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Pharmacological Screening of Aqueous Extract of Parkia biglobosa Leaves: In Vitro Inhibitory Activity Against Key Snake Venom Enzymes

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Abstract

Natural products constitute an invaluable source of bioactive compounds with promising therapeutic potential for the treatment and management of various diseases and disorders. Among such challenges, snake envenomation represents a major health concern due to the complex mixture of biologically active proteins and enzymes in snake venom, which contribute to its potent toxic, inflammatory, and cytotoxic effects. This study investigated the phytochemical composition and enzyme-inhibitory potential of aqueous leaf extract of Parkia biglobosa against key venom enzymes [phospholipase A_2 (PLA2), hyaluronidase, acetylcholinesterase (AChE), and L-amino acid oxidase (LAAO)] of Naja nigricollis (cobra). Phytochemical analyses were performed using standard procedures, while enzyme inhibition assays were conducted in vitro employing spectrophotometric techniques. The phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, phenols, terpenoids, steroids, and saponins. The extract exhibited concentration-dependent inhibitory activity against PLA_2, with an IC_{50} of 62.83 µg/ml. It also demonstrated significant inhibition of hyaluronidase (IC_{50} = 101.3 µg/ml), an enzyme responsible for venom dissemination through degradation of extracellular matrix components. The extract inhibited AChE (IC_{50} = 67.93 µg/ml), suggesting possible neuroprotective effects against venom-induced neurotoxicity. Furthermore, moderate inhibition was observed against LAAO (IC_{50} = 115.3 µg/ml), indicating potential attenuation of oxidative stress and cytotoxicity. Conclusively, the collective inhibition of these enzymes highlights the extract's potential as a natural, multi-target antivenom agent. The results not only substantiate its traditional application in snakebite management but also open avenues for developing phytochemical-based adjunct therapies against envenomation.

Keywords: Aqueous Extract, Parkia Biglobosa Leaves, In Vitro, Enzyme Inhibition, Snake Venom and Antivenom Activity

1. Introduction

Envenomation from snakebite is still a major public health issue, particularly in rural areas of Asia, Latin America, and sub-Saharan Africa as a result of insufficient access to proper treatment [1]. The World Health Organization (WHO) considers it a neglected tropical disease with high rates of morbidity and mortality. Antivenoms are considered the main therapy option [2]. However, they are costly and their use is associated with allergic reactions [2]. Furthermore, they can occasionally be ineffective at counteracting the local tissue damage brought on by venom enzymes such metalloproteinases, hyaluronidases, and phospholipases A_2 (PLA_2) [3].

Traditional medicine has long relied on plant-based remedies for the treatment of snakebites, with various indigenous communities in Africa using parts of Parkia biglobosa (African locust bean tree) for this purpose [4,5]. While some pharmacological properties of Parkia biglobosa have been investigated, scientific evidence supporting its inhibitory effects on specific snake venom enzymes remains sparse and inconclusive. Snake venoms contain a complex mix of enzymes such as phospholipases A2 (PLA2), metalloproteinases, and hyaluronidases, which are responsible for local tissue damage, hemorrhage, and systemic toxicity [6]. A need therefore exists for the leaves of Parkia biglobosa to be scientifically evaluated for its ability to inhibit some of these enzymes that make up snake venom. This would validate the traditional use of the plant and aid in the development of effective adjunct or alternative therapies for snakebite management.

Parkia biglobosa, also known as the African locust bean tree, monkey cutlass tree, or fern tree, is a perennial legume tree that is widespread over the Sahelo-Sudanian region [7]. It is a multipurpose tree that is native to West and Central African countries such as Senegal, The Gambia, Guinea Bissau, Guinea, Sierra Leone, Mali, Burkina Faso, Ghana, Togo, Benin, Niger, Nigeria, Cameroon, Chad, Central African Republic, The Democratic Republic of Congo, Sudan, and Uganda. The tree currently exists within a wide range of natural communities, including tropical rainforests and arid zones, but is most abundant where cultivation is semi-permanent region [7]. It is also present as an introduced species in Australia, South-East Asia, North America, and tropical South America [8]. Several other benefits of P. biglobosa tree have been reported, these include the vital economic role it plays in recycling nutrients from the soil, it is a good source of timber, and a valuable food source [9]. The seeds are used as a condiment for multiple uses; the husks and pods are good feed for livestock; the floury pulp can be made into a refreshing drink, which contains macronutrients, vitamins A and C, and the bark is also used with lemon for wounds and ulcers [10]. Apart from these benefits, its wide adaptability, drought resistance and multifunctional usage make it a sustainable source of its by-products. The different parts of the plant, including the leaves, have been reported to possess activity against inflammation, pain and infections [11,12].

