Methanolic extract of *Nauclea diderrichii* (stem-bark) show anti-microbial, anti-oxidant and anti-inflammatory bioactivities

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**ABSTRACT**

The aqueous stem-bark extract of the tropical plant *Nauclea diderrichii* is used in ethnomedicine to manage symptoms of rheumatism through minimally examined mechanisms. The objective of the study is to examine the scientific bases for the ethnomedicinal use of the plant for the management of rheumatism. As part of this effort to explain its ethnomedicinal efficacy, this study compared and contrasted the anti-microbial, anti-oxidant and anti-inflammatory activities of the methanolic extract with that of the diethyl ether extract. Broth dilution assay, DPPH radical scavenging assay and carrageenan-induced foot swelling of 7-day old chicks were utilized for the experimental assessment of the bioactivities of *Nauclea diderrichii*. Polar methanolic extract exhibited a higher antioxidant status in vitro as estimated quantitative differences in total phenolic content, in total antioxidant capacity and in DPPH and H\textsubscript{2}O\textsubscript{2} radical scavenging converged to show the methanol extract as a more potent anti-oxidant. The methanol extract also possess better in vivo anti-inflammatory activity as demonstrated by the 1.5-fold lower ED50 relative to that of the diethyl ether. The methanolic extract demonstrated better broad-spectrum anti-microbial activity against a panel of six clinical isolates of bacterial and fungi pathogens *in vitro*. The relative strength of the bioactivities of the methanolic extract derives from a higher slew of phytochemical content that is a 3-fold difference larger. The results of this study support the beneficial effect of *Nauclea diderrichii* in its continuing ethnomedicinal use to target rheumatism chemotherapeutically.

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**Keywords**: Ethnomedicine, free radical scavenging, phytochemical, broth dilution, assay, carrageenan-induced inflammatory.

**INTRODUCTION**

*Nauclea diderrichii* is a plant that belongs to the Rubiceae taxonomic class (Romain et al., 2017). The plant is used to manage symptoms of rheumatism through minimally examined mechanisms (Mbiantcha et al., 2020). Among the defining clinical factors that drive rheumatism are microbial infections, oxidative stress and inflammation. Rheumatism is in need of new therapies to limit its immunopathology and the ethnomedicinal ingestion of the aqueous infusion of the stem-bark of *Nauclea diderrichii* offers a promising lead (Taylor et al., 2016). While anti-malaria and the anti-inflammation (Mbiantcha et al., 2020) effects of *Nauclea diderrichii* are well
reported, nearly nothing is known about its anti-microbial, anti-oxidant, time-course of its anti-inflammatory activities and about how these three activities collectively impinge on rheumatism. The hypothesis of this study is that polar stem bark phytochemicals may act as potential modifiers of the pathology associated with rheumatism through simultaneous regulation of microbial, oxidative stress and inflammatory activities.

The goal of this study was to utilize solvent-specific pools of phytochemicals to assess the anti-microbial, anti-oxidant and anti-inflammatory activities via conventional biochemical assays. Together these three properties of anti-microbial, anti-oxidant and anti-inflammatory activities are central to the management of rheumatism and their assessment will lead to the provision of some molecular understanding of the ethnomedicinal basis of disease chemotherapy. An additional objective of this study is to assess the scientific bases for the ethnomedicinal use of the plant for the management of rheumatism.

The choice of solvent determines the pool of extractable phytochemical compounds. The mechanistic principle utilized in this study is that the solvent-specific extractable phytochemical differences in the pool might provide alleviation of rheumatism through the evocation of different levels of anti-microbial, anti-oxidant and anti-inflammation effects.

Using conventional in vitro and in vivo bioactivity-specific assays, this study reports that the methanolic extract evokes a stronger anti-microbial activity and a stronger anti-oxidant activity in vitro as well as induces a more potent anti-inflammatory activity in vivo compared to the diethylether extract. This study provides a deeper insight into the anti-rheumatism effects of Nauclea diderrichii. This understanding may assist in the future drug development potential of the Nauclea diderrichii plant extracts.

MATERIALS AND METHODS

Chemicals
All utilized reagents were of analytical grade. Nutrient broth and nutrient agar, DPPH (2,2-Diphenyl-2-picrylhydrazyl) and Ascorbic Acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA) while Diethylether, Ethanol and Methanol were obtained from Merck (Darmstadt, Germany).

Sample collection
Plant samples were purchased at the central market in Kumasi, Ashanti Region, Ghana. Purchased plant samples were identified based on exomorphic characteristics and on a review of literature as Nauclea diderrichii by the Botanist at the Department of Herbal Medicine, herbarium at KNUST, Kumasi. A sample of the plant was deposited as a voucher at the herbarium of KNUST.

