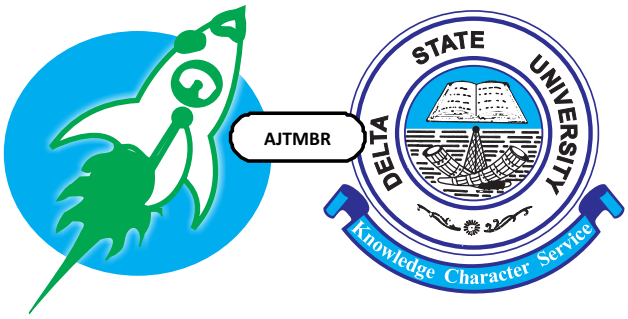
African Journal of Tropical Medicine and Biomedical Research (AJTMBR)



The Journal is the Official Publication of the College of Health Sciences, Delta State University, Abraka, Nigeria.

ISSN: 2141-6397

Vol. 7, No. 1, December 2024

Focus and Scope

The African Journal of Tropical Medicine and Biomedical Research is a multidisciplinary and international journal published by the College of Health Sciences, Delta State University of Abraka, Nigeria. It provides a forum for Authors working in Africa to share their research findings on all aspects of Tropical Medicine and Biomedical Sciences and to disseminate innovative, relevant and useful information on tropical medicine and biomedical sciences throughout the continent. The journal will publish original research articles, reviews, editorials, commentaries, short reports, case reports and letters to the editor. Articles are welcome in all branches of medicine and dentistry including basic sciences (Anatomy, Biochemistry, Physiology, Pharmacology, Psychology, Nursing etc) and clinical sciences (Internal Medicine, Surgery, Obstetrics and Gynaecology, Dental surgery, Child Health, Laboratory Sciences, Radiology, Community Medicine, etc). Articles are also welcome from social science researchers that document the intermediating and background social factors influencing health in countries of Africa. Priority will be given to publication of articles that describe the application of the principles of primary health care in the prevention and treatment of diseases.

Editorial Notices

The journal will be published biannually in the months of March and September. Annual subscription fee in Nigeria is two thousand naira (N2,000) per volume (2issues); One-thousand-naira single copy (N1000). The annual subscription rate for other parts of the world is as follows: United Kingdom £60 (post free). West Africa $60 (post free). The rest of the World and the United States of America $120 (post free). A charge of $60 is made for reprints inclusive of postage. Cheques should made payable to the African Journal of Tropical Medicine and Biomedical Research and addressed to the Editor-in-Chief.

Journal Contact

All correspondence, including manuscripts for publication (in triplicate) should be addressed to:

Professor P.S. Igbigbi
The Editor-in-Chief,
Department of Anatomy,
Faculty of Basic Medical Sciences,
College of Health Sciences,
Delta State University, Abraka,
Delta State, Nigeria.

Or:
Professor Lawrence Omo-Aghoja
Editor
Department of Obstetrics and Gynecology,
Faculty of Clinical Medicine,
Delta State University, Abraka, Nigeria.
Email: journalajtmbr@yahoo.com
Cc: all email to
eguono_2000@yahoo.com
Tel: 08039377043

All authors are advised to submit an electronic copy in CD-ROM along with a hard copy of their manuscript, as this will spare remarkable time in the reviewing and typesetting processes.

In the alternative, authors can submit their articles and covering letter by email attachments. A covering letter (signed by all authors) accompanying the manuscript should certify that the article has not been previously published and is not being considered for publication elsewhere.

Information for Authors

All manuscript are peer-reviewed and accepted with the understanding that the work has not been published or being considered for publication elsewhere. Indeed, the authors would be requested

to sign a copyright form transferring the ownership of the paper to the African Journal of Tropical Medicine and Biomedical Research. All articles must include the correct names and addresses of author(s) including e-mail addresses and telephone numbers. Articles will be subjected to a thorough peer review process before any decision is made to publish or not. Authors should note that the African Journal of Tropical Medicine and Biomedical Research is not under any obligation to publish articles submitted, as decision to publish will be based on recommendations of reviewers and the editorial advisory board.

Manuscripts

Articles submitted for publication should be typed double-spaced with 2.5cm margins with accompanying CD-ROM in Microsoft Word format for easy and quick peer review and typesetting. Each of the following sections should begin in a new page: title page, abstract, introduction, materials and methods, results, discussion, acknowledgment (s), references, tables, legends to figures and illustrations. The manuscript should include:

Title Page

The title page should include the following information: 1. the title and sub-title; 2. the name(s) of the author(s); 3. the affiliation(s) of the author(s); 4. name and address of the corresponding author and 5. three to six key words for indexing and retrieval purposes.

Abstract

The abstract should be structured and not more than 250 words. It should carry the following headings: Introduction, Materials and Methods, Results and Conclusion.

Original Research- The journal welcomes articles reporting on original research, including both quantitative and qualitative studies. Full-length articles should generally not exceed 3000 words, excluding abstract, tables, figures, and references. The subject matter should be organised under appropriate headings and sub-headings as itemized above.

Review Articles- Comprehensive review articles on all aspects of tropical medicine and biomedical sciences will also be considered for publication in the journal. Reviews should provide a thorough overview of the topic and should incorporate the most current research. The length of review articles must not exceed 3,000 words and the organisational headings and sub-headings used are at the author's discretion.

