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Original Article

Neutralizing Effects of *Mimosa pigra* Stem-Bark against Selected Snake Venom from Malaysian Habitat

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ABSTRACT

Introduction: *Mimosa pigra* L. (*M. pigra*) regarded as significant undesirable weed in India, Africa, Australia, South-East Asia and some Pacific islands. Preliminary evidence of the use of the plants by rural community for medical purpose to cure snakebites. The purpose of this research was to validate the traditional claims and usefulness of *M. pigra* L. in the management of envenomation caused by various poisonous snakes found in Malaysia, such as the King Cobra, Malayan Pit Viper, Banded Krait and Cobra. **Materials and Methods:** The powdered stem bark of *M. pigra* L. was subjected to methanolic extraction and the resulting extract was examined for preliminary phytochemical screening. The *in vitro* snake venom neutralization effects of the extract were investigated using the Phospholipase Inhibition Activity (PIA), Venom Induced Hemolysis (VIH) and Adenosine Diphosphate (ADP) induced platelet aggregation methods. In addition, an impregnated disc containing the venom and the stem bark extract was tested to neutralize the lethality induced by the venom using an egg embryo model. **Results:** The *in vitro* assays PIA, VIH and ADP-induced platelet aggregation demonstrated that the methanolic extract of *M. pigra* L. bark exhibited dose dependent neutralization effects against snake venoms from King Cobra, Malayan Pit Viper, Banded Krait and Cobra. A significant neutralization effect was observed in PIA, VIH and ADP activity at a concentration of 640 µg/mL. The LD_{50} of the extract was determined to be 2.419 µg/µL. At an extract concentration of 30 µg/µL to 50 µg/µL, *M. pigra* L. extract was effective in countering the vulnerability of *Ophiophagus Hannah (O. Hannah)* snake venom. The SDS page revealed that the neutralization activity may be attributed to the arrest of the fibrinolytic activity of the cobra venom. **Discussion:** The study confirms that the methanolic extract of *M. pigra* L. stem bark. is effective in neutralizing snake venoms, both *in vitro* and *in vivo* using an egg embryo model. These findings validate the traditional claims regarding the use of *M. pigra* L. in the treatment of snakebites.

Keywords: Egg embryo model, Fibrinolytic, *O. Hannah*, *M. pigra* L., Phospholipase Inhibition Activity, Snake venom.

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INTRODUCTION

Snakebite poses a significant challenge to public health in Malaysia. Historical records indicate that Malaysian hospitals reported around 55,000 cases of snakebite from 1958 to 1980. The venom is known for causing long-lasting health complications.[1-3] Malaysia is home to 26 species of snakes, primarily belonging to the elapids and vipers' families. Medicinal plants have long been a vital source of therapeutic uses, with applications ranging from serving as excipients in pharmaceutical formulations to exhibiting medicinal activities such as anti-cancer and anti-tumor activity, etc.[4,5]

Mimosa pigra L. (*M. pigra*) is classified under the Fabaceae family and is one of the species in the Mimosa genus. Research has demonstrated that the aqueous dried root extract of *Mimosa*

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pudica exhibits inhibitory activity against the lethality and myotoxicity of *Naja kaouthia* (Cobra) venom.[6] *M. pigra* L. is a small thorny plant that invades marshes and is considered a weed in rice fields across the old-world tropics. The plant is capable of invading pastureland, rice paddies, immature oil palm plantations and fruit orchards. Additionally, *M. pigra* L. infestations are more likely to occur on construction sites. The plant has been reported to be used in the peninsular state of Kelantan as folklore medicine, some farmers claimed in 1980 that they had brought *M. pigra* L. from Thailand to treat snake bites. The historical use of *M. pigra* L. in Africa as a treatment for snakebite is also documented in the literature.[1]

The phytochemical analysis found the presence of flavonoids, tannins, phlobatannins, alkaloids and saponins in the methanolic extract of *M. pigra*.[2] Hawwal *et al.*, reported 7 specialized metabolites isolated from the leaves of *M. pigra*, comprising 6 flavonoid-O-glycosides. Interestingly, these isolated flavonoids are a diverse group of bioactive compounds that have displayed inhibitory potential against Phospholipase A2 (PLA_2) enzymes, which are commonly found in snake venoms and are associated with toxic effects. PLA_2 enzymes are prevalent in many snake venoms and significantly contribute to venom toxicity by inducing myotoxicity, hemolysis and coagulopathy. For instance, studies have shown that PLA_2 from Naja species can inhibit coagulation pathways and induce tissue damage, making them a primary target for antivenom development.[2,5]

