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Qualitative Phytoconstituents Determination, FTIR, Toxicity Profiling and Antivenin Potential of *Indigofera Conferta*

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ABSTRACT

The plant from family *fabaceae* has reportedly been used in traditional medicine to treat sore feet and snake bite. This research work is aimed at evaluating the toxicity profile and antivenin potential of the plant using standard procedures. Preliminary phytochemical screening on crude methanol extract revealed the presence of alkaloids, flavonoids, steroids/terpenes, cardiac glycosides, saponin, lipids, carbohydrate and anthraquinones. Hexane fraction showed the presence of steroids and lipids only. Chloroform fraction showed the significant presence of steroids, alkaloids, and lipids while ethyl acetate showed the presence of all the constituents identified in the crude methanol extract. FTIR spectroscopic analysis on the crude methanol extract revealed various characteristic band values related to structural features in complex organic compound and aromatic structures. The toxicity investigation showed that both crude methanol extract and fractions are nontoxic and safe at the tested doses. The antivenin activity evaluated on the chloroform fraction of *I. conferta* yielded significant result with 40% protection at both 125 and 250mg/kg, but no protection was observed at 500 mg /kg. The activity observed might be attributed to individual or combined secondary metabolites identified.

Keywords: Phytochemicals, Toxicity, Antivenin, *Indigofera Conferta*, herbal

1.0 INTRODUCTION

Medicinal plants means species of plant kingdom whose parts (such as leaves, roots, stems, fruits or seeds) are used or prepared as medicine to treat disease. These plants contain secondary metabolites with biological properties and are significant source of bioactive compounds used in various medicinal usages (Weckerle *et al*., 2022; Davis and Choisy, 2024). The term medicinal plant was first used in 1967 in the context of the study of hallucinogenic plants, knowledge of the beneficial properties of medicinal plants to treat diseases represent valuable resources to preserve the biological and cultural diversity of different ethnicities (Davis and Choisy**,** 2024**;** Weckerle *et al*., 2022).

Medicinal plants have been used by indigenous physicians since pre-historical times and are part of the traditional knowledge of humanity. The use of medicinal plants derive from having secondary metabolites with biological properties and some are important source of components for antiseptics, antibiotics, antitumor, antivirals, anti-inflammatory among others. (Hilal *et al.*, 2024; Elshafie *et al* 2023). Antimicrobial, anticancer, antioxidant, enzyme inhibitory, anti-aging, anti-hypertensive, neuroprotective and anticoagulant effect (Lesellier *et al.*, 2021). The most current reports indicate that the planet is home to a diversity of 391,000 vascular plants, of which at least 35,000 species have a potential medicinal use. In addition, 25% of the bioactive compounds used in various medicine come from plants, there are also reports indicating that at least 80% of the world's population depend on traditional herbal remedies (Kew, 2020)

Indigofera conferta belongs to the family fabaceae. Plant commonly found growing in red soil and is an annual herb with sticky hairy leaves and flowers. It can grow up to 2 feet high. Its leaves also possess hairs and is distributed throughout the tropical region of West Africa (Burkil, 1995). *I. Conferta* is used in traditional medicine for various diseases including sore feet, snake bite and infected wound (Abdulkadir *et al*., 2015; Omara *et al*., 2020). The biological activities of this plant includes, antibacterial, antisnake and reduction of bleeding time of envenomed rats (Abdulkadir *et al*., 2015; Isah *et al*, 2026).

One of the basic criteria set by WHO for the use of herbs as medicine is that they should be shown to be non-toxic. Although the use of ethno medicines is widespread in Africa, most of the plants have not been investigated for toxicity. A lot of adverse reactions were reported from locally used herbal medicines. *I. Conferta* have been reported locally to treat numerous diseases such as sore feet, snake bites, infected wounds etc. but yet the toxicity profile of the plant have not been reported. Therefore, this research will investigate the phytoconstituents present, toxicity profile and antivenin potential of *I. conferta* .

