**Phytochemical Screening, GC-MS Analysis and *In Vitro* Antioxidant Studies of Ethanol Extract of *Icicinia mani* (Earth balls) Tubers**

**ABSTRACT**

**Introduction:** *Icicinia mani* also known as *Efik-isong* in Ibibio is a potential feed material for animals including humans. There are reports that commercial producers of *garri,* a major staple food in Southern Nigeria combine large quantities of *Icicinia mani* tubers with cassava to increase their production. In this study, we conducted the phytochemical screening, GC-MS analysis and *in vitro* antioxidant activity of *Icicinia mani* (Earth balls) tubers using standard methods to ascertain the safety or otherwise of this plant material.

**Methodology:** Finely ground *Icicinia mani* tubers (1000 g) was extracted with 80% ethanol and the filtrate was concentrated using a rotary evaporator (LabTech EV400H). The dried extract was refrigerated at 4 oC until required for use. Qualitative and quantitative phytochemical screening were conducted using standard protocols. Analysis of the extract was by gas chromatography-mass spectrometry (GC-MS) to identify the important functional groups and phytochemical constituents. *In vitro* antioxidant activity of the extract was evaluated using ferric-reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, and nitric oxide (NO) scavenging assays.

**Results:** The results of phytochemical screening indicate the presence (g/100g) of alkaloids (29.703), flavonoids (8.911), tannin (5.455), steroids (0.007), saponin (13.269), cardiac glycosides (18.889), cyanogenic glycosides (0.002), oxalate (0.0004) and anthocyanin (10.00). GC-MS analysis revealed the presence of one hundred and five (105) compounds of which 1,2-Benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide had the highest retention time (23.712), Ethyl 2-(2-chloroacetamido)-3,3,3-trifluoro-2-(4-fluoroanilino) propionate had the highest molecular weight (356.70), while n-Hexadecanoic acid the highest peak area (4.92). The FRAP, DPPH, and NO scavenging assays indicated that the extract was capable of neutralizing free radicals.

**Conclusion:** Ethanol extracts of *Icicinia mani* tubers contains useful phytochemicals which synergistically confers strong antioxidant potentials on it, hence, the extract may be considered a potential natural source for developing additives in the food and pharmaceutical industries, offering an alternative to synthetic compounds for enhancing health benefits and mitigating oxidative stress. Further investigations on the minerals and proximate analysis as well as the effects of the extracts on biochemical parameters are on-going in our laboratory.

**Keywords:** *Icicinia mani*, phytochemicals, antioxidants, Gas chromatography-mass spectrometry

**INTRODUCTION**

The ever-increasing population in developing countries places enormous burden on the availability and cost of staple foods. In order to meet the challenging food and nutritional requirements of both animals and humans by the growing population, there has been recourse to unconventional sources in recent times [1]. Research efforts targeted at identifying potential cheap and readily available feed sources have widely been reported [2, 3]. There are unconfirmed reports that commercial producers of *garri,* a major staple food in Southern Nigeria combine large quantities of *Icacinia mani*, a tuber crop that grows in the wildwith cassava to increase their production. However, there are safety concerns regarding the use of these unconventional supplements, hence the need for biochemical and toxicity studies.

*“Icicinia mani* is known as Earth ball in English and *Efik-isong* by the *Efiks* and *Ibibios* of South-south zone of Nigeria” [4]. “The underground part is tuberous and develops into large size tubers. It is an all season plant, locally abundant in the humid tropics of Akwa Ibom and Cross River State, Nigeria. *Icicinia mani* tubers are not used as a food material but have been reported to be added to garri” [5]. “Previous Studies reported that *Icicinia mani* tubers is mainly made up of carbohydrates and small quantity of proteins. Its phytochemical screening indicated the presence of oxalates, alkaloids, hydrogen cyanide, phytic acid and tannins” [6]. However, there is currently no information on the quantitative phytochemicals, gas chromatography-mass spectrometry (GC-MS) analysis and *in vitro* antioxidant potentials of this plant.

**MATERIALS AND METHODS**

**Chemicals and Reagents**

All chemicals and reagents used for this research were of analytical grade. Ethanol, 1- Diphenyl 2-picrylhydrazyl (DPPH) and butylated hydroxytoluene (BHT) were obtained from Sigma-Aldrich, St. Louis, USA.

**Collection of Plant Materials**

Samples of *Icicinia mani* tubers were obtained from locations in Abiakpo Ikot Essien in Ikot Ekpene Local Government Area of Akwa Ibom State in Nigeria between November, 2024 and January 2025, and authenticated by a taxonomist of the Department of Botany and Ecological Studies, University of Uyo. A voucher specimen was deposited in the herbarium of the same University. The tubers were washed under gently running tap water to remove dirt on the tubers and kept for 2 hours for the water to dry off before slicing into smaller pieces using a sharp stainless steel knife. The slices were air-dried at room temperature (25 ± 2 oC) until a constant weight was obtained. After drying, the tubers slices were ground using a desk top grinder (Model No: QBL-18L40, Turinar Corp, Shang-Hai, China) into fine particles and stored in a plastic container with screw cap.

