

Phytochemical and Pharmacological Evaluation of Vitex Negundo Leaf

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ABSTRACT

Nature is always a shining example of the unique occurrence of symbiosis. All of nature's elements—biotic and abiotic, are linked together. Humans cannot exist without plants. *Vitex negundo* Linn. is an herbal medicinal plant commonly found in India. The leaves, roots, fruits and seeds possess hepatoprotective, antioxidant, anti-inflammatory, anti-analgesic, anti-anthelmintic, antipyretic, anticancer and antioxidant properties. This plant is very effective in the treatment of various types of disorders in the ayurvedic, homoeopathic and folklore system of medicine. The leaves intake provides relief in balancing Vata, Kapha, and Pitta. The present study evaluated the in-vivo analgesic activity of *Vitex negundo* leaf aqueous extract in mice using the tail flick method. The result showed a significant dose-dependent increase in the pain threshold, indicating analgesic activity. These findings suggest that *Vitex negundo* leaf aqueous extract possesses analgesic properties, supporting its traditional use in folk medicine for pain management. The bioactive compounds present in the aqueous extract may contribute to its analgesic effects. This study provides evidence for the potential use of *Vitex negundo* leaf aqueous extract as a natural analgesic agent. Further studies are needed to elucidate the mechanisms of action and to evaluate its safety and efficacy in humans.

Keyword: *Vitex negundo*, Physical evaluation, Pharmacological, Analgesic activity, Aqueous extract.

I. INTRODUCTION

Vitex negundo is large aromatic shrub belonging to family Verbenaceae. This plant is commonly known as Nirgundi or five leaved chastetrees.¹ It is woody aromatic shrub which has bitter, pungent, astringent taste. It is commonly found in India. The shrub growing from 2-4cm in height. *Vitex negundo* used as folk medicine in most of the states of India, Bangladesh and South east Asia.² *Vitex*

negundo usually grows in warm areas, including waste places, village area, riverbanks, and deciduous forests, where it is commonly found. The leaves are palmately compound, with a petiole that is 2.5-3.8cm long and 3-5 foliate. The middle leaflets are 5-10cm long, 1.6-3.2cm broad and have a petiolule that is 1-1.3cm long.³ The leaves of *Vitex negundo* contain iridoid glycosides, such as flavonoids and negundosides. Leaves contain a flavonal glycoside called vitexin. Stigmasterol and hentriacontane are present in dried root powder. The triterpene vitextriterpene, glucose, and p-hydroxybenzoic acid are present in the seeds.⁴⁻⁶ The plant finds mention in the verses of the Charaka Samhita which is unarguably the most ancient and authoritative textbook of Indian Ayurveda. *Vitex negundo* has been designated as an anthelmintic and is prescribed as a vermifuge in the exposition on the Charaka Samhita by Sharma.⁷

Plant Profile⁸

Kingdom: Plantae – Plants

Sub Kingdom: Tracheobionta - Vascular plants

Super division: Spermatophyta - Seed plant

Division: Magnoliophyta - Flowering plants

Class: Magnoliopsida - Dicotyledons

Subclass: Asteridea

Order: Lamiales

Family: Verbenaceae

Genus: *Vitex* Linn.



Fig. no.1

Species: *Vitex negundo* Linn. (Chaste tree)

Synonyms⁹

Sanskrit: Indrani, Nilanirgundi, Nilapushpa, Nirgundi, Renuka, Sephalika, Shephali, Shindhooaka, Sindhuvaram.

English: Five Leaved chaste tree

Hindi: Mewari, Ningori, Nirgandi, Nirgunda, Nirgundi, Nisinda, Sambhal, Sambhalu.

Urdu: Sambhalu, Tukhmsambhalu.

Bengali: Nisinda, Sinduari, Beguna, Nishinda, Nishinde.

Kannada: Bile-nekki, Bilenekki, Karilakki, Lakkagida, Lakki, Lakki-gida, Lakkili.

Malayalam: Bem-nosi, Indrani, Karunocci, Nochi, Nochi, Vella-noch-chi.

Marathi: Nirgunda, Nengar, Nirgur, Nirguda, Nirgundi, Lingud, Negumd, Lingur.

Odia: Thinghawilupa, Niligundi.

Tamil: Nallanocci, Nirkkundi, Nirkundi, Nochi, Sinduvaram.

Telgu: Nalla-vavili, Nallavavili, Sindhuvaruma, Sinduvaramu, Tell-vavili.

Habit:

Vitex negundo is a small tree or shrub.

Habitat:

Vitex negundo is commonly found in tropical and subtropical region including India, China, Japan, Southeast Asia.

Materials and methods:

Collection of plant:

The plant was collected from Baidpali, Paikmal in the Bargarh District, Odisha.

