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Anti-oxidant, anti-microbial and anti-inflammatory activities of *Saba thompsonii* fruit extracts

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ABSTRACT

Aqueous extracts of the fruits of *Saba thompsonii* are used topically in ethnomedicine in Ghana for healthy wound healing and wound repair. The objective of this study was to comparatively assess the methanolic and the diethylether extracts of the plant phytochemicals for three bioactivities indicative of wound repairs. Using standard laboratory assays involving broth dilution, carrageenan-induced foot swelling of 7-day old chicks and DPPH radical scavenging, this study assessed the anti-microbial, anti-inflammatory and anti-oxidant activities of the methanolic and the diethylether extracts of the fruits of *Saba thompsonii*. Findings demonstrate that the methanolic extract of *Saba thompsonii* is a potent anti-inflammatory agent that suppresses carrageenan induced swelling of chick feet dose-dependently (with concentrations that ranges from 30 mg/kg through 100 mg/kg to 300 mg/kg). The methanolic extract demonstrated broad-spectrum activity against a panel of clinical isolates of bacterial and fungi pathogens *in vitro* (with MIC ranges of 25-100 mg/mL). Furthermore, the methanolic extract displayed a higher overall anti-oxidant status with demonstrable higher free radical scavenging action in the DPPH radical scavenging assay (IC_{50} of 416.8) and in the H_2O_2 scavenging assay (IC_{50} of 562.1). This methanolic extract's enhanced bioactivity likely resulted from its higher phytochemical content relative to that of the diethylether extract. Altogether, the study supports the ethnomedicinal use of the aqueous extract of the fruits of *Saba thompsonii* for wound repair.

Keywords: *Saba thompsonii*, extract, anti-microbial, anti-inflammatory, anti-oxidant, wound repair.

INTRODUCTION

The fruit of *Saba thompsonii* is a medicinal plant found in tropical West Africa, predominantly in Ghana, Togo, Benin and Nigeria. *Saba thompsonii* belongs to the family of Apocynaceae vascular plants (Houngnon et al., 2021) and is known as 'Bakɔɔ nini' in the Akan language of Ghana. According to anecdotal evidence, aqueous extracts of the fruit of the plant is widely used ethnomedicinally to treat wounds. Current conventional wound healing therapies are limited in efficacy and the efficacious use of the fruits of *Saba thompsonii* in ethnomedicine for wound healing represents a therapeutic potential**.** The scope and the prevalence of use of *Saba thompsonii* (fruit) in ethnomedicine are high enough to warrant scientific investigation of its reputed wound-healing properties.

However, no previous study has been undertaken to examine the anti-microbial, anti-oxidant and anti-inflammatory properties of the

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fruits of *Saba thompsonii*. None of the two reported studies on *Saba thompsonii* involved the use of the fruits (Mireku-Gyimah et al., 2016; Mireku-Gyimah et al., 2018). Understanding the breadth and depth of the mechanism underlying this wound healing effects of the fruits of *Saba thompsonii* is critical to success in its adoption into conventional wound-healing therapy. Consequently, the three bioactive properties of anti-microbial, anti-inflammation and anti-oxidant effects constituted the underlying basis for the scientific investigation reported herein.

Antimicrobial resistance to existing cures has developed in wound healing where co-infections triggered by multiple microbial species, concurrent inflammation of tissues and incipient cellular oxidative stress cause both diagnostic and therapeutic complications (Martin and Nunan, 2015; Su et al., 2019). Ultimately, the ability of *Saba thompsonii* fruit extracts to putatively suppress inflammation, reduce microbial infection, and mitigate oxidative stress improves healing at skin and mucosal surfaces that makes it an ideal wound repair candidate extract.

The objective of this study was to comparatively assess the methanolic and the diethylether extracts of the plant phytochemicals for three bioactivities indicative of wound repairs. Using well-characterized bioassays, the methanolic and diethyether extracts of *Saba thompsonii* (fruits) were evaluated for anti-oxidative activity, anti-inflammatory activity and anti-microbial properties.

MATERIALS AND METHODS

Chemicals

All reagents used were of analytical grade. DPPH (2,2-Diphenyl-2-picrylhydrazyl), Ascorbic Acid and Gallic Acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Methanol, Diethyl Ether, Chloroform and Ethyl Acetate were bought from Merck Chemical Supplies (Damstadt, Germany).

Plant family and plant distribution

Saba thompsonii is a traditional medicinal plant belonging to the family of *Apocynaceae* and located in West Africa particularly in Ghana, Togo, Benin and Nigeria (Figure 1).

Acquisition and authentication of plant material

The fruits of *Saba thompsonii* were collected from Kwahu-Asankraka in the Eastern Region of Ghana in June 2018. The plant material was then authenticated by the Department of Herbal Medicine, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi-Ghana. Voucher specimen (KNUST/2016/S006) had previously been deposited at the herbarium of the Department of Herbal Medicine, KNUST (Mireku-Gyimah et al., 2016; Mireku-Gyimah et al., 2018).

Sample preparation

Foreign matter including soil and stones were removed from the fruit samples. The fruits were then cut into smaller pieces and air-dried for two weeks at room temperature. The dried material was then pulverized and placed in an air tight plastic bag. The bagged powdered material was kept at room temperature until use.

Extraction of phytoconstituents

Soxhlet extractor was used for the extraction of the phytochemicals. Solvents used for the soxhlet extraction were methanol and diethyl ether. For each run, 80 g of the powdered material was placed in the thimble and 250 mL of the solvent was used for extraction. After 5-6 h of extraction run, solvent in the thimble turned colourless indicating the end of extraction. The Rotary evaporator was used to evaporate the solvent and to concentrate the extract. Extracts were placed in vials and frozen until use.

Phytochemical screening

Extracts were examined for phytochemicals using protocols previously reported by Trease and Evans (1983). Qualitative detection of Alkaloids, Steroids, Flavonoids, Tannins, Coumarins, Saponins and Glycosides were performed using standard procedures described by Trease and Evans (1983).

Test for Alkaloids

Alkaloid solutions produce a white-yellowish precipitate when a few drops of Mayer's reagents are added. Most alkaloids are precipitated from a neutral or slightly acidic solution by Mayer's reagent. The extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation.

Test for Steroids

An amount of about 20 mg of the extract was treated with 2.5 mL of acetic anhydride and 2.5 mL of chloroform. A concentrated solution of sulphuric acid was added slowly; a positive test gave a red-violet colour for the presence terpenoids while a green-bluish colour was indicative of the presence of steroids.

Test for Flavonoids

A 4 ml mixture of the extract was treated with 1.5 mL of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and a red color was observed for the presence of flavonoids while the presence of flavones was indicated by an orange color.

