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In vitro anthelmintic, anti-inflammatory, antioxidant activities and FTIR analysis of *Sclerocarya birrea* root

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Abstract
Sclerocarya birrea, has been used as a medicinal plant in folkloric medicine for managing inflammation, stomach disorders, infections and metabolic disorders in Africa. The present study aimed to investigate the *in vitro* anthelmintic, anti-inflammatory and antioxidant activities of ethanol and aqueous root extracts (ERE and ARE) of *S. birrea*. Phytochemical screening was performed using standard methods. *In vitro* anthelmintic activity of both extracts was investigated against *Eudrilus eugeniae* (Earthworms). *In vitro* anti-inflammatory activity of both extracts was evaluated using egg albumin denaturation method. The antioxidant activity was determined by employing H_2O_2 scavenging assay, DPPH radical scavenging assay and total antioxidant capacity (TAC) phosphomolybdenum assay method. FTIR analysis was conducted on both the crude and the purified ethanol extracts. Column Chromatographic separation was performed on the ethanol extract using three different solvent systems of increasing polarity (chloroform, methanol and water) and six fractions (A to F) were collected. The anthelmintic activity of ARE and ERE at test concentrations was observed to be significantly (P<0.001) higher compared to albendazole-treated helminthes. The IC_{50} values of ERE and ARE were 24.66 ± 1.41 and 87.15 ± 2.23 µg/mL, respectively for DPPH radical scavenging assay. The IC_{50} values of ERE and ARE were 142.50 ± 1.67 and 881.90 ± 2.07 µg/mL respectively for H_2O_2 scavenging assay. TAC was determined to be 15.35 ± 2.066 and 22.56 ± 2.240 gAAE/100g for ERE and ARE, respectively. The phytochemical screening revealed the presence of saponins and terpenoids in both extracts, but only steroids in ERE. FTIR analysis indicated the presence of various functional groups in the crude and purified fractions of ERE that confirms the presence of the phytochemicals identified in the screening test. The results suggest that both extracts could be exploited as potential therapeutic candidate for the treatment of helmintic infections, inflammatory diseases and diseases associated with oxidative-stress.

Keywords: *Sclerocarya birrea*, anthelmintic, anti-inflammatory, antioxidant, phytochemical screening

1. Introduction
Natural products such as those from medicinal plants have been a source of medicinal agents for treating human diseases over the centuries in many parts of the globe. Natural products have found many applications in the fields of medicine, pharmacy, bioorganic chemistry, phytochemistry, organic synthesis and natural product chemistry among others. Many plants have been identified to exhibit antimicrobial, antioxidant and anti-inflammatory activities [1, 2]. A considerable number of currently used anthelmintic, anti-inflammatory, antioxidant, antitumor, antiinfectious agents are molecules identified and isolated from plants or their synthetic or semisynthetic derivatives [3]. Some natural compounds have been used as natural remedies to treat human diseases over centuries, though their structural characterization, molecular mechanism of action and exact dosage concentration are not always well understood and established.

Sclerocarya birrea commonly known in English as Marula is a common plant species found in Savannah zones. Its geographical distribution stretches from Gambia, Ghana and Nigeria in West Africa, across Cameroon in Central Africa, to Ethiopia and Sudan in East Africa and to South Africa. All parts of the plant are used: the fruits are eaten or processed into beer or jam; oils are extracted from the kernels; the leaves are used to feed livestock; the stem-bark, root, and leaf of *S. birrea* are used in treatment of human diseases [4]. The stem-bark, roots and leaves of *S. birrea* are used to treat numerous diseases in some African countries. The stem-bark is used for the treatment of diabetes mellitus in Cameroon [5]. In South Africa, the leaves and stem-bark are used to treat diarrhoea, dysentery, proctitis, stomach ailments, ulcers, inflammation, arthritis, hypertension, skin diseases, fever, malaria and diabetes mellitus [6-10]. The methanolic root extracts from *S. birrea* inhibited the growth of *Candida spp.* and

Cryptococcus neoformans. The leaves and roots are used to treat fungal infections and snake poison in Tanzania [11]. In Ghana, leaves are used to treat snakebite, the stem-bark, the root and the fruits are used to treat pharyngitis, splenomegaly and goitre, respectively [5].

Most research studies conducted on the pharmacological potential of *Sclerocarya birrea* have mainly focused on crude extracts of the leaves, fruits and stem-bark [4]. Nevertheless, it is also important to identify the bioactive compounds responsible for each one of the ascribed bioactivities, especially the roots in which at the time of carrying out this research next to nothing had been carried out on. In addition, there are no reports on the anthelmintic activity of this plant.

The aim of this study was to determine the anthelmintic, anti-inflammatory and antioxidant ethanolic and aqueous root extracts of *S. birrea* using *in vitro* assays. Additionally, to identify and confirm the presence of the phytochemicals in the root extract using FTIR analysis.

2. Materials and Methods

2.1. Sample Collection and Identification

The roots of *S. birrea* were collected in the month of October, 2018 at Ejura (latitude: 7º23'0" N and longitude: 1º22'0" W) in the Ashanti region of Ghana. They were taxonomically identified and authenticated at the Department of Herbal Medicine, KNUST, Kumasi, Ghana by Mr. Clifford Osafo Asare, with the voucher specimen (KNUST/HMI/2019/R002) deposited in the herbarium of the same Department 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 roots of *S. birrea* were thoroughly washed first under running water and then distilled water. The roots were further cut into small pieces, air dried under shade for two weeks, pulverized into coarse powder, and stored in a desiccator until analysis.

2.3.1. Preparation of Ethanol Extract of *S. birrea*

A mass of 150 g of the powdered sample of S. birrea was soaked in 1 dm^3 of ethanol and extracted using the soxhlet apparatus. The ethanolic root extract (ERE) was condensed and evaporated to dryness using the rotary evaporator at 50 ºC (BUCHI Rota vapor R -114). The ERE was dried and the percentage yield of extract with respect to powdered plant material determined. The ERE was then stored at 4 ºC in a refrigerator.

2.3.2. Preparation of Aqueous Extract of *S. birrea*

Decoction was carried out according to the method described by Virginie *et al*., with minor modifications [12]. A mass of 300 g of the powdered sample of *S. birrea* was soaked in 3 dm^3 of distilled water and the decoction was carried out with stirring in a water bath at 100 ºC for 2 hours. It was cooled and filtered to obtain the aqueous root extract (ARE). The ARE was oven dried to dryness and the percentage yield of extract with respect to powdered plant material determined. ARE was then stored at 4 ºC in a refrigerator.

2.4. Phytochemical Screening of Extracts

The pulverized sample and the crude extracts (ERE and ARE) obtained were screened to assess the presence of phytoconstituents using the methods described by Trease and Evans [13].

