



Antioxidant Potentials, Phytochemical Profiles and Ameliorating Effects of *Senna siamea* Extract on Arsenic-Induced Hepato-Renal Damage

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRB/2021/v9i130194

Editor(s):

(1) Dr. Mohamed Fawzy Ramadan Hassanien, Zagazig University, Egypt.

Reviewers:

(1) Ajmer Singh Grewal, Guru Gobind Singh College of Pharmacy, India.

(2) Sunil Aggarwal, New York University, USA.

Complete Peer review History: <https://www.sdiarticle4.com/review-history/74802>

Original Research Article

Received 20 July 2021
Accepted 30 September 2021
Published 09 October 2021

ABSTRACT

This study evaluated the *in-vitro* antioxidant, phytochemical constituents and hepato-renal protective efficacy of *Senna siamea* methanolic extract in arsenic-induced oxidative stress. The results of this study show that *S. siamea* has strong antioxidant potentials against 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3 ethylbenzothiazoline-6-sulphonic acids) (ABTS) radicals. The extract scavenges nitric oxide radicals and has strong ferric reducing power. It also inhibited the induction of lipid peroxidation and α -amylase activity in a concentration-dependent manner. Administration of arsenic to rats induced a significant increase in the levels of alanine aminotransferase, aspartate aminotransferase, cholesterol, urea, creatinine, and triglycerides in the plasma, while it decreased superoxide dismutase, glutathione and catalase activities in the liver and kidney. It also significantly reduced the levels of white blood cells, red blood cells, platelet and

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lymphocytes in the blood. However, rats pretreated with *S. siamea* extract before arsenic administration significantly reversed all these arsenic-induced changes. Histological observations showed varying degrees of liver damage in the arsenic group that was untreated, while the pretreatment with *S. siamea* extract significantly improved the general histoarchitecture of the liver. The phytochemical constituents of the extract were determined, the gas-chromatography analysis revealed the possible presence of oxalic acid, barakol, kaempferol, betulin while total phenolics content, crude protein, crude fat, crude fibre are in considerable amount in the plant. This study demonstrated that *S. siamea* extract has antioxidant potentials and ameliorates arsenic-induced hepato-renal toxicity, the effectiveness of *S. siamea* as a medicinal plant could be due to the presence of various phenolics and antioxidant compounds in the plant.

Keywords: *Senna siamea*; arsenic; scavenges; hepato-renal; histological.

1. INTRODUCTION

The plants represent a major substrate of bioactive compounds which could be of pharmacological importance at therapeutic doses [1]. The synthesis of novel drugs and compounds from plants have been used as anti-inflammatory, hepatoprotective, cardioprotective agents etc [2]. Paclitaxel derived from *Taxus brevifolia*, Artemisinin derived from *Artemisia annua* and Silymarin extracted from the seeds of *Silybum marianum* are good examples of drugs derived from plants [3]. Majorly, the effectiveness of medicinal plants in the management of disease state depends on the inhibition of oxidation or donation of hydrogen ion to damaging molecules [4].

Ingestion/ exposure to a substantial amount of arsenic has been a major cause of scientific worry. Although arsenic can be pharmacologically significant especially in the treatment of myeloblastic leukaemia, its exposure activates the prooxidant system and as well reduce the bioactivity of natural antioxidants in the body system [5]. This burdensome act results in oxidative stress causing damage to essential bodily biomolecules, abnormalities to chromosome and in extreme cases death [5].

Although plants are effective in the management of disease state, adverse effects have sometimes been reported from their usage suggesting that plants with minimum side effects and greater therapeutics potentials will be preferable as a medicinal plant. There is growing interest in the use of vegetables, fruits, and tree components because they contain essential phytochemicals such as flavonoids, phenolics among others [6].

One plant that has attracted much attention over the years because of its medicinal potentials is *Senna siamea* which is widely distributed in Africa. *Senna siamea* also refer to as Cassia

siamea belongs to the family Caesalpiniaceae. The plant locally known in Yoruba as 'Odan' and 'Cassod' in English have been used traditionally for their antimalarial, anticancer, antipyretic, sedative, antidepressant and anti-inflammatory potentials [7,8]. Among the phytoconstituents earlier reported to be present in the plant includes anthraquinones, alkaloids, glycosides, coumarins, chromones, terpenoids, tannin, sterols and polyphenols [9]. These phytoconstituents have been reported to be associated with several pharmacological activities for example phenolic compounds are good antioxidant, alkaloids are essential in diseases management, glycosides protect the heart and terpenoids are important in the synthesis of sex hormones [10]. Therefore, this study aimed to investigate the *in vitro* antioxidant potential, chemical constituents and hepato-renal protective efficacy of *Senna Siamea* methanolic extract against arsenic-induced oxidative stress.

2. MATERIALS AND METHODS

2.1 Reagents

Ferric chloride, dinitro salicylic acid reagent, butanol, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ethanol, Trichloroacetic acid (TCA), Folin-Ciocalteu reagent, starch, thiobarbituric acid, sodium carbonate, sodium chloride, sodium nitroprusside, naphthyl ethylenediamine dichloride, α -amylase, glacial acetic acid and potassium hexacyanoferrate, and trichloroacetic acid were obtained from Sigma–Aldrich Chemical Co. Ltd. (England). Nitrobluetetrazolium (NBT) was the product of Fluka (Buchs, Switzerland). All other chemicals used were of analytical grade.

2.2 Plant Materials and Extract Preparation

The leaves of *S. siamea* were obtained from a local farm at Ibadan, Oyo State. The plant

identification and authentication was carried out at the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, by Prof A.J. Ogunkunle. The leaves were air-dried for two weeks at room temperature and pounded into powder. 100g of the powdered *S. siamea* leaves were soaked in 500ml of methanol and shaken for 72 hours, afterwards, it was filtered and the supernatant was concentrated using a rotatory evaporator.

2.2.1 The *in-vitro* antioxidant assays and total phenolic content of *S. siamea*

The *in-vitro* antioxidant potentials of methanolic extract of *S. siamea* was carried out using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity as previously described by Schelesier *et al.*, [11]. Trolox Equivalent Antioxidant Capacity Assay (ABTS) as described by Re *et al.*, [12] Ferric Reducing Antioxidant property (FRAP) Assay described by Oyaizu *et al.*, [13]. Nitric oxide scavenging activity was described by Garrat [14]. Lipid peroxidation Inhibition Assay described by Janero [15] and alpha-amylase inhibition assay described by Bernfeld [16]. Total phenolics of *S. siamea* were determined by the Folin-Ciocalteu method as described by Miliauskas *et al.*, [17].

2.3 Phytochemical Composition Screening

Qualitative analysis of methanolic extract of *S. siamea* was carried out by testing for the presence of flavonoids, terpenoids, tannins, phenols, saponins, phytosterol, alkaloids, phlobotannins and cardiac glycoside. Each assay was carried out following the method of Sofowora *et al.*, [18] for flavonoids, Ejikeme *et al.*, [19] for terpenoids and tannins, Santhi *et al.*, [20] for phenol, Harbone *et al.*, [21] for phytosterol and alkaloids, and Ajiboye *et al.* [22], for phlobotaninns.

2.4 Gas Chromatography Analysis

The gas chromatography (GC) study was done using Shimadzu GC-17A gas chromatography fitted with a flame ionization detector (FID) and an autosampler. GC column used was, fused silica capillary column OV-1, DB-1 (30 m x 0.53 mm, 0.5 μ m film thickness), at 75 °C and programmed to 75 °C at 240 °C/min and 5 min hold. Injector and detector were at 240 and 250 °C respectively. About 1 μ L of each sample was injected and the relative quantity of the chemical

compounds present in the extract of *S. siamea* was expressed as a percentage based on the peak area produced in the chromatogram. The identification of *S. siamea* constituents was carried out by comparison of GC retention times of *S. siamea* with GC retention times of desired standards compounds.

2.5 Proximate Analysis

The proximate composition of *S. siamea* leaf powder samples was determined using standard procedures. Moisture content was determined as described by Udo and Ogunwele [23] with slight modification. Ash was determined by incineration (550°C) of known weights of samples in a muffle furnace [24]. The crude lipid content was determined using the Soxhlet method described by Udo and Ogunwele [23]. The crude fibre was determined after digesting a known weight of fat-free sample with sulfuric acid and sodium hydroxide as described by Udo and Ogunwele [23]. The crude protein percentage was evaluated using the Miro-Kjeldahl method described by AOAC [25] while nitrogen-free extract (NFE) was determined by the addition of all percent of moisture, fat, crude protein, ash and crude fibre subtracted from 100% [26].

