



STUDIES ON THE CHEMICAL AND MEDICINAL VALUE OF *VITEX NEGUNDO* Linn.

^aBrindha, S., ^bThamaraiselvi, T., ^cMaragathavalli, S., ^dAnnadurai, B. & ^dGangwar, S. K.

^aDepartment of Bioinformatics, New Prince Arts and Science College, Chennai,

^bDepartment of Biochemistry, Government Art College for women, Krishnagiri, TN.

^cDepartment of Biochemistry, King Nandi Varman College of Arts and Science, Thellar,

^dDepartment of Crop and Horticultural Sciences, Biotechnology Team, College of Dryland Agriculture and Natural Resources, Mekelle University, Mekelle, Ethiopia.

ABSTRACT

The present study was aimed to study the chemical value and medicinal value of *Vitex negundo* Linn. Herbal medicines are the precursors of many common drugs prescribed in clinical practice modern western industrial countries today. In the present study the selected plant *Vitex negundo* Linn., belongs to the family *Verbenaceae*, which is known to possess several medicinal properties. This plant has been extensively investigated by many scientists for its medicinal activities. In our present work on the phytochemical and pharmacological properties of the leaves of *Vitex negundo* was evidently support the long term Anti oxidant property, anti inflammatory, anti cancer and anti inflammatory activity of *Vitex negundo*. The studies on *Vitex negundo* leaves were showed good potential to utilize this plant for commercial purposes.

KEY WORDS: *Vitex negundo*, phytochemicals, secondary metabolites, Thin layer chromatography.

INTRODUCTION

Plant derived material or preparation with therapeutic or other human health benefits, which contains either raw or processed ingredients from one or more plants. Many of these indigenous plants have shown very significant therapeutic activities like hepatoprotective, anti-inflammatory, antihelmintic etc. (Kokkate, et al., 1988). Principle indigenous systems are homeopathy, herbal medicines (medical herbalism) and aromatherapy. World trade in plant medicines is in billions of dollars. The number of medicinal plants in trade too is astonishing. Germany imports at least 1560 plant species for medicinal purposes (Fransworth, N.K. et al., 1991).

The aim of the present study is phytochemical and pharmacological activities of a medicinally well known plant *Vitex negundo*. Phytochemical studies includes determination of water extractive value of the leaves of *Vitex negundo*, qualitative analysis of the water extract and thin Layer Chromatographic studies of water extract with isolation and quantization of various phytoconstituents from the leaves of *V. negundo*.

Total Glycoside content, Total Tannin content, Total Alkaloid content, Distillation of volatile oil from the leaves of *V. negundo* and identification of its various phytoconstituents through GC-MS analysis. Thin Layer Chromatographic studies of the volatile oil. Pharmacological Studies includes Antioxidant studies of the water extract of the leaves were done by using following methods Superoxide scavenging activity, Hydroxyl scavenging activity, Lipid peroxide scavenging activity, Anti-inflammatory activity of the water extract in Balb/c mice. Antioxidant studies of total Glycosides include Superoxide scavenging activity. Hydroxyl scavenging activity Anti-inflammatory activity of total

Glycosides in Balb/c mice. Anti-inflammatory activity of the volatile oil in Balb/cmice.

MATERIALS AND METHODS

A laboratory experiment was carried out to assess the effect of *Vitex negundo*.

Plant

The leaves of *Vitex negundo* Linn., belong to the family *Verbenaceae*, were collected from Vellore area and the authentication was done at Presidency College Herbarium in Chennai.

Animals

Mice were supplied by Animal Breeding Station, University of Veterinary and Animal Science, Chennai.

Phytochemical evaluation of *Vitex negundo*

Phytochemical investigation of a plant essentially involves a preliminary extraction, separation and isolation of the individual constituents of interest, their purification and characterization.

Extraction

Extraction is the removal of constituents from the drug. The process involves the separation of medically active portion of plant or animal tissue from the inactive or inert components by the use of selective solvent by standard extraction procedures. The principal methods of extraction are.

