Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
https://www.phytojournal.com
JPP 2024; 13(3): 115-124
Received: 21-03-2024
Accepted: 26-04-2024

Akwasi Acheampong
Department of Chemistry, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Silas Ofori Frimpong
Department of Chemistry, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Patrick Buah
Department of Chemistry, University of Maryland, College Park, Maryland, USA

Lydia Tima Sarfo-Mainoo
Department of Dispensing Technology, Sunyani Technical University, Sunyani, Ghana

Adolf Oti-Boakye
Department of Science, Saint Louis College of Education, Kumasi, Ghana

Kennedy Ameyaw Baah
Department of Science, Wesley College of Education, Kumasi, Ghana

Judith Odei
CSIR-Forestry Research Institute of Ghana, Ghana

Corresponding Author:
Akwasi Acheampong
Department of Chemistry, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Antioxidant, anthelminthic, and antimicrobial activities and GC-MS analysis of methanolic stem extract of *Memecylon accedens*

Akwasi Acheampong, Silas Ofori Frimpong, Patrick Buah, Lydia Tima Sarfo-Mainoo, Adolf Oti-Boakye, Kennedy Ameyaw Baah and Judith Odei

DOI: https://doi.org/10.22271/phyto.2024.v13.i3b.14952

Abstract
Memecylon accedens is used in traditional medicine in the treatment of yaws, typhoid, amenorrhea, diarrhea, and piles. This study sought to investigate the phytochemical constituents, antioxidant, antimicrobial, and anthelmintic activity methanolic stem extract of *Memecylon accedens*. Standard methods were employed to determine the phytochemicals present in the extracts. Anthelminthic activity and antimicrobial activity were respectively assessed utilizing *Pheretima posthuma* and the broth dilution method respectively. The antioxidant capacity was assessed using the DPPH radical, Total Antioxidant Capacity, and H_2O_2 scavenging assays. The phytochemical investigation of the extract revealed the presence of alkaloids, phenols, tannins, saponins, steroids, polyphenols, flavonoids, and terpenoids. The extract showed promising activity against *Pheretima posthuma*. The extract recorded an MIC of 20.83 mg/ml and an MBC of 25 mg/ml giving an MBC/ MIC ratio of ≤ 2 (bactericidal) for all the selected bacteria. It also showed higher activity against the fungus, *Candida albicans* with an MIC and MBC of 10.38 mg/ml and 12.5 mg/ml respectively. The IC_{50} values recorded for the extract's DPPH assay was 54.46±0.104 µg/ml. The TAC (gAAE/100 g) for the extract was 217.1712 mg. The GC-MS analysis revealed the presence many important phytochemicals with medical importance. The findings of this study provide insights into the possible use of *Memecylon accedens* as a potential source for the discovery and development of drugs for the treatment of Helminth-related diseases, reactive oxidants, and microbial infections.

Keywords: *Memecylon accedens*, antimicrobial activity, anthelminthic activity, antioxidant activity, *Pheretima posthuma*

Introduction
Natural products have made a substantial impact in the realm of drug discovery and pharmacotherapy [1]. Throughout history, a plethora of plants with medicinal properties have been recorded and employed in addressing various ailments [2]. Recently, there has been heightened interest in utilizing medicinal plants for health maintenance and disease management, spanning both developing and developed nations [3]. Studies suggest that over 40% of the global population depends on traditional medicine or plant-based remedies for their healthcare needs [4]. Notably, extracts from plants such as neem and basil are renowned for their antibacterial properties, with numerous bioactive compounds extracted from these sources [5].

Moreover, various species of *Memecylon* have been extensively employed in treating various ailments, especially in regions like India and Madagascar. Puttaswamy & Achur scrutinized the extract of *Memecylon umbellatum*, unveiling potent antibacterial, antioxidant, and moderate antifungal activities [6]. Similarly, the methanolic extract of *Memecylon terminale* Dalz showcased promising biological activities, including analgesic and antioxidant effects [7]. These advantageous properties of plant extracts predominantly arise from their diverse phytochemical composition. Consequently, plants unquestionably serve as invaluable sources of medicine, playing a pivotal role in drug discovery.

Nevertheless, one limitation of traditional herbal medicine is the utilization of entire plant parts for treating ailments, which may contain only a fraction with efficacy against the specific disease, alongside potentially toxic components. Therefore, it is imperative to pinpoint the specific fraction or portion of the plant that demonstrates potency against a particular ailment.

With *Memecylon accedens* belonging to a genus with plants of known medicinal activities, and there seeming to be a dearth of pharmacological activity information on it, we purposed to delve into the phytochemical constituents and pharmacological activities of the methanolic stem bark extract of *Memecylon accedens*.

2. Materials and Methods

2.1 Chemicals and reagents

The drug standards were generously provided by Tradewinds Chemist and Kinapharma Ghana Ltd. The organic solvents and other chemicals were procured from the BDH Laboratory Supplies and Sigma Aldrich Co. Ltd in UK.

2.2 Sample collection and identification

Akwapim secondary forest and Kwahu Asakraka in Ghana (Eastern Region) were the places where the plants were harvested. It was authenticated in the Kwame Nkrumah University of Science and Technology's Department of Herbal Medicine with voucher specimens (KNUST/HM1/2021/L029) deposited for future reference.

2.3 Extraction of Bioactive Compounds

The stem bark extracts were washed appropriately and then dried under shade for a month and pulverized for extraction [8]. The extraction was carried out using cold maceration with methanol (99.9%) as solvent and at ambient temperature. A volume of methanol (700 mL) was poured onto 100 g of pulverized sample and macerated for 72 hours. The obtained extract was filtered, and the filtrate was concentrated under reduced pressure utilizing a rotary Evaporator (BUCHI Rotavapor R-114). The extract was dried and weighed and the percentage yield calculated. The extract was then refrigerated at 4 °C until needed.

2.4 Qualitative phytochemical analysis

Memecylon accedens methanolic stem extract was screened for phytochemicals such as tannins, flavonoids, alkaloids, cardiac glycosides, steroids, phenolics, saponins, polyphenols, terpenoids, quinones, carotenoids, and phytosterol utilizing the methods of Trease and Evans [9].

2.5. *In vitro* anthelmintic assay

2.5.1. Worm Collection and Authentication

The earthworms were authenticated at KNUST (zoology unit), after their collection near the Wiwi River (latitude 6º35 N- 6º40 N, and longitude 1º30 W-1º35 W). They were transported to the laboratory for authentication and use by being put into glass bottles containing portions of the soil they originally inhabited. Mr. Lawrence Yeboah authenticated the worms.

