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Proximate Composition, Comparative Phytochemical Analysis, and HPLC Profiling of Various Solvent Extracts of *Anthocleista djalonensis* Leaves

Nsofor Benedicta Chineze [a], Nwankwo Valerie Ezinne [a], Obidiegwu Onyeka Chinwuba [a], Eze Peter Maduabuchi [b] and Abba Chika Christiana [a*]

[a] *Department of Pharmaceutical and Medicinal Chemistry, Nnamdi Azikiwe University, Awka 420110, Anambra State, Nigeria.*
[b] *Department of Environmental Health Science, Nnamdi Azikiwe University, Awka 420110, Anambra State, Nigeria.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Choosing the right solvent in herbal preparation is very important for extracting bioactive compounds from medicinal plant, as it greatly affects the yield and quality of phytochemicals obtained. This study aimed to investigate the proximate composition, phytochemical analysis and HPLC profiling of various solvents extracts of *Anthocleista djalonensis* leaves, a medicinal plant

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**Corresponding author: E-mail: cc.abba@unizik.edu.ng;*

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used in herbal remedies. The dried leaves of *Anthocleista djalonensis* were analyzed for their nutritional profile using standard methods. The leaves were then extracted by maceration using three different solvents: 95% analytical ethanol, hot distilled water, and local gin (78% alcohol). Various phytochemicals in the extracts were identified and quantified using standard analytical methods. Additionally, High-Performance Liquid Chromatography (HPLC) was used to compare the chemical compositions of the extracts, using a reverse-phase C_{18} column and UV detection at 254nm. The results of the proximate analysis revealed good nutritional value, with protein (15.90%), moisture content (9.08%), total ash (10.37%), crude fibre (4.00%), crude fat (2.40%), carbohydrate (61.53%), and an energy value of 331.33 KJ. All tested phytochemicals were found in the ethanol and local gin extracts, while the distilled water extract showed the absence of terpenoids and steroids. In the quantitative analysis, the local gin extract had the highest concentrations of alkaloids, terpenoids, flavonoids, and tannins. On the other hand, the HPLC fingerprint analysis identified seventeen (17), twelve (12), and nine (9) compounds in ethanol, local gin and water extracts respectively. Four (4) shared peaks with similar retention time were observed across all extracts, indicating the presence of common phytocompounds. In conclusion, this study shows that *Anthocleista djalonensis* leaves have good nutritional values, suggesting their potential as dietary phytonutrient. Local gin stands out as the best solvent among those tested for extracting beneficial compounds from *Anthocleista djalonensis* leaves supporting its traditional use as solvent in herbal preparation.

Keywords: Proximate composition; Anthocleista djalonensis; extracts; phytochemicals; HPLC; local gin.

1. INTRODUCTION

For centuries, traditional medicine has played a vital role in addressing health challenges, with plants serving as a rich source of natural remedies. Many drugs in use today are either direct products of medicinal plants or their derivatives. Approximately 80% of the world's population still relies on plant-based traditional healthcare products for primary healthcare [1]. These medicinal plants are extracted and processed for direct consumption in various forms, such as essential oils, herbal teas, capsules, and tablets containing a powdered form of the raw herb or its dried extract.

Phytochemicals also referred to as secondary metabolites or phytonutrients, are naturally occurring substances found in medicinal plants. They offer a diverse range of health benefits and serve as prime sources of lead compounds for the pharmaceutical industry due to their therapeutic nature. Phytochemicals, such as phenolic compounds, alkaloids, steroids, flavonoids, tannins, saponins, and glycosides, are examples of phytochemicals that possess curative or preventive properties. These bioactive compounds inspire the development of new drugs and herbal remedies, offering a natural approach to improving health and well-being [2].

In phytomedicine research, laboratory-grade solvents such as acetone, methanol, and ethanol are frequently used to extract plant-based bioactive compounds which are generally unsuitable for human consumption due to toxicity. On the contrary, traditional medicine frequently uses locally available and culturally acceptable solvents, such as oil, locally brewed gin, and water, to prepare herbal treatments. These solvents serve as vehicles, facilitating the extraction of various phytochemicals, which are the bioactive components responsible for the plant's therapeutic effects and suitable for consumption. The deliberate selection of traditional solvents in the extraction of secondary metabolites not only ensures the safety of the process, but also enhances the appeal of the resulting medicinal products to consumers.

Local gin is a traditional solvent widely used in herbal preparation. It is commonly known as kaikai, ogogoro, or akpeteshie. It is a traditional distilled alcoholic beverage commonly produced in West Africa using fermented starchy or sugary materials like sap of various species of palms trees (palm wine). The fermentation process, which converts sugar into ethanol and carbon dioxide, yields a liquid which is then distilled to concentrate the alcohol content. Local gin production is usually in small scale and often unregulated leading to variations in the alcohol content which depends on the specific production methods, fermentation efficiency, and distillation techniques employed.