Short Reports - Brief descriptions of preliminary research findings or interesting case studies will be considered for publication as short reports. The length of the abstract and article should be restricted to 150 and 2,000 words respectively and organisation of short reports are left to the author's discretion.

Commentaries or Editorials- Commentaries or editorials on any aspect of tropical medicine and biomedical sciences in Africa will be considered for publication in the journal. Opinion pieces need not reference previous research, but rather reflect the opinions of the author(s). The length should not exceed 2,000 words.

Tables and Figures

All tables and figures should be submitted on separate sheets of paper and should be clearly labelled. Coloured tables and figures may be reprinted in black and white. Authors should especially take care that all tables are clear and understandable by themselves, independent of

the text. A reader should be able to read only the tables and easily grasp all information without the text.

Acknowledgments

Acknowledgments should be included on a separate sheet of paper and should not exceed 100words. Funding sources should be noted here.

References

References should be in the Vancouver style and numbered consecutively in the order in which they are mentioned in the text. Titles of journals should be abbreviated according to the Index Medicus style. Authors must cross-check and make sure that all information provided in the reference list is complete and correctly written. Reference numbers should be inserted above the line on each occasion a reference is cited in the text, e.g., … as 1-3 reported in other studies. Numbered references should appear at the end of the article and should include the names and initials of all authors. The format of references should be as published by the International Committee of Medical Journal Editors in the British Medical Journal 1988, volume 296, pages 401-405. The following are sample references for an article published in a journal and for a book: Ahmed Y, Mwaba P, Chintu C, Grange JM, Ustianowski A, Zumla A. A study of maternal mortality at the University Teaching Hospital, Lusaka, Zambia: the emergence of tuberculosis as a major non-obstetric cause of maternal death. Int J Tuberc Lung Dis 1999; 3: 675-680. Whitby LG, Smith AF, Beckett GJ. Enzyme Tests in Diagnosis. In: Lecture Notes on Clinical Chemistry. Whitby LG, Smith AF & Beckett GJth (eds). 4 editions. Blackwell Scientific Publications. 1988. 103-127.

Units of Measurement

All measurements should be expressed in SI (Systeme International) Units.

Galley proofs

Corrections of galley proofs should be strictly restricted to Printer's error only. Orders for offprints should be made when the corrected proofs are being returned by the authors. Articles accepted for publication remain the property of the journal and can only be reproduced elsewhere in line with section 5 of the copyright agreement.

Table of Contents

Inhibition of *Naja nigricollis* Venom Phospholipase A2 by Ethylacetate Extract of *Solanum dasyphyllum* Schum and Thonn leaf: An *In-vitro* and *In-silico* Approach

*Adewunmi RF[1], Yesufu HB. [2], Gidado [3], Pudza JS[4]

Abstract

Introduction: Snakebite envenomation is an acute medical emergency, particularly in the tropics, and clinical treatment is through the administration of antivenom. However, given the limitations of conventional antivenoms, the plant kingdom is being explored for possible antivenom compounds. The aim of this study was to evaluate the phospholipase A_2 inhibitory potential of ethylacetate extract of *Solanum dasyphyllum* and to isolate the compound responsible for the antivenom activity.

Materials and Method: The leaves of *S. dasyphyllum* were extracted, phytochemical constituents were screened and phospholipase A_2 enzyme inhibition study was carried using standard methods. The ethylacetate extract was subjected to various chromatographic techniques to isolate the antivenom compound, and the structure was determined using HNMR and CNMR spectra. The proposed structure of the isolated compound was used for molecular docking study with cobra venom phospholipase A_2.

Results: The result of phospholipase A_2 enzyme inhibition shows that different concentrations of ethylacetate extract of *S. Dasyphyllum* significantly inhibited the activity of phospholipase A_2 in *N. nigricollis* venom. Chromatographic investigations of the extract lead to the isolation of methyl linolenate, and a strong interaction with the enzyme active site and a binding energy of -6.60 kca/mol was recorded when docked with cobra phospholipase A_2 enzyme, thereby making it a potential inhibitor of the enzyme.

Conclusion: Conclusively, ethylacetate extract of *S. dasyphyllum* demonstrated a significant inhibition of phospholipids A_2 enzyme and this finding suggests that methyl linolenate may be a potential inhibitor of phospholipase A_2 and could possibly lead to the development of a drug to treat snakebites.

Keywords: *Solanum dasyphyllum*, envenomation, molecular docking, chromatography, NMR, Inhibition.

1Department of Biochemistry, Baba-Ahmed University Kano, Kano State
2Department of Pharmaceutical Chemistry, University of Maiduguri, Borno State.
3Department of Biochemistry, University of Maiduguri, Borno State.
4Department of Biochemistry, Al-Ansar University, Maiduguri, Borno State.
**Email: Adewunmi_rofiat@yahoo.com*

Corresponding author: Rofiat Funmilola Adewunmi Department of Biochemistry, Baba-Ahmed University Kano, Kano State, Email: Adewunmi_rofiat@yahoo.com

INTRODUCTION

According to the World Health Organization, snakebites are a neglected public health problem, especially in tropical countries, where millions of people suffer from snakebite envenomation [(1)]. It is estimated that over 435,000 snakebites occur annually in Africa, while Nigeria reportedly accounts for a fifth of the total West African snakebite burden [(2)]. Snake venom is a mixture of pharmacologically active substances such as acetylcholinesterase, L-amino acid oxidase, phosphodiesterases, phospholipase A_2, etc., which have potent, lethal and debilitating effectson biological system [(3)]. Phospholipase A_2

are esterolytic enzymes found in snake venoms that typically catalyze the breakdown of glycerophospholipids, the main component of biological cell membranes, into lysophospholipids and fatty acids, including arachidonic acid, the precursor of inflammatory cascade, leading to a range of adverse pharmacological effects by altering the physical properties of cell membranes and activating downstream signal transduction pathways [4].