2.5.2 Anthelminthic Assay

The anthelminthic assay was conducted following a modified protocol based on the methods outlined by Ajaiyeoba and colleagues [10] and described by Acheampong and co-workers [11]. Extract solutions of concentrations of 1.250, 2.50, 5, 10, and 20 µg/mL were prepared from a 150 µg/mL stock solution. Mebendazole (0.5 mg/mL) and sterile distilled water served respectively as the standard reference drug and a negative control. The standard drug and test solutions were freshly prepared prior to the commencement of the experiment. Four worms of similar size were individually placed into Petri dishes (separately), each containing 50 mL of the respective test solutions. The time of paralysis (no movement except under vigorous shaking), and death of the worms (no movement after vigorous shaking and exposure to warm water (50 °C), and accompanied body colour fading), were recorded. The results were recorded as the mean ± SEM (standard error of the mean) after triplicate measurements [10, 12].

2.6. Antioxidant activity Determination (In vitro)

2.6.1 Hydrogen Peroxide Scavenging Assay

The extracts' hydrogen peroxide scavenging activity was determined by standard methods of Mukhopadhyay *et al.* [13] with slight modification [14]. Extract solutions (200, 400, 600, and 800 µg/mL) were produced from a stock solution (1000 µg/mL) of the extract. Similar concentrations were prepared for the standard gallic acid. A volume of 0.5 mL of ferrous ammonium sulfate (1 mM) was poured into a series of test tubes. Then 3 mL of test solutions (extracts or gallic acid solutions) were added. A 5 mM Hydrogen peroxide solution (0.13 mL) was poured in and the mixture incubated for 5 minutes at room temperature. Afterward, 3 mL of 1, 10-phenanthroline (1 mM) was poured into each test tube and the mixture was incubated at room temperature for 10 minutes. At 510 nm, the absorbance of the solution was measured utilizing a UV-Vis spectrophotometer. The negative control contained all the solutions of the mixture except the test solutions (extracts and standard). The formula utilized in calculating for the percent scavenging activity was as below:

$$\% \text{ Scavenging} = \frac{A_{test}}{A_{control}} \; x \; 100$$

Where $A_{control}$ is the absorbance of the negative control, and A_{test} is the absorbance of the test samples. IC_{50} values were then obtained from the results and reported.

2.6.2 1, 1 Diphenyl-1-picrylhydrazy (DPPH) Assay

The DPPH assay was performed following the method by Sanchez-Moreno *et al.* [15] with slight modifications [14]. A mass of 2 mg of DPPH was dissolved in methanol to form 100 mL of a solution of concentration 0.02 mg/mL. A Stock solution (200 µg/mL) prepared utilizing double distilled water as solvent, was serially diluted to obtain 20, 40, 60, 80 and 100 µg/mL extract solutions. Similar concentrations were prepared for the stock and diluted solutions of the standard drug. The positive control was Ascorbic acid. A reaction mixture (200 µg/mL) was made by adding 0.02 mg/mL DPPH solution (150 µL) to 50 µL of test solutions (different concentrations). Incubation of the mixtures was performed in the dark for 30 minutes at room temperature. Utilizing a V-730 UV-Vis Spectrophotometer (Jasco, USA), the absorbance of each mixture was measured at 517 nm. Double distilled water was used as negative controls. Measurements were made in triplicates. The calculation of DPPH scavenging activity utilized the formula.

$$\text{DPPH scavenging activity (\%)} = \frac{A_0 - A}{A_0} \; x \; 100$$

Where A_0 is the absorbance of the negative control, and A is the absorbance of the mixture containing the extract.

2.7 Antimicrobial Activity Determination

The extract's antimicrobial activity was determined utilizing the Broth micro-dilution assay.

2.7.1 Sources of Microorganisms

The microorganisms used were two Gram-positive bacteria (*Staphylococcus aureus and S. pyogenes*), two Gram-negative

bacteria (*Salmonella typhi*, *Escherichia coli*), and a fungus (*C. albicans*). The Department of Pharmaceutics (Microbiology section), KNUST, provided the microbial strains. Sub-culturing of the microbial strains was done on nutrient broth slants for 24 hours and incubated at 37 °C.

2.7.2 Inoculum Preparation
Onto nutrient agar plates (Oxoid, United Kingdom) were bacterial isolates streaked at 37 °C and incubated for 24 hours. The organisms were suspended in nutrient broth at 37 °C and incubated overnight (Direct colony suspension method). The colony suspensions in sterile saline were adjusted to 0.5 McFarland standard (for the tests) and diluted further to obtain ~2 × 10^5 CFU/mL utilizing sterile double-strength nutrient broth [16].

2.7.3 Broth Micro-Dilution
The micro-well broth dilution method as described in our previous work was employed [12]. A suspension of 2.0×10^5 CFU/mL was made using serial dilutions of an inoculum of microorganisms made from 24-hour broth cultures. Addition of specific volumes of extract stock solutions to each well containing 100 µL of double-strength nutrient broth led to the preparation of solutions of concentration 50, 25, 12.5, 6.25, 3.125, and 1.6125 mg/mL. A final volume of 200 µL was prepared by adding 20 µL of freshly prepared inoculum into each well. Incubation of the microplates was done at 37 °C for 24 hours. To determine the growth of the microorganisms, 20 µL of tetrazolium salt (3-(4, 5 dimethylimidazole-2yl-2,5-diphenyltetrazolium bromide) (MTT) solution was added and further incubation for 30 minutes was done. The presence of viable microorganisms was indicated by a dark purple colouration of wells while absence of viable microorganisms is indicated by absence of colour change. The concentration (least) at which no colour was observed upon the addition of MTT was determined and recorded as the MIC. Ciprofloxacin was used as the positive control. Triplicate measurements were taken, mean found, and values reported as mean ± SEM.

2.8 Fourier Transform Infrared Spectrophotometer (FTIR) Analysis: The extract was analyzed with Fourier transform infrared spectrophotometer (FTIR) (UATR Two, PerkinElmer) to assess the presence of functional groups in the extract. The region between 400 cm^{-1} and 4000 cm^{-1} was scanned, then followed by baseline correction.