Test for Tannins

To 0.5 mL of a solution of the extract, 1 mL of water and 1-2 drops of ferric chloride solution was added. A blue color was observed for the presence of gallic tannins while a greenish-black color was observed for the presence of catecholic tannins.

Test for Coumarins

To 2 mL of the extract was added 3 mL of 10% NaOH. The formation of a yellow color is indicative of the presence of coumarins.

Test for Saponins

About 2 mL of 1% sodium bicarbonate was added to 1 mL of the extract and shaken. A persistent froth for some time is indicative of the presence of saponins.

Test for Glycosides

A small amount of the extract was taken in a test tube and drops of acetic anhydride were added to it. Then 1-2 drops of concentrated sulphuric acid was added to the mixture. A blue-green color shows the presence of glycosides.

Thin layer chromatography (TLC)

Analytical TLC was performed with an in-house TLC plate consisting of silica gel on a rectangular glass plate (Mensah and Golomeke, 2015). Sample spots on silica gel were developed with an ethanol:petroleum ether:3:2 solvent mixture. Chromatographic bands obtained after TLC fractionations were visualized by brief exposure to Iodine vapour. Retention factor (Rf) were computed by dividing the distance moved by the observed spots by the distance moved by the solvent system.

Microorganism culture and maintenance

Test microorganisms used for the study were provided by Microbiology Laboratory of the Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology-Ghana. A panel of six microorganisms comprising four bacterial species [(*Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogenes* (ATCC 19615), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 4853)] and two fungal species [*Tinea corporis* (ATCC)

and C*andida. albicans* (ATCC 10231)] were used for the assessment of the antimicrobial activity of the plant extracts.

Broth dilution assay

Broth dilution assay was carried out as explained previously (Wiegand et al., 2008; Mensah and Golomeke, 2015; Ogwuche and Amupitan, 2015). Briefly, serial dilutions of samples (extracts and control drugs) were performed and each sample examined for the Minimum Inhibitory Concentration (MIC) for each microorganism. Ciprofloxacin and Clotrimazole, at concentrations presented in Table 3, were used as standard positive control drugs. MICs (mg/mL) were taken as the last concentration in the series that showed no microbial growth in the wells of the microtitre plates as previously described (Mensah and Golomeke, 2015).

Total phenolic content

The total phenolic content in the extracts were determined using a slight modification of the Folin-Ciocalteu phenol method as previously described (McDonald et al., 2001; Mensah and Armah, 2018). Briefly, extracts were prepared at 1000, 500 and 250 mg/L. A 0.01 g of Gallic acid was dissolved in 50 mL volumetric flask to prepare 200 mg/L Gallic acid solution. A 10 mL of Folin-Ciocalteu reagent was diluted with 90 mL distilled water in a 100 mL volumetric flask to prepare a 10 % Folin-Ciocalteu solution. A 0.7 M $NaHCO_3$ was prepared by dissolving 2.94 g of solid $NaHCO_3$ in a 250 mL volumetric flask with distilled water. A 10 mL reaction mixture was prepared by combining 5 mL of 10% Folin-Ciocalteu reagent, 4 mL 0.7 M $NaHCO_3$ solution and 1 mL of the extract being tested for. The reaction mixture was allowed to stand for 5 mins before the 0.7 M $NaHCO_3$ was added. The reaction mixture was then allowed to stand for 30 mins at 25˚C in the dark. Individual absorbance of the solutions was measured at 765 nm and the phenolic contents of the extracts were calculated (in g GAE/100 g) on the basis of a calibration curve generated from the reference compound Gallic acid (12.5, 25, 50, 100, 200 mg/L). As shown below, the equation for calculating the g GAE/100 g masses of the extracts is

$$T = \frac{C \times V}{M} \times 100$$

Where T is the total phenolic content of extracts in g GAE/100 g, C is the concentration of Gallic acid established from the calibration curve, V is the volume of the reaction mixture and M is the mass of the extract in the reaction mixture.

Total anti-oxidant capacity

The phosphomolybdenum method was utilized for the assessment of the total anti-oxidant capacity of the extracts (Mensah and Armah, 2018; Prieto et al., 1999). A 1.043 g of $NaPO_4$ and 0.19601 g of $(NH_4)_2MoO_4$ were measured accurately using the chemical balance. A 8.4 mL of concentrated sulphuric acid was poured on both salts and the mixture was then dissolved in distilled water in a 250 mL volumetric flask to prepare the phosphomolybdenum (PM) reagent. A 1000, 500 and 250 mg/L of the mixtures of the extracts were prepared. A 5 mL of the PM reagent and 5 mL each of the prepared concentrations of the extracts were shaken well in a centrifuge tube. The tubes were then placed in a 95˚C water bath for 90 mins incubation period. Absorbances were taken at 695 nm using the UV-VIS spectrophotometer. Ascorbic acid of prepared concentrations: 20, 40, 60, 80 and 100 mg/L were read by the same procedure to generate a calibration curve. The respective antioxidant capacities in g AAE/100 g of the extracts were obtained via interpolation from the calibration curve obtained. As shown below, the equation for calculating the g AAE/100 g masses of the extracts is

$$TAC = \frac{C \times V}{M} \times 100$$

Where TAC is the total anti-oxidant capacity of extracts in g AAE/100 g, C is the concentration of Ascorbic acid established from the calibration curve, V is the volume of the reaction mixture and M is the mass of the extract in the reaction mixture.

DPPH radical scavenging assay

The DPPH radical scavenging assay was performed using a slight modification of a

method earlier described (Mensah and Armah, 2018; Awal et al., 2010). Briefly, a 0.00979 g of DPPH was dissolved in 100 mL of 100 % methanol to prepare a 0.25 mM DPPH solution. The DPPH solution was freshly prepared just before the experiment and was kept away from light by covering the containing vessel with an alluminium foil. Different concentrations of the extracts were prepared in the range of 200 – 1000 ppm. Different concentrations of Ascorbic acid standard, were also prepared. 150 µL of the DPPH solution was pipetted into designated wells on a microtitre plate. Then, 50 µL of extracts, Ascorbic acid extract vehicle (distilled water) each were pipetted into assigned wells containing the DPPH solution. The mixture was allowed to stand for 30 min in the dark. Colour change from deep violet to light yellow was measured with a UV – VIS spectrophotometer at 517 nm. The results were then reported in percentage DPPH radical scavenging. The IC_{50} (half maximal radical scavenging concentration) was then calculated as previously described (Mensah and Golomeke, 2015; Mensah et al., 2019).