2.0 MATERIALS AND METHODS

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2.1 Plant Collection and Identification

Fresh aerial part of *Indigofera Conferta* was obtained from Samaru Local Government of Kaduna State, Nigeria. The sample was identified and authenticated by Mallam Namadi Sanusi of Herbarium Unit, Department of Botany, Faculty of Life Science, Ahmadu Bello University Zaria, where a voucher specimen number V/N ABUHO/637 was obtained.

2.2 Plant Preparation

The fresh aerial part of *Indigofera Conferta* were properly cleaned and washed carefully with distilled water (3 litres) but not excessive, shade dried, pulverized, labelled and stored at room temperature.

2.3 Plant Extraction

The powdered plant material (786 g) was extracted in an airtight glass bottle containing 3 litres of methanol for three (3) days, the extract was filtered using Whatman No.1 filter paper and solvent was evaporated using rotary evaporator. The resulting crude extract was dried and subsequently weighed to determine the percentage yield. The crude methanol extract (58 g) obtained was then successively subjected to liquid-liquid extraction with hexane (250 cm^3), chloroform (250 cm^3) and ethyl acetate (250 cm^3) in sequential order of polarity, afforded hexane, chloroform and ethyl acetate fractions respectively.

2.4 Qualitative Phytochemical Screening

Each crude extract and fraction (2 g) was subjected to a preliminary phytochemical screening to detect for the presence of secondary metabolites according to the procedures described by (Garba *et al*., 2025).

2.5 FITR Analysis

FTIR spectra of crude methanol extract were recorded in the range of 4000-400 cm^{-1} using a spectrometer. Transmittance was measured to identify the absorption peaks corresponding to various molecular vibrations (Mayuresh and Madhura, 2025)

2.6 Venom Sample

The venom of an adult *Naja nigricollis* was collected by the milking method of Markfalane (Markfalane, 1967; WHO, 2021). It was pooled lyophilized and stored at 4^0C until required.

2.7 Experimental Animals

Swiss albino mice (19-26 g) of both sexes were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Nigeria. They were fed with a laboratory diet and water, then placed in propylene cages at room temperature under standard conditions (12-h light and 12-h dark cycle).

2.8 Median Lethal Dose (LD_{50}) Determination of the Crude Methanol Extract and Fractions

The route of administration was oral using the method described by Lorke (1983; OECD, 2022). In the first stage, exactly nine mice of different sexes were divided into three groups of three mice each. Groups 1, 2 and 3 received 10, 100 and 1000 mg/kg, respectively. In the second phase, three mice were used based on the results of the first phase. Each of the three mice received different doses of the extract (1600, 2900, 5000 mg/kg). The experiment was repeated for hexane, chloroform and ethyl acetate fractions sat the same graded doses. The median lethal dose was calculated as follows:

LD50= $\sqrt{MLDxMSD}$ ……(1)

Where:

MLD –Minimum lethal Dose

MSD –Maximum survival Dose

2.9 Statistical Studies

Statistical analysis was performed by one-way analysis of variance (ANOVA), followed by multiple comparisons of values in control versus treatment groups using post hoc Dunnett's test. The results were considered significant at $p < 0.05$. All the analysis was done using statistical package for the social sciences (SPSS) version 20.0.

3.0 RESULTS AND DISCUSSION

Table 1: Percentage Yield of *Indigofera Conferta* Crude Methanol Extract and Fractions

Fractions	Colour	Weight (g)	Yield (w/w%)
Hexane	Dark green	7.4	21.3
Chloroform	Light green	0.9	0.2
Ethyl acetate	Light brown	1.6	0.5
Methanol (Crude)	Greenish brown	34.7	44.1

The percentage yield of methanol crude extract obtained from (786 g) of pulverized aerial part of *Indigofera conferta* was (58.0 g) which represents (7.4% w/w). Subjecting the crude extract (34.7 g) to liquid-liquid extraction, the percentage yield recorded are 7.4g, 0.9g and 1.6g equivalent to 21.3%, 0.2% and 0.5% respectively.

