Research Article

www.ijrap.net

ISOLATION, PURIFICATION AND CHARACTERIZATION OF ACTIVE COMPOUND FROM *ANDROGRAPHIS PANICULATA* L. AND TESTING ITS ANTI-VENOM AND CYTOTOXIC ACTIVITY BY *IN-VITRO* AND *IN-VIVO* STUDIES

R. S. A. Sorna Kumar[1], Ajit Vincent Joshua[2], M. Sangeetha[3], D. Thilagavathy[4], Sridevi Gnanaiah[4]*

[1]Centre for Biotechnology, Anna University, Chennai, India

[2]Department of Biotechnology, Jeppiaar Engineering College, Chennai, India

[3]Department of Biotechnology, Sathyabama University, Chennai, India

[4]Department of Anti-toxin, King Institute of Preventive Medicine and Research, Chennai, India

Received on: 04/03/14 Revised on: 07/04/14 Accepted on: 15/04/14

***Corresponding author**

Dr.Sridevi Gnanaiah, Deputy Director, Department of Anti-toxin, King Institute of Preventive Medicine and Research, Chennai-32, India

E-mail: gsrijai@gmail.com

DOI: 10.7897/2277-4343.05233

ABSTRACT

Snake bite is a serious problem in tropical and subtropical countries like India with 5 million human fatalities annually. The present study aimed on the shade grown *Andrographis paniculata* L under organic condition. 6 month old plants were collected at dawn; shade dried and extracted with methanol, followed by fractionation to isolate the active compound and characterization of the active compound was done using FT-IR study which was found to be identified as andrographolide. Furthermore, *in vivo* and *in vitro* studies confirm the anti- venom property of the plant and the cytotoxic levels determined by the use of cell lines has its major start towards the cellular level study. These studies and investigations confirms the presence of cobra venom inhibiting factor whose bite pose a huge threat to the life of people. Commercializing the drug against envenomation would prove successful in rural areas and the areas where the health centre is out of reach. Under economic circumstances, this study has the advantage of being very niggardly charged for the anti- venom preparation unlike the present day anti-venom that involves in rearing a horse and collecting the horse plasma that possess the raised anti- bodies which by further purification process would result in ASVS. Thus prepared ASVS holds the main disadvantage of bringing cross reactions like anaphylaxis and many more of the same sort. So, the plant as a drug would prove more effective, with high degree of safety and cost effective.

Keywords: *Andrographis paniculata* L, Andrographolide, medicinal plant, lime flocculation

INTRODUCTION

Snake bite is a serious problem in tropical and subtropical countries like India. According to World Health Organization (WHO), poisonous snakes are responsible for at least 5 million human fatalities annually[1]. *Andrographis paniculata* L. is an erect annual herb extremely bitter in taste in all parts of the plant body. It is known as Kalmegh or Kalamegha, meaning dark cloud in Ayurveda and nila vembu in siddha. *A. paniculata* grows erect to a height of 30–110 cm in moist, shady places. The slender stem is dark green, squared in cross-section with longitudinal furrows and wings along the angles. The leaves are lanceolate having hairless blades measuring up to 8 cm length by 2.5 cm width. The small flowers are borne in spreading racemes. The fruit is a capsule around 2 cm long and a few mm wide. It contains many yellow-brown seeds.[2] Since ancient times, *A. paniculata* is used in traditional Siddha and Ayurvedic systems of medicine as well as in tribal medicine in India and some other countries for multiple clinical applications. The plant extract exhibits anti-typhoid, anti-fungal anti-hepatotoxic, anti-biotic, anti-malarial, anti-hepatitic, anti-thrombogenic, anti-inflammatory, anti-snake venom, and anti-pyretic properties to mention a few, besides its general use as an immune stimulant agent[3]. The herb has shown an ability to reduce inflammation (heat) and fight viral infection, and is used as a principal ingredient in traditional Chinese medicinal formulas for lung support from colds. There are many researches done on anti-venom activity of *A. paniculata,* yet no study was done on the plant grown in shade under organic farming method. Research has been done to study the anti-venom activity of *A. paniculata* plant extracts against venom by in vivo and in vitro methods using the Methanolic extract[3]. Until this day, extraction of andrographolide from *A. paniculata* is usually carried out using liquid organic solvent preferably methanol for more yield. Followed by purification using silica gel chromatography[4,5]. Similarly, lime flocculation, a method generally used for study of the activity of tetanus anti-dote was used to in this study which has been new to the study of anti-venom activity of purified plant extract.