2. Materials and Methods

2.1. Materials

2.1.1. Chemicals and Reagents

The chemicals and reagents used were of analytical grade and include: 1% Thiobarturic acid (BDH, England), Acetone (Sigma Aldrich, Germany), Aluminium chloride (BDH, England), Ammonia (BDH, England), Anticoagulant such as EDTA, heparin (Randox, USA), Ascorbic acid (May and Baker, England), Drangendorff's reagent (May and Baker, England), Lead acetate solution (Merck Darmstadt, Germany), Mayer's reagent (BDH, England), Picric acid (Merck Darmstadt, Germany), Potassium hydroxide (Sigma Aldrich, Germany), Sodium dodecyl sulphate (BDH, England), Sodium hydroxide (May and Beakers, England).

2.1.2. Equipment and Instruments

The following equipment and instruments were used: UV-vis spectrophotometer (Shimadzu UV-160, Japan), Filter paper (Whatman, Maidstone, England), Hot air oven (N30C, Gen lab, England), Vortex mixer (Vortex genie-2, scientific industries Inc., Bohemia, N.Y., USA), Centrifuges (Beckman CS-15, Germany, KA-1000, China), Flasks, Beakers, Test tubes, Measuring cylinders, Rotary evaporator, UNICO UV-2102.

2.2. Methodology

2.2.1. Venom Sample

Lyophilized N. nigricollis (Cobra) venom was procured from the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria, and was preserved at 4°C. Before use, the venom was reconstituted in phosphate buffer, pH 7.2, centrifuged at 2000 rpm for 10 mins and the supernatant was used for anti-venom studies.

2.2.2. Collection and Preparation of Plant Material

The leaves of Parkia biglibosawere harvested from its natural habitat at the bank of Ogede River, Abejukolo Area of Omala, Kogi State, Nigeria. The leaves were identified at the Department of Plant Science and Biotechnology, Prince Abubakar Audu University, Anyigba and a voucher specimen was deposited for future reference.

2.2.3. Extraction of Parkia biglibosa leaves

Fresh leaves of Parkia biglibosa were rinsed with distilled water to remove dirt, shade-dried for 7 days and blended using an electric blender. A quantity (1.5 kg) of the powder was cold-macerated in 7.5 L of absolute ethanol. After 72 h, the suspension was filtered using a mesh, and then Whatman No 1 filter paper. This procedure was repeated twice and all the filtrates were concentrated in a rotary evaporator set at 45 °C to obtain the crude ethanol extract of Parkia biglibosa leaves.

2.2.4. Phytochemical Analysis of the Aqueous Extract of Parkia biglibosa Leaves

The phytochemical analysis of the extract was carried out according to the methods of Harborne and Trease and Evans to identify and quantify its active constituents [13,14].

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2.2.5. Phospholipase A_2 (PLA_2) Inhibitory Assay

Phospholipase A_2assay was determined according to the acidimetric method of Tan and Tan with slight modification. Lecithin suspension was prepared by mixing proportionately 1% lecithin, 18 mM calcium chloride and 8.1 mM sodium deoxycholate [15]. The pH of the suspension was adjusted to 8.0 with 0.02 M sodium hydroxide and stirred for 10 minutes to ensure homogenous mixing. Next, 0.1 ml venom solution was added to 15 ml of lecithin suspension to initiate the hydrolysis. The initial decrease in pH was measured by a pH meter. Inhibition study was carried out by pre-incubating the venom with different concentrations (100-1000 µg/ml) of the plant extract for 45 min at 37°C. The percentage inhibitory effect of the extract on PLA_2 activity was subsequently calculated as follows:

$$\% \text{ inhibition} = \frac{\text{Enzyme activity of test sample}}{\text{Enzyme activity of venom}} \times 100$$

where, Enzyme Activity (mmole/Fatty acid/Min) = $\frac{\text{mmoles of fatty acid released}}{\text{Time taken in minutes}}$

The IC_{50} value was defined as the concentration of extract required to inhibit 50% of the PLA2 activity under the assay conditions and determined by regression analysis of the PLA2 inhibition (%) versus the log of the extract concentration.

