Phytochemical screening, Gas Chromatography Mass Spectroscopy (GC-MS) and in vitro antiplasmodial analysis of Senna siamea leaves as antimalarial, Yobe State, Nigeria

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Abstract
Poverty and lack of access to health facilities had necessitated the less privileged to depend on herbal remedies to treat a number of diseases including malaria. The successes recorded from the discovery of novel antimalarials from plant sources had paved the way for the search of antimalarial compounds from traditional pharmacopeia. Phytochemical analysis as well as GC-MS analysis of crude leaves extract of Senna siamea was conducted, with a view to identifying compounds with biological activities. Further antimalarial assessment of the crude extracts was also performed in an in vitro assay. The phytochemicals; phenols, tannins, anthraquinones, alkaloid and flavonoids were detected from hexane and methanol extracts. GC-MS analysis revealed the presence of Saponins specifically, triterpenoids (Lupeol, α-amyrin), Sesquiterpene (Octadecane), Diterpenes (Eicosane), Esters of pthalic acids (Diethyl phthalate) Squalene, α-Tocopherol (a fat soluble Vitamin E) and hexadecanoic acid butyl ester. A dose dependent suppression of parasite growth was observed for all extracts, with methanolic extract showing less antimalarial potency (IC₅₀ = 3.74 µg/mL) when compared to the hexane extract (IC₅₀ = 4.349 µg/mL). Extracts and compounds detected from the leaves of this plant could be used as novel lead compounds to develop new drugs.

Keywords: Phytochemical analysis; GC-MS; antimalarial; Senna siamea; medicinal plants.

Introduction
Malaria is the leading cause of mortality in children under five years of age in sub-Saharan Africa [1-3]. Until recently, there is no vaccine in clinical use for malaria [4, 5]. Resistance to chloroquine [6-8], the cornerstone for malaria chemotherapy, Artemisin in combination therapies (ACTs) [9-12] the mainstay of malaria treatment in most malaria endemic countries as well as other forms of antimalarials has made it necessary to search for antimalarials. The successes recorded from the discovery and isolation of quinine from Cinchona tree [13], which eventually led to the development of the synthetic drug, chloroquine, as well as the discovery of Artemisinins from sweet wormwood (Artemisia annua) [14] has paved the way for scientist to search for antimalarials from plant sources.

In traditional medicinal practice, the leaves of Senna siamea was reported as a good source of antimalarials [15-17]. In Yobe State, north-eastern Nigeria, a decoction of the leaves of S. siamea alongside leaves of Magnifera indica, Psidium guajava and Citruss spp is believed to cure typhoid and malaria fever. Other medicinal uses attributed to S. siamea are gastrointestinal anthelmintic (the fruit) and prevention of convulsion in children [18]. The stem bark are reported to possess analgesic and anti-inflammatory effects [19] in addition to its antimicrobial activities.

Senna siamea otherwise termed Kassod or Cassod,
tree (English) is a medium sized evergreen tree belonging to the subfamily Caesalpinioideae. The plant is a native of tropical Africa and is cultivated mostly in Egypt, Sudan, and other parts of the tropics [20]. In northern Nigeria, the indigenous Hausa community referred to this plant as Malga/Marga [21] while locals in Yobe State call the plant Dr. Jalo. Here, we evaluate the presence of certain phytochemicals from this medicinal plant as well as define the identity of compounds in crude extracts by Gas Chromatography Mass Spectroscopy (GC-MS). Further assessment of the antiplasmodial effect of crude extracts was performed in vitro.

Materials and methods
Selection criteria
Record of medicinal plants in the folklore of Yobe State was obtained from the traditional medicine section of Yobe State Ministry of Health. The plant was selected on the basis of its frequent use as ethnomedicines for the treatment of typhoid and malaria. Information of the plant parts used, local preparation method, mode of administration and reported side effects (if any) were obtained from traditional herbalists. This was then recorded as previously described [22].

Collection and identification
Scientific identity of the plant was authenticated in the Department of Biological Sciences, Yobe State University, Damaturu, Nigeria. Following identification, voucher specimen was deposited in the herbarium of the same department for record purpose.