2.5. Collection of *Eudrilus eugeniae* for Anthelmintic Activity

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* Anthelmintic Assay

In-vitro anthelmintic activity against *Eudrilus eugeniae* (Earthworms) of both ARE and ERE were examined. The anthelmintic assay was carried out using a modification of the standard methods by Adu *et al.* [1, 14]. Stock solutions of 12 mg/mL of ARE and ERE were prepared using sterile distilled water as a solvent. From the stock solutions, six other concentrations of 6.0, 3.0, 1.5, 0.75, 0.375, and 0.1875 µg/mL were prepared by serial dilution. A concentration of 10.0 mg/mL of albendazole was used as reference drug and normal saline (0.9 %w/v) as a negative control. All test solutions and reference drug solutions were prepared freshly 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 concentrations. 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 when dipped in warm water (50 ºC) followed with 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* Anti-inflammatory Assay by Egg Albumin Denaturation

Anti-inflammatory assay was carried out according to a modification of the standard methods by Kumari *et al*., [15]. Stock solutions of 1600 µg/mL of both ARE and ERE were prepared by using distilled water as a solvent. From the stock solutions, various concentrations of 100, 200, 400 and 800 µg/mL were prepared using distilled water as a solvent. The reaction mixtures of total volume 5 mL were prepared by dissolving 0.2 mL of egg albumin (from egg of a hen), 2.8 mL of phosphate buffer saline (PBS, pH 6.4) and 2 mL of the various concentrations of extract solutions. A volume of 2 mL of 200 µg/mL of diclofenac sodium was used as the standard reference drug and 2 mL of double distilled water solution served as negative control. The mixtures were incubated at 37 ºC in Bio-Oxygen Demand (BOD) incubator for 15 minutes. The mixtures were then heated in a water bath at 70 ºC for 5 minutes to induce denaturation. The absorbance of the solutions was measured in triplicate at 660 nm using UV-vis spectrometer. The procedure was independently repeated to obtain three independent sets of data for the analysis in triplicate.

The percentage inhibition of protein denaturation was calculated as follows:

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

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

2.7. *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 and the Total Antioxidant Capacity assays.

2.7.1. DPPH radical Scavenging Assay

The free radical scavenging activity of ARE and ERE were examined according to a modification of the standard methods previously described [1, 16]. Ascorbic acid was used as reference standard. The absorbances were measured at 517 nm. The experiment was independently repeated to obtain three independent sets of data for the analysis. 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

2.7.2. Hydrogen Peroxide Scavenging Assay

Determination of hydrogen peroxide scavenging potential of ARE and ERE was carried out according to a modification of the standard methods previously described [1, 17]. The assay is based on specific complexation of ferrous ion with 1,10-phenanthroline to form red-orange tri-phenanthroline complex. Gallic acid was used as reference standard. Absorbance was taken at 510 nm using 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 experiment was independently repeated to obtain three independent sets of data for the analysis. The percentage scavenging activity was calculated using the formula below

$$\%\text{ Scavenging} = \frac{\text{A}_{\text{test}}}{\text{A}_{\text{control}}} \text{x } 100\%$$

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}.

2.7.3 Total Antioxidant Capacity (TAC) assay

A modification of the methodology as previously described was used to study the total antioxidant capacity of the ARE and ERE of *S. birrea* [1, 18]. 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 reference standard and distilled water was used as the blank. The absorbance of the solutions was measured in triplicate, using the UV-visible spectrophotometer at 695 nm. The experiment was independently repeated to obtain three independent sets of data for the analysis. From the linear equation of the ascorbic acid concentration-absorbance plot, the corresponding independent variables as ascorbic acid equivalents (AAE) were determined, and the results expressed as gAAE/100g ascorbic acid.

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 ERE and ARE about 1 cm from the bottom edge of plates, with the aid of capillary tubes and allowed to dry [1, 19]. Various solvent systems of methanol, chloroform and ethyl acetate in the ratio of 1:1:1, 5.0:2.5:2.5, 8:0:2 and 10:0:0 respectively were used. The ratio of 10:0:0 (i.e. 100% methanol) gave the best separation of components for the ARE, whereas the 80:0:20 (methanol, chloroform and ethyl acetate respectively) gave the best separation of components for the ERE. 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:

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

This solvent system was then adapted and used in Column Separation.

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 [20]. A mass of 2 g of dry powdered ERE was chromatographed on a column packed with silica gel and eluted with a gradient of solvents $CHCl_3$/MeOH/H_2O (8/42/0; 0/50/0; 0/25/25 50 mL each) to provide 6 fractions, namely A to F. The fractions were monitored by means of TLC (eluent MeOH/EtOAc 4:1). Fractions A, B, D, E and F were positive to the presence of saponins and C to steroids. The fractions were evaporated to dryness using the rotary evaporator, then dried and stored at 4 °C in a refrigerator until their use.

2.10 Fourier Transform Infrared Spectrometer (FTIR) Analysis

The dried fractions (A-F) were subjected to (FTIR) analysis equipment (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 4.12 and 2.13 % for ERE and ARE, respectively.

3.2. Phytochemical Screening

Pharmacological activity of a plant depends on the phytochemical composition of the plant [13]. Of the eleven phytochemicals tested on *S. birrea*, only saponins, terpenoids

and steroids were present in the ERE. Only saponins and terpenoids were identified in ARE (Table 1).

Table 1: Phytochemical constituents of the pulverized sample and the extracts of *S. birrea*

Phytochemical	Pulverized Sample	ARE*	ERE**
Saponins	+	+	+
Tannins	-	-	-
Alkaloids	-	-	-
Glycosides	-	-	-
Terpenoids	+	+	+
Phenols	-	-	-
Flavonoids	-	-	-
Carotenoids	-	-	-
Anthraquinone	-	-	-
Steroids	+	-	+
Coumarins	-	-	-

Key: (+) = presence of secondary metabolite; (-) = absence of secondary metabolite
*ERE – Ethanol root extract of *S. birrea*; **ARE – Aqueous root extract of *S. birrea*

The extracts had two phytochemicals in common, that is saponins and terpenoids, but ERE had steroids in addition. Secondary metabolites of plants which include saponins, terpenoids, and steroids have been shown to possess anti-inflammatory activity [21]. The inhibitory effect of saponins on inflamed cells was revealed by Just *et al.* [22]. Saponins have a wide range of pharmacological properties, including anti-inflammatory, antifungal, molluscicidal and antiparasitic [23]. Saponins found in *Z. joazeiro* have bioactive constituents with potential anthelmintic activity in goats due to the inhibitory action on the hatching of gastrointestinal nematodes (GIN) eggs [24]. Betulinic acid (BA), a triterpenoid found in the bark of Zizyphus joazeiro has been shown to exhibit a variety of biological activities including anthelmintic, anti-inflammatory, antioxidant, antibacterial, antimalarial properties and the inhibition of the replication of the human immunodeficiency virus (HIV) [25]. The presence of these phytochemicals in the ERE and ARE of *S. birrea* indicate that they will play a key role in the prevention of various helminthic infections, inflammatory and diseases associated with oxidative-stress.

3.3. Anthelmintic Activity

Anthelmintic potency of the extracts was 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 [26]. ERE and ARE displayed concentration-dependent anthelmintic activity at concentrations of 12.00 to 0.1875 mg/mL (Figures 1 and 2). The anthelmintic activity of ARE and ERE at test concentrations was observed to be significantly (P<0.001) higher compared to albendazole-treated worms.