MATERIALS AND METHODS

Preparation of venom

Lyophilized snake venom of *Naja naja* (Indian Cobra) was collected from King Institute of preventive medicine and research (KIPM and R). One gram of lyophilised venom was dissolved in 100 ml of 0.90 % saline and centrifuged at 2500 rpm for 10 minutes. The supernatant was used as venom and stored at 4°c for further use.

Isolation of plant extract

Organic shade grown *A. paniculata* leaves collected from the DAT garden were submitted in the sample section of DAT at King Institute of Preventive Medicine and Research for authentication and were assigned the voucher number: DAT020312. The leaves were dried for 7 days under shade, powdered using a homogeniser and separated using a percolator. The plant powder was packed and placed in the thimble of soxhlet apparatus and was extracted with methanol (60-80°C for 72 h) and the

extract was then concentrated in rotary evaporator for further drying purpose. The dried extract was stored in desiccators at room temperature for further use.

Column Chromatography

Column was packed with slurry of silica gel (mesh size, 60-120) with chloroform. Then the dried methanol extract (4 g) of *A. paniculata* was first dissolved in methanol and carefully applied by pipette at the top of prepared column. Immediately after application of sample, a gradient of Chloroform and Methanol (mobile phase) was used as elute to collect fractions of Methanol extract of *A. paniculata*. The column was run with a gradient of Chloroform : Methanol (98:2, 95:5, 90:10, 80:20, 70:30, 50:50, 30:70, 20:80, 10:90, 5:95, 2:98) finally 100 % methanol and 12 fractions (F1-F12) were collected. The optical density of the collected samples was determined using spectrophotometer (HITACHI U-1900) and the values were tabulated. The fraction that corresponds to higher O.D value is taken for further experiments. Thereafter, from all the collected fractions, solvent was removed by evaporation at room temperature and the obtained crystals are set to characterization studies.

Fourier Transform Infra Red Spectroscopy

A total of 5 % (w/w) of sample, with respect to the potassium bromide (KBr) disc was mixed with dry KBr. The mixture was ground into a fine powder before compressing into KBr disc under a hydraulic press at 10,000 psi. Each KBr disc was scanned at 4 mm/s at a resolution of 2 cm over a wave number region of 400-4500 cm^{-1}. The characteristic peaks were recorded using Perkin Elmer (Waltham, MA, USA) spectrophotometer.

Kjeldahl Method of Protein estimation

0.7 g of the sample i.e. the plant powder is weighed and mixed with 10 g of anhydrous ammonium sulphate. A pinch of copper sulphate is added that acts as a catalyst. To this mixture, 30 ml of concentrated sulphuric acid is added which will help the nitrogen to convert into ammonium sulphate. This gets charred due to the organic compounds present in the sample and is heated in a burner. After sufficient heating, the mixture is being cooled by adding distilled water to it and the heat liberated is reduced by exposing the flask to running tap water. The whole contents are transferred to a 2 litre round bottomed flask for further distillation process. To the mixture, 1:1 alkali (NaOH) is added and 30 ml of 0.1 N HCl is added in the condenser with methyl red as an indicator. During the process of distillation, all the nitrogen compounds get converted to ammonium chloride and a titration against 0.1N NaOH confirms the quantity of protein present in the sample using the formula,

Percentage of Protein Content in sample =
(Titre value of Nitrogen * protein conversion factor) / Amount of the sample taken.

Shinoda Test for the confirmation of flavonoids

To 5 mg of the plant sample, few magnesium turnings were added. To this, few drops of conc. HCl is added and left for incubation at room temperature for 2 minutes and is observed for a pink colouration if the sample contains flavonoids.