2.2.6. Hyaluronidase Inhibitory Assay

Hyaluronidase assay of crude venom was determined turbidometrically by the method of Pukrittayakamee et al., [16]. The assay mixture contained buffer of Tris – HCl (pH 8.0), 50 mg hyaluronic acid (0.5 mg/ml in buffer) and venom (1mg/ml) in the same buffer in a final volume of 1.0 ml. The mixture was incubated for 15 min at 37°C and the reaction was stopped by the addition of 2 mL 2.5% (w/v) cetyltrimethyl ammonium bromide in 2% (w/v) NaOH. The absorbance was read at 400 nm (within 10 min) against a blank containing 1mL of the same buffer and 2 ml 2.5% (w/v) cetyltrimethyl ammonium bromide in 2% (w/v) NaOH. Inhibition study was carried out by pre-incubating venom with different concentrations (100-1000 µg/ml) of the plant extract for 45 min at 37°C. Percentage inhibition was calculated as follows;

$$\% \text{ inhibition} = \frac{\text{A Control - AExtract}}{\text{A Control}} \times 100$$

The IC_{50} value was defined as the concentration of inhibitor required to inhibit 50% of hyaluronidase activity under the assay conditions and determined by regression analysis of the hyaluronidase inhibition (%) versus the log of the extract concentration.

2.2.7. Acetylcholinesterase Inhibitory Assay

Acetylcholinesterase enzyme activity was assayed according to the method described by Ellman et al., [17]. 0.1 ml of 0.01 M DTNB was added to 2.6 ml of 0.1 M phosphate buffer (pH 8.0), 0.04 ml of the venom was added to the above mixture followed by incubation for 5 min, after incubation, 0.04 ml of the substrate (0.075 M acetylcholine iodide) was added to the reaction mixture. Absorbance readings were taken at 420 nm continuously for 3 min at 30 s intervals. For the inhibition studies, venom was pre-incubated with different concentrations (100- 1000 µg/ml) of the plant extract for 45 minutes at 37°C. Percentage inhibition was calculated as follows;

$$\% \text{ inhibition} = \frac{\text{A Control - AExtract}}{\text{A Control}} \times 100$$

The IC_{50} value was defined as the concentration of inhibitor required to inhibit 50% of the Acetylcholinesterase activity under the assay conditions and determined by regression analysis of the Acetylcholinesterase inhibition (%) versus the log of the extract concentration.

2.2.8. L Amino Acid Oxidase Inhibitory Assay

The L-amino acid oxidase activity was carried out according to Li et al., [18]. Reaction mixture consisted of 1.0 mL of L-leucine, 2.0 mL of Tris-HCl buffer (pH 8.0), 0.25 mL of 0.1% dianisidine hydrochloride, 0.15 mL of 0.1% horseradish peroxidase and 0.04 mL of 0.5% crude venom solution. It was allowed to stand for ten minutes at room temperature and then the absorbance was measured at 415 nm. One unit (U) was defined as the amount of enzyme that catalyzed the formation of 1 µmol H_2O_2 per minute. For the inhibition studies, venom was preincubated with different concentrations (100- 1000 µg/ml) for 30 minutes at 37°C. Percentage inhibition was calculated as follows;

$$\% \text{ inhibition} = \frac{\text{A Control - AExtract}}{\text{A Control}} \times 100$$

The IC_{50} value was defined as the concentration of inhibitor required to inhibit 50% of the L-amino acid oxidase activity under the assay conditions and determined by regression analysis of the L-amino acid oxidase inhibition (%) versus the log of the inhibitor concentration.

2.2.9. Statistical Analysis

Data were expressed as Mean ± standard deviation. Statistical differences between means were determined by one-way analysis of variance (ANOVA) followed by Duncan's post–hoc test for multiple comparison tests and values were considered significant at $p \leq 0.05$. The IC_{50} values were determined through a nonlinear regression analysis of the log concentration-inhibition curve. All analyses were carried out using GraphPad version 10.0.

3. Results

3.1. Qualitative Phytochemical Analysis of the Aqueous Extract of Parkia biglibosa Leaves

The aqueous extract of Parkia biglibosa was analysed qualitatively for its phytochemical composition. Results showed the presence of all the phytochemicals tested for in varying proportions (Table 1).