H_2O_2 radical scavenging assay

A modification of an earlier described method was used for the quantitative assessment of the H_2O_2 radical scavenging activity (Mensah et al., 2019; Mukhopadhyay et al., 2016). 0.04 g of ferrous ammonium sulphate was dissolved in a 100 mL volumetric flask with distilled water. Then, 5 mL of concentrated sulphuric acid was added to the resolving solution and excess distilled water was added to top it up to the 1000 mL mark. A 0.018 g of 1-10 phenanthroline was dissolved by sonication in a 100 mL volumetric flask with distilled water. A 5 mM hydrogen peroxide solution was prepared by diluting 12 mL of 0.02 M hydrogen peroxide in a 50 mL volumetric flask with distilled water. To each 6 mL of prepared concentration of extracts or Ascorbic acid, 1 mL of ferrous ammonium sulphate was added and 0.26 ml of prepared 5 mM hydrogen peroxide solution was also added. The reaction mixture was left in the dark for at least 5 minutes to incubate. A negative control was prepared in same fashion but without the anti-oxidant species i.e. the prepared extract or the Ascorbic acid standard. A 6 mL of 1-10 phenanthroline was then added to reaction mixture of each tube and kept again in the dark for another 10 min. Absorbance of the solution was taken at 510 nm using a UV-VIS spectrophotometer. The percentage scavenging activities of the extracts and of the standards were calculated using the formula below;

$$\%\ scavenging = \frac{\text{Atest}}{\text{Acontrol}} \times 100$$

Where $Atest$ is absorbance of the test samples and $Acontrol$ is the absorbance of the positive control.

The results were further reported in IC_{50} as previously described (Mukhopadhyay et al., 2016; Mensah et al., 2019).

Animal husbandry

Healthy cockerels (*Gallus gallus*) were obtained from Akati farms, Kumasi-Ghana, one day post-hatch. Chicks were transported to the animal house in KNUST campus and placed in stainless steel cages (34 cm × 57 cm × 41 cm) at a population of 15 chicks per cage. Water and feed were made available to chicks three times daily for six days. Temperature of the animal house was kept at 30 ^{0}C at all times. Chicks were starved 24 hours prior to experiment. Prior to experiments, chicks were randomly grouped in fives by tagging them with 3 different colors in each cage. All experiments were carried out in accordance with international guidelines for the Care and Use of Laboratory Animals and were endorsed by the Animal Care and Use Committee of KNUST.

Carrageenan-induced foot edema in 7-day old chicks

Chicks received a 0.05 mL intra-plantar injection of a 1% w/v carrageenan solution in

0.9% saline in the right foot and the diameter of the foot was measured by means of a micro-calliper, as described by (Mensah and Armah, 2018; Winter et al., 1972). Chicks were then treated orally with extract (30, 100 and 300 mg/kg) or vehicle (saline, 5 ml/kg); and the control chicks injected with Diclofenac (1, 3 and 10 mg/kg) and Dexamethasone (0.1, 0.3 and 1.0 mg/kg). Measurements of the diameter of the oedematous foot was performed at hourly intervals up to 6 h. Percentage inhibition of foot swelling at 1, 2, 3, 4, 5 and 6 h were calculated by reference to vehicle treated animals (0% inhibition). Changes in inflammation within the foot of the 7-day old chicks after ingestion of extracts were plotted against different hourly time points post treatment (pt). The dose-response was estimated as the net anti-inflammatory response at the end of the time-course period. Dose-response data was used for the quantitative estimation of the ED50 (median effective dose).

Statistical analysis

Unless otherwise stated, experimental data were recorded as the mean and standard deviation of triplicate measurements. Data were tested by one-way ANOVA using GraphPad Prism application where needed. All tests were considered statistically significant at $p < 0.05$.

Figure 1: Location of *Saba thompsonii* within West Africa. The plant can be found in Ghana, Togo, Benin and Nigeria.

RESULTS

Phytochemical screening

To determine the phytochemical content of each extract, conventional phytochemical assays were used as stated in the materials and methods section. The results showed that the methanol extract had a higher breadth of phytochemical contents than the diethylether extract with glycosides and alkaloids serving as the phytochemical points of difference. The methanol extract showed the presence of saponins, triterpenoids, anthraquinones, steroids and coumarins in addition to glycosides and alkaloids while the diethylether demonstrated the presence of all the phytochemical groups in methanol except glycosides and alkaloids (Table 1).

TLC

To ascertain whether extracts are composed of multiple chemical constituents, aliquots of extracts were resolved into constituent chemical entities by TLC. TLC results revealed that both extracts are composed of multi-constituent compounds (Figure 2; Table 1).

Broth dilution

To assess the microbiocidal and microbiostasis activities, the standard broth-based dilution assay was used to test for the anti-microbial efficacies of both extracts on four bacterial and two fungal species (*P. aeruginosa, E. coli, S. pyogenes, S. aureus, T. corporis and C. albicans*) selected from a pathogenic panel commonly used for such studies. The results demonstrated that the methanol and the diethylether extracts are both weak anti-bacterial and anti-fungal agents relative to the standard control drugs as recorded by their respective MICs. Both polar and non-polar extracts showed more than a 100-fold higher difference in MIC values against all microbes when compared to that of the control drugs Ciprofloxacin and Clotrimazole (Tables 2-4) ($p<0.05$).

In comparing the two extracts, the diethylether extract was the more potent multi-spectrum anti-microbial agent, demonstrating a 4-fold lower MIC for all but *T. corporis* fungi ($p<0.05$). The MIC of the diethylether extract against *T. corporis* showed a 2-fold lower quantitative value relative to that of the methanol extract ($p<0.05$). Taken together, the diethylether extract demonstrated better broad-spectrum anti-microbial activity against the panel of clinical isolates of bacterial and fungi pathogens in vitro.

Total phenolic content

To estimate the quantitative sum of all phenolic groups that have the capacity to neutralize several distinct classes of reactive oxygen species, extracts were assayed for the Total phenolic content. Higher Total phenolic content, indicative of a higher repertoire of phenolic anti-oxidant-mediated activities, were estimated in the diethylether extract and lower Total phenolic content reflective of a lower catalog of phenolic-based anti-oxidant activities was estimated in the methanolic extract (Table 5) ($p<0.05$). The Total phenolic content of the diethylether extract was 2.5-fold difference higher than that of the methanol extract (Table 5) ($p<0.05$).

Total anti-oxidant capacity

Extracts were individually assayed for Total anti-oxidant capacity. Higher Total anti-oxidant capacity levels suggestive of higher capability of quenching several classes of reactive species was found in the methanol extract (Table 5). And lower Total anti-oxidant capacity reflective of lower antioxidant-redox status was found in the diethylether extract (Table 5). The Total anti-oxidant capacity of the methanol extract was however higher (about 1.08-fold higher) than that of the diethylether (Table 5) ($p<0.05$).

