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Effect of Methanol Leaf Extract of *Costus afer* on Rats Injected With *Naja nigricollis* Venom

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Abstract
Snake envenoming is a neglected tropical diseases mostly affecting rural farmers and their families, leading to morbidity and sometimes mortality. Over the years plants and their products have been useful in the treatment of snakebites. This study investigated the effect of methanol leaf extracts of Costus afer on rats injected with Naja nigricollis snake venom. Phytochemical analysis, plant and venom toxicities (LD_{50}) were determined using standard methods while enzyme assays were carried out using spectrophotometric techniques. The phytochemicals were found to be high with tannins been the highest. The acute toxicity studies (LD_{50}) of the plant extracts were found to be more than 3000mg/kg while that of the venom was 0.28mg/kg body weight. Serum Alanine Aminotransferase (ALT) and Serum Aspartate Aminotransferase (AST) activities in rats injected with 0.2ml reconstituted venom and treated with 50mg/kg, 100mg/kg and 200mg/kg doses of leaf and stem extracts were determined. ALT activity was 69.16±6.47, 61.66±6.54 and 38.16±12.28u/l respectively compared to the control group (70.60±8.96u/l). AST activity was 65.16±6.70, 42.33±3.20 and 32.00±4.66u/l respectively compared to the control group (70.60±8.96u/l). The enzymatic antioxidants assayed showed superoxide dismutase activity of 8.33±0.56, 17.83±5.46 and 7.60±1.98µmol/min when compared to the control group (6.60±3.04µmol/min), catalase activity were 20.88±8.59, 22.31±4.96 and 37.19±9.37µmol/min when compared to the control group (20.57±5.84µmol/min) and glutathione peroxidase activity were 148.04±27.69, 281.99±53.22 and 668.37±103.47µmol/min when compared to the control group (157.93±43.28µmol/min). The activities of these enzymes were significant at $p<0.05$. These findings showed that C. afer contain good nutraceutical properties that support its traditional use against snakebite, hence, their use and consumption by snakebite victims should be encouraged.

Keywords: *Costus afer, Naja nigricollis, Snakebite, Envenomation, Antivenom,*

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I. Introduction

Snakebite envenoming is one of the neglected tropical diseases which require immediate attention from local and international health authorities since it is causing critical public health issues and common medical concern in most countries. According to World Health Organization 2019 report, globally it is estimated that more than 5.4 million people are bitten by snakes each year with up to 2.7 million envenoming, out of which 81,000 to 138,000 snakebite patients die yearly while others may recover with or without amputation and other permanent disabilities (WHO, 2019). In Asia up to 2 million people are envenomed by snakes each year, while in Africa there are an estimated 435,000 to 580,000 snakebites annually (WHO, 2019) that need treatment with about 30,000 deaths. These figures are, however, underestimated because of patients' treatment-seeking behavior that delays access to health centers and increase the risk of death before reaching it. Such a situation results from the high proportion of rural population and the living conditions in sub-Sahara Africa. The population at risk is made up of active people (15-50 years old), mostly farmers (males, females and their children) during agricultural and pastoral activities in poor rural communities in low- and middle-income countries (Chippaux *et al.,* 2019).

Snakes are elongated, legless, carnivorous reptiles of the suborder Serpentes (Reeder *et al.,* 2015). They feed on small animals, snails, fishes, frogs, toads, lizards, chickens, mice, rats and even other snakes. They use their venoms as offensive weapons in incapacitating and immobilizing their prey (the primary function), as defensive tools against their predators (the secondary function) and to aid in digestion (Burke and Dennis, 2009). Snakes unleash neurotoxic, cytotoxic, cardiotoxic and/or haemotoxic effects in order to subdue their prey (Suresh and Balasubramanian, 2016). Bites by venomous snakes can cause acute medical emergencies involving severe paralysis that may prevent breathing, cause bleeding disorders that can lead to fatal haemorrhage, cause irreversible kidney failure and severe local tissue destruction that can cause permanent disability and limb amputation (WHO, 2019).

