In vitro anthelminthic, antimicrobial and antioxidant activities and FTIR analysis of extracts of Alchornea cordifolia leaves

Article · January 2019

CITATIONS
4

READS
215

5 authors, including:

Clement Osei Akoto
Kwame Nkrumah University Of Science and Technology
10 PUBLICATIONS **78** CITATIONS
SEE PROFILE

Akwasi Acheampong
Kwame Nkrumah University Of Science and Technology
29 PUBLICATIONS **101** CITATIONS
SEE PROFILE

Yaw Duah Boakye
Kwame Nkrumah University Of Science and Technology
57 PUBLICATIONS **316** CITATIONS
SEE PROFILE

Michael Okine
1 PUBLICATION **4** CITATIONS
SEE PROFILE

Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(4): 2432-2442
Received: 07-05-2019
Accepted: 09-06-2019

Clement Osei Akoto
Department of Chemistry, Faculty of Physical and Computational Sciences, College of Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Akwasi Acheampong
Department of Chemistry, Faculty of Physical and Computational Sciences, College of Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Yaw Duah Boakye
Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Desmond Akwata
Department of Chemistry, Faculty of Physical and Computational Sciences, College of Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Michael Okine
Department of Chemistry, Faculty of Physical and Computational Sciences, College of Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Correspondence
Clement Osei Akoto
Department of Chemistry, Faculty of Physical and Computational Sciences, College of Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

In vitro anthelminthic, antimicrobial and antioxidant activities and FTIR analysis of extracts of *Alchornea cordifolia* leaves

Clement Osei Akoto, Akwasi Acheampong, Yaw Duah Boakye, Desmond Akwata and Michael Okine

Abstract
Alchornea cordifolia has ethnomedicinal use in the treatment of wounds, gonorrhea, conjunctivitis, diarrhea, urinary disorder and gastrointestinal disorder. The main focus of this study was to verify the biological activities (anthelmintic, antimicrobial and antioxidant) and isolate some of the components in the chloroform, methanol, and petroleum ether leaf extracts. Phytochemical screening, antimicrobial (agar and broth dilution method), anthelminthic and antioxidant [total antioxidant capacity (TAC), DPPH and H_2O_2 scavenging] assays were carried out on extracts. Thin layer and column chromatography were employed in the isolation and purification of the components of the petroleum ether extract. The phytochemical investigation revealed the presence of secondary metabolites such as terpenoids, flavonoids, steroids, saponins, carotenoids, phenols and tannins. All three extracts showed antimicrobial activity against test organisms with MICs ranging from 1.56 – 25 mg/mL. The IC_{50} values for methanol, chloroform and petroleum ether extracts in the DPPH and H_2O_2 assays were 35.22, 94.77 and 93.02 ppm, and 272.0, 626.5 and 898.4 ppm, respectively. The TAC (gAAE/100 g) for chloroform, petroleum ether and methanol extracts were 25.85, 40.0 and 40.08 g, respectively. The anthelminthic activity of the extracts at test concentrations was observed to be significantly (P<0.001) higher compared to albendazole-treated helminthes. By means of thin layer and column chromatography two spots have been isolated and purified. FTIR analysis indicated the presence of various functional groups in the purified fractions that is in agreement with findings of phytochemical screening. The findings of this study suggest that *Alchornea cordifolia* leaves could be exploited as potential therapeutic candidate for the treatment of helminthic and bacterial infections and oxidative-stress diseases.

Keywords: *Alchornea cordifolia*, antimicrobial, antioxidant, anthelminthic, phytochemical

1. Introduction
Medicinal plants are presently in considerable significance view due to their extraordinary attributes as a large source of therapeutic phytochemicals that may lead to the advancement of novel drugs [1]. Medicinal plants have additionally led to the isolation of novel bioactive compounds, which in turn serve as lead compounds for the synthesis of patentable entities with improved activity and reduced toxicity [2]. Many plants have been identified to exhibit antimicrobial, antioxidant and anti-inflammatory activities [3-4]. A considerable number of currently used anthelminthic, anti-inflammatory, antioxidant, antitumor, anti-infectious agents are molecules identified and isolated from plants or their synthetic or semisynthetic derivatives [5].

Alchornea cordifolia of family name Euphorbiaceae, is an essential medicinal plants in traditional medicine which is prevalently used in Africa. *A. cordifolia* is known as Christmas bush which originated from Senegal and is now found in Kenya, Tanzania, West Africa and throughout Central Africa to Angola [6]. It comprises of sixty species, among which only six species occur in Tropical Africa. The leaves, root and stem bark contains terpenoids, steroids, glycosides, flavonoids, tannins, saponins, carbohydrate and alkaloids [7].

The root and stem bark are used in the treatment of jaundice and the powdered leaves of *A. cordifolia* are utilized to treat diarrhea and wounds [8]. The plant is broadly used in Ghana and West Africa for the treatment of conditions like headaches, colds, and control of spontaneous abortion [9]. A slurry of the fruit is administered for the cure of asthma and cough. The treatment of urinary and gastrointestinal disorder forms part of its traditional usage [10]. The leaves and root bark are externally applied to treat Leprosy and as an antidote to snake venom. The sap of the fruit is applied to cure eye problems and skin diseases. A decoction of leafy twigs is applied to remedy fever, malaria and rheumatic pains [11].

A cold infusion of the dried and crushed leaves acts as a diuretic. Leaf and root decoctions are generally used as a mouth wash to treat ulcers of the mouth and toothache [6].

Helminth infection is one of the most common diseases in the world, especially in the tropical regions. Recent estimates of the World Health Organization (2019) suggest that about 1.5 billion people suffer helminthiasis [12]. This infection is most prevalent in tropical and subtropical areas, with the greatest numbers occurring in sub-Saharan Africa, the Americas, China and East Asia and pose a great threat to both human and animal health. Parasitic worm infection may result in many health disorders such as anaemia, malnutrition, diarrhea, eosinophilia and pneumonia as well as organ damage and death in severe cases. Moreover, helminthic drugs resistance is now a global challenge for the control of gastro intestinal nematodes of ruminants and is of great concern for the control of nematode infections in animals. Despite this global challenge, none of the available anthelmintics for humans, which were mostly adopted from veterinary medicine, is optimal for human use [13] and human helminth infections have been under served by drug discovery efforts [14-15]. New strategies and research into screening sources of natural products for novel anthelmintic agents and drug discovery programs are needed to change this situation.

Free radicals, reactive oxygen and nitrogen species (RONS) are produced from various biochemical reaction such as oxidative stress and cell metabolism [16]. The production of these free radicals such as superoxide, hydroxyl, and peroxide radicals, peroxynitrite and nitric oxide is the major cause of several age-related conditions (ie, cardiovascular diseases [CVDs], chronic obstructive pulmonary disease, chronic kidney disease, neurodegenerative diseases, and cancer), including sarcopenia and frailty [17]. Antioxidants ability to scavenge and inhibit RONS, results in protection from oxidative harm and are accordingly viewed as essential therapeutic and prophylactic agents against the development of diseases [18]. However, some synthetic variants may pose a threat to humans due to their associated adverse effects on living cells. Thus, the interest in utilizing and screening sources for novel natural antioxidants from natural products is an urgent need.