DPPH and H_2O_2 Anti-oxidant Activities Based on IC50 Estimates.

DPPH Scavenging Activities and IC_{50}

The methanol extract scavenges DPPH free radicals with an IC_{50} (half maximal radical scavenging concentration) of 416.8 µg/mL (Table 6). The diethylether extract has an IC_{50} of 531.4 µg/mL against DPPH and as a consequence displays approximately 1.5×

lower potency in DPPH scavenging compared to the methanolic extract (Table 6) ($p<0.05$). The Ascorbic acid control has an IC_{50} of 80.66 µg/mL against DPPH and as a consequence displays ~5× greater potency in DPPH compared to the methanol extract ($p<0.05$). The relatively higher potency of the methanol extract vs. diethylether extract (IC_{50} = 416.8 µg/mL vs. 531.4 µg/mL) suggests that other phytochemicals besides phenols are involved in the induction of the anti-oxidant activities (Table 6) ($p<0.05$).

Although the diethylether extract had higher total phenolic content, estimated quantitative differences in total anti-oxidant capacity and in the DPPH and H_2O_2 radical scavenging indicate that the diethylether extract was a less potent anti-oxidant capacity showing 1.5-fold weaker DPPH and 2-fold feebler H_2O_2 scavenging activity than the methanol extract ($p<0.05$). This observation suggests that the anti-oxidant activities evoked by the methanol extract are not solely attributable to phenolic molecular species but were additionally evoked by the repertoire of anti-oxidant phytochemical molecular entities besides phenolics.

The graphical depiction of the DPPH scavenging of samples follows the same quantitative pattern as that of their respective IC_{50}s: the Ascorbic acid control demonstrated the steepest gradient and showed higher quantitative scavenging values; the methanol extract displayed a steeper gradient and manifested an intermediate quantitative scavenging values; and the diethylether extract showed the shallowest gradients and exhibited lower quantitative scavenging values (Figure 3).

H_2O_2 Scavenging Activities and IC_{50}

The methanol extract was the more active peroxide radical scavenger with the half maximal radical scavenging concentration (IC_{50}) of 562.1 µg/mL. The diethylether extract exhibited ca. 2-fold higher IC50 value relative to the methanol extract and consequently showed a considerably lower peroxide radical scavenging activity (Table 6) ($p<0.05$). IC_{50} estimates of the methanol and diethylether extracts were at similarly higher values when compared with that of Gallic acid control (Table 6). Gallic acid displayed greater than 8-fold potency than the methanol extract (Table 6) ($p<0.05$).

Graphically, both fruit extracts showed graded H_2O_2 scavenging responses with increasing extract concentration (Figure 4). However the Gallic acid control demonstrated the steepest curve indicative of the strongest scavenging response. Scavenging responses of the methanol extract were graphically higher than that of the diethylether extract (Figure 4).

Anti-inflammatory assay

To assess the anti-inflammatory potential of extracts, the well-cited carrageenan-induced inflammation of chick foot was used. Anti-oedematous data was analyzed on an hourly interval post treatment (pt) for 6 h to describe the time-course of inflammatory action and was evaluated as a net anti-inflammatory response after 6 pt for the overall dose-response effect.

Time-Course

Methanolic extract

Anti-inflammation was maximally induced at a higher dose of 100 mg/kg at 5 h. Again, at the dose of 100 mg/kg, anti-inflammatory activity was detected as early as 1 h and it peaked at 5 h and declined progressively thereafter to the 6 h termination point (Figure 5). The anti-inflammatory time-course of the extract varied over the 10-fold change in extract concentration (from 30 to 300 mg/kg) as the 300 mg/kg dose elicited significantly lower anti-inflammatory responses relative to the 30 mg/kg. For the 300 mg/kg dose, anti-inflammation levels were barely visible at 1 h, The 300 mg/kg dose of the methanol extract then showed the steepest increase in anti-inflammatory response from 1 h to 2h. The increase reached a maximum point at 5 h and was then sustained up to 6 h where anti-inflammatory levels later fell to vehicle control levels at 6 h (Figure 5).

Diethylether extract

Extracts showed little anti-inflammatory response as all doses displayed

less anti-inflammation action compared to vehicle control from 1 to 3 h pt (Figure 5). The anti-inflammation of the 300 mg/kg dose became higher than that of the vehicle control from 4-5 h pt. At the 6 h termination point only the 300 mg/kg dose showed an anti-inflammtion response that was lower than that of the vehicle control. For the 100 mg/kg dose, anti-inflammation peaked at 4 h, remained elevated at 5 h and subsequently decreased to a level close to vehicle control levels at 6 h. Peak anti-inflammation for the 30 mg/kg dose at 5 h was only suppressed slightly at 6 h (Figure 5).

Diclofenac

Anti-inflammatory effect of 30 mg/kg dose of Diclofenac during the first hourly interval pt was low. During the second hourly interval pt, the levels of inflammation dropped to levels that persisted for 3 h (Figure 6). Subsequently the magnitude of this increased anti-inflammation decreased further until the termination point. Maximal inhibition of the 10 mg/kg dose in this time-course was 60-70% at the 3-hr time point. By contrast diclofenac at 3 mg/kg showed a marked inhibition at both 3 h and at 5 h after the carrageenan injection (Figure 6). Diclofenac at 30 mg/kg dose showed the steepest increase in anti-inflammatory response (Figure 6).

Dexamethasone

The time-course changes in inflammation for the three doses were slightly similar to each other (Figure 6). Inhibitory effect of the 3 mg/kg dose on inflammation started at 3 h and peaked at 5 h and progressively decreased after 5 h to the termination point at 6 h. The 1 mg/kg dose failed to show significant anti-inflammation within the first 2 h. Robust anti-inflammatory activity of this 1 mg/kg dose first becomes detectable 3 h after treatment and was sustained up to 5 h. The lowest dose (0.3 mg/kg) showed anti-inflammatory effect as early as the second hour pt. This 0.3 mg/kg dose triggered substantial anti-inflammatory responses that peaked 4 h and steadily decreased to the 6 h termination point (Figure 6).

Dose-response

Methanolic extract

Oral administration of 30 mg/kg and 100 mg/kg doses of the methanolic extract caused a dose-dependent inhibition of mean inflammation when compared to the vehicle treated group (Figure 7) ($p<0.05$). The methanolic extract inhibited foot swelling by 36.5% at the dose of 30 mg/kg; and suppressed foot inflammation by 57.5% at a dose of 100 mg/kg. Of particular note is the observation that anti-inflammation decreased with the highest dose (300 mg/kg) for the methanolic extract. The 300 mg/kg dose of the methanolic extract produced no greater inhibition (39.7%) than that of 100 mg/kg (Figure 7) ($p<0.05$). Maximal inhibition varied with the extract dose, but inhibition never exceeded 58% (Figure 7).

