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In Vivo Determination of Effects of Fractions of Chrysophyllum Albidum Leaf Extract on Kidney Function of Bitis Arietans Envenomated Wistar Rats.

ENEJI, EMMANUEL A.[1], YAKUBU, O. E.[2], IMO, CHINEDU[3]
[1,2,3]*Department of Biochemistry, Federal University Wukari, Taraba State Nigeria*

Abstract-
Background: ***In many parts of Nigeria, the Puff adder (Bitis arietans) is a serious threat to public health. Its venom often causes severe internal damage, specifically targeting the kidneys. This study set out to see if natural compounds found in African Star Apple (Chrysophyllum albidum) leaves could help protect the kidneys from these toxic effects.***
Methods: ***We started by breaking down the leaf extract into 24 different parts (fractions). After testing them in the lab, we chose the three most powerful fractions (F5B, F6A, and F10B) to test in Wistar rats. We gave the rats a controlled dose of venom and then treated different groups with either the plant fractions or a standard anti-venom. We then monitored their kidney health by checking urea, creatinine, and electrolyte levels, alongside physical exams of the kidney tissues and antioxidant activity.***
Results: ***The results showed that the venom caused immediate and significant kidney damage, spiking waste levels in the blood and causing "oxidative stress" in the tissues. However, the rats treated with the C. albidum fractions showed remarkable recovery. These plant extracts worked similarly to standard anti-venom, bringing kidney function markers back toward normal levels. Detailed analysis (HPLC) revealed that the leaves are packed with protective compounds like myricetin and various beneficial acids. Furthermore, the extracts helped reduce the over-expression of the VEGF gene caused by the venom and physically shielded the kidney tissues from permanent scarring.***
Conclusion: ***Our findings show that Chrysophyllum albidum leaves contain potent ingredients that can fight off the kidney-damaging effects of Puff adder venom. This suggests that these local plants could be a valuable resource for developing new, more accessible treatments for snakebite victims.***

Index Terms- **Chrysophyllum albidum, Bitis arietans,** ***Kidney function, Anti-venom, Nephrotoxicity, Antioxidants***

I. INTRODUCTION

The puff adder (Bitis arietans) is recognized globally as one of the most medically significant snake species (Figure 1). As a large, terrestrial ambush predator, its vast geographic range spans 46 countries across Africa and the Middle East.1,2 Its remarkable camouflage makes it nearly invisible in the savannah regions of sub-Saharan Africa, leading many unsuspecting victims to accidentally step on the snake, resulting in frequent and severe bites to the lower limbs.1 Furthermore, the puff adder's popularity in the exotic pet trade and zoos has extended the risk of envenomation to North America, Europe, and Asia regions where the species does not naturally occur.1

Figure 1: Pictorial view of *Bitis arietans* (Puff Adder).[1]

Despite the heavy burden of these bites, empirical data regarding the exact epidemiological impact and clinical progression of puff adder envenomation remain surprisingly sparse.[3] Clinical observations suggest that *B. arietans* accounts for a staggering proportion of snakebites in certain regions, such as Zimbabwe, where it is responsible for up to 75% of cases.[1] Interestingly, the most detailed clinical study of puff adder envenomation in Nigeria dates back to 1975; nearly fifty years later, this single study

remains the primary academic reference for the species' clinical features.[1] This lack of modern data is concerning, as a narrow understanding of the venom's effects can lead to misdiagnosis, delayed antivenom administration, and poorer patient outcomes.[1,4] This challenge is further complicated by significant "venom heterogeneity" meaning the chemical makeup of the venom varies widely between different regions and even individual snakes.[5] The World Health Organization notes that the puff adder is responsible for a high number of fatalities across Africa due to its potent, cytotoxic venom.[6] Victims typically experience excruciating pain, rapid swelling, and localized tissue destruction (necrosis).[6] Systemically, the venom can trigger hypotension, spontaneous internal bleeding, and long-term organ complications.[6] While the primary treatment is the timely administration of specific or polyvalent antivenom, the effectiveness of these treatments can be hampered by the geographical variation in venom potency.[6,7] Furthermore, in rural areas, limited access to antivenom and supportive care such as fluid resuscitation remains a major barrier to survival.[6]

At a molecular level, *B. arietans* venom is a complex "cocktail" refined by millions of years of evolution for hunting and self-defense.[8] The primary toxin families include snake venom metalloproteinases (SVMPs), snake venom serine proteases (SVSPs), phospholipase A2s (PLA2s), and C-type lectin-like proteins (CLPs).[9] Notably, SVMPs and SVSPs can account for half of the venom's dry weight.[9] These toxins drive the most severe symptoms:

- SVSPs (such as KN-Ba): These cleave fibrinogen and trigger massive inflammation by releasing mediators like TNF and Interleukin-6.[10]
- CLPs (such as Bitiscentin-3): These cause blood platelets to clump together, disrupting normal clotting.[11]
- Short Peptides: Proline-rich oligopeptides and bradykinin-potentiating proteins contribute to the dangerous drop in blood pressure (hypotension) seen in victims.[12]

Research indicates that Nigerian *B. arietans* populations possess venom with significantly higher protease activity compared to those from Malawi, Saudi Arabia, or Zimbabwe.[13] Similarly, studies in Angola found that venom from the Calandula region had much higher PLA2 activity than samples from the Mufuam region.[14] Such variation can drastically affect how well a standard antivenom works.[15] While some studies on the EchiTAb-Plus antivenom showed consistent neutralizing power against both Nigerian and Cameroonian venoms, other regional variations continue to pose a significant hurdle for universal treatment efficacy.[16]

The jump from village remedies to high-tech laboratory analysis is rooted in the impressive versatility of *Chrysophyllum albidum*. For generations, healers have reached for its bark and leaves to treat everything from the high fevers of malaria to the painful swelling of a physical injury.[15,16] These traditional practices aren't just cultural artifacts; they are the first clues pointing toward the plant's deep-seated biological power. When a healer uses a leaf extract to stop a wound from bleeding or to soothe a bruise, they are likely tapping into the plant's natural ability to control inflammation and fight off oxidative damage.[17]

In the context of a puff adder bite, these properties are exactly what the body needs. *Bitis arietans* venom is essentially a biological "fire" that causes rapid tissue death and organ failure.[18] By isolating the most potent parts of the *C. albidum* leaf what we call the active fractions—we are essentially concentrating the plant's "best defenders." Compounds like myricetin and phenolic acids, found in these leaves, act like molecular sponges that soak up the toxic free radicals produced by the venom.19,20

This study, therefore, serves as a scientific handshake between the wisdom of folklore and the precision of modern medicine. We are not just asking *if* the plant works, but *how* its specific chemical fractions shield the kidneys from the devastating systemic storm triggered by one of Africa's most dangerous snakes.
Building on its historical use, modern science has begun to peel back the layers of *Chrysophyllum albidum* to understand exactly what makes it so effective. Comprehensive screenings of the plant's

stems, seeds, and leaves have confirmed a rich "chemical toolbox" containing alkaloids, tannins, phenols, and flavonoids.[21-23] While some variation exists for instance, roots may lack certain glycosides and leaves are particularly dense in tannins the consensus among researchers is that this plant is a powerhouse of bioactive compounds.[21,24]

Methanol extracts of the leaf and seed cotyledons have further revealed the presence of specific markers like terpenoids, saponins, and steroids.[22,25] Beyond these common metabolites, *C. albidum* is a nutritional giant; its fruit contains a concentration of Vitamin C nearly 100 times that of oranges and 10 times that of guavas or cashews.[25,26] This is complemented by significant levels of iron, potassium, and essential vitamins like K and B1, alongside industrial-grade components like anacadic acid and unsaturated fatty acids, which make up roughly 74% of its oil content.[27-31]

The true medicinal potential of *C. albidum* lies in its ability to manage oxidative stress a key factor in surviving a snakebite. When tested against free radicals, various fractions of the leaf (specifically ethanol and ethyl acetate) showed a remarkable ability to "scavenge" or neutralize these harmful molecules.[32,33] Animal studies have demonstrated that the plant doesn't just work on its own; it actually boosts the body's internal defense systems, significantly increasing levels of protective enzymes like catalase (CAT) and glutathione (GSH) while decreasing lipid peroxidation (MDA).[32,34]

Specifically, the compound myricetin rhamnoside has shown radical-scavenging power comparable to standard medical controls, while the alkaloid eleagnine provides additional antioxidant support.[34,35] Because of this, researchers suggest that *C. albidum* acts as a natural "antioxidant booster," making it a perfect candidate for treating disorders where free radicals cause organ damage exactly what happens when *Bitis arietans* venom enters the bloodstream.[32,34]

II. MATERIALS AND METHODS

Materials

Chemical/Reagents

All assays kits were purchased from Randox laboratories Ltd. Ardmore, Co. Antrm UK. Chemicals and reagents used were purchased from Sigma chemical Company St. Louis U.S.A. Folin Ciocalteu Phenol, gallic Acid, quercetin, Carbon tetrachloride (Sigma-Aldrich), sodium hydroxide, sodium carbonate, aluminium chloride, sodium nitrate, sodium chloride.