Anthocleista djalonensis A. Chev (*A. djalonensis)*, a small tree belonging to the Gentianaceae family, has a rich history as a traditional remedy across various African countries like Nigeria, Mali, Cote d'Ivoire, Guinea, South Africa, and Tanzania. The stem bark, seed, leaves, and roots of this plant are valued for their medicinal properties [3]. Traditionally, these parts are prepared as decoctions or macerations using culturally accepted and available solvents like local gin or hot/boiling water and administered orally. Previous studies have shown that different parts of *Anthocleista djalonensis* may have useful medical properties, such as antidiabetic, antimalarial, antipyretic, anthelmintic, antimycobacterial, antibacterial, and wound healing properties [3,4,5]. These medicinal properties of *Anthocleista djalonensis makes its worthy to be explored for both* traditional and modern medical benefits.

The study aimed to investigate the proximate composition and compare the phytochemical profile and HPLC fingerprint profile of leave extract of *Anthocleista djalonensis* using various solvents. The solvents used were hot distilled water, local gin, and analytical ethanol, which shares similar alcoholic properties with local gin, but in a standardised composition and will serve as a benchmark for comparison. These solvents were selected based on their traditional use and scientific relevance. Our primary goal is to identify the best extraction solvent for extracting therapeutic phytochemicals from *Anthocleista djalonensis* leaves. This knowledge can contribute to the development of novel, naturally-derived therapies for various diseases.

2. MATERIALS AND METHODS

2.1 Plant Materials

The fresh leaves of *Anthocleista djalonensis* were sourced from its natural habitat within the environs of Nnamdi Azikiwe University-Main Campus, Agu Awka, Anambra State. The plant leaves were harvested on April 15, 2023, in the morning between 7 a.m. and 10 a.m. The leaves were authenticated by the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, with voucher number PCG/474/9/057.

2.2 Determination of Ethanol Content in Local Gin

The percentage of ethanol (alcohol) in the local gin used for this study was determined using a Soxhlet extraction apparatus. 100 ml of local gin was measured using a graduated cylinder and transferred to a round bottom flask. The flask was connected to a Soxhlet extractor and a condenser; the apparatus was placed on a heating mantle (isomantle) and switched on to heat the local gin. The ethanol (having a lower boiling point than water) evaporated and travelled through the condenser, where it cooled and converted back into liquid. The condensed ethanol dripped into a collection reservoir within the Soxhlet extractor. When the reservoir reached a specific level (before siphoning back), the collected ethanol was transferred to a separate measuring cylinder and sealed to prevent evaporation. Heating continued until no further ethanol dripped into the reservoir. The volume of collected ethanol was recorded.

2.3 Preparation of the Crude Extract

The leaves of *Anthocleista djalonensis* were rinsed twice with tap water to remove dust before detaching from the twigs. They were air-dried at room temperature, away from direct sunlight. Once dried, the leaves were ground mechanically into a fine powder. The powdered leaves were stored in an airtight container to preserve them for extraction. For the extraction process, maceration method was employed using 95% analytical grade ethanol, local gin (78% alcohol content), and hot distilled water as solvents. 150g of the dried powdered leaves was weighed into three separate extraction tanks. Each tank containing the weighed powdered leaves was soaked in 2000 ml of each solvent respectively. The mixtures in each tank were then covered with their respective lids and agitated vigorously for 24 hours at room temperature. The extracts were filtered separately using a muslin cloth and subsequently through a Whatman No. 1 filter paper (Whatman®, England). The filtrate obtained from the ethanol solvent was transferred to a round-bottom flask and concentrated to dryness using a rotary evaporator (Buchi Rotavapor R-200, Switzerland) under reduced pressure. For the distilled water and local gin extracts, lyophilization was performed. The extracts were first frozen in a deep freezer overnight and then freeze-dried using a lyophilizer to remove any water content. The resulting concentrated extracts was transferred into three different well labeled amber glass bottle respectively and stored at 4°C until needed for analysis. The percentage yield of each extract was calculated using the formula:

Percentage yield

$$= \frac{weight\ of\ dried\ extract}{weight\ of\ pulverized\ leave\ used\ in\ maceration} \times 100$$

2.4 Qualitative Phytochemical Screening

Screening for phytochemicals was done on each of the leaves extract of *Anthocleista djalonensis* using standard procedures to identify phytochemical constituents (alkaloids, tannins, flavonoids, saponins, terpenes, phenols, cardiac glycoside). The following qualitative tests were carried out as follows:

2.4.1 Test for alkaloids

Wagner's reagent test: Each solvent extract (0.2 mg) was introduced into dilute HCl (6 ml), respectively, and the mixture was boiled, cooled, and filtered. Pipette 1.0 ml of filtrate into a test tube and add 0.1 ml of Wagner's reagent (1.27g iodine + 2g potassium iodide + distilled water to make a final volume of 100 ml) then add along the sides of the test tube and mix properly. The formation of a reddish-brown precipitate indicates the presence of alkaloids [6].

