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In Vitro Assessment of Anti-Venom Effect of *Polyalthia Korinti* Extracts against Cobra Venom

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Abstract: ***The antivenom potential of Polyalthia korinti leaves and bark extracts were tested against Cobra snake venom using Phospholipase activity and Caseinolytic inhibition methods. P. korinti extracts inhibited PLA2 dependent hemolysis of sheep RBCs induced by Cobra venom in a dose-dependent manner. The concentration of plant extract at which the hemolytic halo (11 mm) was reduced to half its original diameter (5.5 mm) may be used to assess venom phospholipase inhibition. The hemolytic halo of 5.37±0.12 mm and 5.43±0.06 was observed to inhibit 600µg/ml of venom by 3 mg/ml of methanol extracts of leaves and bark respectively. And leaves water extract also reduced the hemolytic halo (11 mm) to half of its diameter (5.39±0.07) at 5 mg/ml concentration. Caseinolytic inhibition assay indicated that all the extracts of P. korinti leaves and bark showed inhibitory activity against the proteolytic activity of the Cobra venom. Out of all the extracts, methanol and water extracts completely inhibited the proteolytic activity of venom at different concentrations. The methanol extract of leaves and bark completely inhibited the proteolytic activity of venom at the ratio of 1: 50 and 1: 60 (w/w) venom: plant extract respectively. At a ratio of 1: 70 (w/w) venom: water extract of leaves and bark also completely inhibited the proteolytic activity of venom. Out of all the extracts, Methanol extracts of leaves and bark of P. korinti showed the most potent phospholipase inhibition and proteolytic activities.***

Key words: Cobra snake venom, Phospholipase activity, Caseinolytic inhibition**,** proteolytic activity

1.Introduction

Snakebite is a serious medical and legal problem in rural India, especially among forest workers and farmers. There are approximately 216 snake species in India, 52 of which are venomous [1]. Poisonous snakes found in India include the cobra (*Naja naja*), krait (*Bungarus caeruleus*), russell's viper (*Daboia russelli*), and saw scaled viper (*Echis Carinatus*) [2]. Snake venom poisoning can only be treated with anti-venom immunotherapy. Antivenom serum can cause anaphylactic shock, pyrogen reaction, and serum sickness [3]. Antivenom is expensive and in short supply in most countries. Antiserum processing in animals is time-consuming, costly, and necessitates optimal storage conditions. Many attempts have been made over the years to develop snake venom antagonists, especially from plant sources. Plant extracts have long been used by traditional healers, particularly in tropical areas where snakebite therapy is common [4]. Several attempts have been made in modern science to research these plants in order to determine their efficacy [5], [6]. The use of medicinal plants has a long history in India. A variety of Indian medicinal plants can be used to treat snakebites [7].

2.Materials and Methods

Collection and Identification of Plant material

The leaves and bark material of *Polyalthia korinti* (Dunal.) Thaw. was collected from the Seshachalam Hills. Seshachalam hill ranges of Eastern Ghats lie between 13°38' to 13°55' N latitudes and 79° 07 to 79° 24' E longitudes and spread over two districts viz., Chittoor and Kadapa of Southern Andhra Pradesh.

The Plant was authenticated by taxonomic expert Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University (SVU), Tirupati, and Andhra Pradesh. Where the voucher specimens were deposited (Herbarium voucher No 566).

The required quantity of plant parts was collected and separated from undesirable materials. Washed with running water followed by distilled water to remove the contaminants. Chopping process was carried out and they were allowed to dry under shade [8]. The dried material was ground into a coarse powder with the help of a suitable pulverizer. The powder was stored in an airtight container and kept in a cool, dark and dry place.

Extraction Technique

The dried powder of the leaves and bark was extracted sequentially [9] using a soxhlet apparatus [10] with various solvents based on their polarity, such as hexane, chloroform, methanol, and water. Using a rotary evaporator, the extracts were concentrated and solvent-free under reduced pressure. To measure the extractive yield, the dried crude concentrated extracts were weighed and stored in an airtight bottle until used for analysis.

In vitro assessment of anti-venom effect of plant extracts

This study looked into the effects of phospholipase activity and caseinolytic inhibition methods on neutralizing *P. korinti*i leaves and bark extracts against Cobra venom.