Treatment of infectious diseases has become a challenge due to the problem of multi-drug resistance. Microorganisms of various species have developed resistance to antimicrobial agents due to the indiscriminate use of the antimicrobial agents commonly used in their treatment [19]. The rapid emergence of multiple drug-resistant strain of microbes to the current antimicrobial agent has generated an urgent need for developing new antibiotics from medicinal plants. The risk of adverse effects encountered with the use of some of the current antibiotics, propel people to seek other alternatives. It is therefore highly desirable to explore plants for new antimicrobial agents [20]. Recognition of traditional medicine as an alternate form of health care has reopened the research domain for the biological activities of medicinal plants[11]. Consequently, the systemic studies of medicinal plants, especially studies involving antimicrobial and antioxidant are very crucial in order to find active compounds and their use as a medicine for curing various diseases [21]. For this reason, screening sources of natural products for novel antimicrobial agents for safer compounds with fewer side effects are needed. Most research studies conducted on the pharmacological potential of *A. cordifolia* are mainly focused on crude extracts of the leaves, fruits, roots and stem-bark. Nevertheless, it is also important to identify the bioactive compounds responsible for each one of the ascribed bioactivities. At the time of carrying out this research, mostly ethnobotanical survey [22] and preliminary report had been carried out on the plant [23] concerning its anthelmintic activity.

The aim of this study was to examine the efficacy of *A. cordifolia* chloroform, methanol, and petroleum ether extracts as an anthelminthic, antimicrobial and antioxidant using *in vitro* assays. Additionally, to isolate, purify and identify the presence of the phytochemicals in the leaves extract eliciting pharmacological activities using column chromatographic purification and FTIR analysis.

2. Materials and Methods

2.1. Sample collection and identification

The fresh matured leaves of *A. cordifolia* were collected in the month of October, 2018 at Mpraeso in the Kwawu South District in the Eastern region of Ghana with the help of a local herbalist. They were taxonomically identified and authenticated at the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi by Mr. Clifford Osafo Asare, with the voucher specimen number (KNUST/HMI/2011/L047) deposited in the herbarium for reference purposes.

2.2. Chemicals and reagents

All chemicals were purchased from Sigma Aldrich Co. Ltd, Irvine, U.K., except the standard drugs. The organic solvents were of analytical grade and procured from BDH Laboratory Supplies (England).

2.3. Extraction of plant material

The leaves of *A. cordifolia* were thoroughly washed first under running water and then distilled water. The leaves were air dried under shade for two weeks, pulverized into coarse powder, and stored in a desiccator until analysis.

2.3.1. Preparation of Extracts

A mass of 1 kg of the powdered sample of *A. cordifolia* was soaked separately in 5 dm^3 of petroleum ether, 2.5 dm^3 of chloroform and 2.5 dm^3 of methanol and extracted using the soxhlet apparatus. The extracts were condensed and evaporated to dryness using the rotary evaporator at 50 ^oC (BUCHI Rota vapor R-114). The extracts were dried and the percentage yield of extracts with respect to powdered plant material determined. The extracts were then stored at 4 ^oC in a refrigerator.

2.4. Phytochemical screening of extracts

The pulverized sample and the crude extracts obtained were screened to assess the presence of phytoconstituents using the methods described by Trease and Evans (2009) [24].

2.5. Evaluation of Anthelminthic Property

Eudrilus eugeniae (Earthworms) were collected from a water logged area behind the Department of Theoretical and Applied Biology within KNUST, Kumasi – Ghana (latitude 6º35 N-6º40 N and longitude 1º30 W-1º35 W). The worm type was authenticated at the Zoology Unit, Department of Theoretical and Applied Biology, KNUST by Mr. Lawrence Yeboah.

2.5.1. *In vitro* Anthelminthic Assay

In vitro anthelminthic activity against *Eudrilus eugeniae* (Earthworms) of the three extracts (petroleum ether, chloroform, and methanol) were examined. The anthelminthic assay was carried out using a modification of the standard methods by Ajaiyeoba *et al*., (2001) [25]. Stock solutions of 12.0 mg/mL of the three extracts were prepared using sterile

distilled water as a solvent. From the stock solutions, four other concentrations of 0.75, 1.50, 3.0, and 6.0 mg/mL were prepared. A concentration of 12.0 mg/mL of albendazole was used as reference standard and sterile distilled water as a negative control. All test solutions and standard drug solutions were prepared fresh before the start of the experiment. Three worms of approximately the same size were released into separate petri dishes containing 50 mL of each of the various test solutions. Determination of time of paralysis and time of death of the worm were recorded. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor dipped in warm water (50 ^{o}C), followed by fading away of their body colors. The experiment was done in triplicate and the results expressed as a mean ± standard error of the mean (SEM).

2.6. *In vitro* Antioxidant Assays

Three main assays were employed for the antioxidant activity determination. They were the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radicals scavenging, Hydrogen Peroxide scavenging (H_2O_2) and the Total Antioxidant Capacity (TAC) assays.

2.6.1. DPPH radical Scavenging Assay

The free radical scavenging activity of the three extracts were examined according to a modification of the standard methods by Sanchez-Moreno *et al*., (1998) [26]. A solution of 0.02 mg/mL DPPH was prepared by dissolving 2 mg of DPPH in 100 mL of methanol. Stock solutions of 200 µg/mL of each extract in methanol was prepared from which various concentrations of 100, 50, 25, 12.5 and 6.25 µg/mL were prepared using methanol as a solvent. A methanolic stock solution of 200 µg/mL ascorbic acid was also prepared from which various concentrations of 100, 50, 25, 12.5 and 6.25 µg/mL were made through serial dilution. Ascorbic acid was used as standard reference drug.

The reaction mixtures of volume 200 µL were prepared by adding 150 µL of 0.02 mg/mL DPPH solution to 50 µL of various concentrations of the test solutions. The mixtures were then incubated in dark at room temperature for 30 minutes. After which the absorbance were measured at 517 nm. DPPH radical scavenging (%) was calculated using the formula:

$$\% \text{ Scavenging} = \frac{A_0 - A}{A_0} \text{ x } 100 \%$$

Where, A_0 = absorbance of control; A = absorbance of test solution

2.6.2. Hydrogen Peroxide Scavenging Assay

Determination of hydrogen peroxide scavenging potential of extracts were carried out according to a modification of the standard methods by Mukhopadhyay *et al*., (2016) [27]. The assay is based on specific complexation of ferrous ion with 1, 10-phenanthroline to form red-orange tri-phenanthroline complex.

Stock solutions (1000 µg/mL) of each extract was diluted in sterile distilled water to produce concentrations of 200, 400, 600 and 800 µg/mL. For the standard, a stock solution of 1000 µg/mL of gallic acid was prepared, from which various concentrations ranging from 200 to 800 µg/mL were obtained by dilution.

To a series of test tubes, 0.5 mL of ferrous ammonium sulphate (1 mM) were added. Then 3 mL of test solutions (various concentrations of both the extracts and gallic acid solutions) were added and mixed. A volume of 0.13 mL hydrogen peroxide at a concentration of 5 mM was added and thereafter incubated at room temperature in the dark (because hydrogen peroxide can be photo bleached) for 5 minutes. After incubation, 3 mL of 1 mM 1, 10-phenanthroline was added to each tube, mixed well and incubated for 10 minutes at room temperature. Finally, absorbance was taken at 510 nm through a UV-vis spectrophotometer. The negative control contained only ferrous ammonium sulphate (0.5 mL, 1mM), distilled water (3 mL) and 1,10-phenanthroline (3 mL, 1 mM). The percentage scavenging activity was calculated using the formula below

$$\% \, Scavenging = \frac{A_{test}}{A_{control}} \times 100$$

Where A_{test} is absorbance of the test samples and $A_{control}$ is the absorbance of the negative control. The results were further reported in IC_{50}.