The inhibition of PLA_2 activity is crucial because it directly correlates with the reduction of venom-induced damage, as demonstrated by the efficacy of various PLA_2 inhibitors, including Varespladib, which has shown potent activity against multiple snake venoms.[4,6] VIH is primarily caused by the action of PLA_2 and other hemolytic components in the venom.[7,8] The ability of antivenoms to prevent or reduce hemolysis is a critical parameter in their evaluation.[9,10] Thus, VIH serves as a vital indicator of the therapeutic potential of antivenoms against the systemic effects of snake venom. ADP-induced platelet aggregation assays are employed to assess the impact of snake venoms on platelet function, which is often compromised following envenomation. Many snake venoms contain components that inhibit platelet aggregation, leading to coagulopathy and increased bleeding risk.[11,12] The assessment of antivenoms in reversing these effects is essential, as demonstrated by studies showing that antivenom can restore platelet function *in vitro*. This method not only evaluates the antivenom's ability to neutralize the venom's anticoagulant properties but also provides insights into the broader hemostatic effects of the venom.

This implies that the flavonoid-O-glycosides identified in *M. pigra* may hypothetically contribute to neutralizing snake venom, taking into account the known activity of similar compounds.[13,14] This study evaluates the snake antivenom efficacy of *M. pigra* against targeted venomous species.

MATERIALS AND METHODS

Materials

Snake venom and anti-venin was purchased from Queen Saovabha Memorial Institute, The Thai Red Cross Society, Bangkok, Thailand. The extracted venom was lyophilized and was kept at -20°C until further use. The venom concentration was expressed in terms of dry weight. *M. pigra* L. was collected from Chemor district in Medan Klebang Prima industrial area (Latitude: 4.6668, Longitude: 101.1223) Ipoh, Malaysia. Lecithin, Calcium Chloride ($CaCl_2$) and Ethylene Diamine Tetra Acetate (EDTA) was procured from Sigma Aldrich (Malaysia). Methanol was procured from Merck (Malaysia). All other chemicals used were of analytical grade.

Methods

Plant collection and authentication

The *M. pigra* L. plant was collected from Ipoh, Malaysia. The voucher specimen was prepared, authenticated, labelled as UniKL/MP-01, submitted and preserved at the UniKL herbarium. The fresh stem bark was thoroughly washed in distilled water to remove contaminants before being chopped into small pieces. These small pieces were sun-dried for four weeks before being pulverized for later use.

100 g of the powdered stem of *M. pigra* L. were macerated in methanol with agitation on an orbital shaker. The method was continued for 7 days. The resulting mixture was filtered through Whatman filter paper, dried with a rotary evaporator (Buchi) and stored at 4°C until further use.

Preliminary phytochemical screening

The methanolic extracts of *M. pigra* L. stem bark was subjected to qualitative tests for phyto-constituent detection.[15]

In vitro studies

Preparation of standard anti-venin solutions

The freeze-dried anti-venin of the King Cobra, Malayan Pit Viper, Banded Krait and Cobra was reconstituted with 10 mL of sterile water provided with the sample.

Preparation of venom solutions

The lyophilized venom of King Cobra, Malayan Pit Viper, Banded Krait and Cobra was suitably diluted by sterile water for injection.

Phospholipase Inhibition Activity

The reaction vessel was filled with a reaction mixture comprising 50 mg of lecithin (dissolved in 25 mL of diethyl ether), 1 mL of drug solution (160, 320 and 640 µg/L), 1 mL of venom solution (400 µg/L) and 0.1 mL of 5% $CaCl_2$ solution. The mixture was homogenized and incubated at room temperature for 4 hr. After

the incubation, 25 mL of alcohol and 0.3 mL of cresol red as an indicator solution were added and the solution was titrated with 0.02 N methanolic NaOH. 100% phospholipase activity was achieved by reacting 400 µg/mL venom with 50 mg lecithin. This served as the control.[16]

Venom induced hemolysis

The preparation of cell suspension was carried out by using venom solution (0.36%, 2 mL), phosphate buffer (0.15 M, pH 7.4; 1 mL) and freshly collected HRBC (1%, 0.5 mL) in test tubes. Varying concentrations of the extract was then added to the above test tubes. Isosaline (0.85%) was used as a control. All the tubes were incubated for 30 min at 37°C before being centrifuged. The colour of the supernatant was measured at 560 nm.[17] The control was taken as 100% lyses and the percentage of prevention of hemolysis of the drug was calculated using the equation:

$$\text{Percentage prevention of hemolysis} = \frac{\text{Absorbance of treated sample}}{\text{Absorbance of the control}} \times 100 \quad \ldots\ldots 1$$

ADP induced platelet aggregation

Blood from healthy adult human volunteers was collected in EDTA tubes to prevent clotting. The platelet rich plasma was separated by centrifugation method. The reaction mixture was prepared with 0.5 mL of drug extract solution with different strengths (160, 320, 640 µg/mL), venom solution 0.5 mL (200 µg/mL) and platelet rich plasma 0.5 mL. The reaction mixtures was stirred for 2 min at 37°C. To the above mixture, 0.5 mL of ADP solution was added, incubated for 4 min and, absorbance was measured at 414 nm. ADP induced platelet aggregation was calculated using the following formula.[16]

$$\%\ \text{ADP induced platelet aggregation} = \frac{(T1-T2)-(T1-T3)}{(T1-T2)} \times 100 \ldots . 2$$

Where, T=ADP+Platelet, T2=ADP+Venom+Platelet and T3=ADP+Venom+Platelet+Plant extract.