**Extraction Procedure**

The finely ground *Icicinia mani* tubers (1000 g) were soaked in 1000 ml of 80% ethanol at room temperature for 24 hours in a 2000 ml reagent bottle with continuous shaking and the extract was filtered using a cheese cloth to obtain the filtrate. The residue was re-suspended in 1000 ml of 80 % ethanol for another 24 hours and filtered as before. The combined extract was evaporated at 45 oC in an open water bath to remove all the solvent and the dried extract of the sample was refrigerated at 4 oC until required for use.

**Phytochemical Screening**

Chemical tests for the identification of bioactive chemical constituents in ethanol extracts of *Icicinia mani* tubers were carried out in the extracts using standard procedures as described by Kokate [7], Sofowara [8], Trease and Evans [9], and Harborne [10].

**Qualitative Phytochemical Analysis**

**Test for Saponins**

**Froth Test: About 0.5 grams of the extracts were separately shaken vigorously with 2 ml of distilled water in different test tubes and allowed to stand for 15 minutes.** Persistent foaming or **the appearance of creamy mist of small bubbles (froth) showed the presence of saponins** [8, 10, 11]

**Test for Alkaloids**

**About 0.5 gram of the extract was added to** 10 ml of 2 % dilute hydrochloric acid and heated for 5 minutes and then filtered and the filtrate (1 ml) used to test for the presence of alkaloids as follows:

Mayer’s Test:Mayer’s reagent (Potassium mercuric iodide solution) was added to 2 ml of the filtrate. After 15 minutes, the formation of yellow precipitate showed the presence of alkaloids.

**Test for Flavonoids**

**Preparation of Test solution**: About 500 mg of the extract was dissolved using 100 ml of the respective solvent and filtered using Whatman filter paper No.1. The filtrate was the test solutions for the following preliminary screening tests.

**Sulphuric Acid (H2SO4) Test:** Dilute aqueous ammonia (5 ml) was pipetted into 2 ml of each of the extracts followed by 1 ml of 2M H2SO4. An orange or yellow colouration that disappears on standing for 10 - 15 minutes indicates the presence of flavonoids.

**Test for Tannins**

**Ferric Chloride (FeCl3) Test: “**About 0.2 g of the extract was dissolved in 10 ml of each of the solvent and then filtered using Whatman filter paper No.1.To about 5 ml of each of the extract in a test tube, 1 ml of 5 % FeCl3 in pyridine was introduced and the formation of dark green colour indicates the presence of tannin” [12].

**Test for Cardiac Glycosides**

**Keller-Killani Test:** About 0.2 g of the extract was added to 10 ml of the solvent and filtered using Whatman filter paper No.1. Glacial acetic acid (1 ml) was pipetted into 2 ml of each of the filtrates and allowed to attain room temperature. Thereafter, 1 ml of 1 % FeCl3 solution was added to the mixture and the contents were transferred to a test tube containing 2 ml of concentrated H2SO4. A reddish brown ring observed at the junction of two layers indicated a positive test.

**Test for Anthraquinones**

**Borntrager’s Test:** About 0.5 g of the extract was successively boiled with 5 ml of 10 % H2SO4 and filtered. Each of the filtrates was shaken with 2.5 ml of benzene, the benzene layer was separated and half its own volume of 10 % NH4OH added. A pink, red, or violet colouration in the ammonia phase indicated the presence of anthraquinones.

**Test for Steroids**

**Salkowski’s Test: “**About 0.5 g of the extract was dissolved in 10 ml of the solvent in a test tube and filtered using Whatman filter paper No.1. Chloroform (2.0 ml) and 2.0 ml of concentrated H2SO4 were added to 2.0 ml of the filtrate. After agitation of the mixture, the formation of a red colour by the chloroform layer and a greenish yellow fluorescence by the acid layer indicated the presence of steroids” [2].

**Test for Oxalate**

“About 0.5 g of the extract was dissolved in 10 ml of dilute hydrochloric acid and Calcium chloride was then added to the resulting solution, shaken and allowed to stand for few minutes. The formation of a white precipitate (calcium oxalate) confirmed the presence of oxalate” [2].

**Test for Cyanogenic Glycosides**

**Borntrager’s Test:** About 0.5 g of the extract in 5 ml of 10 % H2SO4 was heated to boil before filtration and the filtrate was shaken with 2.5 ml of benzene; the benzene layer was separated and half its own volume of 10 % NH4OH added. The formation of a pink colour indicated the presence of glycosides.

**Test for Anthocyanins**

About 0.5 g of the extract was dissolved in 10 ml of solvent and filtered using Whatman filter paper No.1. Thereafter, 2 ml of 2N HCl and 2 ml of aqueous ammoniawas added to the filtrate. The presence of anthocyanins was indicated by the formation of pink red colour which turns blue violet.

