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Antimicrobial, antioxidant, anthelmintic and anti-inflammatory bioactivities of *Sabicea calycina*

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ABSTRACT

Sabicea calycina is broadly used for the treatment of different diseases including sexually transmitted infection, gastrointestinal disorders, wound treatment, and wound dressing. This study is focused on assessing the biological activity (anthelmintic, antioxidant, antimicrobial, and anti-inflammatory) studies of methanol and hexane leaf crude extracts of *S. calycina* on its ethno medicinal uses. Phytochemical analyses revealed the presence of six phytoconstituents in the methanolic extract and by demonstrating four in the hexane extract. *In vitro*, the anthelmintic activity of the crude extracts was examined against *Milsonia ghanensis* (Earthworms) using albendazole as the standard drug. The anthelmintic activity of methanol and hexane extracts at test concentrations was observed to be significantly higher compared to albendazole. The methanolic extract was comparatively the more potent antioxidant as ascertained by its relatively higher antioxidant capacity TAC and its disproportionately lower for the DPPH and H₂O₂. Regularly for each of the group of four bacterial and one fungal pathogenic microbial cell lines, the methanolic extract showed higher antimicrobial activity values, MICs that were quantitatively lower than that of the hexane extract. *In vivo* anti-inflammatory activity of methanol and hexane extracts was assessed against diclofenac as a reference drug, utilizing the carrageenan-induced chick foot edema method using 7-day old chicks. This method indicated the methanol extract showing a dose-dependent decreasing in foot edema and the hexane extract lack of showing measurable anti-inflammatory activity. The results show that the extracts could be employed as a remedial contender for the treatment of helminthic, oxidation, microbial and inflammatory diseases.

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Keywords: *Sabicea calycina*, phytochemical, antimicrobial, antioxidant, antimicrobial and anti-inflammatory.

INTRODUCTION

Sabicea calycina is a plant that belongs to the Rubiceae family (Soladoye et al., 2005). *Sabicea* is used in southern Nigeria as a laxative and is drunk in palm wine. The ground leaves are applied to the limbs of small children to strengthen their bones and help them walk. The crushed leaves are also applied to cuts and wounds. An infusion of the leaves is believed to be good for memory. A member of the

genus, probably *S. Africana*, is used for the treatment of senile dementia. It is an ingredient in the Yoruba agbo medicine. African Names - Ashanti: ananse, ananse ntoroma homa; Basa: gor-vah (*S. lasiocalyx*); Mende: namatei; Yoruba: jiri, ogan-aparo (Borokini and Omotayo, 2012). *Sabicea calycina* is well reported, nearly nothing is known about its antimicrobial, antioxidant, antimicrobial and time-course of its anti-inflammatory activities

and about how these four activities collectively effect on wound treatment and wound healing (Van-Wyk and Wink, 2018). The research suggests that phytochemicals present in polar leaves may function as possible modulators of wound treatment by regulating microbial, oxidative stress and inflammatory activities simultaneously (Felgus-Lavefve et al., 2021). These four properties of antimicrobial, antioxidant, anthelmintic, and anti-inflammatory activities are all important in wound healing treatment, and their evaluation will provide some mechanistic insight into the ethnomedicinal origin of disease treatment (Ghuman et al., 2019). This study showed that the methanol extract has greater antimicrobial, anthelmintic, and anti-oxidant activity *in vitro*, as well as a more effective anti-inflammatory activity *in vivo*, when compared to the hexane extract. *Sabicea calycina* anti-wound properties are explored in further detail in this research. The objective of this research is to use solvent-specific (methanol and hexane) phytochemical pools to assess antimicrobial, antioxidant, anthelmintic, and anti-inflammatory properties using traditional biochemical tests. This knowledge might help with the medication development potential of *Sabicea calycina* plant extracts in the future.

MATERIALS AND METHODS

Chemicals

All of the reagents used were analytical grade. Sigma Chemical Co. (St. Louis, MO, USA) provided the nutritional broth and nutrient agar, as well as DPPH (2, 2-Diphenyl-1-picrylhydrazyl) and Ascorbic Acid, while Merck provided hexane, ethanol, and methanol (Darmstadt, Germany).

Sample collection

The leaves of *sabicea calycina* were taken in September 2020 at Nkawkaw, kwahu (Latitude: 6° 32' 44.664" N and Longitude: 0° 45' 46.0368" W), in the Eastern region of Ghana. Mr. Asare of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, and Prof. Kuffuor of the Department of Biology and Biological Sciences, Kwame Nkrumah University, both authenticated the plant. A sample of the plant

was deposited as a voucher at the herbarium of KNUST.

Sample preparation and extraction

Sabicea calycina leaves were washed under running water and air-dried for fourteen days at room temperature. The dried samples were pulverized into a fine powder and stored in a polythene sack in the refrigerator at four degrees Celsius until needed. Methanol and hexane were used as separate extraction solvents to extract phytochemicals using the cold maceration method. Extracts were reduced to dry mass using air, and the dried extracts were then stored in airtight glass containers and frozen until needed.

Phytochemical screening

Phytochemical analyses revealed the presence of tannins, flavonoids, saponins, steroids, anthraquinones, phenols, and coumarins in the methanolic extract and by demonstrating that alkaloids, tannins, terpenoids, coumarins, and steroids are present in the hexane extract using protocols described by Soni et al. (2013).

Diagnostic TLC

TLC plates were silica gel coated in-house and used with an in-house chromatographic technique to display the estimated number of chemical entities contained in each extract. The existence of chemical entities was determined by using iodine vapor visualization of chromatographic bands. With the use of the estimated Retention factor, chromatographically resolved bands were described (R_f). R_fs were calculated by dividing the sample spot's distance travelled by the solvent's distance travelled. Diagnostics TLC as previously reported by (Mensah et al., 2016).