2.6 Animals

Twenty four male Wistar rats with an average weight of 200g were obtained from the Experimental Animal Unit of Faculty of Agriculture, Ladoke Akintola University of Technology, Ogbomosho, Nigeria. All rats had free access to feed and water and were maintained under standard environmental conditions. The animals were acclimatized for 3 weeks before the commencement of the study. The Faculty of Basic Medical Science, Ladoke Akintola University of Technology, Ogbomoso, Research Ethics committee gave Ethical approval for the study (FBMS2019/012). All the ethical protocols laid by the committee in line with the standard accepted principles for laboratory animal handling and care were followed.

2.6.1 Experimental design

Three weeks after acclimatization the animals were divided into four groups of six animals per group. Group 1 rats received normal saline only and served as control. Group 2 rats received a single dose of arsenic at 10mg/kg body weight intraperitoneally on the 7th day. Group 3 and 4 rats were treated orally with *S. siamea* and

Ascorbic acid at 200mg/kg body weight per day respectively for 7days and on the 7th day, a single dose of arsenic at 10mg/kg body weight was administered intraperitoneally. The rats were sacrificed twenty-four hours after administration of arsenic by cardiac puncture under light ether anaesthesia. Blood, liver and kidney samples were removed from the animals and stored for biochemical analyses

2.6.2 Determination of biochemical parameters in plasma

The concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, urea, creatinine, cholesterol and total triglycerides were determined in the plasma using enzymatic kits.

2.6.3 Preparation of liver and kidney homogenates

The liver and kidney samples are homogenized in phosphate buffer saline (PBS) to give a 10% (w/v) liver and kidney homogenate. The supernatant obtained after the homogenates were centrifuged at 12,000 rpm for 15 min was used for biochemical assay.

2.6.4 Determination of antioxidant enzyme activities and MDA levels

The concentration of reduced glutathione (GSH), superoxide dismutase (SOD), thiobarbituric acid-reactive product malondialdehyde (MDA) and catalase (CAT) in the liver and kidney homogenates were measured, as described by Jollow et al. [27], Misra and Fridovich [28], Buege and Aust [29] and Sinha [30] respectively. All enzyme activities were expressed as per mg of protein.

2.6.5 Haematological study

Fresh blood collected in EDTA bottles were analysed to determine haematological parameters using an automatic haematological assay analyser (ERMA PCE 210, ERMA, Japan).

2.6.6 Histopathological study

The liver tissue was harvested from the sacrificed rats and immediately fixed in formal saline and used for histomorphological studies.

2.7 Statistical Analysis

The results of this study were expressed as mean \pm S.E.M. One-way Analysis of Variance (ANOVA) followed by Turkey's test was used for

statistical analysis and p -value <0.05 were considered statistically significant.

3. RESULTS

3.1 DPPH Radical Scavenging Assay

The extract of *S. siamea* demonstrated a concentration and time-dependent scavenging activity by quenching DPPH radicals and was compared with gallic acid, as a positive control (Fig. 1). The IC_{50} values (defined as the concentration of test compound required to produce 50% inhibition) for DPPH scavenging by *S. siamea* and gallic acid are 319.69 μ g/dL and 16.13 μ g/dL respectively (Figs. 1 and 2).

3.2 Ferric Reducing Antioxidant Property (FRAP) Assay

The reducing abilities of *S. siamea* leaves were evaluated and compared with standard ascorbic acid. The reductive capabilities were found to increase with the increasing concentration of *S. siamea* and standard ascorbic acid. Comparatively, the reducing abilities of *S. siamea* was 72% of that of standard ascorbic acid at the same concentration and experimental conditions (Fig 3).

3.3 Nitric Oxide Scavenging Activity

The nitric oxide scavenging activity of methanolic extract of *S. siamea* was determined at different concentrations of extract and compared with standard Ascorbic acid which has been known for its scavenging property of nitric oxide radical. The IC_{50} values (defined as the concentration of test compound required to produce 50% inhibition) for nitric oxide scavenging by *S. siamea* and ascorbic acid were found to be 149.342 μ g/ml and 167.17 μ g/ml respectively (Fig 4).

3.4 ABTS Radical Cation Scavenging Ability

The scavenging abilities of *S. siamea* leave against ABTS radicals were evaluated and compared with gallic acid. The scavenging capabilities were found to increase with the increasing concentration of *S. siamea* and gallic acid. Comparatively, the scavenging abilities of *S. siamea* was 31.31% of that of standard gallic acid at the same concentration and experimental conditions.

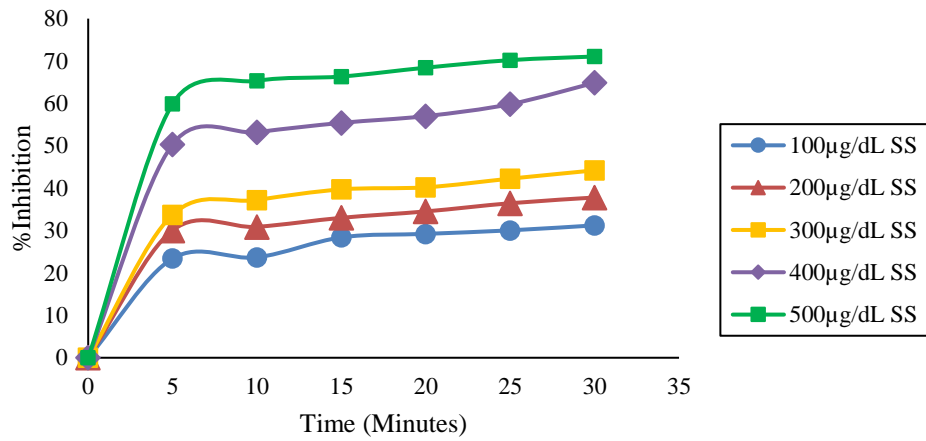


Fig. 1. Effect of time on different concentrations of methanolic extract of *S. siamea* on inhibition of DPPH radical

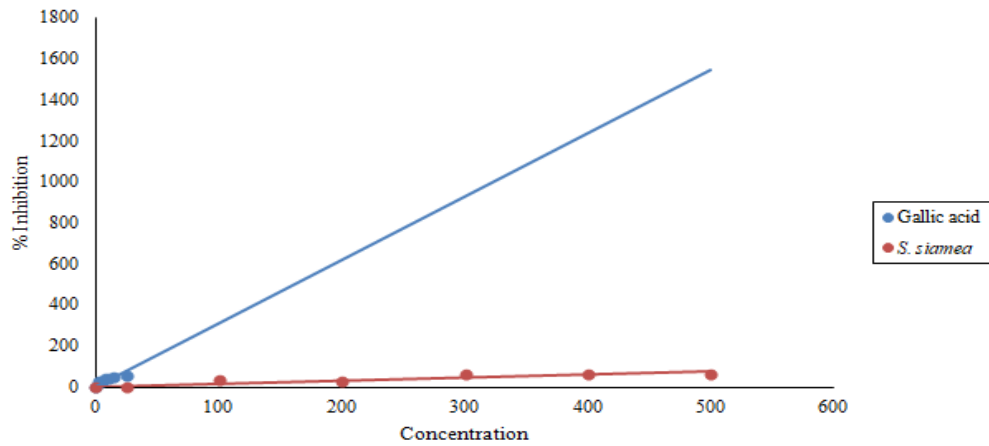


Fig. 2. The effects of different concentrations of *S. siamea* and gallic acid on the inhibition of the DPPH radical

