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Phytochemical screening and evaluation of the anti-venom effect of leaves extracts of *Vitex negundo*

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Abstract

The number of snake envenomation cases is estimated to be more than 5 million per year. Out of these cases, approximately 1,00,000 individuals experience severe complications. The objective of the current research is to investigate the effectiveness of local plant extracts from the western ghats of India in countering snake venom. The medicinal plant *Vitex negundo* has been traditionally used by healers in India to treat snakebite victims. Prior to injection, extract from the *Vitex negundo* plant was incubated at 37°C for 30 minutes. The study revealed that doses ranging from 0.15-0.17 mg of *Vitex negundo* extracts completely neutralized the lethal effects of *V. russelli* venom, while doses of 0.16-0.19 mg neutralized the lethal effects of Najanaja venom. In rats, the minimum edematic dose of the venom was found to be 4µg. Notably, significant inflammation was observed 1 hour after venom injection, with maximum inflammation occurring at 180 minutes. Furthermore, plant extracts administered at doses of 100mg/kg, 200mg/kg, and 400mg/kg exhibited significant activity compared to the control group. The minimum percentage inhibition was 29.13% and 27.1% at 100mg/kg, 58.18% and 57.21% at 240 minutes for 400mg/kg, and 60.89% for Vitex negundo with standard polyvalent antivenom. against V. Russelli and Najanaja venom, respectively.

Keywords: Phytochemical; *Vitex negundo*; Antivenom Activity; Phospholipase; *D. russelli venom.*

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Introduction

Snakebites are a prevalent occupational and environmental hazard, particularly in tropical and subtropical regions. They pose a significant risk to individuals working in agriculture, such as farmers and plantation workers, resulting in a notable rise in both mortality and morbidity rates. The World Health Organization (WHO) officially classified snakebites as a neglected tropical disease in 2009.[1] The study conducted by Swaroop and Grab revealed two intriguing findings: first, there are significant variations in snakebite mortality rates across different regions, and second, regions with similar topography tend to have higher rates of snakebite incidents. Globally, an estimated 30,000-40,000 deaths occur annually due to snakebites, with exceptions in China, the USSR, and Central European countries. The highest number of fatalities were recorded in Asia (25,000-30,000), followed by South America. Conversely, North America, Europe, and Oceania reported lower figures. While an exact estimate for Africa was unavailable, it was noted that annual snakebite deaths ranged from 400 to 10,000.

Vitex negundo, commonly known as the Chinese chaste tree, five-leaved chaste tree, or horseshoe vitex, or nisinda is a large aromatic shrub with quadrangular, densely whitish, tomentose branchlets. It is widely used in folk medicine, particularly in South and Southeast Asia.

Vitex negundo is an erect shrub or small tree growing from 2 to 8 m (6.6 to 26.2 ft) in height. The bark is reddish brown. Its leaves are digitate, with five lanceolate leaflets, sometimes three. Each leaflet is around 4 to 10 cm (1.6 to 3.9 in) in length, with the central leaflet being the largest and possessing a stalk. The leaf edges are toothed or serrated and the bottom surface is covered in hair. The numerous flowers are borne in panicles 10 to 20 cm (3.9 to 7.9 in) in length. Each is around 6 to 7 cm (2.4 to 2.8 in) long and are white to blue in color. The petals are of different lengths, with the middle lower lobe being the longest. Both the corolla and calyx are covered in dense hairs.

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Asterids
Order:	Lamiales
Family:	Lamiaceae
Genus:	*Vitex*

Vitex negundo is native to tropical Eastern and Southern Africa and Asia. It is widely cultivated and naturalized elsewhere. Countries it is indigenous to include Afghanistan, Bangladesh, Bhutan, Cambodia, China, India, Indonesia, Japan, Korea, Kenya, Madagascar, Malaysia, Moza mbique, Myanmar, Nepal, Pakistan, the Philippines, Sri Lanka, Taiwan, Tanzania, Thailand, and Vietnam. *Vitex negundo* are commonly found near bodies of water, recently disturbed land, grasslands, and mixed open forests.