1. Maceration
2. Percolation
3. Digestion
4. Infusion
5. Decoction
6. Continuous hot extraction (soxhlet extraction).

The continuous hot extraction method was used for the experimental work. This is a classical chemical procedure of obtaining organic constituents from dried plant tissues.

In this method the powdered material is continuously extracted in a Soxhlet apparatus with a range of solvent beginning from non polar solvents like petroleum ether and ending with the most polar solvent i.e., water so as to ensure a complete extraction.

In the soxhlet apparatus the vapour passes through the side tube and reflux returns to the extraction chamber where the solution collects. As this takes place the liquid level will also rise in the return tube, when the liquid reaches the top of the return tube a siphon is set up and content of the extraction chamber are transferred to the flask.

Experiment

The coarsely powdered, air dried leaves of *Vitex negundo* was extracted by using the continuous hot extraction method. The solvent used as water. The extract was subsequently used for preliminary phytochemical screening of alkaloid, glycosides, volatile oils, tannins, resins etc

METHODS FOR PHYTOCHEMICAL ASSAY

a. Total glycosides (Stass-Otto method)

Coarsely powdered crude drug (2g) was percolated with dilute ethanol (50%). The tannins were precipitated from the percolate by the addition of a solution of lead acetate until the precipitation was complete. The precipitate was centrifuged. The precipitate of lead tannate was rejected whereas the supernatant was retained. Hydrogen sulphide was bubbled through the supernatant solution to remove the excess of lead as lead sulphide. The solution was filtered to remove the precipitate and then evaporated to dryness to get the residue of total glycosides. (Turner *et al.*,1975 Amount of total glycoside was estimated and result is in Table no:3.

b. Total Tannins

3g of the powder was shaken in 100ml of water for 30min. on a mechanical shaker. The extract was filtered and the residue was washed thoroughly with water. The filtrate was treated with lead acetate to precipitate the tannins as lead tannate. The precipitate was centrifuged, washed with water and suspended in ethanol. Then hydrogen sulphide gas was passed to remove excess of lead. The solution was filtered. The precipitate was discarded and the filtrate was evaporated to dryness on a water bath to a constant weight. Amount of total tannin was estimated and result is given in table no:3

c. Total Alkaloid content

Accurately weighed (2 grams) crude drug was extracted with 95% ethanol in a soxhlet extractor till extraction was complete. The extract was evaporated to dryness on a water bath and treated with dilute HCl (50ml). The solution was filtered and the filtrate was made alkaline with sodium carbonate and extracted with 3 portions of chloroform of 25ml each. The chloroform extracts were collected together and evaporated to a constant weight on a water bath. Amount of total alkaloid was estimated and result is in table no:3

Antioxidant assay of glycosides isolated from the leaves of *Vitex negundo*

a. Determination of superoxide scavenging activity of glycoside

Superoxide scavenging was determined by nitroblue tetrazolium reduction method. Principle, procedure and

calculations of this method were same to that of water extract. One difference was, instead of various concentrations of water extract, here various concentrations of glycosides were taken.

Anti-inflammatory activity of glycosides isolated from the leaves *Vitex negundo*

Acute and chronic anti-inflammatory activity of glycoside isolated from the leaves of *V. negundo* was evaluated by the method of carrageenan and formalin induced paw oedema in mice hind paw.

Anti-inflammatory activity of water extract

Acute and chronic anti-inflammatory activity was evaluated. The former was done by the method of carrageenan induced paw-oedema in mice and the latter by formalin induced oedema in mice hind paw.

a. Carrageenan induced paw oedema in mice

Animals were divided in to four groups with four animals in each group. In all group acute inflammation was induced by sub plantar injection of 0.02ml of freshly prepared 1% suspension of carrageenan in normal saline in right hind paw of mice. One group was kept as the control, the second group kept as the standard reference and they have administered orally with 10mg/kg diclofenac. The third group received 100mg/kg and fourth group 250mg/kg of water extract orally 1 hour before to the sub plantar injection of carrageenan. The paw thickness was measured initially before carrageenan injection and during six consecutive hours (1 hour interval) after carrageenan challenge by using vernier calipers.