Equipment

Jenway 6405 UV/V Spectrophotometer made by Jenway limited, Beacon road, Staffordshire ST15 05A, UK. Sherwood Colorimeter 257 made by Sherwood Scientific, 1 Paddocks Cherry Hinton Road, Cambrige CB1 8DH, UK. Grant JB Aqua 18 Plus Series-Unstirred Water Bath made by Grant Instruments, Shepreth Road, Cambridgeshire SG8 6GB, UK. Heraeus Labofuge 300 Centrifuge made by Thermo Fisher Scientific Company, 81 Wyman Street, Massachusette, USA. RS-232C Electronic Weighing Balance made by Itin Scale Company, 4802 Glenwood Road, New York, USA. Thomas-Wile Laboratory Mill Model 4 made by Thomas Scientific, 1654 High Hill Road, NJ 08085, USA. Hawskley Heamatospin 1300 Centrifuge and Heamatocrit HAE 3102 reader made by Hawksley Medical Laboratory Equipment, Marlborough Road, Sussex BN15 8TN, UK. Plus Incubator INC.200.230T made by Sayo Gallenkamp P.L.C., Monarch Way, Leicestershire, LE11 5XG, UK. Pasteur pipette, Curvettes, Oral gavage, Pair of scissors, Micropipette, Cages, Syringes, Needles, Spatula, Measuring cylinder, Dissecting set, Dissecting table, Mortar and pestle were used.

Methods

Experimental Design

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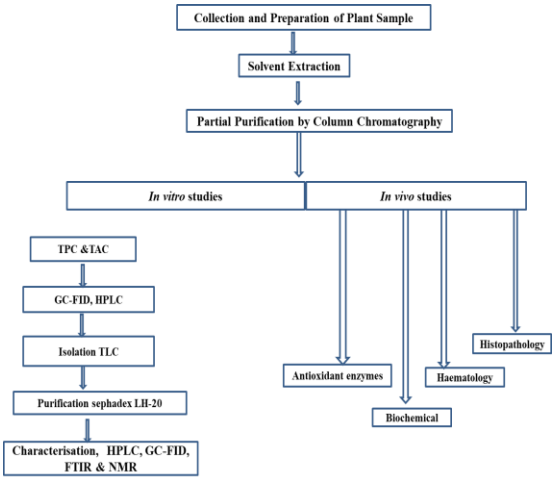

Figure 2: Experimental Design 1

Plant collection and identification

Chrysophyllum albidum (C. albidum), also known as African star apple, healthy leaves were collected from Anyikang forests in Bekwarra Local Government Area of Cross River State. Several leaves from different parts of the tree were taken to ensure representativeness. Both mature and young leaves were collected for comprehensive identification and authentication at the herbarium unit department of Agriculture, University of Calabar, Nigeria with voucher specimen number "UNICAL/AGRI/C.A2024/041".

Preparation and Extraction of Plant Materials

The rinsed leaves were allowed to air dried and Pulverized. 770g of pulverized leaves substance was extracted in 2 L of ethanol for 72 hours and filtered. It was further concentrated under vacuum at 45 ^{o}C using rotary evaporator and water bath, then stored in sealed sterile containers and refrigerated at 2-4 ^{OC} until used.[34]

Partial Purification by Column Chromatography

The ethanol extract was subjected to column chromatography to separate the extract into its component fractions. Silica gel was used in packing the column while different solvent combinations based on increasing polarity were used as the mobile phase as described by [34].

Elution: The ethanol extract (5 g) was dissolved in 5 mL absolute ethanol and the solution was applied to a chromatographic column. Elution of the extract was done with a solvent system of gradually increasing polarity, beginning from chloroform, ethyl acetate, methanol and finally water. The following ratio of the solvent combination was sequentially used in the elution protocol:

Chloroform: Ethyl acetate 100:0, 50:50 and 0:100

Ethyl acetate: Methanol 50:50 and 0:100

Methanol: Water 50:50 and 0:100

A measured volume (400 mL) of each solvent combination was poured into the column each time using a separating funnel. The eluted fractions were collected in aliquots of 400 mL in fraction collection tubes.

High Performance Liquid Chromatography (HPLC) Analysis

The HPLC system (Waters, Milford, MA, USA) that will be used for the analysis consist of a 600 controller pump, a multiple-wavelength UVVisible detector equipped with an in-line degasser AF 2489 and a rheodyne 7725i injector equipped with a 20 mL loop. Quantitative estimation was performed with Empower 2 software programs using the external calibration method. A Milli-Q academic water purification system (Bedford, MA, USA) equipped with 0.22 mm Millipak express filter and Eyela (Tokyo, Japan) rotary vacuum evaporate was used. Membrane filters of 0.45 mm pore size (Millipore) was used for filtration of the mobile phase and Whatman's syringe filters (NYL 0.45 mm) was used for the filtration of the sample.