Diethylether extract

The diethylether extract demonstrated no inhibitory activity in the carrageenan oedema model (Figure 7). Doses of 100 mg/kg or less were inactive, and ingestion of 300 mg/kg of extract yielded anti-inflammation that corresponded quantitatively to that of the vehicle control (no inhibition) ($p<0.05$). For all doses, net inhibition never exceeded 0% (Figure 7).

Diclofenac

Diclofenac produced dose-related suppression of edema in doses of 10-100 mg/kg (Figure 8). Anti-inflammatory response trended toward increased values in all response groups and was significantly increased compared to that of the extracts at 100-fold higher concentrations and to that of Dexamethasone at a 10-fold lower concentration ($p<0.05$). The 30 mg/kg and the 100 mg/kg doses of Diclofenac yielded 61% and 66% anti-inflammatory responses respectively (Figure 8) ($p<0.05$).

Dexamethasone

Dexamethasone also produced a dose related anti-inflammatory response in doses of 0.1-3 mg/kg when compared to the vehicle treated group (Figure 8). Consistent with the changes in anti-inflammatory response, the most notable differences overall were the reduced concentrations of the doses that

evoked a sizable anti-inflammatory response; in fact a response that is quantitatively higher than that of the methanol extract at higher doses. The dose responses are as follows: 0.3 mg/kg yielded 11.2% anti-inflammatory response; 1.0 mg/kg produced 32.6% anti-inflammation while the 3.0 mg/kg generated 60.7% anti-inflammation (Figure 8).

Anti-Inflammatory ED50

The methanolic extract had an ED_{50} (median effective dose) in inhibiting inflammation of 133.2 mg/kg and the diethylether extract had ED_{50} 707.6 mg/kg when extracts were administered orally to chicks with the carrageenan-induced acute inflammation (Table 7). Comparative analyses of ED50 values suggested the diethylether extract provided the least potent anti-inflammation efficacy (5.3-fold higher ED_{50}) while the methanolic extract was the most potent anti-inflammation member of the pair (Table 7) ($p<0.05$).

As earlier noted in Figures 5-6, inhibitory concentrations of either standard drug compound (Diclofenac and Dexamethasone) on inflammation were lower than those of the extracts and were dose-dependent and yielded correspondingly lower ED_{50}s. The ED_{50} for inhibition of inflammation by Diclofenac was 80.96 mg/kg (1.65-fold lower than that of the methanolic extract) ($p<0.05$). The comparatively stronger inhibition of inflammation by Dexamethasone recorded an ED_{50} of 18.02 mg/kg yielding a net 7.4-fold lower ED_{50} value than that of the methanolic extract (Table 7) ($p<0.05$).

Table 1: Total number of chemical entities in extracts as estimated by Thin Layer Chromatography (TLC) and total number of phytochemicals as assessed by phytochemical screening of the extracts of the fruits of *Saba thompsonii*.

	TLC Results		Phytochemicals present
Sample	**Number of Spots**	**Rf Values**	
$(Et)_2O$ Extract	3	0.23, 0.69, 0.92	Saponins, Triterpenoids, Athraquinones, Steroids, Coumarins.
MeOH Extract	4	0.10, 0.38, 0.85, 0.93	Saponins, Glycosides, Triterpenoids, Steroids, Alkaloids, Anthraquinones, Coumarins.

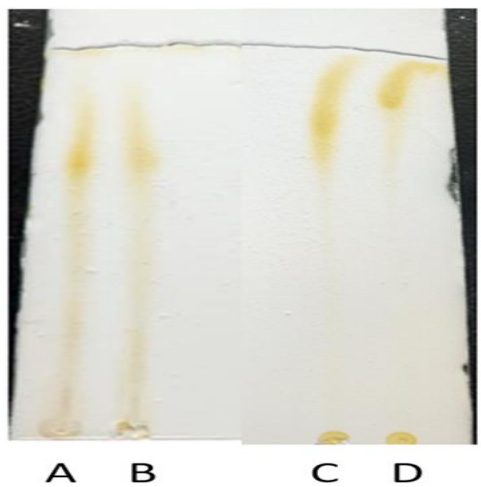

Figure 2: TLC image of the methanol extract (lane A and lane B) and the diethylether extract (lane C and lane D) of *Saba thompsonii*. TLC plates were developed with ethanol:petroleum ether: 2:3.

Table 2: Minimum Inhibitory Concentrations (MIC)s of extracts as estimated by the Broth dilution assay on the fruits of *Saba thompsonii*.

Extracts	Test Organisms	Concentration (mg/mL)							
		100	**50**	**25**	**12.5**	**6.25**	**3.125**	**1.5625**	**0.78125**
Methanol Extract	*P. aeruginosa*	-	+	+	+	+	+	+	+
	E. coli	-	+	+	+	+	+	+	+
	S. pyogenes	-	+	+	+	+	+	+	+
	S. aureus	-	-	-	+	+	+	+	+
	T. corporis	-	-	+	+	+	+	+	+
	C. albicans	-	-	+	+	+	+	+	+
		25	**12.5**	**6.25**	**3.125**	**1.563**	**0.781**	**0.391**	**0.195**
Diethyl Ether Extract	*P. aeruginosa*	-	+	+	+	+	+	+	+
	E. coli	-	+	+	+	+	+	+	+
	S. pyogenes	-	+	+	+	+	+	+	+
	S. aureus	-	-	-	+	+	+	+	+
	T. corporis	-	+	+	+	+	+	+	+
	C. albicans	-	-	-	+	+	+	+	+

+ indicate microbial growth - indicate no microbial growth

Table 3: Minimum Inhibitory Concentrations (MIC)s for the standard control drugs (Ciprofloxacin and Clotrimazole) as estimated by the Broth dilution assay.

Standard	Test Organisms	Concentration (µg/mL)							
		25	**12.5**	**6.25**	**3.125**	**1.563**	**0.781**	**0.391**	**0.195**
Ciprofloxacin	*P. aeruginosa*	-	-	-	-	-	-	+	+
	E. coli	-	-	-	-	-	-	+	+
	S. pyogenes	-	-	-	-	-	-	-	+
	S. aureus	-	-	-	-	-	-	-	+
		100	**50**	**25**	**12.5**	**6.25**	**3.125**	**1.563**	**0.78**
Clotrimazole	*T. corporis*	-	-	-	-	+	+	+	+
	C. albicans	-	-	-	-	+	+	+	+

+ indicate microbial growth - indicate no microbial growth

Table 4: Minimum Inhibitory Concentrations (MIC)s of samples (*Saba thompsonii* fruits extracts and standard drugs) summarized against test organisms and compiled for comparison from earlier results in Table 2 and Table 3.