Authentication:

The herbarium was prepared and was sent to Department of Botany, Gangadhar Meher University, Sambalpur, Odisha-768001 for proper authentication.

The sample was identified to be as *Vitex negundo* Linn.

Drying and pulverization:

The collected plant material (leaves) was shade and dried at room temperature, then they are pulverized in mixture grinder to coarsely powdered drug and passed through mesh size 40 sieve.

Macroscopy:

Leaves: Bright green on top, pale green or white on the bottom

Flower: Purple in colour.

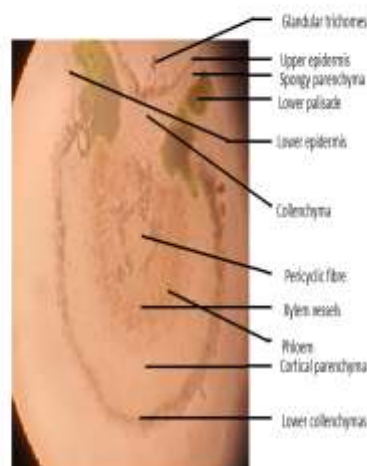
Stem: Green or greyish-green

Fruits: Purple-black when ripe

Bark: Greyish-brown in colour and thin bark

Root: Brown or greyish-brown

Microscopy:



T.S OF VITEX NEGUNDO LEAF

Fig. no. 2

T.S of leaf

Petiole - shows single layered epidermis having a number of unicellular, bicellular and uniseriate multicellular covering trichomes and also glandular trichomes with unicellular to tricellular stalk and unicellular to bicellular head; cortex composed of outer collenchymatous tissue and inner 6 - 8 layers of parenchymatous tissue; collenchyma well developed in basal region and gradually decreases in middle and apical regions; pericyclic fibres absent in basal region of petiole and present in the form of a discontinuous ring in apical region surrounding central horse shoe-shaped vascular bundle; a few smaller vascular bundles present ventrally between arms of central vascular bundle and two, or rarely three, bundles situated outside the arms.

Lamina - shows single layered epidermis having mostly unicellular hairs, bi and multicellular and glandular trichomes being rare; hypodermis 1 - 3 layered interrupted at places by 4-8 palisade layers containing chlorophyll; a large number of veins enclosed by bundle sheath traverse mesophyll; stomata present only on the ventral surface.

Preparation of Extract:

The leaves were collected from a healthy plant. Collected leaves were washed thoroughly with running tap water and dried at room shade for 15 days. Extract was prepared by cold extraction process. After grinding to fine powder of mesh 40 it was successively extracted with distilled water in a 1000ml round bottom flask. The resulting extracts were filtered. Further the solvents were evaporated to dryness and then the residue of extract obtained was taken for the experiment.

Physical evaluation:

The result of the physical evaluation shown in the table no. 2

Phytochemical evaluation:

Table no.3 shows the presence or absence of various phytochemical constituents in Vitex negundo leaf.

Pharmacological screening

Analgesic activity of Vitex negundo leaves

Acute toxicity study:

The experimental procedure will be begun by selecting healthy male albino rats with weights ranging from 200 to 220 grams. These rats will be then divided into four groups, each consisting of six animals. Prior to administration of the test substance, the animals will be fasted overnight to standardize conditions across the groups. Subsequently, different doses of the extract will be administered to the respective groups of animals as per the experimental design. This approach ensures consistency and allows for the systematic evaluation of the effects of the extract across different dosage levels.

Group-I (Control)

The rats assign to group-I will be orally administered with 1 ml per 100 grams of body weight of a 10ml distilled water solution. These rats will be served as the control group for the experiment. This control group allows to compare the effects of the experimental treatment with those of a standard vehicle solution, ensuring that any observed effects are attributable to the administered extract rather than the vehicle itself.

Group- II, III, IV (Test):

The animals design as test groups II, III, and IV will be orally administered a single dose of Vitex negundo leaf extract at concentrations of 200 mg/kg, 700 mg/kg respectively. The extract will be delivered in a 10ml distilled water solution. Subsequently, these test animals will undergo

continuous observation for a period of 24 hours, which will be monitored any physiological changes, potential adverse effects, or mortality. This observation period will extend over 14 days, allowing for a comprehensive assessment of the short-term and longer-term effects of the administer Vitex negundo leaf extract at varying dosage levels. This rigorous observation protocol is crucial for evaluating the safety and potential toxicity of the extract under investigation.

Analgesics are medication that is used to treat pain. They are sometimes referred to as pain relievers or painkillers. In medical terminology, an analgesic is a drug that relieves pain without causing sleepiness or unconsciousness. Many different kinds of medicines have the ability to relieve pain, and medical professionals typically combine medications that function similarly. Nonsteroidal anti-inflammatory medicines (NSAIDs) and opioids (narcotics) are two of the most popular classes of pain relievers, but there are many more.