Animal Study

Animal Care

Sixty (60) healthy Wistar rats weighed between 120–150 g were purchased. The animals kept in conventional laboratory settings (24 ºC, 12/12 h light-dark cycle), fed standard pellet diet and unlimited access to water. All the animals were given two weeks to acclimatize to laboratory conditions prior to the experiment.

Animal Grouping/Treatment

Table 1: Animal Grouping and Treatment

Groups	Dosages
Group 1	Feed and water only.
Group 2	Negative control; 1.25 mL/kg BV
Group 3	Positive control; BV + 0.8mg/kg SVA (IP)
	First fraction of highest concentration of antioxidant activities,
Group 4	BV + 100 mg/kg F5B
Group 5	BV + 200 mg/kg F5B
	Second fraction of highest concentration of antioxidant activities
Group 6	BV + 100 mg/kg F6A
Group 7	BV + 200 mg/kg F6A
	Third fraction of highest concentration of antioxidant activities
Group 8	BV + 100 mg/kg F10B
Group 9	BV + 200 mg/kg F10B
Group 10	BV + 400 mg/kg F5B
Group 11	BV + 400 mg/kg F6A
Group 12	BV + 400 mg/kg F10B

At the end of three days treatments, group 1 to group 9 animals were sacrificed while groups 10 to 12 were given continue treatment for fourteen days before they were sacrificed.

GP: group, BV: Bitis arietans venom, SVA: snake venom antiserum, IP: intra-peritoneally, F5B: Ethylacetate/Ethanol-100:00, F6A: Ethylacetate/Ethanol-50:50, F10B: Methanol/H2O-50:50.

Animal Sacrifice and Samples Collection

At the end of experimental period, animals were fasted overnight and sacrificed by chloroform anaesthesia. Blood was collected through cardiac puncture and serum was separated out. The liver and kidney were immediately removed, washed in normal saline while a portion was reserved for homogenization. Also, small portion of liver and kidney were fixed in 10% formalin for histopathological studies.

In invivo Analysis

Kidney Function Parameters

Determination of serum urea concentration

This was assessed using the method described by Fawcett and Scout (1960). Reagent (1 mL) containing sodium nitropusside and urease was added into three clean test tubes labelled as test sample, standard and reagent blank containing 0.01 mL sample, 0.01 mL standard reagent and 0.01 mL distilled water respectively. The content in each of the test tube was mixed and incubated at room temperature (25-30oC) for 10 minutes. The absorbance of the test sample and standard were read against reagent blank at 578 nm.

Calculation: The serum urea concentration was calculated using the formula below;

$$\text{Urea Conc. (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \; x \; \text{Concentrationof Standard}$$

BUN Concentration (mg/dl) = 0.467 x Urea Concentration (mg/dl).

Determination of serum creatinine concentration

The colorimetric method was used to determined serum creatinine concentration according to Bertels and Bohmer (1973). Working reagent (1 mL) containing picric acid and sodium hydroxide was added into two clean test tubes labelled sample test and standard, containing 0.1 mL of test sample and 0.1 mL of standard solution. The content in each test tube was mixed and after 20 seconds, the absorbance of the standard (ST1) and test sample (TS1) was read at 510 nm. Exactly 80 seconds later, absorbance for (ST2) and (TS2) of the standard and sample were read at 510 nm against distilled water (blank).

Calculation: The Concentration of creatinine in serum (mg/dl) was calculated using the formula below:

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$$\text{Creatinine Conc.}\,(mg/dl) = \frac{TS2 - TS1}{ST2 - ST1}\ x\ \text{Concentration of Standard}$$

(ST= Standard, TS= Test Sample)

Estimation of serum sodium, potassium and chloride ions

A flame photometer Model 143, equipped with an automatic diluter Model 144 (ratio of the dilution of 200:1) (Instrumentation Laboratory, Inc., Lexington, Mass., U.S.A.) was used. The calibration of the flame photometer was performed with twice distilled water and a standard having a Na+ concentration of 140 mequiv/l and a K+ concentration of 5 mequiv/l (Instrumentation Laboratory, Inc., Lexington, Mass., U.S.A). The stability of the instrument was checked with the standard solution after each measurement of a sample.

Statistical Analysis

A one-way analysis of variance (ANOVA) was used for analysis of the data followed by Duncan's multiple comparison test. The data grouped and presented as mean ± standard deviation. The statistical significance of the variances were evaluated using statistical package for social Sciences (SPSS) version 27, with a significance threshold set at ($P<0.05$).

III. RESULTS

Identified Bioactive Compounds in Fraction 5B (Ethylacetate/Ethanol-100:00 fractionation) by HPLC Analysis

Table 2 and figure 3 show the HPLC analysis of fraction 5B of the *Chrysophyllum albidum* leaf extract. It shows three important chemicals and how much of each one is present; 73.1% of coumaric acid, 10.9% of syringic acid and 8.3% of fucoxanthin were present in 5B.