Snake venom is highly modified saliva containing zootoxins that facilitate the immobilization and digestion of prey, and defends against a threat. They are water-soluble and acidic substances secreted by snake oral glands. They can be injected subcutaneously or intravenously through the fangs into the victim's hands, feet, arms, legs (Guimaraes *et al.,* 2014) or any other exposed body parts. The quantity, lethality, and composition of venoms vary with the age and species of the snake, time of the year, geographic location as well as the envenoming snake's diet. Poisonous snake species like *Echis carinatus, Naja nigricollis, Daboia russelli, Bungarus caeruleus, Ophiophagus hannah* among others, account for the majority of the bites and mortality (Gomes *et al.,* 2010).

Some snake venoms are complex mixture of toxic proteins such as cardiotoxins, neurotoxins, acetyl cholinesterase nitrate, metalloproteinases, serine proteinases, phosphomonoesterase, phosphodiesterase, nucleotidases and hyaluronidases (Kang *et al.,* 2011) which are injected to immobilize the victim (Janardhan *et al.,* 2015). The toxins cause haemotoxicity damage to blood vessels resulting in spontaneous systemic and muscle paralysis, myolysis, arrhythmias, cardiac and even renal failure (Omara *et al.,* 2020).

The plant kingdom offers alternative option for the management of snakebites Akah *et al.,* 2019). Over the years, there have been many attempts for the production of snake venom antidotes from plants sources. In most rural areas, some snake bite cases were successfully treated with folk medicines, especially medicinal plants by traditional healers. Several studies have been performed to investigate the action of plant extracts against snake venoms (Adzu *et al.,* 2005; Nunez *et al.,* 2005). Some medicinal plants have been reported to possess antivenom properties; these plants include *Sansevieria liberica* (Akah *et al.,* 2019), *Crinum jagus* (Zadani *et al.,* 2018), *Asystasia gangetica* (Enenebeaku *et al.,* 2018), *Aloysia citriodora* (Caceres *et al.,* 2017), *Albizia lebbeek* (Amog *et al.,* 2016), *Carissa spinarum* (Janardhan *et al.,* 2015), among others. These plants often exhibit a wide range of biological and pharmacological activities. It is generally assumed that the active constituents contributing to these protective effects of plants are the phytochemical constituents, vitamins and minerals (Anyasor *et al.,* 2010). Phytochemicals exhibit a wide range of biological effects resulting in their protective or disease preventive properties (Nimmy *et al.,* 2016). Their possible valuable actions include antioxidant actions, hormonal action, stimulation of enzymes, interference with DNA replication, anti-microbial and physical action (Akpan *et al.,* 2012).

According to Ukpabi *et al.,* (2012), *Costus afer* contained phytochemical constituents which can act synergistically to reduce the toxicity and hepatic oxidative stress induced by Carbon tetra chloride. These suggested that extract of *C. afer* could be exploited as sources of free radical scavengers and bioactive metabolites for nutritional, medicinal and commercial purposes.

Costus afer is a useful medicinal plant that belongs to family *Costaceae*. It is commonly called Ginger lily, bush sugar cane or monkey sugar cane (Nyananyo, 2006; Anaga *et al.,* 2004). It is also known as "Kakizawa" in Hausa, "Okwete" or "Okpoto" in Igboland, "tete-egun" in Yoruba and "Mbritem" in Efik, all in Nigeria. *C. afer* is a monocot and a relatively tall, herbaceous, unbranched tropical plant with creeping rhizome. It is commonly found in moist or shady forest of West and Tropical Africa (Omokhua, 2011). The leaves are arranged spirally, simple and entire. It is a common plant in Africa especially in Nigeria, Ghana, Togo, and Cameroun. *C. afer* could be propagated easily by vegetative means, involving the use offsets at the base of mother plants.

