a control or standard solution.

Procedure: 0.5 grams of the sample were weighed using the electronic balance. 10 mL of 20% acetic acid in ethanol was added. It was heated in a water bath at the temperature of 500°C for 24 hr. After 24 hr, it was filtered and the filtrate collected. The filtrate was then concentrated in a water bath to ½ its original volume. Concentrated NH_4OH was added in drop wise fashion to precipitate saponins. It was allowed to settle and filtered using Whatman filter paper.

Expression of results
The precipitate was weighed, and % saponins was calculated as:

$$\frac{\text{Weight of empty filter paper+Residue-Weight of filter paper}}{\text{Weight of sample}}$$

Determination of tannin content [15]

Principle: When acidified vanillin is in the presence of condensed tannins, it develops a red color with a maximum absorbance at 510 nm.

Apparatus: Whatman filtered paper number one, 250 mL conical flask.
Reagents: 10 mL of 10% acetic acid, 2.5 mL of NH_4OH, 20 mL of ethanol, 10 mL of hexane, and phenolphthalein indicator.

Procedure: The procedure used to determine tannin content was adopted from Dennis's method as described by Person (1974). One gram (1 g) of the sample was weighed. 10 mL of hexane was added. It was covered and allowed for 24 hr. After, it was filtered and allowed to stand for 15 min in the sun so that the hexane evaporates. 10 mL of 10% acetic acid in ethanol was added and allowed to stand for 4 hr. It was filtered to collect the filtrate. 2.5 ml of NH_4OH was added to precipitate and filter out. It was heated to remove NH_4OH still in solution. Phenolphthalein indicator was added. The endpoint was pink.

Expression of results
Tannin content was calculated as:

$$C_1V_1 = C_2V_2, \quad C_1 = \frac{C_2V_2}{V_1}$$

$$\%\ \text{tannic acid content} = \frac{C_1}{\text{EIGHT OF SAMPLE}} \times 100$$

Where,

- C_1 = concentration of tannic acid
- V_1 = Volume of tannic acid
- C_2 = concentration of NaOH
- V_2 = Volume of NaOH

Determination of oxalate content

Principle: The principle for determining oxalate content is based on enzymatic assay using oxalate oxidase. The sample is treated with oxalate oxidase, which converts oxalate to hydrogen peroxide, and the amount of hydrogen peroxide formed is measured colorimetrically, and the oxalate content is determined based on the standard curve.

Procedure: Determination of oxalate by titration methods involves three steps: (1) digestion, (2) oxalate precipitation, and (3) permanganate titration.

Digestion: 1 gram of the sample was weighed. 190 mL of distilled water was added. 10 mL of 6M HCL was added. It was boiled at 100°C for 1 h for digestion to occur. After, it was cooled and made up to 250 mL. It was filtered, and the filtrate was collected.

Oxalate precipitation: 3 drops of methyl red indicator were added. NH_4OH was added in dropwise fashion till the solution changed from pink to faint yellow (pH 4 to 4.5). It was heated to 900°C. After heating to 900°C, it was cooled and filtered to remove the precipitate containing ferrous ions. The filtrate was collected and heated to 900°C. 10 mL of 5% $CaCl_2$. solution was added while stirring continuously. After the solution was heated overnight at 250°C for precipitation to occur. It was centrifuged at 2500 rpm for 5 min. The supernatant was decanted and the precipitate collected. The precipitate was dissolved in 10 mL of 20% (v/v) H_2SO_4.

Expression of results
Oxalate content in milligrams per 100 grams (mg/100g) was obtained using the formula
From

$$C = \frac{CV}{M}$$

Where,

- C=oxalate content in mg/100g
- C = concentration of NH_4OH titer value in mg/100g
- V = volume of extract in mL
- M = mass of the extract in grams (g)

Determination of phytate content

Principle: The principle for determining phytate content is based on spectrophotometric assay using phytase enzyme. The sample is treated with phytase enzymes, which break down phytate, releasing inorganic phosphate, which is measured colorimetrically.

