Phytochemical, antioxidant and anti-inflammatory analysis of dichloromethane extract of *Archonea Cordifolia* leaves

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Abstract

Background: Phytochemical constituents are integral to the therapeutic effectiveness of *A. cordifolia*.

Objectives: This study aims to thoroughly analyze the phytochemical, antioxidant, and anti-inflammatory properties of the dichloromethane extract of *A. cordifolia* leaves.

Results: The percentage yield of the dichloromethane leaf extract was 0.92%, resulting in 6.42g of a brown mass. The extract's essential mineral composition highlights sodium and magnesium as the most prominent macro minerals, while iron and selenium are the most abundant micro minerals. The study revealed that tannins and phenols had the least concentration (0.17±0.02) among the phytochemicals, with alkaloids being the highest concentrated (11.7±0.06). The chromatogram identified various bioactive compounds in the extract, including alkanones, esters, fatty acids, alkanals, phenols, and aromatic compounds. The IC_{50} values of the extract were significantly ($p<0.05$) different compared to the respective standards. The in vitro anti-inflammatory response of the dichloromethane *A. cordifolia* leaf extract was assessed through inhibition of albumin denaturation, membrane stabilization, and anti-proteinase activities, all showing positive, concentration-dependent activities. Notably, the IC_{50} values for albumin denaturation and membrane stabilization were higher for the extract compared to aspirin. The dichloromethane extract of *A. cordifolia* leaves shows significant phytochemical, antioxidant, and anti-inflammatory properties, with high mineral content and potent bioactive compounds.

Conclusion: *A. cordiforlia* dichloromethane extract demonstrated strong in vitro antioxidant and anti-inflammatory activity, outperforming aspirin in albumin denaturation and membrane stabilization, indicating promising therapeutic potential.

Keywords; Phytochemistry, phytomedicine, Anti-inflammatory; *Archoneacordifolia*

INTRODUCTION

Alchornea cordifolia, commonly referred to as the Christmas bush or the African Christmas tree, is a perennial shrub belonging to the Euphorbiaceaefamily [1]. This plant is indigenous to tropical regions of Africa and is widely recognized for its diverse pharmacological properties [2]. Traditionally, *A. cordifolia*has been employed in various cultures for the treatment of ailments such as inflammation, infections, fever, and pain relief. Its leaves, bark, and roots are utilized in folk medicine, often prepared as infusions, decoctions, or poultices[3]. Recent studies have highlighted the plant's rich phytochemical profile, which includes a wide array of secondary metabolites such as flavonoids, tannins, alkaloids, saponins, and triterpenoids, all known for their antioxidant and anti-inflammatory activities.[4,5]

Phytochemical constituents play a crucial role in the therapeutic efficacy of *A. cordifolia*. Flavonoids, a prominent class of polyphenolic compounds found in the plant, are recognized for their ability to scavenge free radicals, thereby mitigating oxidative stress implicated in various chronic diseases[6}. These compounds can inhibit the activity of enzymes involved in the generation of reactive oxygen species (ROS) and enhance the activity of antioxidant enzymes, contributing to cellular protection against oxidative damage.[7]Tannins, another significant group of compounds present in *A. cordifolia*, exhibit anti-inflammatory properties by inhibiting the activity of pro-inflammatory enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX), which are responsible for the production of inflammatory mediators.[8] Furthermore, tannins can bind to proteins and other macromolecules, potentially reducing inflammation by stabilizing cell membranes and preventing the release of inflammatory mediators.[9]The dichloromethane extract of *A. cordifolia*leaves is particularly interesting due to its potential to yield bioactive compounds that may enhance these therapeutic effects.

The antioxidant capacity of *A. cordifolia*has been the subject of several studies, demonstrating significant free radical scavenging activity. For instance, extracts from the leaves have shown promising results in DPPH (1,1-diphenyl-2-picrylhydrazyl) and H_2O_2 assays, indicating their potential as natural antioxidants.[10]The ability of these extracts to inhibit oxidative stress-related damage underscores their relevance in the prevention of diseases associated with oxidative stress, such as cardiovascular diseases, neurodegenerative disorders, and cancer.[11]Oxidative stress arises from an imbalance between the production of ROS and the body's ability to eliminate them, leading to cellular damage[12]. The antioxidants present in *A. cordifolia*can neutralize these free radicals, thus protecting cellular components such as lipids, proteins, and DNA from oxidative damage. Additionally, the presence of phenolic compounds in the extract contributes significantly to its antioxidant properties, as these compounds are known to donate electrons to free radicals, stabilizing them and preventing further cellular damage[13]

In addition to its antioxidant properties, *A. cordifolia*has demonstrated notable anti-inflammatory activities. Various studies have reported that plant extracts can inhibit albumin denaturation and membrane stabilization, which are critical indicators of anti-inflammatory potential.[14] The inhibition of albumin denaturation is particularly relevant, as it indicates ability of plants to prevent the formation of inflammatory mediators such as prostaglandins and leukotrienes.[15]Moreover, the stabilization of cell membranes suggests that *A. cordifolia*extracts can prevent the release of inflammatory enzymes and mediators from damaged cells.[16] This membrane-stabilizing effect is crucial in the context of inflammatory responses, as it can help mitigate

the extent of tissue damage during inflammation. The anti-inflammatory effects of *A. cordifolia*may also be attributed to the presence of triterpenoids, which have been shown to exhibit significant inhibition of inflammatory pathways.[17]

The extraction method employed can significantly influence the yield and composition of bioactive compounds. In this study, dichloromethane is chosen as the extraction solvent due to its ability to selectively extract non-polar and moderately polar compounds, potentially yielding a diverse array of bioactive molecules.[18]This solvent is particularly effective in extracting flavonoids and terpenoids, which are known for their antioxidant and anti-inflammatory properties. The choice of extraction solvent is critical, as it can affect the bioactivity of the extracts and their subsequent therapeutic applications[19, 20].

Given the increasing interest in natural products for therapeutic applications, this study aims to conduct a comprehensive analysis of the phytochemical, antioxidant, and anti-inflammatory properties of the dichloromethane extract of *A. cordifolia*leaves. By elucidating the chemical constituents and their biological activities, this research seeks to contribute valuable insights into the medicinal potential of this plant. The findings may pave the way for future pharmacological studies and applications, reinforcing the importance of *A. cordifolia*in traditional medicine and its potential role in modern therapeutic practices. The exploration of *A. cordifolia*not only highlights its traditional uses but also emphasizes the scientific basis for its medicinal properties. The integration of phytochemical analysis with assessments of antioxidant and anti-inflammatory activities can provide a holistic understanding of the plant's therapeutic potential, ultimately contributing to the development of natural products aimed at promoting health and well-being.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Plant materials were collected in February 2022 from a farm land in Obiaruku. Obiaruku is the headquarters of Ukwuani Local government area of Delta State. With a GPS coordinate of Latitude 5° 50' 48.19" N and longitude 6° 09' 10.44" E. The plant *A. cordifolia* was identified by Prof. Akinnibosun Henry Adewale at the Herbarium unit of Department of Plant Biology and Biotechnology, University of Benin, Benin City Nigeria with a voucher number deposited thus; UBJ-A502