II. Materials And Methods

Plant Sample

Fresh leaf of *Costus afer* Ker (family: *Zingiberaceae*) were collected from bank of the stream at Nnamdi Azikiwe University, Awka, Anambra State. It was authenticated by a Taxonomist from the Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State Nigeria.

Plant Sample Preparation and Extraction

The leaf of *Costus afer* was picked and air-dried at room temperature. The crude methanol leaf extract was obtained by a modification of the method described by Gopi *et al.,* (2015). The dried leaf sample was

pulverized and five hundred grams (500g) was macerated in 1litre of methanol (at the ratio of 1:2 w/v) for 24h with occasional manual shaking. The extraction was repeated for two (2) additional times while changing the solvent in every 24 hours. The mixture was first sieved with muslin cloth and then filtered with Whatman No 1 (125mm) filter paper. The filtrate was concentrated using rotary evaporator under reduced pressure, it was weighed and stored in refrigerator at temperature of 4^{o}C for further use. The concentrated extract was later reconstituted in 10mM phosphate buffer saline (PBS; pH 7.4), centrifuged and the supernatant was used for the studies.

Snake Venom Sample

The venom sample was obtained by using the milking method (Markfarlane, 1967) from locally caught cobra (*Naja nigricollis* Wetch), from the Herpetarium Unit, Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria. The pooled venom was lyophilized, stored in an air-tight container and kept for the intended research use only.

Experimental Animals

Wistar albino rats of both sexes weighing between 180 to 260g were purchased from Department of Biochemistry, University of Nigeria Nsukka, Nigeria. They were kept in well ventilated cages (minding their sex) with sawdust as bedding under conditions of 12:12 hours light and dark cycle and fed with commercial animal feed and water *ad libitum*. The bedding of the cages (sawdust) was changed daily. The animals were allowed to get acclimatized to the experimental environment for two weeks at the Animal House of Applied Biochemistry Department, Nnamdi Azikiwe University, Awka, Nigeria. All animal experiments were conducted in strict compliance with NIH guide for care and use of laboratory animals (National Institute of Health (NIH) (2011) Pub No: 85-23).

Reagents/Chemicals and Assay Kits

All reagents used were of analytical grade and purchased from Sigma-Aldrich Company United Kingdom, through Bristol Scientific Nigeria limited, Lagos State, Nigeria. Aspartate aminotranferase kit (Randox laboratories, UK) and Alanine aminotranferase kit (Randox laboratories, UK) were used for the quantitative *in vitro* assay of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) enzyme activities respectively.

Phytochemical Analysis

Cardiac glycosides were determined according method of Osagie (1998). Alkaloids and Saponin were determined by the method of Harborne (1995). Tannin was determined by titration method described by Pearson (1974). Oxalates were determined by titration method described by Osagie (1998). Phytate was determined according to the method of Lucas and Markaka (1975) while Flavonoids were determined by the method of Boham and Kocipai (1994).

Animal Studies

Acute Toxicity Studies (LD_{50})

The acute toxicity study (Lethal Dose (LD_{50})) of the leaf extract of *C. afer* was determined by the modified method described by Lorke (1983). The acute toxicity of the *N. nigricollis* snake venom was determined according to the method of Meier and Theakston (1986) adopted by Gopi *et al.*, (2015).

Inhibition of Lethality of the Venom

A total of 24 rats grouped into four groups of six rats each weighing 180–260g were used. The rats in the four groups of six rats were separated into three male and three female each. Group 1 (the control group) was administer with saline (10 ml of saline per kg of rat intraperitoneally). Groups 2–4 were administered with graded doses of the extract (50, 100 and 200 mg/kg body weight, i.p., respectively). Reconstituted venom was prepared at the ratio of 2mg of venom per ml of physiological saline. At 24 hrs after administration of the extracts into the animals, 0.2 ml of the reconstituted venom was injected into the animals through the tail vein (Theakston and Reid, 1983). The animals were observed for signs of toxicity and mortality within 6 hrs and recorded.