2.9 Gas Chromatograph - Mass Spectrometer (GC-MS) Analysis
The GC and MS conditions are specified below.

Gas Chromatograph Conditions		Mass Spectrometer conditions	
GC instrument	Gas Chromatograph (PerkinElmer GC Clarus 580)	MS instrument	PerkinElmer Clarus SQ 8 S
GC Column	Fused capillary column (30 x 0.25 µm ID ᵡ 0.25µm DF)	Ionization mode	Electron impact (EI)
Oven temperature	Programmed from 100 °C (isothermal for 2 min), increase of 10 °C/min to 200 °C, then 5 °C/min to 280 °C.	Ion source temperature	220 °C
Carrier gas	Helium gas (99.9999%)	ionization energy	70 eV
Flow rate	1 ml/min	Scan interval	1s
Injection volume	1 µl	Mass range	45 to 500 Da
Solvent Delay	3 min		
Injector temperature	250 ºC		
Run time	43 min		

2.10 Statistical Analysis
GraphPad Prism 5 software version 8.02 (GraphPad Software, San Diego, CA, USA) and Microsoft Excel (Microsoft 365) were used to process and analyze the results.

3. Results and Discussion
3.1 Percentage yield
In calculating the yield of extract with respect to the pulverized sample utilized, the yield was 6.53%. The methanol, being polar, extracted quite a good amount of extract.

3.2 Phytochemical screening
The phytochemical screening indicated the presence of many phytochemicals. A summary of the results is presented in Table 1.

Table 1: Phytochemical Screening Results of Pulverized Sample and Extract

Secondary metabolites	Pulverized sample	Extract
Phenols	+	+
Alkaloids	+	+
Glycoside	-	-
Steroids	+	+
Saponins	+	+
Terpenoids	+	+
Tannins	+	+
Flavonoids	+	+
Polyphenols	+	+
Quinones	-	-
Carotenoids	-	-
Anthraquinone	-	-
Key:(+):Secondary metabolite detected, (-):secondary metabolite undetected		

The pulverized sample and methanol extract of *Memecylon accedens* stem were screened for twelve phytoconstituents. Eight phytochemicals, phenols, alkaloids, steroids, saponins, terpenoids, tannins, flavonoids, and polyphenols were present in the powdered sample.

The crude extract also revealed the same number and types of phytochemicals. The extraction technique employed did not necessarily alter the tested phytoconstituent. In the past, plants not only gave man sustenance but also medicinal properties [17]. Many plants that contain some of these phytochemicals have antioxidant, antimicrobial, and anthelmintic properties. The presence of phytochemicals in plants used in traditional medicine is what gives them their medicinal properties [18].

3.3 GC-MS Analysis of the Methanol Extract: Table 2 presents the phytoconstituents identified in the methanol extract of *Memecylon accedens* using GC-MS. The compounds were tentatively identified by comparing the mass spectra (fragmentation patterns) of the unknown compounds with the mass spectra of known compounds (authentic standards) in the library of the National Institute of Standards and Technology (NIST). Similarity indices of the compounds were used to confirm the identified compounds.

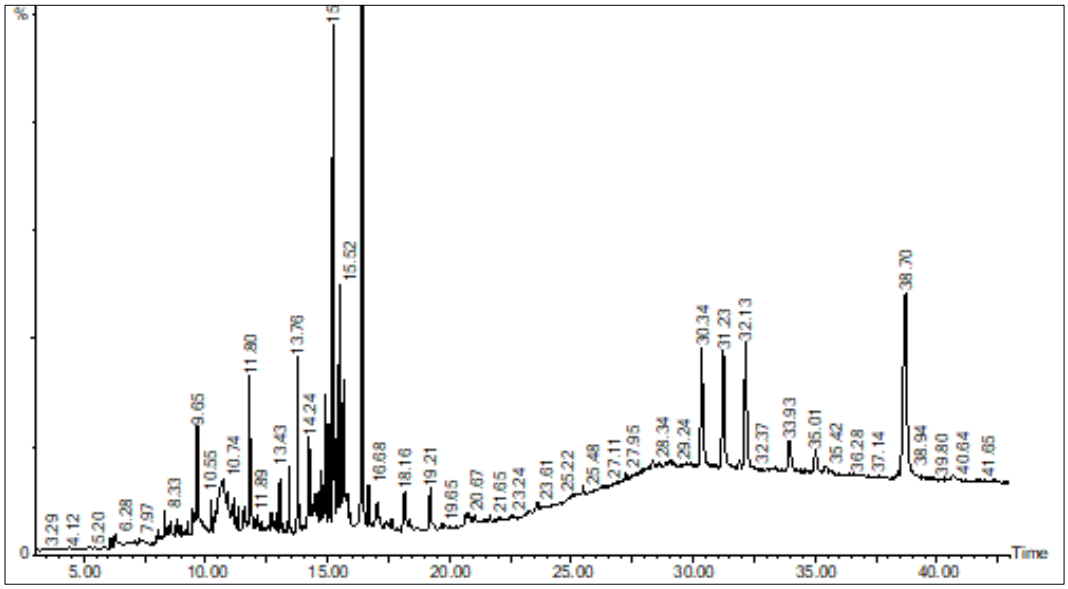


Fig 1: GC-TIC chromatogram of *Memecylon accedens'* methanol extract

In the methanol extract were identified nine major compounds. Figure 1 depicts the GC-TIC chromatogram of the extract while Table 2 presents the compounds, their retention times, and chemical names.

The phytochemicals identified included alcohols, carboxylic acids, ketones, and esters. These identified phytochemicals are likely responsible for the medicinal properties exhibited by the plant.

Table 2: Identified Phytochemicals in *Memecylon accedens* methanol extract

Retention time/mins	Similarity index / %	Compound
10.763	98.45	O- α -D-glucopyranosyl-(1.fwdarw.3)- α -D-fructofuranosyl-α-D-Glucopyranoside
15.228	85.75	9-(3,3-dimethyloxiranyl)-3,7-dimethyl-, methyl ester, (E,E)- 2,6-Nonadienoic acid
15.251	96.08	7-ethyl-9-(3-ethyl-3-methyloxiranyl)-3-methyl-, methyl ester, [2R-[2à(2E,6E),3]- 2,6-Nonadienoic acid
16.402	93.87	4-hydroxy-4a,5-dimethyl-3-methylene-3a,4,4a,5,6,7,9,9a-octahydro-3H-Naphtho[2,3-b]furan-2-one
29.053	96.32	Betamethasone acetate
30.336	97.44	5,5'-(tetrahydro-1H,3H-furo[3,4-c]furan-1,4-diyl)bis-, [1S-(1à,3aà,4á,6aà)]- 1,3-Benzodioxole
31.235	98.85	2,3,3',4'-tetramethoxy-5-(3-methoxyprop-1-enly)stilbene
32.133	91.25	1,4-bis(3,4-dimethoxyphenyl)tetrahydro-, [1R-(1à,3aà,4á,6aà)]- 1H,3H-Furo[3,4-c]furan
33.930	97.98	9,12-dimethyl-15 oxapentacyclo [12.6.0.0(1,6). 0(2,18).0(8,13)]icosa-8(13),9,11-triene-5-carboxylic acid

3.4 FT-IR analysis of extract

To know some of the functional groups present in the plant extract, it was analyzed with an FT-IR instrument. The results yielded a highly deshielded OH group which is likely to have come from phenolic compounds present in the extract. Other groups were carbonyl, alkene, and imine (Figure 2). Table 3 presents the inference of the FT-IR spectrum.