Venom is used to immobilize and digest prey as well as for defensive purposes [5]. However, venomous snakebite is an acute medical emergency characterized by local tissue damage and systemic toxicity such as bleeding disorders, hypotension, severe paralysis, etc. [6,7]. There are over 3000 species of snakes known to science, 30% of which are poisonous and considered dangerous to humans [8]. In Nigeria, *Elapidae* and *Viperidae* are the two snake families that cause the majority of recorded morbidity and mortality in humans. The family *Elapidae* includes the cobras, whose venom are mainly neurotoxic and cytotoxic, while *Viperidae*, such as carpet vipers and saw scale vipers, are mainly hemotoxic [9]. Antivenom immunotherapy is the specific treatment against snakebites, but is associated with various side effects such as serum sickness and pyrogen reactions [10, 11]. Therefore, it is important to explore the plant kingdom for an alternative treatment that involves the use of various venom inhibitors that could replace the action of antivenom.

Solanum dasyphyllum, a semi-woody perennial medicinal plant, belongs to *Solanaceae* family [12]. It has been reported to possess antioxidant, antispasmodic and neuromodulatory activities [13, 14]. In southwestern Nigeria, *S. dasyphyllum* is used ethnomedically to treat snakebite envenomation; however, the effectiveness of the plant as anti-snake venom has not been scientifically studied. In the present study, the isolated compound from the ethyl acetate extract of *S. dasyphyllum* was investigated for possible inhibition of *N. nigricollis* venom phospholipase A_2 enzyme using in vitro and in silico methods.

MATERIAL AND METHODS

Solanum dasyphyllum leaves were collected from Odeomu town (Latitude: 7°32'0\"North; Longitude: 4°24\'0\"East) in Ayedaade local area, Osun State. They were identified and authenticated at the Department of Botany Herbarium, Obafemi Awolowo University, Ile-Ife, Osun State and a voucher specimen was issued with number IFE-17489. The leaves were air dried in the shade for a week and pulverized by manual grinding. 300 g of the powder was then soaked in 3000 mL of ethylacetate for 72 hours with intermittent stirring. Under reduced pressure, the filtrate was evaporated to dryness to give a dark green crude extract.

The ethylacetate extract of *S. dasyphyllum* was evaluated for the presence of alkaloids, tannins, saponin, carbohydrate, flavonoids, terpenes, steroids and cardiac glycosides according to the method described by Sofowora (1993).

Lyophilized *N. nigricollis* venom was obtained from the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria. Before use, the venom was reconstituted in phosphate buffer, pH 7.2, centrifuged at 2000rpm for 10 minutes and the supernatant was used for further studies. The venom was preserved at 4^0C.

Phospholipase A_2 was determined according to the titrimetric method of Adamich et al [20] with slight modification. A lecithin emulsion was prepared by dissolving 4 g of lecithin in 30 mL of 1 M NaCl and 10 mL of 0.1 M $CaCl_2$; the mixture was made up to 200 mL with distilled water. The

reaction mixture containing 15 mL of lecithin emulsion and 1 mL (1 mg/mg) of the reconstituted venom was adjusted to pH 8.0 with 0.02 M NaOH. The volume of 0.02 M NaOH required maintaining pH at 8.0 by titration for 4 minutes was recorded. A decrease of 1 pH unit corresponds to a fatty acid release of 133 µmol. Enzyme activity was expressed in µmol of fatty acid released per minute. For the inhibition study, the extract was pre-incubated with venom at 37 °C for 45 min.

Calculation:

[NaOH] = ml NaOH used Test - ml NaOH used Blank

Units/mg enzyme =
(Molarity of NaOH) × [NaOH] ×1000
(4)× (mg enzyme in the reaction mixture)

1000 = conversion from millimoles to micromoles.

4= Time of assay

Silica gel (100 g) was wet packed into a glass column using 100% chloroform. A portion of the ethylacetate extract (2 g) in fine powder form (pre absorbed with silica in chloroform) was loaded onto the packed column. The eluents (fractions) were collected in 5 mL each while the column was filled with 100% chloroform, chloroform/methanol mixtures (90:10; 70:30; 60:40; 50:50; 40:60; 30:70; 10:90) and methanol (100%). The fractions were analyzed on analytical TLC and similar R_f values from the column were pooled to give Nine (9) fractions designated E_1 to E_9. The fractions E_1 to E_9 were subjected to phospholipase A_2 enzyme inhibition study, the fraction with the best inhibition activity (E_2) was further subjected to preparative thin layer chromatography (TLC) according to the conventional one-dimensional ascending method using a TLC plate, followed by gel filtration with Sephadex LH-20 with 100% methanol as elution solvent, which resulted in the production of a white crystalline substance with the name E_{2i} (26 mg). The isolated compound was subjected to 1H-NMR and 13C-NMR analysis by Agilent Technologies (Jackson_NMR vnmrs 500 spectrometer). The sample was dissolved in the deuterated solvent, filtered and placed in a 5 mm NMR tube. Chemical shifts were recorded in parts per million (ppm).