Extract Preparation

The Fresh leaves of *Alchornea cordifolia* was washed with distilled water to remove debris and were then air dried for two weeks, till a constant weight was obtained. The dried leaves were then reduced to coarse powder using a manual grinder. The coarsely powdered leave was extracted in a ratio of 100g of powdered leaves to 400ml. Hence a total of 700g of the powdered leaf was extracted with 2,800 ml ofdichloromethane using cold maceration for 24hours. The extract was then filtered through cheese cloth with fine pore, and the filtrate was filtered for the second time using Whatman No. 1 filter paper. The resulting extract was then concentrated at 50^{o}C in a rotary evaporator for 2hours. This was then followed by evaporation to dryness in a water bath maintained at 50^{o}C to yield a dark green mass. The obtained extract was stored at 4^{o}C until required for use.

Procedures for Phytochemical and Biological Activity Analysis of *Alchorneacordifolia*

Mineral Analysis

The mineral content of the samples was analysed using a Varian AA240 atomic absorption spectrophotometer, following the method of the American Public Health Association (1995)[21].

Qualitative Phytochemical Screening

Phytochemical screening was conducted using standard methods described by Borokini and Omotayo (2012)[22] and Njoku and Obi (2009) [23] to detect various chemical constituents.

Quantitative Phytochemical Analysis

The methods used for quantitative phytochemical analysis are as described below.

Total Phenols and Tannins

Based on the capacity of Folin-Ciocalteau's reagent to oxidize phenolic substances to phenolates at an alkaline pH in a saturated sodium solution, this was calculated using the Singleton and Rossi[24] approach. Shortly after combining 1.0 ml of extract with the Folin-Ciocalteau's reagent, 1.0 ml of saturated sodium carbonate (Na_2CO_3) was added, and 10 ml of distilled water was added to make up the remaining volume. After 90 minutes of standing in the dark, the mixture's absorbance at 725 nm was measured. In comparison to a standard calibration curve plotted with varying amounts of gallic acid and tannic acid (20 – 100 µg/ml), respectively, the results were represented as mg of gallic acid (GAE) equivalents/g extracts and mg of tannic acid (TAE) equivalents/g extracts.

Total Flavonoids

This is expressed in terms of catechin (CAE) equivalents and calculated using the Jia *et al*.[25] approach. Half a milliliter of the extract was added to a tube that contained 1.25 milliliters of distilled water and 0.075 milliliters of a 5% sodium nitrite ($NaNO_2$) solution. The tube was then let to stand for five minutes. After that, 0.5 ml of 1.0 M sodium hydroxide and 0.15 ml of 10% $AlCl_3$ were added. A spectrophotometer was used to measure the absorbance at 510 nm as soon as the resultant combination was diluted with 0.275 cc of distilled water.

Alkaloids

The Shamsa *et al*.[26] method was used for quantitative estimation, and atropine was generated as the standard equivalent at 40–120 µg/ml for comparison. Five milliliters of Bromocresol green (BCG) solution and five milliliters of pH 4.7 phosphate buffer were added to one milliliter of the extract. The liquid was then shaken with four milliliters of chloroform. Chloroform was used to dilute the extract after it was collected in a 10-ml volumetric flask. At 470 nm, the complex's absorbance in chloroform was measured and contrasted with a blank that was made in the same way but without extract.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS was undertaken using a GC-MS (Agilent 6890 series, China) device in accordance with the procedure that Olasehinde*et* al[27]. previously described. The National Institute of Standards and Technology (NIST) library's mass spectra and those of the chemicals found in the extracts were compared.

In-Vitro Antioxidant Activities

This was carried out following standard methods as described below;

DPPH Radical Scavenging Assay:

Based on the potential of the DPPH (1, 1-diphenyl-2-picryl hydrazyl) radical to be scavenged by an antioxidant by the donation of a proton, resulting in the decreased DPPH, this was ascertained using the approach outlined by Manzocco*et al*.[28] In short, 2 ml of DPPH solution (0.3 mM) was mixed with 0.2 ml of various doses of the A. cordifolia extract (0.020 – 0.10 mg/ml). At 517 nm, the absorbance is measured following a 30-minute dark incubation period. The following formula is used to get the DPPH radical scavenging inhibition percentage:

% inhibition of DPPH radical = $([A_0 - A_1]/ A_0) \times 100$

Where A_0 is the absorbance of the control (blank, without extract) and A_1 is the

absorbance in the presence of the extract. The IC50 value (mg extract/mL) is the effective concentration at which 50 % of DPPH radicals were scavenged and was obtained by interpolation from linear regression analysis.

Nitric Oxide (NO) Radical Scavenging Activity:

Based on the idea that sodium nitroprusside in aqueous solution and at physiological pH spontaneously generate nitric oxide, which combines with oxygen to produce nitrite ions estimated spectrophotometrically at 540 nm, this was also ascertained using the method outlined by Marcocci*et al.* [29]. A. cordifolia extracts in varying doses (0.020–0.10 mg/ml) were combined with 0.5 ml of 10 mM sodium nitroprusside diluted in 0.5 ml of 10 mM phosphate buffer saline (pH 7.4). dichloride (0.1% w/v)], and the mixture was then incubated at 250C. Following 150 minutes of incubation, 0.5 ml of the incubated solution was taken out and combined with 0.5 ml of Griess reagent [1.0 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) combined with 1 ml of naphthyl ethylenediamine dichloride (0.1% w/v) at room temperature for 5 minutes. After 30 minutes of room temperature incubation, the mixture's absorbance at 546 nm was measured against a blank. The following equation was used to determine the % inhibition of nitric oxide radical scavenging:

% inhibition of NO radical = $([A_0 – A_1]/ A_0) \times 100$.

Where A_0 is the absorbance of the control (blank, without extract) and A_1 is the absorbance in the presence of the extract. IC50 value (mg extract/mL) is the effective concentration at which 50 % of nitric oxide radicals were scavenged and was obtained by interpolation from linear regression analysis.

Reducing Power Assay

Oyaizu[30] used a previously published method to determine this. 1.0 ml of the *A. cordifolia* extract (0.020 – 0.10 mg/ml) was mixed with 2.5 ml of K3Fe (CN)6 (1% w/v) and 2.5 ml of 0.2 M phosphate buffer (pH 6.6). After 20 minutes of incubation at 500C, 2.5 ml of 10% w/v trichloroacetic acid was added to the resultant combination. The top layer of the solution (2.5 ml) was collected by centrifuging the mixture for 10 minutes at 3000 rpm. It was then combined with 2.5 ml of distilled water and 0.5 mL of $FeCl_3$ (0.1%, w/v). After that, the absorbance at 700 nm was compared to a blank sample that comprised sodium phosphate buffer and distilled water. As a standard, ascorbic acid (20–100µ/gml) was utilized. The effective concentration at which 50% reduction has taken place is known as the IC50 value (mg extract/mL), and it indicates the reducing power at an absorbance of 0.5. The values were derived using interpolation from linear regression analysis.