Processing plant samples
Fresh leaves of S. siamea was hand-picked, washed in clean tap water and dried under shade at ambient temperature (28-37°C). Dried leaves were then processed in line with standard protocols [20, 23] and extraction of secondary metabolites was performed in accordance with the protocol previously described [24].

Phytochemical screening
Resultant to extraction of bioactive ingredients, qualitative analysis of crude extracts was performed to screen for the presence of phytochemicals using two extraction solvents (n-hexane and methanol). This analysis was conducted in the Chemistry Research Laboratory of Yobe State University, Damaturu, in accordance with Kumar et al [25].

Gas Chromatography: Mass Spectrometry (GC-MS) analysis
In a bid to define the real identity of compounds present in crude extracts, extracts were subjected to further analysis (quantitative) using GC-MS. Powdered extracts (0.5 g) each was macerated in n-hexane (1.5 ml). Samples were filter sterilized using 0.22 μm pore size filter unit and analyzed (GCMS-QP2010 ULTRA, Shimadzu). Column temperature was set at 40°C-300°C with helium gas running as the carrier gas at a flow rate of 4.8 and a run/post run time of 48 minutes. Compounds produced were compared with those in the National Institute Standard and Technology (NIST) database.

Parasite culture:
Preparation of culture medium
Sodium bicarbonate (2.3 g) was dissolved in dH2O and stirred with a magnetic stirrer. The solution was supplemented with RPMI medium 1,640 (gibco) powder, glucose 4 ml (50% w/v in dH2O), Hypoxanthine 2 ml (25 mg/mL in 1M NaOH), gentamicin 2 ml (12.5 mg/mL in dH2O), L-glutamine 1 ml (0.292 g in 10 ml dH2O) and Albumax 25 ml (20% w/v in RPMI IM) to form complete medium (CM). Serum was not used in the culture. The pH was adjusted to 7.25-7.30, volumes of the culture medium was adjusted to 1 liter with dH2O and finally filter sterilized using a 0.22 μM filter unit, labelled and stored at 4°C until use.

Blood preparation
Blood (Group O +) was processed in line with the method of Moon et al [26]. Briefly, The blood was centrifuged at 1,800 rpm for 5 minutes, plasma and the buffy coat was discarded and volume of the pellet (packed RBCs) was measured and resuspended (1:1 v/v) in CM. This was centrifuged as previously described and washing repeated three (3) times. Blood was stored at 4°C in RPMI 1640 (about 50% hematocrit) right after the last wash without discarding the supernatant.

Thawing cryopreserved parasite isolates
Laboratory adapted chloroquine sensitive P. falciparum isolates (3D7) was thawed according to the methods of Ribacke et al [27] with little modifications. Briefly, cryopreserved parasites were removed from liquid nitrogen and immediately thawed for 5 minutes in a water bath set at 37°C, following which it was moved to the biosafety cabinet. Disinfection with 70% ethanol was always carried out before placing any material into the Biosafety Cabinet in line with Orman et al [28]. Volume of parasitized RBCs (iRBCs) was measured, transferred to 15 ml falcon tube and equal volume (1:1 v/v) of thawing solution (3.5% NaCl) was added slowly, dropwise, while shaking gently [29]. The suspension was centrifuged at 1,000 rpm for
5 minutes with slow brake and acceleration, supernatant was removed. Washing repeated 3 times with equal volume of CM until a clear supernatant is obtained [29]. The supernatant was discarded and the final volume of iRBCs was measured and transferred to a culture flask.

**Maintaining parasites in continuous culture**

For continuous culture, parasites were maintained in 2-5% hematocrit. Volume of packed cells (iRBCs and RBCs, 1:1 v/v) was measured, transferred onto a sterile culture flask and the required volume of complete culture medium (CM) was added, gassed (88% Nitrogen, 7% Carbon dioxide and 5% Oxygen) and incubated at 37°C as previously described [30] and modified [31]. Parasitaemia was monitored and medium changed daily [32]. In situations where parasitaemia was found to increase (>20%), culture flask was changed and the volume of culture was increased.