Snake venom collection

The Irula Snake-Catchers Industrial Co-operative Society Limited (ISCICS), Vadanemelli village, Kanchipuram district, Tamilnadu state, provided lyophilized Cobra snake venom for this study. The crude venom was stored in airtight containers at 4ºC until used.

Phospholipase activity

Gutierrez et al., 1988 [11], used an indirect hemolytic assay on agarose-erythrocyte-egg yolk gel plate to assess the function of phospholipase-A2. Increasing doses of Cobra Venom were applied to 3mm wells in agarose gels (0.8 % in PBS, pH 8.1) containing 1.2 % sheep erythrocytes, 1.2 % egg yolk as a source of lecithin, and 10mM $CaCl_2$.

The dimensions of the hemolytic halos were determined after an overnight incubation at 37°C. Control wells contained 15µl of saline. The minimum indirect hemolytic dose is a venom dosage that produces a hemolytic halo of 11mm diameter (MIHD). By combining a constant amount of venom (mg/ml) with varying quantities of plant extracts (mg/ml) and incubating for 30 minutes at 37°C, the efficacy of antivenom (plant extracts) in neutralizing phospholipase activity was measured. The mixtures were then divided into aliquots of 10µl each and placed in wells in agarose-egg yolk sheep erythrocyte gels. Venom without plant extracts is used as a control sample. For 20 hours at 37°C, plates were incubated. As compared to the effect caused by venom alone, neutralization was described as a concentration of plant extract that reduced the hemolytic halo by 50%.

Caseinolytic Inhibition

As previously mentioned, protease activity was measured in 1 % agarose plates with 1 % casein as a substrate [12]. For one hour at 37 ^{o}C, 50 µg of *N. naja* venom was pre-incubated with various concentrations of plant extract. In casein-agarose plates with 3 mm diameter wells (0.1 M Tris-HCl buffer, pH 8.0), the samples were incubated overnight at 37°C. The plates were then stained with Coomassie Brilliant Blue-R 250 for 1 hour before being de stained with a methanol: water: acetic acid solution (50: 40: 10). The clearance zone was measured in the presence of plant extract to determine the percentage of protease inhibition. As a control, the venom clearance zone values obtained without the plant extract were used.

3.Results and Discussion

The antivenom potential of *P. korinti* leaves and bark extracts were tested against Cobra snake venom using Phospholipase activity and Caseinolytic inhibition methods.

Phospholipase activity

Venom phospholipase activity was calculated by measuring the hemolytic halo of 11mm diameter in agarose gel, and that venom concentration is referred to as the minimum indirect hemolytic dose (MIHD) [13]. In table: 1, the concentration of venom was calculated and 600µg/ml of venom produced 11.15±0.06 mm halo in agarose gel. This demonstrates that Cobra venoms have the enzymes (PLA2) that can lyse sheep RBCs (Figure 1).

Table: 1 Phospholipase A2 Activity-Minimum Indirect Heamolytic Dose of Cobra Venom (MIHD)

Conc. of Venom (µg/ml)	1st plate	2nd plate	3rd plate	Me
200	8.1	8.18	8.24	8.1
400	9.6	9.72	9.84	9.7
600	11.08	11.2	11.16	11.
800	12.28	12.34	12.55	12.
1000	13.38	13.48	13.62	13.

*Mean ±SD (n=3) is used to describe each value.

P. korinti extracts have been able to dose-dependently inhibit PLA2 dependent hemolysis of sheep RBCs caused by Cobra venom (Figure 3 and 5). The inhibition of venom phospholipase activity can be determined from the plant extract concentration at which the hemolytic halo (11 mm) has decreased to half of its diameter (5.5 mm). The hemolytic halo of 5.37±0.12 mm and 5.43±0.06 was observed to inhibit 600µg/ml of venom by 3 mg/ml of Methanol extracts of leaves and bark respectively. And leaves water extract also reduced the hemolytic halo (11 mm) to half of its diameter (5.39±0.07) at 5 mg concentration. Out of all the extracts, Methanol extracts of leaves and bark of *P. korinti* showed the most potent phospholipase activity (Figure 2 to 5).