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Table 2: Results of Qualitative Phytochemical Screening of *Indigofera Conferta* crude methanol extract and fractions

Constituents	Test	Methanol Crude	Hexane	Chloroform	Ethyl acetate
Alkaloids	Mayer	++	-	++	+
	Waganer	++	-	++	+
	Hagers	++	-	++	+
Flavonoids	Ferric Chloride	+++	-	-	++
	Alkaline	+++	-	-	++
	Shinoda	+++	-	-	++
Steroids/ Triterpenoids	Salkowski	+++	+++	++	++
	Libermann Burchard	+++	+++	++	++
Tannins	Ferric Chloride	+++	-	-	++
	Lead acetate	+++	-	-	++
Cardiac Glycosides	Killer Killiani	+++	-	+	++
Saponins	Frothing	+++	-	-	+
Anthraquinones	Borntrager	++	-	+	+
Carbohydrates	Molich	+++	-	-	+
	Fehling	+++	-	-	+
Phenols	Ferric Chloride	+++	-	-	++
Lipids	Sudan	+	++	++	+

++ = Significantly present + = Slightly present - = Absent

The qualitative phytochemical screening of *Indigofera conferta* crude methanol extract revealed the presence of secondary metabolites such as alkaloids, flavonoids, tannins, steriods/terpenes, cardiac glycosides and saponins, hexane fraction showed the presence of steroids and lipids only, chloroform fraction showed the significant presence of steroids, alkaloids, and lipids while ethyl acetate revealed the presence of all the constituents identified in the crude methanol extract as shown in Table 2. These components have been reported to be associated with different pharmacological activities of the plant (Muhammad *et al*, 2022)

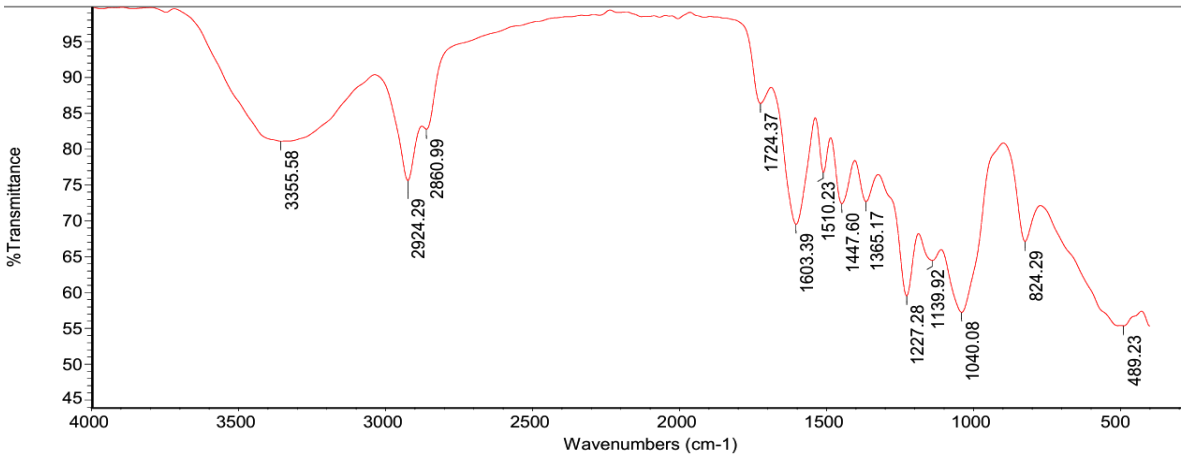


Figure 1: FTIR Spectra of crude methanol extract of *I. conferta*

Table 3: FTIR Peaks of *I. conferta* crude methanol extract

Bond No	Peaks (cm^{-1})	Band Assignment	Interpretation
1	3355.58	N-H	Ammonium Ion
2,3	2924.29 - 2860.99	C-H	Aliphatic Compound
4,	1724.37	C=O	Ester
5,6	1603.39 - 1510.23	N-O	Nitro Compound
7,8	1447.60 - 1365.17	-OH	phenol or Tertiary Alcohol
9	1227.28	N-H	Amine
10,11	1139.92 - 1040.08	C-F	Aliphatic Fluro Compound
12	824.29	C-H	Aromatic Compounds