Total Antioxidant Capacity

Expressed as gallic acid equivalents (GAE) using the method of Prieto *et al.*[31] After putting 1.0 mL (0.20–1.00 mg/ml) of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) into screw-capped tubes, 0.1 mL of A. cordifolia extract. After being sealed, the tubes were incubated for 90 minutes at 95 °C in a thermal block. The absorbance of the aqueous solution in each tube was measured at 695 nm against a blank after it had cooled to room temperature. Gallic acid was employed as a standard (20–100µ/gml). IC50 value (mg extract/mL) is the effective concentration at which 50 % of reduction has occurred showing the total antioxidant capacity at an absorbance of 0.5 and the values were obtained by interpolation from linear regression analysis.

In-Vitro Anti-Inflammatory Activities

The *in vitro* anti-inflammatory activities were based on the protocols of Oyedapo and Famurewa[32] and Eshwarappa*et al.*[33] as described below.

Inhibition of Albumin Denaturation

One milliliter of a 1% aqueous solution of the bovine albumin fraction was mixed with one milliliter of the tested extract to create the reaction mixture. After bringing the reaction mixture's pH down to 6.3, it was incubated for 20 minutes at 37°C and then heated for 30 minutes to 51°C. The absorbance of the sample was measured at 660 nm after it had cooled to room temperature. The following formula was used to determine the percentage inhibition of protein denaturation, and the findings were presented as IC50 values (concentration needed for a 50% inhibition):

Percentage inhibition (%) = $([A_0 – A_1]/ A_0) \times 100$

Where A_0 is the absorbance of the control (blank, without extract) and A_1 is the absorbance in the presence of the extract.

Antiproteinase Activity:

A milliliter of the material was added to a reaction mixture that contained 0.06 mg of trypsin and 1 milliliter of 20 mM Tris HCl buffer (pH 7.4). One milliliter of 0.7% (w/v) casein was then added to the mixture after it had been incubated for five minutes at 37°C. After another 20 minutes of incubation, 2 mL of 70% perchloric acid (HClO4) was added to the reaction mixture to halt it. To extract the supernatant, the reaction mixture was centrifuged for 10 minutes at 6,000 rpm and 4°C. At 210 nm, the absorbance of the supernatant was measured. The percentage inhibition of proteinase was calculated using the following equation, and the results were reported as IC_{50} values:

Percentage inhibition (%) = $([A_0 – A_1]/ A_0) \times 100$.

Where A_0 is the absorbance of the control (blank, without extract) and A_1 is the absorbance in the presence of the extract.

Membrane Stabilization Assay

A healthy human volunteer who has not used any non-steroidal anti-inflammatory medications for two weeks provided a blood sample. The blood cells were reconstituted as a 10% suspension in normal saline after being centrifuged for 10 minutes at 3000 rpm and then rinsed three times with normal saline. One milliliter of the sample was combined with one milliliter of a 10% RBC suspension, and the mixture was incubated for thirty minutes at 56°C to conduct the heat-induced hemolysis assay. After cooling, the mixture was centrifuged for five minutes at 2500 rpm to extract the supernatant. The supernatant was then measured the absorbance at 560nm. The percentage inhibition of haemolysis was calculated using the following equation, and the results were reported as IC_{50} values:

Percentage inhibition (%) = $([A_0 – A_1]/ A_0) \times 100$

Where A_0 is the absorbance of the control (blank, without extract) and A_1 is the absorbance in the presence of the extract.

Statistical analysis

All data were subjected to statistical analysis using the statistical package for the social sciences (SPSS Version 22). Values were reported as Mean ± Standard deviation while one-way ANOVA was used to test for differences between treatment groups. The results were considered significant at p-values of less than 0.05, that is, at 95% confidence level ($p<0.05$). Turkey HSD post- Hoc tool was used for basis of statistical comparison.

RESULTS

Percentage yield of *Alchornea cordifolia* dichloromethane leaf extract

The outcome of the percentage yield of *A. cordifolia* dichloromethane leaf extract recorded a yield of 0.92 % which was a brown mass 6.42g.

Mineral composition of *A. cordifolia*leaf extract

The evaluations of some essential mineral composition of *A. cordifolia* dichloromethane leaf extract is presented in table 1.0. Sodium and magnesium were the most prominent macro minerals, while iron and selenium were relatively more abundant of the micro minerals evaluated respectively.

Table 1: Mineral composition of *A. cordifolia*dichloromethane leaf extract

Minerals	mg/kg
Selenium (Se)	1.09±0.00
Zinc (Zn)	0.26±0.01
Iron (Fe)	1.44±0.02
Calcium (Ca)	0.13±0.00
Copper (Cu)	0.55±0.00
Cobalt (Co)	0.42±0.01
Potassium (K)	2.13±0.04
Manganese (Mn)	0.78±0.00
Magnesium (Mg)	10.6±0.08
Sodium (Na)	11.9±0.00

Values are means ± standard deviations of triplicate determinations.

Qualitative phytochemistry of *A. cordifolia*leaf extract

The phytochemical screening of dichloromethane *A. cordifolia*leaf extracts revealed the presence of saponins, cardiac glycosides and steroid as shown in table 2.

Table 2: Qualitative phytochemical screening of *A. cordifolia* leaf extracts

Phytochemicals	Dichloromethane
Phenol	-
Tannins	-
Flavonoids	-
Alkaloid	-
Terpenes	-
Saponins	+
Phlobatannins	-
Thiols	-
Cardiac glycosides	+
Steroids	+

Key: + = Presence, - = Absent

Quantitative phytochemistry of *A. cordifolia*leaf extract

The result of the quantitative phytochemical analysis of *dichloromethane* leaf extract of *A. cordifolia* is presented in table 3. The result revealed that tannin had the highest concentration and the least was alkaloid.

Table 3: Quantitative phytochemical analysis of *A. cordifolia*leaf extract

Phytochemicals	Concentration
Phenol (mg/gGAE D.W)	0.17±0.02
Flavonoids (mg/gCAE D.W)	0.27±0.03
Alkaloid (mg/gATE D.W)	11.7±0.06
Tannin (mg/g TAE D.W)	0.17±0.02

Values are presented as means ± standard deviations of triplicate determinations.

Key: GAE = Gallic acid equivalent, CAE = Catechin equivalent; ATE = Atropine equivalent, TAE = Tannic acid equivalent.

Gas chromatography-mass spectroscopy analysis of *A. cordifolia* dichloromethaneleaf extract

The chromatogram of the GC-MS analysis of *A. cordiofolia*dichloromethane leaf extract is presented in figure 1. The chromatogram shows the presence of different bioactive compounds at different peaks. Also presented in Table 4 are the identified bioactive compounds in the dichloromethane leaf extract of *A. cordifolia* which includes compounds of different classes of Alkanones, esters, fatty acids, alkanals, phenols and aromatic compounds.