Suppression of Damage to Enzyme Function

At 6 hrs after the venom injection, the animals were anaesthetized with chloroform. Immediately blood samples of the animals were collected using a syringe. The samples were transferred carefully into a centrifuge tube and allowed to clot. The clotted blood samples were centrifuged at 3500 rpm for 10 minutes to obtain the serum (Hsu *et al.*, 1998), which were kept at temperature of 4^{o}C until analysis.

The serum glutamate-oxalate-transaminase (sGOT), (Aspartate aminotransaminase (AST)) and serum-pyruvate transaminase (sGPT), (Alanine aminotransaminase (ALT)) values were estimated according to method of Reitman and Frankel (1957) using Randox kits (Randox Lab Ltd., UK) and measured spectrophotometrically.

Antioxidant Enzyme Assay

The superoxide dismutase (SOD) activity was determined by the ability of the serum SOD to inhibit the auto-oxidation of epinephrine at the absorbance of 480nm as described by Sun and Zigma (1978). The catalase activity was determined using the method described by Goyal *et al.*, (1986). The principle was to measure the catalase degradation of hydrogen peroxide in a mixture of the serum while the glutathione peroxidase activity was determined by the ability of glutathione to react with the serum and hydrogen peroxide thus, reducing serum hydroperoxides to their corresponding alcohols and free hydrogen peroxide to water (Tappel, 1978).

Data Analysis

Data obtained from the study were subjected to statistical analysis using the Statistical Package for Social Science (SPSS) software for windows version 17.0 (SPSS Inc., Chicago, Illinois, USA). The data were represented as mean ± standard error of mean and the statistical significance between the treatment and the control analyzed using one-way ANOVA. The limit of significance was set at $p<0.05$.

III. Results

Phytochemical Constituents

The phytochemical composition of the leaf extract of *C.afer*. The tannins, saponins and alkaloids were higher than other parameters tested (Table 1).

Table 1: Quantitative Phytochemical Composition of Leaf Extract of *C. afer*

Phytochemical	***C. afer* (leaf) (mg/100g)**
Alkaloids	340.13±0.35
Saponins	600.42±4.53
Cardiac glycosides	193.34±1.29
Tannins	653.35±0.47
Phytates	7.15±0.01
Oxalates	103.70±0.45

Acute Toxicity Test (LD_{50}) of the Plant Extracts

The acute toxicity study showed that the methanol leaf extract of *C.afer* was toxic at very high concentration. The oral administration of low doses of 10, 100 and 1000mg/kg body weight of the extract showed no visible sign of toxicity in the experimental animals within 24 hours post administration of the extract. The doses were then increased to oral administered doses of 1000, 1600, 2900 and 5000mg/kg body weight. The animals administered with 2900 and 5000mg/kg showed signs of weakness while at 5000mg/kg there were signs of panting and some death within 24 hours post administration of the extracts (Table 2). The LD_{50} of the aqueous leaf extract of *C. afer* was more than 3000 mg/kg and was estimated to be 3807.89mg/kg body weight.

Table 2: LD_{50} Estimation of Methanol Leaf Extracts of *C. afer*

Group	**No of Death**
Phase I	
Group 1 (10mg/kg)	0/3
Group 2 (100mg/kg)	0/3
Group 3 (1000mg/kg)	0/3
Phase II	
Group 1 (1000mg/kg)	0/3
Group 2 (1600mg/kg)	0/3
Group 3 (2900mg/kg)	0/3
Group 4 (5000mg/kg)	2/3

Acute Toxicity Test (LD_{50}) of *N. nigricollis* Venom

The acute toxicity study of *N. nigricollis* venom showed that the animal administered with different concentrations of reconstituted venom sample showed different signs of toxicities (Table 3). Death of mice were recorded at different times on mice administered with venom concentration of 0.4mg/kg b.w. and above. The LD_{50} was calculated to be 0.28mg/kg body weight.