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FTIR provides a comprehensive qualitative and quantitative analysis of materials based on their unique absorption peaks corresponding to atomic bond vibration (Hong *et al* 2021)

The FTIR result of *I. Conferta* crude methanol extract contrasted with the reference graph recognized the various functional groups. The absorption peak at 3355.58 cm^{-1} revealed the presence of amino groups (Joselin *et al*., 2025). Absorption peaks at 2924.29 and 2860.99 cm^{-3} Corresponds the presence of aliphatic Compounds (C-H bend) (Singh *et al.*, 2022) The peak at 1724.37 cm^{-1} showed the presence of esters group, also the absorption bands at 1603.39 and 1510.23 cm^{-1} are due to N-O stretching associated with the nitro Compounds (Jotiram *et al*., 2025)

The absorptions at 1447.60 and 1365.17 cm^{-1} are due to -OH bending associated with phenol or tertiary alcohol (Joselin *et al*., 2025). The absorption band at 1227.28 cm^{-1} represent C-N stretching of the amine, also absorption peaks at 1139.92 and 1040.08 cm^{-1} Correspond to aliphatic fluoro Compounds of the extract. Band at 824.29 cm^{-1} Signifies aromatic disubstitution (Figure 1)

Table 4a: Determination of Median Lethal Dose (LD_{50}) for *Indigofera Conferta* crude methanol extract and fractions

Extracts (First Phase)

Dose (mg/kg)	Number of Mice used	Mortality
10	3	0/3
100	3	0/3
1000	3	0/3

Table 4b: Determination of Median Lethal Dose (LD_{50}) for *Indigofera Conferta* crude methanol extract and fractions

Extracts (Second Phase)

Dose (mg/kg)	Number of Mice used	Mortality
1600	1	0/1
2900	1	0/1
5000	1	0/1

$LD_{50}= \sqrt{5000\ X\ 5000}$

= 5000mg/kg

The acute toxicity profile of the crude methanol extract and fractions of *Indigofera Conferta* was studied. The median lethal dose (LD50) was found to be greater than 5000 mg/kg each. This suggests that the extract and fractions are nontoxic (Lorke, 1983; OECD, 2022), and are relatively safe administered doses. The minimal lethal dose (LD99) of *Naja nigricollis* venom estimated by probit analysis was 5.75 mg/kg. However, Isah *et al.* (2022) reported higher value (9.7 mg/kg). This variability observed in the minimal lethal dose of venom may be due to differences occur as a result of composition due to geographic location, subspecies, season, age, and diet of snakes.

Table 5: *In vivo* Detoxifying Effect of *Indigofera Conferta* Chloroform Fraction on *Naja nigricollis* Venom

Treatment Components	Control group (LD_{99}+NS)	% Survival of Mice within 24 hours on different treatments		
		(LD_{99} + 125) =T1	(LD_{99} + 250) =T2	(LD + 500) =T3
Chloroform	0	40.0	40.0	0.00

Keys: NS = Normal Saline, T1 = (LD99 + 125) Treatment dose, T2 = (LD_{99} + 250) Treatment dose and T3 = (LD_{99} + 500) treatment dose