**Quantitative Phytochemical Analysis**

**Alkaloids Determination**

“Five grams (5 g) of the sample was weighed into a 250ml beaker and 200ml of 20% acetic acid in ethanol was added, covered and allowed to stand for 4 hours at 250C. The mixture was filtered using Whatman filter paper No. 42 and the filtrate was concentrated using a water bath (Memmert) to one quarter of the original volume. Drop by drop, concentrated ammonium hydroxide was added until the precipitation was observed. The precipitate was collected and washed with dilute NH4OH (1% ammonia solution) after it has settled and then filtered with pre-weighed filter paper. The residue on the filter paper is the alkaloid, which was dried in an oven at 800C (precision electrothermal model BNP 9052 England). The alkaloid content was calculated and expressed as a percentage of the weight of the sample analysed” [10, 13]

Calculation:

Gram % of alkaloid = x

**Flavonoids Determination**

“Ten (10 g) of the plant sample was extracted with 100 ml of 80 % aqueous methanol at room temperature. The whole solution was filtered using Whatmann filter paper No. 42 (125mm). The filtrate was transferred into a crucible, evaporated into dryness using a water bath until a constant weight was obtained” [14].

Calculation:

Gram % of flavonoids = x

**Tannin Determination by Follins Dennis Titration**

The Follins Dennis titrating method as described by Pearson [15] was used. Petroleum ether (100 ml) was added to 20 g of the powdered sample in a conical flask. The flask was covered with foil paper for 24 hours before filtration and filtrate kept for 15 minutes for the petroleum ether to evaporate. This was re-extracted by soaking the pellets in 100 ml of 10 % acetic acid in ethanol for 4 hours before filtration and the filtrate collected.

25 ml of NH4OH was added to the filtrate to precipitate the alkaloids. The alkaloids mixture obtained was dried on an electric hot plate to remove some of the NH4OH still present in solution. The remaining volume was measured to be 33 ml. 5 ml of the solution containing the alkaloids was pipetted into a beaker containing 20 ml of ethanol. The resulting solution was titrated with 0.1M Na0H using phenolphthalene as indicator, which gave a pink colour at end- point. Tannin content was then calculated as follows:

Calculation:

C1V1 = C2V2

Where;

C1 = conc. of Tannic Acid C2 = conc. of NaOH (Base)

V1 = Volume of Tannic acid V2 = Volume of NaOH (Base)

Therefore C1 =

And gram % of Tannic acid =

**Determination of Steroid Content**

1.0 g of the powdered sample was weighed into a conical flask, then 100 ml of distilled water was added and mixed. The mixture was filtered and the filtrate eluted with 0.1N ammonium hydroxide solution. 2 ml of the eluent was pipetted into a test tube, mixed with 2ml of chloroform. Three (3) ml of ice cold acetic anhydride was added to the mixture in the flask. Two drops of (200mg/dl) standard sterol solution was prepared and treated as described for test as blank. The absorbance of standard and test solution was measured, zeroing the spectrophotometer with blank at 420nm.

Calculation (mg/100 ml) = x

**Determination of Saponin**

“Exactly 5g of the sample was measured into a solution of 20 % acetic acid in ethanol and the mixture placed in a water bath at 50 0C for 24 hours, and filtered. The filtrate was concentrated using a water bath to one-quarter of the original volume. Drop by drop of concentrated NH4OH was added to the extract until the precipitation was complete, and the whole solution was allowed to settle and the precipitate collected by filtration and weighed. The saponin content of the filtrate was weighed and recorded for the calculation of the percentage (%) content” [13].

Calculation:

Gram % of saponins = x

**Cardiac Glycosides Determination**

Wang and Filled method was used. To 1ml of extract was added 1ml of 2 % solution of 3,5-DNS (Dinitro Salicylic acid) in methanol and 1ml of 5 % aqueous NaOH. It was boiled for 2 minutes (until brick-red precipitate was observed) and the boiled sample was filtered. The weight of the filter paper was weighed before filtration. The filter paper with the absorbed residue was placed in an oven at 50 0C to dry and the weight of the filter paper and the dried residue was recorded. The cardiac glycoside was calculated in percentage (%) as follows:

Calculation:

Gram % of cardiac glycosides = x

**Oxalate determination by Titration method**

This determination involves three major steps digestion, oxalate precipitation and permanganate titration.

**Digestion**

1. Two (2) grams of sample is suspended in 190 ml of distilled water in a 250 ml volumetric flask.
2. 10ml of 6M HCl was added and the suspension digested at 100 0C for 1 hour.
3. Cool, and then make up to 250 ml mark before filtration.

**Oxalate precipitation**

Duplicate portions of 125 ml of the filtrate were measured into beakers and four drop of methyl red indicator added. This is followed by the addition of NH4OH solution (dropwise) until the test solution changes from salmon pink colour to a faint yellow colour (pH 4 - 4.5). The precipitates containing ferrous ion was removed by heating at 90 0C, cooled and filtered. The filtrate was again heated at 90 0C and 10 ml of 5 % CaCl2 solution added while being stirred constantly. The solution was left overnight at 25 0C before it was centrifuge at 2500 rpm for 5minutes. The supernatant was decanted and the precipitate completely dissolved in 10 ml of 20 % (v/v) H2S04 solution.