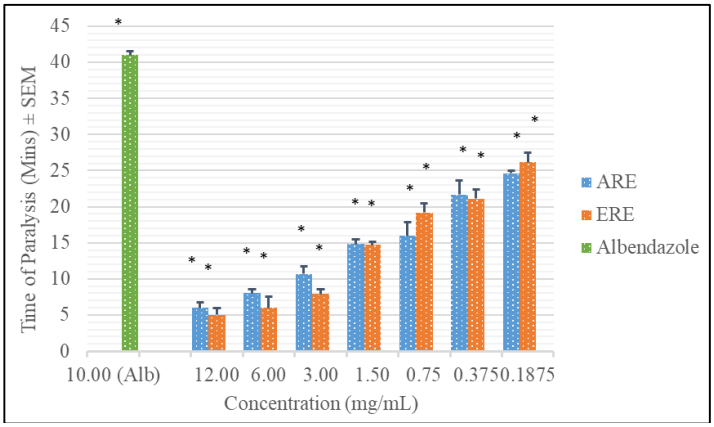

Fig 1: Anthelmintic activity (Paralysis time vs Concentration) results for ERE and ARE of *S. birrea* and Albendazole (reference standard)

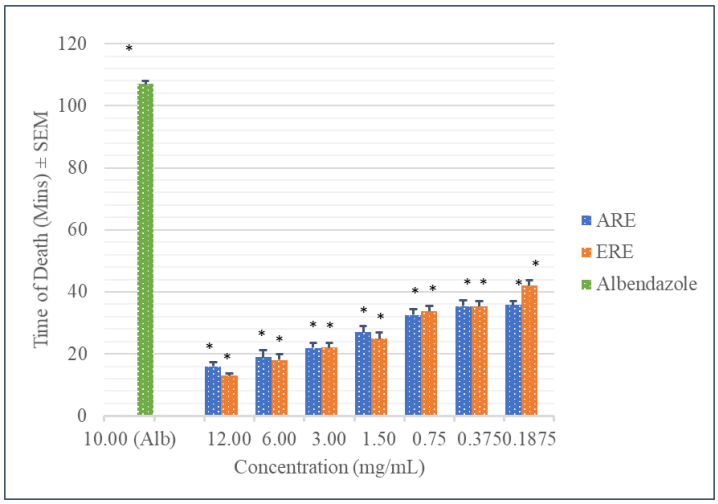

Fig 2: Anthelmintic activity (Death time vs Concentration) results for ERE and ARE of *S. birrea* and Albendazole (reference standard) the worms in the negative control (saline water) were alive after 24 hours. Each time represents mean ± SEM (N=3). The data were analysed using ONE-WAY ANOVA compared 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 [14]. The extracts not only exhibited such potency, but also caused death of the worms at a rate much higher than the reference drug albendazole. The high potency of the extracts could be attributed to the presence of saponins identified in this work which could be exploited as therapy for the treatment of helmintic infection due to their proposed ability to disrupt membrane integrity and make them more permeable [24].

3.4. *In vitro* Anti-inflammatory Assay (Egg albumen denaturation method)

Denaturation of proteins is a well-documented cause of inflammation and rheumatoid arthritis. Several anti-inflammatory drugs have shown concentration-dose-dependent ability to inhibit thermally induced protein denaturation. The ability of plant extract to inhibit thermal denaturation of protein (egg albumin) is a reflection of its anti-inflammatory activity [27].

At the concentration of 200 µg/mL, percentage inhibition of ARE, ERE and the reference drug were 40.94 ± 0.509, 27.22 ± 0.320 and 73.87 ± 0.450% respectively as shown in Figure 3. The anti-inflammatory activity shown by the extracts could be attributed to the presence of saponins, terpenoids and steroids in the ARE, ERE of *S. birrea* which have been reported to exhibit anti-inflammatory activity [28].

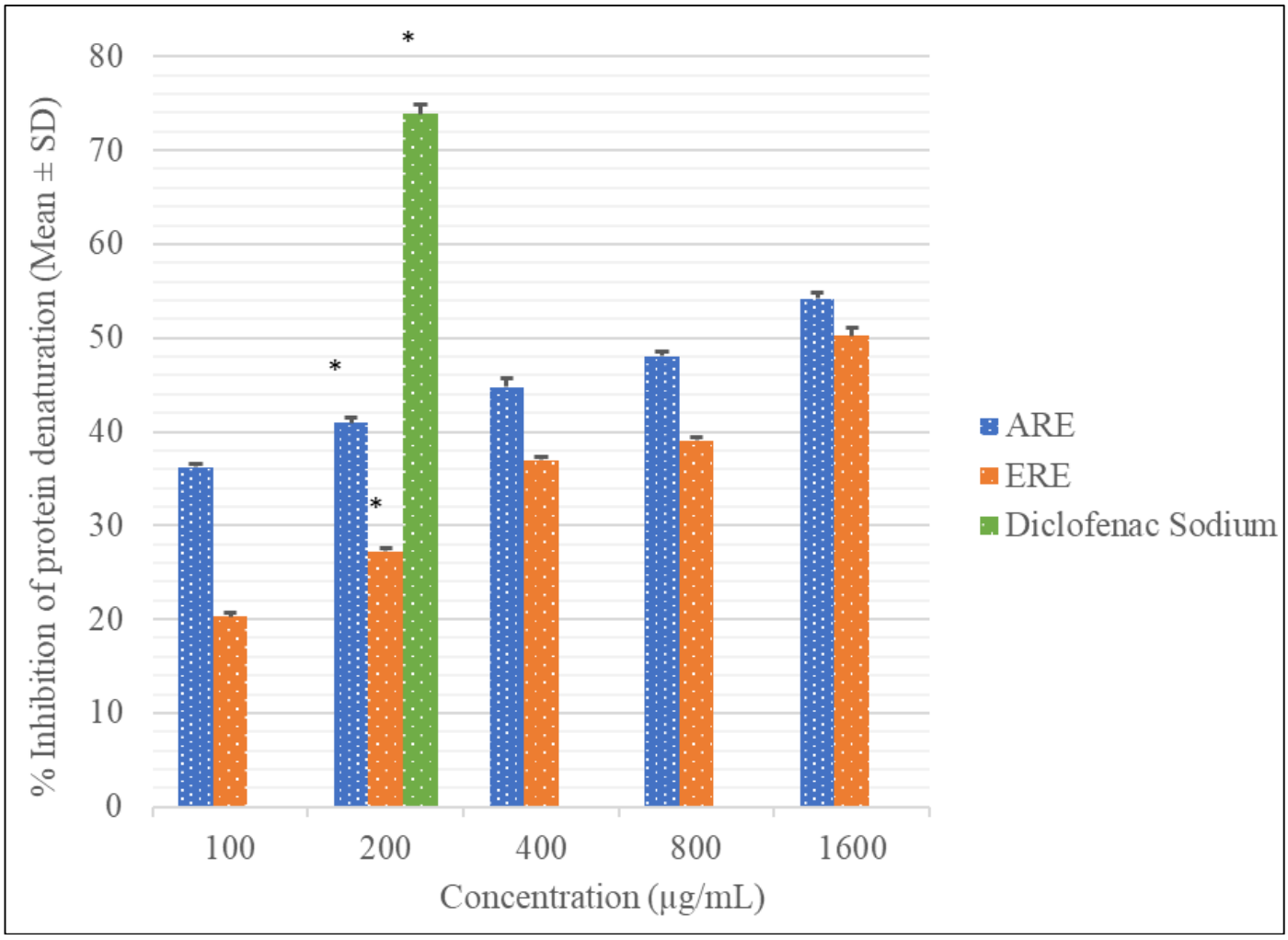

Fig 3: Anti-inflammatory activity results for ERE and ARE of *S. birrea*

Results were expressed as mean (n = 3) ± standard deviation; The data were anaylsed using one-way ANOVA compared to diclofenac (reference drug). * $P < 0.001$.