Assessment of anti-inflammatory activity

Animals

The genotype, feeding, and housing of the day-old chicks used in the anti-inflammatory studies have already been published (Mensah et al., 2019). For each administered extract concentration, each control medication, and each negative control,

five chicks were employed (Mensah and Armah, 2018).

Carrageenan-induced foot edema in chicks

Carrageenan-induced foot edema was previously used to test the anti-inflammatory activity of extracts in 7-day-old chick. Previously, a description of the right foot sub-plantar production of inflammation in chicks, followed by treatment of chicks with extracts and control medicines were reported (Mensah and Armah, 2019). *Sabicea calycina* leaf extracts were given orally at three different concentrations (30, 100, and 300 mg/kg). As positive controls, the drugs diclofenac (1-100 mg/kg) were injected intraperitoneally. Only normal saline was given to the negative control animals (Mensah and Armah, 2019). The hourly serial monitoring of the swollen foot during the 6 h post treatment (pt) time-course and within the 30-300 mg/kg extract dose-range.

Data analysis for the anti-inflammation assay

The data analysis of the carrageenan-induced inflammation was done using a one-way analysis of variance (ANOVA). Dunnett's post hoc test was used to examine differences in inflammation between the chick groups. The whole foot volume for each treatment group was expressed using differences in Area under the Curve (AUC). The percentage inhibition of edema for each treatment group was calculated using the following formula (1):

$$\% \text{ Inhibition} = \frac{\text{AUC}_{\text{control}} - \text{AUC}_{\text{treated}}}{\text{AUC}_{\text{control}}} \quad (1)$$

As previously stated, the 50 percent effective dose (ED50) was calculated (Mensah and Armah, 2019).

Assessment of anti-microbial activity

Culture and maintenance of microorganisms

The extracts were tested against five microorganisms: two Gram negative (*Pseudomonas aeruginosa* and *Escherichia coli*), two gram positive (*Staphylococcus aureus* and *Bacillus subtilis*), and one fungus (*Candida albicans*). ATTC provided all six microorganisms utilized in this study. Taxonomic data on pathogenic bacteria species

and fungi, including genotype, has previously been described (Mensah and Golomeke, 2015). All five microbes have been stored, cultured, and maintained in previous studies (Mensah et al., 2019).

Broth dilution assay

The Broth dilution test was carried out according to a recently reported method (Ajaiyeoba et al., 2001). As shown in Table 2, pathogenic microbiological specimens were treated with varied concentrations of *Sabicea calycina* extracts. The positive control drugs were ciprofloxacin and ketoconazole. The negative controls were inoculated sterile broth medium seeded with or without extracts. Extract minimum inhibitory concentrations (MIC) were calculated in mg/mL and defined as the lowest extract concentration that completely inhibited microbiological growth. The lack of a violet coloring of the reaction mixture following the addition of 0.1 mL of MTT dye was used to identify MICs.

Assessment of the antioxidant activity

DPPH scavenging assay

The 2, 2-diphenyl-1-picrylhydrazil (DPPH) assay was used to determine the extracts' free radical scavenging activity described by Ajaiyeoba et al. (2001). The percentage of DPPH that has been scavenged was calculated by the formula (2):

$$\% \text{ Inhibition} = \frac{A_o - A_i}{A_o} \times 100 \quad (2)$$

The absorbance of the control is A_o , while the absorbance of the sample is A_i . The control drug was ascorbic acid.

Hydrogen peroxide scavenging assay

A previously established protocol (Ajaiyeoba et al., 2001) was used to test H_2O_2 radical scavenging activity. A UV-VIS spectrophotometer was used to measure sample absorbance at 510 nm. Equation (3) used to calculate the results.

$$\% H_2O_2 \text{ scavenging activity} = \frac{A_{\text{test}}}{A_{\text{control}}} \times 100 \quad (3)$$

Where A_{test} is the absorbance of the extract and A_{control} is the absorbance of the control (Ascorbic acid).

Total Phenolic Content (TPC)

The total phenolic content of extracts was determined using a technique described previously by Aliyu et al. (2012). Each extract's Total Phenolic Content was measured in Gallic Acid Equivalents (GAE) per 100 g of extract.

Total antioxidant capacity (Phosphomolybdenum method)

The phosphomolybdenum method was used to determine the total antioxidant capacity of extracts, as previously reported by Mensah and Armah (2019). The Ascorbic Acid Equivalent (AAE) per 100 g of extract was used to calculate each Total Antioxidant Capacity.

Assessment of anthelmintic assay

In this study, adult earthworms (*Milsonia ghanensis*) were employed. Mr. Lawrence Yeboah of the Kwame Nkrumah University of Science and Technology's Department of Biology in Kumasi, Ghana, validated the worms. Before being utilized in the experiment, the worms were cleaned with distilled water.

Adult Worms Mortality Assay

The two extracts were tested in this assay (hexane and methanol). Each extract was produced at the following concentrations: 12, 6, 3, 1.5, and 0.75 mg/mL. Petri dishes were filled with 50 mL of the various extract concentrations. With at least three worms per Petri plate, the actively moving adult worms were put in the pool of extract. The reference drug was albendazole (12 mg/mL), while the control group received distilled water instead of plant extract. The time it took each worm to paralyze and die was measured in minutes. When there was no movement of any kind, even when the worms were violently shaken, the paralysis period was recorded. After determining that worms did not move when shook violently or immersed in heated water (50°C), the time it took for them to die was recorded (Spiegler et al., 2017). Worm counts

were represented as mean \pm SEM in the statistical analysis. Protocol for the *in vitro* anthelmintic activity was used according to Ajaiyeoba et al. (2001).