Procedure: 3 grams of the sample were weighed. It was put in a conical flask. 150 mL of 2% HCl was added. It was soaked for 3 h. After, the solution was filtered, and 50 mL of the filtrate was taken out and made up to 250 mL with distilled H_2O. After, 10 mL of 0.3% ammonium thiocyanide was added. The solution was titrated with standard $FeCl_3$ containing 0.0195 g Fe per mL. The end point is red.

Expression of results

$$\text{Phytic acid contents (mg/100g)} = \frac{\text{Titrevalue} \times 0.00195 \times 1.19}{\text{Weight of sample}}$$

2.10. Determination of antioxidant levels and activity

DPPH (2, 2-diphenyl-pirylhydrazyl) free radical scavenging activity method

Principle: The principle is based on the DPPH assay used to determine the ability of a sample to scavenge free radicals. The sample is mixed with a DPPH solution and incubated. The decrease in absorbance due to the scavenging of DPPH radicals is measured spectrophotometrically.

Procedure: DPPH (2, 2-diphenyl-1-picrylhydrazyl) produces a violet-purple color in methanol solution and fades to shades of yellow in the presence of antioxidants. A solution of 0.1 mM DPPH in methanol was prepared, and a stock solution of 10 µg/mL was prepared. Dilutions of the stock solution were prepared to get 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, up to 90 µg/mL. Absorbance was read at 517 nm. From the extracts, a stock solution was prepared at 25, 50, 70, and 100 µg/mL. To the sample solution of different concentrations, 1 mL of DPPH solution was added. It was incubated at room temperature for 30 min in the dark. A control was prepared by mixing 1 mL methanol and 1 mL DPPH solution. Absorbance of all the solution was measured using a spectrophotometer at 517 nm. Ascorbic acid was used as the standard. IC_{50}, 50% inhibitory concentrations, and IC_{50} values of the extracts were calculated from the graph as concentration (x) versus percentage inhibition (y). The IC_{50} value is the concentration of the sample required to scavenge 50% of DPPH free radicals. Measurements were taken in triplicate [16].

Expression of results

$$(\text{I }\%) = \frac{A_c - A_0}{A_c} \times 100$$

- A_c = Absorbance of control (1 uL methanol + 1 mL DPPH)
- A_0 = Absorbance of sample
- I% = (Percentage of inhibition)

Determination of total phenolic content [15]

Principle: The principle of the Folin-Ciocalteu assay is based on the reduction of the Folin-Ciocalteu reagent (FCR) in the presence of phenolics, resulting in the production of molybdenum-tungsten blue (blue color) that is measured spectrophotometrically at 760 nm, and the intensity increases linearly with the concentrations of the phenolics in the reaction medium.

Preparation of standard gallic acid for calibration curve: A standard gallic acid solution was prepared by dissolving 10 mg of gallic acid in 10 mL of methanol. Various concentrations of the gallic acid solution in methanol were prepared from the stock solution (1 mg/mL). Blank, 25, 35, 50, 65, and 75 mg/mL were prepared. From each concentration, 1 mL was taken out. 5 mL of 10% Folin-Ciocalteu's reagent was added, then 4 mL of 7% Na_2CO_3 was added, making up the final volume to 10 mL. The obtained blue-colored mixture was shaken and incubated for 30 min at 40°C in a water bath. The absorbance of each of the concentrations at 760 nm was recorded.

Preparation of samples: The samples were prepared in triplicate for each analysis, and the average value of absorbance was used to plot the calibration curve to determine the level of phenols in the extract.

Expression of results
Total phenolic content of the extract was expressed as milligrams of gallic acid equivalent per gram of sample in dry weight (mgGAE/g).

$$C = \frac{CV}{M}$$

Where,

- C=total phenolic content in mgGAE/g
- C = concentration of gallic acid (blank) in mg/mL
- V = volume of extract in mL
- M = mass of the extract in grams (g)

Determination of ferric reducing antioxidant power (FRAP)

Principle: The ferric reducing antioxidant power is based on the reduction of the $Fe_3{}^+$ tripyridyltriazine complex (colorless complex) to the $Fe_2{}^+$ tripyridyltriazine complex (blue-colored complex) formed by the action of electron-donating antioxidants at low pH.