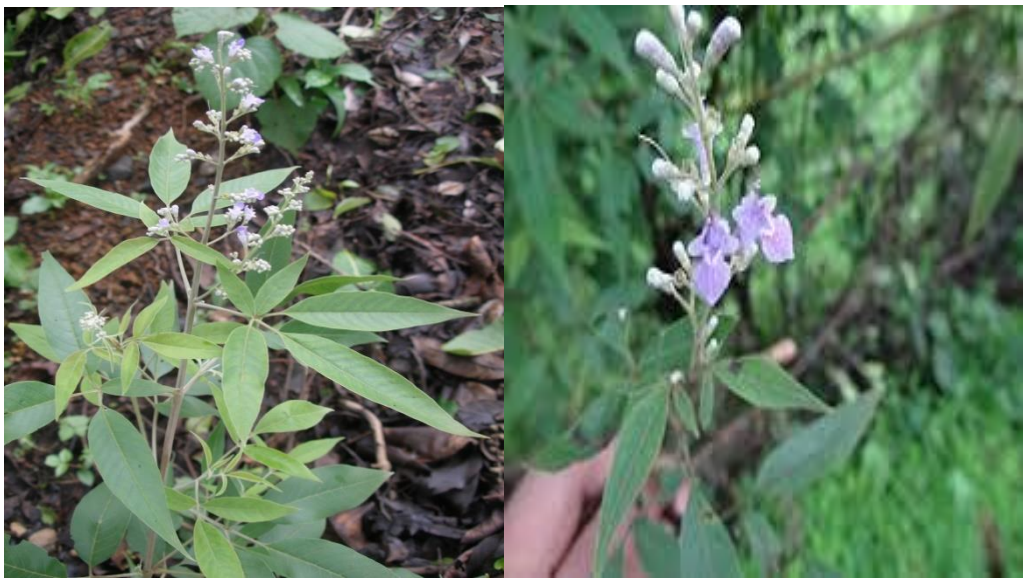

Figure 1: Plant and flowers of *Vitex negundo*

Material and Methods

Plant Selection

Medication revelation from restorative plant has advanced to incorporate various fields of request and different strategies for examination. The procedure normally starts with a botanist, ethno botanist, ethnopharmacologist, or plant scientist who distinguishes the plant of intrigue. Assortment may include species with known natural action for which dynamic compound(s) have not been disengaged (e.g., generally utilized home grown cures). Based on escalated writing overview; *Vitex negundo* was chose for utilized as anti-venom activity in present examination. The writing survey demonstrated that the chose plant has potential as anti-venom activity.

Table 1: Details of plant selected for study

Sr. No.	Botanical Name	Family	Vernacular Name	Parts Used	Activity to be screened
1.	*Vitex negundo*	Lamiaceae	--	Leaves	Anti-venom

Collection and identification

The *Vitex negundo* was identified and authenticated by Bhima Ram choudhary Deputy conservator of forest, Botanical Survey of India, Sikar, Rajasthan India. The voucher specimens were deposited at the respective Institutes. *Vitex negundo* voucher specimen sample number is VN-1. The certificates for the authentication were obtained.

Preparation and microscopical evaluation of plant materials

Leaves of *Vitex negundo*

The Leaves of *Vitex negundo* plant was conceal dried, decreased to coarse powder with the assistance of processor and put away in water/air proof compartment till further use. Powder investigation assumes a huge job in distinguishing proof of rough medication. These characters will help in the recognizable proof of right assortment and quest for adulterants. Powder microscopy is one of the least difficult and least expensive techniques to begin with for building up the right character of the source materials. It is helpful for additional pharmacological and remedial assessment alongside the normalization of plant material. Primer assessment and conduct of the powder with various substance reagents was done and microscopical assessment was done

after treatment with various reagents like Phloroglucinol, Conc. HCl, Ruthenium red, Acetic acid and Iodine solution.[2]

Preliminary test

The leaves powder was characterized by its morphological features like white colour, presence of specific odour and taste.

Microscopical observation of Leaves of Vitex negundo

Microscopical Observation of plant revealed specific structural character for each organ of plant.