Based on the phytochemical result obtained in table 2 above which showed that chloroform, contained significant presence of alkaloids and steroids compared to the other fractions tested. The chloroform fraction was therefore, subjected to antivenin activity. The result showed 40%protection each at 125 and 250mg/kg doses however, no protection was observed at highest dose (500mg/kg). (Table 5). Flavonoids, tannins and terpenoids have been reported to possess important pharmacological activities, including protein-binding, enzyme-inhibitory, antioxidant and anti-inflammatory properties. These activities may contribute to the neutralization of some toxic effects associated with snake venom. Recent studies have also demonstrated that phytochemicals such as flavonoids, terpenoids and sterols can interfere with venom enzymes and other biologically active components involved in snake envenomation (Yusuf *et al*., 2019; Omara *et al*., 2020; Isah *et al*., 2022). Therefore, the observed antivenom activity of the chloroform fraction of *Indigofera conferta* may be attributable to the presence of these bioactive constituents, either individually or through synergistic interactions among different phytochemicals present in the fraction.

4.0 CONCLUSION

The research work showed that *I.Conferta* is nontoxic and relatively safe at administered dose and significantly, demonstrated antivenin activity against *Naja nigricollis* Venom and therefore, confirmed its medicinal claim in the treatment of snake bite. The

observed activity might as a result of the secondary metabolite identified.

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REFERENCES

Abdulkadir, S., Adamu, A.K., Adelanwa, M.A., Ibrahim G. (2015). Effects of *Indigofera Conferia* Whole Plant Petroleum ether, Ethanol and Aqueous Extract Against Naja Nigricollis Venom on Bleeding and Clothing time of Rats, Katsina Journal of Natural and Applied Sciences 4.

Burkill, M.H. (1995). The Useful Plants of Tropical Africa, Families J-L, Vol. 3. Royal Botanical Gardens Kew.

Davis, C. C., & Choisy, P. (2024). Medicinal plants meet modern biodiversity science. *Current Biology*, *34*(4), R158–R173. https://doi.org/10.1016/j.cub.2023.12.038

Dev, M., & Mukadam, M. (2025**).** Functional group profiling of medicinal plants using FTIR spectroscopy. *World Journal of Biology Pharmacy and Health Sciences*, *21*(1), 243–249. https://doi.org/10.30574/wjbphs.2025.21.1.0039

Elshafie, H. S., Camele, I., & Mohamed, A. A. (2023). A comprehensive review on the biological, agricultural and pharmaceutical properties of secondary metabolites based-plant origin. *International Journal of Molecular Sciences*, *24*(4), 3266. https://doi.org/10.3390/ijms24043266

Garba, U., Faruq, U. Z., Uba, A., Kwazo, H. A., & Gayari, S. M. (2025). Phytochemical, antibacterial and characterization of 9-methoxy-12-phenyl-dodec-10-enoic acid-3,4,5-trihydroxy-6-methyl-tetrahydropyran-2-ylester isolated from *Acacia macrostachya* leaves. *International Journal of Science for Global Sustainability*, *11*(3), 97–108. https://doi.org/10.57233/ijsgs.v11i3.925

Hilal, B., Khan, M. M., & Fariduddin, Q. (2024). Recent advancements in deciphering the therapeutic properties of plant secondary metabolites: Phenolics, terpenes, and alkaloids. *Plant Physiology and Biochemistry*, *211*, 108674. https://doi.org/10.1016/j.plaphy.2024.108674

Hong, T., Yin, J.Y., Nie, S.P and Xie M.Y (2021) Applications of Infrared Spectroscopy in polysaccharide Structural analysis: progress, challenge and perspective. *Food Chem,* X(12): 100168.