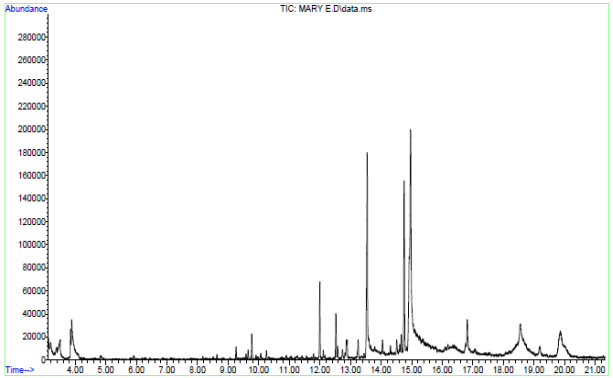

Figure 1a: GC-MS Chromatogram for dichloromethane leaf extract of *A. cordifolia*

Table 4: GC-MS composition of bioactive compounds of *A. cordifolia*dichloromethaneleaf extract

Compounds	RT (min)	Area (%)	Name	Molecular formula	Molecular weight (g/mol)	Biological activities
1	3.510	1.78	2-Hexenoic acid, (E)-	$C_6H_{10}O_2$	114.14	No biological activities reported
2	3.602	0.11	Actinobolin	$C_{13}H_{20}N_3O_6$	300.31	Anti-bacterial[34]
3	3.751	0.09	1-Hexyne, 5-methyl-	C_7H_{12}	96.17	No biological activities reported
4	3.877	4.29	2-Pyrrolidinone, 1-methyl-	C_5H_9NO	99.13	No biological activities reported
5	4.443	0.03	Acetamide, 2-fluoro-	C_2H_4FNO	77.05	No biological activities reported
6	4.649	0.17	Oxirane, 2-butyl-3-methyl-, cis-	$C_7H_{14}O$	114.19	No biological activities reported
7	4.849	0.25	Phenylethyl Alcohol	$C_8H_{10}O$	122.16	No biological activities reported
8	5.244	0.05	Propiolamide	C_3H_3NO	69.06	No biological activities reported
9	5.353	0.08	2-Propenal	C_3H_4O	56.06	No biological activities reported
10	5.519	0.03	Cyclobutanone, 2-methyl-2-oxiranyl	$C_7H_{10}O_2$	126.15	No biological activities reported
11	5.811	0.10	Methanol, (4-amino-1,2,5-oxadiazol-3-yl) (imino)-	$C_3H_5N_3O_2$	115.09	Antibacterial, antiviral and anti-inflammatory[35]
12	5.914	0.17	1-Naphthalenol, 3,4,7,8-tetrahydro	$C_{10}H_{12}O$	148.20	-
13	6.022	0.05	Pyridine, 2,3,4,5-tetrahydro-	C_5H_9N	83.13	No biological activities reported
14	6.183	0.05	Cyclohexanone, 2-(2-propenyl)-	$C_9H_{14}O$	138.20	No biological activities reported
15	6.303	0.14	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	$C_7H_9NO_2$	139.15	No biological activities reported
16	6.434	0.10	Chloroacetic acid, 1-cyclopentylethyl ester	$C_9H_{15}ClO_2$	190.67	No biological activities reported
17	6.606	0.03	Cyclohexene, 4-ethenyl-	C_8H_{12}	108.18	No biological activities reported
18	6.698	0.03	1,6-Heptadiene	C_7H_{12}	96.17	No biological activities reported
19	6.846	0.10	Chloromethyl cyanide	C_2H_2ClN	75.49	No biological activities reported
20	6.995	0.05	1,3,5-Triazine	$C_3H_3N_3$	81.07	Antibacterial, fungicidal, antimalarial, anticancer, antiviral, antimicrobial, anti-inflammatory, antiamoebic, and antitubercular activities[36]
21	7.115	0.14	2,4,6,8-Tetramethyl-1-undecene	$C_{15}H_{30}$	210.39	No biological activities reported
22	7.276	0.04	Ethenamine, N-methylene-	C_3H_5N	55.07	No biological activities reported
23	7.442	0.04	2-Propenamide	C_3H_5NO	71.07	No biological activities reported
24	7.693	0.04	Propiolonitrile	C_3HN	51.04	No biological activities reported
25	7.785	0.12	6-(Dimethylamino) fulvene	$C_8H_{11}N$	121.17	No biological activities reported
26	7.962	0.04	Cyanamide, dimethyl-	$C_3H_6N_2$	70.09	No biological activities reported
27	8.168	0.09	1-Heptyn-3-ol	$C_7H_{12}O$	112.16	No biological activities reported
28	8.294	0.08	1,2-Cyclohexanedimethanol, 3-(acetyloxy)-1,2-dimethyl-, diacetate	$C_{16}H_{26}O_6$	314.37	-
29	8.403	0.05	1-Phenyl-3-methylpenta-1,2,4-trien	$C_{12}H_{12}$	156.22	No biological activities reported
30	8.512	0.13	1H-Pyrrole-2-carboxylic acid, 1-ethenyl-	$C_7H_7NO_2$	137.14	No biological activities reported
31	8.637	0.15	Benzenemethanol,. alpha.,4-dimethyl-	$C_9H_{12}O$	136.19	Antibacterial activities[37]
32	8.861	0.12	(E)-2-Butenylcyclopropane	C_7H_{12}	96.17	No biological activities reported
33	9.061	0.04	2-Methylenecyclohexanol	$C_7H_{12}O$	112.16	No biological activities reported
34	9.261	0.37	s-Triazine, 2,4-diamino-6-(2-furyl)-	$C_7H_7N_5O$	177.16	No biological activities reported
35	9.484	0.05	3-Aminothiophenol, N,N,S-trimethyl	$C_9H_{13}NS$	167.27	No biological activities reported
36	9.656	0.38	4,6-Dimethyl-2-pyrimidone	$C_6H_8N_2O$	124.14	No biological activities reported
37	9.770	0.64	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	$C_{11}H_{16}O_2$	180.24	Analgesic, antidiabetic, antibacterial and antifungal activities[38]
38	9.913	0.16	Oxirane, decyl-	$C_{12}H_{24}O$	184.31	No biological activities reported
39	0.074	0.21	4-Ethyl-5-methylthiazole	C_6H_9NS	127.20	No biological activities reported
40	10.251	0.22	2-Tridecanol	$C_{13}H_{28}O$	200.36	No biological activities reported
41	10.337	0.14	Acetic acid, chloro-, pentyl ester	$C_7H_{13}ClO_2$	164.63	No biological activities reported
42	10.514	0.07	1,5-Pentanediol, 3-methyl-	$C_6H_{14}O_2$	118.17	No biological activities reported
43	10.617	0.03	Acetohydroxamic acid	$C_2H_5NO_2$	75.06	Urease inhibitor[39]
44	10.755	0.12	1,9-Decadiyne	$C_{10}H_{14}$	134.21	No biological activities reported
45	10.903	0.12	Cyclohexanepropanol-	$C_9H_{18}O$	142.23	No biological activities reported
46	11.064	0.13	Pent-2-ynal	C_5H_6O	82.10	No biological activities reported