Determination of venom toxicity by using egg embryo model

Preparation of the embryos

The fertilized eggs were purchased from CP poultry farm, Ipoh. These eggs were incubated in a custom-built incubator for 36 hr in a horizontal position at 37-39°C with 70-80% humidity. The eggs were manually tilted to 45° angle twice or thrice in a day to prevent the embryo from sticking to the eggshells. This was done to ensure that the egg incubation mimicked the natural process. On the third day of incubation, a slit was made on the broad end of the egg using sterile needle and forceps. The eggshell was sterilized after the process with 70% ethanol solution. The eggs were then incubated again for three days before being used in the experiment.

Determination of Acute Toxicity of *M. pigra* L. extract

Four groups of six embryos each were used per extract dilution. A disc of 2 mM was cut from Whatman filter paper using a puncher. Each disc received a total of 2 µL of *M. pigra* L. extract in different concentrations. The discs were placed over the Anterior Vitelline (AV) vein of the embryo. Normal saline (0.9%w/v) was used as control. The embryos were observed for any lethality, until 24 hr. Each test was carried out in triplicate.

Determination of Venom lethality

The lethality of various snake venoms was studied. 2 µL of serially diluted venom in a concentration of 1-5 µg/L was applied to the disc and placed on the AV vein. Saline water was used as control. The LD_{50} was calculated with a confidence limit of 50% probability by the analysis of death occurring within 24 hr of venom injection. All tests were carried as triplicate.

Measurement of anti-venom activity

The venom sample 3 times the extract of LD_{50} was incubated for half an hr at 37°C with an equal volume of varying concentrations of drug extract (concentration used were below the toxic concentration). A disc of 2 mm filter paper was soaked with the mixture and placed on the AV vein of the yolk sac membrane. For the positive control embryo, 2 µL of each venom without the extracts was applied. In the negative control embryo was given 2 µL of normal saline (0.9% w/v). The embryos were observed at 1, 2, 4 and 6 hr intervals, for any lethality. The number of survivors at 6 hr were recorded. The death of the embryo was a clear end point with cessation of the heart beat followed by submergence of the yolk sac membrane into the yolk.

Isolation and fibrinolytic assay using SDS-Page

The isolation and fractionization of *M. pigra* L. stem bark methanol extract was carried out via flash chromatography, which resulted in 21 different fractions. 7 out of the 21 fractions dried up to form the crystallized powder and the remaining fractions were assumed to be the solvent fractions. The consideration was that a minimum of 1 mg per fraction would be required for further analysis. Thus, from the 7 fractions, 4 of the fractions were chosen as they comply with the requirements made for the further analysis process.

Human fibrinogen (4 µg) was incubated for one hr at 37°C with 5 µg King cobra snake venom pre-incubated with *M. pigra* L. extract (1:15, w/w) for 30 min at 37°C. The reaction was stopped with 6 µL of 0.5M Tris-HCl buffer (pH 6.5) containing 2% (w/v) SDS, 3.5% (v/v)-mercaptoethanol and 0.05% (w/v). The protein bands were stained with Coomassie Brilliant Blue R-250 and the staining images were captured using the Bio-Rad ChemDoc Imaging System. Electrophoresis of 12% (w/v) sodium dodecyl Sulfate Polyacrylamide gel was used to analyse the samples (SDS-PAGE).[18]

Plant extract

M. pigra L. methanol extract formed a dark brown color with a fine consistency and a yield of 1.21%.

Phytochemical analysis

Phytochemical analysis of *M. pigra* L. revealed the presence of alkaloids, flavonoids, protein, tannin and saponins.

In vitro studies

Phospholipase Inhibition Activity

Our study, showed that the methanolic extract of *M. pigra* L., exhibited significant activity at an extract of 640 µg/mL against all the venoms (p<0.0001), but the activity was more pronounced against banded krait venom. The results for Phospholipase Inhibition Activity (PIA) are shown in Figure 1.