**Permanganate titration**

Atthis point, the total filtration resulting from digestion of 2 g of powdered sample was made up to 300ml. Aliquots of 125 ml of the filtrate was heated until near boiling and then titrated against 0.05M standardized KMNO4 solution to a faint pink colour which persists for 30 seconds. The calcium oxalate content was calculated using the formula:

Amount of Oxalate (mg/100g) =

Where T is the titre of KMn04(ml), Vme is the volume-mass equivalent (i.e. 1 ml of 0.05M KMn04 solution is equivalent to 0.00225 g anhydrous oxalic acid). Df is the dilution factor Vt/A (2.4 where Vt is the total volume of titrate (300 ml) and A is the aliquot used (125 ml), ME is the molar equivalent of KMn04 in oxalate (KMn04 redox reaction) and Mf is the mass of sample used [10].

**Determination of Anthocyanin using the gravimetric method of Harborne, 1973**

**Principle**

Acid hydrolysed sample when filtered reacts with ethylacetate to enable extraction of anthocyanin. Upon addition of amyl alcohol, anthocyanin was extracted and after drying, the percentage composition was determined in relation to weight of original sample gravimetrically.

**Procedure**

5.0 g of the powdered sample was boiled in 100 ml of 2 MHCl for 30minutes. The hydrolysate was filtered using Whatman filter paper. The filtrate was transferred into separation funnel and equal volume of ethylacetate added, mixed and allowed to separate into two layers. The ethylacetate layer was recovered while the aqueous layer was discarded.

The extract was dried over a steam bath. The dried extract was treated with 10 ml of conc. Amylalcohol to extract the anthocyanin. After filtration, the alcohol extract was dried. The weight of anthocyanin was determined and expressed as percentage of original sample.

Calculation % = x

**Gas Chromatography Mass Spectrum (GC-MS) Analysis**

GC-MS analysis was carried out using 7890A GC system, 5675C Inert MSD with triple-Axis detector. The column has a length of 30 m with an internal diameter of 0.2 µm and a thickness of 250 µm, treated with phenyl methyl silox, ion source temperature (EI), 250 oC , interface temperature; 300 oC, pressure; 16.2 psi out time, 1.8 mm, 1μl injector in split mode with split ratio 1:50 with injection temperature of 300 oC, the column temperature started at 35oC for five minutes and changed to 150 oC at the rate of 4 oC per minutes, the temperature was raised to 250 oC at the 162 rate of 20 oC per minutes and held for five minutes. The total elution was 47.5 minutes. MS Solution software provided by supplier was used to control the system and to acquire the data. Identification of the compounds was carried out by comparing the mass spectra obtained with those of the standard mass spectra from National Institute of Standard and Technology (NIST) database. The identity of the spectra above 95% were selected and used to ascertain the name, molecular weight and structure of the components in the ethanol extracts of *Icicinia mani* tubers.

**Antioxidant evaluation of *Icicinia mani* tubers extracts**

The antioxidant evaluation of the extract was carried out using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, nitric oxide (NO) scavenging and ferric reducing antioxidant power (FRAP) assay.

**Determination of 2, 2-Diphenly-1-Picrylhydrazyl (DPPH) Radical scavenging activity**

The ability of ethanol extractof *Icicinia mani* tubers to scavenge the DPPH radical was tested in a rapid dot-plot screening and quantified using a spectrophotometric assay.

**Principle**

DPPH radical reacts with an antioxidant compound that can donate hydrogen, and gets reduced and when acted upon by an antioxidant, is converted into diphenylpicryl hydrazine, which is identified by the conversion of purple to light yellow colour.

**Dot-plot rapid assay**

The rapid screening assay was performed by the method of Soler-Rivas *et al*.[16].

**Reagents**

1. TLC plates (silica gel 60 F254-Merck)

2. DPPH (0.4mM) in methanol

**Procedure**

Aliquots of sample samples (3:1) were spotted carefully on TLC plates and dried for 3 minutes. The sheets bearing the dry spots were placed upside down for 10 seconds in a 0.4 mM DPPH solution and the layer was dried. The stained silica layer revealed a purple background with yellow spots, which showed radical scavenging capacity.

**DPPH spectrophotometric assay**

The scavenging ability of the natural antioxidants of the ethanol extractof *Icicinia mani* tubers towards the stable free radical DPPH was measured by the method of Mensor *et al*.[17].

**Reagent**

1. DPPH – 2,2-diphenyl-2-picryl hydrazyl hydrate (0.3 mM in methanol)

2. Methanol

**Procedure**

The ethanol extractof *Icicinia mani* tubers (20 μl) were added to 0.5 ml of 0.1 mM methanolic solution of DPPH and 0.48 ml of methanol. The mixture was allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol, without the ethanol extractof *Icicinia mani* tubers samples, served as the positive control while butylated hydroxytoluene (BHT) served as reference. After 30 minutes of incubation, the discolouration of the purple colour was measured at 518 nm in a spectrophotometer (Genesys 10-S, USA). The radical scavenging activity was calculated as follows:

Scavenging Activity % = x

**Nitric Oxide (NO) Scavenging Activity of ethanol extract of *Icicinia mani* tubers**

The extent of inhibition of nitric oxide radical generation *in vitro* was carried out using the method of Green *et al.*[18].

**Principle**

Sodium Nitroprusside in aqueous solution, at physiological pH, spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that are estimated spectrophotometrically at 546 nm.