Molecular docking protocols are often used to predict binding affinities for a range of ligands. The computational docking study was carried out to predict the most favorable binding position of the isolated compound within the binding pocket of the phospholipase A_2 enzyme isolated from Cobra venom. To obtain accurate results, all docking was performed with default parameters. Molecular docking was performed on the three-dimensional structures of Cobra phospholipase (PDB: 1A3F, resolution = 2.65 Å) obtained from the protein database (http://www.rcsb.org/pdb/home/home.do) and the 2D structure of the isolated compound, drawn using ChemDraw.

Intermediate steps, such as pdbqt files for protein and ligand preparation and grid box creation, were performed using the graphical user interface program AutoDock Tools (ADT). ADT assigned polar hydrogen, uniform atom Kollman charges, solvation parameters, and fragment volumes to the protein. AutoDock saved the prepared file in PDBQT format. AutoGrid was used to create the grid map using a grid box. During the docking process, both the protein and the ligands are considered rigid. The results with a positional root mean square deviation (RMSD) of less than 1.0 Å were summarized and represented by the most favorable binding free energy result. The pose with the lowest binding energy was analyzed

by Discovery Studio

RESULTS

The result of the phytochemical screening as shown in Table 1 revealed that presence of Alkaloids, Flavonoids, Tannins, Steroids, Terpenoids and Carbohydrate; Cardiac glycoside was not detected.

The in-vitro enzyme inhibition study of Phospholipase A_2 enzyme activity was carried in triplicates and mean ± standard deviation was calculated. The different concentrations of ethylacetate extract were able to significantly inhibit the activity of phospholipase A_2 enzyme in the treated groups compared to the control group, as shown in Figure 1. However, 200 and 400 µg/ml showed the best inhibitory effect (88.2%), followed by 600, 800 and 1000 µg/ml (76.5%).

The effect of different fractions of ethylacetate leaf extract of *S. dasyphyllum* on phospholipase A_2 activity of *N. nigricollis* venom is presented in Figure 2 where fraction 2 exhibited the least activity, followed by fraction 4. The details of the isolate obtained after series of chromatographic techniques is presented in table 2, table 3, figure 3 and figure 4. 3D structure of proposed isolated compound Methyl linolenate isolated from *S. dasyphyllum* leaf extract serve as ligand used for molecular docking study as presented in Fig 5, 6 and 7. The binding free energy values observed for Methyl linolenate docked with Phospholipase A_2, − 6.60 kcal/mol.

Table 1: **Phytochemical Constituents of Ethylacetate Leaf Extract of Solanum dasyphyllum**

Phytochemicals	**Observation**
Alkaloids	
Dragendorff's	+
Mayer's reagent	+
Flavonoids	
Shinda's test	+
Ferric chloride	+
Tannins	
Ferric chloride	+
Bromine water	–
Steroid	+
Terpenoids	+
Carbonhydrate	
Molish test	+
Cardiac Glycoside	
Keller-kilian	

Key: + = Present - = Absent

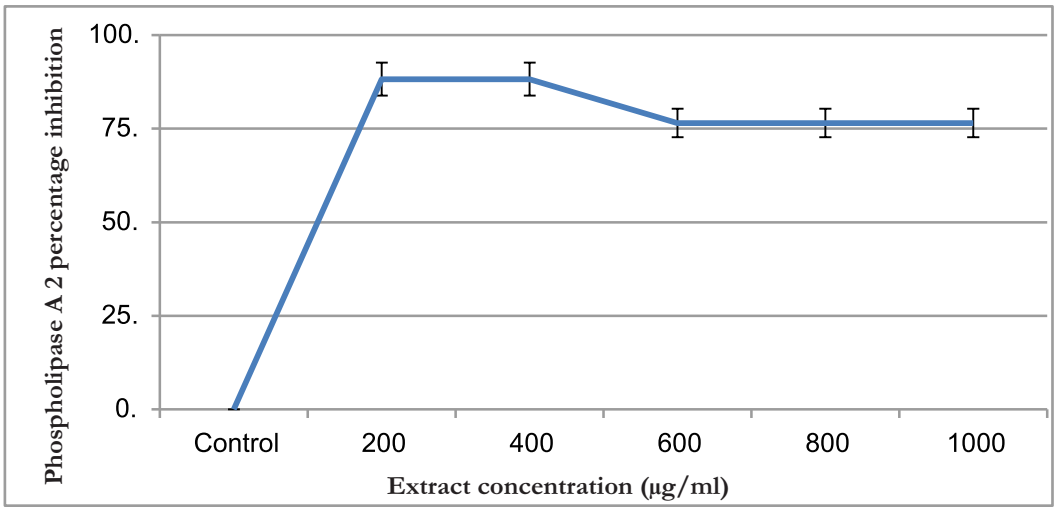


Figure 1: Effect of different concentrations of Ethylacetate leaf extract of *Solanum dasyphyllum* on phospholipase A_2 activity of *Naja nigricollis* venom.