Staining

The fresh leaf of the plant *Vitex negundo* cut and fixed in FAE (Formalin - 5 ml+ Acetic acid 5 ml + 70 % Ethyl alcohol 90 ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary butyl alcohol. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60^{o}C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the section was 10-12 µm. De waxing of the sections was done by customary procedure. The sections were stained with toluidine blue, since toluidine blue is a polychromatic stain. The staining results were remarkably good and some cytochemical reactions were also obtained. The dye rendered pink color to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. Wherever necessary, sections were also stained with safranin and fast green and IKI (for starch). Powdered materials were cleared with NaOH and mounted in glycerin medium after staining.[3]

Photomicrographs

Photographs of different magnifications were taken with Nikon Lab photo 2 Microscopic Unit. For normal observation, bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars.[4]

Quantitative Microscopy

Quantitative microscopical parameters such as fiber length, size of trichome, starch grain dimension, stomatal number, stomatal index, vein-islet number and vein termination and crystal dimension were studied in the present study. These data will help in identifying the plant in powder form from its related species. The quantitative microscopical data were given and discussed in detail.[5]

Powder microscopy analysis

For powder microscopy, the shade dried leaves of *Vitex negundo* were pounded into fine powder with the assistance of electric processor. The fine powder was then exposed to minute assessment according to the standard strategies. The powdered of lentil was assessed all things considered and furthermore by treating independently with various reagents like phloroglucinol, concentrated hydrochloric corrosive, weakened hydrochloric corrosive, glycerine, iodine arrangement, safranin, water, sudan and methylene blue for deciding diverse infinitesimal highlights.[6] All the arrangements were seen under Olympus CH20iBIMF magnifying lens (Trinocular with camera connection). Photomicrographs in both the cases were taken utilizing SONY advanced camera Model No. DSC-350.

Analytical Parameter

Ash Values

The residues remaining after incineration is the ash content of the leaves powder *Vitex negundo.* Ash values are helpful in determining the quality and purity of crude drug, especially in the powdered form. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may also include

inorganic matter added for the purpose of adulteration. Hence, an ash determination furnishes a basis for judging the identity and cleanliness of a drug and gives information regarding its adulteration with inorganic matter. Procedure given in Indian Pharmacopoeia was used to determine the different ash values such as total ash, acid insoluble ash, and water soluble ash.[7]

Determination of total ash value

Accurately weighed about 3 gm of air dried powdered plant parts were taken in a tared silica crucible and incinerated by gradually increasing the temperature to make it dull red hot until free from carbon. Cooled and weighed, repeated for constant value. Then the percentage of total ash was calculated with reference to the air dried drug.

Determination of acid insoluble ash value:

The ash obtained as directed under total ash value was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water, ignited and weighed, then calculated the percentage of acid insoluble ash with reference to the air dried powder.

Sample Preparation

About 5.0 ±0.01 g (for lead, cadmium and arsenic) and 1.0 ±0.005 g (for mercury) dried homogenized powder tests of each ajwain, ginger, neem tree, peppermint, turmeric were weighed precisely in an iodine jar independently, 10 ml of concentrated HNO3 was included into every carafe (for mercury 0.5 ml of H2SO4 was included with this). The iodine flagons were refluxed for 1 h at 95 °C ± 5 °C. The example arrangements were cooled, and 5 ml of concentrated HNO_3 was included into every carafe. The carafes were again refluxed for around 1 h at 95 °C ± 5 °C. Rehashed the procedure until the absorption was finished. Vanished the answer for 5 ml. Arrangements were cooled, and 10 ml of concentrated HCl was included into every flagon. Saved the answers for refluxed for around 15 min to evacuate the nitrous exhaust. Cooled the processed example arrangements, 20 ml of HPLC grade water was included into every jar and separated the processed arrangement through Whatman channel paper no. 41 into 50 ml volumetric flagon and made up to the volume utilizing HPLC grade water. Recuperation study was done by strengthening known centralization of standard into the pre-broke down example.

Instrument conditions

ICP-OES Conditions: Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) with outspread light outfitted with argon immersion get together was utilized for the assurance of lead and cadmium. High immaculateness (99.99%) argon was utilized as plasma, assistant, and nebulizer gas. The gas streams were kept at 15.0 l/min for plasma, 1.50 l/min for assistant, and 0.56 l/min for nebulizer. Radio recurrence (R.F) intensity of the plasma generator was 1.35 kW. The vertical tallness of the plasma was fixed at 7 mm. Test take-up season of 30.0 sec, the defer season of 5 sec, flush season of 10 sec, starting adjustment season of 10 sec and time between reproduce examinations of 5 sec was kept up all through the investigations for ICPOES. All the perception of outflow were recorded at 220.353 nm and 226.502 nm, which relates to the most delicate emanation frequency of lead and cadmium, separately. The instrument was adjusted for different boundaries before the investigations.