Liver Enzymes Assay

There were reductions in the activity of alanine aminotransferase and Aspartate aminotransferase in the treated animals injected with 0.2ml reconstituted venom of *N. nigricollis*. Highest reduction was observed in the animals treated with 200mg of methanol leaf extract of *C. afer* (Table 4). The result in Mean ± Standard Error of Mean was significant at $p<0.05$.

Table 3: LD50 Estimation of N. nigricollis Venom

Group	Conc of Venom (mg/kg b.w)	No of Death	Toxic symptoms
1	Saline (0.2ml)	0/4	No toxic symptoms
2	0.2	0/4	No respiratory failure but signs of weakness and not active.
3	0.4	1/4	Difficulty in breathing, coughly sound in the throat, weakness, limb paralysis before death of one
4	0.6	2/4	Difficulty in breathing, coughly sound in the throat, weakness, limb paralysis eye closure, limb paralysis before death of two.
5	0.8	4/4	Difficulty in breathing, coughly sound in the throat, weakness, Limb paralysis before death
6	1.0	4/4	Difficulty in breathing, coughly sound in the throat, weakness, Limb paralysis before death

No death was recorded on mice administered intraperitoneally with normal saline and venom concentration of 0.2mg/kg b.w.

Table 4: Enzyme Activity in Treated Animals

Treatment	Liver Enzymes Activity	
	ALT (u/l)	AST (u/l)
Control (Normal Saline) + 0.2ml Venom	79.40±4.70	70.60±8.96
50mg of extract per kg bw + 0.2ml venom	69.16±6.47	65.16±6.70
100mg of extract per kg bw + 0.2ml venom	61.66±6.54	42.33±3.20
200mg of extract per kg bw + 0.2ml venom	38.16±12.28	32.00±4.66

Antioxidant Enzyme Assay

There was an increase in the activity of superoxide dismutase enzymes in the treatment animals injected with 0.2ml reconstituted venom of *N. nigricollis* when compared with the control group. The highest activity was observed in the animals treated with 100mg of methanol leaf extract of *C. afer.* There was increase in the activity of Catalase enzymes in the treatment animals injected with 0.2ml reconstituted venom of *N. nigricollis*. Highest activity was observed in the animals treated with 200mg of methanol leaf extract of *C. afer.* There was an increase in the activity of Glutathione peroxidase enzymes in the treated animals injected with 0.2ml reconstituted venom of *N. nigricollis*. Highest activity was observed in the animals treated with 200mg of methanol stem extract of *C. afer.* Animals treated with 50mg of methanol leaf extract of *C. afer* showed a decrease in the Glutathione peroxidase activity below the control group animals. The result in Mean ± Standard Error of Mean was significant at $p<0.05$.

Treatment	Antioxidant Enzyme Activities ($\mu molmin^{-1}$)		
	SOD	CAT	GPx
Control (Normal Saline) + 0.2ml Venom	6.60±3.04	20.57±5.84	157.93±43.28
50mg of extract per kg bw + 0.2ml venom	8.33±0.56	20.88±8.59	148.04±27.69
100mg of extract per kg bw + 0.2ml venom	17.83±5.46	22.31±4.96	281.99±53.22
200mg of extract per kg bw + 0.2ml venom	7.60±1.98	37.19±9.37	668.37±103.47

IV. Discussion

The study of the interaction between plants and humans is invaluable in discovering new herbal medicines and plant-derived drugs. Plant and its constituents have been implicated in neutralizing the effects of snake venoms because of their ability to control infection, stop pain, improve symptoms, correct imbalance, adjust immune system and boost energy for better health and quality of life (Das, 2009).