Statistical Analysis

The statistical comparisons of data for each experimental point were done using ANOVA. At $p < 0.05$, statistical values were considered significant.

RESULTS

Extraction

Because ethnomedicinal use of the plant relies on aqueous infusions, it was critical to use a phytochemical extraction strategy that allowed for maximal bioactive component recovery. Cold maceration was used to extract the phytochemicals in this communication, which resulted in a 12.80% recovery of polar phytochemicals and a 4.88% recovery of non-polar phytochemicals. The extraction of a larger variety of phytochemicals from the stem-bark of *Sabicea calycina* was made possible by using two solvents with different polarities and varying solvent strengths. The dry weight of crude extract obtained by methanol extraction was 12.0 times that of hexane extraction (Table 1). Because the polar extract is used for chemotherapy in ethnomedicine, bioactive phytochemical concentrations in the methanol extract are likely to be below pharmacological limits.

Phytochemical screening

The methanol extract produced the largest quantitative pool of phytochemicals, as shown in Table 1. Tannins, flavonoids, saponins, steroids, anthraquinones, phenols, and coumarins were among the phytochemicals found in the polar methanolic extract of *Sabicea calycina*, which contributed to its chemotherapeutic and chemopreventive effects. Only alkaloids, tannins, terpenoids, coumarins, and steroids phytochemicals were extracted with hexane due to its lower solvent strength.

TLC

On the TLC plates, the methanol extract had six distinct bands that were well-resolved and evenly dispersed. As shown by R_f values, TLC for the hexane extract revealed two resolved bands that were far apart from one another (Table 1).

Anti-inflammatory assay

One of the underlying causes of wounds is inflammation, for which there is no effective treatment. *Sabicea calycina* extract may help individuals with their symptoms by targeting inflammation.

Carrageenan-induced inflammation model in 7-day old chicks was utilized to generate swelling of the feet to investigate the impact of *Sabicea calycina* on inflammation, as stated in Material and Methods. In a 6 h time-course approach and in a net dose-dependent way, swollen feet were then serially evaluated for hourly decrease of inflammation.

As previously indicated, a 6 h post treatment (pt) time-course was used to monitor the *Sabicea calycina*-induced decrease of inflammation in order to simulate the early phase of acute inflammation. For the time-course investigation and the net dose-dependent effects, three different concentrations of extracts expected to contain physiological doses of *Sabicea calycina* phytochemicals were used.

Time course of anti-inflammatory response

The quantitative amount of time course of development of anti-inflammatory response differs between the two solvent specific extracts. Anti-inflammation levels related with extract dose, with the 300 mg/kg dose being the most effective. The maximum inflammatory response was obtained 1 h post-treatment for all three dosages. The anti-inflammation effect then lasted for the remaining 6 h of the trial.

Methanol extract

After 1 h, the levels of anti-inflammation generated by the methanol extract increased for all three concentrations, reaching a maximum two-fold rise by 3 h (Figure 3a). Following the initial anti-

inflammatory peak, anti-inflammation continued to rise rapidly over the next 3 h. Anti-inflammation levels continued to rise until, at 6 h pt, a time course review as increasing anti-inflammation was achieved (Figure 3a).

Hexane extract

The result shows that anti-inflammation levels dropped considerably at 1 h - 2 h pt and then gradually reduced until 6 h (Figure 4b). Anti-inflammation in both the early and late phases was influenced by extract concentration. Increasing the hexane extract concentration from 30 mg/kg to 300 mg/kg resulted in an increase in anti-inflammatory responses that peaked at 5 h and then gradually declined until 6 h (Figure 4b).

Diclofenac control

After 2 h, inflammation drops quickly to 5-20%, then increases sharply to 10-25% at 3 hours pt for the 30 mg/kg concentration (Figure 4a). The anti-inflammatory effect was concentration-dependent, peaking after 5 h and showing persistent decreases in swelling up to 6 h following pt cessation (Figure 4a).

Anti-inflammatory dose response

The data indicates two different types of concentration–response correlations. The anti-inflammatory effect of all samples (methanol extract, hexane extract, and diclofenac control) increased as the sample concentration increased.

Methanol extract

There was only 7% anti-inflammatory effectiveness at low extract concentrations (30 mg/kg) (Figure 5a). When the extract concentration reached 100 mg/kg, the anti-inflammatory magnitude increased substantially to 26%. The highest anti-inflammation was obtained at 300 mg/kg, which was around 63 times greater than the vehicle control value (Figure 5a). The data indicate that the anti-inflammatory effect of the methanol extract is dose and time dependent.

Hexane extract

The extract with the lowest concentration (30 mg/kg) was slightly insensitive to the anti-inflammatory response, eliciting just a 9% increase in effect compared to the vehicle control (Figure 5b). Anti-inflammation rose by 22% when the extract concentration was raised to 100 mg/kg. When chicks were given 300 mg/kg of extract, anti-inflammatory activity reached a high of around 48% of the vehicle control. In all of the instances studied, hexane extracts inhibited inflammation in a concentration-dependent manner.

Diclofenac

At low dexamethasone concentrations, anti-inflammatory efficacy was minimal, with only a 10.26% anti-inflammation effect at 30 mg/kg (Figure 6). Anti-inflammatory magnitude rose dramatically to 35.36% when medication concentration reached 10 mg/kg (Figure 6). At 30 mg/kg of diclofenac, the maximum anti-inflammation was reached, which was 63% higher than the vehicle control value (Figure 6). The results indicate that diclofenac's anti-inflammatory effects are dose and time dependent.