2.6.3 Total Antioxidant Capacity (TAC) assay

A modification of the methodology described by Prieto *et al*., (1999) [28] was used to study the total antioxidant capacity of the extracts of *A. cordifolia*. The method is based on the reduction of phosphomolybdic acid, Mo (VI) to phosphomolybdenum, Mo (V) blue complex by the extracts. Ascorbic acid was used as the standard reference antioxidant drug and distilled water was used as the blank. A stock solution of 100 µg/mL of ascorbic acid was prepared from which concentrations of 50, 25, 12.5 and 6.125 µg/mL were prepared through serial dilution using distilled water as solvent. A test solution of concentration of 500 µg/mL of each extract in distilled water was also prepared.

Reaction mixtures of total volume 10 mL was prepared from 5 mL of test solution mixed with 5 mL phosphomolybdenum (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in series of test tubes. The mixtures were then incubated in a water bath at 95 °C for 90 minutes.

The absorbances of each of the solutions were then measured in triplicate, using the UV-visible spectrophotometer at 695 nm after cooling. A plot of the measured absorbance of the ascorbic acid solutions against their concentrations were made to obtain the calibrated concentration-absorbance curve of the ascorbic acid using Microsoft excel. The absorbances of the extract solutions were then substituted for the dependent variable in the linear equation of the ascorbic acid concentration-absorbance plot to determine their corresponding independent variables as ascorbic acid equivalents (AAE), expressed as gAAE/100g ascorbic acid.

2.7. Antimicrobial activity

Agar well diffusion and Broth micro-dilution (minimum inhibitory concentration) assays were employed to assess the antimicrobial activities of the extracts.

2.7.1. Sources of microorganisms

Four bacteria and one fungi were used as test organisms. These were two Gram positive bacteria which included *Staphylococcus aureus* and *Enterococcus faecalis* and two Gram negative bacteria which included *Escherichia coli*, *Pseudomonas aeruginosa.* The fungi was *Candida albicans*. The microbial strains were provided by the Pharmaceutical

Microbiology Section of the Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Science, KNUST, Kumasi. The microbial strains were sub-cultured on nutrient agar slants and incubated at 37 °C for 24 hours.

2.7.2. Inoculum Preparation. Bacterial isolates were streaked onto nutrient agar (Oxoid, United Kingdom) plates and incubated for 18–24 hours at 37 °C. using the direct colony suspension method, suspensions of the organisms were made in nutrient broth and incubated overnight at 37 °C. For the tests, colony suspensions in sterile saline was adjusted to 0.5 McFarland standard and further diluted in sterile double strength nutrient broth ($\sim 2 \times 10^5$ CFU/mL) [29].

2.7.3. Agar well Diffusion

The antimicrobial activities of the different extracts were determined using the agar well diffusion method as described by Agyare *et al*., (2004) [30]. In this method, the inoculum of bacteria were prepared from a 24 hour broth cultures and inoculum of candida from a 48-hour broth culture and serial dilutions were made to achieve a suspension of 2.0×10^5 CFU/mL. One milliliter of the diluted cultures were inoculated into sterile molten agar at 45 °C and poured into a sterile petri dish. They were swirled gently and allowed to solidify. In each petri dish, 6 wells were bored into the solidified inoculated nutrient agar plates using cork borer number 5. Different concentrations of 20, 10, 5 and 2.5 mg/mL of the extracts, ciprofloxacin (10 mg/mL), clotrimazole (10 mg/mL) and control were introduced into the wells after which the plates were incubated at 37 °C for 24 hours for bacteria and 48 hours for the fungus. At the end of the incubation period, the diameter of the inhibition zone(s) was measured and recorded. The extracts and antibiotics were tested in triplicates and mean zones of inhibition were calculated for each extract and the standard antibiotic.

2.7.4 Broth micro-dilution

In the determination of the minimum inhibitory concentration (MIC), the method used was micro-well dilution described by Agyare *et al*., (2004) [30] with slight modification. The inoculum of microorganisms were prepared from 24-hour broth cultures and serial dilutions were made to achieve a suspension of approximately 2.0×10^5 CFU/mL. The 96-well sterile plates were prepared by dispensing into each well 100 µL of double strength nutrient broth and calculated volumes of the stock solutions of extracts were added to the appropriately labeled wells to achieve concentrations of 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125 and 0.39065 mg/mL. Twenty microliters of the freshly prepared inoculum was then transferred into each well to obtain a final volume of 200 µL. The micro plates were incubated at 37 °C for 24 hours. Growth of the microorganisms was determined by adding 20 µL of a solution of tetrazolium salt (3-(4, 5 dimethylimidazole-2yl-2, 5-diphenyltetrazolium bromide) (MTT) and incubating for a further 30 minutes. Dark purple colouration of wells indicate the presence of viable microorganisms whiles no colour change indicated the absence of viable cells. The MIC was determine as the least concentration which showed no colour change after addition of MTT. Ciprofloxacin and clotrimazole were used as positive control. The experiment was carried out triplicate.

2.8 Thin Layer Chromatography (TLC)

The number of components present in the extracts were determined by the analytical TLC method. The pre-coated silica gel plates (0.25 mm) with a fluorescent indicator (F254) were spotted with the extracts about 1 cm from the bottom edge of plates, with the aid of capillary tubes and allowed to dry [31]. Various solvent systems of petroleum ether/ethyl acetate and hexane/ethyl acetate in the ratio of 9:1 and 8:2 respectively were used. The ratio of 8:2 (hexane/ethyl acetate) gave the best separation of components for all the extracts. The plates were dried and visualized by a 254 nm UV lamp. The separated spots were then marked and their sample and solvent fronts were measured.

The retardation factor (R_f) of the eluted spots was calculated as follows:

$$R_f = \frac{\text{Distance travelled by spot}}{\text{Distance travelled by solvent font}}$$

2.9 Column Chromatographic Separation

Flash chromatography was performed using 40–63 µm silica gel (200 x 400 mesh) to separate the number of components present in the extracts [32]. Dry powdered petroleum ether extract was chromatographed on a column packed with silica gel and eluted with a gradient of solvents hexane, then hexane/EtOAc (10/0; 9.5/0.5; 9/1, 50 mL each) to provide 2 fractions, namely A and B. The fractions were monitored by means of TLC (eluent Hexane/EtOAc 4:1). The fractions were evaporated to dryness using the rotary evaporator, then dried and stored at 4 °C in a refrigerator until the use.

2.10 Fourier Transform Infrared Spectrometer (FTIR) Analysis

The dried fractions (A and B) were subjected to (FTIR) analysis (UATR Two, PerkinElmer) to determine the functional groups present. The regions between 4000 cm^{-1} and 400 cm^{-1} were scanned, then followed by baseline correction.

2.11 Data Analysis

Microsoft Excel 2016 and GraphPad Prism 6.0 for Windows (GraphPad Sofware, San Diego, CA, USA) were used for all data analyses and graphs.

3. Results and Discussion

3.1 Extraction of plant material

The yields of the extract in relation to the powdered plant material were calculated as percentages. The yields were 0.34, 0.67 and 1.02 % w/w for methanol, petroleum ether and chloroform extracts, respectively.

3.2. Phytochemical Screening

Pharmacological activity of a plant depends on the phytochemical composition of the plant [24]. The phytochemical screening revealed the presence of all nine secondary metabolites tested for in the pulverized sample while the methanol extract showed the presence of eight secondary metabolites with the exception of alkaloids. Tannins, saponins, and phenols were absent in the chloroform and petroleum ether extracts (Table 1).