2.4.2 Test for phenol

Ferric chloride test: Phenols were prepared by dissolving 1 ml of the respective solvent extracts in distilled water (5 ml), and then a few drops of a 5% ferric chloride solution were added. A dark green/bluish black coloration indicated the presence of phenolic compounds [7].

2.4.3 Test for flavonoids

Lead acetate test: 1 mL of each solvent extract was added to a test tube, respectively, and a few drops of 10% lead acetate solution were introduced into the same test tube and properly mixed. A yellow precipitate is observed, which indicates the presence of flavonoids [6].

2.4.4 Test for tannins

Braymer's test: Each solvent extract (0.30 g) was weighed into a test tube and boiled for 10 minutes in a water bath containing 30 ml of water. Filtration was carried out after boiling using number 42 (125 mm) Whatman filter paper. 1 ml of the filtrate was pipetted into a test tube, and 3 drops of 0.1% ferric chloride solution were added. A brownish green or a blue-green coloration showed a positive test [6].

2.4.5 Test for saponins

Olive oil test: 2g of each solvent extract was boiled together with 20 ml of distilled water in a water bath and filtered. 10 ml of the filtered sample is mixed with 5 ml of distilled water in a test tube and shaken vigorously to obtain a stable, persistent froth. The frothing is then mixed with 3 drops of olive oil and shaken vigorously again. The formation of emulsion (foam) indicates the presence of saponins [8].

2.4.6 Test for cardiac glycoside

Cardiac glycoside was prepared with 0.5 ml of the respective solvent extracts mixed in a test tube and dissolved in pyridine and sodium nitroprusside. 5 drops of 20% NaOH were added to the test tube. A red colour that fades to brownish yellow indicates the presence of cardinolide glycone, a cardiac glycoside [9].

2.4.7 Test for Phlobatannins

HCl test: 2 ml of the aqueous solution of each extract was added to 2 ml of 1% dilute HCl (boiled), respectively. Observation of a red precipitate indicates the presence of phlobatannins [10].

2.4.8 Test for glycosides

Aqueous NaOH test: 0.5 ml of each solvent extract was dissolved in 1 ml of water, and then a few drops of aqueous NaOH solution were added. The formation of a yellow colour indicates the presence of glycosides [11].

2.4.9 Test for terpenoids

Chloroform and H_2SO_4 test: Each solvent extract (0.30 g) is mixed with 2 ml of chloroform in a test tube, respectively; 3 ml of concentrated tetraoxosulphate (VI) acid (H_2SO_4) is carefully added to the mixture to form a layer. An interface with a reddish brown coloration is formed if terpenoids are present [12].

2.4.10 Test for steriods

Salkowski's test: 0.5g of each extract was dissolved in 10 ml of anhydrous chloroform and filtered, respectively. A few drops of concentrated H_2SO_4 were added to each solution (shaken well and allowed to stand). The acid forms a lower layer, and the interface was observed to have a reddish colour (in the lower layer), which indicates the presence of a steroid ring [11].

2.5 Quantitative Determination of Phytochemicals

The quantitative determination of phytochemical composition of crude leaves extracts of *Anthocleista djalonensis* were carried out using the standard procedures described by Harborne [13], for alkaloid, steroid and phenol; Flavonoid was determined using Boham and Kocipal-Abyazan [14] method, Tannin was done using [15] method. For saponins [16] and for cardiac glycoside [17].

2.6 HPLC Profiling Analysis

The HPLC analysis was carried out on a Shimadzu HPLC system. 10 mg/ml of the extracts were prepared by weighing 20 mg of each sample, dissolving it in 1 mL of distilled water, and then making up to 2 ml. The final solution was filtered with a 0.45 µm Millipore membrane filter before use. An aliquot of 10 µl of each sample solution was injected into the HPLC system for analysis. A binary gradient elution system composed of 0.1% formic acid in HPLC-grade water as solvent A and acetonitrile as solvent B was applied for the fingerprint analysis with the gradient elution as listed in Table 1. The HPLC system comprises a Prominence Auto Sampler (SIL-20A), equipped with Shimadzu Ultra Fast LC-20AB reciprocating pumps connected to a DGU 20A3 degasser, a SPDM20A UV-diode array detector (UV-DAD), a column oven (CTO-20AC), a system controller (CBM-20Alite), and Windows LC Solution Software 1.22 SP1.