**Reagents**

1. Sodium Nitroprusside (100 mM)

2. Phosphate buffered saline (pH 7.4)

3. Griess reagent (1 % sulphanilamide, 2 % H3PO4 and 0.1 % Naphthylethylene diamine dihydrochloride)

**Procedure**

The reaction was initiated by adding 2.0ml of sodium nitroprusside, 0.5 ml of PBS, 0.5ml of ethanol extract of *Icicinia mani* tubers (50mg) and incubated at 25 0C for 30 minutes. Griess reagent (0.5 ml) was added and incubated for another 30 minutes. Control tubes were prepared without the samples. The absorbance was read at 546 nm against the reagent blank, in a spectrophotometer (Genesys 10-S, USA).

**Ferric Reducing Antioxidant Power (FRAP) Assay**

**Principle**

The principle of the assay is the quantification of ferric degradation product by its condensation with the extract.

**Method**

The FRAP activity of the ethanol extractof *Icicinia mani* tubers was determined by the method of Pulido *et al.*[19].

**Procedure**

Various concentrations (20, 40, 60, 80, 100 ug/mL) of the ethanol extractof *Icicinia mani* tubers were mixed individually with the mixture containing 2.5 mL of 0.2 M Sodium Phosphate buffer (pH 6.6) and 2.5 mL of Potassium ferricyanide (K2Fe(CN)6) (1 % w/v). The resulting mixture was incubated at 50 oC for twenty minutes, thereafter, 2.5 mL of trichloroacetic acid (10 % w/v) was added. The resulting mixture was centrifuged at 2000 rpm for ten minutes. The upper layer (supernatant) of the solution (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride (0.1% w/v). The absorbance was measured at wavelength 700 nm against a blank sample and garlic was used as the reference compound.

**RESULTS**

**Phytochemical screening of ethanol extracts of *Icicinia mani* tubers**

The results of the qualitative and quantitative phytochemical profile of ethanol extracts of *Icicinia mani* tubers are presented in Tables 1 and 2.

The results of the qualitative phytochemical screening of ethanol extracts of *Icicinia mani* tubers the indicates the presence of alkaloids, flavonoids, tannin, steroids, saponin, cardiac glycosides, cyanogenic glycosides, oxalate and anthocyanin (Table 1).

The results of the quantitative phytochemical analysis of ethanol extracts of *Icicinia mani* tubers in (Table 2) showed that alkaloids contents (g/100 g) was the highest (29.703), followed by cardiac glycosides (18.889), saponins (13.265), flavonoids (8.911), tannins (5.455), while oxalate was the least (0.0004).

Table 1. Qualitative phytochemical screening of ethanol extracts of *Icicinia mani* tubers

|  |  |  |
| --- | --- | --- |
| S/N | Phytochemicals | Ethanol Extract |
| 1 | Alkaloids | +++ |
| 2 | Flavonoids | ++ |
| 3 | Tannin | ++ |
| 4 | Steroids | + |
| 5 | Saponin | +++ |
| 6 | Cardiac glycosides | +++ |
| 7 | Cyanogenic glycoside | + |
| 8 | Oxalate | + |
| 9 | Anthocyanin | +++ |

Key: + = **+++** = strongly present, **++** = present in high concentration, **+** = trace

Table 2. Quantitative phytochemical analysis of ethanol extracts of *Icicinia mani* tubers

|  |  |
| --- | --- |
| Phytochemicals | Composition  (g/100g) |
| Alkaloids  Flavonoids  Tannin  Steroids  Saponins  Cardiac glycosides  Cyanogenic glycoside  Oxalate  Anthocyanin | 29.703  8.911  5.455  0.007  13.265  18.889  0.0016  0.0004  10.00 |

**GC-MS profile of ethanol extracts of *Icicinia mani* tubers**

The results of the GC-MS analysis of ethanol extracts of *Icicinia mani* tubers at spectra above 95% revealed the presence of ten (10) compounds (Fig. 1). The names, molecular weight and structure of the compounds are shown in Table 3.

Table 3. Identified compounds from GC-MS analysis of ethanol extract of *Icicinia mani* tubers

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| S/N | Retention time | Compound Name | Molecular Formula | Molecular weight | Area (%) |
| 1 | 13.151 | Hexadecanoic acid, methyl ester | C17H34O2 | 270.4507 | 1.16 |
| 2 | 13.913 | n-Hexadecanoic acid | C16H32O2 | 256.4241 | 4.92 |
| 3 | 16.413 | Oleic Acid | C18H34O2 | 282.4614 | 4.45 |
| 4 | 16.413 | trans-13-Octadecenoic acid | C18H34O2 | 282.4614 | 4.45 |
| 5 | 16.413 | cis-13-Octadecenoic acid | C18H34O2 | 282.4614 | 4.45 |
| 6 | 16.866 | Octadecanoic acid | C18H36O2 | 284.4772 | 1.81 |
| 7 | 13.763 | 1,2-Benzenedicarboxylic acid | C8H6O4 | 166.1308 | 0.63 |
| 8 | 15.085 | Cyclopropaneoctanal, 2-octyl- ester | C19H36O | 280.50 | 0.06 |
| 9 | 23.712 | 1,2-Benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide | C₁₃H₁₆N₂O₂S | 264.35 | 0.18 |
| 10 | 13.763 | Ethyl 2-(2-chloroacetamido)-3,3,3-trifluoro-2-(4-fluoroanilino) propionate | C13H13ClF4N2O3 | 356.70 | 0.63 |