3.5. *In vitro* Antioxidant Activity

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 determined and understood by only one method [29]. 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.

3.5.1. DPPH Radical Scavenging Capacity

The DPPH scavenging activity of the extracts was used to determine and study the ability of the ERE and ARE of *S. birrea* to mop up free radicals that may be found in animals and humans. ERE, ARE of *S. birrea* and ascorbic acid (reference standard) scavenged DPPH at concentrations ranging between 31.25 to 500.00 µg/mL as shown in Figure 4.

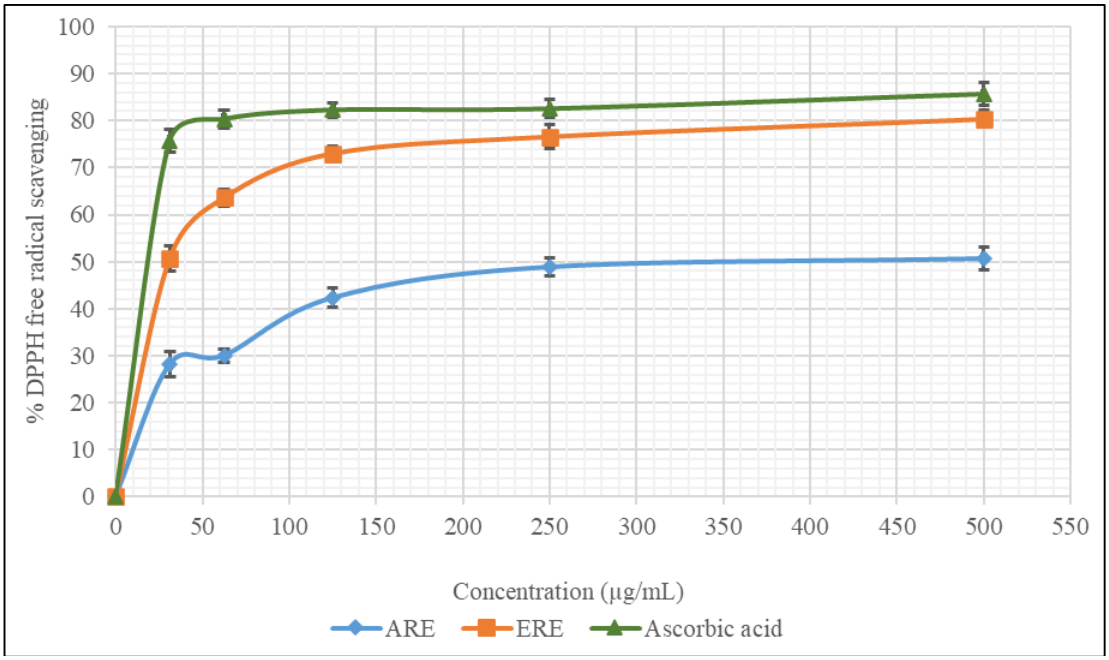

Fig 4: Comparative DPPH radical scavenging activity of the ARE, ERE, of *S. birrea* and ascorbic acid.

The reference antioxidant (ascorbic acid), ERE, and ARE of *S. birrea* showed varying antioxidant activity in the DPPH free radical scavenging assay with IC_{50} ranging from 13.34 ± 1.880 to 87.15 ± 2.230 µg/mL, as shown in Table 2.

Table 2: IC_{50} of DPPH Radical Scavenging Activity for ARE, ERE of *S. birrea* and Ascorbic Acid

Sample	IC_{50} (µg/mL)
Aqueous Root Extract (ARE)	87.15 ± 2.230
Ethanolic Root Extract (ERE)	24.66 ± 1.410
Standard (Ascorbic Acid)	13.34 ± 1.880

The results implied that the effectiveness of the test samples of extracts as antioxidants decreased in the order as follows: ascorbic acid > ERE > ARE (Figure 4). Though ERE and ARE which comprise of a mixture of compounds showed comparable bioactivity as the ascorbic acid, *S. birrea* root 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.

3.5.2 Hydrogen Peroxide Scavenging Assay

Non-radical oxidizing agents scavenging potential of the ARE and ERE of *S. birrea* were evaluated by the use of hydrogen peroxide (H_2O_2) scavenging method. The results are shown in Figure 5.

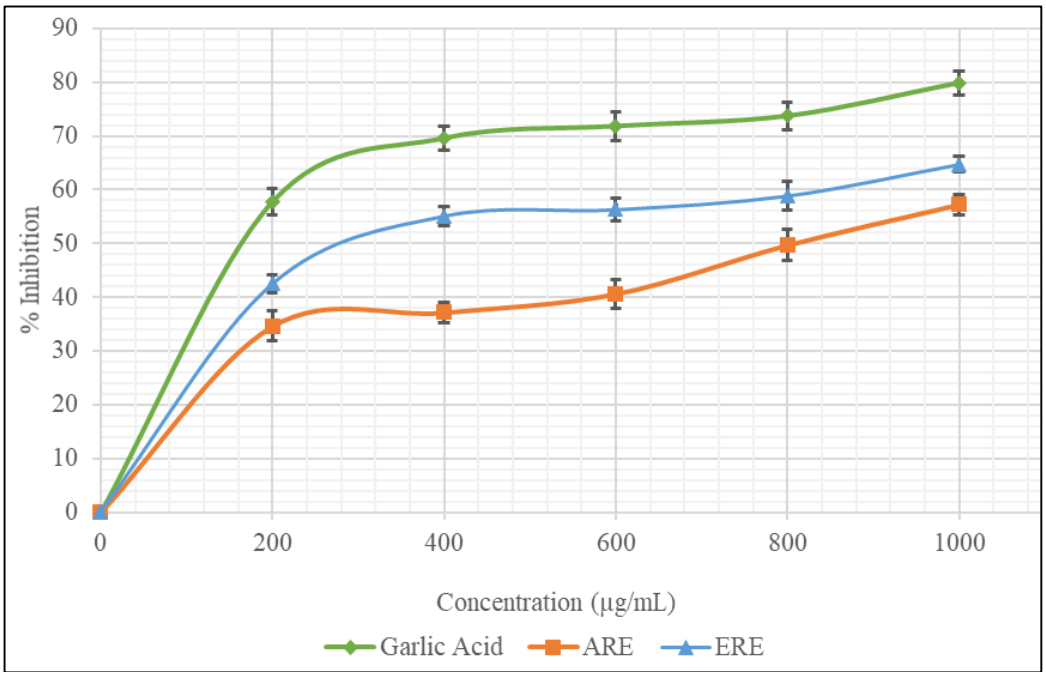

Fig 5: Hydrogen Peroxide scavenging capacity of ARE, ERE and Gallic acid Results were expressed as mean (n = 3) ± standard deviation; P < 0.001

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 [30]. Results showed that, both ARE and ERE demonstrated a significant antioxidant activity in concentration-dose dependent manner. The IC_{50} values ranged from 142.50 ± 1.672 to 881.90 ± 2.070 µg/mL as shown in Table 3.