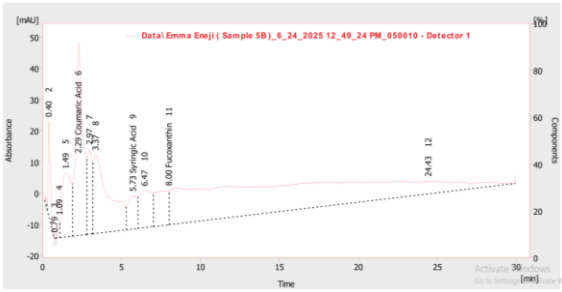

Figure 3: Identified Bioactive Compounds in Fraction 5B (Ethylacetate/Ethanol-100:00 fractionation) by HPLC Analysis

Table 2: Identified Bioactive Compounds in Fraction 5B (Ethylacetate/Ethanol-100:00 fractionation) by HPLC Analysis.

	Reten. Time [min]	Response	Amount [mg]	Amount% [%]	Peak Type	Compound Name
1	0.198	2.235	0.002	0.1		
2	0.398	30.386	0.030	1.8		
3	0.787	0.300	0.000	0.0		
4	1.085	5.843	0.006	0.4		
5	1.490	20.364	0.020	1.2		
6	2.295	1910.800	1.218	73.1	Ordnr	Coumaric Acid
7	2.972	27.303	0.027	1.6		
8	3.370	25.087	0.025	1.5		
9	5.730	436.831	0.182	10.9	Ordnr	Syringic Acid
10	6.468	11.851	0.012	0.7		
11	8.002	646.889	0.139	8.3	Ordnr	Fucoxanthin
12	24.427	4.091	0.004	0.2		
	Total		1.666	100.0		

Identified Bioactive Compounds in Fraction 6A (Ethylacetate/Ethanol-50:50 fractionation) by HPLC Analysis

The HPLC analysis of fraction 6A (ethyl acetate/ethanol, 50:50) in Figure 4 and Table 3 verifies the existence of various phenolic chemicals, a phytosterol, and unexpectedly, an alkaloid. 1.1% of caffeic, morphine (2.1%), salicylic acid (6.3%), syringic acid (8.6%), sitosterol (15.5%) and isorhapotigenin (59.8%) were found in 6A.

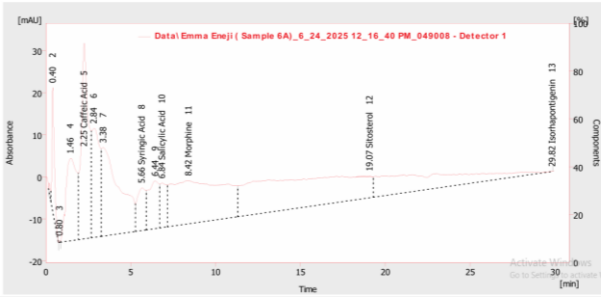

Figure 4: Identified Bioactive Compounds in Fraction 6A (Ethylacetate/Ethanol-50:50 fractionation) by HPLC Analysis

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Table 3: Identified Bioactive Compounds in Fraction 6A (Ethylacetate/Ethanol-50:50 fractionation) by HPLC Analysis.

	Reten. Time [min]	Response	Amount [mg]	Amount% [%]	Peak Type	Compound Name
1	0.195	2.224	0.002	0.1		
2	0.400	29.437	0.029	1.7		
3	0.795	0.450	0.000	0.0		
4	1.455	19.614	0.020	1.2		
5	2.255	1356.767	0.019	1.1	Ordnr	Caffeic Acid
6	2.838	25.944	0.026	1.5		
7	3.378	21.065	0.021	1.2		
8	5.657	352.706	0.147	8.6	Ordnr	Syringic Acid
9	6.437	11.333	0.011	0.7		
10	6.837	277.723	0.107	6.3	Ordnr	Salicylic Acid
11	8.417	2245.977	0.036	2.1	Ordnr	Morphine
12	19.073	3167.978	0.264	15.5	Ordnr	Sitosterol
13	29.825	1523.371	1.016	59.8	Ordnr	Isorhapontigenin
	Total		1.699	100.0		

Identified Bioactive Compounds in Fraction 10B (Methanol/H_2O-50:50 fractionation) by HPLC Analysis

Figure 5 and Table 4 show the HPLC examination of fraction 10B (methanol/water, 50:50). Phenol (39.4%), delphinidins (29.5%), myricetin (24.8%), pinocembrin (2.3%) theaflavins (0.5%) and stigmastanol (0.0%) were present.