Venom Induced Hemolysis

In vitro anti-snake venom activity was carried out by the HRBC membrane stabilization method. The methanolic extract of *M. pigra* L. was proven to be significantly effective in a concentration of 640 µg/mL against all the selected venoms (p<0.0001), limited or no effects were recorded at a lower concentration. The results for VIH are shown in Figure 2.

ADP induced platelet aggregation

The PAL A_2 is present in snake venom and can cause ADP induced platelet aggregation. The methanolic extract significantly decreased the inhibition of platelet aggregation at an extract concentration of 640 µg/mL against all the snake venoms (p<0.0001). The results are depicted in Figure 3.

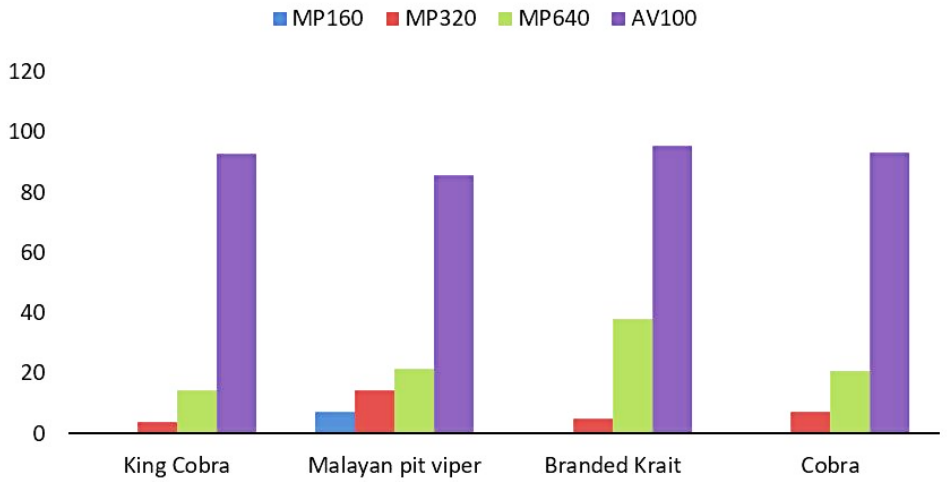

Figure 1: Phospholipase inhibition activity of *M. pigra* stem-bark extract (p<0.0001).

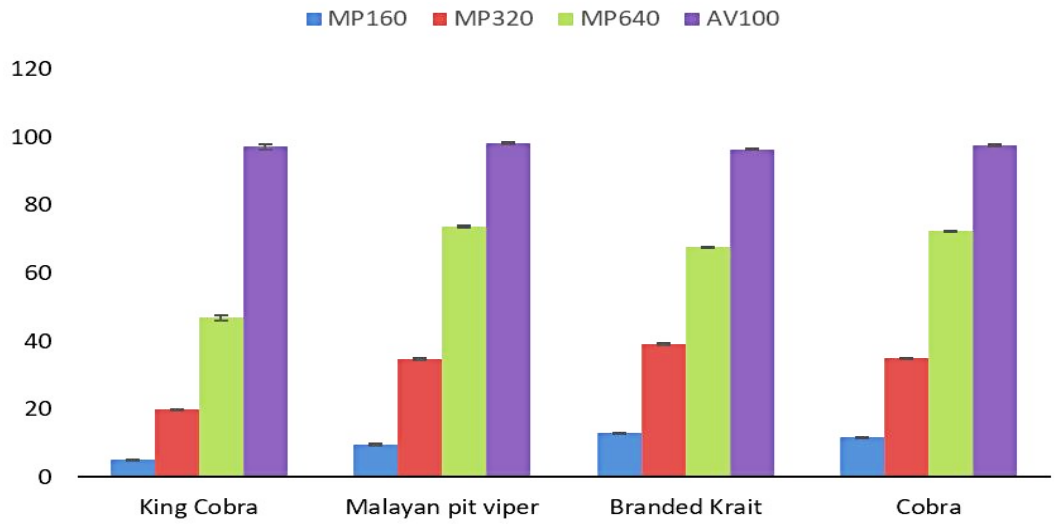

Figure 2: Inhibition of venom induced haemolysis activity of *M. pigra* stem-bark extract (p<0.0001).