AAS-VGA Conditions

Atomic Absorption spectrometer-Vapor Generation Assembly (AASVGA), outfitted with Graphite Thermal Analyzer, was utilized for assurance of arsenic and mercury. High immaculateness acetylene and zero air were utilized. For fume age, 6% Sodium Borohydride arrangement (w/v) and half Hydrochloride arrangement (v/v) were utilized for arsenic, and 25% Stannous Chloride (SnCl2) was utilized for mercury. The gas streams were kept at 13.50 l/min for zero air and 2.10 l/min for acetylene. The light current 10.0 mA and ghastly transfer speed of 0.5 nm was utilized for both the metals. All the perception were recorded at 193.7 nm

and 253.7 nm, which relates to the most delicate frequency of arsenic and cadmium. The EHT volts were recorded 68 and 59 for arsenic and mercury, individually.

Evaluation of antivenom activity

Evaluation of antivenom activity by using *In vitro* anti-venom study was carried out by using five different types of research models. *Viz.* Phospholipase activity, Procoagulant activity, Fibrinolytic activity, Proteolytic activity and Hyaluronidase activity and *in vivo* techniques for different extracts of *Vitex negundo* . *In vivo* antivenom study was carried out for PEEAP, CEAP, EEAP and AEAP.

Venom and experimental animals

The freeze-dried snake venom powder of cobra venom (*Najanaja)* and Russell's viper venom (*Daboia russelli)* were obtained and was stored at 4°C. Male inbreed Swiss albino mice 18-20 g were used for efficacy studies. Institutional Animal Ethics Committee clearance was obtained to conduct the experiment.

***In vivo* anti-venom activity**

In vivo anti-venom study was carried out by using five different types of research models. *Viz.* Determination of Lethal Toxicity, Haemorrhagic activity, Defibrinogenating activity, Edema forming activity and Myonecrotic activity.

Lethal Toxicity determination

The median lethal dose (LD_{50}) of *V.russelli* and *Naja naja* venom was determined according to the method developed by Theakston and Reid 1983. Various doses of venom in 0.2 ml of physiological saline were injected into the tail vein of mice, using groups of 3-5 mice for each venom dose. The LD_{50} was calculated with the confidence limit at 50% probability by the analysis of deaths occurring within 24 h of venom injection. The antilethal potentials of *Vitex negundo* plant extract was determined against $2LD_{50}$ of V.russelli venom. Various amount of plant extracts (µl) were mixed with $2LD_{50}$ of venom sample and incubated at 37°C for 30 minutes and then injected intravenously into mice. 3–5 mice were used at each antivenom dose. Control mice received same amount of venom without antivenom (plant extracts). The median Effective Dose (ED_{50}) calculated from the number of deaths within 24h of injection of the venom/antivenom mixture. ED_{50} was expressed as µl antivenom/mouse and calculated by probit analysis.[8]

Edema forming activity

The minimum edema-forming dose (MED) of *V.russelli* and *Naja naja* venom was determined by the method of Lomonte et al. (1993) and Camey et al. (2002). MED was defined as the least amount of venom which when injected subcutaneously into mice footpad results in 30% edema within 6 hours of venom injection. The thickness of each footpad was measured every 30 min after venom injection with a low-pressure spring caliper (Rojas et al., 2005). The ability of both plant extracts in neutralizing the edema-forming activity were carried out by pre-incubating the constant amount of venom and various dilutions *Vitex negundo* plant extract for 30 minutes at 37°C. Mice were injected subcutaneously in the right footpad with 50µl of the mixtures, containing venom/plant extracts, whereas the left footpad received 50µl of PBS alone. Control mice were injected with venom in the right footpad and 50µl of PBS in the left footpad. 1 hour after injection edema was evaluated as described by Yamakawa et al. (1976). Edema was expressed as the percentage increase in thickness of the right footpad compared to the right footpad of the control mice.[9]

In vitro anti-venom Activity

Phospholipase Activity

Phospholipase A_2 activity was measured using an indirect hemolytic assay on agarose–erythrocyte–egg yolk gel plate by the methods described by Gutierrez et al., 1988. Increasing doses of *V.russelli* and *Naja naja* venom (µg) were added 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 $CaCl_2$. Slides were incubated at 37°C overnight and the diameters of the hemolytic halos were measured. Control wells contained 15µl of saline. The minimum

indirect hemolytic dose (MIHD) corresponds to a dosage of venom, which produced a hemolytic halo of 11mm diameter. The efficacy of antivenom (plant extracts) in neutralizing the phospholipase activity was determined by mixing constant amount of venom (µg) with various amount of plant extracts (µl) and incubated for 30 minutes at 37°C. Then, aliquots of 10µl of mixtures were added to wells in agarose-egg yolk sheep erythrocyte gels. Control samples contain venom without plant extracts. Plates were incubated at 37°C for 20 h. Neutralization was expressed as the ratio of mg antibodies/mg venom which could reduce the diameter of the hemolytic halo by 50% when compared to the effect induced by venom alone.[10]