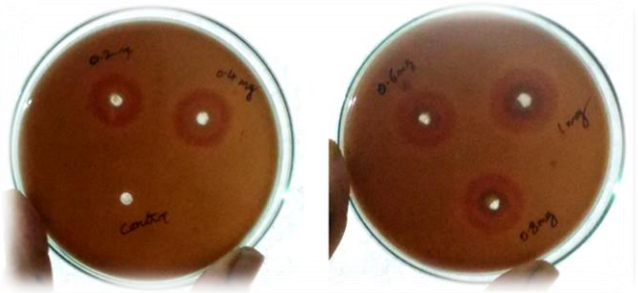

Figure: 1 Phospholipase A2 Activity-Minimum Indirect Heamolytic Dose of Cobra Venom (MIHD)

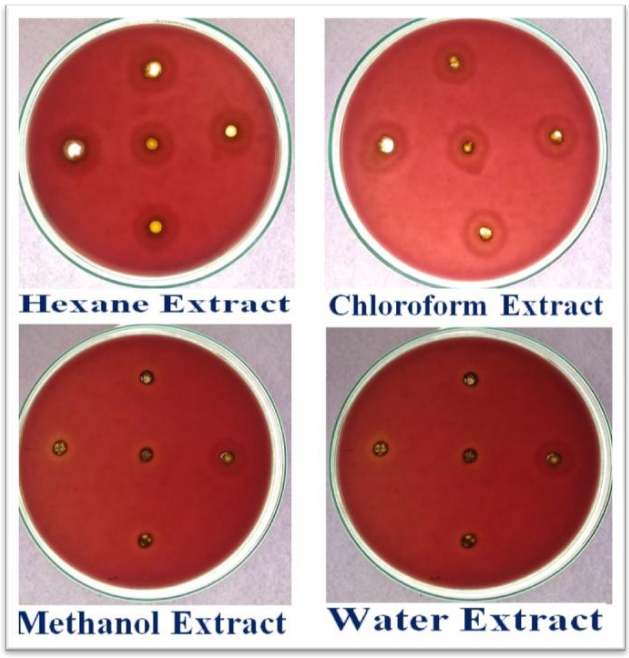

Figure 2: Phospholipase A2 Inhibition Activity of *P. korinti* leaves Extracts

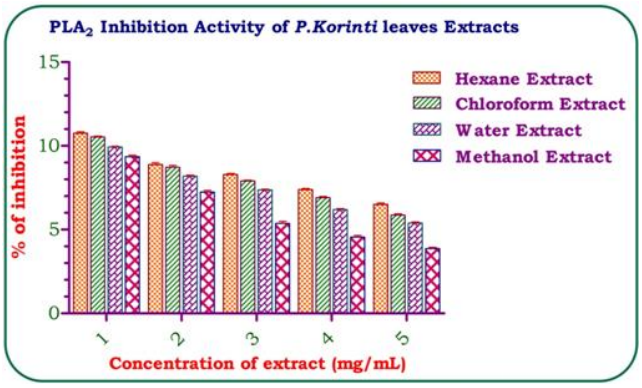

Figure 3: Phospholipase A2 Inhibition Activity of *P. korinti* leaves Extracts

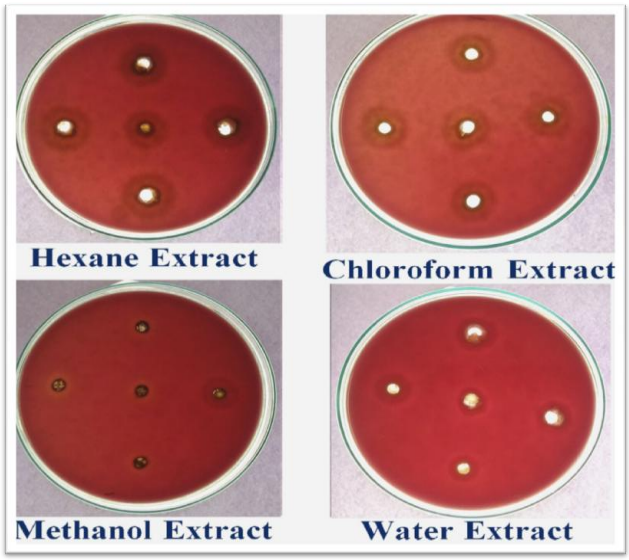

Figure 4: Phospholipase A2 Inhibition Activity of *P. korinti* bark Extracts

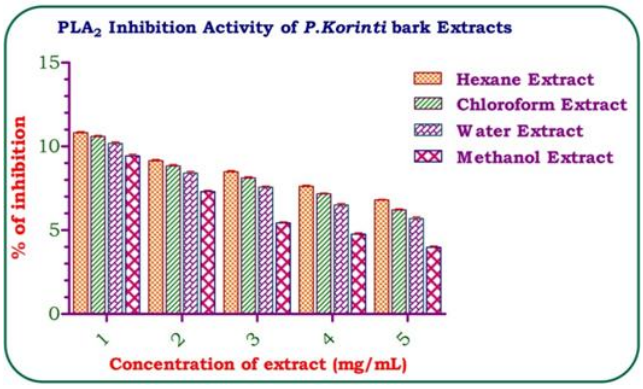

Figure 5: Phospholipase A2 Inhibition Activity of *P. korinti* bark Extracts

The aim of this study is to see whether *P. korinti* has any phospholipase inhibitory properties. Our findings clearly indicated that the methanol extract and water extract of *P. korinti* leaves can be used as an antidote for snake venom, as evidenced by decreased hemolysis activity of snake venom when incubated above said extracts, as evidenced by our findings based on decreased hemolysis activity of snake venom when incubated above said extracts. Our findings also show that reaming extracts of leaves and bark minimize snake venom hemolysis activity, but they require more than 5 mg/ml of extract to reduce the hemolytic halo (11 mm) to half its diameter (5.5 mm).

Phospholipase A2 (PLA2) enzymes in snake venom work on RBC membrane-associated phospholipids to release lysolecithin, causing RBC hemolysis. The cell is more susceptible to secondary damage from free radicals as a result of the injury to the RBC membrane. This study demonstrated the ability of methanol extracts of *P. korinti* leaves and bark, as well as water extracts of leaves, to stabilize the RBC membrane and prevent hemolysis. These results are in accordance with the results published by other studies [14, [15]. The extracts contain active constituents that bind to the Phospholipase A2 enzyme, preventing it from binding to its substrate and thereby inhibiting it [16]. Cobra venom contains multiple proteases that can lyse several essential proteins, causing erythrocyte membrane degradation. A proteolytic inhibition analysis demonstrated the ability of plant extract to significantly inhibit protease activity, thus protecting RBC membranes [17].