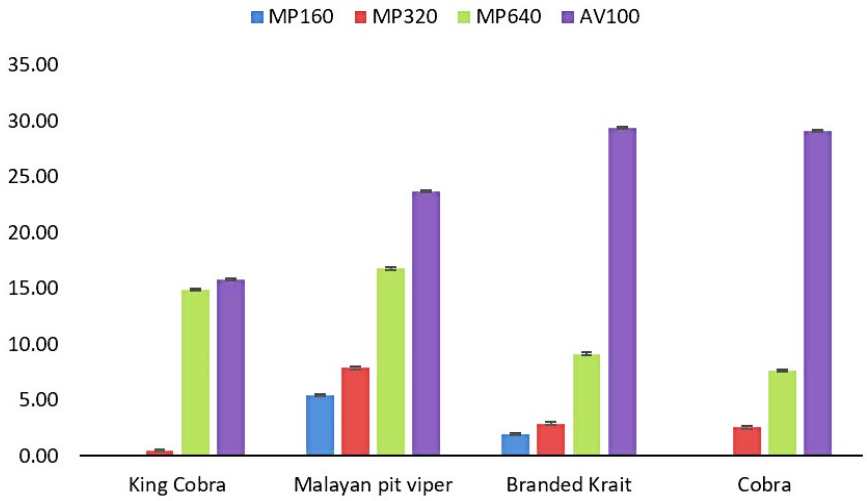

Figure 3: Percentage ADP induced platelet aggregation of venom induced hemolysis of *M. pigra* stem-bark extract ($p<0.0001$).

Table 1: Results of embryos survive after 24 hr of extract treatment.

Extract Concentration (µg/µL)	Embryo Survived after 24 hr
10	4
20	2
30	1
40	0
50	0

Table 2: Results of embryos survive with different venom concentration.

Venom Concentration (µg/µL)	Embryo Survived
1	3
2	2
3	1
4	0
5	0

In vivo studies using egg embryo model

Determination of extract toxicity

The acute toxicity of *M. pigra* L. stem bark extract was measured within 24 hr and found to be completely safe up to 10 µg/mL of extract treatment. 50% of the egg embryos survived 20 µg/mL of the extract treatment. The embryos showed signs of lethality at concentrations beyond 30 µg/mL of the extract. Only 25% of embryos survived at a concentration of 30 µg/mL. We also hypothesized, that the location of the sample disc would also have an impact on the embryo's survivability. The disc should be placed exactly on the Anterior Vitelline Vein (AVV) vessel to minimize any errors. The AVV is a major blood vessel in the embryonic structure that transports blood directly from the yolk sac to the heart. Thus, it was concluded that *M. pigra* L. minimum toxic concentration in the embryo was found to be between 30-50 µg/mL. The negative control group which received saline solution showed no abnormalities or mortality up to 24 hr (Table 1).

Determination of Venom Lethality

The primitive embryonic heart with normal blood circulation was visible in the 6th day embryo with a vascularized yolk sac and its arrest provides a clear endpoint for the lethality test. Each group of embryos had venom concentration ranging from 1-5 µg/mL applied to the AV vein. Embryo lethality was observed within 24 hr at higher concentrations of more than 4 µg/mL (Table 2). The LD_{50} was calculated using probit analysis based on Finney's method and was found to be 2.419 µg/mL.[19]

M. pigra L. anti-venom efficiency

The goal of this study was to see if *M. pigra* L. stem bark exhibited anti-venom activities against selected snake venoms. *O. hannah* venom was selected as the model venom to elucidate the anti-venom efficiency of *M. pigra* L. A pre-incubated 50 µg/mL of the extract successfully neutralized *O. hannah* venom as 100% of the treated embryo survived after 6 hr of incubation. The positive control embryos, which were given 7.26 µg/mL of *O. hannah* alone, died within the first hr of the experiment (Figure 4).

Isolation and fibrinolytic assay using SDS-Page

The extract was further isolated into 4 fractions (Figure 5). The fibrinogenolytic activity of King cobra venom with and without anti-venin or *M. pigra* L. stem bark extracts and its fractions upon human fibrinogen. It was observed in Fibrinogen α-band in Lane 2 shows that the King cobra venom has succeeded in degrading the Fibrinogen α-band. The anti-venin of King cobra venom was applied as treatment in Lane 3, but it does not present enough antagonizing activity towards the degradation of Fibrinogen α-band. However, when *M. pigra* L. stem bark methanol extract

and its fractions were applied as treatment in Lane 4 to Lane 8, the treatment had shown visible antagonizing activity on the degradation of Fibrinogen α-band by King cobra. It was also believed that *M. pigra* L. Fraction B and C in Lane 6 and 7 were the most effective in counteract the fibrinogenolytic activity exhibited by King cobra. Besides that, the protein bands of King cobra venom which were visible in Lane 2 has been degraded successfully by *M. pigra* L. Fraction B and C in Lane 6 and 7 respectively (Figure 6).

Statistical test

A one-way ANOVA was performed to compare the means of across groups to determine if there were statistically significant differences among them.

DISCUSSION

The study use methanol as the extracting solvent as methanolic extract is expected to extract flavonoids and tannin which are known to have antivenin properties. Phospholipase is a pathological enzyme found in snake venom known to be highly toxic to cell membrane and causing major tissue damage.