Table 1. Gradient Composition Used for the HPLC Analysis

Time (min)	Composition of B (%)
0 – 10	15
10 – 15	15 – 20
15 – 20	20 -30
20 -25	30 – 35
25 – 30	35 – 45
30 – 35	45 – 50
35 – 40	50 – 25
40 – 50	25 – 15
50 – 60	15

Chromatographic conditions under gradient elution include: column (C_{18}) (4.6 x 150 mm x 5 µm) mobile phase was 1% formic acid in HPLC graded water (A) and Acetonitrile (B); flow rate was 0.6 mL/min and column temperature was maintained at 40 ^{0}C; injection volume, 3µL and the DAD detector wavelength was set at 254 nm [18].

2.7 Proximate Analysis of *Anthocleista djalonensis* Leaves

The proximate composition of dried leaves of *Anthocleista djalonensis* was determined using standard methods of the Association of Official Analytical Chemists (AOAC) [19,20,21], and each analysis was carried out in triplicate. The moisture content was performed in a dry oven at 105 °C for 24 hours, while total ash was analysed following the calcination of the dried sample in a muffle furnace at 300 °C for 3 hours, and the temperature increased to 600 ^{0}C for 9 hours. The crude fibre was measured by the initial digestion of 2g of the dried sample under reflux with an equal concentration of acid and base (200 ml of a solution containing 1.25g of acid/base per 100 ml), followed by sequential washing with hot water, acetone, and hydroethanol. Petroleum ether was used for the extraction of crude fat using a soxhlet extractor at 60 ^{0}C for 6 hours, following drying in a hot air oven at 105 °C. Total protein in dried leaves was estimated using the micro-Kjeldahl method, and 6.25 were used to convert the nitrogen to protein. Carbohydrate content was estimated by the difference method using the equation below:

Carbohydrate (%) = 100 – [Moisture (%) + Ash (%) + Crude protein (%) + Crude fat (%)]
Total energy value was calculated by Atwater factors:

Energy value (KJ) **=** (% crude protein ×4) + (% carbohydrate ×4) + (% crude fat ×9)

2.8 Statistical Analysis

The experiment results were analysed in triplicate, and the results were expressed as mean ± standard error of the mean (SEM). Statistical analysis was conducted using analysis of variance (ANOVA) to determine the level of significance. Differences in mean values were considered significant at $P < .05$.

3. RESULTS

3.1 Percentage Yield of Extracts

The percentage yield of the crude extracts (local gin, ethanol, and distilled water) of *Anthocleista djalonensis leaves is* presented in Fig. 1. A total of 30.8g (20.5%) of dried local gin crude extract, 25.6g (17.1%) of dried distilled water crude extract, and 14.1g (9.4%) of dried ethanol crude extract are shown in Fig. 1. The local gin extract yielded more than the ethanol and water extracts

3.2 Percentage of Ethanol (Alcohol) in Local Gin Solvent

The analysis of local gin used in this study revealed an ethanol content of 78% (v/v) (Fig. 2) as determined by Soxhlet extraction. The high ethanol content makes local gin a polar solvent. However, the presences of water (22%) can dilute the concentration of ethanol molecules, impacting the overall polarity of the local gin.

3.3 Phytochemical Screening

The results of the qualitative and quantitative determination of phytochemicals in the crude leaves extracts of *Anthocleista djalonensis* screened in this study are shown in Table 2 and Table 3.

3.4 HPLC Profiling

The HPLC profile results obtained from the analysis of crude leaf extracts of *Anthocleista djalonensis* revealed distinct peaks, each characterized by its retention time range of 0 to 60 minutes, accompanied by corresponding area values. The atomized wavelength employed for this analysis was set at 254nm absorbance.

3.4.1 HPLC chromatogram of ethanol crude leave extract of *Anthocleista djalonensis*

The crude extract of *Anthocleista djalonensis* revealed wide variability in its chromatogram, according to the HPLC analysis. About seventeen (17) compounds were visualised in the form of peaks (Fig. 3). There are three (3) prominent peaks with distinct compounds: peak 9 (retention time = 16.577 min, peak area = 1272733 mAU), peak 11 (retention time = 21.171 min, peak area = 800619 mAU), and peak 13 (retention time = 28.629 min, peak area = 166035 mAU). The peak with a retention time of 16.577 minutes has the highest sharp peak.

3.4.2 HPLC chromatogram of local gin crude leaves extract of *Anthocleista djalonensis*

The HPLC analysis of the crude local gin extract of *Anthocleista djalonensis* leaves revealed twelve (12) compounds visualised in the form of peaks (Fig. 4). There are three (3) prominent peaks with retention times of 2.571 minutes, 16.460 minutes, and 21.089 minutes observed. The compound with a retention time of 21.089 minutes has the sharpest peak.