Sample preparation and extraction
Purchased plant were continuously air dried at room temperature (27°C) until successive weighing of a plant sample recorded the same weight. Subsequently plants were pulverized and powdered plant samples were stored in air-tight containers. Phytochemicals were extracted via the Soxhlet method using as separate extraction solvents methanol and diethylether. Extracts were reduced to dry mass via rotary evaporator and dried extracts were then kept in air-tight glass containers which were subsequently frozen until needed.

Phytochemical screening
Extracts were screened for the presence of phytochemicals including saponins, flavonoids, tannins, coumarins, glycosides, triterpenes, steroids, anthraquinones and alkaloids (Trease and Evans, 1983; Ngouana et al., 2021).

Diagnostic TLC
Thin-Layer Chromatography (TLC) plates were silica gel coated in-house and were utilized with an in-house chromatographic system to show for each extract the approximate number of distinct chemical entities that are present. Iodine vapor visualization of chromatographic bands was used for the detection of the presence of chemical entities. Chromatographically
resolved bands were characterized with the aid of the computed Retention factor (Rf). Rf’s were computed as the distance moved by the sample spot divided by the distance moved by the solvent.

**Assessment of anti-microbial activity**

**Culture and maintenance of microorganisms**

Six microorganisms [two gram negative (*Pseudomonas aeruginosa* and *Escherichia coli*), two gram positive (*Staphylococcus aureus* and *Streptococcus pyogenes*) and two fungi (*Candida albicans* and *Tinea corporis*)] were used for the assessment of anti-microbial activity of the extracts. All six microorganisms used were acquired from ATTC. Taxonomic data including genotype on both pathogenic bacteria species and fungi are earlier described (Mensah and Golomeke, 2015). Earlier studies have also reported the storage, culture and maintenance of all six microbes (Mensah et al., 2019).

**Broth dilution assay**

A reported protocol was utilized for the execution of the Broth dilution assay (Mensah and Amarh, 2018; Ogwuche and Amupitan, 2015). Pathogenic microbial specimens were treated with different concentrations of *Nauclea diderrichii* extracts as specified in Table 2. Ciprofloxacin and Clotrimazole were the positive control drugs. Un-inoculated sterile broth media seeded without extracts were the negative controls. Minimum inhibitory concentrations (MIC) of extracts were estimated in μg/mL and were defined as the lowest extract concentration that yielded complete microbial growth inhibition. MICs were detected by the absence of a violet coloration of the reaction mixture after the addition of 0.1 mL of MTT dye.

**Assessment of anti-inflammatory activity**

**Animals**

The genotype, feeding and the housing of the day old chicks utilized for the anti-inflammatory experiment have been reported elsewhere (Mensah and Amarh, 2018). Five chicks were used for each applied extract concentration, each control drug and each negative control (Mensah et al., 2020).

**Carrageenan-induced foot edema in chicks**

The carrageenan-induced foot edema for the assessment of anti-inflammatory activity of extracts in 7-day old chick is previously reported (Mensah and Amarh, 2018).

A description of the right foot sub-plantar induction of inflammation in the chicks followed by the treatment of chicks with extracts and with control drugs has been similarly described earlier (Mensah and Amarh, 2018; Winter et al., 1972). The stem bark extracts of *Nauclea diderrichii* were administered orally at three different concentrations (30, 100, 300 mg/kg). The drugs Diclofenac (1-100 mg/kg) and Dexamethasone (0.3-3.0 mg/kg) were administered via intraperitoneal injection as positive controls. Negative control animals were administered only normal saline. The hourly serial monitoring of the swollen foot within the 6 h post treatment (pt) time-course and within the 30-300 mg/kg extract dose-range were previously reported (Mensah and Amarh, 2018).

**Data analysis for the anti-inflammation assay**

A one-way analysis of variance (ANOVA) was used for the Data analysis of the carrageenan-induced inflammation. Differences in inflammation between the groups of chicks were analysed by Dunnett’s post hoc test. Differences in Area under the Curve (AUC) were used to express the entire foot volume for each treatment group. For each treatment group the percentage inhibition of edema was estimated using the following relationship (1):

\[
%\text{Inhibition} = \frac{\text{AUCcontrol} - \text{AUCtreated}}{\text{AUCcontrol}} \times 100
\]

The 50% of effective dose (ED50) was computed as earlier described (Mensah and Amarh, 2018).