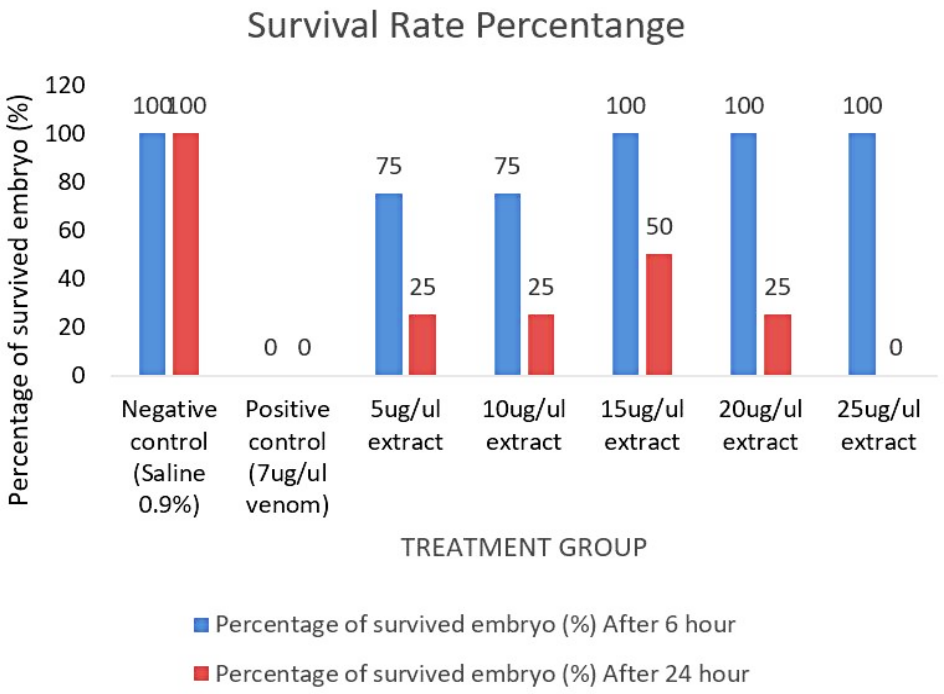

Figure 4: Survival rate of embryos treated with the mixture of King Cobra venom and *M. pigra* extract after pre-incubation.

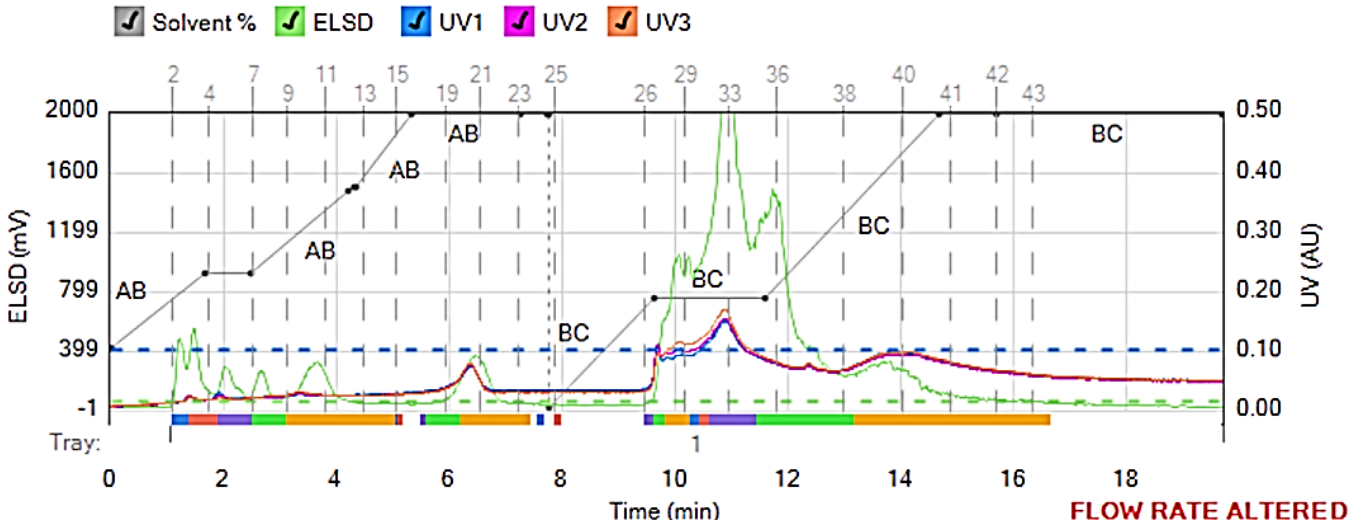

Figure 5: The spectrum of fractions of *M. pigra* stem bark methanol extract.