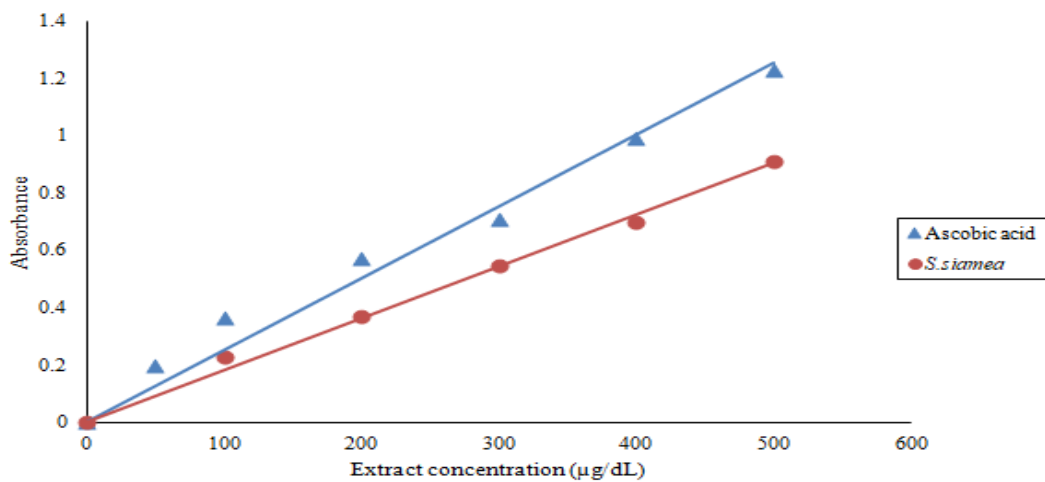


Fig. 3. Ferric reducing power of *S. siamea* and ascorbic acid at different concentrations

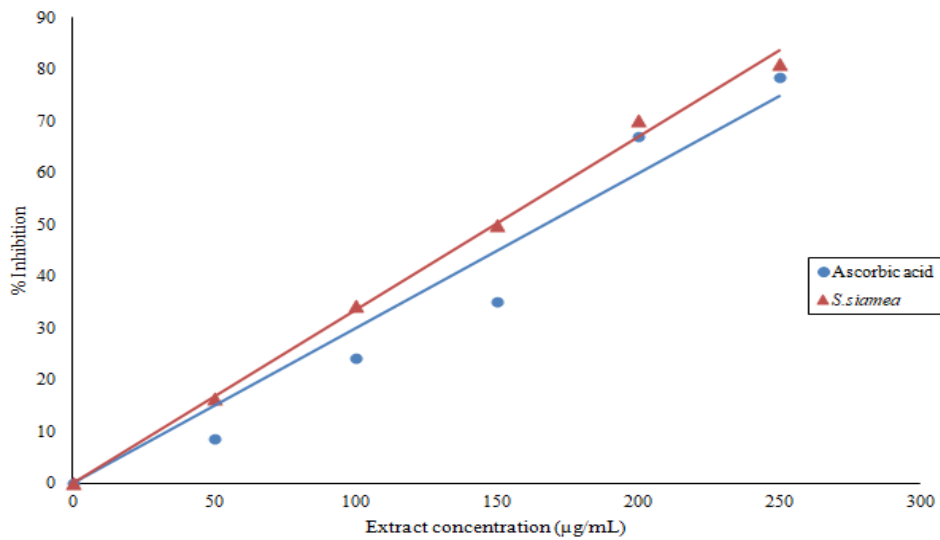


Fig. 4. The effects of different concentrations of *S. siamea* on the inhibition of nitric oxide radical formation

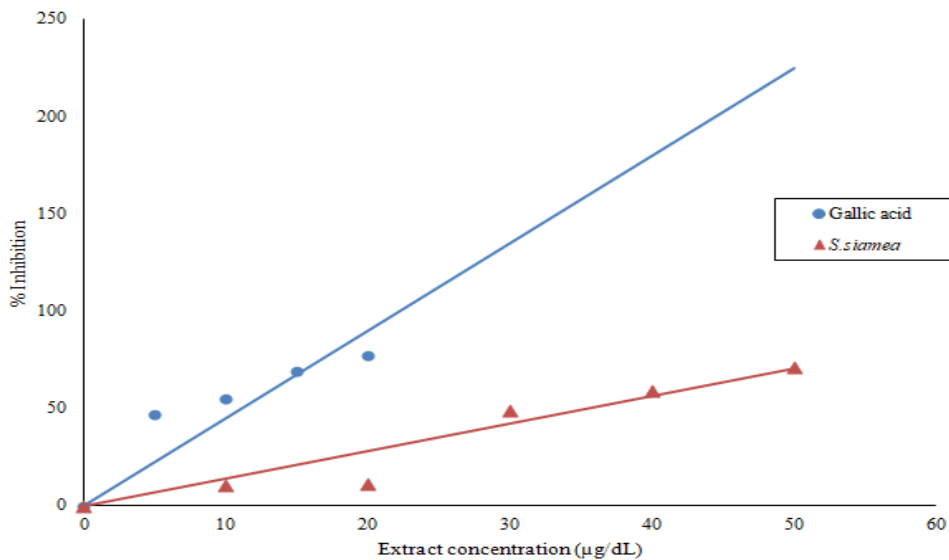


Fig. 5. The effects of different concentrations of *S. siamea* and gallic acid on the inhibition of the ABTS radical

3.5 Lipid Peroxidation Inhibition Assay (TBARS)

The ability of different concentrations of *S. siamea* to inhibit the induction of lipid peroxidation was compared with the control sample where lipid peroxidation induction was 100%. A decrease in the induction of lipid peroxidation was observed with increased concentration of *S. siamea*, 100 µg/ml of the extract reduced lipid peroxidation induction by 40.47% while 500µg/ml

reduced lipid peroxidation induction by 72.10%.

3.6 Inhibition of α - Amylase

The inhibition activities of the extracts obtained from *S. siamea* was investigated on the α -amylase enzyme. The methanolic extract of *S. siamea* significantly inhibited α -amylase activity in this study. The level of inhibition was found to be concentration-dependent and maximum percentage inhibition of α -amylase activity (73.21%) was obtained at 250µg of the extract

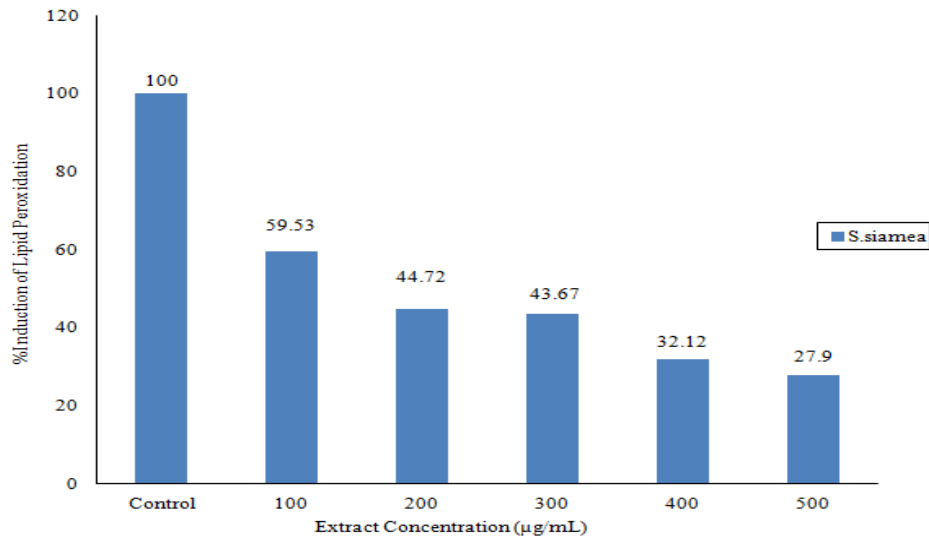


Fig. 6. The effects of different concentrations of *S. siamea* on the induction of lipid peroxidation