Caseinolytic Inhibition

The Agar well diffusion assay showed that both *P. korinti* leaf and bark extracts showed inhibitory activity against the proteolytic activity of the Cobra venom (Figure: 6 and 7). Of all the extracts, the proteolytic activity of venom at various concentrations was completely inhibited by methanol and water extracts. The methanol extract of leaves and bark completely inhibited the proteolytic activity of venom at the ratio of 1: 50 and 1: 60 (w/w) venom: plant extract respectively. The proteolytic activity of the venom was also completely inhibited at a ratio of 1: 70 (w/w) venom: water leaf extract and bark. Figures 6 and 7 indicate that *P. korinti* leaves and bark extracts have dose-dependent inhibition of venom proteolytic activity. Proteases of snake venom primarily impact hemostasis and induce systemic haemorrhage. The chelating property of phenolic

compounds present in the extract is the most probable mechanism involved in the inhibition of these proteases by plant extracts. It has been documented that phenolic compounds form hydrogen bonds with histidine residues present in Zn^{2+} binding motifs of metalloproteases, resulting in a decrease in the enzyme's hydrolytic activity [18]. Melo *et al. (*1994) and Soares *et al. (*2005) suggested that the plant extracts would have compounds that bind to divalent metal ions, which are required for enzymatic activities [19], [20]. As the presence of proper metal ion coordination is a pre-requisite for the hydrolytic activity of metalloproteases, any metabolite that can weaken the protease-metal ion interaction will result in inhibition of the proteolytic activity.