**Assessment of the antioxidant activity**

**DPPH scavenging assay**

The free radicals scavenging activity of extracts was assessed with the 2, 2-diphenyl-1-
picrylhydrazil (DPPH) assay as described earlier (Mensah and Amarh, 2018; Awah et al., 2010). Scavenged DPPH (%) was calculated using the relationship (2):

\[
\% \text{ Inhibition} = \frac{A_0 - A_i}{A_0} \times 100 \tag{2}
\]

Where, \(A_0\) is the absorbance of the control and \(A_i\) is the absorbance of the sample. Ascorbic acid was used as the control.

**Hydrogen peroxide scavenging assay**

\(H_2O_2\) radical scavenging activity was assessed using a previously described protocol (Mensah and Amarh, 2018). Sample absorbance was taken at 510 nm on a UV-VIS spectrophotometer. Results were computed using equation (3).

\[
\% \text{ } H_2O_2 \text{ } \text{scavenging activity} = \frac{A_{\text{test}}}{A_{\text{control}}} \times 100 \tag{3}
\]

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

**Total antioxidant capacity (Phosphomolybdenum method)**

Total Antioxidant Capacities of extracts were assessed with the phosphomolybdenum method as previously described by (Mensah and Amarh, 2018). Each Total Antioxidant Capacity was expressed in Ascorbic Acid Equivalent (AAE) per 100 g of extract.

**Total phenolic content (Folin-Ciocalteu method)**

Total Phenolic Content of extracts were assessed with a protocol previously described (Mensah and Amarh, 2018). The Total Phenolic Content for each extracts was expressed as Gallic Acid Equivalents (GAE) per 100 g of extract.

**Statistical analysis**

ANOVA was used for the statistical comparisons of data for each experimental point. Statistical values were considered significant at \(p<0.05\).

**RESULTS**

**Extraction**

Since ethnomedicinal usage relies on aqueous infusions of the plant, it was essential to utilize an extraction scheme for phytochemicals that would provide maximum recoveries of bioactive compounds. The extraction scheme utilized in this communication is soxhlet and it resulted in 8.23% recovery of polar phytochemicals and a 6.31% recovery of non-polar phytochemicals. The utilization of two solvents of differing polarity and diverging solvent strength enabled the extraction of a wider range of phytochemicals from the stem-bark of *Nauclea diderrichii*. Methanol extraction produced a dry weight of crude extract that was 1.3 times higher than that of diethyl ether extraction (Table 1). Since ethnomedicinal practices utilize the polar extract for chemotherapy, it is likely that bioactive phytochemical concentrations in the methanolic extract are within pharmacological values.

**Phytochemical screening**

As shown in Table 1, the methanolic extract provided the larger quantitative pool of phytochemicals. Saponins, Steroids, Tannins, Flavonoids, Alkaloids and Glycosides provided the phytochemical contribution to the chemotherapeutic and chemopreventive properties of the polar methanolic extract of *Nauclea diderrichii*. On the basis of its weaker solvent strength, diethyl ether extracted only Saponin and Alkaloid phytochemicals.

**TLC**

The methanolic extract displayed three discernible well-resolved and uniformly distributed bands on the TLC plates. TLC for the diethyl ether extract showed two resolved bands widely separated from each other as demonstrated by Rf values (Table 1).

**Anti-microbial assay**

Microbial infection has an etiological link to rheumatism and so the evocation of anti-microbial effects by *Nauclea diderrichii* improves its anti-rheumatoid medicinal effect. To assess the anti-microbial effects of extracts, Broth dilution assay was used. Measures of microbiocidal effects relied on relative
estimates of minimum inhibitory concentration or MIC.

**Broth Dilution Assay**

**Methanol extract**

Growth inhibitions were stronger for the bacteria specimen (with MICs ranging from 195 µg/ml to 390 µg/ml) than for the fungi (MICs 780 µg/ml) (Tables 2-4). Inhibitory concentration for both fungal specimens *Candida albicans* and *Taenia corporis* (MICs 780 µg/ml) were estimated to be at least two fold difference higher that of bacteria (p<0.05). For bacteria, MIC was lowest for *Escherichia coli* and highest for both *Pseudomonas aeruginosa* and *Streptococcus pyogenes*. In all cases, MICs generated by the Methanol extract for pathogenic microbes were at least two-fold difference lower than that of the control drugs Ciprofloxacin and Clotrimazole (Tables 2-4) (p<0.05).