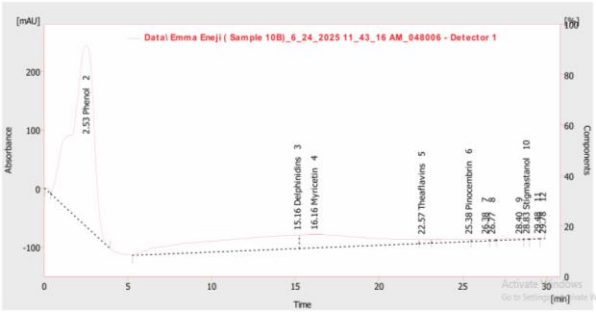

Figure 5: Identified Bioactive Compounds in Fraction 10B (Methanol/H_2O-50:50 fractionation) by HPLC Analysis

Table 4: Identified Bioactive Compounds in Fraction 10B (Methanol/H_2O-50:50 fractionation) by HPLC Analysis

	Reten. Time [min]	Response	Amount [mg]	Amount% [%]	Peak Type	Compound Name
1	0.193	91.919	0.092	0.5		
2	2.527	28016.059	7.004	39.4	Ordnr	Phenol
3	15.158	10495.789	5.248	29.5	Ordnr	Delphinidins
4	16.155	6619.518	4.413	24.8	Ordnr	Myricetin
5	22.573	300.685	0.086	0.5	Ordnr	Theaflavins
6	25.382	729.374	0.417	2.3	Ordnr	Pinocembrin
7	26.377	228.443	0.228	1.3		
8	26.772	67.232	0.067	0.4		
9	28.402	186.459	0.186	1.0		
10	28.830	19.116	0.003	0.0	Ordnr	Stigmastanol
11	29.482	20.067	0.020	0.1		
12	29.782	4.335	0.004	0.0		
	Total		17.769	100.0		

Effects of Active Fractions of Chrysophyllum albidum Leaf Extract on Kidney Function Indices of Bitis arietans Envenomed Wistar Rats

Table 5 compares the nephroprotective effects of different treatments on rats with venom-induced kidney damage. The analysis reveals that fractions F5B, F6A, and F10B from *Chrysophyllum albidum* leaf extract significantly ($p < 0.05$) protect the kidneys in a dose-dependent manner with the highest dose of 400 mg/kg showing comparable or superior results to standard anti-venom (GP3) treatment. Compared to the venom-only group (GP2), SVA and the fractions significantly ($p < 0.05$) lowers the levels of urea and creatinine and helps bring the balance of electrolytes back to normal.

Table 5: Effects of Active Fractions of Chrysophyllum albidum Leaf Extract on Kidney Function Indices of Bitis arietans Envenomed Wistar Rats

Group	UREA (mg/dL)	CREAT (mg/dL)	NA^+ (mEq/L)	K^+ (Mmol/L)	CL^- (mEq/L)
GP 1	90.75±1.30^{d}	4.04±0.03^{d}	358.53±3.83^{e}	9.12±1.10^{d}	195.75±3.55^{d}
GP 2	114.01±2.82^{e}	9.04±0.01^{e}	435.83±16.75^{f}	19.34±0.56^{e}	289.81±0.79^{e}
GP 3	88.10±2.49^{cd}	4.04±0.01^{d}	345.83±1.77^{e}	8.92±0.61^{d}	184.62±1.69^{b}
GP 4	86.03±0.28^{bc}	4.03±0.02^{d}	341.59±10.54^{e}	9.34±0.70^{d}	195.66±2.72^{d}
GP 5	85.50±0.41^{bc}	4.12±0.09^{d}	344.54±1.91^{e}	8.85±0.65^{cd}	192.92±0.34^{d}
GP 6	84.07±1.60^{b}	4.20±0.01^{d}	307.36±8.20^{d}	8.56±0.68^{cd}	191.89±1.02^{c}
GP 7	83.04±0.73^{b}	4.23±0.05^{d}	306.26±1.36^{d}	8.86±0.24^{cd}	191.51±1.57^{c}
GP 8	84.42±0.65^{b}	4.21±0.01^{d}	298.53±6.90^{d}	7.12±0.64^{b}	192.46±1.29^{c}
GP 9	89.67±7.09^{d}	4.21±0.01^{d}	275.52±5.02^{c}	8.05±0.53^{c}	192.64±1.09^{c}
GP 10	68.47±0.34^{a}	3.48±0.02^{c}	221.04±45.83^{a}	5.75±0.44^{a}	116.60±1.39^{a}

GP 11	67.93±0.32[a]	3.04±0.10[b]	204.48±11.26[a]	5.78±0.22[a]	116.89±1.68[a]
GP 12	65.57±0.36[a]	2.39±0.46[a]	231.90±9.76[a]	5.80±0.25[a]	116.89±0.61[a]

N = 5, results are in mean ± standard deviations. Values with different superscript down the column vary significantly at ($p < 0.05$).