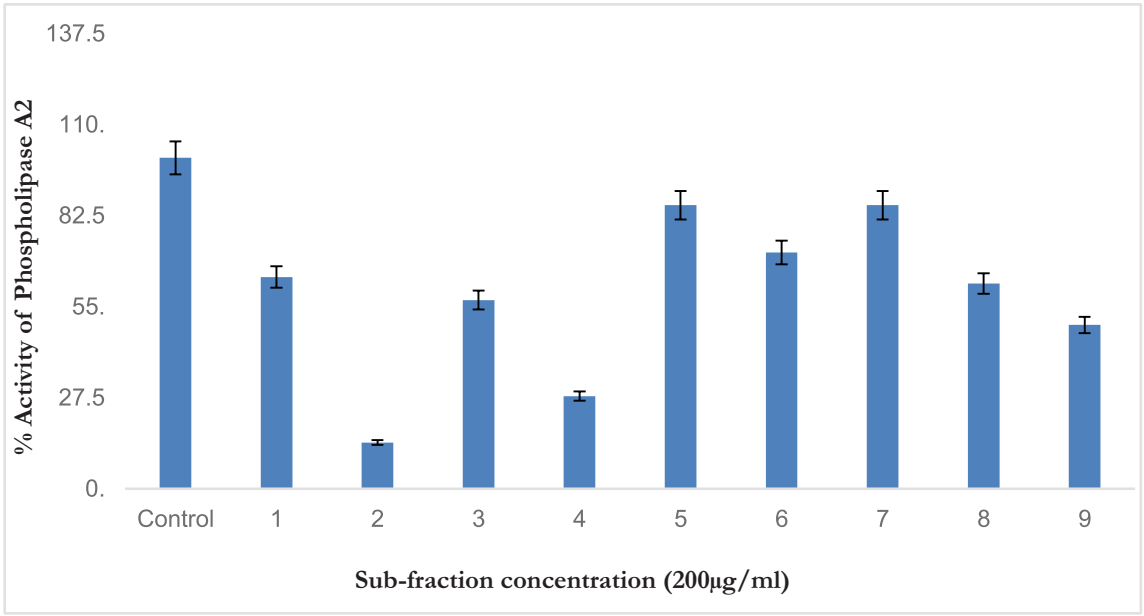


Figure 2: Effect of Different fractions of Ethylacetate Leaf Extract of *S. dasyphyllum* on Phospholipase A_2 Activity of *N. nigricollis* Venom.

Table 2: Physical properties of the isolated compound ($E2_i$)

Colour of isolate	Colour of spot	Solvent system	*Rf* value	Solvent
Crystal white	light purple	Butanol/Acetic acid/Water (5:4:1)	0.77	Chloroform

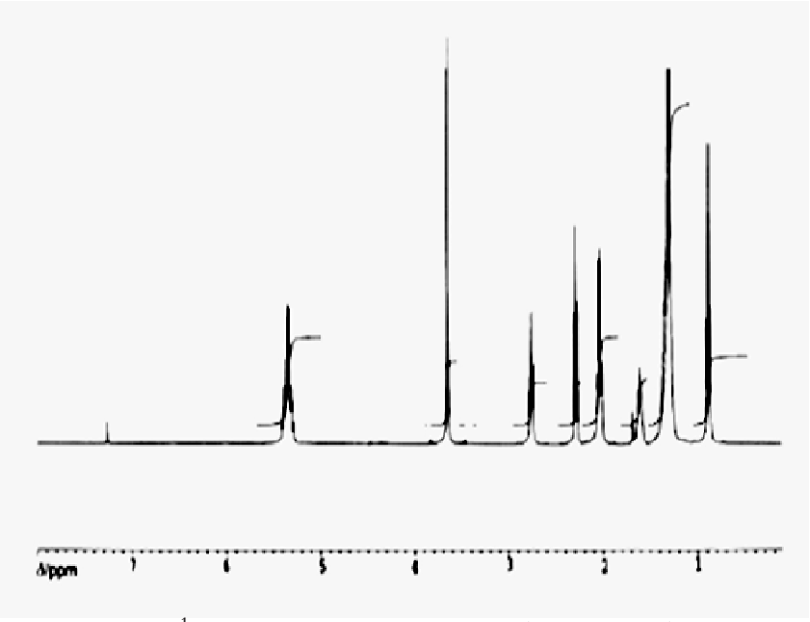


Figure 3: ^{1}H-NMR spectra of isolated compound ($E2_i$).