**Diethylether extract**

The diethyl ether extract generated MICs that were quantitatively higher (at least 16-fold difference higher for bacteria and 4-fold difference higher for fungi) than that produced by the methanolic extract (Tables 2-4) (p<0.05). Specific comparison with the methanolic extract yielded diethyl ether MICs that were 32-fold difference higher for *Pseudomonas aeruginosa* and *Escherichia coli* and 16-fold difference higher for *Streptococcus pyogenes* and *Staphylococcus aureus* as well as 4-fold difference higher for the fungi *Candida albicans* and *Taenia corporis*. For all microbial species, diethyl ether MICs were quantitatively 4-8 fold difference higher than that of Ciprofloxacin and 2-fold difference higher than that of Clotrimazole (Tables 2-4) (p<0.05).

**Anti-oxidant assay**

To examine the anti-oxidant potential of *Nauclea diderrichii*, the methanolic and the diethylether extracts were individually assayed for the Total Phenol Content, for the Total Antioxidant Capacity and for the radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the H$_2$O$_2$ assays.

**Total Phenolic Content**

Total Phenolic Content estimates the sum of all phenolic groups including tannins that have the capacity to neutralize several distinct classes of reactive oxygen species. Higher Total Phenolic Content is indicative of a higher repertoire of phenolic compounds capable of exerting anti-oxidant activities and lower Total Phenolic Content reflects a lower catalog of phenolic-based compounds and its consequent anti-oxidant activities.

Results revealed that the Total Phenolic Content of crude methanolic extract was comparatively higher (1.4-fold higher) than that of the diethylether extract (Table 5) (p<0.05). Thus, the results suggest that phenolic phytochemicals with the higher propensity to scavenge free radicals has a higher representation in the methanolic extract compared to the diethylether extract.

**Total Antioxidant Capacity**

Total antioxidant capacity estimates the sum of protective properties of all antioxidants against oxidative stress. The Total Antioxidant Capacity level is directly associated with antioxidant-redox status in cells and tissues. Higher Total Antioxidant Capacity levels suggest that antioxidants in polar *Nauclea diderrichii* are capable of quenching several classes of reactive species and lower Total Antioxidant Capacity reflects the incapability of the total antioxidants to quench cellular-induced reactive oxygen species.

Estimated quantitative differences in total antioxidant capacity indicate that the diethylether extract was devoid of potent anti-oxidant capacity showing 10.5-fold weaker Total Antioxidant Capacity than the methanolic extract (p<0.05).

**Free radical scavenging activities**

Antioxidant activity of *Nauclea diderrichii* is mediated by the scavenging of reactive oxygen species. To characterize the free radical scavenging activity of *Nauclea*...
diderrichii the DPPH assay and the H₂O₂ assay were separately used.

**DPPH radical scavenging activities**

Results from the DPPH assay revealed that both extracts showed graded dose-response levels that were qualitatively similar but quantitatively different. The radical scavenging activity of the crude methanolic extract was comparatively 4-fold difference higher than that of the diethylether extract (Table 6; Figure 1) (p<0.05). The study unexpectedly demonstrated also that the methanolic extract of *Nauclea diderrichii* scavenged DPPH free radicals far more efficiently than Ascorbic acid, the main pure compound used as positive control (Table 6) (p<0.05). The diethylether extract scavenges DPPH free radicals with an IC₅₀ (half maximal radical scavenging concentration) of 82.55 µg/mL (Table 6). The methanolic extract has an IC₅₀ of 10.2 µg/mL against DPPH scavenging and as a consequence displays approximately 8× greater potency in IC₅₀ in the DPPH scavenging compared to that of the diethylether (Table 6) (p<0.05).

**H₂O₂ radical scavenging activities**

Radical scavenging activity assessed via H₂O₂ assay revealed qualitatively similar extracts dose-response levels (Table 7; Figure 2). The crude methanolic extracts of *Nauclea diderrichii* had higher peroxide radical scavenging activity than the diethylether extract. The difference in the strength of radical scavenging between the methanolic and the diethylether extracts is 1.5-fold. Using the IC₅₀ values as criteria, the methanolic extract was a more active peroxide radical scavenger with an IC₅₀ = 292.9 µg/mL. The diethylether extract had approximately 2-fold lower peroxide radical scavenging activity as recorded by its IC₅₀ (Table 7). Furthermore the Gallic acid control also showed dose-response levels but one that surpassed the diethylether extract in quantitative extent (Table 7; Figure 2) (p<0.05). The pre-eminence of the peroxide radical scavenging activity of the methanolic extract became more obvious as the extract surpassed the Gallic acid control in free radical scavenging action (Table 7) (p<0.05).