Procedure: The ferric reducing antioxidant power of the sample was estimated spectrophotometrically following the procedure of [16]. 1 mL of the filtrate was taken out from the sample solution. 2.5 mL of phosphate buffer saline was added, bringing the pH to 3.6. It was mixed well. 2.5 mL of potassium ferricyanide solution was added. The mixture was vortexed well and incubated at 50°C in a water bath for 20 min. 2.5 mL of 10% trichloroacetic acid was added. The mixture was centrifuged at 3000 rpm for 10 min. 2.5 ml of the supernatant was taken out. 2.5 mL of deionized water was added. 0.5 mL of 0.1% ferric chloride solution was added. A bluish color was produced. Absorbance was read at 593 nm against the blank and compared using the standard calibration curve of ferrous sulfate absorbance. The calibration curve was prepared by plotting the absorbance at 593 nm versus different concentrations of $FeSO_4$. The concentration of $FeSO_4$ was plotted against standard antioxidant.

Expression of results
The ferric reducing antioxidant power (FRAP) was obtained by comparing the absorbance changes in the test mixture with those obtained from increasing concentrations of $Fe_3{}^+$ and expressed as mg of $FeSO_4$ equivalents per gram of sample.

$$C = \frac{CV}{M}$$

Where,

- C = ferric reducing antioxidant power in mg $FeSO_4$ E/g
- C = concentration of $FeSO_4$ in mg/mL
- V = volume of extract in mL
- M = mass of the extract in grams (g)

Determination of flavonoids content [15]

Principle: Aluminum chloride forms acid-stable complexes with C-H keto groups, either with the C-3 or C-5 hydroxyl group of flavones or flavonoids. It also forms acid-labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids.

Procedure: The total flavonoid content was measured by a colorimetric assay as described by [17]. 100 µL of ethanolic extract of the sample was added to 4 mL of distilled water. 0.3 mL of 5% sodium nitrite was added. After 5 min, 0.3 mL of 10% aluminum chloride was added. After 6 min, 2 mL of 1M sodium hydroxide was added. The mixture was then immediately diluted by adding 3.3 mL of distilled water and mixed thoroughly. The absorbance was read at 510 nm versus a blank. Quercetin (QE) was used as the standard for the calibration curve.

Expression of results
Total flavonoid content of the extract was expressed as mg quercetin equivalents per gram of sample (mgQE/g).

$$C = \frac{CV}{M}$$

Where,

- C = total flavonoids content in mgQE/g
- C = concentration of quercetin in mg/mL
- V = volume of extract in mL
- M = mass of the extract in grams (g)

2.11. *In vitro* anti-diabetic effects of *X. monospora* extracts

Determination of α-Amylase Inhibition Activity

Principle: The principle is based on the DNSA assay used to measure the inhibition of alpha-amylase enzyme activity by a sample. Alpha-amylase is incubated with the sample and a substrate solution containing starch. After incubation, a DNSA solution is added, which forms a color complex with the remaining starch. The decreasing intensity of the color indicates the inhibition of alpha-amylase activity.

Procedure: The α-amylase inhibition assay was performed using the 3.5-dinitrosalicyclic acid (DNSA) method for screening. Extracts of *X. monospora* were dissolved in buffer (Na_2HPO_4/NaH_2PO_4 (0.2 M), NaCl (0.006 M) at pH 6.9) to give concentrations ranging from 25 to 800 µg/mL. A volume of 200 µL of α-amylase solution (Molychem, India) (2 units/mL) was mixed with 200 µL of the extract and was incubated for 10 min at 30°C. 200 µL of starch solution (1% in water (w/v)) was added to each tube and incubated for 3 min. This was terminated

by the addition of 200 µL DNSA reagent (12 g sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM of 3.5-dinitrosalicylic acid solution) and was boiled for 10 min in a water bath at 85°C. The mixture was cooled to ambient temperature and was diluted with 5 mL of distilled water, and the absorbance was measured at 540 nm using a UV-visible spectrophotometer (Agilent Technology, Malaysia). The blank with 100% enzyme activity was prepared by replacing the plant extract with 200 µL of the buffer. A blank reaction was similarly prepared using the leaf extracts at each concentration in the absence of the enzyme solution. A positive control sample was prepared using acarbose (Bayer, Germany), and the reaction was performed similarly to the reaction of the plant extract as mentioned above.