Salkowski Test

To 0.5 g each of the extract, was added 2 ml of chloroform. 3 ml of conc. Sulphuric acid is carefully added over to form a layer. A reddish brown colouration at the interface indicates the presence of terpenoids in the active drug.

***In vitro* studies**

Limes Flocculation Test

5 mg of the plant extract was weighed and dissolved in 5 ml of DMSO; making the concentration 1 mg/ml. The mixture was distributed in 5 tubes each containing 1 ml and the tubes were labelled from T_1 to T_4 with one tube as the control. To the tubes labelled from T_1 to T_4, venom was mixed at different concentrations i.e. 0.1 %, 0.01 %, 0.001 % and 0.0001 %. The water bath was set at 37°C and the tubes were incubated in the water bath for 30 minutes to observe any flocculation present in the tubes due to the neutralizing effect of Andrographolide against snake venom.

Procoagulant Activity

Human blood was collected and sodium citrate was added to the blood to prevent coagulation, mixed gently and centrifuged for 15 min at 5000 rpm. The resulting supernatant contain the citrated plasma and the rest of that was discarded. 0.5 ml of the citrated plasma was distributed in different tubes and different concentrations of venom i.e. 1 %, 0.1 %, 0.01 % and 0.001 % was added and the coagulating time was noted with help of timer. The minimum coagulating dose (MCD) was determined as the concentration of the venom to coagulate the plasma within 60 seconds. Plasma incubated with PBS alone served as a control. In case of the neutralization assays, constant amount of venom was mixed with various dilutions of plant extract. The mixture was incubated for 30 minutes at 37°C. 0.1 ml of the mixture was added to 0.3 ml of citrated plasma and the clotting times were recorded. In control tubes, plasma was incubated with venom alone and plant extracts alone. In neutralization assays, constant amounts of venom was mixed with various dilutions of the active drug contained in the plat extract which was pre incubated at 37°C for 30 minutes. Neutralization was expressed as effective dose (ED), defined as the ratio amount of anti-venom (plant extracts) needed per mg of venom by which the clotting time increases three times when compared with clotting time of plasma incubated with two MCD of venom alone.

Hemolytic Assay

Blood was collected from a healthy volunteer. Sodium citrate was added to the collected human blood, mixed gently and centrifuged for 15 minutes at 5000 rpm. The supernatant was discarded and the settled RBCs were taken for the study. 1 % of agarose was added to 25 ml of PBS, heated and allowed to dissolve. 0.25 ml of RBCs were mixed with 0.25 ml of the egg yolk and poured in the dissolved agarose solution at 55°C. The mixture was poured onto a petri plate and allowed to solidify. After

solidification, 2 wells were punched wherein the 1st well was loaded with 0.1 % venom, 2nd well loaded with the pre incubated mixture of plant extract (1 mg/ml) and venom. The plate was incubated at 37°C for 24 h and was observed for hemolytic hallows corresponding to the wells.

Cell Toxicity Studies

The cell toxicity was assessed for the drug in non cancerous cell lines like L6. The sub cultured cell lines were washed with PBS to remove any dead or old cells. 1 % TPVG was added to the monolayer and incubated in a CO_2 incubator for 5 minutes and then washed off. Fresh MEM media was added to the culture flask and flushed well so as to completely suspend all the cells and plate them on a micro titre plate. The plate was incubated for 48 h until monolayer of cells developed. After the formation of monolayer, drug dilution was performed to assess the toxicity of the active compound. 2 mg of the drug was weighed and dissolved in 0.25 ml of DMSO and was homogenized well for 5 minutes. 9 ml of MEM was added to the drug solution and filtered using a syringe filter and the dilutions were prepared. The diluted plant sample was added to all the wells having a drug control and cell control as negative and positive controls. The cells contained in the wells were incubated at 37°C in a 5 % CO_2 incubator. The cell morphology was assessed having an interval of 24 h, 36 h and 48 h after which MTT assay was performed.