Procoagulant Activity

Procoagulant activity was assayed according to the method described by Theakston and Reid, 1983 as modified by Laing et al., 1992. Various amounts of venom dissolved in 100µl PBS (pH 7.2) was added to human citrated plasma at 37°C. Coagulation time was recorded and the minimum coagulant dose (MCD) was determined as the venom dose, which induced clotting of plasma within 60 seconds. Plasma incubated with PBS alone served as control. In neutralization assays constant amount of venom was mixed with various dilutions of plant extracts. The mixtures were incubated for 30 minutes at 37°C. Then 0.1ml of mixture was added to 0.3ml of citrated plasma and the clotting times recorded. In control tubes plasma was incubated with either venom alone or plant extracts alone. Neutralization was expressed as effective dose (ED), defined as the ratio µl antivenom (plant extracts)/mg venom at which the clotting time increased three times when compared with clotting time of plasma incubated with two MCD of venom alone. [11,12]

Result and Discussion

Snakebites are an occupational and environmental disease. It is a common occurrence in tropical and subtropical countries (Warrell, 2010). It is common in agricultural countries affecting farmers and plantation workers leading to significant increase in mortality and morbidity.

Based on escalated writing overview; *Vitex negundo* was chose for utilized as anti-venom activity in present examination. The writing survey demonstrated that the chose plants have potential as anti-venom activity. The *Vitex negundo* was identified and authenticated by Bhima Ram choudhary Deputy conservator of forest, Botanical Survey of India, Sikar, Rajasthan India.

The Leaves of *Vitex negundo* plant was conceal dried, decreased to coarse powder with the assistance of processor and put away in water/air proof compartment till further use. The leaves powder was characterized by its morphological features like white colour, presence of specific odour and taste.

Microscopical Observation of plant revealed specific structural character for each organ of plant. Quantitative microscopical parameters such as fiber length, size of trichome, starch grain dimension, stomatal number, stomatal index, vein-islet number and vein termination and crystal dimension were studied in the present study.

For powder microscopy, the shade dried leaves *Vitex negundo* were pounded into fine powder with the assistance of electric processor. The fine powder was then exposed to minute assessment according to the standard strategies. The residues remaining after incineration is the ash content of the leaves powder *Vitex negundo* .Ash values are helpful in determining the quality and purity of crude drug, especially in the powdered form. 10 gms of the air-dried coarse leaves powder of *Vitex negundo* were separately macerated with 100 ml of 90% ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18 hours. Loss on drying is the loss in weight in % w/w determined.

WHO indicates that restorative plant material ought to be liberated from any perilous or harmful unfamiliar issue and beyond what many would consider possible liberated from harmless unfamiliar issue.

The leaves powder of *Vitex negundo* plant was extracted by Hot extraction method with petroleum ether, chloroform, ethanol (90%) and water utilizing hot extraction strategy and These extracts were labeled as PEEAP, CEAP, EEAP and AEAP.

The total content of phenols in the crude all extract was determined using a modified Folin-Ciocalteu colorimetric method with Gallic acid as a standard, TLC was performed on 4 × 10 cm^2 plates covered with 0.25 mm layer of silica gel 60 F254 (Merck, Germany).

Crude drugs will undoubtedly defile with the pathogenic or non-pathogenic microorganisms. For the rough medications prepared for inner utilize the maximum furthest reaches of specific microorganisms like *E. coli*, *Salmonella*, complete high impact exercise has been given by WHO. The fluorescence examination of the powdered samples of *Vitex negundo* with different solvents and concoction reagents.

In the assessment and evaluation of the toxic characteristics of a substance, determination of acute oral toxicity is usually an initial step. It provides information on health hazards likely to arise from a short-term exposure by the oral route. Assurance of deposits of harmful substantial metals essentially lead, cadmium, arsenic and mercury by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) and Atomic Absorption.