**Anti-inflammatory assay**

Inflammation is considered to be one of the underlying causes of rheumatism for which effective treatment is lacking. Targeting inflammation with *Nauclea diderrichii* extract may relieve patients of symptoms. To examine the effect of *Nauclea diderrichii* on inflammation, carrageenan-induced inflammation model in 7-day old chicks was used to induce swelling of the feet as described in Material and Methods. Swollen feet were then serially examined for hourly loss of inflammation in a 6 h time-course approach and in a net dose-dependent manner.

To model the early phase of acute inflammation, a 6 h post treatment (pt) time-course, as earlier stated, was chosen to monitor the *Nauclea diderrichii*-induced loss of inflammation. Three different concentrations of extracts anticipated to contain physiological doses of *Nauclea diderrichii* phytochemicals were utilized for the assessment of the time-course study and for the net dose-dependent effects.

**Time course of anti-inflammatory response**

The two solvent specific extracts differ in their quantitative extent of time course of development of anti-inflammatory response. Levels of anti-inflammation correlated with the dose of extract and were highest in the 300 mg/kg dose. For all three doses, maximal inflammatory response was obtained 1 h pt (p<0.05). Anti-inflammation effect then persisted throughout the remaining 6 h study period.

**Methanolic extract**

The levels of anti-inflammation induced by the methanolic extract for all three doses increased after 1 h reaching concomitantly a maximal two-fold increase by 3 h (Figure 3a). After the initial anti-inflammatory peak, anti-inflammation persisted, increasing sharply throughout the next 3 h pt. The increase in anti-inflammation continued until a time course
best described as increasing anti-inflammation is reached at 6 h pt (Figure 3a).

**Diethylether extract**

The data demonstrates that anti-inflammation levels markedly decreased sharply after 1 h - 2 h pt and then declined slowly afterwards to 6 h (Figure 3b). Both early and latter phase anti-inflammation were dependent of extract concentration. Increasing the diethylether extract concentration from 30 mg/kg to 300 mg/kg led to increases in levels of anti-inflammatory responses that reached a maximal level at 5 h and to progressive decreases afterwards to 6 h (Figure 3b) \((p<0.05)\).

**Diclofenac control**

Inflammation decreases sharply to 5-20% after 2 h and then rises sharply afterwards to 10-25% at 3 h pt (Figure 4a). Anti-inflammatory response was concentration-dependent \((p<0.05)\) and was maximal after 5 h and was consistent in demonstrating reductions in swelling up to 6 h pt termination (Figure 4a) \((p<0.05)\).

**Dexamethasone control**

The 0.3 mg/mL concentration consistently showed lower responses and recorded the maximal anti-inflammatory response up to 5 h pt (Figure 4b) \((p<0.05)\). Increasing concentrations of Dexamethasone produced time-dependent parallel changes in anti-inflammatory response at 6 h pt (Figure 4b).

**Anti-inflammatory dose response**

Inspection of the data reveals two types of concentration–response relationships. For all samples (the methanolic extract, the diethylether extract, and diclofenac control, and dexamethasone control), anti-inflammatory action increased progressively as the sample concentration increased.

**Methanolic extract**

There was little (3%) anti-inflammatory activity at low extract concentrations (30 mg/kg) (Figure 5a). When the extract concentration reached 100 mg/kg, anti-inflammatory magnitude increased sharply to 22%. The maximal anti-inflammation achieved was about 60% above the vehicle control value and occurred at 300 mg/kg (Figure 5a) \((p<0.05)\). Taken together, the data suggest that the anti-inflammatory action of the methanolic extract is both dose- and time-dependent.

**Diethylether extract**

The extract with the lowest concentration (30 mg/kg) was mildly sensitive to the anti-inflammatory response triggering a meager 9% effect (Figure 5b). By increasing the extract concentration to 100 mg/kg, anti-inflammation increased markedly to 34%. Anti-inflammatory action reached a peak of approximately 46% of the vehicle control when chicks were treated with 300 mg/kg of extract \((p<0.05)\). In all examined cases, diethylether extracts caused concentration-dependent inhibition of inflammation (Figure 5b).

**Diclofenac**

Anti-inflammatory responses to utilized doses were relatively high although the utilized concentrations were 10-fold order of magnitude lower than that of extracts. For the 3 mg/kg and the 10 mg/kg concentrations, anti-inflammations were recorded at 50% and 60% respectively, whereas for 30 mg/kg it occurred at 67% (Figure 6a) \((p<0.05)\). Although diclofenac is the strongest inflammation-inhibitor in this study, it failed to completely abolish the inflammation induced by the carrageenan.