Table 1: Phytochemical constituents of the pulverized sample and the extracts of *A. cordifolia*

Phytochemical	Pulverized sample	Petroleum ether extract	Chloroform extract	Methanol extract
Flavonoids	+	+	+	+
Steroids	+	+	+	+
Saponins	+	-	-	+
Terpenoids	+	+	+	+
Carotenoids	+	+	+	+
Glycosides	+	+	+	+
Phenols	+	-	-	+
Tannins	+	-	-	+
Alkaloids	+	+	+	-

Key: (+) = presence of secondary metabolite; (-) = absence of secondary metabolite

The petroleum ether and chloroform extracts had six phytochemicals in common, that is alkaloids, glycosides, carotenoids, terpenoids, steroids and flavonoids. The absence of tannins, phenols and saponins (which are polar due to their polyphenolic OH and many alcohol functional groups) in the chloroform and petroleum ether extracts might be due to the fact that these solvents are more non polar hence could not extract these phytochemicals from the powdered plant material. Methanol extract, however, showed the presence of tannins, phenols and saponins due to its polarity. Secondary metabolites of plants which include terpenoids, flavonoids, phenolic acids, alkaloids and fatty acids steroids have been shown to exhibit various pharmacological activities such as wound healing, anti-inflammation, anticancer, antioxidant, immunomodulation, antidiarrhoeal, antimicrobial, antidepressant, hepatoprotective, antiplasmodial and anxiolytic [33]. The roots and stem bark have been found to contain terpenoids, steroids, glycosides, flavonoids, tannins, saponins, carbohydrates and the imidazopyrimidine alkaloids such as alchorneine, alchornidine and several guanidine alkaloids [34]. The presence of these phytochemicals in the extracts of *A. cordifolia* leaves indicate that they will play a key role in the prevention of various helminthic and bacterial infections and oxidative-stress diseases.

3.3. Anthelminthic Activity

Anthelminthic potency of the extracts were examined using earthworms owing to their anatomical and physiological resemblance with that of intestinal roundworm parasites of human beings as well as their ease of availability [35]. Methanolic, petroleum ether and chloroform extracts displayed concentration-dependent anthelminthic activity at test concentrations of 12.00 to 0.75 mg/mL (Table 2). The anthelminthic activity of methanolic, petroleum ether and chloroform extracts at test concentrations was observed to be significantly (P<0.001) higher compared to albendazole-treated worms.

Table 2: Anthelminthic activity of methanolic, petroleum ether and chloroform leaf extracts of *A. cordifolia*

Conc (mg/mL)	Mean Paralysis and Death Time (Min) ± SEM					
	Chloroform		Methanol		Pet ether	
	Paralysis time	Death time	Paralysis time	Death time	Paralysis time	Death time
12	10.18 ± 0.080*	93.54 ± 2.540*	26.28 ± 0.575*	57.30 ± 0.370*	21.17 ± 0.08*	89.72 ± 0.720*
6	52.75 ± 0.560*	132 ± 0.500*	35.19 ± 0.260*	70.23 ± 0.225*	36.28 ± 0.195*	119 ± 0.315*
3	121 ± 1.450*	305 ± 0.500*	63.0 ± 2.575*	138.5 ± 2.480*	57.31 ± 1.530*	210.5 ± 1.460*
1.5	193 ± 1.500*	320.1 ± 1.600*	75.54 ± 0.040*	258 ± 0.945*	66.33 ± 1.615*	260.3 ± 0.880*
0.75	229 ± 2.000*	440 ± 1.015*	92.27 ± 2.540*	282 ± 1.795*	82.33 ± 1.250*	294 ± 1.245*

Albendazole (12.00 mg/mL): Paralysis time: Death time - 135±2.6: 285±2.0

Each time represents mean ± SEM (N=6). The data were analyzed using ONE-WAY ANOVA compared to the albendazole treatment.

*P-value < 0.001

The mode of action of some anthelminthes like piperazine citrate is to cause paralysis of worms such that they can be expelled in the faeces of man and animals [25]. The extracts not only exhibited such potency, but also caused death of the worms at a rate much higher than the standard drug albendazole. The high potency of the extracts could be attributed to the presence of terpenoids, tannins and saponins identified in the extracts of the leaves which could be exploited as therapy for the treatment of helminthic infection due to their proposed ability to disrupt membrane integrity and make them more permeable [36].

3.4. *In Vitro* Antioxidant Capacity

The total antioxidant potential of a plant extract depends largely on both the constituent of the extract and the test system. Different factors can also influence the activity of the extract, and therefore antioxidant capacity cannot be fully determine and understand by only one method [37]. Considering the various mechanisms of antioxidant actions, the antioxidant properties of the extracts were evaluated by (DPPH) free radicals scavenging, Hydrogen Peroxide scavenging and the Total Antioxidant Capacity assays.

3.4.1. DPPH radical scavenging capacity

The DPPH scavenging activity of the extracts was used to determine and study the ability of the extracts of *A. cordifolia* to mop up free radicals that may be found in animals and humans.

Methanol, petroleum ether and chloroform extracts of *A. cordifolia* and ascorbic acid (standard drug) of concentrations ranging between 6.25 to 100.00 µg/mL, scavenged DPPH at an absorbance ranging between 0.248 to 0.707 nm, 0.695 to 0.829 nm, 0.669 to 0.837 nm and 0.474 to 0.848 nm respectively. Figure 1 shows the percentage inhibition of petroleum ether, chloroform and methanol extracts and standard drugs over a range of concentrations.

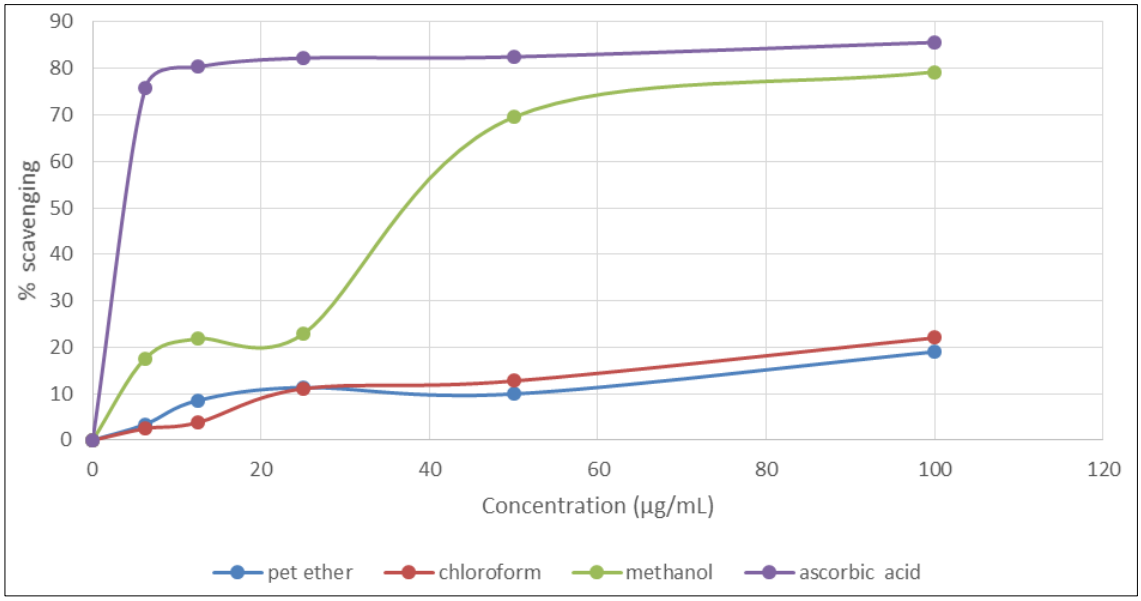

Fig 1: Comparative radical scavenging activity of the petroleum ether, chloroform and methanol extracts and ascorbic acid.