**Parasite synchronization and seeding**

A sterile 96 wells flat bottom plate was used for in vitro antimalarial sensitivity assay of crude hexane and methanolic *S. siamea* leaves extract. In this assay, parasites were synchronized by density gradient method with histodenz as previously described [29]. Synchronized (rings and trophozoites) parasite cultures (1% parasitaemia, 2% hematocrit) was pipetted (180 μL) into duplicate wells (Columns 1-10) of a chloroquine pre-coated sterile 96 wells flat bottom plate. To duplicate wells (Columns 1-8), an aliquot, 20 μL of various concentrations (500, 250, 125, 62.5 μg/ml) respectively of crude plant extracts was added [33] to yield a final concentration (50, 25, 12.5 and 6.25 μg/mL) of each extract in the respective wells [34]. Columns 9 and 10 received 20 μL of the culture medium to serve as negative controls, while Columns 11 and 12 previously coated with the reference antimalarial (chloroquine) received 200 μL of synchronized parasite cultures to serve as positive control. Microplates were covered, placed in a gas chamber, gassed (88% Nitrogen, 7% Carbon dioxide and 5% Oxygen) and incubated at 37°C for 48 hours. Gassing was repeated after the first 24 hours and gas chamber placed back in the incubator for another 24 hours.

Following incubation parasite culture was harvested as previously described [35] and percentage parasitaemia as well as growth suppression was calculated using the formula:

\[
\text{Growth suppression (%) = } \frac{\text{Mean parasitaemia}_{\text{Negative control}} - \text{Mean parasitaemia}_{\text{Treated group}}}{\text{Mean parasitaemia}_{\text{Negative control}}} \times 100
\]

**Results**

Results of phytochemical analysis of two solvent extracts of *S. siamea* are presented in Table 1.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Phytochemicals</th>
<th>Type of Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hexane</td>
</tr>
<tr>
<td><em>S. siamea</em></td>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Anthraquinones</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Flavonoids</td>
<td>+</td>
</tr>
</tbody>
</table>

**Figure 1.** GC-MS chromatogram of methanol extract of *S. siamea* leaves.

**Table 1.** Phytochemicals identified in crude Hexane and Methanol leaves extracts of *S. siamea*.

**Data analysis**

Data obtained was analyzed using Microsoft excel 2010 and then Graphpad prism 5.0. For IC50 calculations, data was normalized so that the largest value in the data set corresponded to 100% and the smallest value corresponded 0%. Log-transformed drug/crude extract concentrations were then plotted against the dose response and the IC50 values were determined using nonlinear regression. The log (inhibitor) vs. Normalized response-Variable slope option was selected for this analysis.

**Key:** + = present; – = absent.

Percentage parasitaemia (%) = \[
\frac{\text{Number of infected Red Blood Cells (iRBCs)}}{\text{Total number of Red Blood Cells (RBCs)}} \times 100
\]
Gas chromatography Mass Spectrometry (GC-MS)

Compounds in crude extracts were identified by GC-MS. Based on the results obtained, a total of 33 and 34 compounds were detected from the methanol and hexane extracts respectively. Of these, certain compounds such as saponins specifically, triterpenoids (Lupeol, α-amyrin), Sesquiterpene (Octadecane), Diterpenes (Phytol, Eicosane), Esters of phthalic acids (Diethyl phthalate) Squalene, Stigmasterol, α-Tocopherol (a fat soluble Vitamin E), hexadecanoic acid butyl ester and Isopropyl myristate were previously reported to possess potent biological activities. Other compounds identified, their chemical formulae, molecular weight as well as concentrations (ng/L) are not presented.

**Table 2.** Summary of some bioactive compounds in phytochemicals of Hexane extract of *S. siamea* as revealed by GC-MS analysis.