Table 3: Inference of the FT-IR Spectrum

Wavenumber/ cm^{-1}	Inference
3371.59	OH stretching (polyphenols)
2853.77-2925.65	C_{SP^3}-H stretching
1713.53	C=O stretching
1621.35	C=C
1253.14	C=N

These functional groups, OH, C=C, C=O, and C=N are present in most of the phytochemicals detected by GC-MS to be present in the extract.

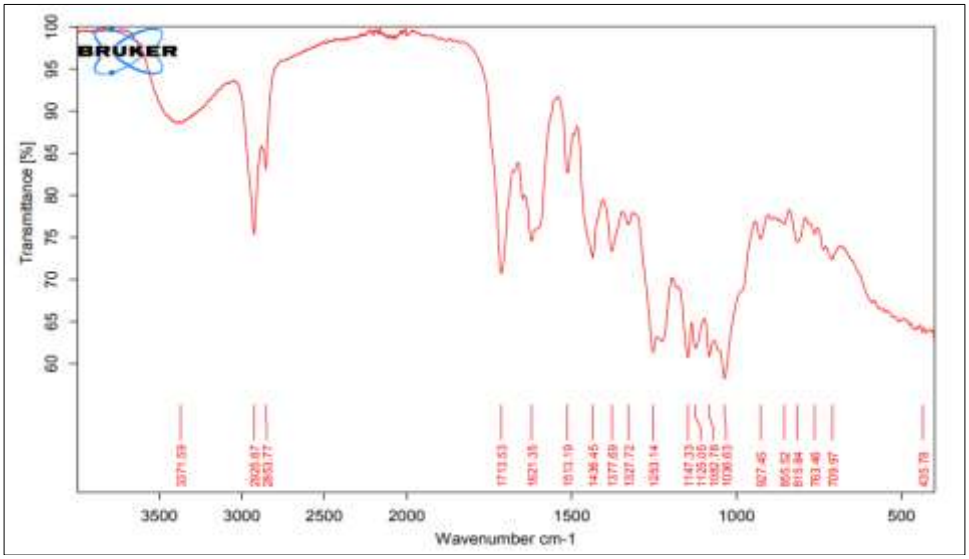


Fig 2: FT-IR spectrum of *Memecylon accedens'* methanolic stem extract

3.5 Antioxidant Activity of Extract

The extract's DPPH radical scavenging activity was evaluated as one of the antioxidant activity assays, the others being total antioxidant capacity (TAC), and H_2O_2 scavenging activity.

3.5.1 DPPH radical scavenging activity of extract

The results of the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity is presented in Table 4.

Table 4: Antioxidant activities (DPPH, H_2O_2, and TAC) of the extract and standards expressed as IC_{50}

Sample	IC_{50} (µg/ml) ± SEM		
	DPPH	H_2O_2	TAC
Standard	45.23±0.06[a]	151.80±0.09[a]	-
Extract	54.46±0.10[b]	178.63±0.15[b]	217.17±0.91

DPPH is purple when in solution, and antioxidants can reduce the DPPH to DPPH-H by the transfer of a proton to it. This reduction changes the purple color of the DPPH to pale yellow. The scavenging activity of the extract through the annihilation of the DPPH radicals was investigated. In the 96-well microtiter plate, the scavenging property of the extract after the 30 min incubation period was concentration-dependent.

The absorbances decreased with increasing concentration of the extract. An increase in DPPH scavenging activity was observed as the concentration of both the extract and standard drug increased (Figure 3). The IC_{50} values recorded were 54.46±0.104 µg/ml for the extract and 45.23 ± 0.058 µg/ml for the ascorbic acid (Table 4). IC_{50} is the concentration of the test sample at which it could inhibit 50% of the targeted free radical. It means the concentration above is the concentration needed for the extract and standard to inhibit 50% of free radicals. The lower IC_{50} value of 45.23 ± 0.058 µg/ml for the ascorbic acid makes it a stronger antioxidant agent. The 54.46 ± 0.104 µg/ml IC_{50} value of the extract is relatively lower and its closeness to that of the standard makes it also a strong and a good alternative antioxidant to the standard. The ability to quench free radicals could be a result of some phytoconstituents present in the extract having the capacity to donate electrons or hydrogen atoms to stabilize radicals. The extract is said to exhibit antioxidant activity. Natural phenolic compounds are well known for their capacity to chelate metals, reduce pressure, donate hydrogen, and most impressively, trap free radicals and halt chain reactions [19]. According to a study, phenolic compounds' hydroxyl groups may support antioxidant activity directly, and play a crucial part in free radicals scavenging [20]. As a result, plants with high polyphenol content have gained more attention as natural antioxidants across the globe [21]. The activity of the standard (ascorbic acid) was observed to be significantly different (p<0.0001) from the extract.

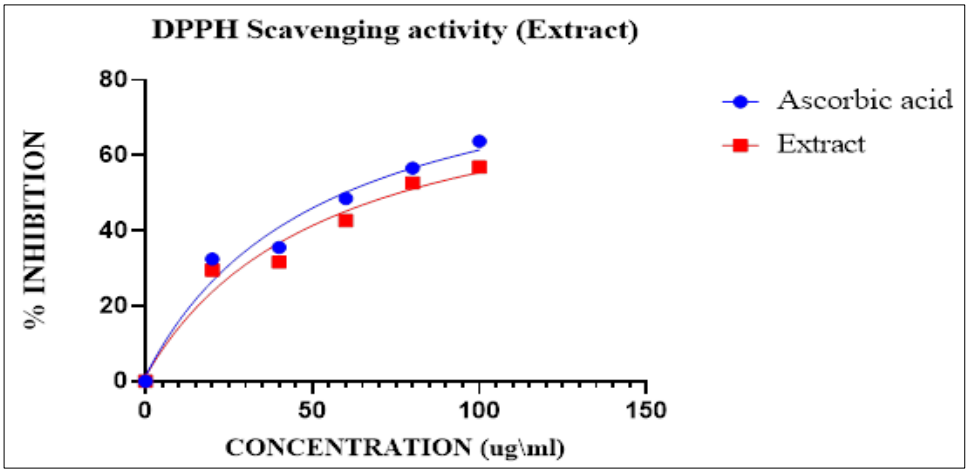


Fig 3: Graph of DPPH scavenging property of extract vs. ascorbic acid (standard drug)

3.5.2 Hydrogen peroxide scavenging activity

The H_2O_2 scavenging assay examines an extract's capacity to remove hydrogen peroxide. In an organism, hydroxyl radicals produced by the dissociation of H_2O_2 can destroy essential biochemicals like proteins and DNA. Therefore, the purpose of this test was to assess the extract's capacity to bind with these peroxide radicals.