Anti-inflammatory ED₅₀ values

The anti-inflammatory potency assessed in the dose-response graphs corresponded to the samples' ED₅₀ (median effective dosage). The control drug, diclofenac, was very effective as an anti-inflammatory agent, with an ED₅₀ of 4.590 mg/kg, which were two orders of magnitude lower than the two extracts (Table 8). None of the extracts inhibited inflammation more than 70% as well as the vehicle control, which is backed up by their high ED₅₀ in the hundreds. The calculated ED₅₀ of the two extracts have a significant positive association with their respective inflammation suppressive effects, with the ED₅₀ of the methanol extract being 2.5-fold lower than that of the hexane. The hexane extract had the least powerful efficacy (ED₅₀ =

101.30 mg/kg), whereas diclofenac was the most active of the tested samples (Table 6).

Anti-oxidant Assay

The methanol and hexane extracts were separately tested for Total Antioxidant Capacity and radical scavenging activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and H₂O₂ assays to determine *Sabicea calycina*'s anti-oxidant potential.

Total antioxidant capacity

The sum of all antioxidants' defensive qualities against oxidative stress is calculated as total antioxidant capacity. Antioxidant-redox state in cells and tissues is closely related to total antioxidant capacity. Lower total antioxidant capacity represents the total antioxidants' inability to quench cellular-induced reactive oxygen species, whereas higher total antioxidant capacity indicates that antioxidants in polar *Sabicea calycina* are capable of quenching various types of reactive oxygen species. The hexane extract was found to be devoid of significant anti-oxidant ability, with a total antioxidant capacity 2.5-fold lower than the methanol extract.

Free radical scavenging activities

Sabicea calycina antioxidant action is achieved through the scavenging of reactive oxygen species. The DPPH test and the H₂O₂ assay were performed independently to assess *Sabicea calycina* free radical scavenging activity.

DPPH radical scavenging activities

Both extracts demonstrated graded dose-response levels that were subjectively comparable but quantitatively different, according to the DPPH assay results. The hexane extract has a 2-fold greater radical scavenging activity than the pure methanol extract (Table 6; Figure 1). The methanol extract of *Sabicea calycina* scavenged DPPH free radicals considerably more efficiently than Ascorbic acid, the major pure component employed as a positive control, according to

the study (Table 6). With an IC_{50} of 28.80 g/mL, the hexane extract scavenges DPPH free radicals (Table 6). The IC_{50} of the methanol extract against DPPH scavenging is 10.24 g/mL, indicating that it has about 2.6 times the efficacy of the hexane extract in DPPH scavenging (Table 6).

H₂O₂ radical scavenging activities

The H₂O₂ test indicated that the dose-response values of the extracts were qualitatively similar (Table 7; Figure 2). *Sabicea calycina* crude methanol extracts exhibited greater peroxide radical scavenging efficacy than hexane extracts. The strength of radical scavenging differs 1.5 times between the methanol and hexane extracts. Using IC_{50} values as a criterion, the methanol extract was shown to be a more active peroxide radical scavenger, with an IC_{50} value of 142.42 g/mL. According to the IC_{50} value, the hexane extract showed a 2-fold reduced peroxide radical scavenging activity (Table 7). Additionally, the gallic acid control demonstrated dose-response levels, but one that was quantitatively superior to the hexane and methanol extract (Table 7; Figure 2). As the extract IC_{50} value approached that of the gallic acid control in free radical scavenging action, the pre-eminence of the peroxide radical scavenging activity of the methanol extract became clearer (Table 7).

Total phenolic content

Total phenolic content analyzes the total of all phenolic groups, including tannins, which may neutralize a variety of reactive oxygen species. A greater total phenolic content indicates a larger range of phenolic compounds with anti-oxidant properties, whereas a lower total phenolic content indicates a smaller catalog of phenolic-based compounds with anti-oxidant properties. The total phenolic content of crude methanol extract was found to be much greater (1.2-fold) than that of hexane extract (Table 5). As a result, the findings imply that phenolic phytochemicals with a higher tendency to

scavenge free radicals are more abundant in the methanol extract than in the hexane extract.

Anti-microbial assay

Due to the etiological connection between microbial infection and wound infection, *Sabicea calycina* evocation of anti-microbial properties increases its wound healing medicinal action. Broth dilution test was performed to evaluate the antimicrobial properties of extracts. Relative estimates of the minimum inhibitory concentration, or MIC, were used to assess microbiocidal effects.

Broth dilution assay

Methanol extract

The MICs produced by the methanol extract are presented in Tables 2. The bacterium specimen had stronger growth inhibitions (MICs ranging from 0.39 mg/mL to 0.78 mg/mL) (Table 2). The inhibitory concentration for *Candida albicans* (MICs 1.56 mg/mL) was determined to be at least two times greater than that of bacteria. In terms of bacteria, *Escherichia coli* had the lowest MIC, while *B. subtilis* had the highest. In every case, the Methanol extract yielded MICs for pathogenic microbes that were at least three times lower than the control drugs ciprofloxacin and clotrimazole (Table 3). Both control medicines had MIC values that didn't vary much from one microbial species to another (Table 3).