**Dexamethasone**

There was meager anti-inflammatory activity at low dexamethasone concentration as the 03 mg/kg concentration evoked only a 11.16% anti-inflammation (Figure 6b). When the drug concentration reached 1.0 mg/kg, anti-inflammatory magnitude increased sharply to 32.6% (Figure 6b). The maximal anti-inflammation achieved was about 60% above the vehicle control value and occurred at 3.0 mg/kg of dexamethasone (Figure 6b) \((p<0.05)\).
Taken together, the data suggest that the anti-inflammatory action of dexamethasone is both dose- and time-dependent.

Anti-inflammatory ED50 values
The ED50 (median effective dose) of samples correlated with the anti-inflammatory potency estimated in the dose-response graphs. Diclofenac and Dexamethasone, the two control drugs were highly effective as anti-inflammatory agents with corresponding ED50s (Diclofenac-5.534 mg/kg; Dexamethasone-4.441 mg/kg) two orders of magnitude lower than that of the two extracts (Table 8). None of the extracts produced an inhibition of inflammation greater than 70% of the vehicle control and this observation is amply supported by their respectively high ED50s reported in the hundreds. There is a strong positive correlation between the relative inflammation suppressive effects of the two extracts and the estimated ED50s as the ED50 of the methanolic extract was recorded to be 1.5-fold lower than that of the diethylether (p<0.05). The diethylether extract provided the least potent efficacy (ED50 = 381.3 mg/kg) and Dexamethasone was the most potent member of the series of examined samples (Table 8).

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 stem-bark of Nauclea diderrichii.

<table>
<thead>
<tr>
<th>Sample</th>
<th>% yield</th>
<th>TLC Results</th>
<th>Phytochemicals Present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic</td>
<td>8.23</td>
<td>Three 0.05, 0.58, 0.99</td>
<td>Saponins, Steroids, Tannins, Flavonoids, Alkaloids, Glycosides.</td>
</tr>
<tr>
<td>Diethylether</td>
<td>6.31</td>
<td>Two 0.39, 0.91</td>
<td>Saponins, Alkaloids.</td>
</tr>
</tbody>
</table>

Table 2: Broth dilution-based estimation of MICs for extracts of the stem-bark of Nauclea diderrichii.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Test Organisms</th>
<th>Concentrations (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Methanolic</td>
<td>P. aeruginosa</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>S. pyogenes</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>S. aureus</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>C. albicans</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>T. corporis</td>
<td>-</td>
</tr>
<tr>
<td>Diethylether</td>
<td>P. aeruginosa</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>S. pyogenes</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>S. aureus</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>C. albicans</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>T. corporis</td>
<td>-</td>
</tr>
</tbody>
</table>

+ indicates microbial growth; - indicates no microbial growth
Table 3: Broth dilution-based estimation of MICs for the standard control drugs (Ciprofloxacin and Clotrimazole).

<table>
<thead>
<tr>
<th>Standard</th>
<th>Test Organisms</th>
<th>Concentration (µg/ml)</th>
<th>100</th>
<th>50</th>
<th>25</th>
<th>12.5</th>
<th>6.25</th>
<th>3.12</th>
<th>1.56</th>
<th>0.78</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td><em>P. aeruginosa</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>S. pyogens</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>S. aureus</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Clotrimazole</td>
<td><em>C. albicans</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>T. corporis</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

+ indicates microbial growth; - indicates no microbial growth

Table 4: Summary of MIC values of samples (*Nauclea diderrichii* extracts and standard drugs) estimated against test organisms and compiled for comparison from earlier results in Table 2 and Table 3.

<table>
<thead>
<tr>
<th>Test Organisms</th>
<th>Methanolic MIC (µg/ml)</th>
<th>Diethyl Ether MIC (µg/ml)</th>
<th>Ciprofloxacin MIC (µg/ml)</th>
<th>Clotrimazole MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aeruginosa</em></td>
<td>390</td>
<td>12500</td>
<td>1560</td>
<td></td>
</tr>
<tr>
<td><em>S. pyogens</em></td>
<td>390</td>
<td>6250</td>
<td>780</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>195</td>
<td>6250</td>
<td>1560</td>
<td></td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>390</td>
<td>6250</td>
<td>780</td>
<td></td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>780</td>
<td>3125</td>
<td>6250</td>
<td></td>
</tr>
<tr>
<td><em>T. corporis</em></td>
<td>780</td>
<td>3125</td>
<td>6250</td>
<td></td>
</tr>
</tbody>
</table>