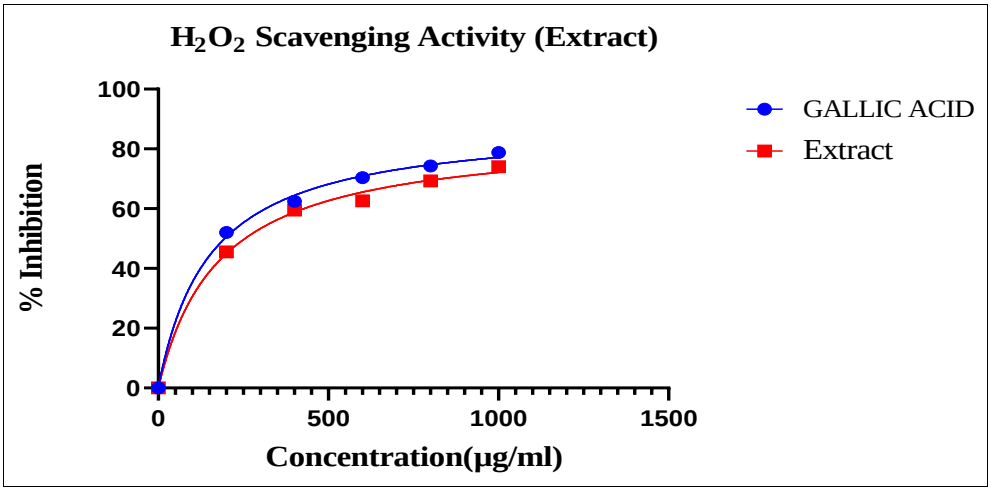


Fig 4: H_2O_2 scavenging activity of the gallic acid standard drug and the methanol extract

The results revealed that the absorbance measured decreased with decreasing concentration. The percent inhibition also decreased with decreasing concentration of extracts (Figure 4). The IC_{50} obtained for this assay for the extract and gallic acid (standard drug) are 178.63±0.152 µg/ml and 151.80±0.094 µg/ml respectively (Table 4). The extract demonstrated high antioxidant activity in this assay, even though it was lower than that of the standard, and this further confirmed the methanolic stem extract's antioxidant activity. The activity of the standard (gallic acid) was significantly different ($p< 0.001$) from the extract.

3.5.3. Total Antioxidant Capacity of the extract

Total antioxidant capacity is an assay that quantifies the number of total antioxidants in the plant extract. This was carried out using the phosphomolybdenum assay with ascorbic acid as the standard antioxidant drug. This assay is hinged on the reduction of Mo (VI) to Mo (V) by the extract [22].

The total antioxidant capacity of the extract was 217.17±0.905 mg (Table 4). This implies that 217.17±0.905 mg of the extract is acting as 100 g of ascorbic acid. Current research has shown that certain phytochemicals of medicinal plants such as phenols, carotenoids, and terpenoids may be used to manage oxidative stress diseases due to their potent antioxidant properties [23-25]. Delay of aging by neutralizing free radicals has been indicated to be the mechanism of action for vitamins C and E, and phenols. The antioxidant capacity of *Memecylon accedens* could be due to the terpenoids, phenols, and polyphenols present in the extract.

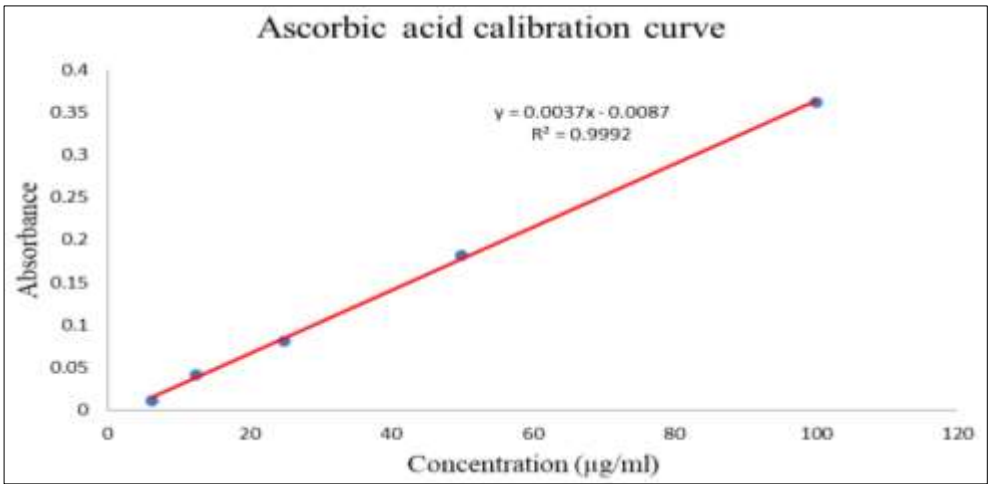
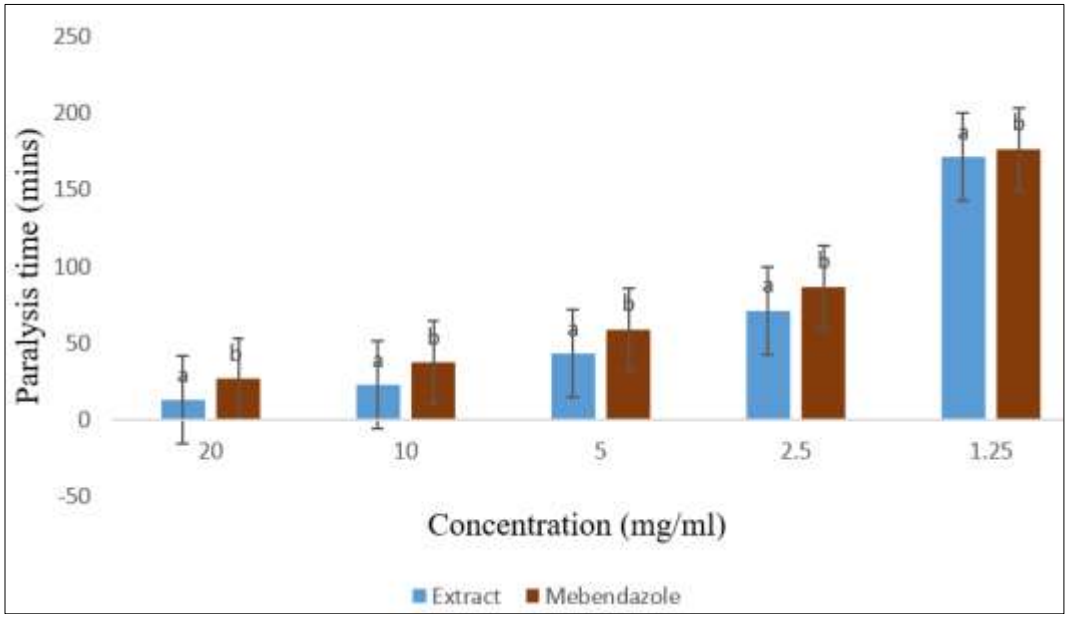