47	11.270	0.22	2-Octyn-1-ol	$C_8H_{14}O$	126.19	No biological activities reported
48	11.418	0.12	Tridecanal	$C_{13}H_{26}O$	198.34	No biological activities reported
49	11.561	0.09	9-Octadecene, (E)-	$C_{18}H_{36}$	252.47	Antifungal, antioxidant, anticarcinogenic and antimicrobial activity[40]
50	11.682	0.04	Pent-3-enylamine	$C_5H_{11}N$	85.15	No biological activities reported
51	11.796	0.23	Undec-10-ynoic acid	$C_{11}H_{18}O_2$	182.25	No biological activities reported
52	11.893	0.04	Aziridine, 1-(2-methyl-1-propenyl)	$C_6H_{11}N$	97.15	No biological activities reported
53	11.996	1.65	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7 a-tetrahydrobenzofuran-2(4H)-one	$C_{11}H_{16}O_3$	196.24	Antidiabetics activities[41]
54	12.111	0.34	2-Decene, 7-methyl-, (Z)-	$C_{11}H_{22}$	154.29	No biological activities reported
55	12.334	0.17	Octenyl tiglate, 5Z-	$C_{13}H_{22}O_2$	210.32	No biological activities reported
56	12.437	0.13	1,9-Decadiyne	$C_{10}H_{14}$	134.21	No biological activities reported
57	12.523	1.42	Neophytadiene	$C_{20}H_{38}$	278.51	Anti-inflammatory agent and antimicrobial activities[42].
58	12.649	0.05	Ethanimidamide, N-cyano-	$C_3H_5N_3$	83.09	No biological activities reported
59	12.734	0.33	6-Nonen-1-ol, acetate, (Z)-	$C_{11}H_{20}O_2$	184.27	No biological activities reported
60	12.895	1.20	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.53	Precursor of synthetic forms of vitamin E and vitamin K1[43]
61	13.083	0.12	3(2H)-Thiophenone, dihydro-, oxime, 1,1-dioxide	-	-	-
62	13.249	0.75	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	Antibacterial activities [44].
63	13.364	0.11	3-Tetradecyn-1-ol	$C_{14}H_{26}O$	210.35	No biological activities reported
64	13.444	0.16	Hexadecane, 1-chloro-	$C_{16}H_{33}Cl$	260.88	No biological activities reported
65	13.547	8.41	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	Antifungal, Antioxidant, HypocholesterolemiaHemolytic 5-alphareductase Inhibitor, Potent Antimicrobial Agent, Antimalarial and Antifungal[45]
66	13.793	1.96	2,4-Pentadien-1-ol, 3-propyl-, (2Z)-	$C_8H_{14}O$	126.20	No biological activities reported
67	14.050	1.33	Estra-1,3,5(10)-trien-17.beta.-ol	$C_{18}H_{24}O$	256.38	precursor to the hormone estrogen[46]
68	14.308	0.96	1-(3-Benzyl-2-thioureido)-1-deoxy-.beta.-d-glucopyranose 2,3,4,6-tet raacetate	-	-	-
69	14.422	0.30	1-Dodecanol, 3,7,11-trimethyl-	$C_{15}H_{32}O$	228.41	No biological activities reported
70	14.508	0.97	17-Pentatriacontene	$C_{35}H_{70}$	490.93	Anti-inflammatory, anticancer, antibacterial and antiarthritic activities[47]
71	14.663	1.55	1,3-Cyclooctadiene	C_8H_{12}	108.18	No biological activities reported
72	14.754	4.33	Phytol	$C_{20}H_{40}O$	296.5	Anti-microbial, Anti-inflammatory, antinociceptive activities[48, 49]
73	14.966	15.84	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	$C_{18}H_{32}O$	264.44	Antioxidant and antibacterial activities [50]
74	15.058	6.76	1-(2-Propenyl) cyclopentene	C_8H_{12}	108.18	No biological activities reported
75	15.384	3.82	Pentanoic acid, 10-undecenyl ester	$C_{16}H_{30}O_2$	254.41	No biological activities reported
76	15.681	0.62	2-Pentadecyn-1-ol	$C_{15}H_{28}O$	228.4	Antioxidant, antimicrobial, and anti-inflammatory activities[51]
77	15.796	1.57	Pentafluoropropionic acid, nonyl ester	$C_{12}H_{19}F_5O_2$	290.27	Antimicrobial activities [52]
78	15.893	0.21	6-Dodecyne	$C_{12}H_{22}$	166.30	No biological activities reported
79	16.191	3.32	8-Oxabicyclo [5.1.0] octane	$C_7H_{12}O$	112.16	No biological activities reported
80	16.820	2.94	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281.47	Anti-inflammatory and antibacterial activity[53]
81	17.072	0.92	1-Octadecyne	1-Octadecyne	250.46	No biological activities reported
82	17.192	0.86	3-Decyn-2-ol	$C_{10}H_{18}O$	154.24	Antimicrobial activities[54]
83	17.398	0.57	Alantolactone, 4, alpha.,4A,alpha-- epoxy-	$C_{15}H_{20}O_3$	248.32	No biological activities reported
84	17.552	0.75	11-(2-Cyclopentenyl) undecanoic acid, ethyl ester	$C_{18}H_{32}O_2$	280.44	Antimicrobial activities[55]
85	17.873	0.75	Oxirane, (7-octenyl)-	$C_{10}H_{18}O$	154.249	No biological activities reported
86	18.073	0.77	Benzofurazan, 4,5,6,7-tetrahydro-	$C_6H_8N_2O$	124.14	No biological activities reported
87	18.559	8.64	Tetradecanoic acid, dodecyl ester	$C_{26}H_{52}O_2$	396.68	No biological activities reported
88	18.828	0.98	1,2,3,4,4a,5,6,8a-Octahydro-naphthalene	$C_{10}H_{16}$	136.23	No biological activities reported