Test Organisms	MIC (mg/mL)			
	Methanol Extract	**Diethyl ether Extract**	**Ciprofloxacin**	**Clotrimazole**
P. aeruginosa	100	25	0.000781	
E. coli	100	25	0.000781	
S. pyogenes	100	25	0.000391	
S. aureus	25	6.25	0.000391	
T. corporis	50	25		0.0125
C. albicans	50	6.25		0.0125

Table 5: Extract specific quantitative differences in the Total Phenolic Content (gGAE/100g) and in the Total Antioxidant Capacity (gAAE/100g) of the fruits of *Saba thompsonii*. Data on Gallic acid and Ascorbic acid were included as respective controls.

Sample	Total Phenolic Content (mg GAE/100 g)	Total Antioxidant Capacity (mg AAE/100 g)
MeOH Extract	4.783	2.718
$(Et)_2O$ Extract	4.408	2.630

Table 6: DPPH and H_2O_2 anti-oxidant IC_{50}s evoked by the methanol and the diethylether the fruit extracts of *Saba thompsonii* and by their respective Ascorbic acid and Gallic acid standard controls.

Sample	H_2O_2 Scavenging	DPPH Scavenging
	IC_{50} Values	
MeOH Extract	562.1	416.8
$(Et)_2O$ Extract	637.1	531.4
Ascorbic Acid	N/A	80.66
Gallic Acid	64.16	N/A

N/A = Not Applicable

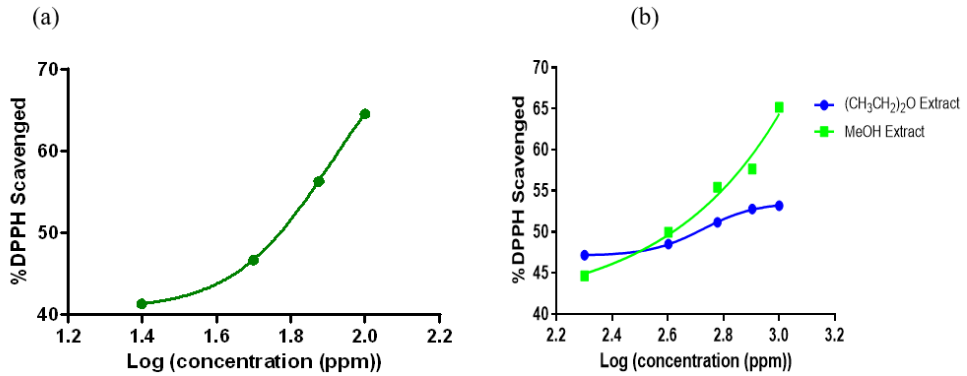

Figure 3: Graph depicting the DPPH radical scavenging activities of (a) the Ascorbic acid control and (b) the methanol and the diethylether extracts of the fruits of *Saba thompsonii*.

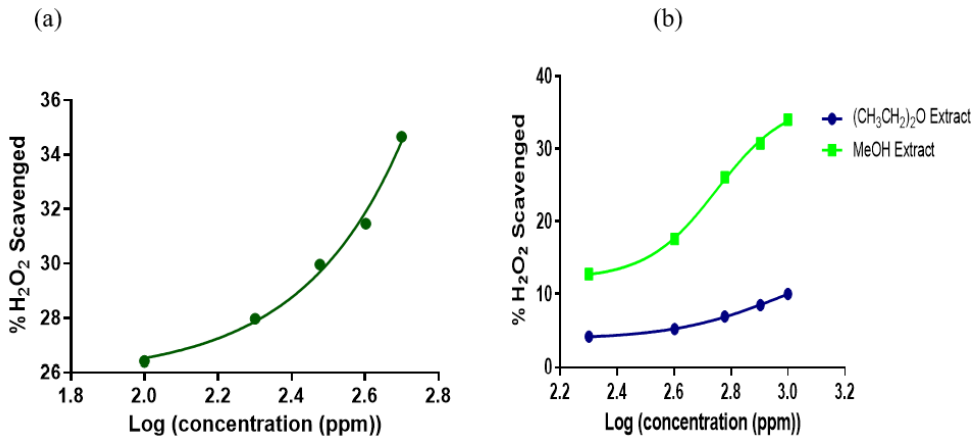

Figure 4: Graph depicting the H_2O_2 radical scavenging efficacies of (a) the Gallic Acid control and (b) the methanol, diethylether extracts of the fruits of *Saba thompsonii*.

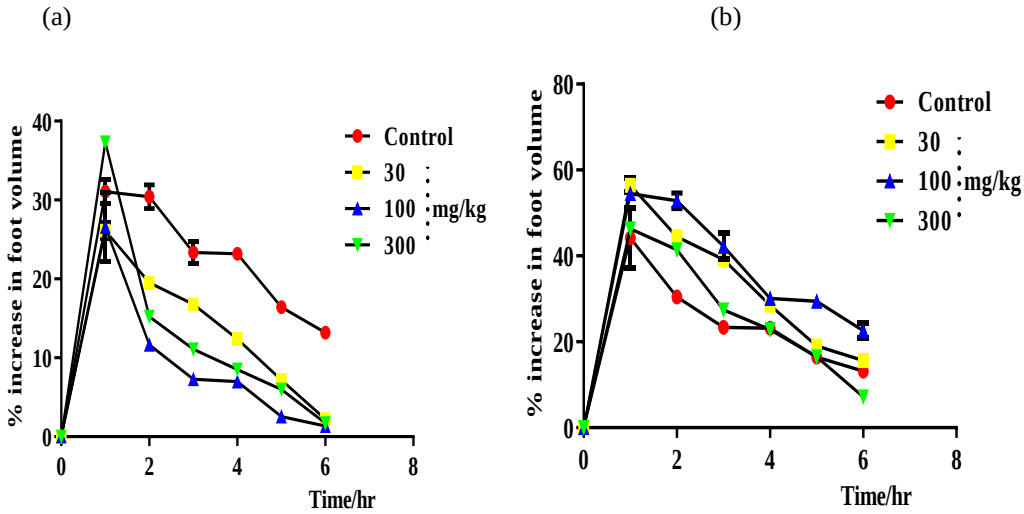

Figure 5: Time course of inflammation-inhibitory effects evoked by *Saba thompsonii* fruits extracts on the carrageenan-induced inflammation of the 7-day old chick foot. Inhibitory effects on swelling were estimated as percentage (%) increase in foot volumes post treatment (pt) time per hour (h) for: (a) the methanolic extract of the fruit of *Saba thompsonii* and (b) the diethylether extract of the fruit of *Saba thompsonii*. Different colors represent dose-induced inhibitory effects on swelling. Graphical trend lines represent mean values across time-points. Each time-point represents the mean ± S.E.M. of 5 animals.