There have been search for alternatives therapy for snake bite due to reported adverse reactions associated with antisnake serum (Morais and Massaldi, 2009). Serious attention has been shifted to herbal remedies mainly due to the acclaimed successes by the traditional healers in the management of snakebites with herbs. In this modern time, many locals still rely on the use of medicinal plants by traditional healers for the treatment of snakebites (Akah *et al.,* 2019). A good number of plants have been reported to show good potentials for the treatment of snakebites (Gomes *et al.,* 2010), and many procedures are been used to investigate plants with antisnake bite activity (Harvey, 2003; Adzu *et al.,* 2005; Ode and Asuzu 2006; da Silva *et al.,* 2012; Felix-Silva *et al.,* 2014). Though, there are conventional anti-venom drugs due to their high cost, not readily available, their adverse effects and other disadvantages, a systematic investigation of plant-based remedies for snake bite is justified.

This study was carried out to establish the scientific basis for the traditional application of *Costus afer* Ker Gawl in the treatment of victims of snake bite since the traditional use of plants to treat snakebites is still quite common in our society. The results of this study have shown that the methanol leaf extract of *C. afer* may be adopted for treating snakebites. Phytochemical qualitative screening of leaf methanol extracts of *C. afer* revealed the presence of some of the plants' secondary metabolites that have been implicated in antisnake venom activities in varied concentrations. According to Momoh *et al.,* (2011), the major phytochemical constituents of *C. afer* leaf were flavonoids, phenols, alkaloids, glycosides and tannins. The results of the phytochemical screen were in line with the findings of Arhoghro *et al.,* (2014).

Adzu *et al.,* (2005) related the activity of *Annona senegalensis* plant extract in the treatment of snake bite to the plant's tannin contents. He further stated that phytochemical constituents of plants used for snakebites tend to have similar (isoflavone skeleton, acidic nature and deoxygenated) functions, just as Abubakar *et al.,* (2000) also implicated the *in vitro* snake venom detoxifying action of the leaf extract of *Guiera senegalensis* to the high composition of tannins. Ijioma *et al.,* (2014) attributed the antinociceptive property of leaf extracts of *C. afer* to the phytochemical constituents of the plant, thus the plant may relief the sense of pain (like snake bite pain) without loss of consciousness. The folkloric use of *C. afer* in the treatment of sore throat, hemorrhage and wound healing might be due to presence of tannins (Okwu and Okwu, 2004).

According to Chatterjee *et al.,* (2006) the root extracts of *Sarsaparilla hemidesmas indicus* neutralized the phospholipase A2 (PLA2) activity; as such,inhibited the lethality, edema and haemorrhagenation effects of *Doboia kauthia* venom owing to the plant's lupeol acetate (an alkaloid) content. Some phytochemical constituents such as terpenoids, flavonoids, polyphenol, xanthene, and quinonoids has the ability to inhibit snake venom phospholipase A2 (PLA2) activities of both viper and cobra venom since they possess protein binding and enzyme inhibiting properties (Selvanayagam *et al.,* 1996).

The liver is a major producer for most of serum proteins. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) are largely used in the assessment of liver damage by drugs or other hepatotoxins (Moke *et al.,* 2015).

The result of Alanine Aminotransferase and Aspartate aminotransferase activities showed a dose dependent reduction ($p<0.05$) in the treated animals injected with 0.2ml reconstituted venom of *N. nigricollis*. The plant extract could interact and inhibit most hepatocellular injuries that could be unleashed by the venom

enzymes. Thus, the dose dependent ALT and AST activity reduction could be as a result of the inhibitory action of the extract on the venom enzymes since according to Moke *et al.,* (2015) aqueous extracts of *C. afer* were less likely to cause hepatocellular damage but could act synergistically to alleviate possible damage to the liver. Also Ezejiofor *et al.,* (2014) reported that *C. afer* have the potential as a renoprotective agent against nephrotoxic medications through its antioxidant, anti-inflammatory, and anti-apoptotic actions. According to Bhattacharjee and Bhattacharyya (2013), ALT and AST contents of serum of animals treated with different doses of aqueous extract of the root of *Aristolochia indica* in venom intoxicated rats showed a dose dependent decrease in their activities. In line with the present study, the findings of Asmari *et al.,* (2015) demonstrated serum biomarkers for acute hepatotoxicity of *Echis pyramidum* snake venom in rats, showed that snake venoms have the potency to increase the activity of ALT level above the control animals especially when left untreated with any available antivenin agents.