Fig 5: Calibration curve for Ascorbic Acid

3.6 Anthelmintic Activity of Extracts

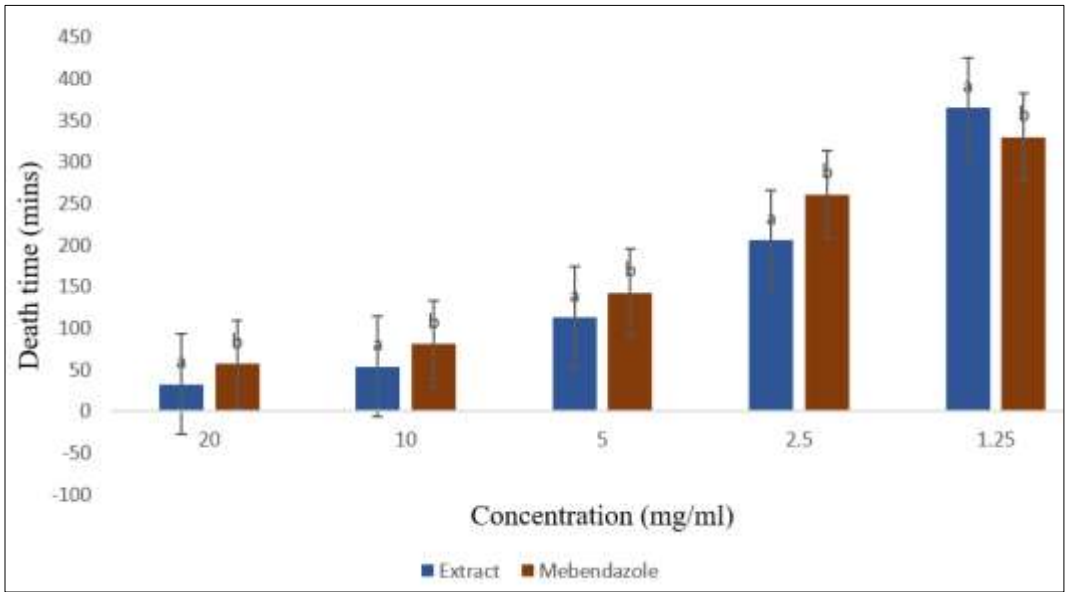
3.6.1 Adult earthworm (Pheretima posthuma) mortality activity: The results of the anthelminthic assay are presented in Figures 6 and 7.



Different alphabets on bars at the same concentration mean they are statistically different at 5% probability level using Turkey's M.C.T.

Fig 6: Paralysis time of worm for extract and Mebendazole drug (standard)

Generally, anthelmintics cause the parasites' energy metabolism to be disturbed. The crucial glycolytic enzyme lactate dehydrogenase (LDH), which is found in anaerobic helminth parasites, is essential for the parasites' metabolism of carbohydrates which, in the presence of NADH, converts pyruvate to lactate in addition to re-oxidizing the reduced NADH created by maintaining the cytosolic redox potential via the glycolytic process (NAD/NADH) [26]. Most Anthelmintics work by altering the activity and inhibiting the parasite's LDH activity of catalyzing the oxidation of lactate [27]. Due to *Pheretima posthuma*'s anatomical and physiological similarity to *Ascaris lumbricoides*, a human intestinal roundworm, it was chosen for this study [28].



Different alphabets on bars at the same concentration mean they are statistically different at 5% probability level using Turkey's M.C.T.

Fig 7: Death time of worm for the extract and mebendazole drug (standard)

The *in-vitro* anthelmintic assay provides a enables a rapid screening of different plant extracts for potential anthelmintic activities. Possible mechanisms involved in the active compounds and parasites interactions are also analyzed with this assay. Various concentrations of the methanolic stem extract of *Memecylon accedens* were compared with mebendazole (standard). The extract recorded a shorter paralysis and death time as compared to the mebendazole. The shorter the time, the higher the anthelmintic activity of the test solution. The high activity of the extract may be the presence of several secondary metabolites acting synergically. The combined effects of the extracts are the sum of the actions of each of their parts [29]. The standard, however, recorded a shorter death time than the extract at the lowest concentration of 1.25 mg/ml. This implies that the extract is needed in a higher concentration for the best activity. This may be due to its crude nature and the presence of components that are not anthelmintic. Worms remained active

in the negative control using distilled water. Plant extract exhibited anthelmintic activity as a result of the phytochemicals like terpenoids and tannins present in the extracts [30]. Medicinal herbs' anthelmintic effect is mostly brought about by phytochemicals like alkaloids, tannins, flavonoids, and triterpenoids contained in the extract [31] which obstruct the energy-generating pathways and cause paralysis and death [27]. The extract's activity was significantly higher (p<0.0001) than that of the standard drug (mebendazole).