Phytochemicals	Extract
Alkaloids	++
Phenols	+
Flavonoid	++
Saponin	++
Glycosides	+
Tannins	+++
Terpenoids	+
Steroids	++

Key: + slightly Present, ++moderately present, +++ highly Present

Table 1: Qualitative Phytochemical Composition of the Aqueous Extract of Parkia Biglibosa Leaves

3.2. Phospholipase A_2 (PLA_2) Inhibitory Activity of Aqueous Extract of Parkia biglibosa Leaves

A concentration-dependent inhibitory effect on PLA_2 was observed with the extract and the standard inhibitor-Varespladib (Table 2). The Median Inhibitory Concentration (IC_{50}) of the extract for PLA_2 was estimated to be 62.83 µg/ml while that of the standard was 48.36 µg/ ml (Figure 1).

Concentration (µg/ml)	Extract % Inhibition	Varespladib % Inhibition
10	5.83±0.08	9.42±1.45
20	9.71±0.32	15.26±1.80
30	18.64±1.26	27.45±1.85
40	22.30±1.45	38.33±1.99
50	31.92±1.21	47.26±2.01
60	40.15±2.11	53.84±2.42
70	56.08±2.90	70.70±2.01
80	63.77±2.31	81.94±2.36
90	75.29±2.57	84.26±2.51
100	90.34±2.71	93.02±2.81

n=3

Table 2: Phospholipase A_2 (PLA_2) Inhibitory Activity of Aqueous Extract of Parkia Biglibosa Leaves

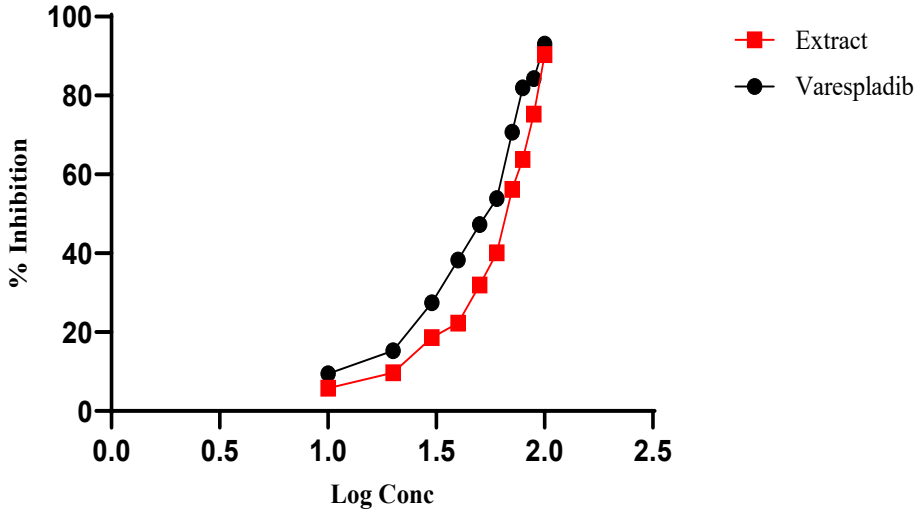

Figure 1: Log Concentration of Aqueous Extract of Parkia biglibosa Leaves and Varespladib vs Percentage Inhibition Curve

3.3. Hyaluronidase Inhibitory Activity of Aqueous Extract of Parkia biglibosa Leaves

For Hyaluronidase, a concentration-dependent inhibitory effect was also observed with both the extract and sodium aurothiomalate (Table 3). The Median Inhibitory Concentration (IC50) of the extract for hyaluronidase was estimated to be 101.3 µg/ ml while that of the standard was 151.9 µg/ ml (Figure 2).