89	18.983	0.46	7-Oxabicyclo [4.1.0] heptane, 1-methyl-4-(2-methyloxiranyl)-	$C_{10}H_{16}O_2$	168.23	No biological activities reported
90	19.200	1.20	Phthalic acid, monodecyl ester	$C_{18}H_{26}O_4$	306.39	No biological activities reported
91	19.515	0.23	Oxalic acid, allyl hexadecyl ester	$C_{21}H_{38}O_4$	354.5	Causes Central NS paralysis [56]
92	19.681	0.05	2-Nonen-1-ol	$C_9H_{18}O$	142.23	Antifungal activity, antimicrobial activity, sex pheromone and antibacterial quorum sensing [57]
93	19.864	4.86	Squalene	$C_{30}H_{50}$	410.7	Anti-oxidant and cardio protective activities[58]
94	20.413	0.10	1,8-Cyclopentadecadiyne	$C_{15}H_{22}$	202.33	No biological activities reported
95	20.522	0.03	Piperidine, 3-(bromomethyl)-	$C_6H_{12}BrN$	178.07	No biological activities reported
96	20.631	0.05	Cyclopropanemethanol, 2-isopropylidene-. alpha. -methyl-	$C_8H_{14}O$	126.0	No biological activities reported
97	20.722	0.04	Acetonitrile, 2,2'-iminobis-	$C_4H_5N_3$	95.10	No biological activities reported
98	20.837	0.17	4-Hexenoic acid, 6-hydroxy-4-methyl-, methyl ester, (E)-	$C_8H_{14}O_3$	158.19	No biological activities reported

In-vitro free radical scavenging activities of *A. cordifolia*leaf extract

The result in table 5 presents the *in vitro* Totalantioxidant capacity of *A. cordifolia* dichloromethane extract. The result is indicative that there was a concentration dependent increase on the total antioxidant capacity of the extract. However, in comparison with the gallic acid standard, the standard performed better in a concentration dependent manner except at 0.6 mg/ml concentration where the extract had a better performance compared to the standard.

Table 5: Total antioxidants capacity of *A. cordifolia*leaf extract

Conc. (mg/ml)	Extract 695nm	Gallic acid 695nm
0.2	0.23±0.01	0.21±0.00*
0.4	0.47±0.03	0.44±0.01*
0.6	0.68±0.01	0.57±0.01#
0.8	0.72±0.01	0.74±0.01*
1.0	0.86±0.01	0.94±0.03*

Values are means ± standard deviations of triplicate determinations. Values not sharing common superscript on the same column differ significantly ($p<0.05$).

Table 6 presents the ferric reducing power, nitric oxide and 1, 1-diphenyl-2-picryl hydrazyl scavenging activities in comparison with different standards of Ascorbic acid and catechin. It was observed that ferric reducing power increased in a concentration dependent manner. However, the standard ascorbic acid had a better reducing power at all concentration. Similarly, the inhibition of NO by the dichloromethane extract of *A. cordifolia* also increased in a concentration dependent manner with the standard catechin having a better inhibition at all concentration. DPPH inhibition followed a similar trend as in NO, except for the standard ascorbic acid that had a lower % inhibition at a concentration of 0.04 mg/ml. Put together, the Standard anti-oxidants ascorbic acid and catechin had a better *in vitro* antioxidant property relative to the dichloromethane extract of *A. cordifollia* as depicted by lower IC50 values of the standards as presented in table 7

Table 6: Ferric reducing, 1,1-diphenyl-2-picryl hydrazyl and nitric oxide scavenging properties of *A. cordifolia*leaf extract

Conc. (mg/ml)	Extract	Ascorbic acid	Extract	Catechin	Extract	Ascorbic acid
	RP 700 nm		NO (% Inhibition)		DPPH Inhibition)	(%
0.02	0.04±0.00	0.17±0.01*	4.70±0.01	17.7±0.85*	27.2±0.06	15.5±0.66#
0.04	0.05±0.00	0.31±0.00*	11.1±0.58	34.2±0.89*	27.7±0.10*	24.6±0.66#
0.06	0.07±0.00	0.54±0.01*	16.2±0.51	41.9±0.89*	28.4±0.38*	37.7±0.31*
0.08	0.09±0.00	0.72±0.00*	27.9±1.18	50.3±2.54*	28.6±0.08*	42.7±0.70*
0.10	0.10±0.01	0.89±0.00*	28.2±1.21	53.2±1.85*	29.2±0.20*	55.0±0.35*

Values are means ± standard deviations of triplicate determinations. Values followed by * indicates that the standard had a better antioxidant activity when compared to the plant, while values followed by # indicates that the plant extract had a better antioxidant activity.

Table 7:IC_{50} values of the *in-vitro* free radical scavenging activities of *A. cordifolia*

Parameters	Extract	Standard
	mg/ml	mg/ml
TOAC	0.48±0.00	0.05±0.00*
Reducing power	0.62±0.04	0.06 ±0.00*
Nitric oxide	0.17±0.01	0.08±0.00*
DPPH	0.95±0.08	0.09±0.00*

Values are means ± standard deviations of triplicate determinations. Values not sharing common superscript on the same row differ significantly ($p<0.05$). TOAC = Gallic acid; Reducing power & DPPH = Ascorbic acid; Nitric oxide = catechin.

In-vitro anti-inflammatory activities of *A. cordifolia*leaf extract

Table 9 presents the *in vitro* anti-inflammatory activities of the dichloromethane extract of *A. cordifolia* in comparison to standard anti-inflammatory drug Aspirin. The result showed that there was a dose dependent increase in the percentage inhibition across albumin denaturation, anti-proteinase and membrane stabilization assays. It also showed that at all concentrations, that Aspirin performed better for albumin denaturation while the plant extract performed better in the ani-proteinase inhibition assay. On the other hand, there was

no significant difference between the extract and the standard drug except at 0.20 mg/ml concentration. Put together, it was also observed that the plant extract had a better ani-inflammatory activity compared to the standard drug aspirin based on the IC_{50} as presented in table 9

Table 8: Anti-inflammatory properties of *A. cordifolia* leaf extract

Conc. (mg/ml)	Extract	Aspirin	Extract	Aspirin	Extract	Aspirin
	% Inhibition					
	Albumin denaturation		Anti-proteinase		Membrane stabilization	
0.20	9.69±1.43	33.4±0.63*	13.1±1.11	5.70±1.04#	11.5±1.12	14.3±1.63*
0.40	17.7±0.78	39.7±0.94*	22.9±0.80	13.4±0.80#	26.0±1.19	26.3±1.19
0.60	24.3±0.79	45.9±0.94*	29.2±1.02	23.2±0.66#	35.2±1.57	35.0±1.57
0.80	30.0± 1.13	54.8±1.10*	39.7±1.01	32.6±0.84#	41.2±1.63	41.4±1.63
1.00	35.5±1.09	61.8±0.36*	48.4±1.92	42.1±0.95#	48.7±1.96	48.5±1.96

Values are means ± standard deviations of triplicate determinations. Values with * indicates that the standard drug had a better anti-inflammatory activity in relation to the extract while values followed by # is indicative that the plant extract was better in relation to the standard drug.

Table 9: IC_{50} values of *in-vitro* anti-inflammatory activities of *A. cordifolia*

Parameters	Extract (mg/ml)	Aspirin (mg/ml)
Albumin Denaturation	1.43±0.05	0.68±0.02#
Anti-proteinase	1.17±0.25*	1.20±0.02
Membrane Stabilization	0.98±0.05*	1.00±0.02

Values are means ± standard deviations of triplicate determinations. Values with * indicates that the plant extract had a lower IC_{50} while values followed by # is indicative that the standard drug aspirin was lower respectively for the different assays.