3.7 Antimicrobial Analysis
3.7.1 Broth microdilution

Table 5: Summary of MIC and MBC results of the extract

Test organisms	Minimum Inhibition Concentration (MIC)			MBC of Extract (mg/ml)
	Extract (mg/ml)	Ciprofloxacin (µg/ml)	Fluconazole (µg/ml)	
Escherichia coli	20.83	7.8×10^{-1}	NA	25
Streptococcus pyogenes	20.83	9.8×10^{-2}	NA	25
Staphylococcus aureus	20.83	9.8×10^{-2}	NA	25
Salmonella typhi	20.83	7.8×10^{-1}	NA	25
Candida. albicans	10.38	NA	1.25×10^{1}	12.5

NA: Not Applicable

The results of the antimicrobial activity, reported in MIC and MBC, are presented in Table 5. The MIC is the lowest concentration of an extract that would inhibit the growth of microorganisms while MBC is the lowest concentration in which the test sample will be lethal to the microorganism. After the 24 hrs incubation period, the MIC was determined by the addition of MTT [32]. Wells without purple colorization after the addition of MTT indicate that the organisms have been inhibited by the extract.

The antimicrobial assay results (Table 5) showed that the extract was able to inhibit both the Gram-positive and Gram-negative bacteria, as well as the fungus used in the test. The extract inhibited all the tested bacteria at 28.83 mg/ml and the fungus at 10.38 mg/ml. The recorded fungicidal and bactericidal concentrations of the extract were 12.5 and 25 mg/ml respectively. The extract exhibited similar activity on all the selected bacteria. The fungus was however inhibited at a lower concentration than the bacteria. The extract, therefore, showed stronger antifungal activity than antibacterial activity. The selected microorganisms were influenced by the diseases that the local people use the plant to treat. Also, these microorganisms are among the common pathogens that mostly infect humans.

The high susceptibility of *E. coli* to the extract further supports the use of the plant for the treatment of diarrhoea since *E. coli* is a common pathogen in most diarrhoea infections [33].

The MIC values indicated that the reference drugs had higher antimicrobial activity than the extract. The reference drug, being a single compound and made specifically as an antimicrobial, worked without any other compound antagonizing it's actions, hence the higher activity. However, the extract, being a mixture of phytoconstituents, had some of the constituents possessing antimicrobial activity but their activity could be antagonized by other constituents present leading to lower antimicrobial activity. The other constituents could also have worked in synergy with the antimicrobial constituents to increase their antimicrobial activity. Researchers have proven that terpenoids [34], saponins [35], tannins [36, 37], and alkaloids [38] identified in this work possess certain antimicrobial properties. The antibacterial action of the plant extract was once again found by numerous investigations to be due to a variety of phytochemicals, including terpenoids, flavonoids, steroids, tannins, alkaloids, and certain phenolic compounds [39]. The antimicrobial activities of the methanol extract of *Memecylon accedens* could be due to the phytoconstituents present in it.

4. Conclusion
This study was carried out to investigate the phytochemical constituents, antioxidant, anthelmintic activity, and antimicrobial activity of the methanol extract of *Memecylon accedens*. The phytochemicals present in the extract and pulverized sample were saponins, tannins, alkaloids, phenols, steroids, carotenoids, flavonoids, and terpenoids. GC-MS analysis of the extract identified nine major phytoconstituents including O- α -D-glucopyranosyl-(1.fwdarw.3)- α -D-fructofuranosyl-α-D-Glucopyranoside, 9-(3,3-dimethyloxiranyl)-3,7-dimethyl-, methyl ester, (E,E)- 2,6-Nonadienoic acid, 7-ethyl-9-(3-ethyl-3-methyloxiranyl)-3-methyl-, methyl ester, [2R-[2à(2E,6E),3]- 2,6-Nonadienoic acid, and the commonest being Betamethasone acetate which is indicated for being an anticancer and anti-inflammatory agent. The extract showed antioxidant, antimicrobial, and anthelmintic activities which gave credence to the use of the plant for medicinal purposes by the local people. Compared to the standard drugs used in the study, the extract showed pronounced anthelmintic activity. The extract also exhibited better antifungal activity than antibacterial activity which is consistent with its use in combating fungal infections.

5. Data Availability
All the data that supports this research are included in the manuscript. However, if further details are required, the authors are willing to provide them.

6. Conflict of Interest
There is no conflict of interest (professional, financial, or personal) for the authors to declare.

7. Funding Statement
The research was funded from the authors' own resources, and no funding was received from any funding agency.

8. Acknowledgment
The authors acknowledge Stepanie Adu Amoako (Microbiology laboratory), technicians of Department of Chemistry, and the Central laboratory of KNUST for their immense help in the research.

References
1. Lahlou M. The success of natural products in drug discovery. Pharmacology and Pharmacy, 2013, 4(3A). ID:33502. DOI:10.4236/pp.2013.43A003.