Evaluation of antivenom activity by using *In vitro* anti-venom study was carried out by using five different types of research models. *Viz.* Phospholipase activity, Procoagulant activity, Fibrinolytic activity, Proteolytic activity and Hyaluronidase activity and *in vivo* techniques for different extracts of *Vitex negundo*. *In vivo* anti-venom study was carried out for PEEAP, CEAP.

Isolation and Characterization of bio potent Extract, The isolation was started with hexane and polarity increased with ethyl acetate, The elution up to hexane: DCM (7:3) were mixed together and refrigerated overnight with addition of di-ethyl ether, which yielded compounds (EEAPF1 and as a yellowish powder. Further elution up to hexane: DCM (2:8) yielded compound (EEAPF2).

Characterization of isolated phytoconstituent from ethanolic extract (EEAP and EEMA) by Spectral analysis Tools like Fourier transform infrared spectroscopy (FTIR) Agilent Cary 630 FTIR spectrometer , mass spectrometer (MS) Agilent 6520 (Q-TOF) , and nuclear magnetic resonance spectroscopy (NMR) Bruker II Avance 400 FT-NMR . With these tools the structures of most natural products can be determined.

For the Leaves powder of *Vitex negundo* was characterized by morphological features like light yellowish green colour, presence of specific characteristic and characteristic taste. T.S. of leaf showed its typical dorsoventral nature. Upper and lower epidermis, lamina, mesophyll, and midrib region were observed as important diagnostic characters. Palisade tissue appeared in double layer just below upper epidermis in lamina region. Midrib shows central nonlignified phloem, lignified xylem with well-defined xylem fibers, vessels, and parenchyma. Vascular bundles are phloecentric and surrounded by endodermis and crystal sheath. Well-defined patches of collenchyma were observed above and below the vascular bundles in the midrib area. Trichomes are present and stomata (anomocytic) were observed on both epidermal surfaces. In the mesophyll region, 2-3 lignified vein lets on either side of midrib in lamina portion and loosely arranged spongy parenchyma were observed.

Analytical parameters of *Vitex negundo* powder Moisture content (Loss on drying) 6.02± 0.05, 7.23± 0.04 Total ash value 10.13 ± 0.06, 11.12 ± 0.05 Acid insoluble ash value 0.51 ± 0.08, 0.65 ± 0.01 Water soluble ash value6.02 ± 0.04, 5.04 ± 0.03 Alcohol extractive value 9.74 ± 0.06, 9.92 ± 0.04 and Water extractive value19.14 ± 0.12, 18.10 ± 0.10 respectively.

Foreign matter of *Vitex negundo* powder 2.81 g and 2.16 g were obtained. The PEEAP and PEEMA were found to contain fats and oils, volatile oil, flavonoids, alkaloids, tannins and phenolic compounds. The EEAP, EEMA, AEAP and AEMA were found to contain carbohydrates, proteins, amino acids, glycosides, alkaloids, tannins and phenolic compounds. The CEAP and CEMA was found to contain fats and oils, steroids, volatile oil, saponins, glycosides, flavonoids, alkaloids, tannins, phenolic compounds and triterpenoids.

Phytochemical screening of the ethanol concentrate and water concentrate of organic product powder of *Vitex negundo* indicated the nearness of different phytoconstituents, for example, Carbohydrates, phenolics, tannins sap, and protein. Substantial metal substance was found beneath discovery limit.

Acute toxicity studies conducted revealed that the administration of graded doses of both the crude ethanol extracts (up to a dose of 5000 mg/kg) of leaf powder of *Vitex negundo* did not produce significant changes in behaviors such as alertness, motor activity, breathing, restlessness, diarrhea, convulsions, coma and appearance of the animals. No death was observed up to the dose of 5 g/kg body weight. The mice were physically active. These effects were observed during the experimental period (72 hrs).

The heavy metals determined i.e. Pb, Cd, As and Hg were seen as straight with connection coefficient (r) of more than 0.990 and 100.0, 10.0, 5.0, 5.0 for Pb, Cd, As and Hg; 2) 200.0, 20.0, 10.0, 10.0 for Pb, Cd, As and Hg; 3) 400.0, 40.0, 20.0, 20.0 for Pb, Cd, As and Hg The recuperations of Pd and Cd in tests are run between 85% to 98% and 80% to 97% separately for ICP-OES strategy and the recuperations of As and Hg in tests is extended between 78% to 98% and 76% to 98% individually.