Table 5: Quantitative differences in the Total Phenolic Content (gGAE/100g) and the Total Antioxidant Capacity (gAAE/100g) of the methanolic and diethylether extracts of the stem-bark of *Nauclea diderrichii*. Gallic acid and Ascorbic acid were used as the respective controls.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total Phenolic Content (gGAE/100g)</th>
<th>Total Antioxidant Capacity (gAAE/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic</td>
<td>9.08</td>
<td>19.12</td>
</tr>
<tr>
<td>Diethylether</td>
<td>6.44</td>
<td>1.82</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>% DPPH Scavenging</th>
<th>IC50 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>61.08</td>
<td>21.12</td>
</tr>
<tr>
<td>Methanolic</td>
<td>75.41</td>
<td>10.20</td>
</tr>
<tr>
<td>Diethyl ether</td>
<td>18.84</td>
<td>82.55</td>
</tr>
</tbody>
</table>
Table 7: Percentage peroxide scavenging activities in the H₂O₂ assay computed along with the IC50s of extracts of *Nauclea diderrichii* and with that of the Gallic acid control.

<table>
<thead>
<tr>
<th>Sample</th>
<th>% H₂O₂ Scavenged</th>
<th>IC₅₀ µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>32.50</td>
<td>332.7</td>
</tr>
<tr>
<td>Methanolic</td>
<td>40.53</td>
<td>292.9</td>
</tr>
<tr>
<td>Diethyl ether</td>
<td>26.62</td>
<td>507.6</td>
</tr>
</tbody>
</table>

Figure 1: Graphical depiction of the DPPH radical scavenging activities of (a) the methanolic and the diethylether extracts of *Nauclea diderrichii* aligned with that of (b) the Ascorbic acid control.

Figure 2: Peroxide radical scavenging efficacies of the methanolic and the diethylether extracts of *Nauclea diderrichii* in the H₂O₂ assay plotted alongside that of the Gallic Acid control.
Figure 3: Time course of inflammation-suppressive effects evoked by *Nauclea diderrichii* 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 methanolic extract of the stem-bark of *Nauclea diderrichii* and (b) the diethylether extract of the stem-bark of *Nauclea diderrichii*. Dose-induced suppressive effects on oedematous volumes are represented by different colors. Each point represents the mean ± 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: (a) Diclofenac: 3, 10, 30 mg/kg and (b). Dexamethasone: 0.3, 1.0, 3.0 mg/kg pt are shown by differently colored curves. Each point represents the mean ± 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) methanolic extract and (b) diethylether extract of Nauclea diderrichii. 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 and (b). Dexamethasone: 0.3, 1.0, 3.0 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 (methanolic and diethylether extracts, Diclofenac and Dexamethasone controls) estimated pt from the carrageenan-induced inflammation of the foot of the 7-day old chicks.

<table>
<thead>
<tr>
<th>Sample</th>
<th>ED50 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic Extract</td>
<td>261.4</td>
</tr>
<tr>
<td>Diethyl ether</td>
<td>381.3</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>5.534</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>4.441</td>
</tr>
</tbody>
</table>
DISCUSSION

Studies were performed to assess: i) the dose-dependence inflammation protective effects of orally administered *Nauclea diderrichii* in the carrageenan-induced model of inflammation in 7-day old chick; ii) the anti-oxidant effect of *Nauclea diderrichii* in four different *in vitro* assays; iii) the broad-spectrum anti-microbial effect of *Nauclea diderrichii* on a panel of pathogenic microbes *in vitro*.

Bioactive properties of extract are attributable to the activities of the slew of *Nauclea diderrichii* phytochemical that were collectively identified in the phytochemical screen (Chindo et al., 2010). Extraction solvent-based divergence in phytochemical compositions shaped the magnitude and the duration of the bioactive responses of the two extracts (Ngouana et al., 2021). All the polar bioactive phytocompounds in *Nauclea diderrichii* have well authenticated history of medicinal action (Mbiantcha et al., 2020; Romain et al., 2017). Such bioactive phytochemicals in the polar *Nauclea diderrichii* extract likely acts through a multi-pronged mechanism at multi-sites in its prevention and in its management of rheumatism. Since microbial infection, inflammatory response and oxidative stress are associated with disease progression in rheumatism, the hypothesis that *Nauclea diderrichii* will reduce symptoms of rheumatism by increasing microbial attenuation, by decreasing immunoinflammation processes and by reducing oxidative stress are relevant.