Concentration (µg/ml)	Extract % Inhibition	Na-aurothiomalate % Inhibition
10	4.50±0.43	2.01±0.03
20	6.78±0.68	3.45±0.07
30	10.95±1.34	7.01±0.99
40	15.43±1.47	10.28±1.23
50	19.81±1.94	12.91±1.85
60	26.77±1.74	18.31±1.84
70	32.40±1.74	20.45±1.99
80	38.96±1.53	26.03±2.02
90	45.12±1.91	30.22±2.50
100	50.80±2.04	33.26±3.26

n=3

Table 3: Hyaluronidase Inhibitory Activity of Aqueous Extract of Parkia Biglibosa Leaves

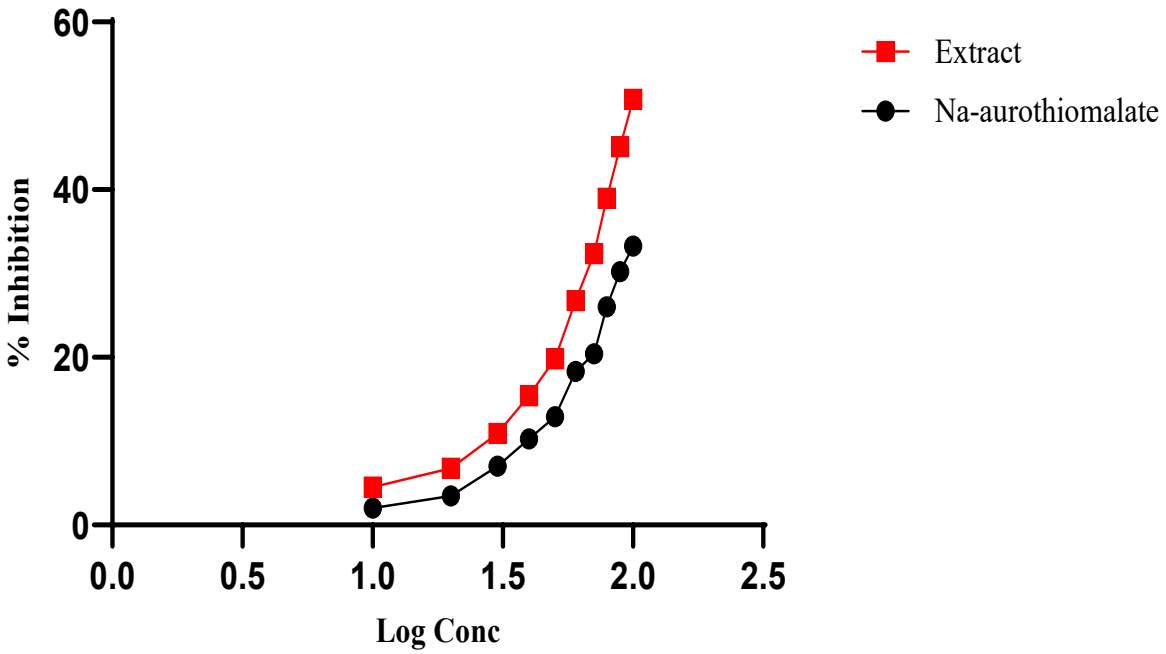

Figure 2: Log Concentration of Aqueous Extract of Parkia biglibosa Leaves and Sodium Aurothiomalate vs Percentage Inhibition Curve

3.4. Acetylcholinesterase (AChE) Inhibitory Activity of Aqueous Extract of Parkia Biglibosa Leaves

In this study, a concentration-dependent inhibitory effect on AChE was observed with the extract and the standard-edrophonium (Table 4). The Median Inhibitory Concentration (IC50) of the extract for the enzyme was estimated to be 67.93 µg/ ml while that of edrophonium was 100.9 µg/ ml (Figure 3).

Concentration (µg/ml)	Extract % Inhibition	Edrophonium % Inhibition
10	6.42±0.82	2.46±0.34
20	11.08±0.99	7.51±0.43
30	17.95±1.65	11.06±1.57
40	23.13±1.83	19.21±1.01
50	29.84±1.32	22.05±1.63
60	37.66±1.83	26.31±1.46
70	46.21±1.34	35.29±1.53
80	58.39±1.90	36.93±1.83
90	69.07±1.98	45.24±1.83
100	80.10±2.61	52.58±1.26

n=3

Table 4: Acetylcholinesterase (AChE) Inhibitory Activity of Aqueous Extract of Parkia Biglibosa Leaves