Conversely, findings from the work of Adzu *et al.*, (2005), indicated a dose dependent increase in the activities of serum ALT and AST in which the activities of the two enzymes were enhanced in *N. nigricollis* envenomated rats treated with *Annona senegalensis*. Adzu and colleagues were of the opinion that the liver may be involved directly in the action of neutralizing the effects of venom enzymes in the treated rats, but the actions could perhaps be on a cumulative basis and other possible symptomatic and physiological antagonism by the plant extract. Importantly, findings of this present study and some other reported articles could establish the fact that the activities of liver enzymes ALT and AST in envenomed and plant extract-treated rats may be related to the phytochemical constituents of the plant, the nature and potency of the snake venom.

Antioxidant enzymes play good role in scavenging free radicals released into the blood due to some oxidative stress activities. Superoxide dismutase, catalase and glutathione peroxidase are among the major antioxidant enzymes that differ from each other in structure, tissue distribution and cofactor requirement (Shahjahan *et al*., 2005).

Superoxide dismutase (SOD) has an antitoxic effect against the superoxide anion (O^{2-}) and accelerates dismutation of superoxide radicals like hydrogen peroxide (H_2O_2) which is subsequently removed by haem-containing catalase that catalyses the conversion of hydrogen peroxide (H_2O_2) to water and molecular oxygen, thereby protecting cells from the toxic effects of hydrogen peroxide. It has been proposed that glutathione peroxidase is responsible for the detoxification of H_2O_2 in low concentration, whereas catalase come into play when glutathione peroxidase is saturated with substrate (Okpuzor *et al.*, 2009).

The results showed that action of methanol leaf extract of *C. afer* on animals injected with *N. nigricollis* venom could increase SOD, catalase and glutathione peroxidase activities. Akaninwor *et al*. (2014) pointed out that the methanol extract of *Costus afer* could serve as electron donor, terminating the free radical chains in treatment animals since it showed potent hydroxyl radical scavenging activity. They also reported that the hydroxyl radical scavenging ability of the extract was effective in order of concentration dependent manner.

Some compounds from plants used for inflammation were known to inhibit enzymes from snake and scorpion venoms (Hutt and Houghton, 1998) by complexing with the venom constituents, thereby rendering them unable to act on receptors (Hutt and Houghton, 1998). Although the mechanism by which the extract achieves its effect remains unclear, a plausible speculation at this point is likelihood of the existence of an active component, which might be responsible for the observed effects. The various classes of compounds identified in the phytochemical study of the extract could be studied closely. Tannins are also known to unspecifically inactivate proteins (Abubakar *et al*., 2000). The activity of the extract may, therefore, be linked to its tannin contents.

These results as well as other reported findings, especially the anti-nocicepive and anti-inflammatory effects of *C. afer* (Adzu *et al*., 2003a) indicate the basis for the success claimed by traditional healers in the use of *C. afer* as one of the antidotes to treat victims of snakebite.

V. Conclusion

Costus afer has shown to be one of the most important plants to humans especially to snakebite victims. The methanol leaf extract of the plant have shown to reduce the mortality and significantly inhibited the onset, and severity of neurotoxic signs induced by *Naja nigricollis* venom. Since the traditional healers sometimes administered the leaf by making it into paste and place them over incisions made at the point of bites, and giving the juice to the snakebite patient it is possible that the toxic venom enzymes might be neutralized through the processes since some plant constituents have the ability to bind to venom proteins. Thus, *C. afer* may help to provide the basic health-care services to the greater part of the rural population in many parts of Nigeria and other countries of the world.

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