3.4.3 HPLC chromatogram of distilled water crude leaves extract of *Anthocleista djalonensis*

The HPLC chromatogram of the crude distilled water extract of *Anthocleista djalonensis leaves* in Fig. 5 revealed the presence of nine (9) compounds visualised in the form of peaks, with four (4) prominent compounds at peak numbers 1, 7, 8, and 9 with retention times of 2.684 minutes, 10.880 minutes, 16.537 minutes, and 21.066 minutes, respectively. The sharpest peak was seen in peak 1, followed by peak number 8.

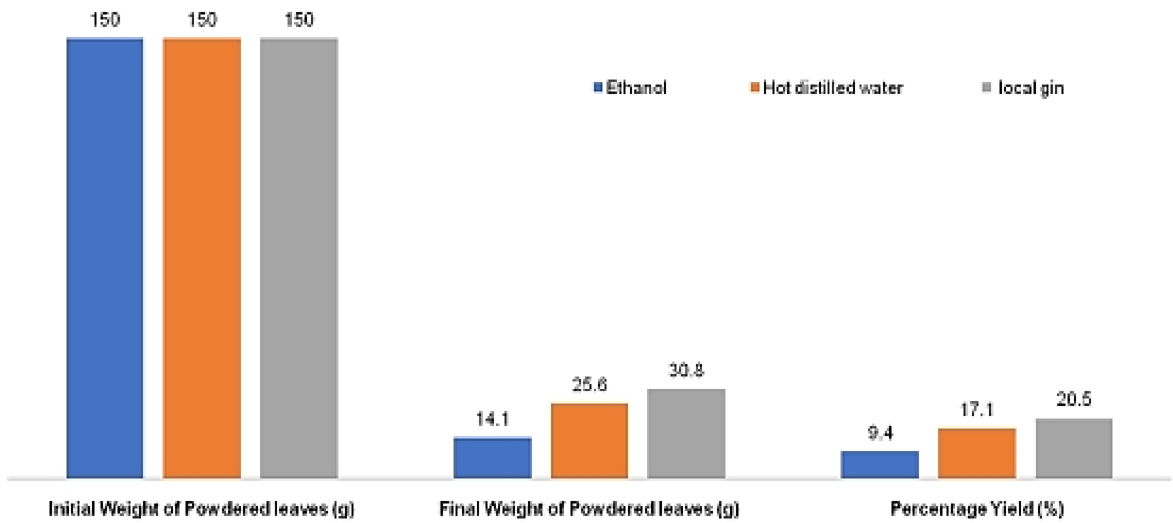

Fig. 1. Percentage Yield of *Anthocleista djalonensis* Crude Leaf Extracts

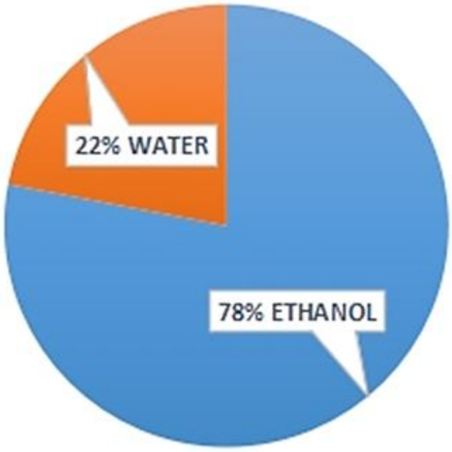

Fig. 2. Pie Chart of Percentage of Ethanol in Local Gin

Table 2. Qualitative Phytochemical Analysis of Crude Leave Extracts of *Anthocleista djalonensis* Using Different Solvents

Phytochemical Test	Observation	Ethanol	Local Gin	Distilled Water
Alkaloids *(Wagner's reagent)*	Reddish brown precipitate	++	++	++
Phenol *(Ferric chloride test)*	Dark green colouration	+	++	++
Flavonoid *(Lead acetate test)*	Yellow colour precipitate	++	++	++
Tannins *(Braymer's test)*	Blue-green colouration	+	+	+
Saponnins *(Olive oil test)*	Persistence foaming	+	+	++
Cardiac glycosides (*Test for cardenolides)*	Red colour that fades into brownish yellow colour	++	++	++
Phlobatannins *(HCl test)*	Red colour precipitate	+	+	+
Glycosides (*Aqueous NaOH test)*	Yellow colouration	+	+	+
Terpenoid *(Chloroform and H_2SO_4 test)*	Reddish brown colour	+	+	-
Steroids *(Salkowski's test)*	Red colour at interface	++	++	-

Keys: ++ indicate moderately present, + indicate trace/mildly present, - indicate absent

Table 3. Quantitative Determination of Phytochemical Components of the Crude Leaves Extracts of *Anthocleista djalonensis*