Hexane extract

The MICs produced by the hexane extract were much greater (0.39-3.12 mg/mL and 6.25 mg/mL, at least a 4-fold difference, for bacteria and fungus respectively) than those produced by the methanol extract (Table 2). Hexane MICs were 4-fold higher for *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*, and 16-fold higher for fungus (*Candida albicans*) when compared to the methanol extract. Hexane MICs were 4 times higher than ciprofloxacin MICs for all microbiological species and 4.5 times higher than clotrimazole MICs for all microbial species (Table 3).

Anthelmintic assay**Adult worms' mortality assay**

In contrast to a standard drug albendazole (12 mg/mL), methanol and hexane extract with concentrations of 12, 6, 3, 1.5 and 0.75 mg/mL were used. The anthelmintic level was evaluated using paralysis and death (mortality) time. The time it takes for an extract to paralyze and die shows how effective it is as an anthelmintic agent (Table 9).

Methanol extract

According to the observations, when compared to albendazole (12 mg/mL), all two extracts (methanol and hexane) at a concentration of 12 mg/mL caused faster paralysis and death. Even when the worms were exposed to lower concentrations of the extracts (6 and 3 mg/mL), the worms were paralyzed and died faster than when they were subjected to albendazole (12 mg/mL). The worms were particularly sensitive to the methanol extract. This is because the methanol extract (12 mg/mL) had a paralysis time of 26 minutes and a dead time of 57 minutes, while the hexane extract (12 mg/mL) had a paralysis time of 32 minutes and a dead time of 60 minutes, while albendazole (12 mg/mL) had a paralysis and death time of 127 minutes and

265 minutes, respectively. As a result, the methanol extract had greater activity than the hexane extract. The presence of phytochemicals such as tannins and terpenoids in the two extracts resulted in anthelmintic action. The efficacy of tannins is dependent on their structure, condensed tannins exhibit anthelmintic action (Table 9).

Hexane extract

The hexane extract had a paralysis and death time which is 5-fold higher than that of methanol extract and 10-fold lower than the standard albendazole drug. Both the hexane and methanol had a paralysis and a death time which sensitive than the control albendazole which is at a concentration of 12 mg/ml had a paralysis and a death time of 127 and 265 minutes than that of the hexane with paralysis and death time of 10 and 99 minutes at the same concentration (Table 9).

Albendazole control

The standard albendazole drug at the highest concentration of 12 mg/ml had a paralysis and a death time of 127 minutes and 265 minutes which is way higher than both extracts (Table 9).

Table 1: Estimation of total number of chemical entities in extracts based on analytical Thin Layer Chromatography (TLC) and assessment of phytochemical contents based on standard phytochemical analyses of extracts of the leaves of *Sabicea calycina*.

Sample	% yield	TLC Results	
		Number of spots from TLC and Rf values	Phytochemicals Present
Methanol	12.80	Six 0.10, 0.18, 0.32, 0.36, 0.56, 0.75	Saponins, Steroids, Tannins, Flavonoids, Alkaloids, Anthraquinones.
Hexane	4.88	Three 0.10, 0.56, 0.75	Saponins, Alkaloids, Terpenoids, Coumarins

Table 2: Broth dilution-based estimation of MICs for extracts of the leaves *Sabicea calycina*.

Sample	Test Organisms	Concentrations (mg/ml)							
		25	12.5	6.25	3.12	1.56	0.78	0.39	0.19
Methanol extract	<i>P. aeruginosa</i>	-	-	-	-	-	-	+	+
	<i>B. subtilis</i>	-	-	-	-	-	-	+	+
	<i>E. coli</i>	-	-	-	-	-	-	-	+
	<i>S. aureus</i>	-	-	-	-	-	-	+	+
	<i>C. albicans</i>	-	-	-	-	-	+	+	+
Hexane extract	<i>P. aeruginosa</i>	-	-	-	-	+	+	+	+
	<i>B. subtilis</i>	-	-	-	-	+	+	+	+
	<i>E. coli</i>	-	-	-	-	-	-	-	+
	<i>S. aureus</i>	-	-	-	-	+	+	+	+
	<i>C. albicans</i>	-	-	-	+	+	+	+	+

+ indicates microbial growth; - indicates no microbial growth

Table 3: Broth dilution-based estimation of MICs for the standard control drugs (Ciprofloxacin and Clotrimazole).

Standard	Test Organisms	Concentration (mg/ml)							
		50	25	12.5	6.25	3.12	1.56	0.78	0.39
Ciprofloxacin	<i>P. aeruginosa</i>	-	-	-	-	-	-	+	+
	<i>B. subtilis</i>	-	-	-	-	-	-	+	+
	<i>E. coli</i>	-	-	-	-	-	-	-	+
	<i>S. aureus</i>	-	-	-	-	-	-	-	+
		50	25	12.5	6.25	3.12	1.56	0.78	0.39
Clotrimazole	<i>C. albicans</i>	-	-	-	-	+	+	+	+

+ indicates microbial growth; - indicates no microbial growth

Table 4: Summary of MIC values of samples (*Sabicea calycina* extracts and standard drugs) estimated against test organisms.

Test Organisms	MIC (mg/ml)		MIC (µg/ml)	
	Methanol	Hexane	Ciprofloxacin	Clotrimazole
	<i>P. aeruginosa</i>	0.78	3.12	1.560
<i>B. subtilis</i>	0.78	3.12	1.560	
<i>E. coli</i>	0.39	0.39	0.781	
<i>S. aureus</i>	0.78	3.12	0.781	
<i>C. albicans</i>	1.56	6.25		6.250

Table 5: Quantitative differences in the Total Phenolic Content (gGAE/100g) and the Total Antioxidant Capacity (gAAE/100g) of the methanol and hexane extracts of the stem-bark of *Sabicea calycina*, with Gallic acid and Ascorbic acid as the respective controls.