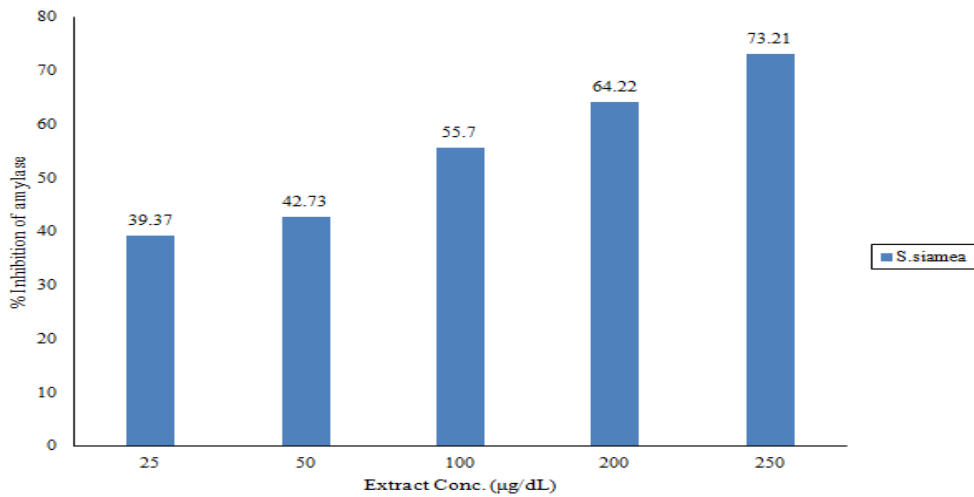


Fig. 7. The effects of different concentrations of *S. siamea* on inhibition of α-amylase activity

3.7 Effect of *S. siamea* on ALT and AST Activities

Administration of arsenic significantly ($p < 0.05$) increased enzymatic activity of ALT and AST by 3.22 fold, and 5.68 fold respectively when compared with the normal rats. However, pre-treatment of normal rats with 200 mg/kg body weight of extract *S. siamea* and 200 mg/kg ascorbic acid before arsenic administration significantly reduced plasma ALT activity by 35.95 % and 58.90 % respectively, and AST activity by 49.61 % and 66.25 % respectively when compared with rats treated with arsenic only.

3.7.1 Effect of *S. siamea* on plasma urea, Creatinine, total protein and triglycerides

Administration of arsenic significantly increased plasma urea concentration by 2.5 fold, plasma creatinine by 5.6 fold, cholesterol by 4.4 fold and triglycerides by 3.5 fold while it reduced total protein content by 24.97 % when compared with normal rats. Treatment of normal rats with *S. siamea* and ascorbic acid before arsenic administration significantly reduced urea, creatinine, cholesterol and triglycerides concentration while it increased total protein content when compared with rats treated with arsenic only.

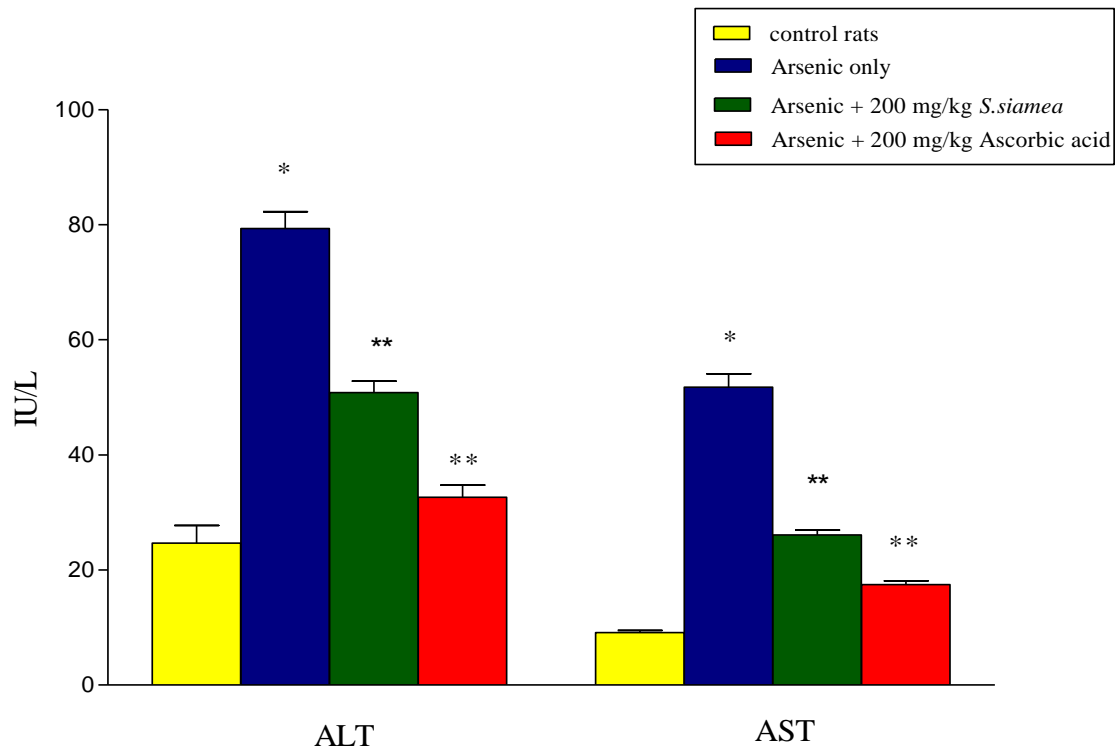


Fig. 8. Effect of methanolic extract of *S. siamea* on activities of ALT and AST in arsenic-treated rats. Values are mean \pm SEM (n=6). *(p < 0.05) Groups 2 (arsenic-treated rats) compared with group 1 (control rats). **(p < 0.05) Groups 3 and 4 (*S. siamea* and ascorbic acid-treated rats respectively) compared with group 2 (arsenic-treated rats)

Table 1. Effect of methanolic extract of *S. siamea* on urea, creatinine, total protein, cholesterol and triglycerides in arsenic-treated rats

Parameter	Control	Arsenic only	Arsenic + 200mg/kg <i>S. siamea</i>	Arsenic + 200mg/kg Ascorbic acid
Urea (mg/dL)	25.77 \pm 2.06	65.72 \pm 3.40 *	42.29 \pm 2.87**	31.63 \pm 3.56**
Creatinine (mg/dL)	0.37 \pm 0.07	2.10 \pm 0.24*	0.83 \pm 0.11**	0.91 \pm 0.14**
Total protein (g/dL)	7.65 \pm 0.69	5.74 \pm 0.23*	5.82 \pm 0.65**	7.50 \pm 0.62**
Cholesterol (mg/dL)	55.89 \pm 2.72	246.80 \pm 28.60*	158.7 \pm 7.15 **	92.72 \pm 10.23**
Triglycerides (mg/dL)	47.52 \pm 2.93	168.50 \pm 9.12 *	73.86 \pm 9.85**	103.0 \pm 7.48**

Values are mean \pm SEM (n=6). *(p < 0.05) Groups 2 (arsenic-treated rats) compared with group 1 (control rats). ***(p < 0.05) Groups 3 and 4 (*S. siamea* and ascorbic acid-treated rats respectively) compared with group 2 (arsenic-treated rat).

3.7.2 Effect of *S. siamea* on hepatic and renal GSH levels

Administration of arsenic significantly decreased glutathione level in both liver and kidney when compared to the control rats. However, pretreatment of rats with 200 mg/kg *S. siamea* and ascorbic acid significantly increased (p < 0.05) glutathione levels in the liver by 73.20 % and 118.85 % respectively and in the kidney by

50.13% and 49.62 % respectively when compared with rats in the arsenic-treated group only.

3.7.3 Effect of *S. siamea* on hepatic and renal SOD Activity

Administration of arsenic caused a significantly decreased in SOD activities in both liver and kidney homogenate by 65.11 % and 65.03 %

when compared to the control rats. Pretreatment of rats with 200 mg/kg *S. siamea* and ascorbic acid significantly increased ($p < 0.05$) SOD activities in the liver by 105.83 % and 145.12 %

respectively and kidney by 124.56 % and 167.73 % respectively when compared with rats in the arsenic-treated group only.