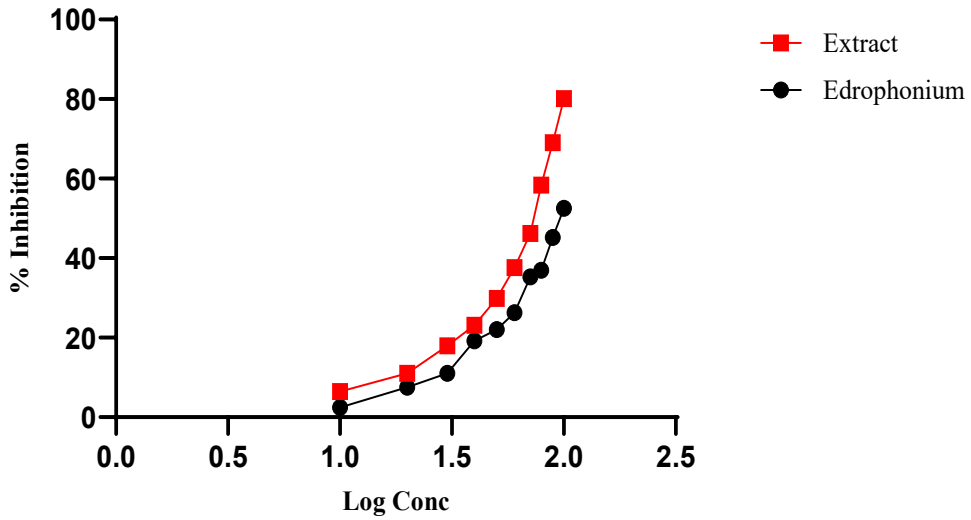

Figure 3: Log Concentration of Aqueous Extract of Parkia biglibosa Leaves and Edrophonium vs Percentage Inhibition Curve

3.5. L-Amino Oxidase Inhibitory Activity of Aqueous Extract of Parkia Biglibosa Leaves

Results of this study showed a concentration-dependent inhibitory effect on L-Amino oxidase was observed with the extract and aristolochic acid (Table 5). The Median Inhibitory Concentration (IC_{50}) of the extract for the enzyme was estimated to be 115.3 µg/ mL while that of aristolochic acid was 99.38 µg/ mL (Figure 4).

Concentration (µg/ml)	Extract % Inhibition	Aristolochic acid % Inhibition
10	4.78±0.56	4.21±0.26
20	7.56±0.89	8.91±0.52
30	10.91±1.45	11.26±1.31
40	15.47±1.28	17.45±1.41
50	19.03±1.45	25.26±1.29
60	25.68±2.12	30.09±1.51
70	30.12±2.74	32.33±1.33
80	35.89±2.81	40.06±2.18
90	40.76±2.37	48.11±2.91
100	45.23±2.28	51.25±2.23

n=3

Table 5: L-Amino Oxidase Inhibitory Activity of Aqueous Extract of Parkia Biglibosa Leaves

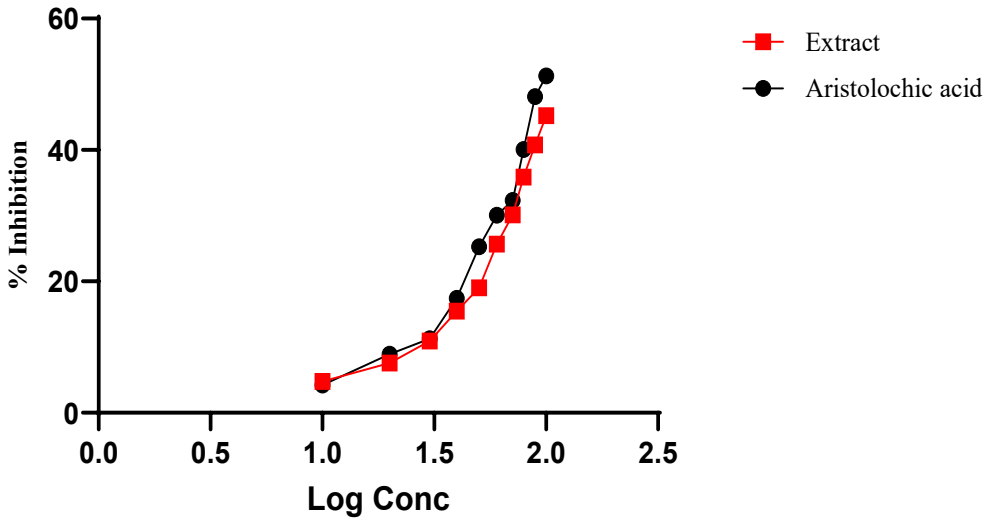

Figure 4: Log Concentration of Aqueous Extract of Parkia biglibosa Leaves and Aristolochic Acid vs Percentage Inhibition Curve