SAMPLE	Total Phenolic Content (gGAE/100g)	Total Antioxidant Capacity (gAAE/100g)
Methanol	7.18	28.54
Hexane	5.54	13.62

Table 6: Percentage DPPH radical scavenging efficacies computed along with the respective IC₅₀s of *Sabicea calycina* extracts and with that of the Ascorbic acid control.

Sample	% DPPH Scavenging	IC ₅₀ µg/ml
Ascorbic acid	70.20	4.71
Methanol	60.50	10.24
Hexane	40.20	28.80

Table 7: Percentage peroxide scavenging activities in the H₂O₂ assay computed along with the IC₅₀s of extracts of *Sabicea calycina* and with that of the Gallic acid control.

Sample	% H ₂ O ₂ Scavenged	IC ₅₀ µg/ml
Gallic acid	70.20	4.71
Methanol	60.50	10.24
Hexane	40.20	28.80

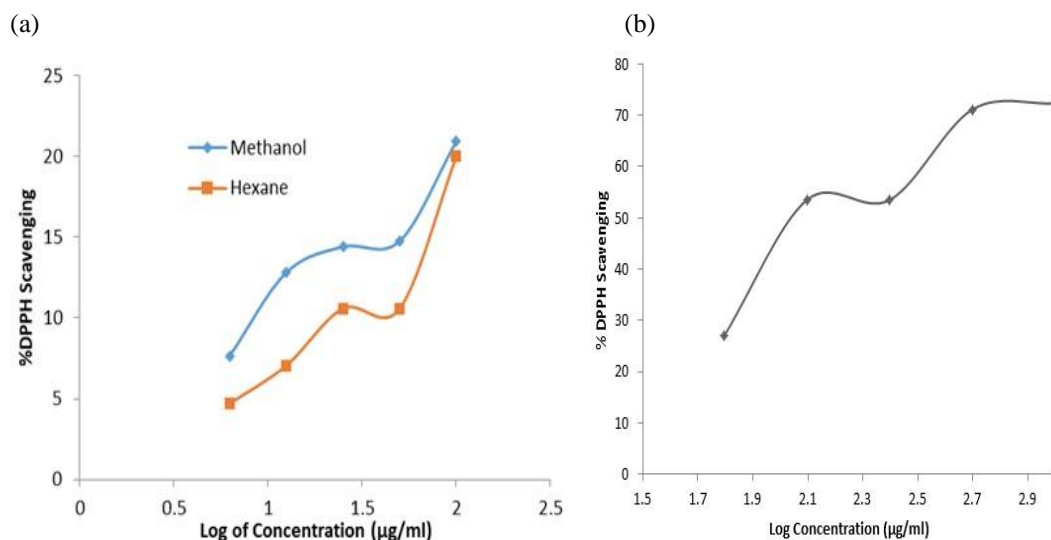


Figure 1: Graphical depiction of the DPPH radical scavenging activities of (a) the methanol and the hexane extracts of *Sabicea calycina* aligned with that of (b) the ascorbic acid control.

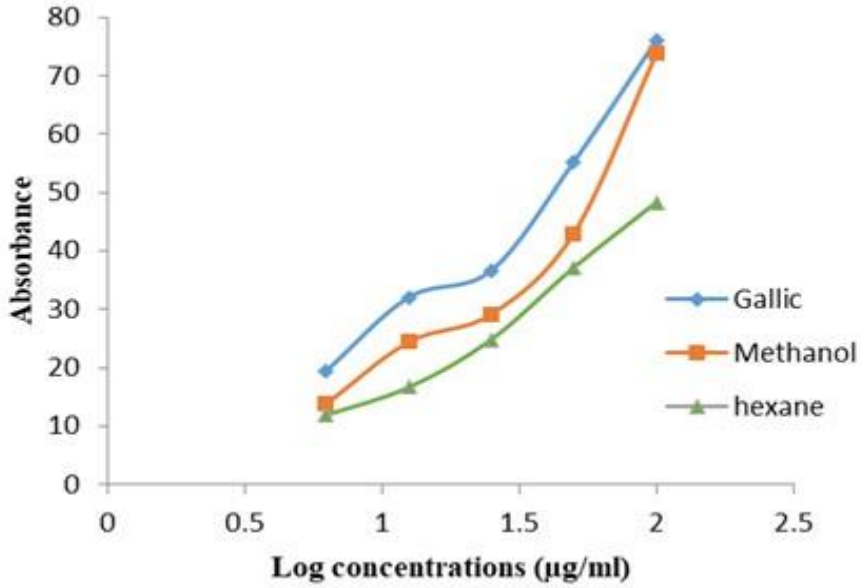


Figure 2: Peroxide radical scavenging efficacies of the methanolic and the hexane extracts of *Sabicea calycina* in the H₂O₂ assay plotted alongside that of the gallic acid control.