Table 3: IC_{50} of Hydrogen Peroxide Radical Scavenging Activity of extracts of *S. birrea*

Sample	IC_{50} (µg/mL)
Aqueous Root Extract (ARE)	881.90 ± 2.070
Ethanolic Root Extract (ERE)	142.50 ± 1.672
Standard (Gallic Acid)	203.50 ± 2.087

From the results ERE was a more effective antioxidant than ARE and gallic acid (reference standard) although, they are all good antioxidants. Bioactive isolates from these extracts responsible for antioxidant activity could be attributed to the triterpenoid saponins present in *S. birrea* and could be exploited for the treatment of diseases associated with oxidative-stress [25].

3.5.3. 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 electron to free reactive radicals and thus quench their reactivity. Ascorbic acid is also known for its hydrogen donating ability [31].

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 6).

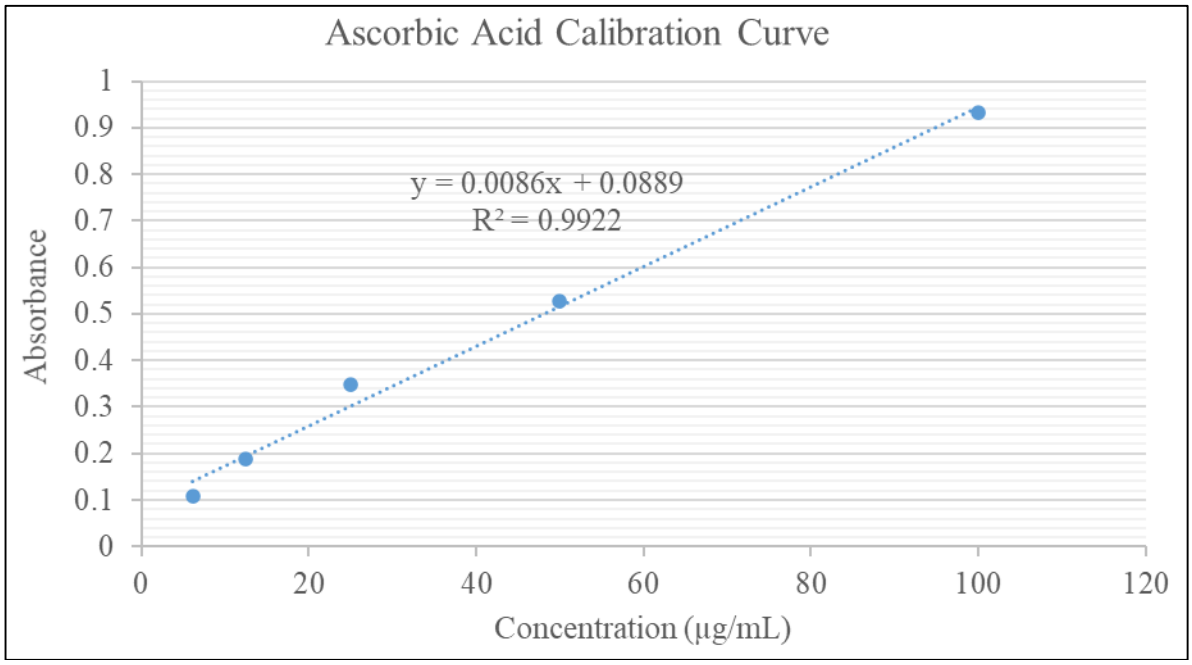

Fig 6: Mean absorbance of $PMo^{V}_{4}Mo^{VI}_{8}O_{40}^{7-}$ (formed in ascorbic acid solution) against concentration of ascorbic acid solution.

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 ARE and ERE had 15.35 ± 2.066 and 22.56 ± 2.240 gAAE/100g respectively, (Table 4).

Table 4: Total antioxidant capacity of ARE and ERE expressed as gAAE

Sample	TAC[a] (gAAE[b]/100g)
Aqueous Root Extract (ARE)	15.35 ± 2.066
Ethanolic Root Extract (ERE)	22.56 ± 2.240

[a]TAC - Total Antioxidant Capacity; [b]AAE - Ascorbic Acid Equivalent

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

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 5 gives the results of the TLC analysis of extract showing various components and their retardation factor using methanol/ethyl acetate (4:1) as mobile phase.

Table 5: Constituents of *S. birrea* extracts as determined using TLC.

Components	Retardation factor, R_f	
	ERE	ARE[a]
A	0.13	0.13
B	0.75	0.77
C	0.77	0.84
D	0.89	ND

ND - Not detected. [a]ARE showed only 3 spots

The ERE and ARE showed four and three distinct spots with R_f values between 0.13 to 0.89 and 0.13 to 0.84 respectively.

The number of spots indicating the separated components in both the ERE and ARE were more when compared to the phytoconstituents identified to be present in each root extract. This means that some of the components could exist as different isomers or have similar functional groups and it may be necessary to employ two dimensional TLC, column chromatography or High Pressure Liquid Chromatography (HPLC) to achieve complete separation of the components.

3.7. Column Chromatographic Separation

Column chromatography was employed to separate the components of the ERE. Six fractions namely A to F were separated after elution with a gradient of solvents $CHCl_3$/MeOH/H_2O (8/42/0; 0/50/0; 0/25/25) as indicated in Table 6.

Table 6: Chromatographic separation of the fractions of constituents of *S. birrea* ERE

Components	Retardation factor, R_f
A	0.89
B	0.89
C	0.89
D	0.77
E	0.75
F	0.13

The column chromatography results confirm the analytical TLC results of the components either being existed as different mixtures, isomers or co-eluted. The fractions were monitored by means of TLC (eluent, MeOH/EtOAc 4:1).

3.8 Phytochemical Screening of Purified Fractions

The purified fractions from column chromatography were subjected to phytochemical screening to ascertain the various bioactive components in the ERE of *S. birrea*, Table 7.

Table 7: Phytochemical Screening result for collected fractions of ERE of *S. birrea*

Components	Saponins	Terpenoids	Steroids
A	+	-	-
B	+	-	-
C	-	-	+
D	+	-	-
E	+	-	-
F	+	-	-

Fractions A, B, D, E and F were positive to the presence of saponins and C to steroids. This result corroborates with the earlier screening of the crude ERE albeit fractions presents as terpenoidal saponins, steroidal saponins, or saponins require further investigations. Ongoing research is on the separation of all the components present in the extracts that will lead to isolation, purification, identification and bioactivity determination of each component. The presence of these identified secondary metabolites supports the fact that the root of *S. birrea* could be exploited as a therapeutic candidate for helmintic infections, inflammatory diseases and diseases associated with oxidative-stress. This results also give credence to the ethnomedicinal uses of the root of *S. birrea* against human ailments [32].

3.9. FTIR Analysis

FTIR analysis was performed on both the crude ARE and ERE as well as the purified components obtained from ERE column chromatographic separation. The results were presented in Figures 7 to 13 and Tables 8 to 11 below.