The reference antioxidant (ascorbic acid), petroleum ether, chloroform and methanol extracts of *A. cordifolia* showed anti-oxidant activity in the DPPH free radical scavenging assay with IC_{50} of ascorbic acid, petroleum ether, chloroform and methanol being 2.92, 105.40, 94.77 and 93.02 µg/mL, respectively as shown in Table 3.

Table 3: IC_{50} of DPPH Radical Scavenging Activity for Petroleum ether, Chloroform and Methanol extracts and Ascorbic Acid

Sample	IC_{50} (µg/mL)
Standard (Ascorbic acid)	2.92
Petroleum ether	105.40
Chloroform	94.77
Methanol	93.02

The results implied that the potency of the test samples of extracts as antioxidants decreased in the order: ascorbic acid > methanol > chloroform> petroleum ether (Figure 1). Though petroleum ether, chloroform and methanol extracts which comprise of a mixture of compounds were not as potent as the ascorbic acid, *A. cordifolia* leaf extracts may be useful in the manufacture of drugs to help prevent or cure health problems that could arise from the systemic actions of oxidative agents.

3.4.2 Hydrogen Peroxide Scavenging Assay

Non-radical oxidizing agents scavenging potential of the petroleum ether, chloroform and methanol extracts of *A. cordifolia* were evaluated by the use of hydrogen peroxide (H_2O_2) scavenging method.

The IC_{50} of a sample is the concentration of the sample required to scavenge 50% of the peroxide in a system. It is used to evaluate the antioxidant capacity of a sample. The lower the IC_{50}, the better the antioxidant potential of the sample under examination [38]. Results showed that, petroleum ether, chloroform and methanol extracts demonstrated a significant antioxidant activity in concentration-dose dependent manner. The IC_{50} values of Gallic acid (standard drug), petroleum ether, chloroform and methanol extracts ranged from 204.4 to 898.6 µg/mL as shown in Table 4.

Table 4: IC_{50} of Hydrogen Peroxide Scavenging Activity

Sample	IC_{50} (µg/mL)
Standard (Gallic acid)	204.4
Petroleum ether	898.6
Chloroform	626.5
Methanol	272.0

From the results methanol extract was a more effective anti-oxidant than chloroform and petroleum ether but comparable to gallic acid (standard drug) although, they are all good antioxidants. Bioactive isolates from these extracts responsible for antioxidant activity could be attributed to the terpenoids, flavonoids and phenols in *A. cordifolia* and could be exploited for the treatment of oxidative-stress diseases [33].

3.4.3. Total Antioxidant Capacity (TAC)

Ascorbic acid also known as Vitamin C is an electron donor antioxidant and this property is responsible for all its known functions. Vitamin C is a potent reducing agent and scavenger of free radicals in biological systems. It can donate an electron to free reactive radicals and thus quench their reactivity. Ascorbic acid is also known for its hydrogen donating ability [39].

Concentrations of ascorbic acid ranging between 6.125 to 100 µg/mL showed antioxidant activity and mean absorbances between 0.059 ± 0.003 to 0.932 ± 0.002 at wavelength of 695 nm (Figure 2). The TAC values of the extracts were calculated by substituting the mean absorbances corresponding to the various extract concentrations into the linear equation ($y = 0.0091x + 0.0818$) of the mean absorbance versus concentration plot for the ascorbic acid (Figure 2), and finding their respective equivalence of ascorbic acid concentrations.

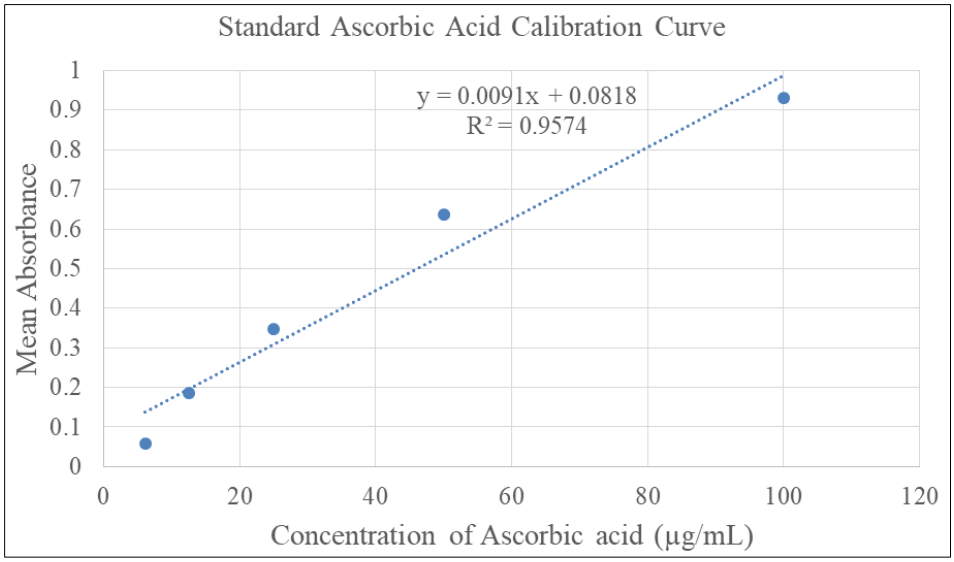

Fig 2: Absorbance of $PMo^{V}_{4}Mo^{VI}_{8}O_{40}^{7-}$ (formed in ascorbic acid solution) against concentration of ascorbic acid solution.

The TAC was found to be proportional to the concentration of extract. TAC of the extracts were examined by Phosphomolybdenum method and the results were expressed as gram ascorbic acid equivalent per 100 grams (gAAE/100g). The gAAE/100g, represents the fraction of the plant extract that can act as ascorbic acid in 100 g of the extract. The petroleum ether, chloroform and methanol extracts had 40.00, 25.85 and 40.08 gAAE/100g, respectively, (Table 5).

Table 5: Total Antioxidant Capacity of Petroleum ether, Chloroform and Methanol extracts expressed as gAAE/100g

Extract	TAC (gAAE/100g)
Petroleum ether	40.00
Chloroform	25.85
Methanol	40.08

TAC - Total Antioxidant Capacity; AAE - ascorbic acid equivalent

Generally, the TAC increased with increasing concentration, thus the higher the TAC, the better the activity of the sample. All the extracts demonstrated appreciable antioxidant activities.

3.5. Antimicrobial Assay

3.5.1 Agar well diffusion

The antimicrobial activities of the extracts were determined at three concentrations levels of 25, 50 and 100 mg/mL for the agar well diffusion assay as shown in Table 6.