Expression of results

The inhibition of α-amylase was expressed as a percentage of inhibition and was calculated by the following equation:

$$\text{Inhibition } (\%) = \frac{(A_c - A_{cb}) - (A_s - A_{sb})}{(A_c - A_{cb})} \times 100$$

Where

- A_c = absorbance of control
- A_{cb} = absorbance of control blank
- A_s = absorbance of sample
- A_{bs} = absorbance of sample blank

The % α-amylase inhibition was plotted against the extract concentration, and the IC_{50} values were obtained from the graph.

2.12. Statistical analysis

Statistical procedures were carried out using IBM SPSS version 23.0. Normality was checked for continuous variables using the Kolmogorov-Smirnov test. Descriptive data has been presented as mean ± standard deviation. Comparison of means of nutrients, antioxidants, and anti-nutrients between the two extracts was carried out using one-way ANOVA, using Dennett's test for multiple comparisons. The comparison of mean blood glucose level, lipid concentrations, and glycogen concentration across the treatment days was carried out using a univariate general linear model. Multiple comparisons were carried out using the Bonferroni method. Statistical significance was considered at $p < 0.05$.

3. Results

3.1. Nutritional and antinutritional contents of aqueous (AqSE) and ethanol (EtE) extracts of *Xymalos Monospora*

Table 1: Nutritional and antinutritional contents of the aqueous and ethanol extracts of *Xymalos Monospora*

Extract	Nutritional contents						Antinutritional contents			
	Fe (mg/100)	Zn (mg/100g)	Mg (g/100)	K (mg/100g)	Vit A (β -carotene) (µg/ml)	Vit C (Ppm)	Saponin (mg/100g)	Tannin (mg/100g)	Phytate (mg/100g)	Oxalate (mg/100g)
AqsE	12.81± 0.02^a	20.12± 0.10^a	24.50± 0.61^a	18.44± 0.50^a	3.643± 0.01^b	3.06± 0.05^a	0.37± 0.07^a	0.65± 0.04^a	0.80± 0.04^a	5.08± 0.28^a
EtE	12.83± 0.04^a	20.22± 0.01^a	24.52± 0.06^a	20.21± 0.08^b	1.45± 0.16^a	4.93± 0.01^b	0.39± 0.05^a	0.69± 0.01^a	1.24± 0.01	3.06± 0.29^a

Values represent Mean ± SD of three samples per extract based on one-way ANOVA using the Dunnett's test for comparison between different groups. Values not sharing the same superscript letters (a-b) differ significantly at $p < 0.05$.

3.2. Nutritional contents of aqueous and ethanol extracts of *Xymalos Monospora*

The results for mineral and vitamin contents of aqueous and ethanol extracts of *Xymalos Monospora* are presented in Table 1. As seen on this table, the iron, zinc, magnesium, and potassium contents were similar in the ethanol extract (70%) than the iron, zinc, magnesium, and potassium in the aqueous extracts (70%). No significant difference ($p < 0.05$) was observed for iron, zinc, and magnesium between ethanol and aqueous extracts.

Also, the vitamin A and C content were similar in aqueous extract than for ethanol extract.

3.3. Antinutrient contents of aqueous and ethanol extracts of *Xymalos Monospora*

The results for saponins, tannins, phytate, and oxalate contents for aqueous and ethanol extracts are presented in Table 1. As seen on that table, these saponin, tannin, and phytate contents were similar than ethanol extract content. Thus, no significant difference ($p < 0.05$) was observed for saponins and tannins between ethanol and aqueous extracts.