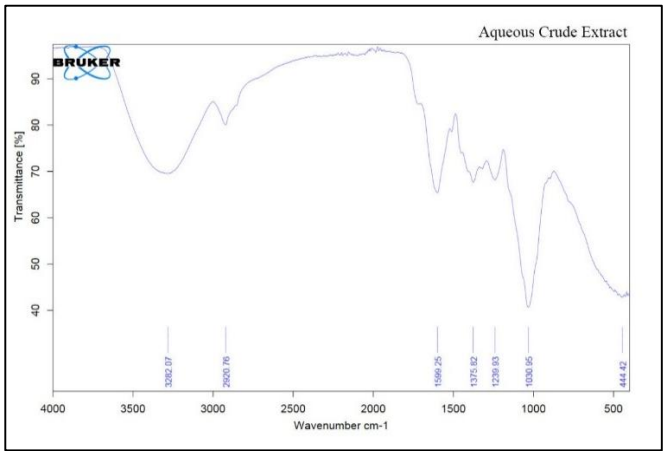

Fig 7: FTIR spectrum of the crude ARE of *S. birrea*

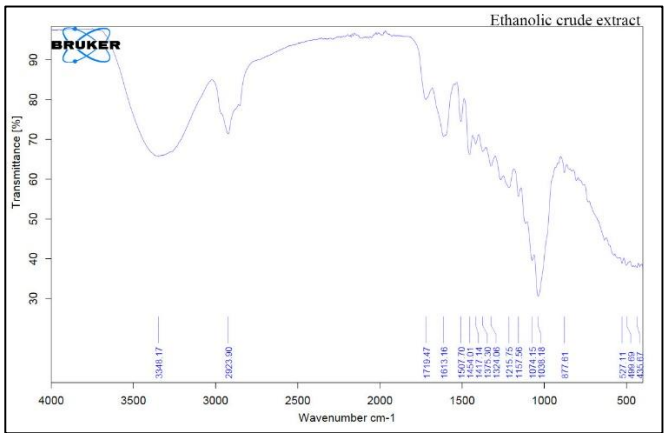

Fig 8: FTIR spectrum of the crude ERE of *S. birrea*

Table 8: FTIR Interpretation of the crude ERE and ARE of *S. birrea*

S. birrea ERE			*S. birrea* ARE		
Peak value (cm^{-1})	**Functional group**	**Inference**	**Peak value (cm^{-1})**	**Functional group**	**Inference**
3348	O-H stretch	These functional groups confirm the presence of saponins, steroids and terpenoids identified in the screening test.	3282	O-H stretch	These functional groups confirm the presence of saponins, and terpenoids identified in the screening test.
2923	C-H stretch		2920	C-H stretch	
1719	C=O carbonyl group		1599	C=C stretch	
1613, 1507	C=C stretch		1375, 1030	C-O stretch	
1417, 1375, 1215, 1038	C-H, C-C, C-O bend / stretch		1239	C-C stretch	

The wave numbers around 3348 to 3282 cm^{-1}, 2923 to 2920 cm^{-1}, 1719 cm^{-1}, 1613 to 1507 cm^{-1}, and 1417 to 1030 cm^{-1} positions of the spectra are characteristic of alcohol (O-H), aliphatic (C-H), carbonyl (C=O), olefinic/aromatic (C=C)

stretches and C-O, C-C, C-H stretching, bending functional groups. These functional groups confirm the presence of saponins, steroids and terpenoids identified in the screening test, as shown in Table 1.