2. Ernest M, Grace OM, Saslis-Lagoudakis CH, Nilsson N, Simonsen HT, Rønsted N. Global medicinal uses of *Euphorbia* L. *(Euphorbiaceae*). Journal of ethnopharmacology. 2015;176:90-101.
3. Banjari I, Misir A, Šavikin K, Jokić S, Molnar M, De Zoysa HKS, *et al*. Antidiabetic effects of *Aronia melanocarpa* and its other therapeutic properties. Frontiers in Nutrition. 2017;4:53.
4. Murugesan S, Vijayakumar R, Panneerselvam A. Evaluation of phytochemical constituents from the leaves of *Memecylon umbellatum Burm. f.* Research journal of pharmaceutical, biological and chemical sciences. 2011;4(2):1145-1151.
5. Serafini M, Bellocco R, Wolk A, Ekström AM. Total antioxidant potential of fruit and vegetables and risk of gastric cancer. Gastroenterology. 2002;123(4):985-991.
6. Puttaswamy R, Achur RN. The medicinal value of *Memecylon umbellatum* leaf extract. Journal of pharmacy research. 2013;6(4):447-451.
7. Puttaswamy R, Padukone S, Achur RN. Pharmacological and gross behavioral studies on *Memecylon terminale* Dalz, a medicinal plant from Western Ghats in southern India. World Journal of Pharmaceutical Sciences, 2013 Aug 23:81-92.
8. Baah KA, Acheampong A, Amponsah IK, Jibira Y, Osei Owusu M, Tima LA, *et al*. Evaluation of the anti-inflammatory, antimicrobial, and antioxidant activities of the stem bark extract of Croton penduliflorus Hutch (Euphorbiaceae). Journal of Pharmacognosy and Phytochemistry. 2017;6(6):1705-1710.
9. Trease GE, Evans WC. Pharmacognosy, 16th edition. W.B. Sanders Co Ltd., New York; c2009. p. 1-604.
10. Ajaiyeoba EO, Onocha PA, Olarenwaju OT. *In vitro* anthelminthic properties of *Buchholzia coriaceae* and *Gynandropsis gynandra* extracts. Pharmaceutical Biology. 2001;39(3):217-220.
11. Osei Akoto C, Acheampong A, Boakye YD, Asante B, Ohene S, Amankwah F, *et al*. Anthelminthic, Anti-inflammatory, Antioxidant Activities and FTIR Analyses of Vernonia camporum Stem-Bark. Journal of Chemistry. 2021;ID 3328073
12. Osei Akoto C, Acheampong A, Boakye YD, Takyi S, Garba R. Phytochemical screening and *in vitro* antioxidant and antimicrobial activities of the extracts of the stem-bark of *Psydrax peruviana*. Journal of Medicinal Plants Studies. 2019;7(5):28-34.
13. Mukhopadhyay D, Dasgupta P, Roy DS, Palchoudhuri S, Chatterjee I, Ali S, *et al*. A sensitive *in vitro* spectrophotometric hydrogen peroxide scavenging assay using 1, 10-phenanthroline. Free Radicals and Antioxidants. 2016;6(1):124-132.
14. Osei Akoto C, Acheampong A, Boakye YD, Naazo AA, Adomah DH. Anti-inflammatory, antioxidant, and anthelmintic activities of *Ocimum basilicum* (Sweet Basil) fruits. Journal of Chemistry; c2020. DOI: 10.1155/2020/2153534.
15. Sanchez-Moreno C, Larrauri JA, Saura-Calixto F. A procedure to measure the antiradical efficiency of polyphenols. Journal of the Science of Food and Agriculture. 76(2);270-276.
16. Wiegand I, Hilpert K, Hancock REW. Agar and Broth dilution methods to determine the minimum inhibitory concentration (MIC) of antimicrobial substances. Nature protocols. 2008;3(2):163-175.
17. Aiyeloja AA, Bello OA. Ethnobotanical potentials of common herbs in Nigeria: A case study of Enugu state. Educational Research Review. 2006;1(1):16-22.
18. Ayodele SQ. The effects of herbal remedies. Paper presented at the 12th Annual Conference of the Botanical Society of Nigeria (BOSON), University of Lagos; c2003. p. 21-29.
19. Hurrell R. Influence of vegetable protein sources on trace element and mineral bioavailability. Journal of Nutrition. 2003;133(9):2973S-2977S.
20. Elmastas M, Isildak O, Turkekul I, Temur N. Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. Journal of Food Composition and Analysis. 2007;20(3-4):337-345.
21. Akter KN, Karmakar P, Das A, Anonna SN, Shoma SA, Sattar MM, *et al*. Evaluation of antibacterial and anthelmintic activities with total phenolic contents of Piper betel leaves. Avicenna journal of phytomedicine. 2014;4(5):320.
22. Leelaprakash G, Dass SM. *In vitro* anti-inflammatory activity of methanol extract of Enicostemma axillare. International Journal of Drug Development and Research. 2011;3(3):189-196.
23. Jan S, Khan MR, Rashid U, Bokhari J. Assessment of antioxidant potential, total phenolics and flavonoids of different solvent fractions of *Monotheca buxifolia* fruit. Osong Public health and research perspectives. 2013;4(5):246-254.
24. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. Proceedings of the National Academy of Sciences. 1993;90(17):7915-7922.
25. Boniface PK, Ferreira SB, Kaiser CR. Recent trends in phytochemistry, ethnobotany and pharmacological significance of *Alchornea cordifolia* (Schumach & Thonn) Muell. Arg. Journal of Ethnopharmacology. 2016;191:216-244
26. Marchat L, Loiseau PM, Petek F. Purification and characterization of lactate dehydrogenase isoenzymes 1 and 2 from Molinema dessetae (Nematoda, Filarioidea). Parasitology Research. 1996;82:672-680.
27. Veerakumari L, Munuswamy N. *In vitro* effect of some anthelmintics on lactate dehydrogenase activity of *Cotylophoron cotylophorum* (Digenea, Paramphistomidae). Veterinary Parasitology. 2000;91:129-140.
28. Basha SN, Rekha R, Saleh S, Yemane S. Evaluation of *In vitro* anthelmintic activities of Brassica nigra, *Ocimum basilicum* and Rumex abyssinicus. Pharmacognosy Journal. 2011;*3*(20);88-92.
29. Rates SMK. Plants as source of drugs. Toxicon. 2001;*39*(5):603-613.
30. Hoste H, Torres-Acosta JFJ, Sandoval-Castro CA, Mueller-Harvey I, Sotiraki S, Louvandini H, *et al*. Tannin-containing legumes as a model for nutraceuticals against digestive parasites in livestock. Veterinary parasitology. 2015;212(1-2):5-17.
31. Niezen JH, Waghorn TS, Charleston WAG, Waghorn GC. Growth and gastrointestinal nematode parasitism in lambs grazing either lucerne (*Medicago sativa*) or sulla (*Hedysarum coronarium*) which contains condensed tannins. Journal of Agricultural Science. 1995;125:281-289
32. Acheampong A, Okyem S, Osei Akoto C, Baah AB. Antioxidant, antimicrobial, and FTIR analysis of

methanol root extract of *Cnestis ferruginea* and ethanol root extract of Citrus limon. Journal of Pharmacognosy and Phytochemistry. 2018;*7*(4):2938-2946.
33. Fleckenstein JM, Hardwidge PR, Munson GP, Rasko DA, Sommerfelt H, Steinsland H, *et al*. Molecular mechanisms of enterotoxigenic Escherichia coli infection. Microbes and infection. 2010;12(2):89-98.
34. Kurmukov AG. Phytochemistry of medicinal plants. In Medicinal Plants of Central Asia: Uzbekistan and Kyrgyzstan. New York, NY: Springer New York; c2012 Jul 20. p. 13-14.
35. Faizal A, Geelen D. Saponins and their role in biological processes in plants. Phytochemistry reviews. 2013;12(4):877-893.
36. Okuda T, Ito H. Tannins of constant structure in medicinal and food plants - Hydrolyzable tannins and polyphenols related to tannins. Molecules. 2011;16:2191-2217.
37. Yadav RNS, Agarwala M. Phytochemical analysis of some medicinal plants. Journal of phytology, 2011, 3(12).
38. Berlinck RG, Bertonha AF, Takaki M, Rodriguez JP. The chemistry and Biology of guanidine natural products. Natural product reports. 2017;34(11):1264-1301.
39. Sule WF, Okonko IO, Omo-Ogun S, Nwanze JC, Ojezele MO, Ojezele OJ, *et al*. Phytochemical properties and *in-vitro* antifungal activity of *Senna alata* Linn. Crude stem bark extract. Journal of medicinal plants research. 2011;5(2):176-183.