Table 6: Mean zones of inhibition (ZI) for petroleum ether, chloroform and methanol extracts of *A. cordifolia* and standard drugs ciprofloxacin and clotrimazole in agar well diffusion assay

Sample/Drug	Concentration (mg/mL)	Mean zone of inhibition (mm)				
		E. faecalis	*P. aeruginosa*	*E. coli*	*S. aureus*	*C. albicans*
Ciprofloxacin	50×10^{-3}	27	27	36	37	NA
Clotrimazole	50×10^{-3}	NA	NA	NA	NA	27
Petroleum ether	100	20	18	20	24	20
	50	16	13	18	-	16
	25	12	-	16	-	-
Methanol	100	26	26	21	25	21
	50	20	24	18	18	18
	25	12	12	12	15	15
Chloroform	100	17	16	20	22	27
	50	15	14	17	18	19
	25	-	-	-	-	16

NA=Not Applicable, Diameter of cork borer = 10 mm

The agar well diffusion is carried out to test for the sensitivity of the organisms to the antimicrobial agent (plant extract). The diameter of the zone of inhibition determines the effectiveness of the extract against the microorganism. The larger the diameter, the greater the sensitivity of the microorganism to the extract. The sizes of the zone of inhibition are compared to standards to determine if the microorganism is sensitive or resistant to the plant extract.

From the results obtained, the lowest concentration at which all three extracts recorded a zone of inhibition was 25 mg/mL. For concentrations of 12.5 and 6.25 mg/mL, no zone of inhibition was recorded. The methanol extract recorded the largest zones of inhibition and also at a concentration of 25 mg/mL the extract was able to inhibit the growth of all the microbes to some extent. The petroleum ether extract showed inhibition against *E. faecalis* and *E. coli* at a concentration of 25 mg/mL while the chloroform showed inhibition against *C. albicans* at the same concentration.

3.5.2 Broth microdilution

The extracts showed broad spectrum antimicrobial activity against the test organisms. The methanol extract showed a better antimicrobial activity (at concentrations of 1.56 mg/mL to 3.13 mg/mL) against the test organisms than the chloroform and petroleum ether extracts (at concentrations of 1.56 to 25 mg/mL). The results are shown in Table 7.

Table 7: Minimum inhibitory concentrations (MIC) of extracts and reference drugs against test organisms

Test organisms	Minimum Inhibitory Concentration (mg/ml)				
	Petroleum ether (mg/ml)	Chloroform (mg/ml)	Methanol (mg/ml)	Ciprofloxacin (mg/ml)	Clotrimazole (mg/ml)
E. faecalis	3.13	3.13	1.56	0.63×10^{-3}	NA
P. aeruginosa	12.50	25.00	3.13	2.50×10^{-3}	NA
E. coli	1.56	1.56	1.56	5.00×10^{-3}	NA
S. aureus	6.25	1.56	1.56	0.63×10^{-3}	NA
C. albicans	12.50	12.50	1.56	NA	1.25×10^{-3}

NA=Not Applicable

The broth micro dilution uses the 96-well microtitre plate to determine the Minimum Inhibitory Concentration (MIC). The MIC is the lowest concentration of an extract that would inhibit the growth of microorganisms. The MIC was determined by the addition of MTT. After the addition of MTT, a purple coloration indicates the presence of microorganisms as the MTT which is a tetrazolium salt will react with dehydrogenase enzyme in the live microorganism to form a dark purple complex [4]. Wells with no color change after addition of MTT indicates the inhibition of the organisms by the extract.

The results from the antimicrobial assay performed showed that the three extracts of *A. cordifolia* leaves exhibited varying inhibitory effects against the five selected microorganisms (two Gram-positive, two Gram-negative and one fungus). The best results were observed with the use of the methanol extract against all the selected microorganisms. The minimum inhibitory concentrations (MICs) were between the range of 1.56 mg/mL to 3.13 mg/mL. The highest activity observed with the use of methanol extract was against *E. faecalis, E. coli, S. aureus* and *C. albicans* with MIC of 1.56 mg/mL.

3.6. Thin Layer Chromatography (TLC)

The number of components present in the extracts were determined by the analytical TLC method. The chromatographic spots which were representative of compounds in the various extracts were observed and their R_f values determined. Table 8 gives the results of the TLC analysis.

Table 8: TLC results of extract showing various components and their retardation factor using hexane/ethyl acetate (4:1) as mobile phase.

Components	Retardation factor, R_f		
	Petroleum ether	Chloroform	Methanol
A	0.9431	0.9434	-
B	0.7643	0.7643	0.7643
C	0.5447	0.5450	0.5450
D	0.4156	0.4156	0.4156
E	0.1807	0.1808	0.1810

The petroleum ether and chloroform extracts showed five spots and methanol four spots with R_f values between 0.1807 to 0.9431, 0.1808 to 0.9434 and 0.1810 to 0.7643, respectively. The number of spots indicating the separated components in the three extracts were less when compared to the phytoconstituents identified to be present in each leaf extract. This means that some of the components existed as isomers or co-eluted in mixtures and it may be necessary to employ two dimensional TLC, HPLC or column chromatography to achieve complete separation of the components.

3.7. Column Chromatographic Separation

Column chromatography was employed to separate the least polar components of the petroleum ether extract. Two fractions namely A and B were separated after elution with a gradient of solvents Hexane/EtOAc (10/0; 9.5/0.5; 9.0/1.0) as indicated in Table 9.

Table 9: Chromatographic separation and the fractions collected using hexane/ethyl acetate (4:1) as mobile phase.

Components	Retardation factor, R_f
A	0.9697
B	0.7647

The column chromatography results were further analyzed using FTIR.

3.8. FTIR Analysis

FTIR analysis was performed on the purified components obtained from petroleum ether extract using column chromatographic separation. The results are presented in Figures 3 and 4 and Table 10 below.