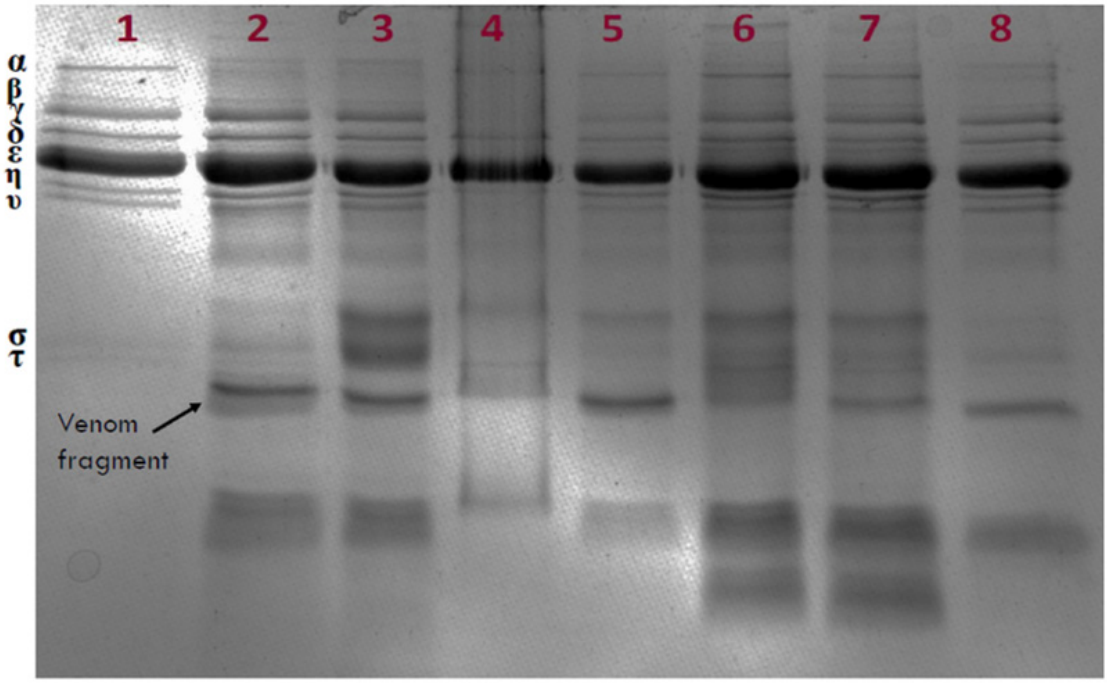

Figure 6: SDS-PAGE of the fibrinogenolytic activity of Malayan Pit Viper venom with and without anti-venin or *M. pigra* stem bark extracts and its fractions upon human fibrinogen.Lanes: 1. Fibrinogen control; 2. Fibrinogen+Venom; 3. Fibrinogen+Venom+Anti-venin; 4. Fibrinogen+Venom+*M. pigra*; 5. Fibrinogen+Venom+*M. pigra* A; 6. Fibrinogen+Venom+*M. pigra* B; 7. Fibrinogen+Venom+*M. pigra* C; 8. Fibrinogen+Venom+*M. pigra D.*

Researchers suggest the first line of treatment of snake bite could be inhibiting the circulating venom Phospholipase enzyme.[20] Moreover, it is also envisaged that the presence of venom phospholipase enzyme may also be accountable for excruciating pain, particularly in banded krait.[21] The study acknowledged that the extracts of *M. pigra* L. exhibit appreciable inhibition of venom phospholipase enzyme in a concentration dependent manner with the maximum inhibition evident in banded krait. Tannins or flavonoids present in the extract may be responsible for the neutralization of snake venoms. More research is needed to understand the explicit mechanism.[14,22]

Studies propounds that most of the snake venoms are very potent in destabilizing the cellular components of human blood, particularly the red blood cells. Lysis of HRBC is considered as an expression of the digestive action of snake venom rather than lethality.[23] It is necessary to stabilize the cellular components of human blood to reduce the detrimental effects of snake venom. The study was done to supervise the effects of plant extracts on the stabilization of HRBC. The extracts of *M. pigra* L. showed conclusive and dogmatic results substantiating the neutralization activity of the selected plant. The stabilization effects were concentration dependent against the selected venoms and showed it maximum intensity at a concentration of 640 µg/mL. The precise mechanism of action responsible for membrane stabilization needs to be investigated further, but it is possible that the presence of polyphenolic flavonoid may contribute to membrane stabilization of HRBC.[17]

Studies have also suggested various mechanisms of ADP induced platelet aggregation effects of snake venom. Snake venom may be composed of various components such as aggretin, glycoprotein, arachidonic acid, PLA A2 may actuate the fatal platelet aggregation process.[24,25] Heparin and hirudin have been successfully tested with complete inhibition of rabbit platelets aggregation.[26] Plants containing lignin, tannins and flavonoids can also be successfully employed to reduce ADP induced platelet aggregation in blood. The extracts of *M. pigra* L. exhibited excellent activity against all the selected venoms with a superlative degree of inhibition in branded krait and Malayan pit viper owing to its satisfying inhibition of phospholipase activity, particularly due to the high content of tannins and flavonoids.[27] Our findings indicates that *M. pigra* L. stem bark possesses satisfying neutralizing effects against snake venoms commonly found in Malaysia.