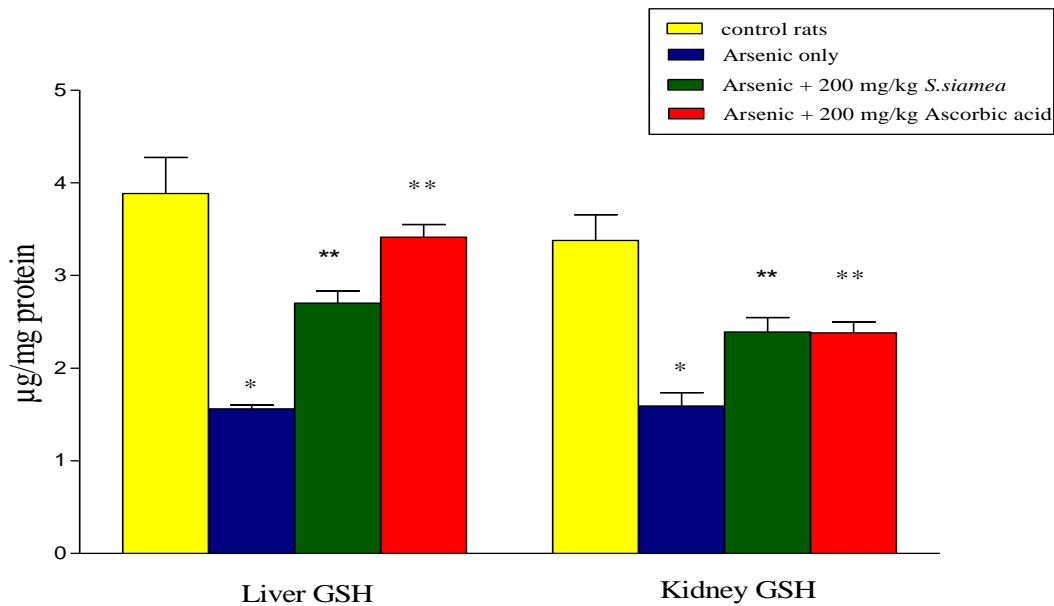


Fig. 9. Effect of methanolic extract of *S. siamea* on hepatic and renal GSH levels in arsenic-treated rats; Values are mean \pm SEM (n=6). *($p < 0.05$) Groups 2 (arsenic-treated rats) compared with group 1 (control rats). **($p < 0.05$) Groups 3 and 4 (*S. siamea* and ascorbic acid-treated rats respectively) compared with group 2 (arsenic-treated rats)

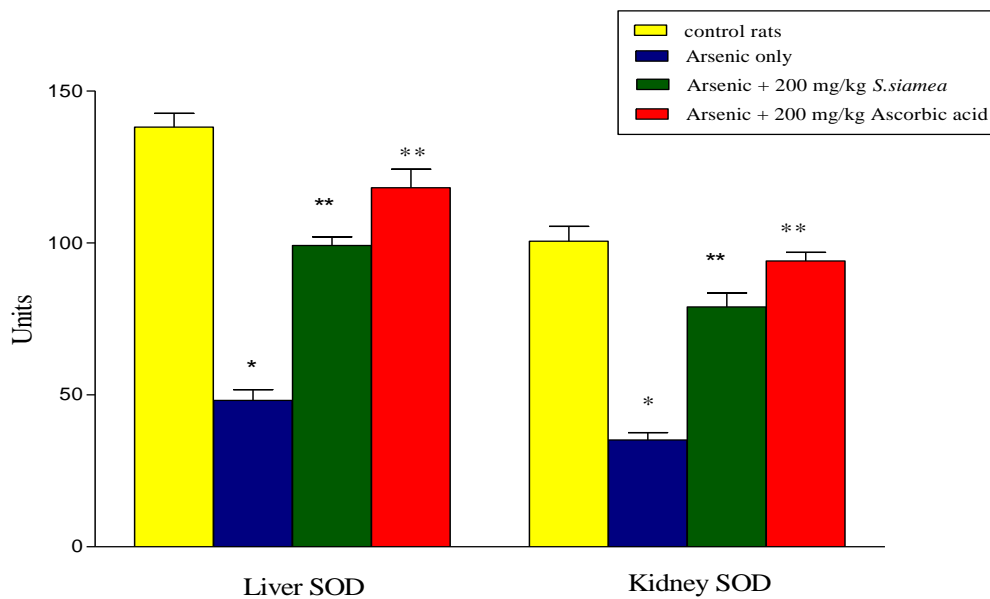


Fig. 10. Effect of methanolic extract of *S. siamea* on hepatic and renal SOD activities in arsenic-treated rats; Values are mean \pm SEM (n=6). *($p < 0.05$) Groups 2 (arsenic-treated rats) compared with group 1 (control rats). **($p < 0.05$) Groups 3 and 4 (*S. siamea* and ascorbic acid-treated rats respectively) compared with group 2 (arsenic-treated rats)

3.7.4 Effect of *S.siamea* on hepatic and renal malonaldehyde levels

Arsenic treatment significantly raised MDA levels in the liver and kidney when compared with control rats. Pretreatment of rats with 200mg/kg *S. siamea* and ascorbic acid before arsenic administration significantly reduced ($p < 0.05$) MDA levels in the liver by 54.10 % and 60.90 % respectively and kidney by 40.83% and 63.19 % when compared with rats in the arsenic-treated group only.

3.7.5 Effect of *S.siamea* on hepatic and renal catalase activity

Administration of arsenic caused a significantly decreased in CAT activities in both the liver and kidney homogenate by 66.99 % and 66.87 % when compared to the control rats. Pretreatment of rats with 200mg/kg *S. siamea* and 200mg/kg ascorbic acid significantly increased ($p < 0.05$) CAT activities in liver and kidney when compared with rats in the arsenic-treated group only.

3.8 Haematological Parameters

The effects of arsenic administration on haematological parameter were depicted in Table 2. No significant changes in the parameters of Haemoglobin (HGB), Haematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), red blood cell distribution width-standard deviation (RDW-SD), red blood cell distribution width- coefficient of variation (RDW-CV), Platelet large cell ratio (P-LCR), Mean Platelet Volume (MPV), Platelet distribution width (PDW), Mean Corpuscular Haemoglobin Concentration (MCHC) and Platelet crit (PCT) were found but the values of White Blood Cell (WBC), Red Blood Cells (RBC), Platelet (PLT) and Lymphocytes (LYM) were significantly lowered when compared with control animals. However, pretreatment of rats with 200 mg/kg *S. siamea* and ascorbic acid before arsenic administration significantly increased ($p < 0.05$) the values of all lowered parameters when compared with rats in the arsenic-treated group only.

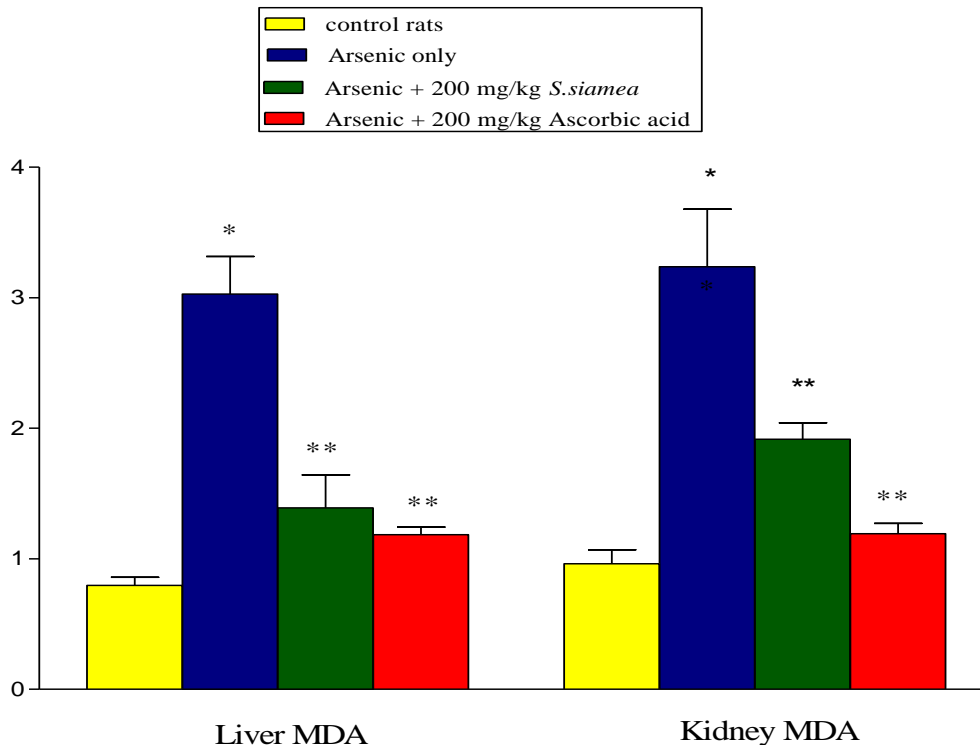


Fig. 11. Effect of methanolic extract of *S. siamea* on hepatic and renal MDA levels in arsenic-treated rats; Values are mean \pm SEM (n=6). *($p < 0.05$) Groups 2 (arsenic-treated rats) compared with group 1 (control rats). **($p < 0.05$) Groups 3 and 4 (*S. siamea* and ascorbic acid-treated rats respectively) compared with group 2 (arsenic-treated rats)