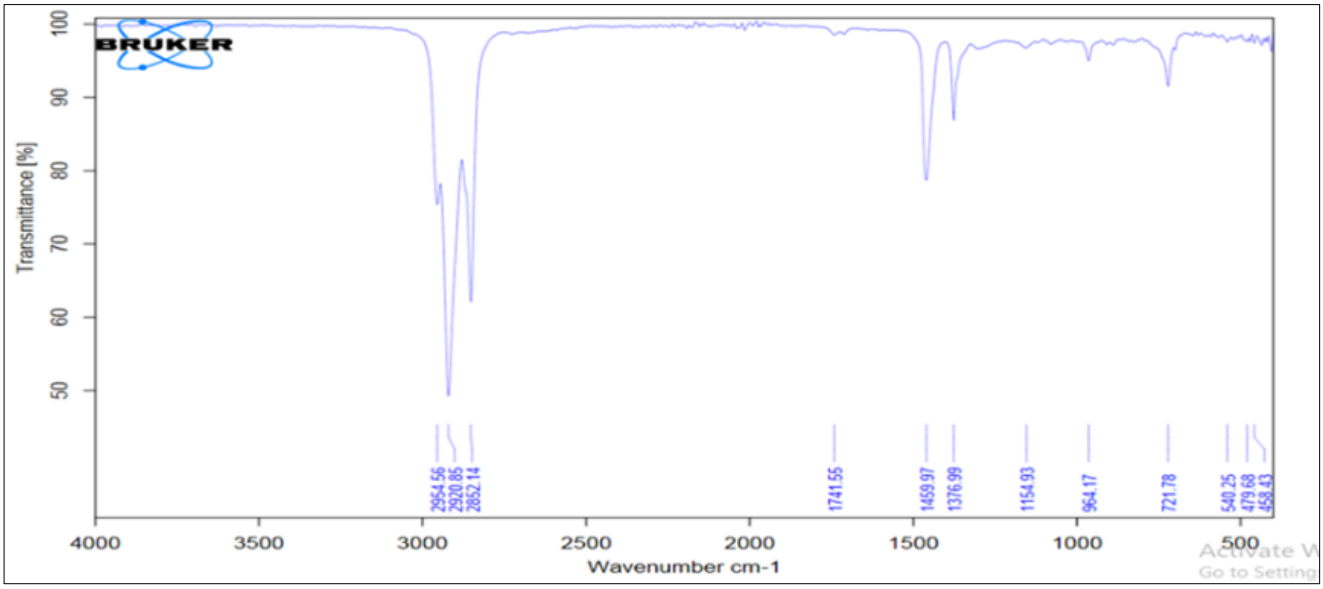

Fig 3: FTIR spectrum of petroleum ether extract of Fraction A of *A. cordifolia*

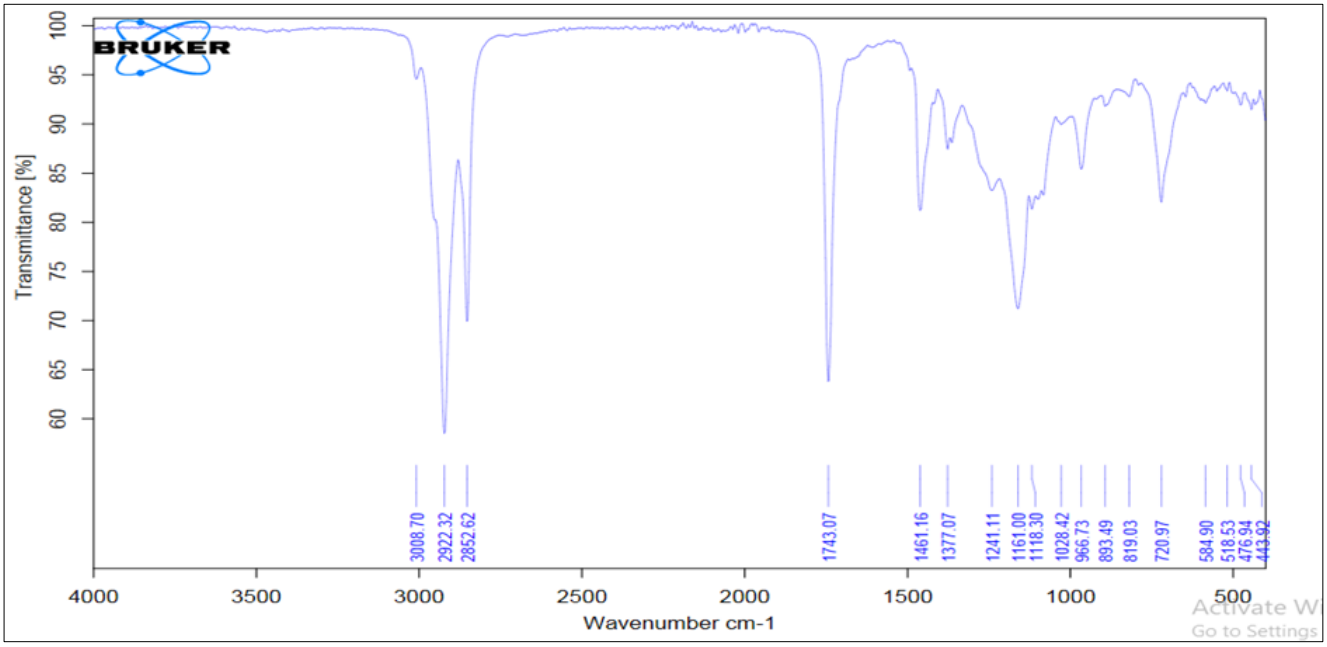

Fig 4: FTIR spectrum of petroleum ether extract of Fraction B of *A. cordifolia*

Table 10: FTIR analysis of purified Fractions A and B of petroleum ether extract

Fraction A			Fraction B		
Peak value (cm^{-1})	**Functional group**	**Inference**	**Peak value (cm^{-1})**	**Functional group**	**Inference**
2954 – 2852	C-H (sp^3) strecth	The functional groups shows highly saturated compound.	3008	C=**C-H** (sp^2) strecth	Presence of carbonyl, olefinic functionalities confirms the identified phytochemicals from the screening test.
1459	C-H bend / strecth		2922, 2852	C-H (sp^3) strecth	
1376	C-O, C-C strecth		1743	C=O carbonyl group	
1154	C-O strecth		1451, 1377, 1241, 1161	C-H, C-C, C-O bend / stretch	

The wave numbers around 3008 cm^{-1}, 2954 to 2852 cm^{-1}, 1743 cm^{-1} and 1451 to 1154 cm^{-1} positions of the spectra are characteristic of olefinic (C=C-H, sp^2) strecth, aliphatic (C-H, sp^3), carbonyl (C=O) and C-O, C-C, C-H stretching, bending functional groups [40]. These functional groups confirm the presence of the identified secondary metabolite from the screening test.

4. Conclusions

The petroleum ether, chloroform and methanol extracts of *A. cordifolia* contain promising bioactive phytocomponents against helminthic, infectious and oxidative-stress diseases, and could become a potential therapeutic agent for their treatment. Column Chromatographic separation revealed two purified fractions, A and B. FTIR studies on the purified fractions revealed the presence of olefinic carbon-hydrogen bond, carbonyl, ether and aliphatic carbon-carbon/hydrogen/oxygen functional groups characteristics of identified phytochemicals Further studies are ongoing in our laboratory towards isolation, characterization, identification and determination of biological activities present in the leaves of *A. cordifolia*.

5. Disclosure

Part of this work was presented as a poster at the "8th Ghana Science Association. Research Seminar and Poster Presentations" held at the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, in May 2019.

6. Conflicts of Interest

The authors declare no competing financial, professional, or personal interests that might have influenced the performance or presentation of the work described in this manuscript. The authors declare that there is no conflict of interests regarding the publication of this paper.

7. Acknowledgments

The authors are grateful to the Departments of Chemistry and Pharmaceutical Microbiology as well as the Central Laboratory of KNUST for the use of their facilities for this study. The authors acknowledge Mr. Francis Amankwaah of the Department of Pharmaceutical Microbiology, KNUST, for technical support.