GP1: Normal control; GP2: 1.25 mL/kg BV; GP3: BV + 0.8mg/kg SVA (IP); GP4: BV + 100 mg/kg F5B; GP5: BV + 200 mg/kg F5B; GP6: BV + 100 mg/kg F6A; GP7: BV + 200 mg/kg F6A; GP8: BV + 100 mg/kg F10B; GP9; BV + 200 mg/kg F10B. GP10: BV + 400 mg/kg F5B; GP11: BV + 400 mg/kg F6A; GP12: BV + 400 mg/kg F10B. 5B: Ethylacetate/Ethanol-100:00, 6A: Ethylacetate/Ethanol-50:50, 10B: Methanol/H_2O-50:50. UREA: Blood Urea Nitrogen (BUN), CREAT: Creatinine, NA: Sodium ion, K: Potassium ion, Cl: Chloride ion. GP: group, BV: *Bitis arietans* venom, SVA: snake venom antiserum, IP: intra-peritoneal

Identified Bioactive Compounds in Fraction 5B (Ethylacetate/Ethanol-100:00 fractionation) by HPLC Analysis

This research gives clear proof of the exact phytochemicals that are responsible for the biological activity shown in this fraction. Coumaric acid is the main component of this fraction, making up around three-quarters of the total. It is a form of hydroxycinnamic acid and a well-known phenolic chemical that is very good at fighting cancer, inflammation, and oxidation.35 it is likely that it is a major cause of the biological effects shown in fraction 5B, especially the antioxidant activity, because it is so concentrated. Syringic acid is a type of phenolic acid that has been shown to be an antioxidant, an antibacterial, and an anti-inflammatory agent. Its presence in fraction 5B increases the fraction's total antioxidant and anti-inflammatory power even more, working with coumaric acid to provide a synergistic effect.35 Fucoxanthin is a carotenoid that has strong effects against diabetes, obesity, inflammation, and free radicals. Carotenoids are usually found in less polar fractions, so it's noteworthy that this one is here. This shows that the ethyl acetate solvent mixture did a good job of extracting this useful component. The extract's high carotene scavenging activity was probably due to its high antioxidant capacity. The HPLC analysis verifies that the therapeutic effects of Fraction 5B are predominantly influenced by a mixture of phenolic acids (coumaric acid and syringic acid) and a carotenoid (fucoxanthin). Because it has a lot of coumaric acid, it is expected to be the main anti-inflammatory and antioxidant agent in this fraction.36 These findings align closely with the outcomes of the rat envenomation investigation, wherein Fraction 5B exhibited protective effects on red and white blood cells. The HPLC analytical data offers more accurate and conclusive evidence of the phytochemicals involved, in contrast to the GC-MS data, which also detected fatty acids. This indicates that both categories of chemicals may be influencing the fraction's bioactivity.

5.10 Identified Bioactive Compounds in Fraction 6A (Ethylacetate/Ethanol-50:50 fractionation) by HPLC Analysis

The HPLC analysis of fraction 6A (ethyl acetate/ethanol, 50:50) verifies the existence of various phenolic chemicals, a phytosterol, and unexpectedly, an alkaloid. The combination of these chemicals forms a strong chemical basis for the biological activity seen in this fraction. Isorhapotigenin (59.8%) is the compound that was found in the largest quantity in fraction 6A. Isorhapotigenin is a stilbenoid, which is a type of phenolic chemical that has strong antioxidant, anti-inflammatory, and anticancer effects. Its high concentration shows that it plays a big role in the protective effects seen in the rat trial, especially the robust anti-inflammatory response.37 Sitosterol (15.5%) a well-known phytosterol (plant-derived sterol) that has anti-inflammatory, antioxidant and cholesterol-lowering effects, it makes the fraction more anti-inflammatory and good for the heart.38 Syringic acid (8.6%) a phenolic acid that has been shown to have antioxidant, antibacterial and anti-inflammatory effects. It is a subsidiary part, yet it nevertheless has a big effect on the fraction's bioactivity. Salicylic acid (6.3%) a plant hormone and active metabolite of aspirin that is renowned for its anti-inflammatory, antibacterial, and pain-relieving properties.39 The fact that it is there strengthens the anti-inflammatory effect of this component. Finding morphine, an alkaloid with strong pain-relieving effects, in C. albidum is a very important and surprising discovery. Even though