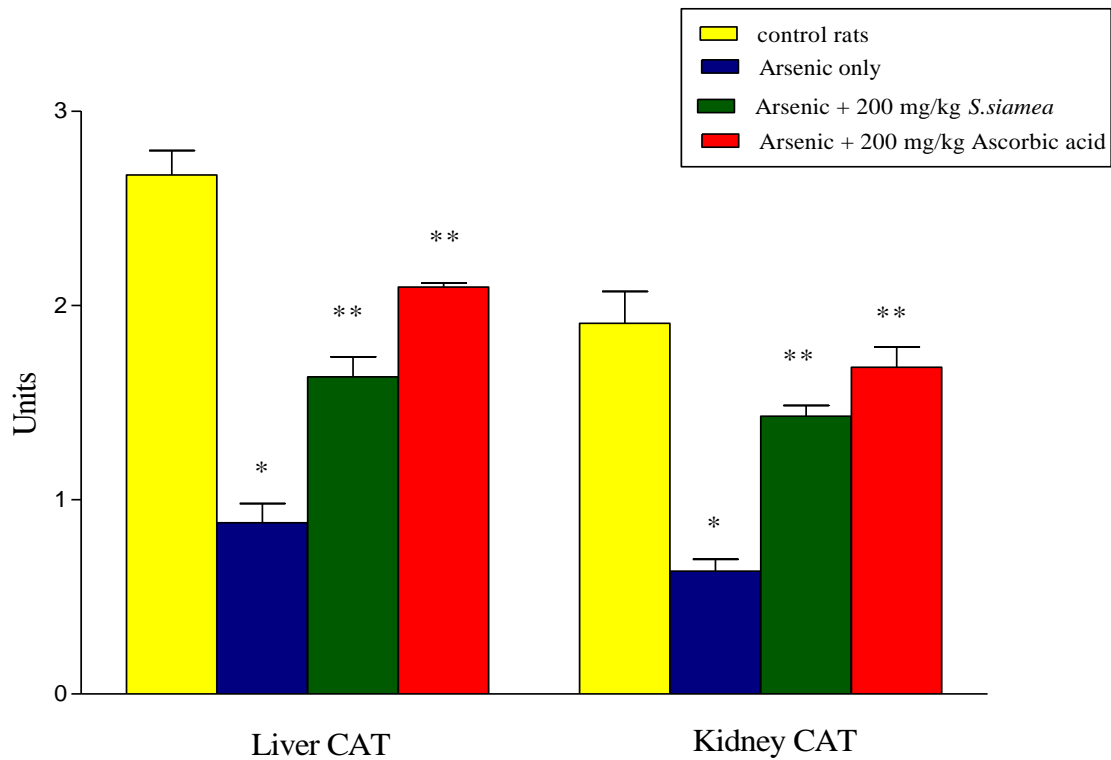


Fig. 12. Effect of methanolic extract of *S. siamea* on hepatic and renal CAT activities in arsenic-treated rats. Values are mean \pm SEM (n=6). *(p < 0.05) Groups 2 (arsenic-treated rats) compared with group 1 (control rats). **(p < 0.05) Groups 3 and 4 (*S. siamea* and ascorbic acid-treated rats respectively) compared with group 2 (arsenic-treated rats)

Table 2. Effect of arsenic administration on haematological parameters of rats

Parameter	Control	Arsenic only	Arsenic + 200mg/kg body weight <i>S. siamea</i>	Arsenic + 200mg/kg body weight Ascorbic acid
WBC	10.13 \pm 1.48	4.26 \pm 0.71*	7.41 \pm 3.80**	8.13 \pm 2.56**
RBC	8.45 \pm 0.26	5.31 \pm 0.57*	5.67 \pm 0.47**	7.02 \pm 0.20**
HGB	13.65 \pm 0.44	12.00 \pm 0.54	14.10 \pm 1.18	14.04 \pm 0.86
HCT	46.05 \pm 1.54	39.80 \pm 1.44	37.85 \pm 3.60	41.88 \pm 3.25
LYM	85.05 \pm 2.16	60.10 \pm 2.67*	67.07 \pm 2.90**	78.18 \pm 2.05**
PLT	740.50 \pm 98.50	341.70 \pm 26.61*	598.0 \pm 73.95**	833.00 \pm 51.41**
MCV	57.24 \pm 1.07	59.82 \pm 1.88	37.85 \pm 3.60	72.62 \pm 1.06
MCH	16.70 \pm 0.53	17.96 \pm 0.49	28.13 \pm 0.92	17.06 \pm 1.460
MCHC	29.18 \pm 0.55	30.04 \pm 0.20	43.69 \pm 3.54	37.29 \pm 2.07
RDW-SD	27.06 \pm 0.49	30.56 \pm 1.40	25.71 \pm 0.25	27.78 \pm 0.25
RDW-CV	10.54 \pm 0.77	11.92 \pm 0.44	10.80 \pm 0.26	10.08 \pm 0.24
PDW	7.18 \pm 0.11	7.70 \pm 0.26	9.50 \pm 0.66	10.09 \pm 1.10
MPV	6.54 \pm 0.20	6.76 \pm 0.08	5.18 \pm 0.49	6.90 \pm 0.26
P-LCR	8.72 \pm 0.46	9.50 \pm 0.59	11.47 \pm 0.68	10.77 \pm 1.84
PTC	0.42 \pm 0.07	0.39 \pm 0.29	0.47 \pm 0.05	0.50 \pm 0.04

Values are mean \pm SEM (n=6). *(p < 0.05) Groups 2 (arsenic-treated rats) compared with group 1 (control rats). ***(p < 0.05) Groups 3 and 4 (*S. siamea* and ascorbic acid-treated rats respectively) compared with group 2 (arsenic-treated rats)

3.9 Histopathological Studies of the Liver in the Control and Treatment Groups

The hepatic cells from the arsenic only treated group relative to the control group and other treatments was characterized by severe infiltration of cytoplasm (blue arrow), necrosis (green arrow), and congested sinusoids (slender arrow) (Fig 12(1)). The hepatic cells from the *S. siamea* extract (Fig 12(6)) and ascorbic acid (Fig 12(9)) treatment groups showed similar morphological organization to the control group (Fig 12(2)).

3.10 Qualitative Phytochemical Analysis of Powder Sample of *S. siamea*

The result of the qualitative analysis of the phytochemicals in *S. siamea* extract is presented in Table 3. The study revealed the presence of alkaloids, flavonoids, tannins, phenol, terpenoids,

phlobotanins, cardiac glycosides and saponin, in the *S. siamea* extract while phytosterols was absent.

3.11 Quantitative Determination of Total Phenolics Compound and Proximate Analysis

The proximate analysis result reveals the percentage composition of moisture, crude protein, crude fat, crude fibre, total ash content and nitrogen-free fat content of methanolic extract of *S. siamea* to be 19.10, 15.29, 20.20, 1.09, 9.61, 34.61 respectively as shown in Table 3 below. The phenolic content of *S. siamea* extract was also determined in this study. The total amount of phenolic compounds presents in the methanolic extract of *S. siamea* was found to be 29.87 mg in gallic acid equivalent (GAE) (Table 3).

