

Screening the Bioactive Compound and Analyze the Membrane Stabilization Property of *Vitex negundo* Aqueous and Alcoholic Extract

Puspal De^{1*}, Subhradeep Sarkar¹, Madhumita J Mukhopadhyay²

¹Department of Genetics, Institute of Genetic Engineering, 30 Thakurhat Road, Kolkata- 700128, West Bengal, India.

²Department of Biotechnology, Institute of Genetic Engineering, 30 Thakurhat Road, Kolkata- 700128, West Bengal, India.

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ABSTRACT

Since the ancient time nature is providing us with several beneficial herbal phyto-chemicals. *Vitex negundo* is one of the common and valuable herbs in traditional medicine. In the current study, qualitative screening of different bioactive compounds and membrane stabilization property was checked in aqueous and alcoholic extract of *Vitex negundo*. Three different concentration 50, 75, 100 microgram/ml was taken for membrane stabilization method using 62.5 microgram/ml sodium diclofenec as a reference drug. The study revealed that *V. negundo* have several bioactive compounds and significant membrane stabilization property. Presence of several bioactive phytochemicals may help in scavenging the reactive oxygen and potent membrane destabilizing in human. This study aims to provide a better understanding of the phytoscience and scope for adopting novel phytomedicine for membrane destabilization related disease in human.

Keywords: *Vitex negundo*, membrane stabilization, bioactive compound, plant extract

INTRODUCTION

Inflammation is a localized physical condition in which part of the body becomes reddened, swollen, hot, and often painful, especially as a reaction to injury or infection and it is frequently associated with pain. The inflammatory process involve occurrence of protein denaturation, increase of vascular permeability and membrane destabilization¹. In broad aspect inflammation is categorized mainly into two categories- acute and chronic. But, according to histological features, causative agents, predominant component, the inflammation is categorized in categories like specific, nonspecific, aseptic, septic, alterative, exsudative and proliferative. Generally acute inflammation occurs due to microcirculation but persisting infection or prolonged exposure to irritants, repeated acute inflammation, autoimmune reaction are the main causes of chronic inflammation². The management of inflammation related diseases is a big challenge to the medical practitioner especially for chronic inflammatory disease like arthritis as there are huge side effects for the long term consumption of anti-inflammatory drug³. Phytomedicines come as alternative therapy for these types of chronic problems along with improvement of clinical research and proper analysis.

Nature provides huge medical agents for thousands of years and a significant numbers of modern human drug are isolated from natural resources. Traditional medicine, folk medicine and ayurvedic medicine now become an important source of chemotherapeutic agent⁴. So there is an urgent need to developed new plant based drug with better bioactive potential and without or less side effects.

So, the present study emphasizes on the qualitative phytochemicals screening and *in vitro* anti-inflammatory property of aqueous and ethanolic extract of *Vitex negundo* by membrane stabilization method.

Vitex negundo, commonly known as *nisindha* in Indian sub continental region, are native to east and South Africa and Asia. It is an erect shrub with reddish brown bark, digitate leaves with five lanceolate leaflets belongs to the family of verbenaceae found throughout the India⁵. Different parts of *nisindha*, have been used in traditional Indian ayurvedic medicines as sedative, chronic bronchitis and cold and have a great value as constituents of ayurvedic preparations such as “Vishagarbha thaila” which is widely used to treat rheumatism in India⁶. It is also frequently used in Chinese herbal medicines. Leaves are also possessed potent vermifuge, febrifuge, astringent property as well as mosquito repelling activity and anti tumor activity⁷.

MATERIALS AND METHODS

Collection of Sample

Fresh leaves of *Vitex negundo* were collected from a local horticultural garden during the month of March and the plant was identified by Prof. Sandip Mukhopadhyay, Professor and Head, Department of Botany, University of Calcutta.

Preparation of Extract

Fresh leaves were washed twice through running tap water followed by distilled water and air dried. After proper drying leaves were blended to make a fine powder. The shade dried powder of leaves was stored in room temperature for future use. The dried powdered 1 gm

leaves were taken in two different pre-labeled conical flask and pour 40 ml of double distilled de-ionized water and ethanol separately. Then the mixtures were kept in the BOD shaker incubator at 30°C temperature in 120 rpm for overnight. Next day both the mixture was filtered through Whatman filter no- 1. During the membrane stabilization assay every time freshly prepared aqueous and alcoholic extracts were used.

Membrane stabilization assay

To study the anti-inflammatory activity, the HRBC membrane stabilization method was adopted after Gandhisan 1991¹¹. Fresh blood was collected from healthy donors without the history of NSAIDS administration for at least two weeks prior to the experiment. The equal volume of sterilized Alsever solution and blood were mixed, the mixture was centrifuged at 3000 rpm and packed cell were washed twice with isosaline. The washed packed cell was made a 10% (v/v) suspension with isosaline to make a HRBC suspension. The assay mixture contained 1ml PBS (pH 7.4), 2ml of hyposaline (0.36% KCL), 0.5ml HRBC suspension and 1 ml of Test solution. Sodium diclofenec was used as reference drug and distilled water was used as control. All the assay mixtures were incubated at 37°C in an incubator for 30 minutes and after that centrifuged at 1000 rpm for 2 minutes. The supernatant containing haemoglobin was estimated using spectrophotometer at 560 nm.