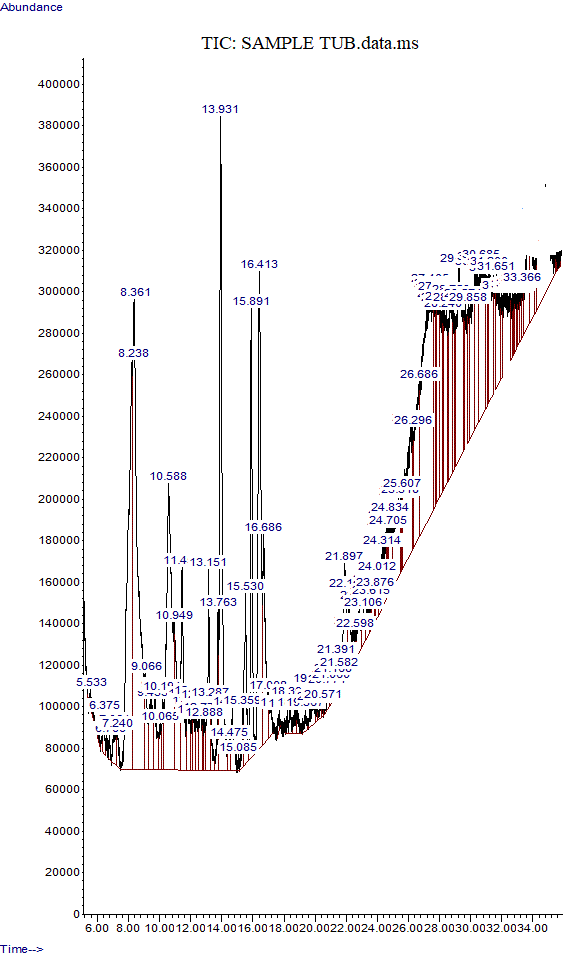
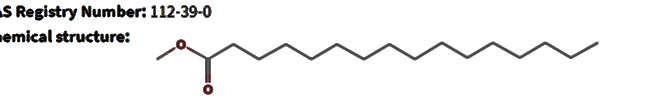
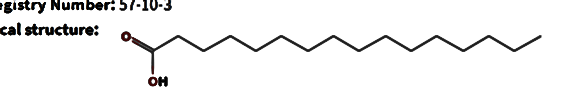


Fig.1. GC-MS spectra of ethanol extracts of *Icicinia mani* tubers

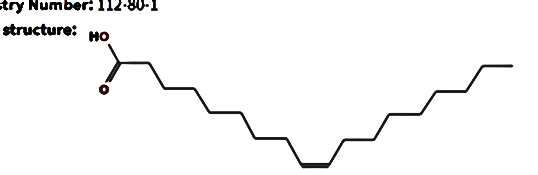
**Chemical structures of compounds from the ethanol extract of *Icicinia mani* tubers**

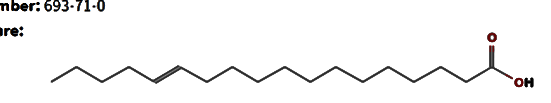
The chemical structures of the compounds found in ethanol extract of *Icicinia mani* tubers

Are as shown in figure 2 below.

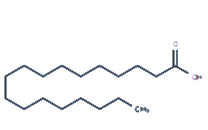


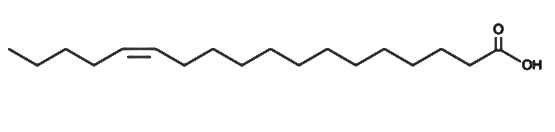
1. Hexadecanoic acid, methyl ester b. n-Hexadecanoic acid



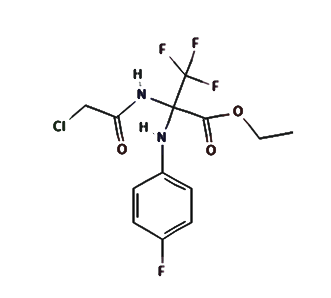


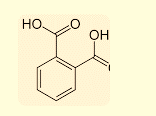
1. Oleic Acid d. Trans-13-Octadecenoic acid



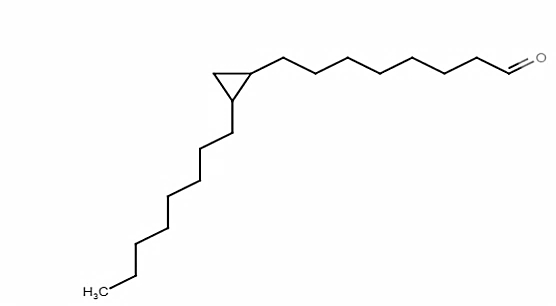


1. Cis-13-Octadecenoic acid f. Octadecenoic acid



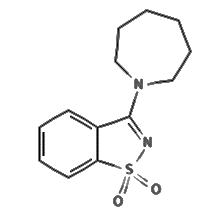


1. 1,2-Benzenedicarboxylic acid



h. Ethyl 2-(2-chloroacetamido)-3,3,

3-trifluoro-2-(4-fluoroanilino) propionate



1. Cyclopropaneoctanal, 2-octyl-

j. 1,2-Benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-,

1,1-dioxide

Fig. 2. Chemical structures of compounds identified from GC-MS analysis of ethanol extract of *Icicinia mani* tubers.