Oedema was calculated as the difference between the two measurements. Reduction of swelling was determined by comparing the changes in hind paw volume in drug treated, standard and control mice.

b. Formalin induced chronic paw oedema in mice

The animals were divided in to four groups with four animals in each group. In all groups, chronic inflammation was induced by sub- plantar injection of 0.02ml of 2% formalin in the right hind paw of mice. One group was kept as control while the second group referred as standard, which is treated with 10mg/kg diclofenac orally one hour prior to the sub plantar injection. The third group received 100mg/kg and fourth group received 250mg/kg of water extract orally one hour before to the formalin injection. The administration of the diclofenac and extract was continued for six consecutive days. Degree of inflammation was measured using vernier calipers before and 6 days after formalin challenge.

Thin Layer Chromatography (TLC)

TLC of the volatile oil was performed using silica gel poured on a glass plate as stationary phase and Benzene: Ethylacetate (95:5) as a mobile phase and Rf value was calculated.

Gas chromatograph/mass spectrometer (gc/ms) analysis

Gas chromatograph is the ideal technique for the separation of thermally stable and volatile compounds. The separated compounds are converted to ions in the mass spectrometer and are separated according to mass to charge ratio. The mass spectrum is plotted as detector output against mass.

RESULTS

The results obtained by the evaluation of leaves extract and the percentage of phytochemicals given in the table 1 and 2. The preliminary phytochemical screening indicated the presence of glycosides, alkaloids and tannins are showed in the table 2. There are various kinds of complex secondary metabolites such as glycosides, alkaloids, flavonoids, volatile oils are having pharmaceutical significance. (Ramawat, *et al.*, 1999). Increased antioxidant intake may help to reduce oxidant stress and to minimize the development of asthmatic symptoms. (Miric, M. *et al.*, 1991) A series of acylation reactions were performed on this compound to increase its cytotoxic potency. One of these derivatives viz 5-3'-dihexanoyloxy-3,6,7,4- tetramethoxy flavone has comparative cytotoxic potency to vitexicarpin this indicate the presence of anticancer property of *vitex negundo* (Diaz, F. *et al.*, 2003) Table 3 fig. 1 shows the oral administration of the water extract close to 100mg/kg and 250mg/kg was found to inhibit the carrageenan and formalin induced paw oedema this was indicated by the presence of anti-inflammatory

activity of *Vitex negundo* (Chawla, A. S. *et al.*, 1992) & Green Wald, R.A., 1991). Water extract of *V. negundo* was found to scavenge the superoxides generated by photo reduction of riboflavin. The concentration of water extract of *V.negundo* needed for 50% inhibition (EC50) of superoxide radicals were found to be **99**µg/ml this indicate the presence of antioxidant property of *Vitex negundo*. Degradation of deoxyribose mediated by **hydroxyl radical** generated by the Fe³⁺/ascorbate/EDTA/H₂O₂ system was also found to be inhibited by the *V. negundo* extract. The concentration of water extract needed for 50% inhibition (EC50) was found to be **80**. Water extract of was found to inhibit **lipid** peroxides generated by the induction of Fe²⁺/ascorbate/Fe³⁺/ADP/ascorbate in rat liver homogenates. The concentration of the water extract needed for the 50% inhibition (EC50) was found to be **95**µg/ml

TABLE 1. Extractive Value As per Hot Soxhlet Extraction

Solvent	Percentage
Water	26%

Preliminary Phytochemical Screening

The results obtained in the qualitative chemical examination of the extract was showed in Table No: 2

Plant Constituents	Water extract	
Test/Reagents	Colour	Result
1. Alkaloids -Dragendorff's reagent	Yellow	+ve
2. Tannins- Ferric Chloride Solution	Greenish black	+ve
3. Sterols- Concentrated Sulphuric acid	Red ring	+ve
4. Glycosides -Liebermann's – Burchard reagent	Pink Colour	+ve
5. Flavonoids- Ethanolic extract + Magnesium ribbon + Concentrated HCl	Orange Colour	+ve
6. Resins- concentrated Nitric acid	No colour change	-ve
7. Carbohydrates- Molisch's reagent	Pinkish ring	+ve
8. Test for polysaccharides- Anthrone test	Green ring	+ve
9. Protein- Pottassium Iodide + Iodine solution	Yellow colour	+ve