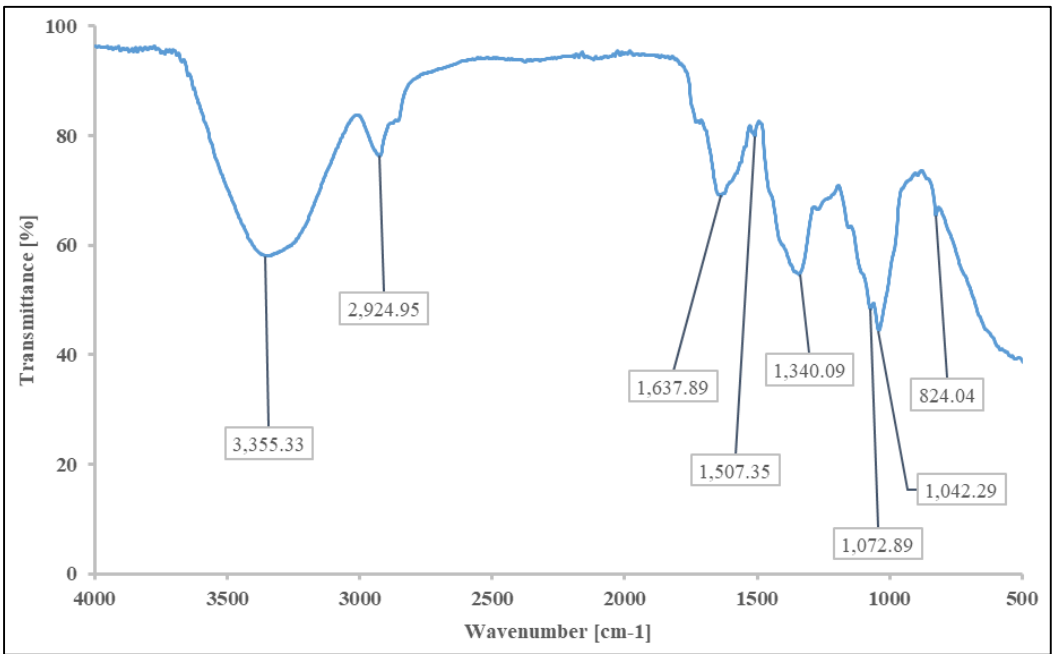

Fig 9: FTIR spectrum of Fraction A from the ERE of *S. birrea*

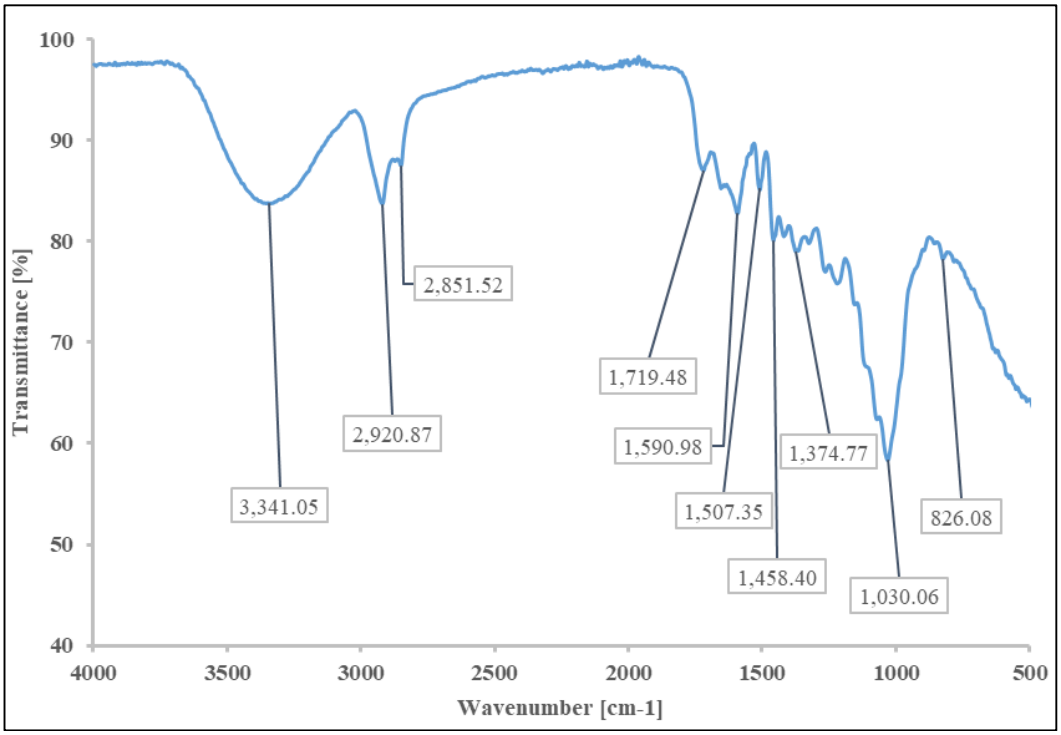

Fig 10: FTIR spectrum of Fraction B from the ERE of *S. birrea*

Table 9: FTIR Interpretation of Purified Fractions A and B of ERE of *S. birrea*

Fraction A			Fraction B		
Peak value (cm^{-1})	**Functional group**	**Inference**	**Peak value (cm^{-1})**	**Functional group**	**Inference**
3355.33	O-H stretch	Absence of C=O stretch for carbonyl functional group which is present in Fraction B.	3341.05	O-H stretch	Presence of C=O stretch for carbonyl functional group which is absent in Fraction A.
2924.95	C-H stretch		2920.87, 2851.52	C-H stretch C-H stretch	
1637.89	C=C stretch		1719.48	C=O stretch	
1340.09	C-O, C-H bend		1590.98	C=C stretch	
1042.29	C-C stretch		1374.77, 1030.06	C-C, C-O stretch	

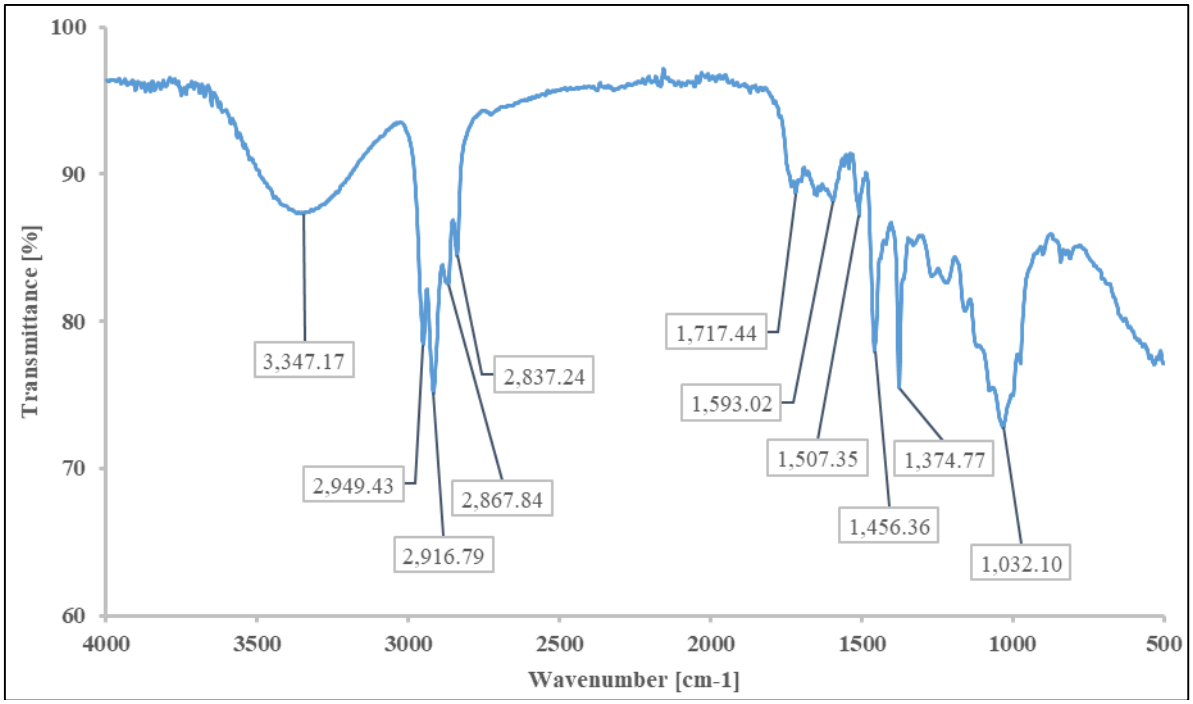

Fig 11: FTIR spectrum of Fraction D from the ERE of *S. birrea*

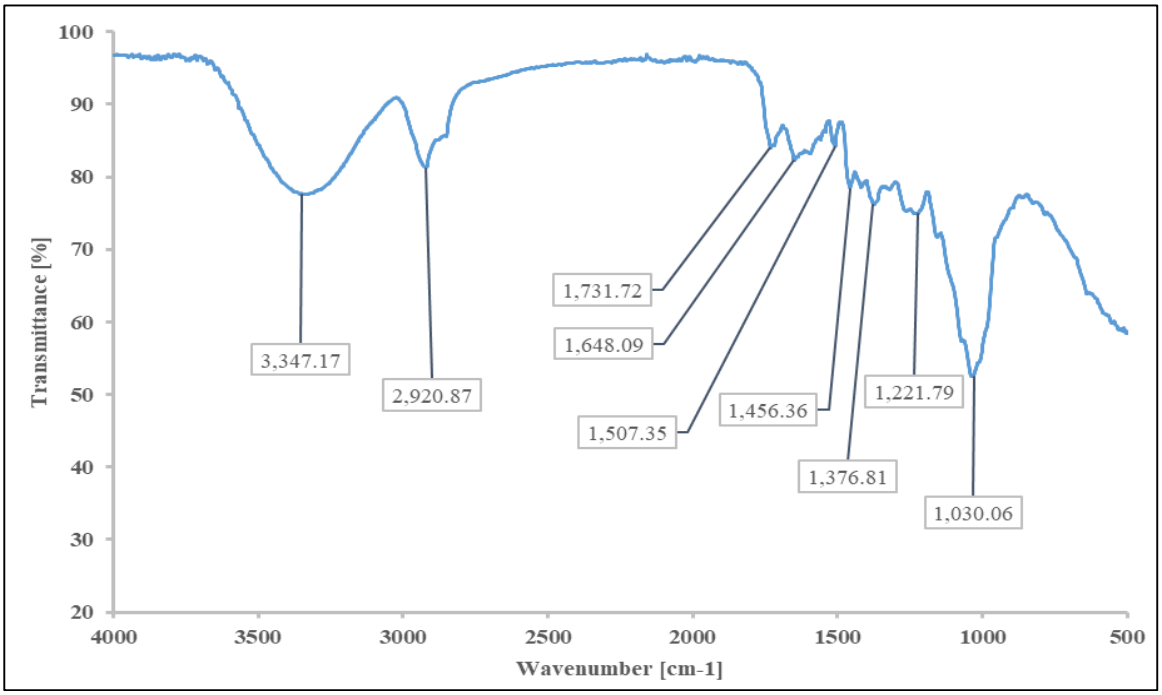

Fig 12: FTIR spectrum of Fraction E from the ERE of *S. birrea*

Table 10: FTIR Interpretation of Purified Fractions D and E of ERE of *S. birrea*