***In vivo* studies**

The *in vivo* studies were carried out in an Ethical committee approved institute with the permission grant number 006/IAEC/KIPMR/2013 dated 22/10/13

LD_{50} Potency

LD_{50} is calculated as the 50 % probability by the analysis of deaths occurring within 24 h of venom injection. 6 numbers of mice weighing 18 – 20 g were taken in each batch; having 5 batches on the whole to assess the lethal potency test. Various concentrations of venom were made up to 5 ml with normal saline. The venom under different concentrations were injected via the intra peritoneal (tail vein) site to the mice. The mice were observed after 24 h to assess the death ratio. To this dose of venom, different dilution of the extract was added and the death rate was checked after 24 h.

Edema Forming Activity

The edema forming activity of *Naja naja* venom was determined by Camey *et al*[6]. Group of 2 mice were injected subcutaneously in the right foot pad with 0.1 ml of venom dissolved in PBS. The right foot pad of the mice was injected with 0.1 ml of PBS alone to serve as a control. Edema was calculated as a percentage of increase in the thickness of the right foot pad injected with venom compared to the control in the left foot pad. The thickness of each foot pad was measured for every 30 minutes after the venom injection using a screw gauge. The ability of the *A. paniculata* extract to reduce the Edema thus formed is also determined. The plant extract is pre incubated with venom for 30 minutes at 37°C. Then a group of 2 mice were injected subcutaneously in the right foot pad with 0.1 ml of the mixtures whereas the left foot pad was injected with 0.1 ml of PBS alone. The control mice were injected with 0.1 ml of venom in the right foot pad and 0.1 ml of PBS in the right foot pad. After 1 h, the Edema was calculated as described by Yamakawa *et al*[7].

RESULTS AND DISCUSSION

Preparation of the crude extract

The dried powder was placed in the thimble of soxhlet apparatus with methanol as the solvent and run for 72 h with 16 cycles to complete. The methanolic solution was condensed and further dried using rotary vacuum evaporator to get the crude extract and stored in desiccators for further use. (Figure 1)

Isolation of the active compound

Column Chromatography

The active compound of was isolated by means of column chromatography with silica gel as the solid phase and methanol: chloroform being the mobile phase. 10 fractions were eluted which were read by using a spectrophotometer (HITACHI U-1900) to identify the fractions showing maximum absorbance. The 4th and 5th fractions showed high peaks at 214 nm in a spectrophotometer that confirms the presence of Andrographolide in the fractions. The 4th and the 5th fractions containing the drug were dried to form crystals and stored for further use.

Characterization studies

FT – IR

The –OH stretching vibration was observed at 3319-3406 cm^{-1} due to presence of three –OH groups. The aliphatic C-H stretching vibration was observed at 2848- 2990 cm^{-1} whereas the lactone carbonyl of cyclopentane ring was observed at 1728 cm^{-1}. The =CH2 was observed at 1674 cm^{-1}. Furthermore, C-C and C-O stretching were observed at 1031 cm^{-1} and 1220.9 cm^{-1}, respectively. (Figure 2)

Phytochemical analysis

The protein content present in the sample was analyzed by kjeldahl's method of protein estimation and was found out to be 6.6 %. Shinoda test was performed to confirm the presence of flavonoids. After the addition of magnesium turnings and con. HCl to the sample, a pink coloration was observed indicating the presence of flavonoids. Salkowski test was carried out to confirm the presence of terpenoids in the sample. A reddish brown coloration at the interface was observed after the addition of chloroform and con. sulphuric acid to 0.5 mg of the drug.