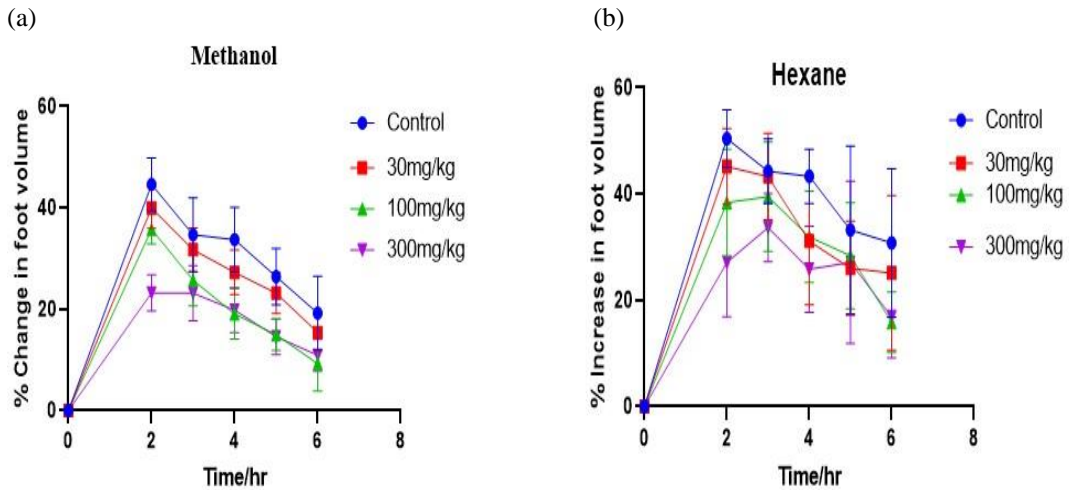


Figure 3: Time course of inflammation-suppressive effects evoked by *Sabicea calycina* extracts on the carrageenan-induced inflammation of the 7-day old chick foot. Suppressive effects on oedema volumes were estimated as percentage (%) increase in foot volumes post treatment (pt) time per hour (h) for: (a) the methanol extract of the leaves of *Sabicea calycina* and (b) the hexane extract of the leaves of *Sabicea calycina*. Dose-induced suppressive effects on oedematous volumes are represented by different colors. Each point represents the mean \pm S.E.M. of 5 animals.

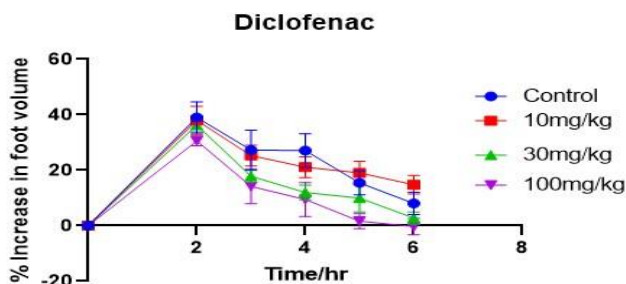


Figure 4: Time course of inflammation-suppressive effects evoked by control drugs on the carrageenan-induced foot oedema volumes in 7-day old chicks. Suppression of foot oedema volumes evoked by the three different dose intraperitoneal injections of: diclofenac: 10, 30, 100 mg/kg and are shown by differently colored curves. Each point represents the mean \pm S.E.M. of 5 animals.

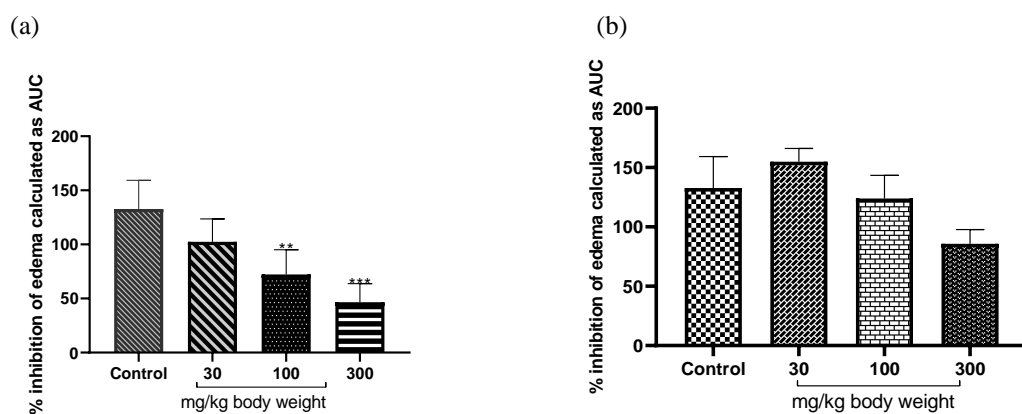


Figure 5: Anti-inflammatory dose-response of oedematous chick foot evoked by increasing concentrations (30, 100, 300 mg/kg) of: (a) methanol extract and (b) hexane extract of *Sabicea calycina*. Anti-oedematous responses to extract doses for 6 h pt were graded and are graphically depicted as area under the curve (AUC) on the Y-axis. Significance levels are indicated by asterisks. (Significance levels: *** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$ compared to the saline-treated control group).

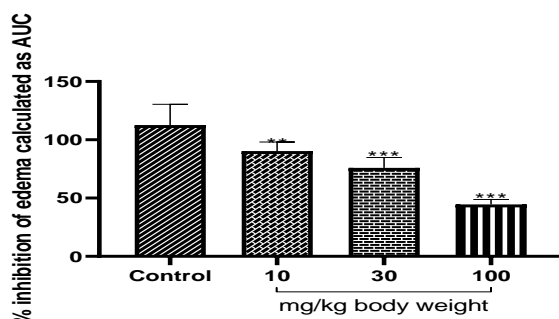


Figure 6: Anti-inflammatory responses (%) induced by graded doses of control drugs: (a). Diclofenac: 3, 10, 30 mg/kg. Individual anti-inflammatory effects induced by the three doses are presented as bar graphs after a net 6 h pt time. AUC in the Y-axis of the bar graphs refer to area under the curve. Significance levels are indicated by asterisks. (Significance levels: *** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$ compared to the saline-treated group).

Table 8: ED50s for the samples (methanol and hexane extracts, Diclofenac control) estimated pt from the carrageenan-induced inflammation of the foot of the 7-day old chicks.