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Chemical formula</th>
<th>Mol. Weight</th>
<th>Retention Time</th>
<th>Peak No. (M/Z)</th>
<th>Conc. (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lupeol</td>
<td>C30H50O</td>
<td>426</td>
<td>46.448</td>
<td>218.20</td>
<td>0.479</td>
</tr>
<tr>
<td>Stigmasterol</td>
<td>C29H48O</td>
<td>412</td>
<td>44.133</td>
<td>55.15</td>
<td>0.334</td>
</tr>
<tr>
<td>Squalene</td>
<td>C30H50O</td>
<td>410</td>
<td>38.016</td>
<td>69.15</td>
<td>2.145</td>
</tr>
<tr>
<td>Hexadecanoic acid, butyl ester</td>
<td>C20H40O</td>
<td>312</td>
<td>27.654</td>
<td>56.15</td>
<td>1.467</td>
</tr>
<tr>
<td>9-Octadecenamide, C28H35NO</td>
<td>281</td>
<td>30.826</td>
<td>59.10</td>
<td>5.923</td>
<td></td>
</tr>
<tr>
<td>Octacosanol</td>
<td>C20H42</td>
<td>410</td>
<td>41.723</td>
<td>57.15</td>
<td>0.415</td>
</tr>
<tr>
<td>Eicosane</td>
<td>C20H42</td>
<td>282</td>
<td>18.011</td>
<td>57.15</td>
<td>2.688</td>
</tr>
</tbody>
</table>

**Table 3.** Summary of some bioactive compounds in phytochemicals of Methanol extract of *S. siamea* as revealed by GC-MS analysis.

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Chemical formula</th>
<th>Mol. Weight</th>
<th>Retention Time</th>
<th>Peak No. (M/Z)</th>
<th>Conc. (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Amyrin</td>
<td>C30H50O</td>
<td>426</td>
<td>45.651</td>
<td>218.20</td>
<td>0.720</td>
</tr>
<tr>
<td>Stigmasterol</td>
<td>C29H48O</td>
<td>412</td>
<td>44.072</td>
<td>44.131</td>
<td>0.756</td>
</tr>
<tr>
<td>Lupeol</td>
<td>C30H50O</td>
<td>426</td>
<td>42.657</td>
<td>46.457</td>
<td>0.863</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>C29H50O</td>
<td>430</td>
<td>42.303</td>
<td>46.043</td>
<td>1.312</td>
</tr>
<tr>
<td>Lupenyl acetate</td>
<td>C24H32O</td>
<td>468</td>
<td>46.043</td>
<td>55.15</td>
<td>0.212</td>
</tr>
<tr>
<td>Diethyl Phthalate</td>
<td>C12H14O</td>
<td>222</td>
<td>15.561</td>
<td>149.00</td>
<td>25.013</td>
</tr>
<tr>
<td>Squalene</td>
<td>C30H50</td>
<td>410</td>
<td>38.012</td>
<td>69.15</td>
<td>3.393</td>
</tr>
<tr>
<td>Eicosane</td>
<td>C20H42</td>
<td>282</td>
<td>14.141</td>
<td>57.15</td>
<td>1.049</td>
</tr>
</tbody>
</table>

Sensitivity tests were carried out to establish the effect of crude hexane and methanolic *S. siamea* leaves extracts against chloroquine sensitive *P. falciparum* (3D7) culture *in vitro* and the IC₅₀ for each extract and the reference antimalarial drug (Chloroquine) was determined. Tables 4 below summarizes the percentage parasitaemia, percentage growth suppression and the IC₅₀ values of extracts investigated. Results of the percent parasitaemia is presented as Mean ± Standard error of mean (M±SEM) except for the percentage growth suppression and the IC₅₀ values. Briefly, a dose dependent suppression of parasite growth was observed for all extracts, with methanolic extract showing less antimalarial potency (IC₅₀ = 3.74 μg/mL) and hexane showing the highest (IC₅₀ = 4.349 μg/mL) at microgram per mills (μg/mL) concentrations.

**Figure 2.** GC-MS chromatogram of hexane extract of *S. siamea* leaves.
Discussion

Result of phytochemical analysis conducted in this study indicates the presence of phenols, tannins, anthraquinones, alkaloid and flavonoids in all solvent extracts. This finding corroborate with the result of Mohammed et al [36]. A good number of phytochemicals, of which, flavonoids and alkaloids put forth their effects by way of scavenging free radicals generated during the course of infection or by activating a series of self-protective proteins. Blocking oxidation in aerobic organisms is a vital process [37] as decreased antioxidant protection may lead to cytotoxicity. In reaction to malaria infection for example, activation as well as up regulation of immune cells produce substantial sums of Reactive Oxygen and Reactive Nitrogen Species (ROS and RNS) to fight infection, thus causing inconsistencies between the formation of oxidizing species and the activity of antioxidants. This imbalance has been reported to be responsible for oxidative stress, an important mechanism of human hosts in response to infections [38]. In the case of malaria infection, oxidative stress can result in parasites death. Percário et al [38] further reported that P. falciparum infected red blood cells generates (free OH• and H2O2) radicals almost two times higher than uninfected erythrocytes.