Fraction D			Fraction E		
Peak value (cm^{-1})	**Functional group**	**Inference**	**Peak value (cm^{-1})**	**Functional group**	**Inference**
3347.17	O-H stretch	Presence of splitting C-H stretching for saturated sp^3 C-H functional groups are absent in Fraction E.	3347.17	O-H stretch	Presence of a weak C-H stretch for saturated sp^3 C-H functional group which is not observed in Fraction D.
2916.79, 2837.24	C-H stretch C-H stretch		2920.87	C-H stretch	
1717.44	C=O stretch		1731.72	C=O stretch	
1593.02, 1507.35	C=C stretch C=C stretch		1648.09, 1507.35	C=C stretch C=C stretch	
1456.36, 1374.77	C-O, C-H bend		1456.36, 1376.81	C-O, C-H bend	
1032.10	C-C, C-O stretch		1221.79, 1030.06	C-C, C-O stretch	

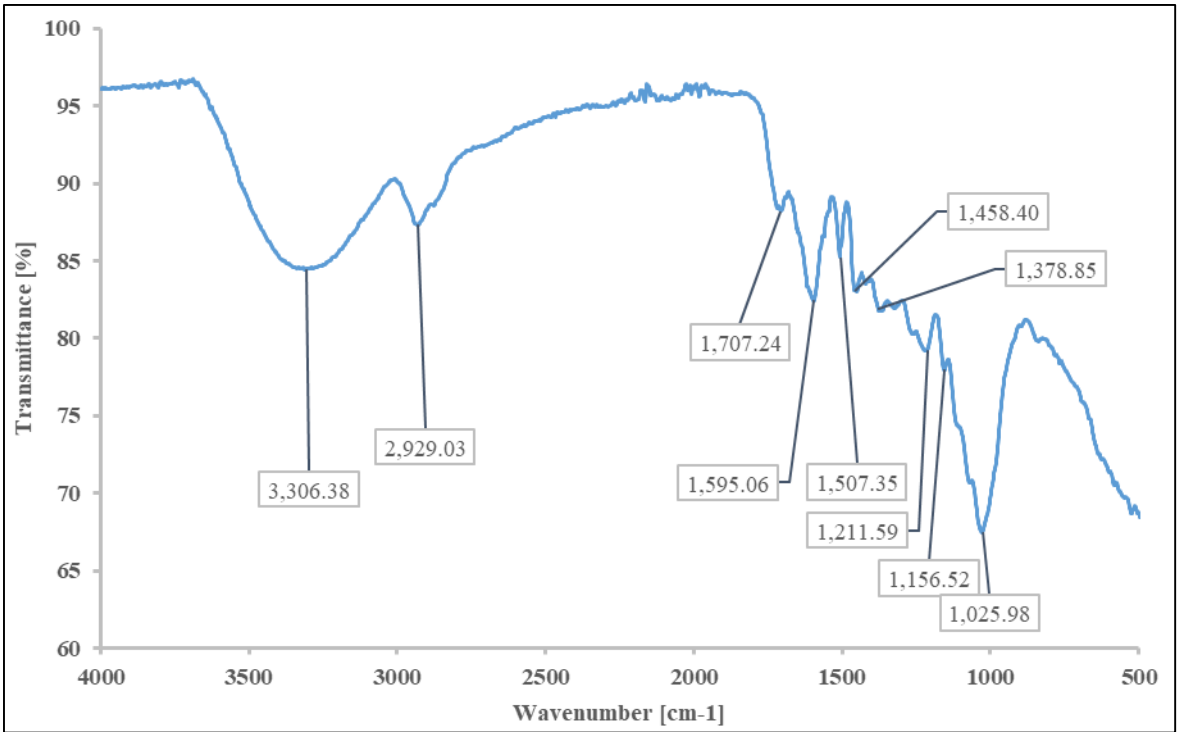

Fig 13: FTIR spectrum of Fraction F from the ERE of *S. birrea*

Table 11: FTIR Interpretation of Purified Fraction F of ERE of *S. birrea*

Fraction F		
Peak value (cm^{-1})	**Functional group**	**Inference**
3306.38	O-H stretch	The observed functional groups confirm the presence of saponin identified in the screening test. The Fraction F, is a mixture of E and the most hydrophilic component.
2929.03	C-H stretch	
1707.24	C=O stretch	
1595.06, 1507.35	C=C stretch	
1458.40, 1211.59, 1025.98	C-H, C-C, C-O bend / stretch	

Similar wave numbers ranging 3355 to 3306 cm^{-1} (Figures 9-13 and Tables 9-11) were found in all the fractions (A, B, D, E and F) showing characteristic broad peaks representing alcohol and or phenol (OH) functional groups. This is typical of saponins representing the glycosidic chains with multiple O-H functional groups. Sharp peaks (Figures 9-13 and Tables 9-11) indicating C-H stretching frequencies were showed around 2949 cm^{-1} to 2837 cm^{-1}. This is typical of the saturated sp^3 C-H stretching frequencies for the aglycone and glycosidic backbone of the identified saponins. Weak Carbonyl (C=O) with wave numbers around 1731 to 1707 cm^{-1} showed in the spectrum of Fractions (B, D, E, and F), are typical of a common keto-enol functionality in the aglycones of saponins. Aromatic, alkene and or enol carbon double bonds (C=C) with wave numbers around 1648 to 1507 cm^{-1} showed in all the spectrum of the Fractions, confirms a typical functional group in the aglycones of saponins. The wave numbers around 1458 to 1340 cm^{-1}, 1221 to 1211 cm^{-1} and 1072 to 1025 cm^{-1} positions of the spectrums are characteristic C-H bending, C-C stretch, and C-O stretching frequencies [33].

The FTIR analysis of all the fractions A, B, D, E and F confirms the identified secondary metabolite saponins from the screening test.

Conclusions

The aqueous (ARE) and ethanol (ERE) root extracts of *S. birrea* exhibited anti-inflammatory, antioxidant and anthelminthic activity which may be due to the phytochemicals identified. However, a higher activity was observed in the ERE than ARE. FTIR studies on the purified fractions revealed the presence of alcohol, carbonyl, ether, carbon-carbon double bonds and aliphatic carbon-carbon/hydrogen/oxygen functional groups characteristics of identified saponins which may be therapeutically responsible for the medicinal use of *S. birrea*. Work is thus ongoing in our laboratory towards isolation, characterization, identification and biological activity of anthelmintic, anti-inflammatory and antioxidant compounds present in the roots of *S. birrea*.

Data Availability

The data used to support the findings of this study are included within the article.

Disclosure

Part of this work was presented as a poster at the "8th Ghana Science Association, Research Seminar and Poster Presentations" and "8th College of Health Sciences & 12th Convention of Biomedical Research Ghana joint Scientific Conference held at the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, in May and July 2019.

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.

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