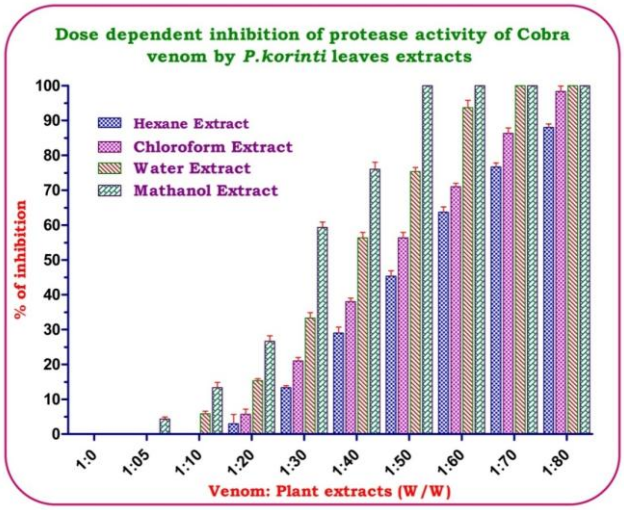

Figure 6: Dose dependent inhibition of Cobra venom by *P. korinti* leaves extracts

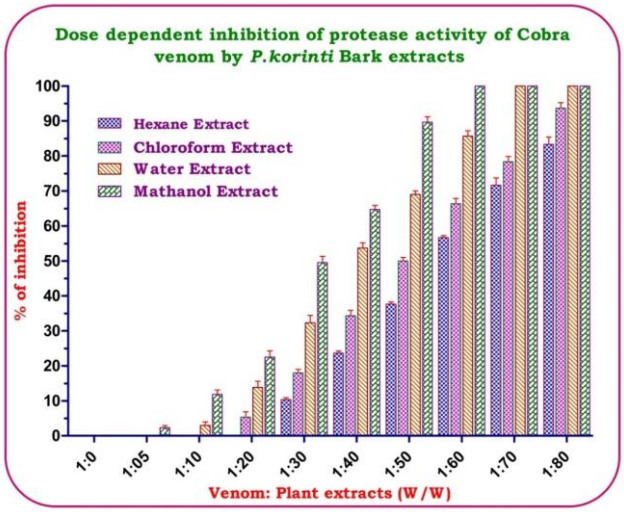

Figure 7: Dose dependent inhibition of Cobra venom by *P. korinti* bark extracts

These studies were conducted to provide a theoretical foundation for indigenous peoples' traditional use of herbal remedies to treat snakebite victims. The test is used to identify agents that may be toxic to zoological systems (cytotoxic) and to predict potential biological activity in pharmaceutical samples that are unknown. It's likely that the toxic enzymes were neutralized in the process because conventional healers applied these agents as a paste and bound them over incisions made at the point of bites. Certain plant constituents can bind venom proteins [21]. The toxicological properties of snakebite are thought to be related to enzymes, as described by Stocker in 1990 [22], [23], especially phospholipase A2 (PLA2) and protease, which are thought to be the most toxic components involved in haemorrhage [24], [25]. As a result, the extract's effects on the venom can be linked to its action against toxic enzymes, the extract's anti-venom effects can be traced back to its ability to inhibit toxic enzymes [26].

Mahanta and Mukherjee hypothesized in 2001 that neutralizing these enzymes would result in venom lethality being inhibited at the application site [27]. The findings of the in-vitro detoxification test show that the extract would operate by neutralizing the venom's role at the bite site, reducing the magnitude of the toxic effects. Another possibility may be asymptomatic or physiological antagonism by the extract in which the toxic features, such as convulsion which lead to eventual suffocation and death could be attenuated, thereby reducing the fatality of the venom a symptomatic relief. The various classes of compounds identified in the phytochemicals study of the extract [28] could be studied closely. Some previous research had linked antivenom activity to alkaloidal glycosides found in the plant under study. Tannins are also known to inactivate proteins in a non-specific manner. The activity of the extract may, therefore, be linked to its tannin contents.

4.Conclusion

From this study, it was concluded that *P korinti* extracts has anti-venom properties by neutralizing Phospholipase and Caseinolytic activity of the Cobra venom. The efficacy of these extracts also justifies the traditional use of plant extracts in the treatment of snake bite. However, further research including the isolation of antivenom compounds and *in vivo* assays is needed to establish these plants as a remedy for snakebite.

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