Isah, M., Hamza, A., Nnadozie, N. T., Bebe, E. A., Tajuddeen, N., & Musa, A. (2026). Isolation and characterization of a dihydrostilbene from the aerial parts of *Indigofera conferta*. Recent Advances in Natural Sciences, 4(1), 246. DOI: 10.61298/rans.2026.4.1.246

Isah M. Salima, M., Sakyanh, M.A., Asmau., N.H., Nasir, T., Muammar, L.D., Mzozoyana, V. & Musa, A.M. (2022). A Bioactive Chalcone from the Aerial Parts of *Indigofera Conferta* Gillet. Natural Product Research. https://doi.org/10.1080/ 14786419. 2022.20984 93

Isah, M., Hamza, A. N., Abdullahi, M., Elrashedy, A., Soliman, M., & Musa, A. M. (2022). A bioactive chalcone from the aerial parts of *Indigofera conferta* Gillet. *Journal of Ethnopharmacology*, *296*, 115464. https://doi.org/10.1016/j.jep.2022.115464

Joselin, J., Benilla, B,S., Shynin Brintha, T.S. and Jeeva, S (2025) phytochemical Profiling, FTIR spectroscopy, and antioxidant evaluation of select Lamiaceae species. *Science Direct* 3(2): 111-117.

Jotiram, D,S., Gulab, M.R and Srushti, S.K (2025) Qualitative phytochemical Screening and FTIR spectroscopic analysis of *Sesamum Indicum L*. *Journal of Medicinal plant-s Studies* 13 (4): 104-108.

Kew, (2020). Royal Botanic Gardens**,** *State of the world's plants and fungi 2020*

Lesellier, Eric; Lefebvre, Thibault; Destandau, Emilie. (2021). Recent developments for the analysis and the extraction of bioactive compounds from Rosmarinus officinalis and medicinal plants of the Lamiaceae family. Trends in Analytical Chemistry, 135, 116158.

Lorke, D.A. (1983). A New Approach to Practical Acute Toxicity Testing. Archive of Toxicology, 54(4), 275-287.https://doi.org/10.1007/ bfo1234480

Markfalane, R.G. (1967). Russell's Viper Venoms. British Journal of Haematology, 13(s1), 437-451.https://doi.org/10.1111/j.1365-2141.1967.tb00754.x

Muhammad M.U, Gayari, M.S., Idris, B, Bello M., & Boyi M. Y., (2023) Anti-Snake Venom Activity of Indigofera Hirsuta Extracts Against Naja Nigricollis Venom, European Journal of Theoretical and Applied Sciences, 1(2), 237-244.

Omara, T., Kagoya, S., Openy, A., Omute, T., Ssempijja, F., & others. (2020). Antivenin plants used for treatment of snakebites in Uganda: Ethnobotanical reports and pharmacological evidences. *Tropical Medicine and Health*, *48*, 6. https://doi.org/10.1186/s41182-019-0187-0

Organisation for Economic Co-operation and Development. (2022). *Test No. 425: Acute oral toxicity: Up-and-down procedure*. OECD Publishing. https://doi.org/10.1787/9789264071049-en

Salvaraju, R., Sakuntala, P. & Jaleeli, K.A. (2021) GC-MS and FTIR Analysis of Chemical Compounds in Ocimum Gratissimum Plant. Biophysics 66, 401-408. https://doi.org/10.1134/S0006350921030167.

Weckerle, C. S., de Boer, H. J., Puri, R. K., van Andel, T., Bussmann, R. W., & Leonti, M. (2022). Recommended standards for conducting and reporting ethnopharmacological research. *Journal of*

DOI: https://doi.org/10.57233/ijsgs.v12i3.1197 ISSNp: 2488-9229; ISSNe: 3027-1118

Ethnopharmacology, 287, 114952. https://doi.org/10.1016/j.jep.2022.114952

World Health Organization. (2021). *WHO guidelines for the production, control and regulation of snake antivenom immunoglobulins* (Updated version). World Health Organization. WHO snake antivenom guidelines

Yusuf, A.J. Abdullahi, M.I., Musa, A. M., Haruna, A.K, Mzozoyana, V. & Abubakar, H. (2019). Antisnake venom Activity and Isolation of Quercetin from the Leaf of Neocarya Macrophylla (Sabine) prance ex. F. white (Malpighiales: Chrysobalanaceae). Brazillian Journal of Biological Sciences, 6(13), 381-389. https://doi.org/10.21472/bjbs.061306