DISCUSSION

The selection of the best solvent for phytochemical extraction is crucial due to the diverse chemical structures and polarities of phytochemicals, which can affect their solubility in the chosen solvent.[59,60] Plants contain a variety of phytochemicals with different solubility levels, influenced by their molecular charges and polarity.[61] In this study, the phytochemical, antioxidant, and anti-inflammatory properties of *A. cordifolia* leaves were analysed using dichloromethane extract.The percentage yield of *A. cordifolia* dichloromethane leaf extract was 0.92%, resulting in a brown mass of 6.42g. This yield is significantly lower than the 8.34% obtained by Effo*et al.*[62] from an aqueous extract of *A. cordifolia* leaves. The differences in yield may be related to the fact solvent dichloromethane extracts non-polar compounds which may be volatile in nature as compared to polar compounds extracted by aqueous.It has been previously reportedthat higher contents of phenolic compounds and antioxidant activity in plant materials are obtained from aqueous organic solvents rather than absolute solvents.[63].

The essential mineral composition of *A. cordifolia* dichloromethane leaf extract reveals significant findings, as shown in Table 1.0. Sodium (Na) and magnesium (Mg) are the most prominent macro-minerals, with concentrations of 11.9 mg/kg and 10.6 mg/kg, respectively. The high concentration of sodium indicates that *A. cordifolia* leaves could be a good source of this mineral, which is vital for maintaining fluid balance, nerve function, and muscle contractions. Similarly, magnesium is essential for biochemical processes, including muscle and nerve function, blood glucose control, and bone health.Among microminerals, iron (Fe) and selenium (Se) are relatively abundant, with concentrations of 1.44 mg/kg and 1.09 mg/kg, respectively. Iron is crucial for oxygen transport in the blood and various enzymatic reactions, while selenium is essential for thyroid function and the immune system.[64] Other minerals present in lower concentrations include potassium (K) at 2.13 mg/kg, important for cardiovascular health,[65] and zinc (Zn) at 0.26 mg/kg, essential for immune function and wound healing.[66]

Copper (Cu) and cobalt (Co) are found in moderate amounts, playing roles in red blood cell formation and enzymatic functions.[67] Manganese (Mn) and calcium (Ca) are also present, important for bone formation and health.[68] When compared to other studies, the mineral composition of *A. cordifolia* differs in several ways. For instance, Effo*et al*.[62] found higher concentrations of potassium and calcium in aqueous extracts of *A.cordifolia* leaves. In contrast, Alikwe and Owen[69]highlighted similar levels of magnesium and sodium in *A. cordifolia*, but reported lower iron content. The differences in mineral concentrations could be attributed to variations in extraction methods such as use of polar solvents like methanol, ethanol and water,use of whole plant material as against crude extract, or environmental factors affecting mineral content.The mineral composition of *A. cordifolia* dichloromethane leaf extract highlights its rich nutritional content, suggesting it could serve as a valuable dietary supplement.[70]

The qualitative phytochemical screening of dichloromethane *A. cordifolia* leaf extracts reveals the presence of saponins, cardiac glycosides, and steroids, as shown in Table 2. The absence of phenols, tannins, flavonoids, alkaloids, terpenes, phlobatannins, and thiols in the extract provides insights into the specific phytochemical profile of *A. cordifolia*.Saponins are glycosides known for their surfactant properties, which can affect the permeability of cell membranes.[71] They have been documented to possess various biological activities, including anti-inflammatory, immunomodulatory, and anti-cancer properties.[72] The presence of saponins in *A. cordifolia* suggests potential therapeutic uses and bioactivity related to these properties. Cardiac glycosides are compounds that influence cardiac muscle contraction and are commonly used in the treatment of heart failure and arrhythmias.[73] They act by inhibiting the Na+/K+ ATPase enzyme, leading to increased intracellular calcium levels and improved cardiac output.[74] The presence of cardiac glycosides in *A. cordifolia* indicates that the plant may have cardiotonic effects, which could be of interest for developing heart-related therapies. Steroids are a diverse group of compounds with various physiological effects, including anti-inflammatory, immunosuppressive, and hormonal activities.[75] The presence of steroids in *A. cordifolia* may contribute to its therapeutic properties and could be relevant for treating conditions related to inflammation or hormonal imbalances.[76] The absence of phenols, tannins, flavonoids, alkaloids, terpenes, phlobatannins, and thiols suggests that *A. cordifolia* dichloromethane extract may not possess the typical antioxidant, antimicrobial, or antidiabetic properties associated with these compounds.[77] This selective phytochemical profile highlights the unique bioactive potential of *A. cordifolia*.The quantitative phytochemical analysis of *A. cordifolia* dichloromethane leaf extract is summarized in Table 3. The analysis revealed that tannins were present in the highest concentration, while alkaloids were the least abundant.Phenolic compounds in *A. cordifolia* were found at 0.17 mg/g (Gallic Acid Equivalent, GAE). Phenols are known for their antioxidant properties, which can help protect cells from oxidative damage and play a role in reducing the risk of chronic diseases.[61] The relatively low concentration of phenols in this extract may suggest limited antioxidant capacity compared to other phytochemicals.

Flavonoids were quantified at 0.27 mg/g (Catechin Equivalent, CAE). Flavonoids are a diverse group of polyphenolic compounds with strong antioxidant activities and potential anti-inflammatory, anti-viral, and anti-cancer properties.[78] The moderate concentration indicates that *A. cordifolia* may offer some degree of these health benefits. The concentration of alkaloids was the highest at 11.7 mg/g (Atropin Equivalent, ATE).

Alkaloids are known for their wide range of biological activities, including analgesic, antimalarial, and antimicrobial effects.[79] Their high concentration suggests that *A. cordifolia* might have significant pharmacological potential.Tannins were present at 0.17 mg/g (Tannic Acid Equivalent, TAE), the same concentration as phenols. Tannins are polyphenolic compounds known for their astringent properties and potential to reduce inflammation and inhibit microbial growth.[80] Their high concentration in this extract underscores their potential role in the plant's therapeutic effects.The concentration of tannins in *A. cordifolia* is relatively high compared to other plants, which often show varied levels of tannins depending on the species and extraction method.[81] Alkaloids, while abundant in this extract, are generally found in varying concentrations across different plants and can be influenced by the extraction process and plant part used.[82]

The GC-MS analysis of *A. cordifolia* dichloromethane leaf extract reveals a complex profile of bioactive compounds, as depicted in Figure 1. The chromatogram illustrates the presence of several classes of compounds, indicating the diverse chemical nature of the extract. Alkanones are ketones with a long aliphatic chain, which often exhibit biological activities such as antimicrobial and antioxidant properties.[83] Their presence in the extract suggests potential health benefits related to these properties.Esters are known for their pleasant odours and flavours, and they also possess various biological activities, including antimicrobial and anti-inflammatory effects.[84] Their detection in the extract could contribute to its therapeutic potential and aroma.Fatty acids are essential components of lipids in plants and are involved in various physiological functions, including anti-inflammatory and antioxidative effects.[85] The presence of fatty acids in *A. cordifolia* may suggest potential benefits related to cardiovascular health and cellular protection.