Phytoconstituents	Ethanol Extract	Local Gin Extract	Distilled Water Extract
Alkaloid (%)	6.95	11.29	8.92
Phenol (mg/g)	4.21	6.54	9.32
Flavonoid (%)	10.58	12.62	9.92
Tannin (%)	3.61	4.82	3.47
Saponnin (%)	1.92	2.78	6.08
Cardiac glycoside (mg/g)	9.02	11.32	12.78
Glycoside (mg/100)	118.56	150.60	182.50
Terpenoid (%)	4.00	8.00	ND
Steroid (mg/g)	10.36	9.24	ND

ND = not detected

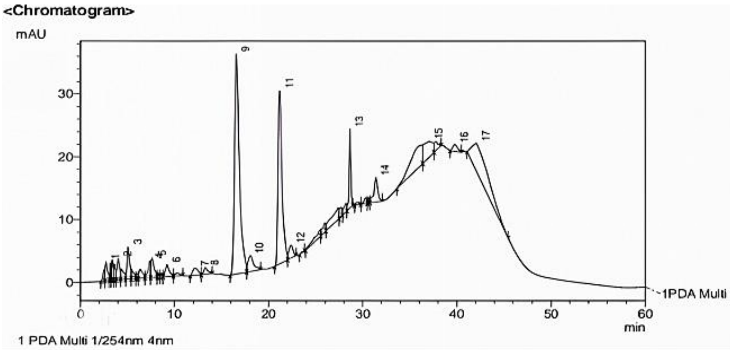

Fig. 3. HPLC Chromatogram Resulting from the Analysis of Ethanol Crude Leaves Extract of *Anthocleista djalonensis* at 254 nm Absorbance

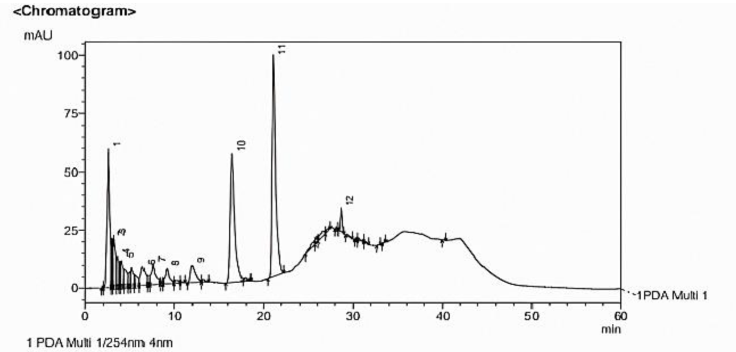

Fig. 4. HPLC Chromatogram Resulting from the Analysis of Local Gin Crude Leaves Extract of *Anthocleista djalonensis* at 254 nm Absorbance

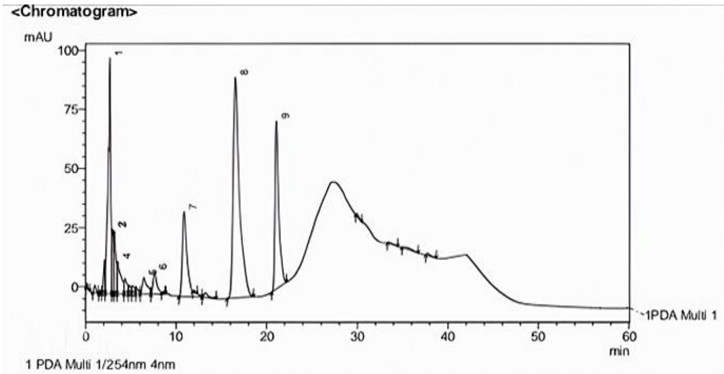

Fig. 5. HPLC Chromatogram Resulting from the Analysis of Distilled Water Crude Leaves Extract of *Anthocleista djalonensis* at 254 nm Absorbance

3.4.4 Comprehensive comparison of HPLC analysis of *Anthocleista djalonensis* crude extracts

The HPLC data extracted using ethanol, local gin, and distilled water solvent extracts were aligned based on their retention time values for comprehensive comparison (Table 4). Peaks that were visible in all extracts at similar retention time values were called “common peaks." These peaks were sequentially numbered based on their elution order, resulting in the identification of four (4) common peaks across all extracts, three (3) common peaks between ethanol and local gin extracts, and four (4) common peaks between local gin and distilled water extracts, as shown in Table 4. The peak areas corresponding to similar retention times across all extracts exhibited significant differences from each other ($P \leq .05$), establishing their statistical significance. Also, the peak areas associated with similar retention times in both ethanol and local gin ($P \geq .99$) and local gin and distilled water ($P \geq .31$) demonstrated no significant differences in the peak areas of their shared compounds.