***In vitro* studies**

Limes Flocculation Test

5 tubes that were distributed with the drug dissolved in DMSO in a concentration of 1 mg/ml along with various concentrations of venom were incubated at 37°C in water bath and were observed for any floccules as an inference for neutralization. The first tube was set as blank which contained only the plant extract. After an incubating the samples for 30 minutes at 37°C, flocculation were

observed in all the tubes except for blank. This proved the neutralization effect of the compound present in the plant against the venom. The concentration of flocculation varied with different tubes. The increase in concentration was directly proportional to the increase in the venom concentration, i.e. more amount of flocculation was observed in the second tube having a venom concentration of 0.1 % than the other tubes, 0.01 %, 0.001 % and 0.0001 % which had relatively low flocculation. This dilution where neutralization is maximum i.e. the tube containing the drug concentration as 1 mg/ml and the venom concentration as 0.1 % is taken for further *in vitro* and *in vivo* studies.

Pro coagulant activity

The minimum coagulant dose was determined and was found to be 100 µg of *N. naja* venom that was able to coagulate the citrated plasma within 60 seconds. The ED_{50} of *A. paniculata* was found to be 1 mg which was capable of inhibiting the fibrinolytic activity induced by the cobra venom.

Hemolytic assay

The plates after incubation were observed for the presence of hallows indicating the lyses of RBCs by the snake venom. After an incubation of 24 h, hallows were formed and the diameter of the zones were measured. The hemolytic zone formed around the snake venom was measured to be 1.9 cm. The zone formed around the pre-incubated sample of the plant extract and venom was reduced considerably showing the neutralization effect which when measured was found to be 0.7 cm. This reduction in the hemolytic zone formation was about 60 % of neutralization by the plant extract.

Cell Toxicity Studies

The cell toxicity study assessed the concentrations from which the plant was toxic to the cell line – L6 was taken for study. After plating the cells, drug dilutions were performed and added on to the plated cells. The cells were observed for toxicity at an interval of 24 h, 36 h and 48 h. The cells after 36 h were assayed with MTT, wherein the cytotoxicty was perfectly established at each concentration. There was a cytotoxicty observed at the dilutions starting from 70 and continue till the 250^{th} dilution in L6 cell lines. Therefore there were formazin crystals observed in the wells containing cell control and dilutions starting from 10 to 60. After the addition of DMSO to the wells, the viable cells were able to take up the dye and give the out a pink coloration; wherein the neat and other wells having dilutions above 70 retained the yellow color of MTT dye. The optical density of the samples was read at 620 nm using an ELISA plate reader and a graph was plotted depicting the cytotoxicity of the drug against the cell line. From the graph plotted, the Inhibitory concentration value (IC Value) was found to be 60 µg/ml. (Figure 5).

***In vivo* tests**

LD_{50} potency test

The lethal toxicity (LD_{50}) of Cobra venom was assessed using 18 g, Balb/c strain mice. Based on the study the LD_{50} value for cobra venom used was found to be 9.4 µg/ mouse The neutralization of lethality was done by mixing constant amount of venom ($2LD_{50}$) with various dilutions of Plant extracts and incubated at 37°C for 30 minutes prior to injection. It was found that 0.38 mg of plant extract was able to completely neutralize the lethal activity of $2LD_{50}$ of Cobra venom.

Edema forming activity

After the injection of venom, the pre incubated mixture containing the drug in a concentration of 1 mg/ml and venom in a concentration of 0.1 % at 37°C for 30 minutes and the control sample having PBS into the foot pad of mice (Figure 6). In case of edema formation, the purified sample had a much better neutralizing activity with increase in time. Screw gauge was used to measure the edema formation. The pre incubated extract and venom mixture showed a reduction of 0.006 mm in the edema formation when compared to pure venom that was injected. In case of PBS that was used as a control, there was a minimal swelling in the footpad of mice irrespective of time interval.