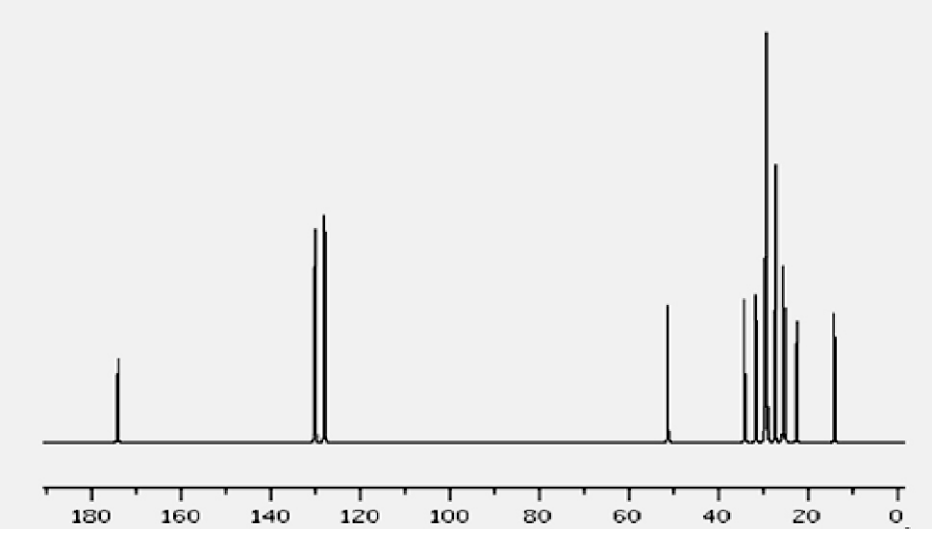


Figure 4: ^{13}C-NMR Spectrum of Isolated Compound (E_{2i})

Table 3: ^{1}H-NMR and ^{13}C-NMR Chemical Shift of the Isolated Compound

Position	^{13}C chemical shift	^{1}H chemical shift
1	14.02	0.86
2	22.63	1.24
3	31.75	1.2
4	29.11	1.25
5	27.21	1.96
6	130.4	5.47
7	128.4	5.48
8	25.81	2.25
9	128.4	5.49
10	130.4	5.50
11	27.2	2.63
12	29.1	1.26
13	29.4	1.28
14	29.4	1.42
15	29.4	1.43
16	24.84	1.55
17	33.88	1.20
18	173.4	-
19	52.21	3.63

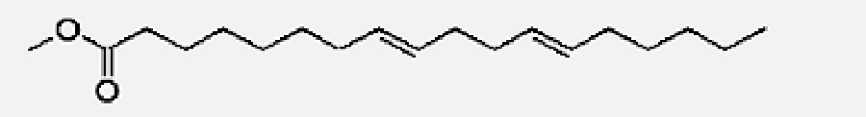


Figure 5: Proposed structure of isolated compound ($E2_i$): Methyl linolenate

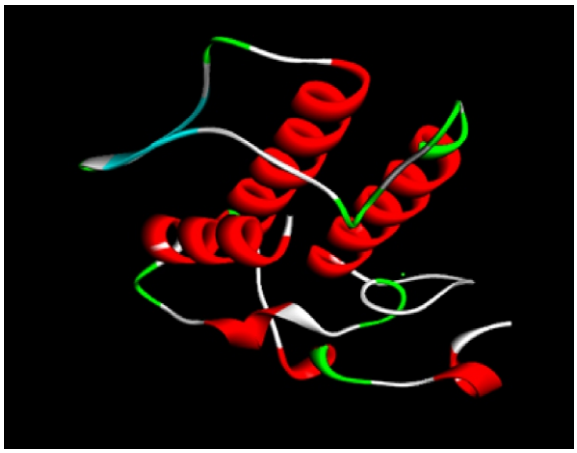


Figure 6: 3-D structure of Phospholipase A_2 (Protein data bank)

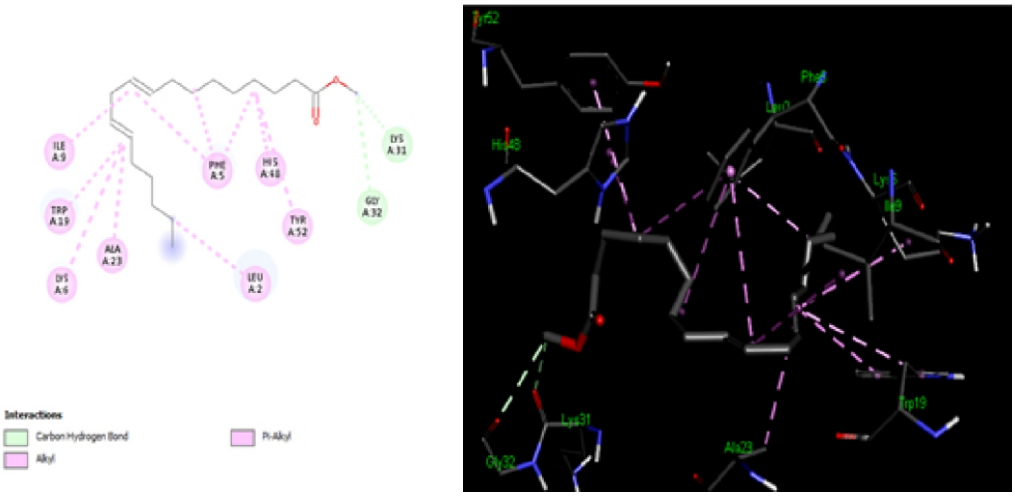


Figure 7: Outline of the molecular docking process. (A) The two-dimensional structure of Phospholipase A_2- Methyl linolenate complex with intermolecular interaction; (B) 3-dimensional conformation of Methyl linolenate docked into the active site of the Phospholipase A_2 enzyme.