4. Discussion

This study investigated the inhibitory effects of the aqueous leaf extract of Parkia biglobosa against key enzymatic components of Naja nigricollis venom, namely phospholipase A_2 (PLA_2), hyaluronidase, acetylcholinesterase (AChE), and L-amino acid oxidase (LAAO). These enzymes are responsible for many of the local and systemic manifestations of envenomation, including tissue necrosis, inflammation, neurotoxicity, and oxidative stress [19,20]. The findings demonstrated a concentration-dependent inhibition of all the studied enzymes, suggesting that the extract possesses bioactive constituents capable of interfering with multiple venom-induced biochemical pathways. PLA_2 enzymes are among the most toxic constituents of elapid venoms, catalyzing the hydrolysis of phospholipids in cell membranes to release lysophospholipids and free fatty acids, particularly arachidonic acid, which serve as precursors for inflammatory mediators [21]. The extract exhibited a pronounced and dose-dependent inhibition of PLA_2 activity, with an IC_{50} of 62.83 µg/mL compared to 48.36 µg/mL for the standard inhibitor Varespladib. Although slightly less potent than the reference compound, the extract's inhibitory strength is significant considering its crude nature. The observed effect could be attributed to secondary metabolites such as flavonoids, tannins, terpenoids, and phenolic compounds detected during phytochemical screening. These molecules are known to form complexes with proteins and metal ions, thereby disrupting catalytic sites or interfering with calcium binding, which is crucial for PLA_2 enzymatic activity [22]. Similar inhibitory trends have been reported for other medicinal plants. For instance, extracts of Andrographis paniculata and Annona senegalensis have been shown to suppress PLA_2 activity through antioxidant and metal-chelating mechanisms [23]. The anti-PLA_2 action of P. biglobosa may therefore contribute to its folkloric use in treating snakebites by limiting local inflammation, edema, and myonecrosis typically associated with elapid envenomation.

Hyaluronidase, often termed the "spreading factor," degrades hyaluronic acid in the extracellular matrix, facilitating rapid diffusion of venom toxins through tissues [23,24]. The aqueous extract of P. biglobosa inhibited hyaluronidase activity in a concentration-dependent manner, with an IC_{50} of 101.3 µg/mL relative to Na-aurothiomalate (IC_{50} = 83.6 µg/mL). Though the extract's potency was lower than that of the standard inhibitor, the effect remains biologically meaningful. Inhibition of this enzyme can delay toxin dissemination, allowing natural defense mechanisms or therapeutic interventions more time to act [25]. Polyphenolic compounds, notably flavonoids and condensed tannins, are known inhibitors of hyaluronidase [26]. They exert their effects through hydrogen bonding with the enzyme's active site residues or by scavenging reactive oxygen species that stabilize the enzyme-substrate complex [27]. The presence of such metabolites in P. biglobosa likely explains its observed activity. Previous studies on Mangifera indica, Carica papaya, and Moringa oleifera extracts have similarly linked hyaluronidase inhibition to phenolic content [26]. Therefore, the moderate inhibition observed in this study underscores the contribution of plant-derived antioxidants in countering venom-induced tissue degradation and hemorrhage.

Neurotoxicity is a major hallmark of cobra venom, primarily arising from the interference of postsynaptic neurotoxins with acetylcholine transmission and secondary enzymatic modulation by AChE [28,29]. The extract of P. biglobosa produced dose-dependent inhibition of AChE activity, with an IC_{50} value of 67.93 µg/mL, compared to 56.72 µg/mL for edrophonium. Although AChE inhibition is often associated with increased acetylcholine levels, which could aggravate toxicity in certain contexts, partial inhibition in venom systems can reduce excessive neurotransmitter degradation and mitigate neuromuscular blockade [28]. Flavonoids, alkaloids, and phenolic acids have been documented to inhibit AChE through reversible interactions at the

enzyme's catalytic triad [29]. Compounds such as quercetin and catechin, previously identified in P. biglobosa, might contribute to this activity by stabilizing the enzyme's active gorge via π–π interactions or hydrogen bonding. Comparable observations have been made for Ginkgo biloba and Ocimum gratissimum, both rich in similar phytochemical classes [30]. Thus, the moderate inhibition seen here may underlie neuroprotective effects of the extract against venom-induced synaptic dysfunctions.