DISCUSSION

Based on older literature such as Castro *et al*[8], it was found that methanolic extraction was the most effective and we have used it in our studies to isolate the extract using soxhlet method. After running the extract in column chromatography using methanol and chloroform, the product that we got was similar to the one done by Borooah *et al*[5] and the studies showed that unlike the colour appearance, the 6^{th} component that had a much lighter colour showed a greater absorbance which concluded that it had active component and we crystallized it. By FT IR studies we were able to confirm that the active compound was andrographolide. This result was similar to the work done by Handa *et al*[9] and Mulukuria *et al*[4]. The confirmation test such as Shinoda test, Salkowski test confirmed the presence of flavonoid group in our purified product. The protein content of the dry leaf was 6.6 % of the total weight which was found out by substituting the protein factor value to the nitrogen value found out by Kjeldhal method. This result was similar to the presence of flavonoid in the active compound done by Sato *al*[10]. In case of *in vitro* studies of the neutralizing activity of the active compound against snake venom, was done similar to the test done in Lime Flocculation test of tetanus. Dark green microscopic flocculation was observed. The most suitable concentration of extract to neutralize 0.1 % venom was found to be 1 mg/ml. In case of pro coagulant activity, the ED_{50} value of *A. paniculata* was found to be 1 mg. Haemolytic assay was done to determine the effect of venom and plant extract on RBCs. The zone thickness of venom was found to be 0.9 cm, wherein the plant had no zone formation. In half an hour pre incubated venom extract mixture, the zone was 0.15 cm and in case of fresh mixture, it was found to be 0.4 cm. In *in vivo* studies carried out, the LD 50 potency rate of cobra venom was found to be 9.4 µg/mouse, which was very close to the result done by Meier. J *et al*[11]. In case of Edema formation, the purified sample had a much better

neutralizing activity with increase in time. Screw gauge was used to measure the Edema formation. The pre incubated extract and venom mixture showed a reduction of 0.006 mm in the Edema formation when compared to pure venom that was injected. In case of PBS that was used as a control, there was a minimal swelling in the footpad of mice irrespective of time interval. This result was similar to the work done by Meenatchi sundaram *et al*[3], Lomonte *et al*[12] and Yamakawa *et al*[7]. In studies to determine the cytotoxic effect of plant extract on cell line, L6 and the cytotoxic/inhibitory concentration for L6 was found to be 60 µg/ml. All these studies gave a positive result that *A. paniculata* possess a potent anti-venom property due to its major component Andrographolide and they were found to have no haemolytic and cytotoxic activity up to 60 µg/ml concentration.

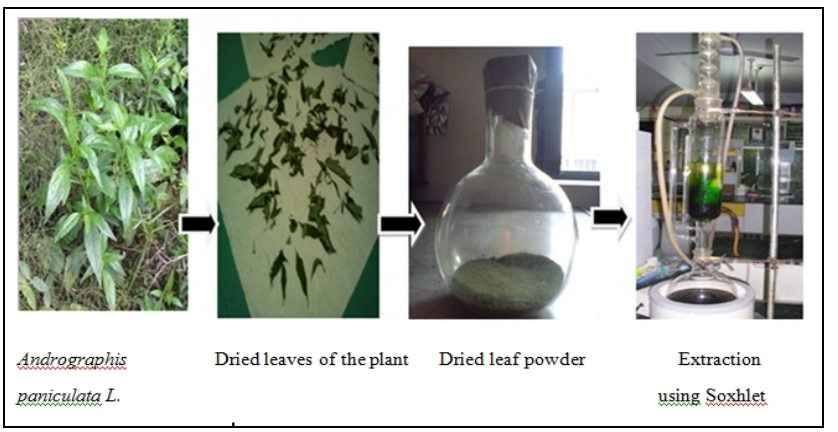

Figure 1: Preparation of the crude extract

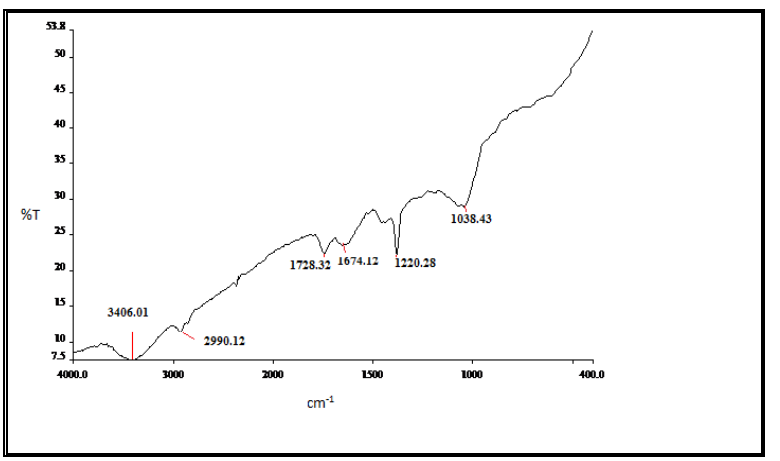

Figure 2: FT IR Spectrum for the isolated Andrographolide from the purified extract