In this work, GC-MS analysis was performed in a bid to identify active ingredients or compounds in phytochemicals from crude extracts. The gas chromatograms (Figures 1 and 2) obtained from this analysis showed the relative concentrations of various compounds getting eluted as a function of retention time. These mass spectra are fingerprints of those compounds which can be identified from the NIST data library [39]. Most of the compounds identified have been reported to show some degree of biological activities. Lupeol, for example, identified in both hexane and methanol leaves extract of S. siamea with a concentration of 0.863 and 0.479 ng/L respectively (Tables 2 and 3) was reported to show antimalarial activities in 3D7 strain of P. falciparum, in vitro [40]. Similarly, Siddique and Saleem [41] reported other beneficial health effects of Lupeol to include; antimicrobial, antiprotozoal, antiarthritic, anti-cancer, anti-diabetic, anti-inflammatory, cardio-protective, hepatoprotective and nephroprotective effects. Squalene, on the other hand, present in the hexane and methanolic leaves extracts of this plant was reported to possess antioxidant and antitumor properties in addition to skin hydration when applied topically [42]. This compound also serves as an intermediate in the biosynthesis of cholesterol. Other triterpenes with potent biological activities identified in this research are the amyrins (α-amyrin) identified in Hexane extracts, the presence of which was also reported in several plants; Shea tree, Vitellaria paradoxa (Sapotaceae) [43]; Cereals [44] and the pure compounds isolated have been reported to show anti-microbial, anti-inflammatory, and other interesting biological activities [43]. Likewise, Diethyl-phthalate was also identified. The compound was reported in Achillea tenuifolia [45]; Plantago major (leaves and roots) [46] and the presence of which was attributed to induction of inflammation,

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Type of extract</th>
<th>Dose (μg/mL)</th>
<th>Parasitaemia (%) Mean ± SEM</th>
<th>Growth suppression (%)</th>
<th>IC50 (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. siamea</td>
<td>Hexane</td>
<td>6.25</td>
<td>2.85 ± 0.05</td>
<td>59.82</td>
<td>4.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.5</td>
<td>2.33 ± 0.11</td>
<td>67.17</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>1.92 ± 0.11</td>
<td>72.85</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>1.28 ± 0.06</td>
<td>81.96</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>6.25</td>
<td>2.50 ± 0.24</td>
<td>64.75</td>
<td>3.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.5</td>
<td>1.89 ± 0.28</td>
<td>73.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>1.80 ± 0.22</td>
<td>74.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>1.43 ± 0.39</td>
<td>79.76</td>
<td></td>
</tr>
<tr>
<td>Chloroquine</td>
<td>–</td>
<td>15.625</td>
<td>2.85 ± 0.59</td>
<td>59.84</td>
<td>11.79</td>
</tr>
<tr>
<td>(Positive</td>
<td>–</td>
<td>31.25</td>
<td>1.55 ± 0.03</td>
<td>78.12</td>
<td></td>
</tr>
<tr>
<td>control)</td>
<td>–</td>
<td>62.50</td>
<td>0.74 ± 0.06</td>
<td>89.63</td>
<td></td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>125</td>
<td>0.68 ± 0.1</td>
<td>90.40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>250</td>
<td>0.41 ± 0.09</td>
<td>94.28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>500</td>
<td>0.21 ± 0.05</td>
<td>97.03</td>
<td></td>
</tr>
<tr>
<td>CM only</td>
<td>–</td>
<td>–</td>
<td>0 ± 0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>(Negative</td>
<td>–</td>
<td>–</td>
<td>7.08±1.39</td>
<td>0.00</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 4. In vitro antimalarial effect of hexane and methanol leaves extracts of S. siamea against P falciparum (3D7).
early puberty in girl child and oxidative stress in human. Although this compound was thought to be a pollutant, its presence in plants is well documented. Diethylphthalate was reported to be used as a plasticiser, and plays a significant role in antitumour activity against mice sarcoma 180 cell lines [47]. Essentially, Eicosane (a Diterpene), identified in this study, have been previously reported to show antitumour activity against the human gastric SGC-7901 cell line [47]. Another compound, $\alpha$-tocopherol identified in hexane extract was also reported in Prunus persica. The biological effects (antitumor-promoting effects as well as treatment of atherosclerosis, premenstrual syndrome and threatened abortion in early pregnancy) produced by Prunus persica was related with the presence of $\alpha$-tocopherol [48]. Similarly, hexadecanoic acid butyler had been reported to possess antimicrobial and antioxidant activities [49]. The presence of these bioactive compounds in the plant extract may likely be responsible for some of the pharmacological properties of the plants and thus could be of considerable interest to the development of new drugs.