Phytochemical Screening

Freshly prepared extract of *Vitex negundo* was screened for the presence of bioactive compounds like alkaloids, flavonoids, tannin, carbohydrate, amino acids and proteins, terpenoids, saponin, sterols etc. The qualitative analysis was done by the standard method of Harbone⁸.

RESULT

In the current study, presence of different bioactive compound in the extract of *Vitex negundo* was depicted in Table-1 and the membrane stabilization property of aqueous and ethyl alcoholic extract was depicted in Table-2. Concentration of reference drug and the experimental samples were also mentioned in the same table.

DISCUSSION

In the modern age of pharmaceutical research use of animal models are associated with certain problems like ethical issues and different mechanism of body homeostasis during adverse condition. So, other suitable methods on the view point of basic mechanism are necessary⁹. Keeping this in mind, in the present study the membrane stabilization assay methods are selected for assessment of *in vitro* anti-inflammatory property of *Vitex negundo*. Membrane de-stabilization is one of the key features of inflammatory tissue and it was a well documented cause of inflammation related disease like arthritis. Agents that can help in membrane stabilization a potential may be used as a potential anti-inflammatory drug in future.

The literature study revealed that a number of plant extracts showing significant amount of membrane stabilization property. Some of these include extract of

*Gynandropis gynendra*¹⁰, *Lanea coromandelica*¹¹, *Gmelina asiatica*¹², *Lantana camara*¹³, *Albuca setosa*⁹, *Wrightia tinctoria*¹⁴. This present study also agreed with other survey of *Vitex negundo* showing antimicrobial activity⁴, antioxidant activity¹⁵ and reduction of carrageenan induced paw edema of wister rats¹⁶.

During inflammation there was excess stimulation of phagocyte trigger the production of reactive oxygen species. like O_2^- , H_2O_2 , $\bullet OH$ ¹⁷, which are normally produced during respiration as 5% of inhaled oxygen was converted to direct oxidizing superoxide¹⁸ and indirectly as with hydrogen peroxide (H_2O_2) and hydroxyl radicals ($\bullet OH$) formed from O_2^- . This reactive oxygen harms the surrounding tissue by lipid peroxidation which leads membrane destruction and provokes inflammatory response by the production of mediators and chemotactic factors¹⁹. It has been observed in various arthritic disorders that the reactive oxygen species are also known to activate matrix metalloproteinase (e.g. collagenase) causing increased destruction of tissues²⁰.

In the present study the *in vitro* membrane stabilization effect of *Vitex negundo* was evaluated against hypotonicity induced erythrocyte membrane destabilization. The present finding exhibits concentration dependant membrane stabilization by the selected plant extract. The inflammatory response was generated by the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases which leads more tissue inflammation by extra cellular release. As erythrocyte membrane is analogues to lysosomal membrane so, the stabilization of erythrocyte membrane implies exact mechanism as to stabilize lysosomal membrane.

Qualitative analysis revealed presence of several phytochemicals like alkaloids, flavonoids, polyphenols, sterols, carbohydrate, tannin in *Vitex negundo* extract. Among these bioactive compounds several showed well known potential biological properties. The membrane stabilization property of nisindha (*Vitex negundo*) may be due to the presence of these bioactive compounds. The effect may be synergistic rather than single one.

CONCLUSION

It has been reported that several non-steroidal anti-inflammatory drugs have the ability to stabilize induced membrane destabilization. Therefore, from the findings of the present preliminary experiment it can be concluded that the ethyl alcoholic and aqueous extract of *Vitex negundo* possess membrane stabilization effect *in vitro*. So, the anti-inflammatory effect of this plant should be further evaluated in pursuit of newer phytotherapeutics against inflammatory diseases.

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Table 1: Bioactive compound analysis in the extract of *Vitex negundo*.

Perticulars	Observation
Alkaloids	+++
Flavonoids	+++
PolyPhenols	+++
Tannin	+++
Carbohydrate	+++
Saponin	---
Carbonyls	+++
Terpenoids	---
Proteins	+++
Sterols	+++
Triterpensess	+++
Anthraquinone	---

+++ =Positive, --- =Negative

Table 2: *In vitro* membrane stabilization activity of aqueous and ethanolic extract of *Vitex negundo*.

Treatment	Concentration (µg/ml)	% Inhibition
Control	-	-
Ethyle	50	43.40
Alcohol	75	44.60
Extract	100	46.40
Aqueous	50	34.10
Extract	75	34.90
	100	47.00
Sodium	50	48.99
Diclofenec		

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing interests.

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