FIGURE-1

Chronic Anti-Inflammatory Activity of Water Extract of *V.negundo* in Formalin Induced Inflammation

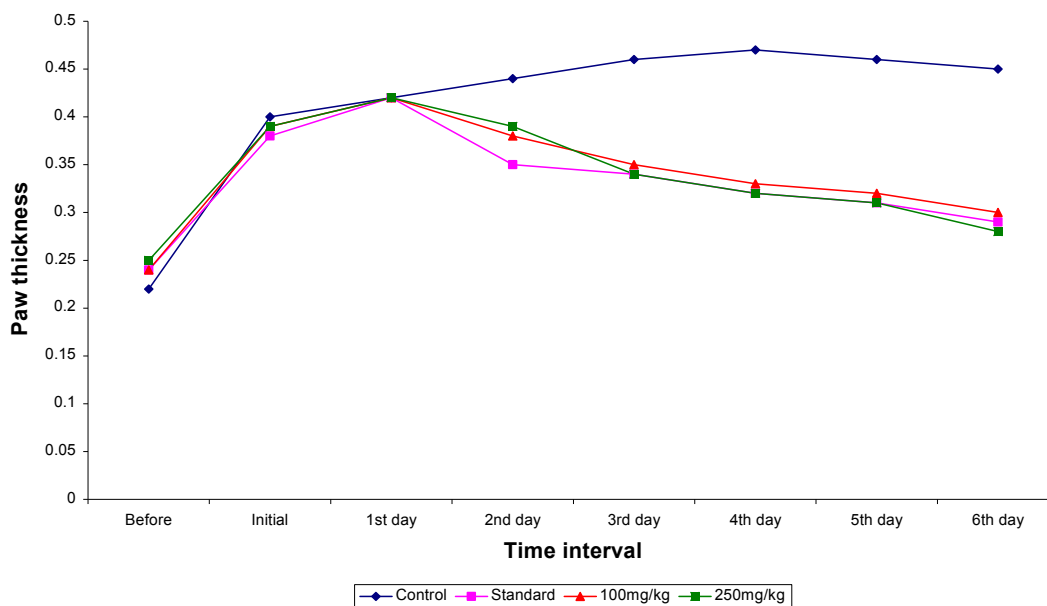


TABLE 2. Chronic anti-inflammatory activity of Glycosides

Groups	Paw thickness before formalin nection (cm)	Paw thickness after 6days (cm)	Increase in paw thickness(cm)	% of inhibition
Group-I Control	0.21 ± 0.01	0.445 ± 0.006	0.225 ± 0.008	-
Group-II Standard - Diclofenac(10mg/kg)	0.238* ± 0.017	0.285*** ± 0.013	0.047 ± 0.005	79%
Group-III Glycosides (250mg/kg)	0.224* ± 0.012	0.243*** ± 0.005	0.019 ± 0.002	91%

TABLE: 3 Amount of total glycoside, Tannins and Total Alkaloids was estimated

Constituent	Percentage w/w
Total glycosides	25%
Total Tannins	15%
Total Alkaloids	10%

DISCUSSION

Medicinal plants contribute nearly 25% of the prescribed drug in the world market. In recent years screening of such plants for biological activities has resulted in the development of therapeutics used in the treatment of cancer, AIDS and others. A large number of medicinal plants are exploited from natural flora for the commercial production of the drugs. In recent study revealed that the phytochemical and pharmacological activities of a medicinally well known plant *Vitex negundo*, the results of present phytochemical and pharmacological observations may evidently support the long term Anti oxidant property, anti inflammatory, anti cancer and anti inflammatory activity of *Vitex negundo*. The studies on *Vitex negundo* leaves showed good potential to utilize this plant for commercial purposes.

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