Alkanals, or aldehydes with a long aliphatic chain, are known for their antibacterial and antifungal properties.[86] Their presence indicates that the extract might have applications in antimicrobial therapies. Phenolic compounds are well-known for their antioxidant properties, which can help in reducing oxidative stress and inflammation.[87] The detection of phenols highlights the extract's potential antioxidant capacity. Aromatic compounds often have diverse biological activities, including anti-inflammatory, antimicrobial, and anticancer effects.[88] Their presence in the extract may contribute to the overall bioactivity of *A. cordifolia*.The GC-MS analysis of *A. cordifolia* dichloromethane leaf extract reveals a rich profile of bioactive compounds with various potential therapeutic applications. The diverse classes of compounds detected—alkanes, esters, fatty acids, alkanals, phenols, and aromatic compounds-suggest that the extract may have multiple beneficial properties, including antioxidant, antimicrobial, and anti-inflammatory effects. This comprehensive chemical profile supports the potential use of *A. cordifolia* in traditional and modern medicine.

The in-vitro antioxidant activities of dichloromethane leaf extract of *A. cordifolia* were assessed using several assays, including total antioxidant capacity, ferric reducing power, nitric oxide scavenging, and 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging. The results are summarized in Tables 5, 6, and 7. Table 5 shows that *A. cordifolia* leaf extract exhibits a concentration-dependent increase in total antioxidant capacity, with values reaching 0.86±0.01 at 1.0 mg/ml. This indicates that the extract's ability to neutralize free radicals increases with higher concentrations. The extract's antioxidant activity is lower compared to gallic acid, a well-known antioxidant, which demonstrates higher activity across all concentrations ($p<0.05$).

The results align with a previous study showing that plant extracts can have significant antioxidant potential, though gallic acid remains a more potent standard.[89] Table 6 indicates that the ferric reducing power of *A. cordifolia* leaf extract increases with concentration, showing a maximum value of 0.10±0.01 at 0.10 mg/ml. The extract's reducing power is lower compared to ascorbic acid, a standard antioxidant, which demonstrates stronger reducing capability ($p<0.05$). This suggests that while the extract has reducing properties, it is less effective compared to ascorbic acid.[90].

The nitric oxide scavenging activity of *A. cordifolia* extract, as shown in Table 6, demonstrates a concentration-dependent increase, with a maximum inhibition of 29.2% at 0.10 mg/ml. The extract's activity is lower compared to catechin, a potent nitric oxide scavenger, which achieves up to 55.0% inhibition ($p<0.05$). This indicates that while the extract can inhibit nitric oxide, its efficacy is less than that of catechin, which is known for its strong antioxidant properties.[91]

The DPPH radical scavenging activity of *A. cordifolia* leaf extract increases with concentration, with an IC_{50} value of 0.95±0.08 mg/ml (Table 7). This value is significantly higher compared to ascorbic acid, which has an IC_{50} of 0.09±0.00 mg/ml. The lower IC50 value of ascorbic acid indicates that it is a more effective DPPH radical scavenger compared to the *A. cordifolia* extract.[92] The in-vitro antioxidant assays suggest that *A. cordifolia* dichloromethane leaf extract has notable antioxidant properties, but these are generally less potent compared to standard antioxidants such as gallic acid, ascorbic acid, and catechin. The concentration-dependent increases in antioxidant activity observed in the extract indicate its potential to neutralize free radicals, though its effectiveness is lower compared to the standards used in the assays.

The anti-inflammatory properties of *A. cordifolia* dichloromethane leaf extract was evaluated using assays for albumin denaturation, membrane stabilization, and anti-proteinase activity. The results are summarized in Tables 8 and 9. Table 8 demonstrates that *A. cordifolia* leaf extract inhibits albumin denaturation in a concentration-dependent manner, reaching 35.5±1.09% inhibition at 1.0 mg/ml. Albumin denaturation is a model for screening anti-inflammatory agents, as it mimics the protein denaturation seen in inflammatory conditions.[93] The extract shows effective inhibition, though less potent compared to aspirin, which achieves 61.8±0.36% inhibition. The IC_{50} value for the extract is 1.43±0.05 mg/ml, significantly higher than aspirin's 0.68±0.02 mg/ml (Table 9). This suggests that while *A. cordifolia* has anti-denaturation activity, its efficacy is lower compared to the standard anti-inflammatory drug implying that the solvent used may not have been potent enough to extract all compounds that confers these anti-inflammatory properties on *A. cordifolia.*[19, 94] Membrane stabilization is another key indicator of anti-inflammatory activity. Table 8 shows that the extract stabilizes membranes with a maximum inhibition of 48.7±1.96% at 1.0 mg/ml. This activity is indicative of the extract's potential to prevent cellular damage associated with inflammation.[95] Although the extract shows considerable membrane stabilization, it is comparable to aspirin, which achieves 48.5±1.96% inhibition. The IC_{50} value of the extract is 0.98±0.05 mg/ml, slightly lower than aspirin's 1.00±0.02 mg/ml (Table 9), indicating that while the extract has comparable activity to aspirin, it is marginally more effective. Anti-proteinase activity reflects the extract's ability to inhibit proteolytic enzymes involved in inflammation. The extract demonstrates anti-proteinase activity with a maximum inhibition of 48.4±1.92% at 1.0 mg/ml (Table 8). This suggests that *A. cordifolia*like other plant extracts can inhibit the enzymatic processes that contribute to inflammatory responses.[95]

The IC_{50} value of 1.17±0.25 mg/ml for the extract (Table 9) is comparable to aspirin's 1.20±0.02 mg/ml, indicating similar efficacy in this aspect. The in-vitro anti-inflammatory activities of *A. cordifolia* dichloromethane leaf extract indicate significant anti-inflammatory potential, though it is generally at a comparable level of potency with the standard anti-inflammatory drug aspirin based on their IC_{50}, the effective inhibition shown in albumin denaturation, membrane stabilization, and anti-proteinase activitysuggests its potential as a therapeutic agent for inflammation-related conditions. However, further optimization or combination with other compounds may enhance its efficacy.

Conclusion

Findings from this study suggests that the dichloromethane is not the best solvent for extraction of bioactive components of *A. cordifolia* leaves in relation to previous reports. Likewise, it reports that aside its poor yield, it contains a select number of macrominerals, bioactive compounds such as saponins, cardiac glycosides, and steroids while standard antioxidants and anti-inflammatory drug ascorbic acid, catechin and gallic acid performed better in the in vitro anti-oxidant assays while it remained comparable with aspirin for the *in vitro* anti-inflammatory assay.

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