**In vitro antioxidant assessment of ethanol extracts of *Icicinia mani* tubers**

The results of the in vitro antioxidant assessment of ethanol extracts of *Icicinia mani* tubers are presented in Tables 4 - 6.

The DPPH [free radical](https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/free-radical) scavenging activity was expressed as percentage inhibition (Table 4). The percentage of DPPH activities of the ethanol extract of *Icicinia mani* tubers ranged from 77.50 - 87.90 %. These values were lower compared to the percentage DPPH activity of the standard butylated hydroxytoluene (BHT) at 10 mg/ml which was 98.50%. The highest percentage DPPH inhibition of the extracts was at 80 mg/ml where the percentage inhibition was 87.9 %. The IC50 of the ethanol extract of *Icicinia mani* tubers was 6.6 mg/ml which was less than that of the standard (4.8 mg/ml).

Table 4. DPPH radical scavenging activity of ethanol extracts *Icicinia mani* tubers

|  |  |  |
| --- | --- | --- |
| Dose (mg/ml) | DPPH Scavenging Activity Percentage Inhibition (PI) % | |
| *Icicinia mani* tuber extract | BHT |
| 10 | 79.89 | 98.50 |
| 20 | 80.92 |  |
| 40 | 77.50 |  |
| 80 | 87.90 |  |
| IC50 (mg/ml) | 6.6 | 4.8 |

The Nitric oxide scavenging activity of ethanol extracts *Icicinia mani* tubers (Table 5) revealed a dose-dependent rise in its scavenging ability with the highest scavenging activity 80 mg/ml. The IC50 of the ethanol extract of *Icicinia mani* tubers was 6.6 mg/ml which was less than that of the standard (4.8 mg/ml). The IC50 of 84 was obtained for nitric oxide scavenging activity of ethanol extract of *Icicinia mani* tubers against 18 for the garlic standard. This indicates that the extract has a moderate antioxidant potential against nitric oxide radicals, meaning it requires a relatively high concentration (84 mg/ml) to inhibit 50% of nitric oxide activity.

Table 5. Nitric Oxide scavenging activity of ethanol extracts *Icicinia mani* tubers

|  |  |  |
| --- | --- | --- |
| Doses (mg/ml) | Nitric Oxide (NO) Scavenging Activity | |
| *Icicinia mani* tuber extract | Garlic |
| 10 | 2.30 | 1.82 |
| 20 | 2.15 | 3.19 |
| 40 | 2.60 | 4.52 |
| 80 | 2.90 | 5.48 |
| IC50 (mg/ml) | 84 | 18 |

The Ferric Reducing Antioxidant Power (FRAP) of ethanol extracts *Icicinia mani* tubers showed a dose-dependent increase (Table 6). The highest FRAP activity was at a dose of 40 mg/ml. The IC50 for Ferric Reducing Antioxidant Power of the extract was 15.00, which was greater than that of the garlic standard (6.46).

Table 6. Ferric Reducing Antioxidant Power (FRAP) of ethanol extracts *Icicinia mani* tubers

|  |  |  |
| --- | --- | --- |
| Doses (mg/ml) | Ferric Reducing Antioxidant Power (FRAP) | |
| *Icicinia mani* tuber extract | Garlic |
| 10 | 45.74 | 76.95 |
| 20 | 53.19 | 77.48 |
| 40 | 58.16 | 87.99 |
| 80 | 54.26 | 93.62 |
| IC50 (mg/ml) | 15.00 | 6.46 |

**Discussion**

The results of the qualitative and quantitative phytochemical analysis of ethanol extract of *Icicinia mani* tubers (Tables 1 and 2) revealed the presence of nine (9) phytochemical compounds thus: alkaloids (29.703 g/100g), cardiac glycosides (18.889 g/100g), saponins (13.265 g/100g), anthocyanin (10.000 g/100g), flavonoids (8.911 g/100g), tannins (5.455 g/100g), steroids (0.007 g/100g), cyanogenic glycosides (0.0016 g/100g) and oxalate (0.0004 g/100g).

“Phytochemical constituents of plants have been reported to possess numerous therapeutic activities such as anti-HIV, anti-plasmodial, anti-diarrheal, anti-septic, anti-bacterial, anti-viral, anti-inflammatory, anti-microbial, hypoglycemic, antioxidant, analgesic and hepatoprotective properties as well as other physiological activities” [8, 20, 21, 22]. “They exhibit structure related biochemical and pharmacological actions capable of reducing the risk of multiple diseases” [23, 24]. “Flavonoids have been utilized to improve human health via their multiple biological functions including anti-inﬂammatory” [25], antimicrobial [20], antioxidant [26], anticancer activities [27] and the prevention of osteoporosis [28]. “Alkaloids have been used as an analgesic, antispasmodic or bactericidal agents” [29]. “They are known to inhibit certain mammalian enzymic activities such as those of phophodiesterase, prolonging the action of cAMP. They also affect glucagons and thyroid stimulating hormones” [30]. “Saponins have been reported to be useful in reducing inflammation of upper respiratory passage and also chiefly as foaming and emulsifying agents and detergents” [29]. “Tannins have astringent properties that hasten the healing of wounds and prevention of decay. Tannins also possess antimicrobial activities responsible for preventing and treating urinary tract infections and other bacterial infections” [21].