The anti-plasmodial potentials of hexane and methanol leaves extracts of S. siamea was also evaluated against a chloroquine sensitive P. falciparum (3D7) (Table 4). Findings of the in vitro studies revealed that, at the lowest dose, the methanol extract was the most potent ($-65\%$ suppression of Plasmodium growth), while both extract showed similar activities ($\geq 80\%$ suppression) at the highest dose levels was observed. A pretty good IC$_{50}$ values; hexane (IC$_{50}$ = 4.23 $\mu$g/mL) and methanol (IC$_{50}$ = 3.74 $\mu$g/mL) was obtained for both extracts. In Cameroon, Ntandou et al [17] examined the anti-plasmodial activities of the crude stem bark extract of this plant on P. falciparum isolates collected from patients and on chloroquine resistant (FcM 29) strain of P. falciparum. Ntandou and co-workers reported an IC$_{50}$ of 0.4 $\mu$g/mL for field isolates and 18.2 $\mu$g/mL for FcM 29 strain. This biological activity was then attributed to the presence of triterpenes which are part of the majority of chemical groups in S. siamea bark extracts. Although not detected in this study, four alkaloids, cassiarins G, H, J, and K were isolated from the leaves of S. siamea [16]. When evaluated against P. falciparum 3D7, moderate antimalarial activities were observed for cassiarins J and K (IC$_{50}$ = 0.3 $\mu$M; 1.4 $\mu$M) at micro molar ($\mu$M) concentrations while both cassiarins G and H showed poor activities (IC$_{50}$ > 50 $\mu$M) [16]. The chloroform, methanol, and hydro alcoholic extract of the leaves of this plant was tested on W2 clone of P. falciparum and weak activities with similar IC$_{50}$ values up to 10 $\mu$g/mL was obtained for all extracts while the aqueous extract showed the lowest activity with IC$_{50}$ value of 23.15 $\mu$g/mL [50]. The selectivity indexes of aqueous extracts of some plant species including the root bark of S. siamea for 3D7 and W2 strain of P. falciparum was reported, with all extracts being selective on 3D7 (selectivity indexes (SIs $\geq$ 3.48) but only that of S. siamea was selective for the W2 strain (SI $>$ 2.1) [51]. A bioassay guided fractionation of the stem bark of S. siamea led to the identification of two compounds with R, spotting of 0.63 and 0.7 respectively on silica gel [15]. Further animalarial screening of these compounds on the chloroquine resistant stain (K1) of P falciparum revealed an IC50 of 5 $\mu$g/mL for all compounds. The finding in this research is consistent with that reported for stem bark fraction of S. siamea [15]. This finding further validates the traditional claim of the pharmacological potency of this plant in typhoid [19, 20] and malarial fevers.

**Conclusion**

*Senna siamea* is a medicinal plant used by the indigenous people of Yobe State to treat malaria and typhoid fevers. Extracts and compounds detected from the leaves of this plant could be used as novel lead compounds to develop new drugs. We recommend further fractionation and identification of bioactive metabolites from various fractions using GC-MS, LC-MS and HPLC. Subsequent evaluation of the antimalarial potencies of compounds detected could yield promising outcomes and may pave ways for the development of novel antimalarial compounds.

**Acknowledgements**

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**Conflict of interest**

The authors declare that we do not have conflict of interest regarding the publication of this manuscript.

**References**