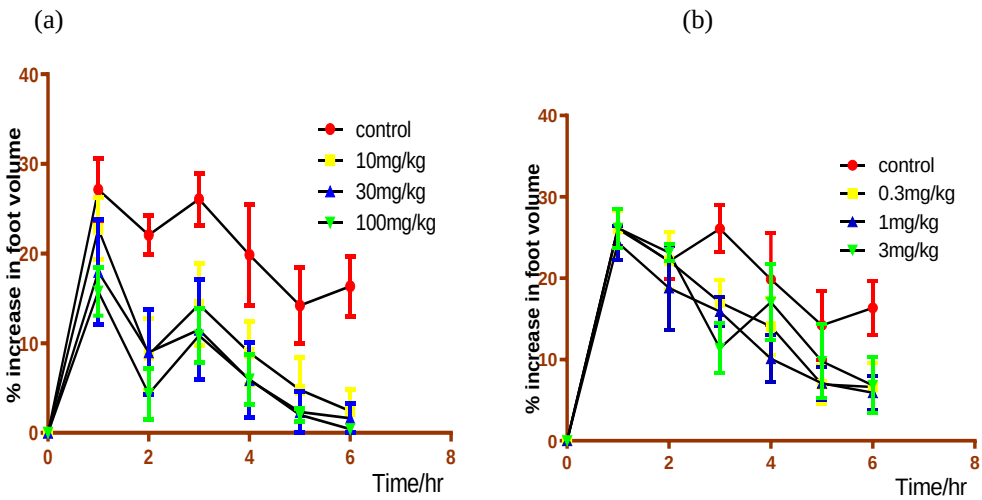

Figure 6: Time course of inflammation-inhibitory effects evoked by control drugs on the carrageenan-triggered foot swelling in 7-day old chicks. Inhibition of foot swelling evoked (a) Diclofenac: and (b). Dexamethasone are depicted by curves that are differently colored by dose. Trend lines in the graph depicts mean values across time points and each point represents the mean ± S.E.M. of 5 animals.

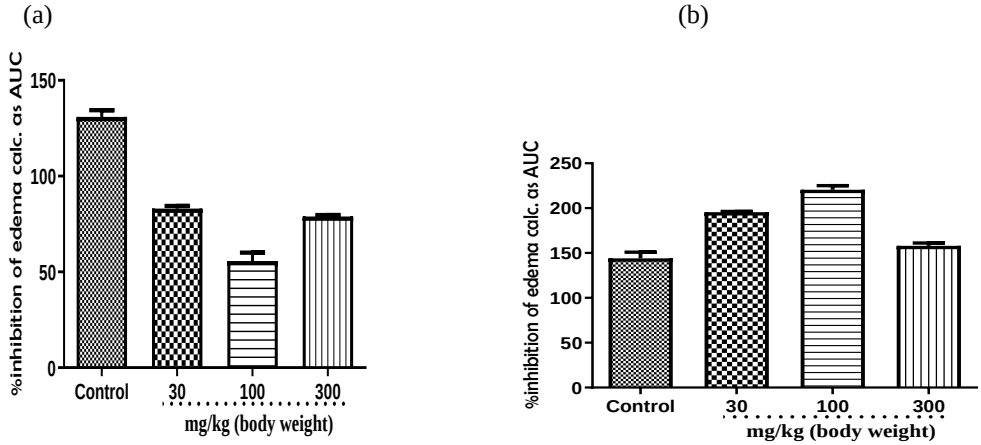

Figure 7: Dose-responses (%) in anti-inflammatory effects of graded concentrations (30, 100, 300 mg/kg) of: (a). methanol extract and (b) diethylether extract of the fruits of *Saba thompsonii*. Reduction in swelling of oedematous foot after 6 h pt were graded and are graphically depicted as area under 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).

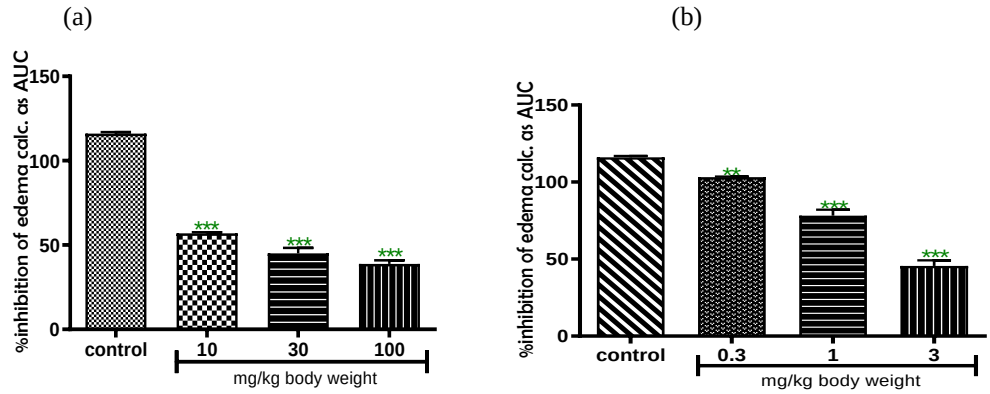

Figure 8: Dose-responses (%) in anti-inflammatory effects induced by graded doses of control drugs: (a). Diclofenac: 10, 30, 100 mg/kg and (b). Dexamethasone: 0.3, 1.0, 3.0 mg/kg. Reduction in swelling of oedematous foot induced by the three doses are presented as bar graphs after a net 6 h pt time. 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 7: Anti-inflammatory ED50s triggered by samples (methanol and diethylether extracts of the fruits of *Saba thompsonii*, Diclofenac and Dexamethasone controls) calculated after 6 h pt from the carrageenan-induced foot oedema of the 7-day old chick.