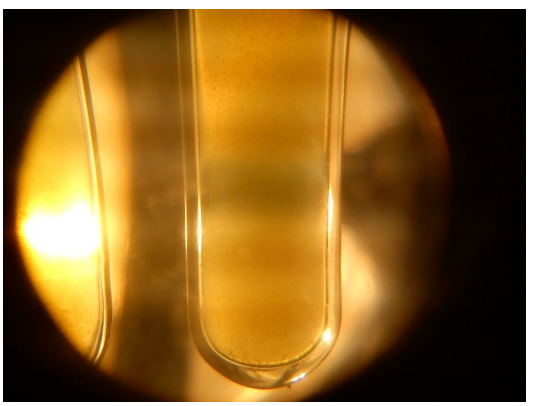

Figure 3: Limes Flocculation test

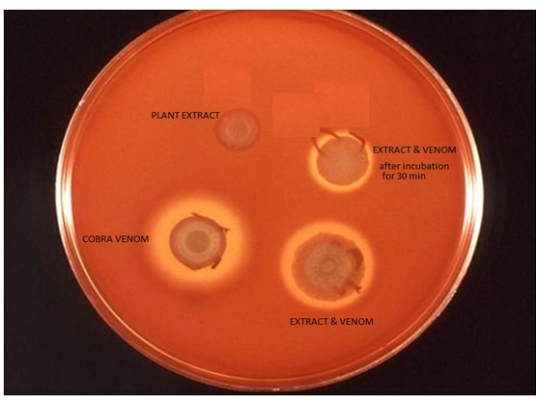

Figure 4: Haemolytic assay

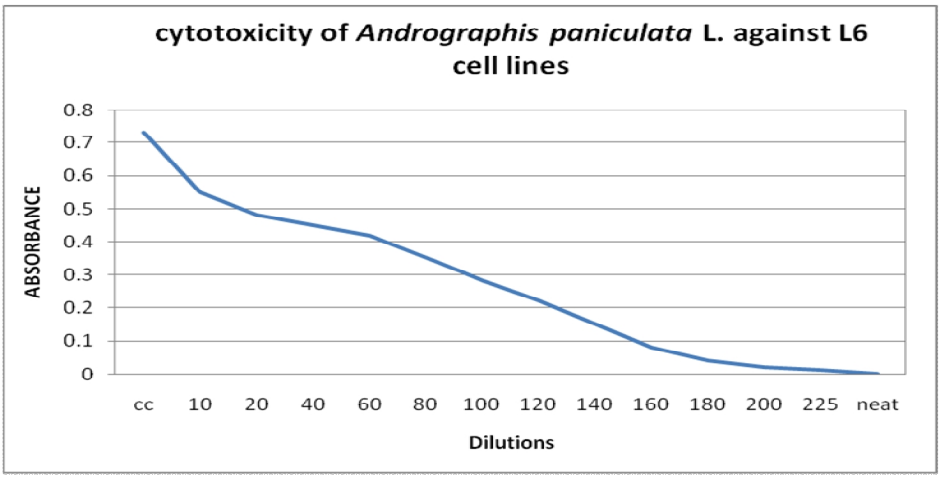

Figure 5: Graph exhibiting the cytotoxicity of *Andrographis paniculata* L. against L6 cell lines