The results of the GC-MS analysis of ethanol extracts of *Icicinia mani* tubers at spectra above 95% revealed the presence of ten (10) compounds (Table 3). These are: Hexadecanoic acid methyl ester, n-Hexadecanoic acid, Oleic Acid, trans-13-Octadecenoic acid, cis-13-Octadecenoic acid, Octadecanoic acid, Cyclopropaneoctanal, 2-octyl-ester, Ethyl 2-(2-chloroacetamido)-3,3,3-trifluoro-2-(4-fluoroanilino) propionate, 1,2-Benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide and1,2-Benzenedicarboxylic acid. Three of these have been reported to possess biomedical potentials[31].

Ethyl 2-(2-chloroacetamido)-3,3,3-trifluoro-2-(4-fluoroanilino) propionate has potential biomedical applications, particularly in medicinal chemistry, as its unique structure makes it a candidate for drug development. This compound can be a potential inhibitor or modulator of specific biological targets [31]. Its presence in plant extracts, suggests potential antiviral activity and cytotoxicity [32]. The compound 1,2-benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide has therapeutic potential for diseases related to Erythropoietin (EPO) insufficiency, Such as renal anemia by agonizing HIF-2 stabilization thus improving EPO production [33]. 1, 2-Benzenedicarboxylic acid is an allelochemical with strong allelopathic effect. It has antimicrobial properties particularly against extended-spectrum beta-lactamases (ESBL) [34].

The results of *in vitro* antioxidant assessment of ethanol extracts of *Icicinia mani* tubers showed that the extract had the highest percentage DPPH inhibition of 87.90 % at 80 mg/ml which was lower compared to the standard butylated hydroxytoluene (98.50 %). The IC50 of the ethanol extract of *Icicinia mani* tubers was 6.6 mg/ml which was less than that of the standard, BHT (4.8 mg/ml) (Table 4). The Nitric oxide scavenging activity of ethanol extracts *Icicinia mani* tubers revealed a dose-dependent rise in its scavenging ability with the highest scavenging activity 80 mg/ml and an IC50 of 6.6 mg/ml which was less than that of the garlic standard (4.8 mg/ml) (Table 5). Also, the Ferric Reducing Antioxidant Power (FRAP) of ethanol extracts *Icicinia mani* tubers showed a dose-dependent increase with the highest FRAP activity at a dose of 40 mg/ml and an IC50 of 15.00 which was greater than that of the garlic standard (6.46) (Table 6). Phongpaichit *et al*. [35] reported that the lower the IC50 value of a plant extract, the higher the antioxidant activity. Hence, the observed IC50 values indicate that the extract has strong antioxidant potential.

DPPH radical has been used extensively to test the reductive ability of plant extracts and foods and to evaluate their antioxidant activities [36]. “The scavenging effect of plant extracts on DPPH has been shown to be related to the phenolic concentration of the extracts” [37, 38]. “Therefore, the free radicals scavenging activities of the extract could be attributed to the presence of flavonoids. Antioxidant effects of nitric oxide radical (NO•) occurs when it reacts with alkoxy and peroxyl radical intermediates during lipid peroxidation thereby stabilizing the inhibition of LDL oxidation” [39]. “The ability of the extract to scavenge NO• is beneficial in biological system as peroxynitrite (ONOO•) and some other NO• metabolites have been implicated in various pathological conditions such as malaria, cardiovascular diseases, inflammation, cancer and diabetes. FRAP assay had been used to determine antioxidant activity as it is simple and quick. Besides that, the reaction is reproducible and linearly related to molar concentration of the antioxidants. Higher FRAP values give higher antioxidant capacity because FRAP value is based on reducing ferric ion, where antioxidants are the reducing agent” [40].

**Conclusions**

Ethanol extracts of *Icicinia mani* tubers contains useful phytochemicals which synergistically confers strong antioxidant potentials on it, hence, the extract may be considered a potential natural source for developing additives in the food and pharmaceutical industries, offering an alternative to synthetic compounds for enhancing health benefits and mitigating oxidative stress.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

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 the writing or editing of this manuscript.

**AUTHORS CONTRIBUTION**

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Prof. Monday Akpanabiatu and Dr. Joseph Ubon conceptualized and designed the study. Joseph Ubon, Utibe Evans, Rose Esen Blessing Joseph Jumbo, and Nsisongabasi Victor Michael executed the work and collected the data under the supervision of Prof. Monday Akpanabiatu and Usenobong Ufot. Joseph Ubon, Utibe Evans, Rose Esen and Usenobong Ufot analyzed the data while Joseph Ubon wrote the manuscript. All authors read, corrected and approved the manuscript for publication.

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