Vitex negundo plant extracts and incubated at 37°C for 30 minutes prior to injection. We found that 0.15-0.17 mg of *Vitex negundo* plant extracts were able to completely neutralize the lethal activity of $2LD_{50}$ of *V. russelli* venom and 0.16-0.19 mg of *Vitex negundo* plant extracts were able to completely neutralize the lethal activity of $2LD_{50}$ of *Naja naja* venom.

The minimum edematic dose of the venom was found to be 4µg in rat. Significant inflammation was seen after 1hr of venom injection and maximum inflammation was seen at 180 min. Plant extracts at dose level 100mg/kg, 200mg/kg and 400mg/kg showed significant activity when compared with control and minimum % inhibition 29.13 27.1 at 100mg/kg, at 60 min 58.18 and 57.21 at 240 min for 400mg/kg, and std polyvalent antivenom 60.89.

Vitex negundo plant extracts 400 mg/kg were effectively antagonised the *V. russelli* and *Naja naja* venom induced defibrinogenating activity. The effective dose was found to be 1.3-1.6 mg and 1.7 to 1.9 mg .

The minimum haemorrhagic dose (MHD) of *V. Russelli* and *Naja naja* were found to be 24µg/200g rat. The plant extract showed significant neutralization of haemorrhage at all the dose levels, when compared with the standard polyvalent antivenom was Venom + extract 400 mg/kg highest 2.328±0.7023 AEAP, 2.339±0.4510 AEAP and lowest 2.667±0.8819 and 2.650±0.8014 PEEAP for *Vitex.*

Plant extracts were capable of inhibiting PLA_2 dependent hemolysis of sheep RBC's induced by *V. russelli* venom in a dose dependent manner. We found that that 0.11 to 0.13 mg.

The minimum coagulant dose (MCD) was determined as the venom dose inducing clotting of plasma in 60s. We found that that 0.11 to 0.13mg and 0.14 to 0.16 of plant extracts were able to completely inhibit PLA_2 dependent hemolysis of sheep RBC's induced by *V. russelli* and *Naja naja* venom respectively and 0.14 to 0.17mg and 0.13 to 0.19mg of plant extracts were able to completely inhibit PLA_2 dependent hemolysis of sheep RBC's induced by *V. russelli* venom and *Naja naja* respectively.

We found that that 0.11 to 0.18 mg and 0.10 to 0.13mg of plant extracts were able to completely inhibit fibrinolytic activity (modified plaque assay) induced by *V. russelli* and *Naja naja* venom. The *Vitex negundo* 0.13 to 0.18 mg of plant extracts was able to completely inhibit fibrinolytic activity (modified plaque assay) induced by *V. russelli*.

Spectral characteristics for isolated compounds (EEAPF1) showed absorption bands characteristics of flavanol indicating the presence of hydroxyl and tinsoporin functionalities at 3449, 1375 and 1079 cm^{-1} respectively, indication of double bond were represented by 1689 cm^{-1}. peak at 1375 cm^{-1} represented C-H bending where as NMR spectra was also characteristic of phytotaxanol, exhibiting the hydroxyl proton signal at δ 5.21as a multiplet, which helped us to characterize this compound as taxaterone, tinsoporin. Compound no (EEAPF2) tested positive for polyphenol by giving dark intensy violet color with ferric chloride reagent. The ^1H NMR of this compounds exhibited signals in range of 6.89- 7.97 and 3.4- 6.34 ppm that belongs to phenolic hydroxyls and D- glucose molecules respectively, and signals in the region 6.23- 7.97 ppm assigned to Gallic acid protons. Compound no (EEMAF1) tested positive for polyphenol by giving dark intensy violet color with ferric chloride reagent. The phenolic hydroxyls and D-glucose molecules respectively, signals in the region 6.23-7.97 ppm assigned to prove that it is ellagitannin, showing structural similarity to trimeric hydrolysable

nobotannins. Compound no (EEMAF2) tested positive for polyphenols. It presented IR spectra in the regions 3417, 2915, 2847, 1729, 1662, 1460, 1364, and 1259 cm $^{-1}$ indicating the presence of characteristics features for polyphenols. The ^{1}H NMR of this compound exhibited signals in the range of 6.89-7.97 and 3.4-6.34 ppm that belonging to phenolic hydroxyls and D-glucose molecules respectively, signals in the region 6.23-7.97 ppm assigned to betahydroxy protons.

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