8. References

1. Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. Molecules. 2016; 21:559-577.
2. Kanlayavattanakul M, Lourith N. Plants and Natural Products for the Treatment of Skin Hyperpigmentation – A Review. Planta Med. 2018; 84:988-1006.
3. Acheampong A, Badu M, Agyemang AY. Comparative Total Phenolics and Antioxidant Activities of *Xanthosoma colocasia*, *Solanum torvum* and *Allium ascalonicum* L. Int. J Chem. Biomol. Sci. 2016; 2:73-79.
4. Acheampong A, Okyem S, Osei Akoto C, Baah AK. Antioxidant, antimicrobial and FTIR analysis of methanol root extract of *Cnestis ferruginea* and ethanol root extract of *Citrus limon*. J Pharmacogn. Phytochem. 2018; 7:2938-2946.
5. David B, Wolfender JL, Dias DA. The pharmaceutical industry and natural products: historical status and new trends. Phytochem. Rev. 2014; 14:299-315.
6. Adeyemi IA, Omonigbehin AE, Stella S, Oluwatosin O, Jumoke S. Antibacterial activity of extracts of *Alchornea cordifolia* (Schum and Thonn) Mull.Arg., *Boerhavia diffusa* (L) and *Bridellia micranthaI* (Hoscht) Baill. used in traditional medicine in Nigeria on Helicobacter pylori and four diarrhoeagenic bacterial pathoge. African J Biotechnol. 2008; 7:3761-3764.
7. Nnamdi A, Ettebong E, Davis K. Antiplasmodial and antioxidant activities of methanolic leaf extract and fractions of *Alchornea cordifolia*. J HerbMed Pharmacol. 2017; 6:171-177.
8. Agbor GA, Léopold T, Jeanne NY. The antidiarrhoeal activity of *Alchornea cordifolia* leaf extract. Phytotherapy Research. 2004; 18(11):873-876.
9. Bayor MT, Ansah C, Duwiejua M, Abaitey AK. *Alchornea cordifolia* (Euphorbiaceae), the Major Constituent of Antiasthmatic Herbal Formulations in Ghana, Stimulates β-adrenoceptors. J Ghana Sci. Assoc. 2008; 10:1-11.
10. Okwu DE, Ukanwa N. Isolation, Characterization and Antibacterial Activity Screening of Anthocyanidine Glycosides from *Alchornea cordifolia* (Schumach. and Thonn.) Mull. Arg. Leaves. E-Journal Chem. 2010; 7:41-48.
11. Ebenyi LN, Nwakaeze AE, Moses IB, Iroha IR, Uzoeto JO, Ugochukwu JI *et al*. Antibacterial activity of *Alchornea cordifolia* leaves found in Ebonyi state, Nigeria. IJAPBC. 2017; 6:46-51.
12. Soil-transmitted helminth infections. Available at: https://www.who.int/news-room/fact-sheets/detail/soil-transmitted-helminth-infections. (Accessed: 12th July 2019)
13. Geary TG, Chibale K, Abegaz B, Andrae-Marobela K, Ubalijoro E. A new approach for anthelmintic discovery for humans. Trends Parasitol. 2012; 28:176-181.
14. Lindquist HAD, Cross JH. Helminths. Infect. Dis. (Auckl). 2017; 8:1763-1779.e1.
15. Peña-Espinoza M. Drug resistance in parasitic helminths of veterinary importance in Chile: Status review and research needs. Austral J Vet. Sci. 2018; 50:65-76.
16. Moharram HA, Youssef MM. Methods for Determining the Antioxidant Activity: A Review. Alexandria J Food Sci. Technol. 2014; 11:31-42.
17. Liguori I, Gennaro Russo G, Francesco Curcio F, Bulli G, Aran L, Morte DD *et al*. Clinical Interventions in Aging Dovepress Oxidative stress, aging, and diseases. Clin. Interv. Aging. 2018; 13:757-772.
18. Afaq H, Mukhtar F. Photochemoprevention by Botanical Antioxidants. Skin Pharmacol. Appl. Skin Physiol. 2002; 15:297-306.
19. Ahmed D, Khan MM, Saeed R. Comparative Analysis of Phenolics, Flavonoids, and Antioxidant and Antibacterial Potential of Methanolic, Hexanic and Aqueous Extracts from *Adiantum caudatum* Leaves. Antioxidants. 2015; 4:394-409. DOI:10.3390/antiox4020394
20. Amos-Tautua BMW, Jonathan G, Angaye SS. Phytochemical screening and antimicrobial activity of the methanol and chloroform extracts of *Alchornea cordifolia*. J Emerg. Trends Eng. Appl. Sci. 2011; 2:445-447.
21. Boadu A, Asase A. Documentation of herbal medicines used for the treatment and management of human diseases by some communities in southern Ghana. Evidence-based Complement. Altern. Med, 2017, 1-12.
22. Ndob BBI, Mengome LE, Bourobou BHP, Banfora LY, Bivigou F. Ethnobotanical survey of medicinal plants used as anthelmintic remedies in Gabon. J Ethnopharmacol. 2016; 191:360-371.
23. Koné WM, Atindehou KK, Dossahoua T, Betschart B. Anthelmintic activity of medicinal plants used in northern Côte d'Ivoire against intestinal helminthiasis. Pharm. Biol. 2005: 43:72-78.
24. Trease GE, Evans WC. Pharmacognosy 16th edition. W.B. Sanders Co. Ltd., New York, 2009, 1-604.
25. Ajaiyeoba EO, Onocha PA, Olarenwaju OT. *In vitro* Anthelmintic Properties of *Buchholzia coriaceae* and *Gynandropsis gynandra* Extracts. Pharm. Biol. 2001; 39:217-220.
26. Sánchez-Moreno C, Larrauri JA, Saura-Calixto F. A procedure to measure the antiradical efficiency of polyphenols. J Sci. Food Agric. 1998; 76:270-276.
27. Mukhopadhyay D, Dasgupta P, Roy SD, Palchoudhuri S, Chatterjee I, Ali S *et al*. *In vitro* Spectrophotometric Hydrogen Peroxide Scavenging Assay using 1, 10-Phenanthroline. Free Radicals Antioxidants. 2016; 6:124-132.
28. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a *Phosphomolybdenum* Complex: Specific Application

to the Determination of Vitamin E. Anal. Biochem. 1999; 269:337-341.
29. Wiegand I, Hilpert K, Hancock REW. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat. Protoc. 2008; 3:163-75.
30. Konning GH, Agyare C, Ennison B. Antimicrobial activity of some medicinal plants from Ghana. Fitoterapia. 2004; 75:65-67.
31. Zhao Y, Slepkov DA, Osei Akoto C, McDonald R, Hegmann FA, Tykwinski RR. Synthesis, Structure, and Nonlinear Optical Properties of Cross-Conjugated Perphenylated iso-Polydiacetylenes. Chem. Eur. J. 2005; 11:321-329.
32. Osei Akoto C, Rainier JD. Harnessing glycal-epoxide rearrangements: The generation of the AB, EF, and IJ rings of adriatoxin. Angew. Chemie - Int. Ed. 2008; 47:8055-8058.
33. Boniface PK, Ferreira SB, Kaiser CR. Recent trends in phytochemistry, ethnobotany and pharmacological significance of *Alchornea cordifolia* (Schumach. & Thonn.) Muell. Arg. J Ethnopharmacol. 2016; 191:216-244.
34. Obadoni BO, Ochuko PO. Phytochemical Studies And Comparative Efficacy of The Crude Extracts of Some Haemostatic Plants In Edo and Delta States of Nigeria. Glob. J Pure Appl. Sci. 2002; 8:203-208.
35. Gaikwad SA, Kale AA, Jadhav BG, Deshpande NR, Salvekar P. Anthelmintic activity of *Cassia auriculata* L extracts- *In vitro* study. J Nat. Prod. Plant Resour. 2011; 1:62-66.
36. Gomes DC, Lima D, Goncalves HV, Santos AV, Santos NS, Dias FO *et al*. *In vitro* anthelmintic activity of the *Zizyphus joazeiro* bark against gastrointestinal nematodes of goats and its cytotoxicity on Vero cells. Vet. Parasitol. 2016; 226:10-16.
37. Song FL, Gan YR, Zhang Y, Xiao Q, Kuang L, Li BH. Total Phenolic Contents and Antioxidant Capacities of Selected Chinese Medicinal Plants. Int. J Mol. Sci. 2010; 11:2362-2372.
38. Ibrahim T, Muluh K, Alexander A. Phytochemical Screening, Antioxidant and Antibacterial Activities of *Commiphora kerstingii*. Int. Biol. Biomed. J. 2016; 2:127-133.
39. Du J, Cullen JJ, Buettner GR. Ascorbic acid: Chemistry, biology and the treatment of cancer. Biochim. Biophys. Acta - Rev. Cancer. 2012; 1826:443-457.
40. Osei Akoto C, Rainier JD. Concise Seven-Membered Oxepene/Oxepane Synthesis – Structural Motifs in Natural and Synthetic Products. Synthesis (Stuttg), 2019, 51. DOI: 10.1055/s-0037-1611838.