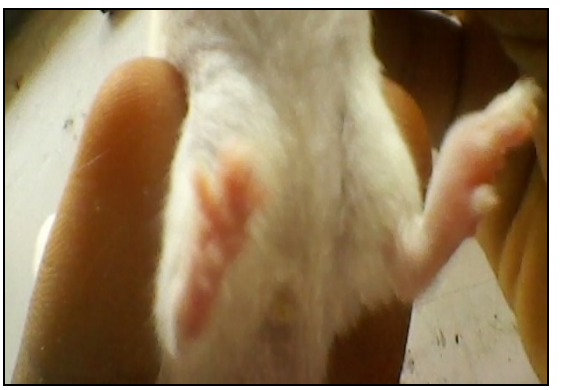

Figure 6: Edema Forming Activity (Left: Edema when injected with 0.1 % of Venom; Right: Edema when injected with venom pre incubated with extract)

REFERENCES

1. Chippaux JP. Snake-bites: appraisal of the global situation. Bull WHO 1998; 76: 5-524.
2. Chakravarti RN, Chakravarthi D. Andrographolide, the active constituent of *Andrographis paniculata* nees, a preliminary communication. Indian medical gazette 1951; 86: 96-97.
3. Meenakshi Sundaram S. Anti-venom activity of *Andrographis paniculata* and *Aristolochia indica* Plant extract against *Daboia russelli* venom. Indian Journal of Science and Technology 2009; 2: 76-79.
4. Sirisha Mulukuri NVL. Isolation of Diterpenoid Lactones from the Leaves of *Andrographis paniculata* and its Anti-cancer Activity 2011; 3: 39-42.
5. Dimpy Das Borooah. Column chromatography for isolation of andrographolide from *in vitro* grown medicinal plant-*Andrographis paniculata*. International Journal of Applied Biology and Pharmaceutical Technology 2011; 2(3).
6. Camey Kyoko U, David T Velarde and Eladio F Sanchez. Pharmacological characterization and neutralization of the venom used in the production of *Bothropicanti*-venom in Brazil. Toxicon 2002; 40: 501–509. http://dx.doi.org/10.1016/S0041-0101(01)00245-8
7. Yamakawa M, Nozaky M and Hokama Z. Fractionation of sakishimahabu (*Trimeresurus elegans*) venom and lethal, hemorrhagic and edema-forming activities of the fractions. In: Toxins: Animal, Plant and Microbial. Eds. New York: Plenum Press; 1976. p. 97-109. http://dx.doi.org/10.1007/978-1-4684-0886-7_10
8. Luque de Castro MD and Garcia Ayuso LE. Soxhlet Extraction of Solid Materials': An Outdated Technique with a Promising Innovative Future. Anal. Chim. Acta 1998; 369: 1-10. http://dx.doi.org/10.1016/S0003-2670(98)00233-5
9. Handa SS Sharma A and Krishna L. Standardization of the Indian crude drug Kalmegh by high pressure liquid chromatographic determination of andrographolide. Phytochem Ana 1992; 3: 129-131. http://dx.doi.org/10.1002/pca.2800030308
10. Sato M and Kuroyanagi M. Flavonoids from *Andrographis paniculata*', Chem pharm bull 1087; 35: 4429-4435.
11. Meier J, Theakston RG. Approximate LD_{50} determination of snake venom using eight to ten experimental animals, Toxicon 1986; 20: 395. http://dx.doi.org/10.1016/0041-0101(86)90199-6
12. Lomonte B, Tarkowski A, Hanson HA. Hostresponse to Bothropsasper snake venom: analysis of edema formation, inflammatory cells, and cytokine release in mouse model. Inflammation 1993; 17: 95–105.

Cite this article as:
R. S. A. Sorna Kumar, Ajit Vincent Joshua, M. Sangeetha, D. Thilagavathy, Sridevi Gnanaiah. Isolation, purification and characterization of active compound from Andrographis paniculata L. and testing its anti-venom and cytotoxic activity by in-vitro and in-vivo studies. Int. J. Res. Ayurveda Pharm. 2014;5(2):163-168 http://dx.doi.org/10.7897/2277-4343.05233

Source of support: Nil, Conflict of interest: None Declared