3.4. Antioxidant contents and α-Amylase Inhibition Activity of aqueous and ethanol extracts *Xymalos Monospora*

Table 2: Antioxidant contents and α-Amylase Inhibition Activity of the aqueous ethanol extracts of *Xymalos Monospora*

Extracts	**Antioxidant contents**			**In-vitro anti-diabetic effect**	
	Total phenol (mg/g)	Flavonoid (mg/g)	FRAP (mg/g)	DPPH IC_{50} (μg/ml)	Amylase inhibition IC_{50} (μg/ml)
AqsE	0.68 ± 0.06^{a}	70.00 ± 11.31^{a}	1.73 ± 0.10^{a}	30.86 ± 2.08^{b}	661.06 ± 10.01^{b}
EtE	4.45 ± 0.38^{b}	116.67 ± 3.79^{b}	14.00 ± 1.31^{b}	20.10 ± 0.64^{a}	493.10 ± 10.01^{a}

Values represent Mean ± SD of three samples per extract based on one-way ANOVA using the Dunnett's test for comparison between different groups. Values not sharing the same superscript letters (a-b) differ significantly at $p < 0.05$.

3.5. FRAP (Ferric Reducing Antioxidant Power), DPPH (2, 2-diphenyl-pirylhydrazyl)

The results for total phenolic, flavonoids, ferric reducing antioxidant power, and 2,2-diphenyl-1-picrylhydrazyl (IC_{50}) are presented in Table 2. Total phenolic and flavonoid contents and ferric reducing antioxidant power were observed to be higher in the ethanol extract than in the aqueous extract, as shown in that table. Thus, there was a significant difference ($p<0.05$) in total phenolic, flavonoid, and ferric reducing antioxidant power contents between ethanol and aqueous extracts. In addition, 2,2-diphenyl-1-picrylhydrazyl (IC_{50}) was observed to be higher in aqueous extract compared to ethanol extract. However, there was a significant difference ($p > 0.05$) in 2,2-diphenyl-1-picrylhydrazyl between aqueous and ethanol extracts.

3.6. In-vitro α-Amylase Inhibition Activity of aqueous and ethanol extracts of *Xymalos Monospora* (Anti-diabetic effects *in vitro*)

Results on the antidiabetic effects *in vitro* of leaf extracts of *X. monospora* using α-amylase are presented in Table 2. As seen in this table, the result of α-amylase for aqueous extract was observed to be higher than the result for ethanol extract. However, there was a significant difference at $p < 0.05$ between the aqueous and ethanol extracts.

4. Discussion

Plants and their extracts have been investigated for their nutritional, antinutritional, and antioxidant activities in recent scientific developments throughout the world. Many of these extracts have been found to exert antidiabetic effects against different diseases [18], including type II diabetes mellitus, and their abilities to alleviate toxic effects are caused by free radicals from reacting oxidizing species (ROS) and environmental pollutants. This study aimed to evaluate the nutritional, antinutritional, and antidiabetic properties of extracts of *X. monospora*. Specifically, it was to produce the aqueous and ethanol extracts with the high nutritional, anti-nutritional, and antidiabetic content of *Xymalos Monospora*. Phytochemical constituents are responsible for most medicinal activities attributed to medicinal plant species [19]. The analysis of these phytochemicals is paramount, as the World Health Organization (WHO) has specified the need to determine the composition of biologically active substances considered for nutritional, antinutritional, antioxidant, and medicinal purposes [20].

The preliminary nutritional, antinutritional, antioxidant, and *in vitro* anti-diabetic analysis carried out on aqueous and ethanol extracts of *X. monospora* leaves showed the presence of some bioactive compounds in the plant. Results on the nutritional contents showed that the presence of a slight increase in the mineral contents in iron, zinc, magnesium, and potassium concentrations in ethanol leaf extract may have resulted from a high solubility and the processing method used since ethanol can enhance the extraction efficiency, resulting in an increased presence of these minerals in the extract. Likewise, the decrease in the mineral content in the aqueous leaf extract may be attributed to factors such as pH, temperature, and solubility of the minerals in water. These results are in agreement with those of [21], who evaluated the phytochemicals, antioxidant activity, and elemental content of Adiantum capillus-veneris leaves.