These multi-faceted bioactivities also suggest that the polar *Nauclea diderrichii* extract is a rich and untapped source of pharmacologically active compounds that could potentially be novel. Therefore, the methanolic extract generated from *Nauclea diderrichii* can serve as the source material for anti-rheumatism drug discovery efforts. Although some phytocconstituents have been isolated by earlier workers, a future goal along this line will be to identify additional phytocconstituent(s) that contribute specifically to these diverse activities. To accomplish this goal, future studies can perform bioassay-guided fractionation of the polar extract leading to the structure elucidation of novel active compounds. Subsequently, the full range of comprehensive Medicinal Chemistry approaches can be utilized to synthesize and test analog compounds for anti-rheumatism effects *in vitro* and *in vivo* in animal models of rheumatism and eventually to identify the proximate molecular target(s) by which bioactive *Nauclea diderrichii* phytocompounds elicit their biological effect and to elucidate their mechanism of action.

The relevance of inflammation and oxidative stress to the etiology of Rheumatism cannot be understated (Veselinovic et al., 2014). Mechanistically, plant-derived polyphenols exert antioxidant activity that prevents or attenuate rheumatism by countering inflammation, an associated etiologic feature of rheumatism. Carragenan-induced inflammation is reported to be mediated by genes in the Nf-kB pathway (Borthakur et al., 2012). As a consequence, specific induction of select genes under the control of NF-kB could be a mechanism by which *Nauclea diderrichii* exerts its immunoinflammation protective actions. Other *Nauclea diderrichii* phytochemicals likely activates/deactivates key genes within the NF-kB signaling pathway and thereby neutralize reactive oxygen species (ROS) second messengers (Veselinovic et al., 2014). The structurally diverse array of *Nauclea diderrichii* bioactive constituents can also act as biocides and/or fungicides via direct antimicrobial effects, via quorum quenching and via inhibitors of efflux pump.

Critical genes within this immunorheumatoid arthritis framework can be identified and targeted with isolated phytochemicals, particularly genes within the immuno-genome and the immune-microbiome, in order to increase the overall chemotherapeutic and chemopreventive effects of *Nauclea diderrichii* phytochemicals (Chikara et al., 2018).
In light of these data there is a strong possibility that constituent(s) of *Nauclea diderrichii* may be developed as novel Rheumatism chemopreventive and chemotherapeutic agents. Together, these studies could strengthen the possibility that isolated phytochemicals could be a significantly useful anti-rheumatism drug.

**Conclusion**

*Nauclea diderrichii* is a multi-purpose plant used for the ethnomedicinal management of rheumatism. Understanding the anti-microbial, anti-oxidant and anti-inflammatory effects of *Nauclea diderrichii* is critical to the provision of a solid scientific basis for the continuing ethnomedicinal use of its aqueous extract to target rheumatism chemotherapeutically. In this study, the *in vitro* anti-microbial, *in vitro* anti-oxidant and the *in vivo* anti-inflammatory activity of the methanol extract of *Nauclea diderrichii* was compared with that of the diethylether. The methanolic extract contains more than 2-fold more putative bioactive phytochemical types when compared to the diethylether extract. And such differences in solvent-specific phytochemical compositions account for the observed differences in the strengths of examined bioactivities. The methanolic extract demonstrates more potent (lower MICs) broad-spectrum activity against a panel of clinical isolates of bacterial and fungi pathogens *in vitro*. The methanolic extract also demonstrated more potent slew of *in vitro* anti-oxidant activities characterized by higher total anti-oxidant capacity, higher total phenolic content and lower IC\(_50\)'s in the DPPH and H\(_2\)O\(_2\) anti-oxidant assays. The methanolic extract displayed higher concentration-dependent reduction in foot-swelling in the carrageenan induced chick feet oedema assay. Such reduction of inflammation in the chick model offers experimental evidence for the use of the polar extract of the stem-bark as an immune-inflammatory agent in rheumatism in humans. Based on these findings, it could be concluded that single therapeutically efficacious *Nauclea diderrichii* constituent(s) can be developed for both prevention and management of rheumatism. The findings of this study partly confirm the reliability of this ethnomedicinal chemotherapeutic strategy for the management of rheumatism.

**COMPETING INTERESTS**

The authors declare no competing interests.

**AUTHORS’ CONTRIBUTIONS**

JKM Conceived the research idea, guided and instructed Ahmed Sala on the bench work and wrote the manuscript. AS Performed most of the experimental work that generated the data for this manuscript. YJ Performed the work on the anti-inflammatory section.

**ACKNOWLEDGEMENTS**

The authors thank Mr. Francis Amankwah for providing technical assistance for the anti-microbial assay.

**ETHICAL STATEMENT**

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

**REFERENCES**