Table 4. Comparative Result of the HPLC Analysis *Anthocleista djalonensis* Crude Leaves Extracts

Ethanol Extract			Local Gin Extract			Distilled Water Extract		
Peak Num.	**Retention Time (min)**	**Area (mAU)**	**Peak Num.**	**Retention Time (min)**	**Area (mAU)**	**Peak Num.**	**Retention Time (min)**	**Area (mAU)**
1**	2.715	52458	1	2.571	1259709	1	2.684	1987208
-			2•	2.995	249269	2	3.000	324505
-			3•	3.184	396773	3	3.176	407279
-			4•	3.602	219395	4	3.560	384202
2$^{\Phi}$	4.019	62478	5	4.056	202432	-		
-			6•	6.433	287468	5	6.446	253408
3	5.068	94453	-			-		
4	7.374	50936	-			-		
5**	7.624	70751	7	7.573	323255	6	7.612	277771
6$^{\Phi}$	9.225	56165	8	9.161	224809	-		
7	12.250	44531	9	11.975	255408	7	10.880	1339186
8	13.290	30228	-			-		
9**	16.577	1272733	10	16.460	1998862	8	16.537	4501282
10	18.050	101277	-			-		
11**	21.171	800619	11	21.089	2697533	9	21.066	2079314
12	22.372	58937	-					
13$^{\Phi}$	28.629	166037	12	28.621	124096			
14	31.401	111310						
15	37.068	174962						
16	39.769	35693						
17	42.022	570936						

P values:
**P = .05
$^{\Phi}P$ = .99
•P = .31

Table 5. Proximate composition (g/100) of dried healthy leaves of *Anthocleista djalonensis*

Proximate composition (g)	Percentage (%)
Ash	10.37 ± 0.18
Moisture	9.80±0.11
Crude fat	2.40±0.10
Crude protein	15.90 ± 0.46
Crude Fiber	4.00 ± 0.46
Carbohydrate	61.53 ± 0.50
Total energy (KJ)	331.33 ± 0.80

Values are presented as mean ± SEM of triplicate determinations (n =3)

3.5 Proximate Composition

The nutritional value of the dried powdered leaves of *Anthocleista djalonensis* was assessed through proximate analysis, and the results are presented in Table 5. The figure revealed the composition of *Anthocleista djalonensis* leaves on a dry basis, expressed in percentages (%). The average of three determinations was used for each data point.

4. DISCUSSION

The effectiveness of using traditional solvents in extracting phytocompounds for herbal preparations has not been scientifically established. Extraction solvents have been shown to have an effect on the extraction yield and the content of bioactive compounds, thus significantly affecting the biological activity of the extracts [22]. In the present study, we compared the efficacy of local gin, distilled water, and ethanol for extracting phytochemicals from *Anthocleista djalonensis leaves. These solvents* were chosen because of its widespread use in herbal preparation and accessibility. Analytical ethanol was included as a comparative solvent due to its similar alcoholic properties with local gin, but in a standardised composition. The cold maceration extraction technique was utilised for the local gin and ethanol solvents, whereas hot maceration was used for the distilled water solvent, as it replicates the method that is widely used by the locals. The decision to investigate *Anthocleista djalonensis* leaves was influenced by its established use in traditional medicine [3].

The findings showed that different solvents resulted in various extraction yields. Local gin extract demonstrated a higher extraction yield in comparison to ethanol and distilled water extracts. Factors such as the polarity of the solvents, the purity of the solvents, the solubility of compounds in the solvents, the extraction method used, and the duration of the extraction impact the percentage yield of the extracts. To understand the solvent's effect on extraction yield, qualitative and quantitative analysis was carried out to show the presence and concentration of bioactive compounds in the extracts.

The phytochemical analysis unveiled a rich composition of medicinal active phytocompounds that varied based on the extractive abilities of each solvent, as detailed in Tables 2 and 3. The local gin solvent was able to extract alkaloids, flavonoids, terpenoids, and tannins in higher concentrations than other tested solvents. The distilled water extract demonstrated the absence of terpenoids and steroids, which aligns with the findings of Okenwa et al. [23] and Oluwayomi et al*.* [24]. These phytochemicals present in the extracts have proven to be valuable in the development of modern medicine and may act singly or in synergism. The high extractive yield and high concentration of phytochemicals in local gin extract suggest that local gin is best for extracting bioactive compounds in *Anthocleista djalonensis* leaves compared to the other tested solvents and may account for its use as a solvent in herbal preparation. This study is the first to report the phytochemical screening of the local gin extract of *Anthocleista djalonensis* leaves.