Table 4: Molecular docking studies of Methyl linolenate isolated from ethylacetate extract of *Solanum dasyphyllum* on venom Phospholipase A_2 enzyme

S/No	Protein	Binding energy (Kcal/mol)	No of H-bond	Inhibition Constant: Ki (uM)	Amino acids involved in H-bond interaction	Amino acids involved in Hydrophobic bond interaction
1	Phospholipase A_2	-6.60	2	14.14	LYS A:31; GLY A:32	ILE A:9; TRP A:19; LYS A:6; ALA A:23; LEU A:2; PHE A:5; HIS A: 48; TYR A:52

DISCUSSION

The use of herbal remedies in the treatment of various diseases is increasing at a rapid pace worldwide; the low toxicity and easy accessibility contribute significantly to this global acceptance [15]. Various researchers have studied the bioactivity of the plant *S. dasyphyllum*; However, the active ingredient responsible for some of these biological activities is not precisely known. The venom of *N. nigricollis* contains proteases, phospholipase A_2, phosphodiesterase, acetylcholinesterase and hyaluronidase enzymes, also known as major enzyme toxins (METs). The phytochemical screening of the ethylacetate leaves extract of *S. dasyphyllum* revealed the presence of alkaloids, tannins, flavonoids, terpenoids, steroids and carbohydrates, but it was devoid of cardiac glycosides. These result correlated with those previously reported by Sodeinde et al. [18] and Manal et al [22], who worked

on *S. dasyphyllum* and *S. incanum L*, another species of the Solanaceae family. Mors et al. [23] reported that phenolic compounds, saponins, flavonoids and tannins can bind to proteins and can directly act on venom constituents. Similarly, Lans et al. [21] reported that plant alkaloids are effective against snake bites. Aristolochic acid, 8-methyloxy-6-nitophenanthro, 12-methoxy-4-methylvoachalotine and atropine are all isolated alkaloids compounds that inhibit the lethal effect of snake venom [24]. Although the quantitative estimate of phytochemicals in the extract was not evaluated, the inhibition of the enzyme suggested that the leaf extract may contain phytochemicals that are likely to bind to the enzyme, thus preventing it from binding to its substrate, leading to its inhibition.

The results of the inhibition study of phospholipase A_2 enzyme activity by different concentrations of ethylacetate leaves extract of *S. dasyphyllum* showed that the extract is potential Phosplipase A_2 inhibitor. This was similar to the results of Akindele et al. [16] where they reported that the ethyl acetate fraction of *M. oleifera* was a potential source of effective compounds against pathologies caused by the venom of *N. katiensis* [16].Furthermore, Ajiboye et al. [12] reported that the ethyl acetate fraction of the leaves of *Solanum macrocarpon* L., a plant belonging to the nightshade family, has an inhibitory effect on cholinergic enzyme activities as well as radical scavenging activity [12].

Chromatographic separation of the ethyl acetate extract resulted in the isolation of the compound methyl linolenate, whose properties, NMR spectra and chemical structure are shown in Table 3, Figures 3, 4 and 5. The compound was confirmed to be a fatty acid ester with the molecular formula $C_{19}H_{32}O_2$ and a molecular weight of 292.5 g/mol.

Molecular docking techniques, a tool used for structure-based drug design strategies to develop novel drugs against the inhibition of therapeutic targets, and several researchers have reported that a negative and low binding energy value indicates strong binding efficiency [17]. In this study, molecular docking investigation was carried out to evaluate the likelihood of inhibition of cobra venom phospholipase A_2 by methyl linolenate, a compound isolated from the ethyl acetate fractions of methanolic extract of *S. dasyphyllum*.Therefore, the docking of the isolated compound drawn with the aid of chemdraw was successfully carried out with cobra phospholipase A_2 obtained from protein data bank as shown in figure 5,6 and 7. The docking of methyl linolenate to the target enzyme was examined with regard to interacting amino acids, atoms involved in hydrogen bonding, predicted binding energy and inhibition constant (Ki) as shown in Table 4. The docking studies revealed the involvement of H-bonds between amino acid residues in the prominent binding site associated with other non-covalent interactions such as alkyl, pi-alkyl, pi-sigma interactions and vander Waals forces. The binding energy of methyl linolenate to the enzyme phospholipase A_2 is -6.60 kcal/mol. This suggests that methyl linolenate may inhibit phospholipase A_2 in snake venom, thereby attenuating the effects of snake venom in a biological system.

CONCLUSION

The present study revealed that the ethylacetate leaf extract of *S. dasyphyllum* contains bioactive components that can inhibit phospholipase A_2 enzyme in*N. nigricollis* venom. Therefore, methyl linolenate isolated from *S. dasyphylum* is worth both in vitro and in-vivo evaluation to assess and optimize its therapeutic efficacy.