Sample	ED_{50} (mg/kg)
MeOH Extract	133.20
$(Et)_2O$ Extract	707.60
Diclofenac	80.96
Dexamethasone	18.02

DISCUSSION

The lack of effective wound healing therapy provides compelling impetus to search for alternative treatment strategies that includes the use of botanicals with proven chemopreventive and chemotherapeutic activities. Due to its demonstrated beneficial and efficacious ethnomedicinal usefulness to stimulate healthy wound healing, this study assessed the differential ability of the phytochemicals of the polar and the non-polar extracts of the fruits of *Saba thompsonii* to mediate biochemical activities that enhance wound repair. The rationale behind this approach is that a change in bioactive efficacy can result from a change in extraction solvent due to differing individual solvent-extracts' composition of phytochemicals.

The polar extract was the more efficacious accounting for the higher observed potency of phytochemical-mediated effects and bioactivities of the fruits of *Saba thompsonii* and this observation gives credence to the use of its aqueous extract in ethnomedicine. The methanol extract offers a higher number of phytochemical and thus show proportionally higher contributions to the chemotherapeutic and chemopreventive properties of the polar extract of the fruits of *Saba thompsonii*. Nevertheless it needs to be stressed that the wound repair effects of the polar extracts of *Saba thompsonii* relies more on its anti-oxidant and its anti-inflammation activities. Microbiocidal and microbiostasis effects of its ethnomedicinal wound repair activities are minimal as MIC values showed high quantitative numbers relative to drug controls in the broth dilution assay.

The demonstration by TLC that both extracts contain multiple compounds agreed with the phytochemical analyses that indicated that each extract contained multiple functional groups including saponins, triterpenoids, anthraquinones, coumarins, steroids and alkaloids. This observation suggested that the ethnomedicinally observed wound repair activities of the aqueous extract of the plant is likely due to the combined multiple biochemical mechanisms of multiple compounds at multiple wound repair target sites. Simply stated: multi-mechanisms at multi-sites.

Recent advances in epigenomics and microbiomics have shed light on microbial and cellular reprogramming in wounds that occurs through the induction of genes in mechanistic pathways that enhances healthy wound healing (Martin and Nunan, 2015; Su et al., 2019). Extracted phytochemical constituents likely modulate gene expression to induce pathways that increase wound repair and decrease unhealthy wound healing that results from the development of microbial infections, the occurrence of oxidative stress and the elicitation of inflammatory responses (Martin and Nunan, 2015; Su et al., 2019). Individual phytochemical constituents of the polar *Saba thompsonii* extracts may be at the nexus of the three examined bioactivities and may interact with multiple specific genes to regulate downstream genetic and epigenetic signaling pathways and to modulate global gene expressions that lead eventually to the amelioration of inflammation, to the mitigation of oxidative stress and to the inhibition of fungal and bacteria growth and to the diminution of its associated virulence leading to healthy wound healing (Martin and Nunan, 2015; Su et al., 2019).

The results from the anti-oxidant study suggest that the methanolic extract has the higher ability to quench cellular-induced reactive oxygen species and, also, has a higher propensity to restore redox balance in cells (Dunnill et al., 2017). The results from this study also implied that specific induction of anti-oxidative effect by the individual methanolic extract phytochemicals could also be a mechanism by which *Saba thompsonii* exert their inflammatory-protective actions. Neutralization of reactive oxygen species (ROS) can specifically block NF-kB signaling pathways evoked via carrageenan induction in mechanisms previously explained elsewhere (Hussain et al., 2016; Borthakur et al., 2012).

As shown by the data, anti-inflammatory activities appear to be one of the key mechanisms through which *Saba thompsonii* exert its healthy wound repair activities. This observation agreed with reports that anti-inflammatory responses to wounds are important early components of proper wound healing. Since carrageenan induces

inflammation through the NF-kB pathway, the anti-inflammatory response of this aqueous extract is mediated through upregulation/downregulation of NF-kB pathway genes (Borthakur et al., 2012). Using NF-kB activation/deactivation as an initial measure of inflammation perturbation, future studies can incorporate mechanism-specific *in vivo* anti-oxidant assays to extend the investigation to isolated and structurally characterized phytochemicals from *Saba thompsonii* (Chindo et al., 2010).

Antimicrobial efficacies of polar extracts against both bacterial and fungal pathogens demonstrated in this study were low and yet encouraging when viewed in the context of synergy of bioactive effects (Malongane et al., 2017). The polar extract phytochemicals can be assessed in future studies at low concentrations to study its effects on virulence properties of wound-specific pathogenic bacteria/fungi in specific wound microbiomes (Ngouana et al., 2021). Such a study could identify potential lead compounds with activity against multi-drug resistant chronic wound-specific bacterial and fungal pathogens and could enhance wound repair.

Future instructive studies can also focus on: deciphering the role of individual isolated extract phytochemicals in quenching oxidative stress-induced inflammatory signaling in wounds and examining the role that such isolated *Saba thompsonii* phytochemicals play in its attenuation; establishing the ability of the specific isolated phytochemicals to prevent oxidative stress in wounds using animal model of wound healing; identifying novel targets for intervention based on the mechanistic understanding of the role of the identified phytochemicals in attenuating ROS during wound healing and; testing the efficacy of phytochemical isolates to reduce fungal/bacterial burden in human and animal models of wounds to improve wound healing (Martin and Nunan, 2015; Su et al., 2019).

Conclusion

Saba thompsonii is a botanical with ethnomedicinal wound-repair properties. Although use of the polar extract in ethnomedicine for wound healing represents a wound repair therapeutic potential, no underlying scientific elucidation has been undertaken. Two solvent-specific extracts of *Saba thompsonii* (methanolic and diethylether) were comparatively assessed for anti-microbial, anti-oxidant and anti-inflammatory activities. The data showed that the methanolic extract is the more potent anti-oxidant and the stronger anti-inflammation agent but a less potent anti-microbial agent. Taken together, the methanolic extract's stronger bioactivity is likely attributable to the comparatively higher number of phytochemical contents that included glycosides, alkaloids saponins, triterpenoids, anthraquinones, steroids and coumarins. The study suggested that the polar extract stimulate healthy wound healing by inhibiting inflammation-driven cellular activities, by mildly attenuating mucosal microbial activities or by minimally reducing fungal/bacterial burden in wounds and by mitigating cellular oxidative stress. Future studies can chemically characterize extracts constituents by spectroscopic methods and assess single purified individual constituents for bioactivity using *in vitro* and *in vivo* bio-assay guided fractionation. Such studies will be the critical first steps towards drug discovery using *Saba thompsonii* as the source for bioactive phytochemicals.

COMPETING INTERESTS

The authors declare that they have no competing interest.

AUTHORS' CONTRIBUTIONS

JKM: Conceived the research idea, guided and instructed Philip Teye Thompson on the bench work and wrote and edited the final manuscript. PTT: Performed all the experimental work that generated the data for this manuscript and wrote the draft of the manuscript.

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ETHICAL STATEMENT

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

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