The HPLC fingerprint profiling conducted in the study enhanced the understanding of the chemical profile of various solvent extracts of *Anthocleista djalonensis* leaves by revealing both similarities and differences in their chemical composition without specifically identifying individual compounds. The results of the analysis revealed that the choice of solvents greatly influences the extraction efficiency of phytocompounds, with the polarity of solvents playing a major part in this process [25]. Ethanol, being a relatively polar solvent, interacts with both polar and non-polar compounds during the extraction process, leading to the extraction and detection of a diverse range of phytocompounds, resulting in the highest number of detectable peaks in the chromatogram compared to local gin and distilled water extracts. Similarly, the high ethanol content in the local gin (78%) used as solvent in this study is the primary factor contributing to it overall polarity. Local gin has a moderate polarity that lies between analytical ethanol and water. This intermediate polarity allows local gin to dissolve and extract a broad range of phytocompounds detected in the chromatogram. The ethanol content enhanced the solubility of various compounds, leading to a higher number of detectable peaks compared to distilled water extract. Also, because distilled water is a very polar solvent, it extracts mostly polar compounds. This results in a less diverse spectrum of phytocompounds, with fewer peaks visible in the chromatogram. Previous studies have identified specific compounds in *Anthocleista djalonensis* leaves, such as isovitexin, septicine, vanillin, 3-methyl-2,3,4-pentanetriol, indo-3-carboxylic acid, cerebroside, and bromohexylamide [26], as well as loganic acid, swertiamarin, sweroside, p-coumaric acid, isovitexin, and 3,5-dicaffeoylquinic acid [27]. Also, Abba et al. [28] reported isovitexin and its derivatives to be the major phytocompounds responsible for the therapeutic properties of *Anthocleista djalonensis* leaves. These findings suggest that similar compounds may be present in the extracts, which could contribute to the plant's observed therapeutic effect.

Furthermore, the results in Table 4 demonstrated that phytocompounds eluted in a consistent order with similar retention times across the extracts. This suggests a potential close relationship and shared chemical similarities among these compounds. The presence of shared peaks with similar retention time observed between local gin and distilled water extracts, as well as between ethanol and local gin extracts indicates the presence of common compounds. These shared peaks could be compounds that are readily soluble in all three solvents or core bioactive constituents present in *Anthocleista djalonensis* leaves, regardless of the extraction solvent used. The relatively close polarity of local gin and ethanol likely contributes to their ability to extract similar phytocompounds leading to shared peaks. The ability of local gin to share peaks with both ethanol and water extracts indicate its effectiveness in extracting compounds with diverse solubility profile. The distilled water extract showed higher peak area values among all shared peaks, likely due to the enhanced solubility of these compounds in distilled water as a result of its polarity, resulting in higher concentrations. The statistical analysis revealed that the peak area associated with common peaks shared between the solvent pairs showed no significant (P ≥.05) differences in the peak area of their shared compounds, as shown in Table 4.

The proximate analysis of dried *Anthocleista djalonensis* leaves, as detailed in Table 5, reveals their rich nutritional compositions. The leaves demonstrated a significant ash content of 10.37%, indicating substantial mineral deposition. This finding aligns with previous research by Okenwa et al. [23] and highlights the potential of these leaves as a source of essential minerals. The high carbohydrate content of 61.53% contributes greatly to its caloric value of 331.33 KJ/100g, which exceeds the values reported in previous studies [23,24,29]. The leaves possess a crude fibre content of 4.00%, which falls within the recommended nutritional range. Additionally, it has a favourable low moisture content of 9.08%, which enhances stability and shell life [30]. Furthermore, the leaves contain a crude fat content of 2.40%, indicating their potential as a source of nutritious fat. This surpasses the values reported by Okenwa et al. [23] and Oluwayomi et al. [24]. The crude protein content of 15.90% aligns closely with the value reported by Okenwa et al. [23], suggesting their benefit as a dietary supplement. The variability in nutritional composition may be attributed to differences in species, environmental conditions, and harvesting factors.

The findings in the study contributes to the understanding of solvent extraction efficiency; however, one key limitation that should be addressed in future research is the inconsistency in the composition and alcohol content of locally brewed gin which can influence the extraction efficiency, amount of phytochemicals extracted and bioactivity of the extracts compared to a standardized solvent.

5. CONCLUSION

Anthocleista djalonensis leaves have beneficial nutritional compositions and are rich in active phytochemical constituents that possess therapeutic properties. The local gin was the best extraction solvent among other solvents tested based on the extract yield and the presence of therapeutic phytochemicals in the extract at high concentrations. These findings offer scientific validation for the traditional use of local gin as a solvent in herbal preparations. Further studies could evaluate the biological activity of the extracts, identify, characterise, and elucidate the structures of these biologically active compounds, particularly in the local gin extract. These phytochemicals will provide insight into the medicinal application of *Anthocleista djalonensis* leave or serve as precursors for modern medicine.

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 writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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