Fig. no.3

Materials:

- Healthy Albino rats
- Thermometer
- Aqueous extracts
- Diclofenac sodium
- Gastric tube

Method:

Tail-flick Method:

Healthy Wister strain albino rats will take. A half hour is given to the animals in their cages to adjust before testing. A stopwatch was used to record the reaction time. Gastric tubes were used to provide the standard test substances to the animals. After the administered of the medication, the reaction time was recorded every 30,60, 120 and 240 minutes. The average reaction time was calculated and compared with the value of standard drug.

Group-I (Control):

Animals in group-I will be received an oral administration of 10ml/100gm of body weight in distilled water and the serve as control.

Group-II (Test):

Animals in test groups II will be orally given the aqueous extract of Vitex negundo at

doses of 80-250mg/kg body weight in distilled water. After drug administration. the temperature of the ratsdifferent groups will be recorded at 0,1,2,and 3 hours. Subsequently, the mean temperature of the test groups will be compared with the standard group to evaluate the analgesic effect of the test substance.

Table no. 1 Analgesic activity

Sl. No	Treatment	0min	30min	60min	120min	240min
1.	Control	5.2±0.15	5.6±0.10	5.3±0.22	6.1±0.17	5.8±0.24
2.	Standard (Diclofenac)	6.1±0.20	7.8±0.23	9.5±0.19	10.8±0.34	12.3±0.35
3.	Test (Aqueous extract)	5.5±0.32	7.2±0.42	10.2±0.39	11.5±0.32	12.6±0.43

Table no.2 Physical evaluation

Sl. No.	Parameters	Values (%)w/w
1	Loss on drying	3.8
2	Foreign matter	2%
3	Ash value	-
	A. Total Ash	0.12
	B. Acid insoluble	1%
	C. Water soluble	2%
4	Extractive Values	-
	A. water soluble extractive	21%
	B. Methanol soluble	2%
	C. Ethanol soluble	16%
	D. Chloroform soluble	4%
	E. Pet-ether soluble	2%
	F. Ethyl acetate soluble	5%
G. n-hexane soluble	1%	
5	Swelling index	0.1
6	Foaming index	0.4

Table no.3 Phytochemical evaluation

Plant constituentstest/reagent used	Powdered drug	Aqueous extract
Test for carbohydrates		
Molisch's Test	-	-
Fehling's Test	-	-
Benedict's Test	-	+
Test for proteins		
Millon's Test	-	-

Xanthoproteic Test	+	+
Test for alkaloids		
Dragendroff's Test	+	+
Hager's Test	+	-
Wagner's Test	-	+
Test for glycoside		
Legal's Test	+	+
Test for tannin and phenolic compounds		
5% FeCl ₃ solution	-	+
Gelatin solution	-	-
Acetic acid	-	+

II. RESULT AND DISCUSSION:

The macroscopic characters of vitex negundo leaf, such as bright green & pale green leaves, purple flowers, greyish brown with thin bark, fruits are purple black when ripe. The microscopical characters are- single layered epidermis having mostly unicellular hairs, paracytic stomata, unicellular & glandular trichomes with unicellular to tricellular stalk. Overall, the results of this study provide a detailed description of the macroscopic and microscopic characters of Vitex negundo leaf, which can be useful in botanical identification and pharmacognostic studies. Vitex negundo is one of the major plants which has wide applications in traditional systems of medicines practiced in different countries.

Physical evaluation of the powder drug showed 0.12% w/w, acid insoluble ash 1% w/w and water-soluble ash 2% w/w. 21% water soluble extractive, 2% methanol soluble extractive, 16% ethanol soluble extractive, 4% chloroform soluble extractive, 2% petroleum ether soluble extractive, 5% ethyl acetate extractive, 1% n-hexane soluble extractive, loss on drying 3.8%, foreign matter, swelling index 0.1%, foaming index 0.4%.

The phytochemical results show that the aqueous, ethanol extract and powder drugs of leaves contain alkaloids, glycoside and phenolic compound.

The aqueous extract of Vitex negundo leaf showed significant anti-analgesic activity in the tail

immersion method, with a maximum inhibition of 40% at a dose of 200mg/kg.

III. CONCLUSION:

Traditional medicine, sometimes referred to as indigenous medicine or folk medicine, is the medical use of traditional knowledge that, prior to the development of modern medicine, developed over many centuries within the folk beliefs of various communities, including indigenous peoples. In conclusion, our study demonstrates that the aqueous extract of Vitex negundo leaf exhibits significant analgesic activity. The extract showed a dose-dependent reduction in pain perception, indicating its potential as a natural pain reliever.

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