Also, results on the antinutrient content showed that the analysis of the presence of tannins, saponins, oxalate, and phytate in both the aqueous and ethanol extracts was positive, but high color intensity was observed in the ethanol extract compared to the aqueous extract. This could be attributed to the presence of higher extracting potentials found in the ethanol extract than in the aqueous extract [22].

However, phenolic compounds are important plant constituents with redox properties responsible for antioxidant activity. The hydroxyl groups in plant extracts are responsible for facilitating free radical scavenging. The phenolic content in ethanol extract had the greatest value (4.45±0.38 mgGAE/g), while the smallest phenolic content was observed in aqueous extract (0.68±0.06 mgGAE/g). The difference in the values of the two extracts may have resulted from the solubility of the organic solvent used. Thus, antioxidants like phenol have been known to prevent oxidative damage in living systems. Moreover, flavonoids are secondary metabolites with antioxidant activity, the potency of which depends on the number and position of OH groups. The significant decrease in flavonoid contents for aqueous extract compared to the ethanol extract might have resulted from genetic diversity and biological, environmental, seasonal, and year-to-year variations of the plant leaves. In addition, the greatest ferric reducing antioxidant power was recorded in the ethanol extract (14.00±1.31) compared to the aqueous extract with the least ferric reducing antioxidant power (1.73±0.10). Since the transformation ability of the compounds from the $Fe_3{}^+$/ ferricyanide complex to the $Fe_2{}^+$/ ferrous form acts as a potential indicator for antioxidant activity, the yellow test solution changes to green and blue depending on the reduction of extracts or compounds. Thus, the least antioxidant power in aqueous extract might have resulted in the inability or decreased ability in the test solution to reduce $Fe_3{}^+$ to $Fe_2{}^+$. The DPPH assay is a simple and widely used technique to evaluate the radical scavenging potency of plant extracts. DPPH is a stable organic free radical, which loses its absorption spectrum band at 515-528 nm when it accepts an electron or a free radical species. Thus, the greatest DPPH scavenging potency with a minimum IC_{50} value was recorded for ethanol extract (20.10±0.64 mg/mL) compared to the aqueous extract (30.86±2.08 mg/mL). All these findings are similar to those obtained by [23], who investigated the total phenolic content, flavonoid content, and antioxidant potential of wild vegetables from West

Nepal for their antioxidative potential using 2,2-dyphenyl-1-1-picrylhydrazyl (DPPH) scavenging, hydrogen peroxide (H_2O_2), and ferric reducing antioxidant power (FRAP) methods.

Results with the aqueous extract of *X. monospora* possess the highest inhibitory activity against α-amylase (IC_{50} value of 661.06±1.01 µg/mL). This analysis revealed that the aqueous extract of this plant inhibited the α-amylase completely but displayed a mixed noncompetitive mode of inhibition toward the ethanol extract (IC_{50} value of 493.10±1.01 µg/mL). This could be attributed to the fact that aqueous extract of *X. monospora* exhibited the best inhibitory activity on the enzyme studied, and the presence of phytochemicals like flavonoids, saponins, and tannins may have contributed greatly to the inhibitory activity of the plant extract [24].

5. Conclusion

The aim of this study was to investigate the nutritional, antinutritional, and antidiabetic effects of extracts of *Xymalos Monospora in vitro*.

The results obtained from this study revealed that both AqsE(S) and EtE(S) contain the nutritional, antinutritional, and antioxidant flavonoids, phenolics, saponins, oxalate, and tannins that could be responsible for the antidiabetic effects of this plant. Therefore, *X. monospora* leaves have the nutritional, antinutritional, and antidiabetic contents that could help diabetic patients to lower their blood glucose levels, could improve blood lipid profiles, and could reduce the risk of developing type 2 diabetes.

Article Information

Conflict of Interests: The authors declare no conflict of interest.

Disclaimer (Artificial Intelligence): The author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.), and text-to-image generators have been used during writing or editing of manuscripts.

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