REFERENCES

1. Gbolade AA. Nigerian Medicinal Plants With Anti-Snake Venom Activity- a Review. J

Malar Res Phytomedicine. 2021;4 (March):29–44.
2. Adetutu A, Morgan WA, Corcoran O. Ethnopharmacological survey and in vitro evaluation of wound-healing plants used in South-western Nigeria. J Ethnopharmacol. 2011 Sep;137(1):50–6.
3. Alam MI, Gomes A. Snake venom neutralization by Indian medicinal plants (Vitex negundo and Emblica officinalis) root extracts. J Ethnopharmacol. 2003;86(1):75–80.
4. Urs NAN, Yariswamy M, Joshi V, Nataraju A, Gowda T V., Vishwanath BS. Implications of phytochemicals in snakebite management: Present status and future prospective. Toxin Rev. 2014;33(3):60–83.
5. Adrião AAX, dos Santos AO, de Lima EJSP, Maciel JB, Paz WHP, da Silva FMA, et al. Plant-Derived Toxin Inhibitors as Potential Candidates to Complement Antivenom Treatment in Snakebite Envenomations. Front Immunol. 2022;13(May):1–28.
6. Abubakar MS, Sule MI, Pateh UU, Abdurahman EM, Haruna AK, Jahun BM. In vitro snake venom detoxifying action of the leaf extract of Guiera senegalensis. J Ethnopharmacol. 2000;69(3):253–7.
7. Abubakar IS, Abubakar SB, Habib AG, Nasidi A, Durfa N, Yusuf PO, et al. Randomised controlled double-blind non-inferiority trial of two antivenoms for Saw-scaled or carpet viper (Echis ocellatus) envenoming in Nigeria. PLoS Negl Trop Dis. 2010;4(7):8–17.
8. Asuzu IU, Harvey AL. The antisnake venom activities of Parkia biglobosa (Mimosaceae) stem bark extract. Toxicon. 2003 Dec;42(7):763–8.
9. Coriolano de Oliveira E, Alves Soares Cruz R, de Mello Amorim N, Guerra Santos M, Carlos Simas Pereira Junior L, Flores Sanchez EO, et al. Protective Effect of the Plant Extracts of Erythroxylum sp. against Toxic Effects Induced by the Venom of Lachesis muta Snake. Molecules. 2016;21(10):1–14.
10. Gopi K, Renu K, Raj M, Kumar D, Muthuvelan B. The neutralization effect of methanol extract of Andrographis paniculata on Indian cobra Naja naja snake venom. J Pharm Res. 2014;4(4):1010–2.
11. Dalhat MM, Muhammad H, Abubakar SB, Garba I, Yola IM, Habib AG. 236. Determinants of High Cost of Care Among Victims of Snake Bite in Kaltungo, Gombe State, Nigeria, 2009. Toxicon. 2012;60(2):216–7.
12. Ajiboye BO, Akalabu MC, Ojo OA, Afolabi OB, Okesola MA, Olayide I, et al. Inhibitory effect of ethyl acetate fraction of Solanum macrocarpon L. leaves on cholinergic, monoaminergic, and purinergic enzyme activities. J Food Biochem. 2018 Dec; 42(6):e12643.
13. Oyinloye E, Aderinola A, Muritala A, Olooto W. Protective role of dichloromethane extract of solanum dasyphyllumin in gentamicin induced nephrotoxicity in mice. Acta Pharm Sci. 2020;58(4):471–9.
14. Funmilola AR, Abubakar G, Hassan Z. In-vitro Antivenom Potential of Solanum dasyphyllum Methanolic Leaf and Fruit Extracts against Naja nigricollis Venom. European J Med Plants. 2020;31(9):38–45.
15. Funmilola AR, Gidado A, Zanna H. Antivenom activities of methanolic leaf extract of Solanum dasyphyllum Schum & Thonn against Naja nigricollis venom mice-induced envenomation. 2021;12(3):465–79.
16. Akindele A. J., Ibe I. F. AOO. Analgesic and antipyretic activities of drymaria cordata (linn.) wild (caryophyllaceae) extract. Afr J Tradit Complement Altern Med. 2012; 9(1):25–35.

17. Ruyck J De, Brysbaert G. Molecular docking as a popular tool in drug design, an in silico travel. 2016; 1–11.
18. Sodeinde OA., Salawu KM., Ogbole OO, Ajaiyeoba E O. Phytochemical, antioxidant, brine shrimp lethality and antiproliferative analyses of *Solanum dasyphyllum schum. & thonn.* leaf and fruit extracts. *Savannah Veterinary Journal* 2019; 2(2): 13-17.
19. Sofowora A. *Medicinal Plants and Traditional Medicine in Africa*, 3rd edn, Spectrum Books Limited, Ibadan. 1993; Pp 191-289.
20. Adamich M, Roberts M, Dennis E. Phospholipid activation of cobra venomphospholipase A2: characterization of the phospholipid-enzyme interaction, Biochemistry 1979; 18: 3308-3316.
21. Lans C, Harper T, Georges K. Medicinal and ethnoveterinary remedies of hunters in Trinidad, *BMC Complimentary and alternative Medicine* 2001; 1: 1-10.
22. Manal AH, Eltohami MS, Ghada MA. The phytochemical screening and antimicrobial activity of *Solanum incanum L. International Journal of Innovative Pharmaceutical Sciences and Research*. 2016; 4(2): 87– 92.
23. Mors WB, Nascimento MC, Pereira NA, Pereira BM. (2000). Plant natural products active against snake bite: the molecular approach. *Journal of Phytochemistry* 2000; 55(6):627–642.
24. Singh P, Mohammad Y, Risha H, Sunisha S, Rahul S. A review on venom enzymes neutralizing ability, *Journal of Pharmacopuncture* 2017; 20(3): 173-178.

Adewunmi RF, Yesufu HB, Gidado A[3], Pudza JS. Inhibition of *Naja nigricollis* Venom Phospholipase A_2 by Ethylacetate Extract of *Solanum dasyphyllum* Schum and Thonn leaf: An *In-vitro* and *In-silico* Approach. Afr. J. Trop. Med. & Biomed. Res. 2024; 7(1) 40 -50

https://dx.doi.org/10.4314/ajtmbr.v7i1.7