The results of phytochemical screening revealed the presence of alkaloids, carbohydrates, protein, amino acid, tannin in *M. pigra* L. extract. Phospholipases A_2 constitute major components of snake venom and exhibit a wide range of pharmacological actions such as neurotoxicity; cardiotoxicity; myotoxicity; anticoagulant, hemorrhagic, edematous, hemolytic, convulsive and hypotensive activities As tannins are known to precipitate proteins, it was expected that activities of PLA_2 present in *O. hannah* venom would be inhibited, thus preventing lethality.[20,28] The most likely mechanism for anti-venom activities by these plant extracts is the binding of venom proteins with polyphenolic and tannin-like substances in the extracts. Since, venom is proteinaceous in

nature, the extract may neutralize these proteins. Flavonoids can bind to the active sites of PLA_2, effectively blocking their enzymatic activity and thereby mitigating the venom's harmful effects.[29] After 6 hr of observation, the results revealed that preincubation of *M. pigra* L. with *O. hannah* venom before inoculation in embryos was able to maintain a 100% survival rate in a dose-dependent manner. However, after 24 hr of incubation, there was an overall reduction in the survival rate percentage of embryos at all concentrations. On the other hand, the *in vivo* test of *M. pigra* L. stem bark extracts after 24 hr of incubation was unable to completely rescue embryos from the lethal effects of *O. hannah* venom. This is due to the presence of tannin in stem bark extract, which non-specifically binds to venom protein and may interact with plasma proteins. Therefore, there is a decrease in binding to *O. hannah* venom proteins, thus unable to provoke total anti-lethal effects.[22] Previous study also confirms that *M. pudica* of the same species was found to effectively antagonize the fibrinolytic activity, the findings suggest that the *M. pigra L.* and its isolated fraction can be further explored for its neutralization activity.[30]

Based on the study results, we discovered that *M. pigra* L. stem bark extract has the potential to be an antagonist to *O. hannah* venom, as demonstrated by our studies. *M. pigra* stem bark shows promising antivenom properties, warranting further investigation for use in snakebite management.

CONCLUSION

The findings from this study envisaged that the methanolic extracts of *Mimosa pigra* L. stem bark extract may be a promising candidate for neutralizing the toxic effects of snake venom. The study showed that the extract demonstrated significant inhibitory activity against PLA A2, venom-induced hemolysis, and ADP-induced platelet aggregation, thereby addressing key pathological mechanisms of snakebite envenomation. The study also demonstrated a promising antivenom efficacy through an *in vivo* embryo model. However, the it was observed that the anti-venom activity reduced its effectiveness over extended incubation periods. This suggests the need for further refinement of the extract's formulation to enhance its bioavailability and specificity. Additionally, fractionation and isolation of active compounds from *M. pigra* L. provide a foundation for future research into its molecular mechanisms and potential therapeutic applications. In conclusion, *M. pigra* L. exhibits remarkable potential as a plant-based antivenom, aligning with the broader goal of exploring traditional medicinal resources for innovative healthcare solutions.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

***M. pigra*:** *Mimosa pigra* L.; ***O. Hannah*:** *Ophiophagus hannah;* **PLA_2:** Phospholipase; **PIA:** Phospholipase inhibition activity; **VIH:** Venom Induced haemolysis; **ADP:** Adenosine Diphosphate; **$CaCl_2$:** Calcium Chloride; **EDTA:** Ethylene Diamine Tetra Acetate.

SUMMARY

The objective of the research work was to validated the folkrole claims that *M. pigra* has the neutralizing property against major snakes found in Malaysian habitat.

The phytochemical analysis of *M. pigra* L. revealed the presence of flavonoids, tannins, phlobatannins, alkaloids and saponins in the methanolic extract.

The research involved investigating the possible mechanism of the inhibitory activity of plant extracts against selected snake venom.

The results indicated that *M. pigra* L. successfully inhibited the lethality and myotoxicity of *O. hannah* venom at higher concentration.

The study's findings suggest that the methanolic extract of *M. pigra* L. could be a potential therapeutic agent for snakebite treatment due to its significant inhibitory activity against Phospholipase A (PLA) enzymes, which are commonly found in snake venoms and contribute to their toxic effects.

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