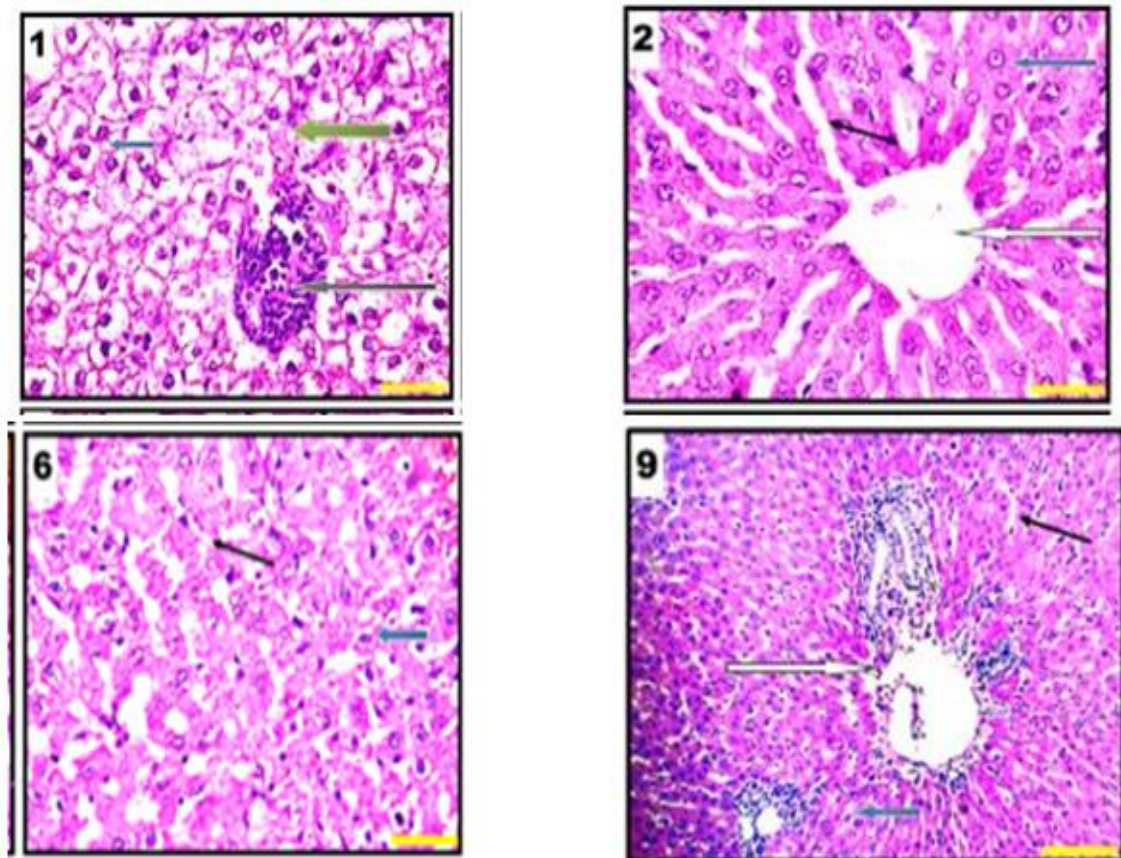


Fig. 13. Photomicrographs of liver section demonstrated by Haematoxylin and Eosin staining at low magnification (200 μ m); The hepatocytes, sinusoids, portal triad (hepatic vein, hepatic artery and bile duct) are all visible across the various groups. 1 = Group treated with Arsenic only, 2 = Group treated with normal saline (control), 6 = Group treated with *S. siamea* extract and Arsenic, 9 = Group treated with ascorbic acid and Arsenic

Table 3. Showing the result of phytochemical analysis on powder sample of *S. siamea*

Constituents	Test	Observation	Inference
Terpenoid	Chloroform test	Brown ring formation	+
Saponins	Foam test	Foam for some minute	+
Phenols	Ferric Chloride	Bluish Black	+
Phlobotanins	Hydrochloric acid	Deposit of red precipitate	+
Phytosterols	Chloroform test	No brown ring formation	-
Alkaloids	Mayer`s test	Cream colour precipitate	+
Cardiac glycosides	Acetic acid test	Violet-green ring	+
Flavonoids	Alkaline reagent	Yellow colouration formation	+
Tannins	Ferric chloride test	Brownish green colouration	+

Table 4. Proximate analysis of *S. siamea*

Sample	<i>S. siamea</i>
Moisture	19.10
Crude protein % (%n x 6.25)	15.29
Crude fat %	20.20
Crude fiber %	1.09
Total ash %	9.61
NFE %	34.61
Total phenolic content	29.87

3.12 GC Analysis

The compounds present in methanolic extract of *S. siamea* leaves are shown in Table 5. Their identification and characterization were based on their elution order in a GC column. The elution time and the amount of these compounds were also presented. Based on abundance, the top two major compounds present in the methanolic extract of *S. siamea* are barakol (10.14%) and oxalic acid (9.32%). The GC chromatograms of the extract presented in Fig 13 shows the retention time in the column and the detected peaks which correspond to the compounds present in the extract.

4. DISCUSSION

The development of oxidative stress which results as a consequence of excessive free radicals production and insufficient antioxidant level could be deleterious to man [31]. This condition could unbalance the homeostatic condition and as well instigate the onset of biochemical disorders which could affect the hepatic, renal, cardiac or cardiovascular system [31]. Pharmacologically, the significance of plants is majorly due to the presence of one or more bioactive compounds, metabolites, and or phytochemical thus ensuring that plants are of biochemical and medical significance to man.

Many antioxidant assays have been notable in estimating the antioxidant capabilities of

pharmacologically active plants. These assays as carried out in this study includes, DPPH, ABTS, lipid peroxidation, nitric oxide assay and ferric reducing power. As revealed in the results section, *S. siamea* in a concentration and time-dependent manner scavenges DDPH and ABTS radicals. Antioxidant generally possesses the ability to donate a hydrogen atom to free radicals, *S. siamea* leaves extract in this study most likely act as an antioxidant by donating hydrogen to free radicals [32]. The ability of *S. siamea* to reduce ferric-tripyridyltriazine (Fe^{3+} -TPTZ) complex to ferrous-tripyridyltriazine (Fe^{2+} -TPTZ) in a concentration-dependent manner further highlights that it possesses antioxidant potential in the amelioration of free radicals into stable and less deleterious products. This is in agreement with a similar study carried out by Adedoyin et al., [33].

The synthesis of nitric oxide radicals, which naturally reacts with oxygen is of physiological importance. The methanolic extract of *S. siamea* in a concentration-dependent cycle was able to minimize the synthesis of nitrite ions and this antioxidant potential of the extract against nitrite ion formation was found to be very strong when compared with the standard ascorbic acid [2]. Lipid peroxidation/ malondialdehyde formation is generally believed to be a strong signal in free radicals impounded cells, because its formation could trigger a cycle of toxicological cascades which if not put to check would disrupt

biomembranes and wreak havoc [34]. The extract of *S. siamea* successfully prevented malondialdehyde formation in a concentration-dependent manner in this study, while maximum induction of 100% malondialdehyde was observed in the control group.

As earlier pointed, oxidative stress triggers the onset of several biochemical disorders, and this

includes diabetes mellitus. However, inhibitors of α -amylase; a hydrolyzing enzyme has been revealed as a hypoglycemic drug for the control of excessive blood glucose levels in humans [35]. Cell-free antidiabetic assay carried out in this study showed that *S. siamea* is effective in inhibiting α -amylase activity which might be attributed to the presence of phenolic compounds in the plant extract [36].