LAAO catalyzes the oxidative deamination of L-amino acids, producing hydrogen peroxide, ammonia, and corresponding α-keto acids. Its activity contributes significantly to venom-induced cytotoxicity, apoptosis, and platelet aggregation [31,32]. The aqueous extract of P. biglobosa inhibited LAAO in a concentration-dependent manner with an IC_{50} of 115.3 µg/mL, compared to 98.2 µg/mL for the standard inhibitor aristolochic acid. Although the inhibitory potency was moderate, it indicates the extract's ability to attenuate oxidative and pro-apoptotic events elicited by the enzyme. Phenolic antioxidants are known to neutralize hydrogen peroxide and interact with the flavin adenine dinucleotide (FAD) cofactor of LAAO, leading to catalytic suppression [26]. Therefore, the presence of phenolic and flavonoid constituents in the extract may explain the observed inhibitory trend. This finding aligns with reports by Li et al. [18], who showed that natural phenolic compounds could effectively modulate LAAO activity from Ophiophagus hannah venom. Collectively, inhibition of LAAO by P. biglobosa may protect tissues from oxidative stress and secondary inflammatory responses following envenomation.

The multi-target enzyme inhibition demonstrated by P. biglobosa underscores its broad-spectrum anti-venom potential. Unlike antivenoms that neutralize systemic toxins through immunoglobulin binding, plant-based inhibitors act at biochemical levels by preventing enzymatic catalysis or scavenging venom-induced radicals [33]. The combined suppression of PLA_2, hyaluronidase, AChE, and LAAO suggests synergistic mechanisms involving direct enzyme binding, metal-ion chelation, and antioxidant effects. The observed IC_{50} values, though generally higher than those of purified reference inhibitors, are notable considering the crude nature of the extract. Fractionation and isolation of active constituents could yield more potent compounds with defined pharmacokinetic properties. Furthermore, the concentration-dependent inhibition across all assays reflects genuine pharmacological activity rather than nonspecific interactions. Comparatively, extracts from plants such as Erythrina variegata, Vitex doniana, and Azadirachta indica have exhibited similar antivenom potentials through inhibition of PLA_2 and hyaluronidase [34]. P. biglobosa, widely used in West African ethnomedicine for wound healing and anti-inflammatory purposes, now gains additional pharmacological justification through these findings [35].

The presence of alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, and phenols in the extract aligns with established phytochemical profiles of P. biglobosa [36,37]. Many of these metabolites exhibit potent antioxidant and enzyme-modulatory properties [26]. Flavonoids and phenolic acids, for instance, possess planar aromatic structures that enable π-stacking interactions with enzyme residues, while tannins form stable hydrogen-bonded complexes that can denature toxic proteins [26, 38]. The antivenom efficacy of the extract may therefore be synergistically driven by multiple phytochemical groups acting at different stages of envenomation- neutralizing toxins, stabilizing cell membranes, and reducing oxidative damage. The findings lend scientific credence to the traditional use of P. biglobosa leaves in snakebite management among rural communities in Nigeria and across West Africa [39]. By inhibiting critical venom enzymes, the extract may mitigate both local and systemic pathologies associated with envenomation [40]. Its multi-mechanistic mode of action is particularly valuable in regions where poly-enzymatic venoms and limited access to commercial antivenoms contribute to high mortality rates.

Moreover, the plant's rich polyphenolic composition suggests additional pharmacological benefits such as anti-inflammatory, analgesic, and wound-healing effects, which could complement its antivenom activity. Future studies should focus on bioassay-guided fractionation, identification of lead compounds, and elucidation of molecular interactions through docking and kinetic analyses. In vivo validation using animal models will further confirm the therapeutic relevance observed in vitro.

5. Conclusion

Conclusively, the aqueous leaf extract of Parkia biglobosa exhibits significant inhibitory activity against four major Naja nigricollis venom enzymes- PLA_2, hyaluronidase, AChE, and LAAO in a concentration-dependent manner. The collective inhibition of these enzymes highlights the extract's potential as a natural, multi-target antivenom agent. The results not only substantiate its traditional application in snakebite management but also open avenues for developing phytochemical-based adjunct therapies against envenomation.

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