there isn't much of it, its presence could help with pain relief after being bitten by a snake. This necessitates additional inquiry to verify and measure its existence, since it may significantly affect the plant's therapeutic attributes and safety assessment. Caffeic acid a hydroxycinnamic acid fights inflammation, bacteria, and free radicals and contributes to the therapeutic potential of the fraction even in little amounts.40 Fraction 6A is very different from Fraction 5B, which was mostly made up of fatty acids and a carotenoid. Fraction 6A has a lot of phenolic chemicals, including Isorhapotigenin. It also has a phytosterol (sitosterol) and an alkaloid (morphine). This shows that the solvent gradient works well to separate different types of molecules. The various compositions probably account for the different biological activities seen in the rat study.40 The high levels of isorhapotigenin, salicylic acid, and morphine in F6A probably make it especially good in reducing inflammation and pain. The HPLC analysis of Fraction 6A elucidates a definitive phytochemical foundation for the documented biological activity, especially the pronounced anti-inflammatory and analgesic effects.39 This fraction is a prospective treatment for snakebite envenomation since it has a lot of isorhapotigenin and other phenolic chemicals, sitosterol, and morphine. The presence of morphine necessitates further investigation to elucidate its role and consequences for the safety and pharmacology of the extract.

5.11 Identified Bioactive Compounds in Fraction 10B (Methanol/H_2O-50:50 fractionation) by HPLC Analysis

HPLC examination of fraction 10B (methanol/water, 50:50), the most polar fraction, shows that it has a lot of different phenolic chemicals, which makes sense because the solvent is polar. The discovered chemicals, such as flavonoids, anthocyanins, and phenol, form a solid phytochemical basis for the antioxidant and anti-inflammatory effects seen in this fraction.41 Phenol (39.4%) is a key phenolic component that helps the fraction's antioxidant activity a lot. Phenolic chemicals are recognized for their ability to get rid of free radicals, which is in line with the significant antioxidant activity seen in the carotene and (H_2O_2) scavenging assays.52 Delphinidins (29.5%) are a kind of anthocyanidin, which is a form of flavonoid that is renowned for being a strong antioxidant, anti-inflammatory, and heart-protective. Their substantial concentration in this fraction shows that they constitute a major part of its biological activity, which includes the protective properties seen in the rat study.42 Myricetin (24.8%) is a flavonol that has strong qualities that fight cancer, inflammation, and oxidative stress. Its presence increases the antioxidant capability of the fraction and may help protect cells from harm induced by snake venom. Pinocembrin (2.3%) a flavonoid that is known to be anti-inflammatory, antioxidant and antibacterial, even though it's there in tiny proportions, it nevertheless adds to the fraction's overall therapeutic potential.42 Theaflavins (0.5%) are polyphenols that protect cells from damage and are usually present in black tea. Their presence, even in modest numbers, is significant and enhances the fraction's antioxidant capacity.43 Stigmastanol (0.0%) a plant sterol that probably came from earlier fractions because it has a low polarity. the fact that it is present in such little amounts shows that it is not a big part of this fraction's bioactivity.44 The phytochemical profile of fraction 10B is very different from that of fractions 5B and 6A. Fraction 10B has a lot of highly polar chemicals, like phenolic acids, anthocyanidins (delphinidins), and flavonols (myricetin). The previous fractions had more fatty acids, esters, and stilbenoids. This shows how well the solvent gradient works to separate different types of molecules dependent on how polar they are. The high levels of delphinidins and myricetin in Fraction 10B give it a strong chemical basis for the antioxidant and anti-inflammatory effects.45

Effects of Active Fractions of Chrysophyllum albidum Leaf Extract on Kidney Function Indices of Bitis arietans Envenomed Wistar Rats

Plant extracts have shown significant potential in mitigating the nephrotoxic (kidney-damaging) effects of Bitis arietans (puff adder) venom in rat models, primarily by reducing oxidative stress, inhibiting venom enzymes, and restoring normal kidney function markers. Bitis arietans venom causes oxidative stress, increasing malondialdehyde (MDA) and depleting antioxidants like glutathione and catalase in kidneys.[46] The venom also causes significant renal damage, including increased serum creatinine, blood urea nitrogen (BUN) and

electrolytes levels, tubular necrosis, and acute renal failure.[47] Plant extracts often work by inhibiting specific components of the venom, such as phospholipase (PLA2) and proteases, which are responsible for damage to tissues.[46]

In the present study the C. albidum fractions F5B, F6A, and F10B have demonstrated the ability to prevent structural damage, such as renal tubular dilation and focal atrophy of glomeruli, which are common in B. arietans envenomation. This observation was evident in the significant reductions of the levels of creatinine, urea and the electrolytes (sodium, potassium and chloride ions) in the kidney following the administration of the selected C. albidun fractions indicating their protective effects. This finding is in tandem with earlier report of Chinyere et al.[48] where aqueous root extract of Annona senegalensis was able to inhibit Bitis arietans venom protease and phospholipase A2 activities to reverse the altered renal function.

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