Sample	ED ₅₀ (mg/kg)
Methanol Extract	44.64
Hexane Extract	101.30
Diclofenac	4.590

Table 9: Summary of the paralyzed and death time recorded for the anthelmintic assay of test samples (methanol, hexane, and Albendazole control).

Concentration (mg/mL)	Hexane		Methanol		Albendazole	
	Paralyzed Time (mins)	Death Time (mins)	Paralyzed Time (mins)	Death Time (mins)	Paralyzed Time (mins)	Death Time (mins)
12	30.33	99.36	26.28	57.32	127	265
6	52.29	158	33.37	72.26		
3	125	298	59.27	135		
1.5	190	336	83.05	261		
0.75	219	438	98.08	285		

DISCUSSION

Studies were performed to assess: i) the anti-inflammatory effects of orally administered *Sabicea calycina* in a carrageenan-induced model of inflammation in a 7-day-old chick; the dose-dependent anti-inflammatory effects of orally administered *Sabicea calycina* in a carrageenan-induced model of inflammation (ii) *Sabicea calycina* anti-oxidant activity was tested in four distinct *in vitro* assays.; iii) the broad-spectrum antimicrobial effect of *Sabicea calycina* on a panel of pathogenic microbes *in vitro*. Iv). The anthelmintic effect of *sabicea calycina in vitro*.

The activities of a plethora of *Sabicea calycina* phytochemicals discovered in the phytochemical screening are responsible for the extract's bioactive characteristics. The intensity and duration of the bioactive reactions of the two extracts were influenced by the extraction solvent-based divergence in phytochemical contents. In the prevention and treatment of wound infection, such bioactive phytochemicals (Sudheer et al., 2021) in the polar *Sabicea calycina* extract presumably operate through a multi-pronged mechanism at

many locations. The hypothesis that *Sabicea calycina* will reduce symptoms of wound infection by increasing microbial attenuation, decreasing inflammation processes, and reducing oxidative stress is relevant because microbial infection (Yadav et al., 2017), inflammatory response, and oxidative stress are all linked to disease progression in. The methanol extract shows a better activity in all the four bio-assays which supports the ethnomedicinal usage of *Sabicea calycina*. Ulcers, wound healing, rheumatism, inflammation, dementia, arthritis and venereal disease are among disorders that herbal medicines have the ability to treat and cure (Shahrajabian et al., 2019). Disinfection, debridement, and creating a moist environment that encourages the establishment of an adequate natural healing climate are all examples of herbal medicines used in wound therapy or care (Moeini et al., 2020). A large range of plants are used in folklore cultures to cure cuts, wounds, and burns (Budovsky et al., 2015; Yuan et al., 2016). A wound is a physical, chemical, electrical, or microbiological risk to living tissue that

disrupts its cellular, anatomical, and functional integrity (Shama et al., 2021). Patients with chronic or non-healing wounds, as well as those with nutritional inadequacies, require special nutrients (Molnar et al., 2014). The healing process is influenced by the metabolism of energy, carbohydrates, proteins, lipids, vitamins, and minerals (Guo and DiPietro, 2010).

Conclusion

The goal of this study was to investigate if the Ghanaian medicinal plant *Sabicea calycina* has any antimicrobial, antioxidant, anti-inflammatory, and anthelmintic properties. The plants were screened for phytochemical constituents in the first process of the study. Alkaloids, tannins, phenols, saponins, steroids, anthraquinones, proteins, coumarins, flavonoids, and terpenoids were found in the pulverized plant materials after the phytochemical screening. The results of this research also revealed that both two extracts had antimicrobial activity against a variety of Gram-positive and Gram-negative bacteria, as well as the fungus *Candida albicans*. The methanol extract inhibited the microorganism with the greatest zone of inhibition, with *E. coli* being the most sensitive. The methanol extract had the greatest antimicrobial results against *E. coli* with MICs of 0.1953 mg/mL. After being tested for DPPH radical scavenging activity, hydrogen peroxide scavenging activity, and total antioxidant content, the whole crude methanol extract of the plant materials showed strong antioxidant activity. The methanol extract had the greatest DPPH radical scavenging activity, with an IC₅₀ of 10.24 ppm and a higher total phenolic content of (7.18 gAAE/100g). The methanol extract also had the lowest IC₅₀ (72.42 µg/ml) peroxide scavenging activity and the greatest total antioxidant content (28.54 gAAE/100g) of the two extracts. *In vivo* anti-inflammatory activity of methanol and hexane extracts was assessed against diclofenac sodium as a reference drug with an ED₅₀ of 4.590 mg/kg, utilizing the carrageenan-induced chick feet edema method using 7-day old chicks. This method indicated

the methanol extract showing a dose-dependent decreasing in foot edema with an ED₅₀ of 44.65 mg/kg and the hexane extract with an ED₅₀ of 101.30 mg/kg lack of showing measurable anti-inflammatory activity. The existence of secondary metabolites in the plants may be responsible for the reported biological effects. The ethnobotanical use of the selected medicinal plant for the treatment of various disease conditions has been demonstrated to be supported to some extent by the findings of this study. *S. calycina* exhibits potential anthelmintic action, as evidenced by the paralysis and death times observed for both extracts.

COMPETING INTERESTS

The authors declare no competing of interest.

AUTHORS' CONTRIBUTIONS

All authors have made adequate effort on all parts of the work necessary for the development of this manuscript according to his expertise. All authors read and approved the final manuscript.

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Animal Facility

The school of Pharmacy of KNUST adheres to the ethical treatment of animals protocols.

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