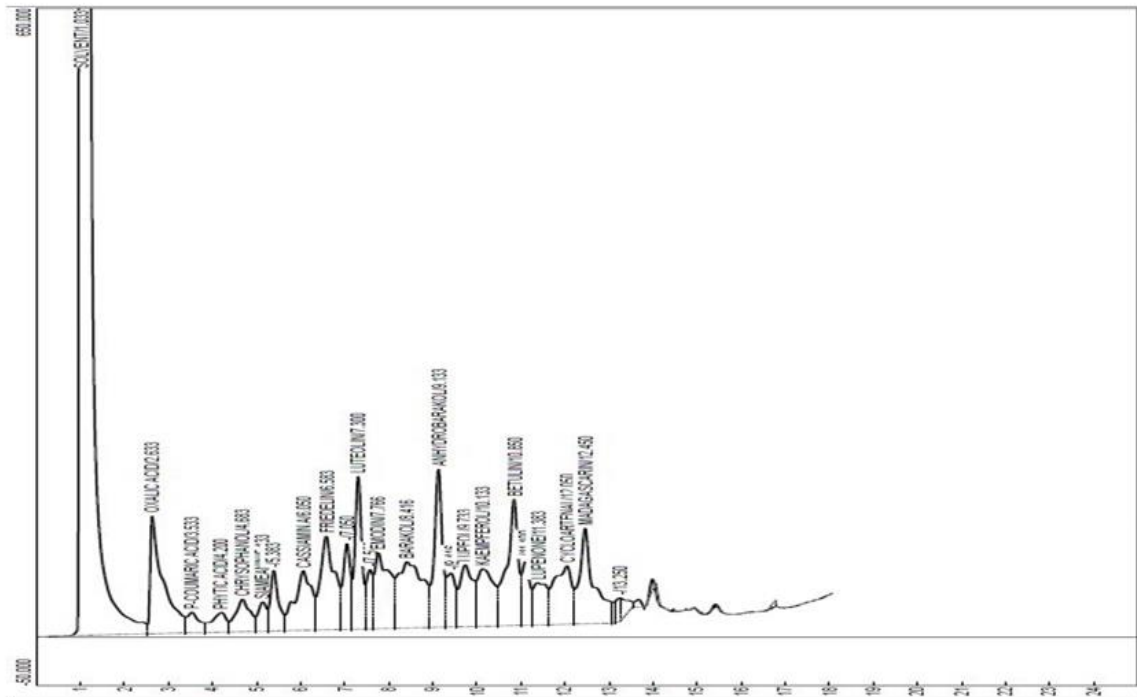


Fig. 14. GC chromatogram of *S. siamea* leaves methanolic extract

Table 5. Biologically active chemical compounds present in *S. siamea* leaves

Component	Retention	Relative (%)
Oxalic acid	2.63	9.32
P-coumaric acid	3.53	1.50
Phytic acid	4.20	1.73
Chrysophanol	4.68	2.89
Siameanin	5.13	1.51
Cassiamin A	6.05	6.01
Friedelin	6.58	8.24
Luteolin	7.30	6.50
Emodin	7.76	6.77
Barakol	8.41	10.14
Anhydrobarakol	9.13	7.73
Lupeol	9.73	4.84
Kaempferol	10.13	5.73
Betulin	10.85	8.73
Lupenone	11.38	3.53
Cycloartenal	12.05	6.35
Madagascarin	12.45	8.46

The ingestion of arsenic above the recommended limits has been found to result in many biochemical deficits. This deficit includes chromosomal abnormalities, epigenetic changes and damage to neuronal cells [36]. This worry has necessitated the development of therapeutic products, majorly synthesized from natural products in a quest to negate the adverse effects of arsenic-induced oxidative damage. Evaluation from this study revealed that *S. siamea* proved effective in lowering hepatic AST and ALT levels. Extrusion of the hepatic enzymes to the plasma of high concentration majorly signifies damage to hepatic cells [37], as observed in arsenic untreated rats. Although, upregulating the antioxidant system via pre-treatment with *S. siamea* and ascorbic acid lowers ALT and AST levels towards control. Thus, proving the hepatoprotective efficacy of *S. siamea* [11].

Nephrotoxic profile was evaluated by measuring plasma urea levels while creatinine levels were evaluated as a measure of the glomerular filtration rate. The significant increase ($p < 0.05$) in plasma urea and creatinine levels observed in arsenic untreated rats when compared with *S. siamea* treated rats shows it has an effective phytochemical capable of protecting renal cells against arsenic toxicity [38]. Arsenic administration significantly increases plasma cholesterol and triglycerides levels when compared with the control group a strong indication of disturbances in lipid metabolism, a result that agrees with a similar study by Bhattacharya *et al.*, [39]. There was a reduction in cholesterol and triglycerides levels in the blood of rats treated with *S. siamea* and ascorbic acid before arsenic administration when compared with the arsenic group only, probably due to modulation of lipoprotein lipase activity [40].

The liver synthesizes most serum protein. Decreased serum total protein content may be a useful index of severity of hepatocellular damage [41]. In this study, administration of arsenic reduced total protein level when compared with control rats. However, rats pre-treated with *S. siamea* and ascorbic acid have their total protein significantly increased ($p < 0.05$) to near normal, when compared with arsenic-treated rats which indicates repair of hepatocellular damage caused by arsenic administration [41]. To further buttress the previous pharmacognostic study carried out by Esiebo *et al.* [42], *in-vivo* antioxidant assays were evaluated. Arsenic administration induced depletion in the levels of hepatic and renal glutathione, superoxide

dismutase (SOD) and catalase (CAT). The decrease in the activity of SOD, GSH and CAT levels during arsenic-induced oxidative stress may be due to excessive production of reactive oxygen species [43]. The levels of liver and kidney GSH, as well as SOD and Catalase activities, were brought to near normal in rats pre-treated with *S. siamea* and ascorbic acid. The stabilization of these enzymes by the extract is an indication of the improvement of the functional status of the liver and kidney. This can probably indicate that the *S. siamea* extract either increases the biosynthesis of SOD, catalase and GSH or reduces the extent of oxidative stress leading to less degradation, or it may have both effects.

Enhanced lipid peroxidation expressed in terms of malondialdehyde contents was observed in arsenic-treated rats in our study which indicates the damage to the hepatic and renal cells this agrees with a previous study [44]. However, a significant reduction was obtained in malondialdehyde levels of rats pre-treated with *S. siamea* and ascorbic acid before arsenic administration when compared to the arsenic group only, suggesting that the extract inhibits lipid peroxidation and its propagation in the liver and kidney [28]. In this study, *S. siamea* administration increased the haematological parameters of WBC, LYM, PLT and RBC to near normal when compared with arsenic-treated rats. This result is at variance with the result obtained from arsenic rats which showed a significant decrease and could thus indicate either haemolysis compromised WBC, compromised blood clotting factor, as well as a suppressed immune system [45]. The histopathological study carried out on hepatic cells of an arsenic-treated rat's revealed the presence of severe steatosis, infiltration of cytoplasm and necrosis which are characteristics of the severe pathological lesion. However, the administration of *S. siamea* and ascorbic acid before arsenic treatment significantly improved the general histoarchitecture of the liver when compared with the arsenic-treated group.

Plants have pharmacological activities attributed to the secondary metabolites which are responsible for essential bioactivities. Screening of the leaves of methanolic extract of *S. siamea* revealed the presence of terpenoid, phlobotannins, alkaloids, phenols, glycosides, and flavonoids. Several studies have explained the biochemical and biological potentials of these metabolites which include their pathogen

mobbing abilities, immune-boosting capabilities, diuretics potential, analgesics and antioxidant potentials [46-48]. The generation of standard chromatogram via the injection of concentrated extract into the GC revealed the possible presence of several biologically active compounds, including oxalic acid, para-coumaric acid, phytic acid, chrysophanol, siameanin, cassianin A, frudlin, luteolin, emodin, barakol, anhydrobarakol, lupeol, kaempferol, betulin, lupenone, cycloartenal and madagascarin. Barakol, is a flavonol with multiple hydroxyl groups, although several studies have shown contradictory results on the pharmacological efficacy of Barakol, the compound has been affirmed to possess strong sedative and anxiolytic effects, decreases blood pressure as well as maintaining liver morphology [49-51]. Similarly, betulin is a triterpenoid that has proved to be effective against certain tumours, preventing atherosclerosis and possess antioxidant potentials [52].

Proximate analysis of the leaves of *S. siamea* done in this study revealed that the plant has a substantial amount of moisture, crude protein, crude fat, crude fibre, total ash content and nitrogen-free extract. From the result, nitrogen-free fat has the highest value, while crude fibre has the smallest value. The ash content of *S. siamea* leaves shows that the plant is rich in the mineral element while the moisture content of the leaves would prevent microbial growth and increase the storage span [53]. The crude fibre in *S. siamea* has the potential to reduce serum cholesterol levels, preventing coronary heart diseases, lowering constipation and reduce the risk of hypertension [54]. Thus, the result obtained in this study offers a scientific basis that methanolic leaves extract from *S. siamea* contains certain nutritional values that could be significant in managing malnutrition and multifactorial disorders.

5. CONCLUSION

This study analysed the bioactive compounds present in *S. siamea* leaves and emulated their anti-oxidant, haematological, histopathological, hepatoprotective and nephroprotective potentials. It has therefore been shown that *S. siamea* could provide a new dimension to various scientific concerns especially in the management of diseases. While the challenge to conform *in-vivo* studies to human application still exist, future studies should explore the effect of *S. siamea* metabolites on various clinical trial phases.

ETHICAL APPROVAL

The Faculty of Basic Medical Science, Ladoko Akintola University of Technology, Ogbomoso, Research Ethics committee gave Ethical approval for the study (FBMS2019/012). All the ethical protocols laid by the committee in line with the standard accepted principles for laboratory animal handling and care were followed.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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